Volume 670, Number 1 / July 2011

Hydrobiologia

Marine Ecosystems and Sustainability

G.J. Pierce, V.D. Valavanis,
M.B. Santos & J.M. Portela

Hydrobiologia (2011) 670:1–4
DOI 10.1007/s10750-011-0739-8

ECOSYSTEMS AND SUSTAINABILITY

Preface: European Commission's Marie Curie Action ECOSUMMER

Graham J. Pierce · Vasilis D. Valavanis ·
M. Begoña Santos · Julio M. Portela

Published online: 24 May 2011
© Springer Science+Business Media B.V. 2011

Marie Curie (born Marja Skłodowska) was the first person to win Nobel Prizes in two sciences (in Physics-1903 and in Chemistry-1911). She moved from her native Poland to study and pursue a scientific career in France and became well known for her dedication, persistence and passion for work. Her scientific achievements are all the more remarkable for taking place at a time in history when conditions for research and in particular for women in science were often difficult. She was well liked by both her colleagues and friends, to whom she was able to communicate the passion she felt for her work.

Hence, it is fitting that the European Commission named its programme of training awards for young researchers after Madame Curie. Marie Curie Actions provide the opportunity for young researchers to improve their research skills and enhance their career prospects through competitive and intensive efforts, mobility and dedication; characteristics that will always contribute to the establishment of a promising scientific career. Specifically, Marie Curie Initial Training Networks (ITN) promote the mobility of young researchers, between different European countries and into Europe from other parts of the world, an important step that exposes them to a variety of established research centres and universities in order to meet, interact with, and collaborate with other researchers, expand their research ideas and horizons, and develop those character attributes required in the increasingly competitive field of research.

This issue presents some of the scientific output of one such network in the field of marine sciences. ECOSUMMER (ECOsystem approach to SUstainable Management of the Marine Environment and its living Resources) was a 4-year (2006–2009) multi-host Training Site for Early-Stage Research Training (EST). Eight universities and research institutes collaborated in the project: the University of Aberdeen (UABDN, UK), the Fisheries Research Services Marine Laboratory (FRS, UK), the Scottish Association for Marine Science (SAMS, UK), the Hellenic Centre for Marine Research (HCMR, GR), the Instituto Español de Oceanografía (IEO, ES) with

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

G. J. Pierce
School of Biological Sciences, University of Aberdeen,
Aberdeen, UK

V. D. Valavanis (✉)
Marine GIS Lab, Institute of Marine Biological
Resources, Hellenic Centre for Marine Research,
Heraklion, Greece
e-mail: vasilis@her.hcmr.gr

M. Begoña Santos · J. M. Portela
Centro Oceanográfico de Vigo, Instituto Español de
Oceanografía, Vigo, Spain

its Centro Oceanográfico in Vigo, the University of Vigo (UVIGO, SP), the University of the Aegean (UAEGEAN, GR) and the Instituto de Investigaciones Marinas (IIM/CSIC, ES). This network of research and education centres provided research topics and training for 23 early-stage researchers for training periods lasting between 3 months and 3 years. These early-stage researchers were selected from a total of 89 applications from all over the world, financing researchers from the UK, France, Spain, Italy, Greece, Portugal, Germany, Denmark, the Netherlands, Chile and Mexico. Now, more than 1 year after the completion of their training and research, all trainees continue to work in the academic and private sectors in Europe and overseas.

The special issue includes 19 scientific papers on marine subjects, including topics in biodiversity and genetics, species–environment interactions, fisheries, aquaculture, morphometrics and ecosystem modelling. The project held a final conference on “Marine Ecosystems and Sustainability (MESS)” in Aberdeen in December 2009. In addition to papers from the Marie Curie researchers themselves, the special issue includes contributions based on invited talks at the MESS conference and contributions from other students and colleagues who participated in project activities. These papers are broadly focused on the diversity of marine ecosystems and their sustainable utilization.

We wish to acknowledge our Project Officer in the Directorate-General for Research (Brussels, Belgium), Frank Marx, for his extensive help and support over 3½ years, also Kamila Partyka, Przemyslaw Jankowski, Evert Van den Broeck and Monika Dudek, who handled the later stages of the project. We thank the many scientists, acting as anonymous referees, who provided constructive comments on earlier versions of the articles in this issue (reviewers’ names are listed below). We are grateful to Martine van Bezooijen (Springer) and Koen Martens (Royal Belgian Institute of Natural Sciences) for providing the opportunity to publish this special issue in *Hydrobiologia*. Finally, we would like to thank all the ECOSUMMER researchers and their supervisors for their contributions to the project and to this special issue (researchers’ names and their projects are listed below). We hope that readers of this special issue of *Hydrobiologia* will find it useful and that it inspire further work on the sustainable use of marine ecosystems.

List of reviewers

Jurgen Alheit
Paula Alvarez
Alexander Arkhipkin
Christos Arvanitidis
Daniela Banaru
Alberto Basset
Janet Brown
Ratana Chuenpagdee
Alberto Garcia
Elizabeth Gosling
Angel Guerra
Belgin Hossu
Jorgen Hylleberg
Stelios Katsanevakis
Giorgos Koumoundouros
Vladimir Laptikhovsky
Evgenia Lefkaditou
Colin MacLeod
Athanassios Machias
Corinne Martin
Paloma Martin
Joan Moranta
Ada Natoli
Ioannis Nengas
Andreas Palialexis
Miguel Palmer
Kim Parsons
Joao Pereira
Benjamin Planque
Andrew Pullin
Dionysios Raitsos
Javier Ruiz
Ana Sabates
Konstantinos Stergiou
Allan Stoner
George Tserpes
Athanassios Tsikliras
Roger Villanueva
Esin Yalçin
Alain Zuur

List of researchers

List of researchers, their country of origin, project titles, and institutions involved under the Marie Curie Action ECOSUMMER (2006–2009)

Fellow name	Project title	Hosts
Angela Ribeiro (Portugal)	Effective population size of the Atlantic salmon (*Salmo salar*) population inhabiting the river Eo (Spain)	UVIGO
Beatriz Guijarro (Spain)	Assessment of the slope trawl fishery resources off the Balearic Islands (western Mediterranean) using experimental survey data: from single to multi-species approach	HCMR
Christian Lønborg (Denmark)	Resolving the importance of dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) in Marine coastal waters	SAMS IIM
Consuelo Hermosilla (Chile)	Population structure of *Octopus vulgaris*: combination of ageing, morphometric, genetics and GIS methods	IIM HCMR UAEGEAN UVIGO
Evangelina Gontikaki (Greece)	The role of organic matter quality in deep-sea carbon cycling	UABDN SAMS
Fiona Read (UK)	Understanding marine mammal interactions with fisheries in Galician waters, NW Spain	IIM IEO
Fleur Visser (Netherlands)	Spatial distribution of cetaceans at the Azores: Species' and niche diversity in a remote marine area	UAEGEAN
Gema Martínez (Spain)	Spatio-temporal distribution of pelagic fisheries in Galician coast using GAMs and other statistical tools	UAEGEAN HCMR
Iñigo Martinez Saez (Spain)	Fished Grounds to Oil Platform Refugium: Temporal changes in Fish Assemblage	FRS
Isidora Katara (Greece)	Atmospheric and oceanic teleconnection patterns and their impact on fisheries–environment interactions	UABDN
Katie Longo (Italy)	Predator–prey interactions and indicators of the impact of fisheries on fish species diversity	UABDN HCMR
Martina Stritmatter (Germany)	Molecular biology of the *Ectocarpus*/*Eurychasma* host-pathogen interaction	SAMS UAEGEAN
Neil Fletcher (UK)	Spatio-temporal analysis of pelagic species in the Aegean Sea in relation to environmental factors and feeding preferences	HCMR UAEGEAN
Patrícia Joana Dias (Portugal)	Evolutionary genetics of the *Mytilus edulis* complex in Scotland	FRS HCMR UABDN
Rebeca Rodriguez (Mexico)	Population structure of *Helicolenus dactylopterus dactylopterus* in the North East Atlantic and Mediterranean using traditional and geometric morphometric techniques	IIM IEO
Ricardo Fernandes (Portugal)	Development of a GIS-based tool for interfacing and manipulation of time series 4D marine datasets	HCMR
Ruth Fernandez (Spain)	Bottlenose dolphin, *Tursiops truncatus* (Montagu 1821), trophic ecology and population structure in Galicia, NW Spain	UAEGEAN HCMR UABDN
Sabine Goetz (Germany)	Analysis of the interactions of cetaceans with fisheries and aquaculture in Galicia	IEO

continued

Fellow name	Project title	Hosts
Sarah Faulwetter (Germany)	Biodiversity resources management: virtual information depository	HCMR UAEGEAN
Sonia Mehault (France)	Integrating the reproductive potential concept into stock assessment and implication for management	IIM IEO
Susana Rivero Rodriguez (Spain)	Evaluation of the environmental impacts of Mediterranean fish farming using life cycle assessment	HCMR
Tania Santos Diniz (Portugal)	Identifying and mapping the overlap in resource exploitation between marine mammals and fisheries along the Galician coast	UVIGO
Vagelis Spyrakos (Greece)	Water quality analysis methods aimed to study and detect HABs on the Galician coast	UVIGO

Hydrobiologia (2011) 670:5–22
DOI 10.1007/s10750-011-0675-7

ECOSYSTEMS AND SUSTAINABILITY

Ontogenetic allometry of the bluemouth, *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae), in the Northeast Atlantic and Mediterranean based on geometric morphometrics

Rebeca Rodríguez-Mendoza · Marta Muñoz · Fran Saborido-Rey

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The bluemouth, *Helicolenus dactylopterus*, is a deep-sea scorpionfish widely distributed in the Atlantic Ocean and the Mediterranean. It is a common by-catch associated to many demersal fisheries. However, there is little information about the stock structure, stock dynamics and life history parameters of the bluemouth. From the perspective of stock identification, it is important to study growth in fish populations to better understand the possible morphological differences among populations and when and why do they arise. Thus, the aim of this study was to determine the growth (allometric) trajectories of shape for several bluemouth populations in Northeast Atlantic and Mediterranean using landmark-based geometric morphometric techniques. In this study, ontogenetic allometry was present in all of the bluemouth samples. Ontogenetic shape changes were most evident in the head and pectoral area, affecting the position of the snout, preopercular spines and pectoral fins, but changes in body depth and length were also important. However, the degree to which these ontogenetic shape changes were present in bluemouth from each of the studied areas was different, indicating that their growth trajectories are not homogeneous. The importance of this finding for size-correction of the shape variables in morphometric studies for stock identification is also discussed.

Keywords *Helicolenus dactylopterus* · Bluemouth · Morphometrics · Growth · Ontogenetic allometry

Introduction

The bluemouth, *Helicolenus dactylopterus dactylopterus* (De la Roche 1809) (Teleostei: Scorpaenidae), is a benthic scorpionfish widely distributed in the Atlantic Ocean and Mediterranean Sea. It dwells on continental shelf edges and upper slopes at depths between 200 and 1,000 m (Whitehead et al., 1986). Although there has been little commercial interest in this species, partially due to its low level of accessibility, it is currently of growing commercial interest as new resources need to be found by fishing fleets because of the depletion of traditional resources. This species is caught by artisanal longline and gillnet fisheries near the Strait of Gibraltar and along the Portuguese continental coast and the Azores (Esteves et al., 1997; Santos et al., 2002; Czerwinski

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

R. Rodríguez-Mendoza (✉) · F. Saborido-Rey
Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain
e-mail: rrodriguez@iim.csic.es

M. Muñoz
Departament de Ciències Ambientals, Universitat de Girona, Campus Montilivi, 17071 Girona, Spain

et al., 2008). In the western Mediterranean, bluemouth are mostly caught as by-catch in bottom trawl fisheries targeted at deep-sea crustaceans (Moranta et al., 2000; Sánchez et al., 2004). However, in areas such as the Catalonian coast, the bluemouth is the most commercially viable scorpionfish species, with important economic value (Ribas et al., 2006). In other fisheries, such as the Irish demersal fisheries that target prawns (*Nephrops norvegicus*) and/or whitefish (e.g., cod *Gadus morhua* and haddock *Melanogrammus aeglefinus*), bluemouth are caught regularly by otter trawlers, but they are mostly discarded (Borges et al., 2005).

Since the late 90s, the biology of the bluemouth has been studied in the NE and NW Atlantic, the Mediterranean and the North Sea, focusing mainly in the distribution, age, growth, and reproduction of the species (Heessen et al., 1996; Esteves et al., 1997; White et al., 1998; Kelly et al., 1999; Muñoz et al., 1999, 2000; Massutí et al., 2000, 2001; Allain, 2001; Sequeira et al., 2003; Abecasis et al., 2006; Mendonça et al., 2006; Ribas et al., 2006; Mamie et al., 2007; Vila et al., 2007). From these studies, the bluemouth has been characterized as a slow growing and long-lived species reaching up to 30 years of age (Massutí et al., 2000). The growth of male and female bluemouth has been studied systematically in most studies with respect to age and growth, but results among the different reports are not consistent. In the western Mediterranean, Massutí et al. (2000) and Ribas et al. (2006) observed that males grew faster than females. A study in the Azores was not conclusive about possible differences in growth rates between sexes because growth curves estimated using whole otolith readings showed that males grew faster, but growth curves estimated by sliced otoliths failed to show differences between the sexes (Abecasis et al., 2006). Moreover, a very recent study by Sequeira et al. (2009) on bluemouth on the Portuguese continental slope found no significant differences between sexes when comparing female and male growth curves.

At present, most reports regarding the growth of bluemouth include only age–length growth curves with no information on the morphological changes that take place as fish increase in size. Body form in fishes is a product of ontogeny (Cadrin, 2005). It is affected by the genetic makeup of an individual, but it also reflects adaptation to environmental factors such as temperature, food availability, feeding mode, swimming behavior, or habitat use (Barlow, 1961; Wimberger, 1992; Swain et al., 2005). During the growth of fishes, body proportions change as the larvae and juvenile fish adapt to transitions in habitat and diet until they reach adulthood. The change in proportions related to variation in size (i.e., growth) is termed ontogenetic allometry, and it has been studied in fishes for quite some time (e.g., Barlow, 1961; Strauss & Fuiman, 1985; Klingenberg & Froese, 1991). Traditionally, changes in proportion are represented as growth trajectories that describe the growth of an organism from its inception to its mature form (Alberch et al., 1979), and more recently, the tools of geometric morphometrics have allowed us to visualize shape changes to identify what happens during the growth of fish and other organisms (e.g., Loy et al., 1996, 1998; Frost et al., 2003; Mitteroecker et al., 2004; Kouttouki et al., 2006; Drake & Klingenberg, 2008). The study of allometric growth also has an important application for size correction of morphological variables when comparisons of multiple groups of specimens with different size compositions are made (e.g., Burnaby, 1966; Mosimann, 1970; Humphries et al., 1981; Thorpe, 1983; Claytor & MacCrimmon, 1987; Klingenberg & Froese, 1991; Klingenberg, 1996).

The aim of this study was to characterize the shape changes that occur during the growth of bluemouth to better understand the biology and ecology of this species. Thus, we determined ontogenetic shape trajectories for bluemouth from several geographical areas in the NE Atlantic and western Mediterranean and examined the variation of growth patterns in the different environments of the study areas. Growth patterns of males and females were also analyzed to determine if sexual dimorphism exists. Finally, we discuss the implications of our results in the context of phenotypic identification of bluemouth stocks in the NE Atlantic and western Mediterranean.

Materials and methods

Specimens and locations

For this study, a total of 1,012 specimens of bluemouth were caught around the Iberian Peninsula and the Porcupine Bank in Spanish bottom trawl surveys (Table 1). The study areas were divided taking into account their oceanographic characteristics. In the NE Atlantic, specimens were sampled from the Galician shelf (from the Miño River to Cape Finisterre), the Cantabrian Sea (from Cape Estaca de Bares to the mouth of the Bidasoa River), the Gulf of Cadiz and the Porcupine Bank (Irish continental margin). Galicia and the Cantabrian Sea are considered to be divided by Cape Estaca de Bares, which is described as a biogeographic limit (boundary effect) and a larval retention area of mesoscale hydrographic anomalies (i.e., anticyclonic eddies) (Sánchez & Gil, 2000). The other two locations in the NE Atlantic are also interesting in terms of their oceanographic characteristics. The Gulf of Cadiz is the first basin where the dense (i.e., salty and warm) Mediterranean outflow encounters the open ocean after crossing the Strait of Gibraltar, and water mass circulation along its continental shelf results in warm and biologically productive waters that are particularly suitable for the reproduction of many fish species (García-Lafuente, 2006; García-Lafuente et al., 2006). The Porcupine Bank is a submarine shelf break bank that is partly attached to the Irish continental shelf, and it has a high productivity due to closed circulation patterns around the bank that promote the retention of organic matter over it. It is also worth noting that it hosts an important number of deep cold-water ecosystems (White et al., 2005).

Specimens from the Mediterranean were caught in the Alboran Sea close to the coast of Alicante (southwest of the Balearic Sea) and along the Catalonian coast (Fig. 1). These locations were selected considering studies by Massutí et al. (2000) and Ribas et al. (2006), which indicated several well-defined areas that can be found in terms of oceanographic conditions in the western Mediterranean: (1) the southwestern basin (Alboran Sea), (2) the northwestern basin (Catalonian coast) and (3) the transition zone, from Cape Palos to Sagunto (Alicante sector).

Data acquisition

After collection, the fish were immediately frozen at −20°C and stored in a horizontal position to avoid any deformation of the body until the time of the analysis. Thirteen homologous landmarks were defined (Fig. 2) to provide an adequate coverage of the body shape based on a previous study by Garabana (2005) on similar species (*Sebastes* spp.). To ensure an accurate localization of the selected points, black-headed entomological pins were placed on each landmark. Once the landmarks were located, each fish was placed on its left side on a white polystyrene board with a ruler with 1-cm gradations. A photograph was taken with a Nikon D1X digital camera (Nikon Corporation, Japan) using a focal length of 35 mm to avoid optic distortions of the images. The images were digitized using TpsDig software version 2.10 (Rohlf, 2006) to obtain the *x*,*y* coordinates of the landmarks.

Morphometric and statistical analysis

Size

To quantify the size of a specimen, centroid size (CS) was computed from the raw coordinates of the landmarks (Dryden & Mardia, 1998) using the MorphoJ software package (Klingenberg, 2008). Centroid size is a measure of geometric scale,

Table 1 Number of bluemouth specimens analyzed in each location

Study area	*N*	Size range (CS, cm)	Mean ± SD (CS, cm)	Source (research survey)
Mediterranean Sea				
Alboran Sea	238	6.73–31.59	17.34 ± 5.70	Medits 2007
Alicante	134	6.61–26.14	13.09 ± 4.06	Medits 2007
Catalonian coast	73	5.30–20.13	10.71 ± 3.01	Medits 2007
NE Atlantic				
Gulf of Cadiz	75	5.92–36.81	21.29 ± 6.35	ARSA 2009
Galicia	191	5.23–34.52	15.08 ± 4.42	Demersales 2007
Cantabrian Sea	119	9.42–39.58	18.65 ± 5.37	Demersales 2007
Porcupine Bank	182	8.54–35.02	24.25 ± 6.03	Porcupine 2008

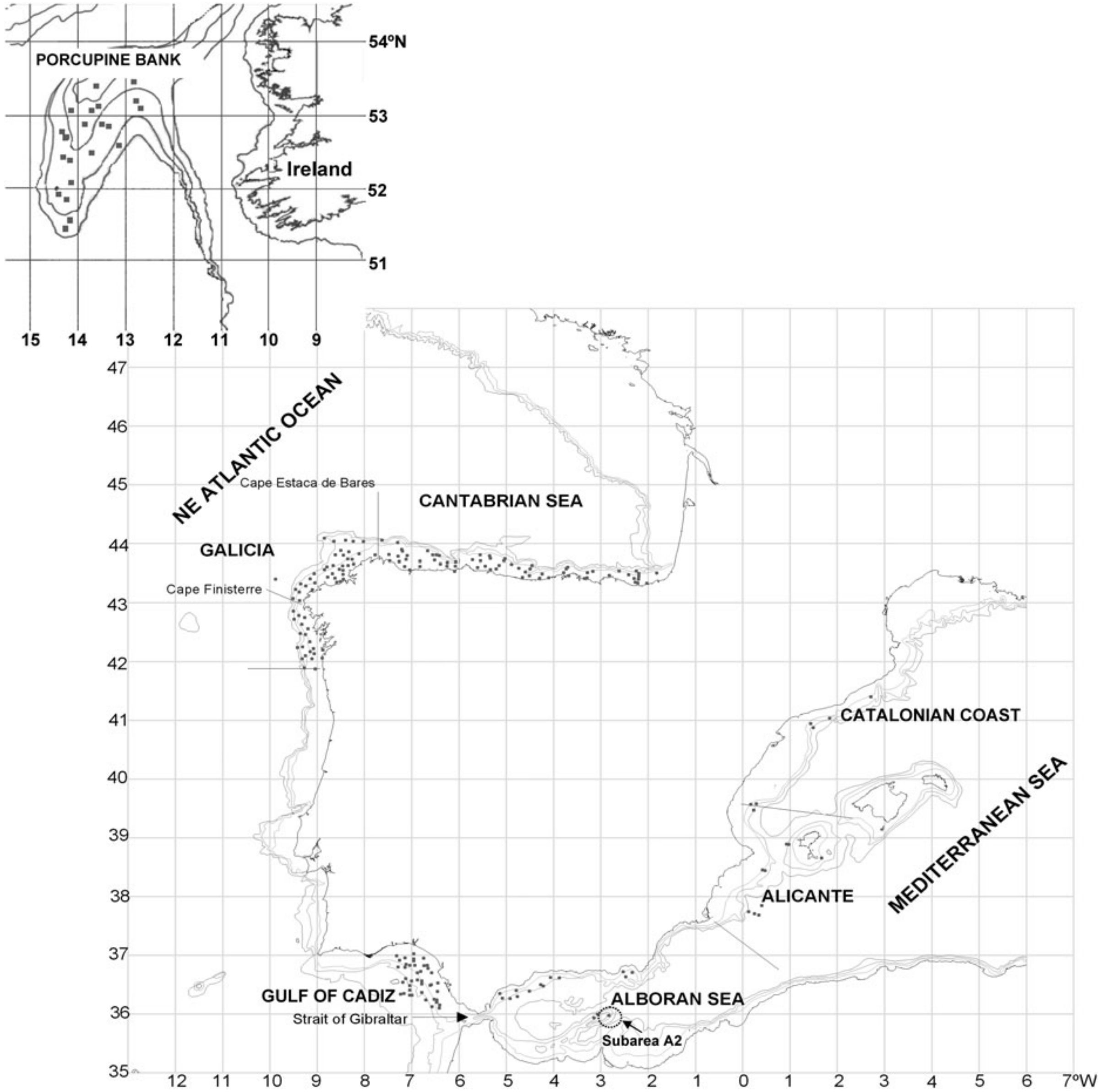


Fig. 1 Map of the study area with the sampling sites in the Northeast Atlantic and Mediterranean

calculated as the square root of the summed squared distances of each landmark from the centroid of the landmark configuration. In the present study, CS was highly correlated with total length of the specimens ($r^2 = 0.9935$, $P < 0.01$) (Fig. 3).

Shape

Body shape was analyzed using landmark-based geometric morphometric methods (Rohlf, 1990; Bookstein, 1991). To remove non-shape variation, a generalized Procrustes analysis (GPA) was carried out using MorphoJ software (Klingenberg, 2008). The first step of this procedure is to scale all of the specimens to unit centroid size. The landmark configurations are then superimposed to have a common centroid and rotated to minimize the distances between the corresponding landmarks of all of the configurations. Once the specimens are aligned, the mean configuration of landmarks is

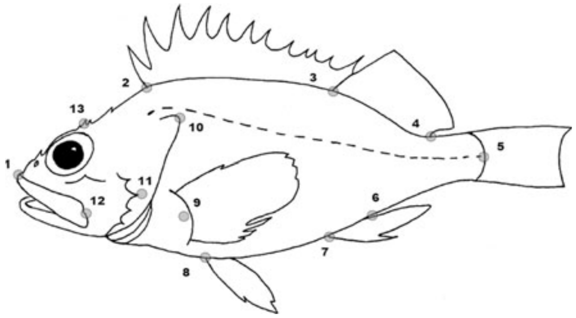


Fig. 2 Schematic representation showing the location of the 13 landmarks used in the analysis

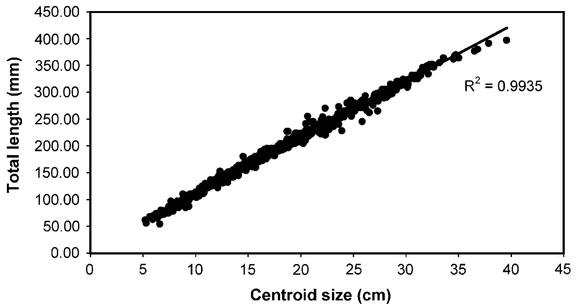


Fig. 3 Relationship between centroid size (CS) and total length (TL) for all the specimens in this study ($r^2 = 0.9935$, $P < 0.01$)

computed, and the specimens are projected to a linear shape tangent space. The mean configuration is usually called the *consensus* or *reference* shape because it is the configuration of landmarks that corresponds to the point of tangency between the exact curved shape space and the approximating tangent space in which the linear multivariate statistical analyses are performed (Rohlf & Slice, 1990; Rohlf, 1999; Slice, 2001). The coordinates of the aligned specimens are the Procrustes coordinates, and they were used as shape variables in the statistical analyses.

Growth trajectories

A multivariate regression of the Procrustes coordinates on the logarithm of centroid size was used to determine growth trajectories and characterize morphological changes in response to size. The amount of shape variation for which each regression accounted was expressed as a percentage of the total variation around the sample means. A permutation test using 10,000 runs (Good, 1994) was used to test the null hypothesis of independence between shape and size.

To visualize the strength of the association between size and shape, we calculated shape scores according to Drake & Klingenberg (2008) and plotted them against log centroid size. A shape score is defined by projecting the shape data onto a line in the direction of the regression vector for the independent variable (centroid size). If the regression model is written as $\boldsymbol{y} = \boldsymbol{\beta} x + \boldsymbol{\varepsilon}$ (where $\boldsymbol{y}$ is the row vector of shape variables; $\boldsymbol{\beta}$ is the regression vector; x is the independent variable; and $\boldsymbol{\varepsilon}$ is the row vector of error terms), the shape score s can be computed as $s = \boldsymbol{y}\boldsymbol{\beta}' (\boldsymbol{\beta}\boldsymbol{\beta}')^{-0.5}$. This shape score is the shape variable associated with the shape changes predicted by the regression model, but it also includes the residual variation in that direction in shape space (Drake & Klingenberg, 2008). These analyses were carried out with the MorphoJ software package (Klingenberg, 2008). The similarity of growth trajectories between sexes and among areas was evaluated following the approach explained in Zelditch et al. (2003a, b) using the VecCompare program (IMP software) (Sheets, 2000). To compare each pair of regression vectors, this program first calculates the angle between these vectors (i.e., between-group angle). That angle is obtained as the arccosine of the signed inner products between normalized regression vectors. Then, the between-group angle is compared with the upper 95% confidence interval of within-group angle ranges assessed by a bootstrapping approach with 900 runs. The null hypothesis is that the observed angle could have been produced by two independent samplings of a single group (i.e., area or sex). If the between-group angle exceeds the 95% confidence interval of the two within-group angles, the difference is judged statistically significant at the 5% level.

Visualization of ontogenetic shape changes

To visualize the shape changes associated with the growth of bluemouth specimens, warped outline drawings were made using the thin-plate spline interpolation function (Bookstein, 1989). Visualizations were made in the MorphoJ software package (Klingenberg, 2008).

Results

Growth trajectories by area

Ontogenetic allometry was present in all of the bluemouth samples, as the multivariate regressions were statistically significant (Table 2). Additionally, the scatter plots used to visualize growth trajectories show an association of the shape scores with centroid size for all of the study areas (Fig. 4). However, the amount of shape variation accounted for by the regressions differed considerably among the studied areas, ranging from 4.57% for the Catalonian coast to 24.13% for the Gulf of Cadiz. For the Catalonian coast, the growth trajectory might not be accurately represented despite the significant relationship between shape and size ($P = 0.0136$) because the sample for this area consisted mainly of small specimens with a mean size of 10.71 cm CS. Thus, the results for this area should be interpreted with some caution. Additionally, some areas from the Iberian Peninsula showed a considerable amount of dispersion around the growth trajectory (e.g., the Cantabrian Sea, the Alboran Sea and Alicante). We examined these locations more closely to determine if there was any pattern indicating a possible substructure of the bluemouth sample within these areas that could explain the observed dispersion and, thus, be considered in the study. In the case of the Alboran Sea, it appears that there are two different growth trends (Fig. 5), one exhibited by bluemouth specimens caught mainly along the coastline (subarea A1, $N = 171$) and another one characteristic of specimens caught off the coast along the slopes of Alboran Island at 35°58.44′N, 2°49.53′W (subarea A2, $N = 67$). Thus, we carried out separate regressions of shape on size for each of the subareas in the Alboran Sea (A1 and A2), and both were statistically significant ($P < 0.0001$). However, the amount of shape variation accounted for by the regressions was noticeably different (7.77% for subarea A1 and 22.82% for subarea A2).

Table 2 Results of the multivariate regression of shape on size for bluemouth specimens within the studied locations

Area	*N*	% Predicted	*P*-value
Alboran Sea	238	9.0962	<0.0001
Subarea A1	171	7.7699	<0.0001
Subarea A2	67	22.8231	<0.0001
Alicante	134	11.2923	<0.0001
Catalonian coast	73	4.5719	0.0136
Gulf of Cadiz	75	24.1378	<0.0001
Galicia	191	8.9842	<0.0001
Cantabrian Sea	119	16.2398	<0.0001
Porcupine Bank	182	12.0634	<0.0001

Growth trajectories for males and females

Multivariate regressions of shape on size were also carried out for males and females separately within the study areas (Table 3 and Fig. 6). Ontogenetic allometry was detected for both sexes from all of the NE Atlantic samples, since the relationship between shape and size was statistically significant ($P < 0.0001$), but no statistical differences between growth trajectories of males and females within these locations were found at the 5% level (Table 4). In the Mediterranean locations, the analysis could not be done for the sample from the Catalonian coast because the number of males and females present in the sample was too low (6 males and 9 females). For the Alboran Sea, the regressions for both sexes from subarea A2 were significant ($P < 0.0001$), but those for subarea A1 were not ($P = 0.2671$ and 0.1036 for males and females, respectively). In the case of subarea A1, the growth trajectories were probably not well defined (and therefore not significant) because the size range of the sexed specimens was very limited. From the 114 males and females in the sample, there was only one specimen smaller than 15 cm CS or 2.7 log centroid size (Fig. 5). Thus, we did not compare the growth vectors for males and females from subarea A1, and we decided to use sexed and unsexed specimens together ($N = 171$) to determine the growth trajectory for comparison with other areas. For subarea A2, the angle between the ontogenetic vectors of males and females was of 44.8° and the 95th percentile of the ranges of the within-sex angles, were 40.9° for females and 44.6° for males. Although the inter-sex angle was significant at the 5% level, its value was very close to the 95th percentile of the range of angles for the males, and this result should also be interpreted with caution. As with subarea A1, we also used all of the available specimens from subarea A2 ($N = 67$) to determine the growth trajectory for comparison with the other

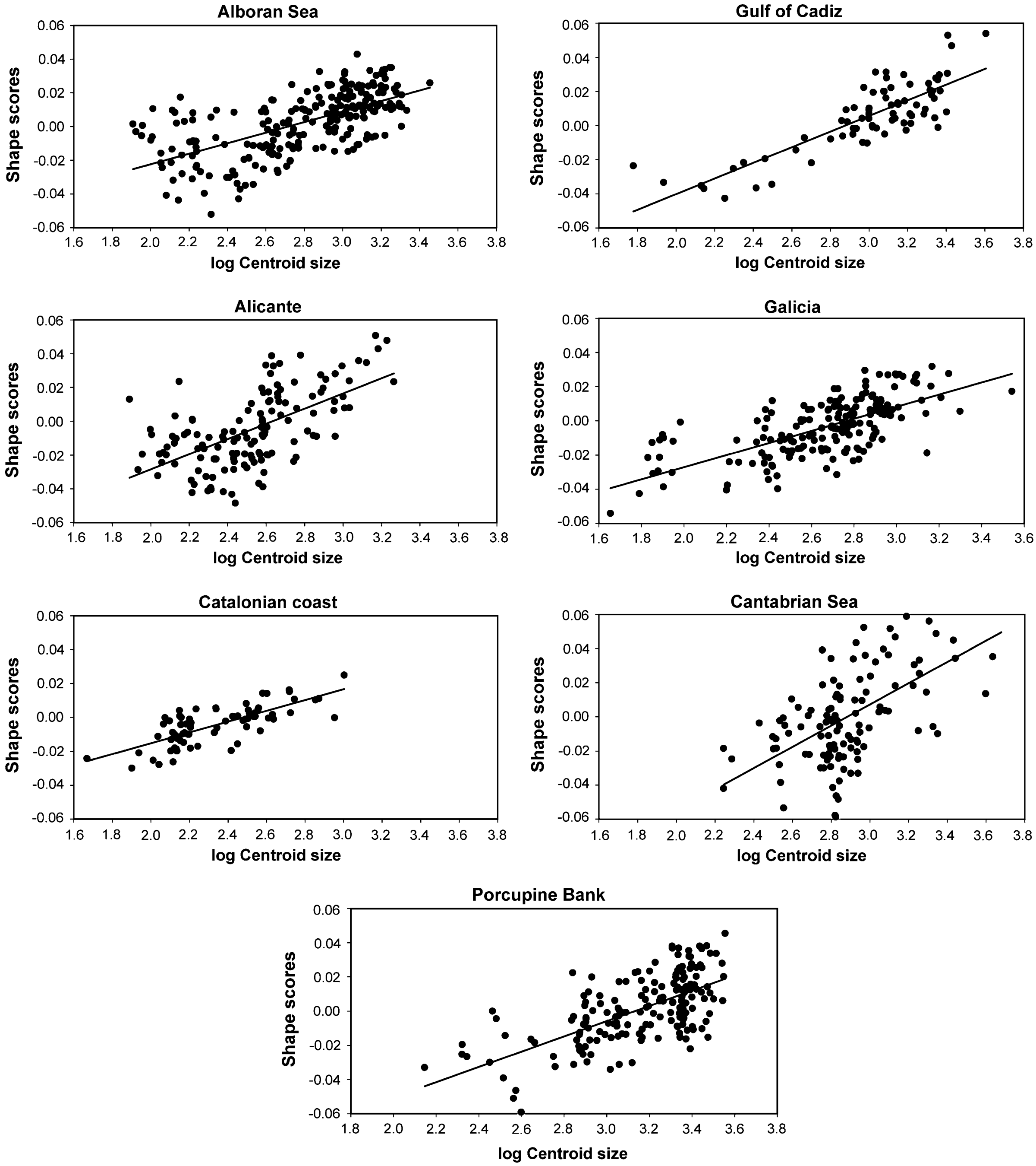


Fig. 4 Ontogenetic allometry for bluemouth from the studied areas. The growth trajectories are represented with shape scores as a function of log-centroid size

areas. For Alicante, the regressions of shape on size for males ($P = 0.0092$) and females ($P = 0.0130$) were significant, and the growth trajectories were similar for males and females in this area (Table 4).

Comparison of growth trajectories between areas

Growth trajectories were compared pairwise by calculating the angle between the regression vectors of the

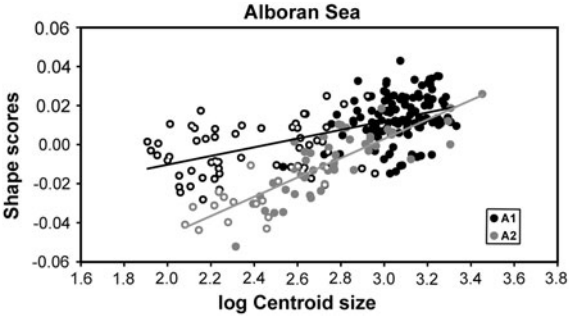


Fig. 5 Growth trajectories for bluemouth from the two subareas in the Alboran Sea (*A1* and *A2*). The trajectories are represented with shape scores as a function of log centroid size. *Filled circles* indicate sexed specimens (males and females) and *open circles* indicate unsexed specimens

studied areas (Table 5). We did not find any clear pattern of geographical variation for the differences between growth trajectories. The pairwise comparisons indicated that bluemouth from the Gulf of Cadiz underwent similar ontogenetic shape changes to those from Galician waters, subarea A2 in the Alboran Sea, Alicante and the Catalonia coast, indicating that there are no growth patterns specific to only the NE Atlantic or the Mediterranean Sea. Interestingly, the growth trajectories for the two subareas within the Alboran Sea differed considerably. Only the growth trajectory for bluemouth from the Cantabrian Sea was different than all others.

Table 4 Angle between growth trajectories of males and females by area

Area	Angle
Alboran Sea–Subarea A2	44.8*
Alicante	28.8
Catalonian coast	–
Gulf of Cadiz	34.1
Galicia	32.4
Cantabrian Sea	18.3
Porcupine Bank	32.8

Growth trajectories for males and females from the Catalonian coast were not determined due insufficient sexed specimens. The comparison between the growth trajectories of males and females from subarea A1 was not performed because the regressions for males and females were not significant at the 5% level

* Significant at the 5% level

Shape changes during growth

The patterns of shape changes during the growth of bluemouth are shown in Fig. 7 for the Mediterranean locations and in Fig. 8 for the NE Atlantic locations.

Table 3 Results of the multivariate regression for males and females within the studied locations

Area	Sex	*N*	Predicted shape variation (%)	*P*-value
Alboran Sea	Females	78	20.77	<0.0001
	Males	89	12.35	<0.0001
Subarea A1	Females	54	3.17	0.1036*
	Males	60	1.99	0.2671*
Subarea A2	Females	24	23.71	0.0001
	Males	29	20.96	<0.0001
Alicante	Females	35	9.48	0.0130
	Males	27	11.94	0.0092
Catalonian coast	Females	9	–	–
	Males	6	–	–
Gulf of Cadiz	Females	35	30.38	<0.0001
	Males	31	11.33	0.0038
Galicia	Females	87	9.69	<0.0001
	Males	75	11.26	<0.0001
Cantabrian Sea	Females	64	21.18	<0.0001
	Males	48	15.64	<0.0001
Porcupine Bank	Females	67	9.95	<0.0001
	Males	108	15.26	<0.0001

The shape variation predicted by each regression is expressed as a percentage of the total shape variation. The regressions for the Catalonian coast were not performed due to insufficient sexed specimens in the area

* Not significant at the 5% level

Table 5 Results for the pairwise comparisons of growth vectors for bluemouth from the studied areas

	Alboran Sea (A1)	Alboran Sea (A2)	Alicante	Catalonian coast	Gulf of Cadiz	Galicia	Cantabrian Sea	Porcupine Bank
Alboran Sea (A1)	0							
Alboran Sea (A2)	62.5*	0						
Alicante	57.9*	27.4	0					
Catalonian coast	54.5	66.4*	51.1	0				
Gulf of Cadiz	57.4*	20.5	20.9	57.0	0			
Galicia	40.2*	38.8*	35.7*	45.1	34.7	0		
Cantabrian Sea	76.1*	26.3*	29.7*	70.1*	29.3*	49.6*	0	
Porcupine Bank	51.8*	30.9*	31.9*	60.9*	20.5	38.8*	41.8*	0

* Growth trajectories are significantly different at the 5% level

In general, the shape changes associated with increases in size in bluemouth specimens consisted of: (a) a relative expansion of the area comprised by landmark 9 (midpoint of the insertion of the pectoral fin), landmark 11 (tip of the second-preopercular spine) and landmark 12 (midpoint of the end of the jaw), (b) a contraction of the head area in relation to body size accompanied in most cases by an upward shift of the tip of the snout and (c) a dorsoventral expansion together with a relative shortening of the body. Thus, as expected, we observed a trend towards a more robust body morphology as the fish become larger. Still, some specific shape changes were identified in bluemouth specimens from the Cantabrian Sea. The estimated shape for large specimens showed a considerable up-rightward displacement of landmark 10 (end of the operculum), an up-leftward displacement of landmark 5 (insertion of the hypural plate) and a larger downward displacement of landmarks 8 (insertion of the ventral fin) and 9 (insertion of the pectoral fin).

Discussion

Body form in fishes is a product of their ontogeny (Cadrin, 2005). It is affected by the genetic makeup of an individual, but it also reflects adaptation to environmental factors such as temperature, food availability, feeding mode, swimming behavior and habitat use (Barlow, 1961; Wimberger, 1992; Swain et al., 2005). During the growth of fishes, body proportions change as the larvae and juvenile fish adapt to habitat and diet transitions until they reach adulthood. According to our results, bluemouth specimens from both NE Atlantic and Mediterranean locations seem to follow a pattern of ontogenetic shape changes that is probably related to the changing ecology of the species over the course of its life history: bluemouth juveniles have a streamlined body shape during their pelagic stage (Furlani, 1997 and references therein), while adults have robust but flexible muscular bodies typical of benthic sit-and-wait predators (Webb, 1984; Uiblein et al., 2003). For most of the studied areas, ontogenetic shape changes were most evident in the head and pectoral area, affecting the position of the snout, preopercular spines and pectoral fins, but changes in body depth and length were also important (Figs. 7, 8). Changes in body depth and length are mostly related to swimming capacity and locomotor adaptations to food capture and escape from predators (Webb, 1984). Functionally, mouth shape changes also have many repercussions in the life of fish because mouth morphology plays an essential role in determining the type of prey consumed, and morphological variations can lead to changes in foraging/predation ability and subsequently differential exploitation of food resources (Karpouzi & Stergiou, 2003). Thus, the observed changes in mouth shape and position are very likely to be related to ontogenetic changes in the diet of bluemouth. In general, their diet consists of benthic decapod crustaceans (Natantia, Brachyura, and Macrura), demersal fish and sometimes pyrosomes, polychaetes and echinoderms (Macpherson, 1979, 1985; Nouar & Maurin, 2000; Serrano et al., 2003), but the proportions of these prey types in their diet vary according to the size of the fish. For example, Macpherson (1979) reported that the diet of small bluemouth individuals from 4 to 9 cm in the

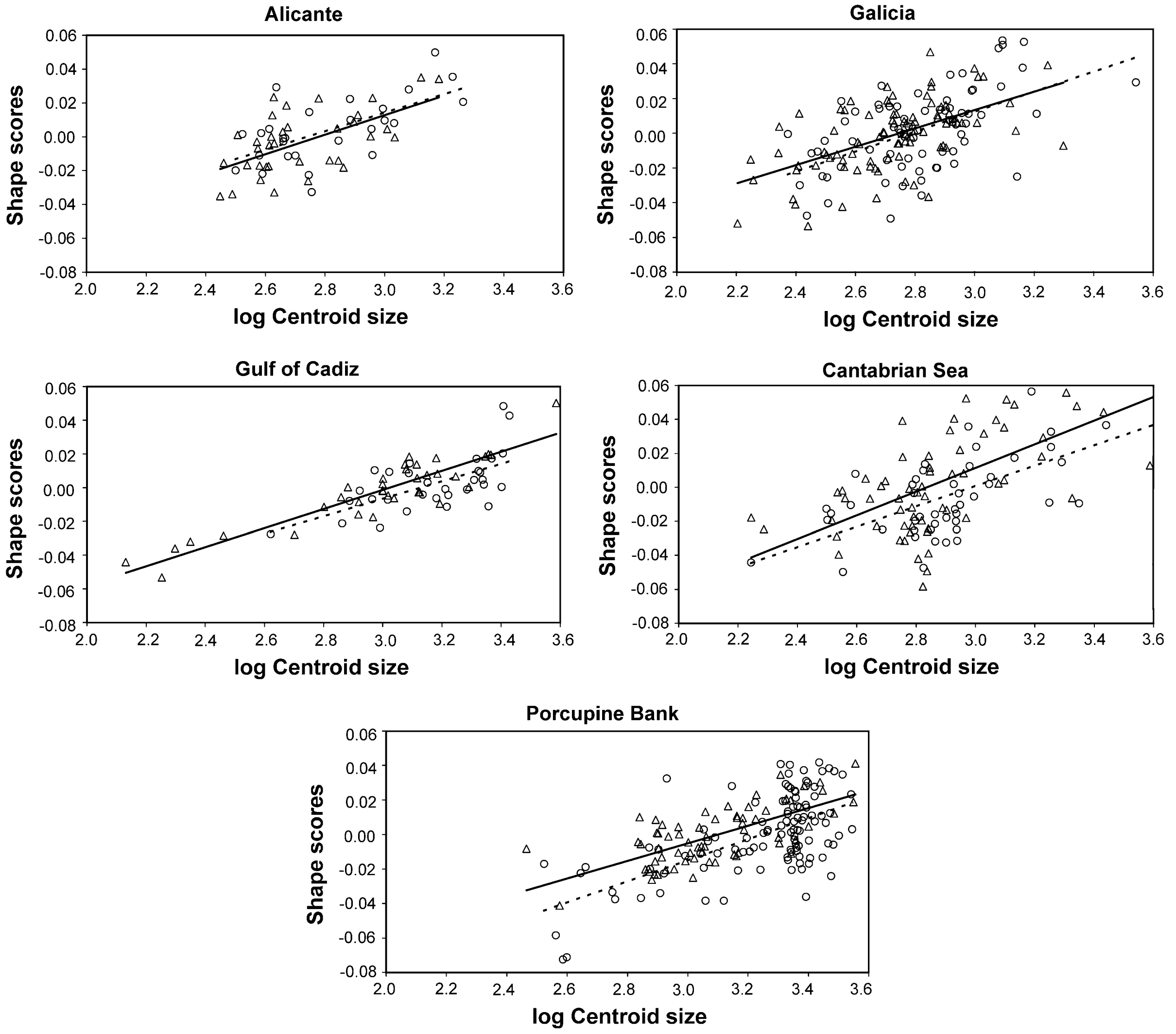


Fig. 6 Growth trajectories for bluemouth males (*open black circles* and *dashed line*) and females (*open black triangles* and *solid line*) for some of the studied areas in the NE Atlantic and Mediterranean. The growth trajectory for the Alboran Sea is shown separately in Fig. 5 (see "Growth trajectories for males and females" section)

Mediterranean consisted mainly of fish (51.9%) such as silvery pout (*Gadiculus argenteus argenteus*) and gobies (*Deltentosteus quadrimaculatus* and *Lesueurigobius friesii*) and decapods including *Alpheus glaber* (20.9%), *Calocaris macandreae* (5.9%), and *Goneplax rhomboides* (4.2%). In contrast, the main prey of adult specimens (20–29 cm in length) was the decapod crustacean *Goneplax rhomboides* (49.4%), followed by other decapods, such as *Calocaris macandreae* (17.6%) and *Alpheus glaber* (14.1%), and a small percentage of pyrosomes (9.4%) and fish (8.2%). In the case of *Helicolenus percoides*, ontogenetic diet changes have been also observed, as the proportions of Crustaceans and fish are inversely related as length increases. For this species, Brachyura were the single most important prey in fish of less than 20 cm, but they were replaced by *Pyrosoma atlanticum* and teleosts in larger size classes (Blaber & Bulman, 1987).

However, the degree to which the above described ontogenetic shape changes were present in bluemouth from each of the studied areas was different, reflecting the differences in growth trajectories that we found in this study. The factors that cause these growth differences are likely to be complex. Phenotypic variation can result from either genetic differentiation or

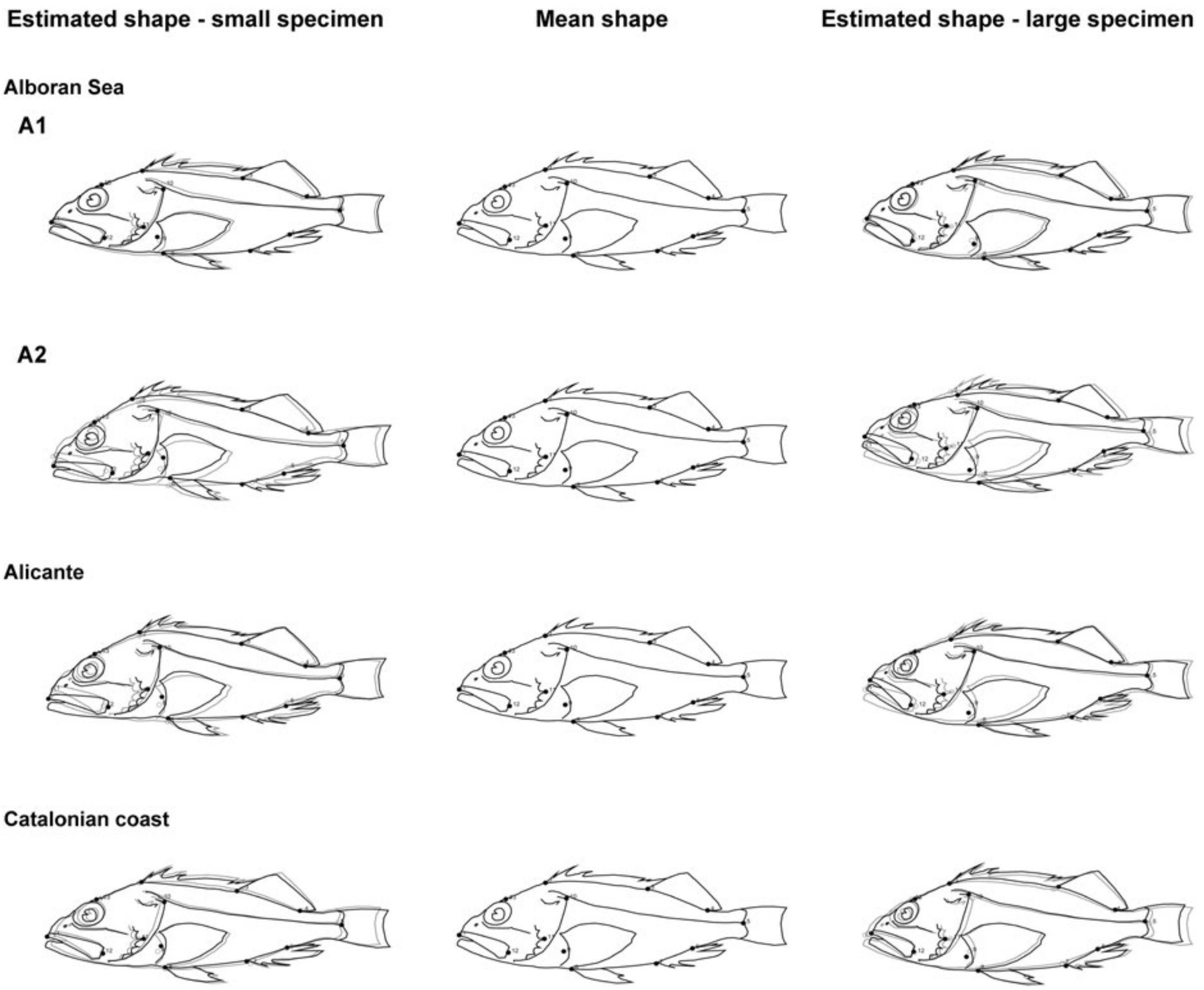


Fig. 7 Visualization of shape changes associated with growth for bluemouth from the western Mediterranean. The mean shape is shown in the center and is also represented as *light gray outlines* drawn in the figures in the *left* and *right columns*. *Left column*: the *black outline* shows the shape change for an arbitrary decrease in log centroid size by 1.5 units, representing the shape for a small specimen. *Right column*: the *black outline* shows the shape change for an arbitrary increase in log centroid size by 1.5 units, representing the shape for a large specimen

phenotypic plasticity. Genetic information on bluemouth populations is still scarce. To our knowledge, only one study has focused on the genetic population structure of the bluemouth in the north Atlantic (the Azores, Madeira and Cape Verde, the coast of Portugal (Peniche) and the northwest Atlantic off the coast of South Carolina, USA) (Aboim et al., 2005). In that study, no significant genetic differentiation was detected between populations within the NE Atlantic region (Azores, Peniche, and Madeira).

Phenotypic plasticity is the ability of a genotype to produce different phenotypes in response to different environmental stimuli (Wimberger, 1992). In fishes, as with most indeterminately growing organisms, the influence of the environment on life history traits is realized primarily through factors that affect body size and the rate at which body size changes throughout an individual's life (Swain et al., 2005). Therefore, fish growth and survival depend on many components of the habitat in which fish live (i.e., prey resources, predation risk, temperature, sediment type, water depth, etc.) (Hayes et al., 1996). In addition to environmental factors, growth in fish can be affected by population density and fishing mortality (Rochet, 1998; Law, 2000; Sánchez Lizaso et al., 2000). In general, size structure differences (and therefore

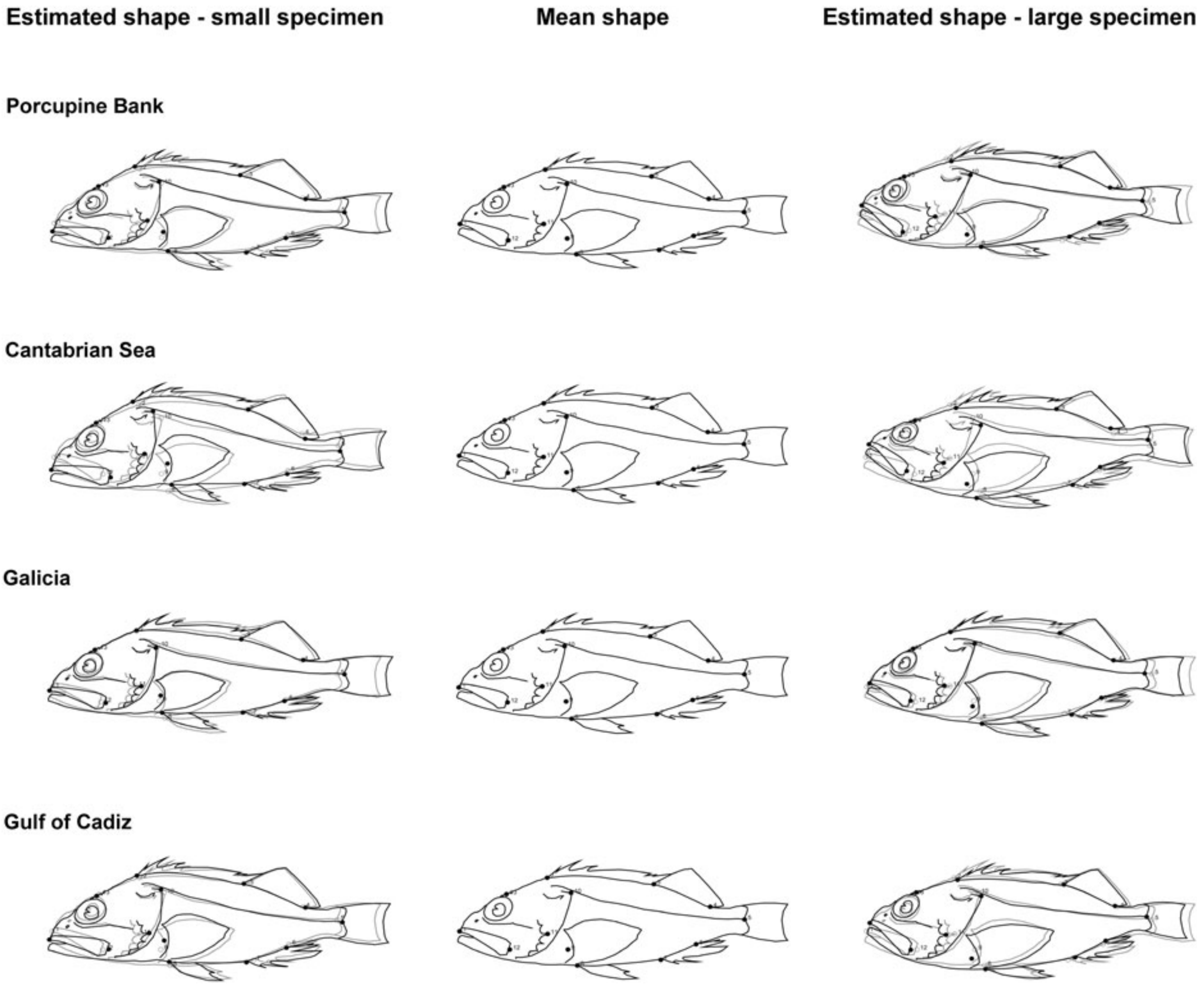


Fig. 8 Visualization of shape changes associated with growth for bluemouth from the NE Atlantic. The mean shape is shown in the center and is also represented as *light gray outline* drawn in the figures in the *left* and *right columns*. *Left column*: the *black outline* shows the shape change for an arbitrary decrease in log centroid size by 1.5 units, representing the shape for a small specimen. *Right column*: the *black outline* shows the shape change for an arbitrary increase in log centroid size by 1.5 units, representing the shape for a large specimen

differences in growth) of deep-sea fishes occur between the NE Atlantic and the Mediterranean (Tortonese, 1960; Stefanescu et al., 1992). More recently, Massutí et al. (2004) compared the deep-sea fish assemblages between these areas, and they also found evidence that for almost all species, those in the Mediterranean tended to grow to a smaller adult size. As a consequence, these fish will have smaller mouths and will, therefore, utilize a different component of the available food resources (Massutí et al., op. cit.). The authors of that study suggested that the primary cause of the differences they observed in size structure were a result of adaptations at both the species and ecosystem level to different trophic relationships between these two areas. However, they also indicated that a high temperature in the Mediterranean (~13°C, compared to ~10°C in the eastern Atlantic areas) could also play an important part in explaining size structure differences. In the same study, size differences between NE Atlantic and Mediterranean bluemouth were found, as the minimum size for locations in the NE Atlantic was at least double than that found in the Mediterranean, and the maximum size was found in the Porcupine Seabight (west of Ireland, NE Atlantic). In our study, bluemouth from the NE Atlantic generally reached larger sizes, but we did not find that NE Atlantic growth patterns were clearly differentiated from those presented by bluemouth from Mediterranean locations. For example, bluemouth specimens from the Gulf of Cadiz, which is located next to the Strait of Gibraltar, exhibited similar ontogenetic shape changes to bluemouth from Galicia and the Porcupine Bank (NE Atlantic) but also to bluemouth from subarea A2 in the Alboran Sea and Alicante (western Mediterranean). In this study, bluemouth from the Cantabrian Sea presented a unique growth pattern that is probably caused by a combination of factors (i.e., food availability along with a low fishing mortality and unique environmental conditions). The Cantabrian Sea is a well delimited area in the Bay of Biscay with particular characteristics that differentiate it from the rest of the Atlantic (Sánchez, 1993), and it also supports an important demersal ecosystem (Le Danois Bank) where no regular fishery operates, allowing for a well-preserved bluemouth spawning stock (Sánchez et al., 2008). In the Cantabrian Sea at the summit of Le Danois Bank where bluemouth are more abundant (400–550 m depth), some of the decapods that are considered to be the main prey of adult bluemouth (i.e., the crab *Goneplax rhomboides*, and the shrimp *Calocaris macandreae* and *Alpheus glaber*) are scarce or even absent due to the low proportion of mud in the sediments, which is required by these burrowing species (Cartes et al., 2007). Therefore, morphological adaptations of the snout in bluemouth from the Cantabrian Sea could arise as the fish use other food resources in the area.

In contrast, these decapods are very abundant in other areas considered in this study such as the southern part of the Galician shelf and the upper slope, where there are fine sediments due to outwelling from the Rías Baixas (Fariña et al., 1997). On the Mediterranean coasts of the Iberian Peninsula, the abundance of *Goneplax rhomboides*, *Calocaris macandreae* and *Alpheus glaber* also varies in the different geographical sectors, with the most abundant regions being found in the Alboran Sea and the northern Catalonia (Abelló et al., 2002). In general, the Alboran Sea has been described as an area with particular hydrographical characteristics due to the influence of Atlantic waters and with a high productivity within the general oligotrophic context of the Mediterranean (Massutí et al., 2001; Abad et al., 2007). Interestingly, the growth trend presented by bluemouth from subarea A1 in the Alboran Sea was different than the one exhibited by bluemouth from adjacent areas. Massutí et al. (2001) have suggested the existence of a well-developed bluemouth spawning stock in the Alboran basin, contrary to what they found in areas with high fishing pressure north of the Alboran Sea, where older fish are poorly represented. In a more recent study, Abad et al. (2007) also found a high abundance of bluemouth on the small seamount Seco de los Olivos in the eastern Alboran Sea, which is an area where trawled sandy bottoms are interspersed with rocky bottoms, and food is readily available due to strong localized currents and upwelling. Thus, food availability on the continental slope of the Alboran Sea in combination with a lower fishing mortality and the oceanographic conditions in the area are likely to produce a different growth pattern than the patterns observed in adjacent areas. Bluemouth caught in subarea A2 in the Alboran basin showed a similar growth pattern to the ones observed in contiguous areas (i.e., Gulf of Cadiz or Alicante). There is a possibility that a group of individuals from these areas migrated to subarea A2 because occasional migrations of adult specimens may occur (Aboim et al., 2005) or that especially particular environmental conditions exist in that location that affect the growth of these individuals. In any case, further study is needed to determine the factors that cause different growth patterns within the Alboran basin, and the temporal and spatial stability of the observed patterns has to be confirmed.

In this study, we also compared growth trajectories between males and females. Information about sexual dimorphism is required for understanding the ecology, behavior, and life history of a fish species (Kitano et al., 2007), and allometry has been suggested to be a main component of sexual shape dimorphism because it accounts for size dimorphism (Gidaszewski et al., 2009). Up to the present, only differences in sexual size dimorphism and growth rates between sexes have been studied for the bluemouth in the NE Atlantic and Mediterranean (White et al., 1998; Kelly et al., 1999; Massutí et al., 2000; Abecasis et al., 2006; Ribas et al., 2006; Sequeira et al., 2009). However, both of these topics are still being studied for bluemouth, as some of these authors have found that females grow faster and achieve a larger asymptotic length, while others have found the opposite trend, and in some studies, no differences in growth rates were detected at all.

Recently, Sequeira et al. (2009) suggested that these discrepancies in results could be related to differences in the length ranges sampled in the various studies. For other species of the same genus, such as *Helicolenus percoides* in southeastern Australian waters, Withell & Wankowski (1988) found that the growth rates of the sexes seemed to be comparable, though females attained a larger size, but a recent study found that males grew slightly faster than females (Paul & Horn, 2009). In the case of *Helicolenus lengerichi*, no differences in growth rates were observed between males and females (Petrova & Chekunova, 1979, as cited in Withell & Wankowski, 1988).

Regarding ontogenetic shape changes, no difference in the growth patterns of males and females was observed within any of the NE Atlantic locations. However, in the Mediterranean, we could only compare growth trajectories for males and females from two of the four areas included in this study: Alicante and subarea A2 in the Alboran Sea (see "Results" section). For Alicante, we did not find differences in allometric growth between sexes. However, our study was inconclusive about possible differences between sexes for subarea A2 due to a relatively low sample size and because the angle between the regression vectors was marginally significant. Perhaps in future studies, a combination of the study of growth rates and allometric shape changes between sexes can be used to better understand sexual dimorphism in bluemouth populations.

In fisheries, differences in life history parameters between groups of fish are assumed to be evidence that populations are geographically and/or reproductively isolated and can be considered discrete stock units for management purposes (Ihssen et al., 1981; Begg, 2005). Thus, the information provided in the present study can be used to complement further studies regarding stock identification of bluemouth around the Iberian Peninsula. Moreover, in the context of stock identification, morphological discrimination among groups of fish is often difficult because samples may differ in size composition and because allometric growth is taking place. This situation implies the risk of confounding real differences between fish populations with accidental differences in size composition of the samples. Thus, in morphometric studies, it is necessary to eliminate shape variation associated with size before we can compare multiple groups (Burnaby, 1966; Mosimann, 1970; Thorpe, 1976, 1983; Humphries et al., 1981; Rohlf & Bookstein, 1987; Klingenberg, 1996). According to our results, bluemouth from around the Iberian Peninsula and the Porcupine Bank exhibit allometric growth. Therefore, this fact has to be taken into account if morphological comparisons of bluemouth from different areas are to be made for the purpose of stock identification in Iberian waters.

In geometric morphometrics, one of the preferred methods for size-correction of variables is to use the residuals of a pooled-within group regression as 'size-free' variables (Klingenberg, 2008) because in this way, we only remove shape variation that is due to size variation, in contrast to what happens with other methods, such as principal components analysis (PCA) (Jolicoeur, 1963) or Burnaby's method (Burnaby, 1966), where an entire dimension is removed from the analysis. In addition to this issue, PCA may not work well for geometric morphometric data because during the generalized Procrustes analysis (see "Materials and methods" section), isometric size is factored out from the samples in the rescaling step. In this way, only in cases where allometric growth is substantially present will the first Principal component be associated with size (Slice & Stitzel, 2004). However, the central assumption of all methods for size-correction is that the groups in the analysis share the same allometric trajectories (Klingenberg, 1996), and in this study, we found evidence that the growth trajectories for bluemouth among the study areas are not homogeneous, representing a problem for size-correction of the shape variables that should be addressed prior to morphometric analysis. Another way to avoid size effects would be to compare samples with similar size compositions or to only use fish of the same size (selective sampling). However, for demersal species like the bluemouth, it is not easy to obtain homogeneous samples from all of the study areas because most of the time, sampling depends on fisheries that target other species (e.g., European hake, *Merluccius merluccius*, or Blue and red shrimp, *Aristeus antennatus*), and the size range of the captured specimens in each area can be affected by factors such as depth and the type of bottom of the fishing area and the fishing gear used (i.e., trawling nets, long-lines or gill-nets) (Demestre et al., 2000; Massutí et al., 2001; Santos et al., 2002). In addition, bluemouth samples from trawling research surveys also vary in size composition, as in this study. In the case of size selective sampling, shape variation outside the chosen size range is ignored, the covariance is reduced; and the ability to distinguish groups is, therefore, weakened (Cadrin, 2000). Another potential drawback of size selective sampling is that if the growth rate is very different among putative populations and it is uncoupled from shape changes, we could be comparing specimens of very different ages, thus resulting in confounding effects.

Conclusions

In this study, geometric morphometric techniques allowed us to determine and visualize ontogenetic shape trajectories for bluemouth specimens from around the Iberian Peninsula and the Porcupine Bank. The general pattern of ontogenetic changes seemed to be related to the changing ecology of the species (i.e., ontogenetic diet and habitat adaptations) and consisted of a relative expansion of the area between the second-preopercular spine and the pectoral fin, a relative deepening and shortening of the body and an upward shift of the snout as the head becomes more compact in relation to the body. However, the degree to which the above described ontogenetic shape changes were present in bluemouth from each of the studied areas was different, indicating that the growth trajectories are not homogeneous. The factors that cause these growth differences are likely to be complex, but a combination of factors such as food availability along with a low fishing mortality and unique environmental conditions is likely to produce distinctive growth patterns such as the ones that we found in areas including the Cantabrian Sea and the Alboran Sea. For the purpose of fisheries management, these observed differences in the way that bluemouth grow could be an indicator that different populations exist and should be further studied. However, if morphological comparisons are to be used as a tool to identify phenotypic stocks, the fact that growth differences exist should be considered because most size-correction methods assume equal or parallel growth trajectories to remove the effect of size from shape variables. Finally, this kind of shape information could be also used to complement traditional growth curves, showing what shape changes occur and when they take place during growth.

Acknowledgments RRM’s work was funded by a Marie Curie fellowship from the Early Stage Training site ECOSUMMER (MEST-CT-2005-020501) and a CONACyT-Mexico scholarship. This research was also supported by the project CTM2006-13964-C03-00 of the Spanish Ministry of Science and Innovation. We would like to thank the anonymous reviewers whose comments and suggestions helped to improve this manuscript.

References

Abad, E., I. Preciado, A. Serrano & J. Baro, 2007. Demersal and epibenthic assemblages of trawlable grounds in the northern Alboran Sea (western Mediterranean). Scientia Marina 71: 513–524.

Abecasis, D., A. R. Costa, J. G. Pereira & M. R. Pinho, 2006. Age and growth of bluemouth, *Helicolenus dactylopterus* (Delaroche, 1809) from the Azores. Fisheries Research 79: 148–154.

Abelló, P., A. Carbonell & P. Torres, 2002. Biogeography of epibenthic crustaceans on the shelf and upper slope off the Iberian Peninsula Mediterranean coasts: implications for the establishment of natural management areas. Scientia Marina 66: 183–198.

Aboim, M. A., G. M. Menezes, T. Schlitt & A. D. Rogers, 2005. Genetic structure and history of populations of the deep-sea fish *Helicolenus dactylopterus* (Delaroche, 1809) inferred from mtDNA sequence analysis. Molecular Ecology 14: 1343–1354.

Alberch, P., S. J. Gould, G. F. Oster & D. B. Wake, 1979. Size and shape in ontogeny and phylogeny. Paleobiology 5: 296–317.

Allain, V., 2001. Reproductive strategies of three deep-water benthopelagic fishes from the northeast Atlantic Ocean. Fisheries Research 51: 165–176.

Barlow, G. W., 1961. Causes and significance of morphological variation in fishes. Systematic Zoology 10: 105–117.

Begg, G. A., 2005. Life history parameters. In Cadrin, S. X., K. D. Friedland & J. R. Waldman (eds), Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, San Diego, CA: 119–150.

Blaber, S. J. M. & C. M. Bulman, 1987. Diets of fishes of the upper continental slope of eastern Tasmania: content, calorific values, dietary overlap and trophic relationships. Marine Biology 95: 345–356.

Bookstein, F. L., 1989. Principal warps: thin-plate splines and the decomposition of deformations. IEEE Transactions on Pattern Analysis and Machine Intelligence 11: 567–585.

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data: Geometry and Biology. Cambridge University Press, Cambridge.

Borges, L., E. Rogan & R. Officer, 2005. Discarding by the demersal fishery in the waters around Ireland. Fisheries Research 76: 1–13.

Burnaby, T. P., 1966. Growth-invariant discriminant functions and generalized distances. Biometrics 22: 96–110.

Cadrin, S. X., 2000. Advances in morphometric identification of fishery stocks. Reviews in Fish Biology and Fisheries 10: 91–112.

Cadrin, S. X., 2005. Morphometric landmarks. In Cadrin, S. X., K. D. Friedland & J. R. Waldman (eds), Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, San Diego, CA: 153–172.

Cartes, J. E., A. Serrano, F. Velasco, S. Parra & F. Sanchez, 2007. Community structure and dynamics of deep-water decapod assemblages from Le Danois Bank (Cantabrian Sea, NE Atlantic): influence of environmental variables and food availability. Progress in Oceanography 75: 797–816.

Claytor, R. R. & H. R. MacCrimmon, 1987. Partitioning size from morphometric data: a comparison of five statistical procedures used in fisheries stock identification research. Canadian Technical Report of Fisheries and Aquatic Services No. 1531.

Czerwinski, I. A., J. C. Gutiérrez-Estrada, M. C. Soriguer & J. A. Hernando, 2008. Morphometric relations for body size and mouth dimensions for four fish species in the Strait of Gibraltar. Acta Ichthyologica et Piscatoria 38: 81–90.

Demestre, M., P. Sánchez & P. Abelló, 2000. Demersal fish assemblages and habitat characteristics on the continental shelf and upper slope of the north-western Mediterranean. Journal of the Marine Biological Association of the United Kingdom 80: 981–988.

Drake, A. G. & C. P. Klingenberg, 2008. The pace of morphological change: historical transformation of skull shape in St Bernard dogs. Proceedings of the Royal Society B 275: 71–76.

Dryden, I. L. & K. V. Mardia, 1998. Statistical Shape Analysis. John Wiley & Sons, New York.

Esteves, E., J. Aníbal, H. Krug & H. Marques da Silva, 1997. Aspects of age and growth of bluemouth, *Helicolenus dactylopterus dactylopterus* (Delaroche, 1809) from the Azores. Arquipélago. Life and Marine Sciences 15A: 83–95.

Fariña, A. C., J. Freire & E. González-Gurriarán, 1997. Megabenthic decapod crustacean assemblages on the Galician continental shelf and upper slope (north-west Spain). Marine Biology 127: 419–434.

Frost, S. R., L. F. Marcus, F. L. Bookstein, D. P. Reddy & E. Delson, 2003. Cranial allometry, phylogeography and systematics of large-bodied papionins (Primates: Cercopithecinae) inferred from geometric morphometric analysis of landmark data. Anatomical Record 275: 1048–1072.

Furlani, D. M., 1997. Development and ecology of ocean perch larvae, *Helicolenus percoides* (Richardson, 1842) (Pisces: Scorpaenidae), from southern Australian waters, with notes on the larvae of other sympatric scorpaenid genera. Marine and Freshwater Research 48: 311–320.

Garabana, D., 2005. The genus *Sebastes* Cuvier, 1829 (Pisces, Scorpaenidae) in the North Atlantic: species and stock discrimination using traditional and geometric morphometrics. PhD Thesis, University of Vigo, Spain.

García-Lafuente, J., 2006. Topical studies in oceanography: the Gulf of Cadiz oceanography: a multidisciplinary view. Deep Sea Research Part II 53: 1141–1143.

García-Lafuente, J., J. Delgado, F. Criado-Aldeanueva, M. Bruno, J. del Río & J. Miguel Vargas, 2006. Water mass circulation on the continental shelf of the Gulf of Cadiz. Deep Sea Research Part II 53: 1182–1197.

Gidaszewski, N. A., M. Baylac & C. P. Klingenberg, 2009. Evolution of sexual dimorphism of wing shape in the *Drosophila melanogaster* subgroup. BMC Evolutionary Biology 9: 110.

Good, P., 1994. Permutation Tests: A Practical Guide to Resampling Methods for Testing Hypotheses. Springer, New York.

Hayes, D. B., C. P. Ferreri & W. Taylor, 1996. Linking fish habitat to their population dynamics. Canadian Journal of Fisheries and Aquatic Sciences 53: 383–390.

Heessen, H. J. L., J. R. G. Hislop & T. W. Boon, 1996. An invasion of the North Sea by blue-mouth, *Helicolenus dactylopterus* (Pisces, Scorpaenidae). ICES Journal of Marine Sciences 53: 874–877.

Humphries, J. M., Jr., F. L. Bookstein, B. Chernoff, G. R. Smith, R. L. Elder & S. G. Poss, 1981. Multivariate discrimination by shape in relation to size. Systematic Zoology 30: 291–308.

Ihssen, P. E., H. E. Booke, J. M. Casselman, J. M. McGlade, N. R. Payne & F. M. Utter, 1981. Stock identification: materials and methods. Canadian Journal of Fisheries and Aquatic Sciences 38: 1838–1855.

Jolicoeur, P., 1963. The multivariate generalization of the allometry equation. Biometrics 19: 497–499.

Karpouzi, V. S. & K. I. Stergiou, 2003. The relationships between mouth size and shape and body length for 18 species of marine fishes and their trophic implications. Journal of Fish Biology 62: 1353–1365.

Kelly, C. J., P. L. Connolly & J. J. Bracken, 1999. Age estimation, growth, maturity, and distribution of the blue-mouth rockfish *Helicolenus d. dactylopterus* (Delaroche 1809) from the Rockall Trough. ICES Journal of Marine Science 56: 61–74.

Kitano, J., S. Mori & C. Peichel, 2007. Sexual dimorphism in the external morphology of the threespine stickleback (*Gasterosteus aculeatus*). Copeia 2: 336–349.

Klingenberg, C. P., 1996. Multivariate allometry. In Marcus, L. F., M. Corti, A. Loy, G. J. P. Naylor & D. E. Slice (eds), Advances in Morphometrics. Plenum Press, New York: 23–49.

Klingenberg, C. P., 2008. MorphoJ Software/Documentation. Version 1.00i. Faculty of Life Sciences, University of Manchester, UK. http://www.flywings.org.uk/MorphoJ_page.htm.

Klingenberg, C. P. & R. Froese, 1991. A multivariate comparison of allometric growth patterns. Systematic Zoology 40: 410–419.

Kouttouki, S., E. Georgakopoulou, P. Kaspiris, P. Divanach & G. Koumoundouros, 2006. Shape ontogeny and variation in the sharpsnout seabream, *Diplodus puntazzo* (Cetti 1777). Aquaculture Research 37: 655–663.

Law, R., 2000. Fishing, selection, and phenotypic evolution. ICES Journal of Marine Science 57: 659–669.

Loy, A., S. Cataudella & M. Corti, 1996. Shape changes during the growth of the sea bass, *Dicentrarchus labrax* (Teleostei: Perciformes), in relation to different rearing conditions. In Marcus, L. F., M. Corti, A. Loy, G. J. P. Naylor & D. E. Slice (eds), Advances in Morphometrics. Plenum Press, New York: 399–405.

Loy, A., L. Mariani, M. Bertelletti & L. Tunesi, 1998. Visualizing allometry: geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). Journal of Morphology 237: 137–146.

Macpherson, E., 1979. Estudio sobre el régimen alimentario de algunos peces en el Mediterráneo occidental. Miscelánea Zoológica 5: 93–107.

Macpherson, E., 1985. Daily ration and feeding periodicity of some fishes off the coast of Namibia. Marine Ecology Progress Series 26: 253–260.

Mamie, J. C. J., D. J. Beare, E. G. Jones, M. Kienzle, H. Dobby, M. R. Heath & D. G. Reid, 2007. Aspects of the distribution and growth of bluemouth (*Helicolenus dactylopterus*, Delaroche 1809) since its invasion of the northern North Sea in 1991. Fisheries Oceanography 16: 85–94.

Massutí, E., B. Morales-Nin & J. Moranta, 2000. Age and growth of blue-mouth, *Helicolenus dactylopterus* (Osteichthyes: Scorpaenidae), in the western Mediterranean. Fisheries Research 46: 165–176.

Massutí, E., J. Moranta, L. Gil de Sola, B. Morales-Nin & L. Prats, 2001. Distribution and population structure of the rockfish *Helicolenus dactylopterus* (Pisces: Scorpaenidae) in the western Mediterranean. Journal of the Marine Biological Association of the United Kingdom 81: 129–141.

Massutí, E., J. Gordon, J. Morata, S. Swan, C. Stefanescu & N. Merrett, 2004. Mediterranean and Atlantic deep-sea fish assemblages: differences in biomass composition and size-related structure. Scientia Marina 68: 101–115.

Mendonça, A., E. Isidro, G. Menezes, M. Rui Pinho, O. Melo & S. Estácio, 2006. New contribution to the reproductive features of bluemouth *Helicolenus dactylopterus dactylopterus* from northeast Atlantic (Azores Archipelago). Scientia Marina 70: 679–688.

Mitteroecker, P., P. Gunz, M. Bernhard, K. Schaefer & F. L. Bookstein, 2004. Comparison of cranial ontogenetic trajectories among great apes and humans. Journal of Human Evolution 46: 679–698.

Moranta, J., E. Massutí & B. Morales-Nin, 2000. Fish catch composition of the deep-sea decapod crustacean fisheries in the Balearic Islands (western Mediterranean). Fisheries Research 45: 253–264.

Mosimann, J. E., 1970. Size allometry: size and shape variables with characterizations of the lognormal and generalized gamma distributions. Journal of the American Statistical Association 65: 930–945.

Muñoz, M., M. Casadevall & S. Bonet, 1999. Annual reproductive cycle of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaeniformes) with special reference to the ovaries sperm storage. Journal of the Marine Biological Association of the United Kingdom 79: 521–529.

Muñoz M., M. Casadevall, S. Bonet & I. Quagio-Grassiotto, 2000. Sperm storage structures in the ovary of *Helicolenus dactylopterus dactylopterus* (Teleostei: Scorpaenidae): An ultrastructural study. Environmental Biology of Fishes 58: 53–59.

Nouar, A. & C. Maurin, 2000. Régime alimentaire de *Helicolenus dactylopterus dactylopterus* (Pisces: Scorpaenidae) des fonds chalutables des côtes algériennes. Cahiers de Biologie Marine 41: 313–320.

Paul, L. J. & P. L. Horn, 2009. Age and growth of sea perch (*Helicolenus percoides*) from two adjacent areas off the east coast of South Island, New Zealand. Fisheries Research 95: 169–180.

Ribas, D., M. Muñoz, M. Casadevall & L. Gil de Sola, 2006. How does the northern Mediterranean population of *Helicolenus dactylopterus dactylopterus* resist fishing pressure? Fisheries Research 79: 285–293.

Rochet, M. J., 1998. Short-term effects of fishing on life history traits of fishes. ICES Journal of Marine Science 55: 371–391.

Rohlf, F. J., 1990. Morphometrics. Annual Review of Ecology and Systematics 21: 299–316.

Rohlf, F. J., 1999. Shape statistics: Procrustes superimpositions and tangent spaces. Journal of Classification 16: 197–223.

Rohlf, F. J., 2006. TpsDig. Version 2.10. SUNY, Stony Brook, NY.

Rohlf, F. J. & F. L. Bookstein, 1987. A Comment on shearing as a method for "size correction". Systematic Zoology 36: 356–367.

Rohlf, F. J. & D. Slice, 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Zoology 39: 40–59.

Sánchez, F., 1993. Las comunidades de peces de la plataforma del Cantábrico. Publicaciones Especiales. Instituto Español de Oceanografía 13: 1–137.

Sánchez, F. & J. Gil, 2000. Hydrographic mesoscale structures and poleward current as a determinant of hake (*Merluccius merluccius*) recruitment in the southern Bay of Biscay. ICES Journal of Marine Science 57: 152–170.

Sánchez Lizaso, J. L., R. Goñi, O. Reñones, J. A. García Charton, R. Galzin, J. T. Bayle, P. Sánchez Jerez, A. Pérez Ruzafa & A. A. Ramos, 2000. Density dependence in marine protected populations: a review. Environmental Conservation 27: 144–158.

Sánchez, P., M. Demestre & P. Martín, 2004. Characterisation of the discards generated by bottom trawling in the northwestern Mediterranean. Fisheries Research 67: 71–80.

Sánchez, F., A. Serrano, F. Sánchez, A. Serrano, S. Parra, M. Ballesteros & J. E. Cartes, 2008. Habitat characteristics as determinant of the structure and spatial distribution of epibenthic and demersal communities of Le Danois Bank (Cantabrian Sea, N. Spain). Journal of Marine Systems 72: 64–86.

Santos, M. N., M. B. Gaspar, C. C. Monteiro & P. Vasconcelos, 2002. Gill net and long-line catch comparisons in a hake fishery: the case of southern Portugal. Scientia Marina 66: 433–441.

Sequeira, V., I. Figueiredo, M. Muñoz & L. S. Gordo, 2003. New approach to the reproductive biology of *Helicolenus dactylopterus*. Journal of Fish Biology 62: 1206–1210.

Sequeira, V., A. Neves, A. R. Vieira, I. Figueiredo & L. S. Gordo, 2009. Age and growth of bluemouth, *Helicolenus dactylopterus*, from the Portuguese continental slope. ICES Journal of Marine Science 66: 524–531.

Serrano, A., F. Velasco, I. Olaso & F. Sánchez, 2003. Macrobenthic crustaceans in the diet of demersal fish in the Bay of Biscay in relation to abundance in the environment. Sarsia 88: 36–48.

Sheets, H. D., 2000. VecCompare6 Software (Integrated Morphometrics Package Series). Department of Physics, Canisius College, Buffalo, NY.

Slice, D. E., 2001. Landmark coordinates aligned by Procrustes analysis do not lie in Kendall's shape space. Systematic Biology 50: 141–149.

Slice, D. E. & J. Stitzel, 2004. Landmark-based geometric morphometrics and the study of allometry. In S.A.E. Conference on Digital Human Modeling for Design and Engineering (DHM), June, 2004, Oakland University, Rochester, MI. Pub. No.: 04DHM-36.

Stefanescu, C., J. Rucabado & D. Lloris, 1992. Depth-size trends in western Mediterranean demersal deep-sea fishes. Marine Ecology Progress Series 81: 205–213.

Strauss, R. E. & L. A. Fuiman, 1985. Quantitative comparisons of body form and allometry in larval and adult Pacific sculpins (Teleostei: Cottidae). Canadian Journal of Zoology 63: 1582–1589.

Swain, D. P., J. A. Hutchings & C. J. Foote, 2005. Environmental and genetic influences on stock identification characters. In Cadrin, S. X., K. D. Friedland & J. R. Waldman (eds), Stock Identification Methods: Applications in Fishery Science. Elsevier Academic Press, San Diego, CA: 45–85.

Thorpe, R. S., 1976. Biometric analysis of geographic variation and racial affinities. Biological Reviews 51: 407–452.

Thorpe, R. S., 1983. Morphometric studies in inbred and hybrid House mice (*Mus* sp.): multivariate analysis of size and shape. Journal of Zoology 199: 421–432.

Tortonese, E., 1960. General remarks on the Mediterranean deep-sea fishes. Bulletin de l'Institute Océanographique, Monaco, 1167: 1–4.

Uiblein, F., P. Lorance & D. Latrouite, 2003. Behaviour and habitat utilisation of seven demersal fish species on the Bay of Biscay continental slope, NE Atlantic. Marine Ecology Progress Series 257: 223–232.

Vila S., M. Sàbat, M. R. Hernandez & M. Muñoz, 2007. Intraovarian sperm storage in *Helicolenus dactylopterus dactylopterus*: Fertilization, crypt formation and maintenance of stored sperm. The Raffles Bulletin of Zoology 14: 21–27.

Webb, P. W., 1984. Body form, locomotion and foraging in aquatic vertebrates. American Zoologist 24: 107–120.

White, D. B., D. M. Wyanski & G. R. Sedberry, 1998. Age, growth, and reproductive biology of the blackbelly rosefish from the Carolinas, U.S.A. Journal of Fish Biology 53: 1274–1291.

White, M., C. Mohn, H. de Stitger & G. Mottram, 2005. Deep-water coral development as a function of hydrodynamics and surface productivity around the submarine banks of the Rockall Trough, NE Atlantic. In Freiwald, A. & J. M. Roberts (eds), Cold-water Corals and Ecosystems. Springer Verlag, Berlin: 503–514.

Whitehead, P. J. P., M. L. Bauchot, J. C. Hureau, J. Nielsen & E. Tortonese, 1986. Fishes of the North-eastern Atlantic and Mediterranean. UNESCO, Paris.

Wimberger, P. H., 1992. Plasticity of fish body shape. The effects of diet, development, family and age in two species of *Geophagus* (Pisces: Cichlidae). Biological Journal of the Linnean Society 45: 197–218.

Withell, A. F. & J. W. Wankowski, 1988. Estimates of age and growth of ocean perch, *Helicolenus percoides* (Richardson), in south-eastern Australian waters. Australian Journal of Marine and Freshwater Research 39: 441–457.

Zelditch, M. L., B. L. Lundrigan, D. H. Sheets & T. Garland, 2003a. Do precocial mammals develop at a faster rate? A comparison of rates of skull development in *Sigmodon fulviventer* and *Mus musculus domesticus*. Journal of Evolutionary Biology 16: 708–720.

Zelditch, M. L., D. H. Sheets & W. L. Fink, 2003b. The ontogenetic dynamics of shape disparity. Paleobiology 29: 139–156.

Hydrobiologia (2011) 670:23–34
DOI 10.1007/s10750-011-0672-x

ECOSYSTEMS AND SUSTAINABILITY

Variability in behaviour of four fish species attracted to baited underwater cameras in the North Sea

**Iñigo Martinez · Emma G. Jones ·
Sarah L. Davie · Francis C. Neat ·
Ben D. Wigham · Imants G. Priede**

Published online: 30 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Baited underwater camera (BUC) systems to estimate demersal fish abundance are becoming increasingly considered as an alternative to traditional survey methods, particularly in environments that contain sensitive habitats or protected species. Based on 27 replicate deployments of BUCs at 100 m depth in the northern North Sea, in rank order of abundance, hagfish (*Myxine glutinosa*), flatfish mainly dabs (*Limanda limanda*), whiting (*Merlangius merlangus*) and haddock (*Melanogramus aeglefinus*) were observed consistently at baits. Higher maximum numbers ($N_{\max}$) occurred during daytime in all species with the most significant effect in flatfish, 18 in daytime and 5 at night-time. Bottom current had no significant effect on numbers of whiting, flatfish or haddock. The $N_{\max}$ of hagfish was strongly related to current speed in a non-linear way with an increase in numbers up to 10 cm s^{-1} and then decrease in $N_{\max}$ at higher water speeds. Understanding and accounting for such species-specific influences is important in the design of long term monitoring surveys using baited cameras.

Keywords Baited camera · Current · Demersal fish · North Sea · Fisheries

Introduction

Baited underwater cameras (BUC) are a potential non-extractive alternative to traditional sampling tools, such as trawling and long lines, for generating metrics of abundance and diversity in inaccessible areas or where the habitat and/or populations are protected. This sampling method has been used successfully in deep sea environments (Priede & Bagley, 2001; Jones et al., 2003), marine protected areas (Willis et al., 2003), shallow temperate and

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

I. Martinez (✉) · E. G. Jones · S. L. Davie · F. C. Neat
Marine Scotland-Science, Marine Laboratory,
375 Victoria Road, Aberdeen AB11 9DB, UK
e-mail: i.martinez@abdn.ac.uk

Present Address:
E. G. Jones
National Institute of Water & Atmospheric Research Ltd.
(NIWA), 41 Market Place, Auckland Central,
1010 Private Bag 99940, Newmarket Auckland 1149,
New Zealand

B. D. Wigham
Dove Marine Laboratory, Newcastle University,
Cullercoats, North Shields NE30 4PZ, UK

Present Address:
S. L. Davie
Marine Institute, Rinville, Oranmore, Co. Galway, Ireland

I. G. Priede
University of Aberdeen, Oceanlab, Newburgh, Aberdeen
AB11 6AA, UK

tropical habitats (Ellis & Demartini, 1994; Cappo et al., 2004; Harvey et al., 2007) and juvenile fish habitats (Laurel et al., 2009).

All sampling methods have inherent selectivity bias related to the specific behaviour of different fish, and the design and efficiency of the gear. BUCs are static systems based on attracting fish to bait in the same way as fishing pots, traps and longlines. Fish attracted to the bait are photographed but do not need to be hooked or trapped. This reduces some of the "near field" selective bias compared to extractive methods but not the "far field" biases. The attraction of fish to bait depends on a variety of environmental and biological factors that affect the detection and reaction thresholds of fish to the odour plume from the bait. The active space of the bait (Bossert & Wilson, 1962) will be defined by the chemical properties of the bait itself, the olfactory detection thresholds of different species and environmental factors that influence the propagation of the odour plume such as current speeds, tidal patterns and seabed topography (Olsen & Laevastu, 1983; Engås & Løkkeborg, 1994). The reaction threshold of fish, the point at which it chooses to search for the source of the odour, will be influenced by factors such as time of day, current speeds in relation to general activity level and swimming ability, hunger level and the presence of other potential prey and predators (e.g. Løkkeborg et al., 1995; Stoner, 2003). The active area will also shrink over time as rate of release of attractants declines (Løkkeborg, 1990), and most fish will be attracted within the first few hours (Løkkeborg & Pina, 1997). The influence of these factors on the performance of baited fish gears was reviewed by Stoner (2004) who concluded that temperature, light level and current speed were likely to have large impacts on fish catchability. Most baited camera studies to date have either been carried out during daylight hours in shallow, well lit environments, or depths well beyond the level of sunlight penetration and variation in light levels has not been investigated. The impact of current speed has also received limited attention, although Heagney et al. (2007) demonstrated an effect on fish abundance estimates and assemblage structure in a pelagic environment using an area-based approximation for plume dispersal.

A number of metrics can be derived from baited camera studies including first arrival time, maximum number of fish observed at any one time, time to maximum number and variations on initial arrival rate at the bait. First arrival time has successfully been used in deep sea studies (Priede & Merrett, 1996) where fish densities are relatively low and arrival times are in the range of tens of minutes to hours. In shallower environments with higher fish densities, this metric has been found to be less useful where arrival times are consistently short (Willis et al., 2000; Stoner et al., 2008). Stoner et al. (2008) also found time to TFA (time of first arrival) was not as well correlated with comparative seine net catches as $N_{\max}$ or NFA; total number of fish observed arriving within a fixed time period. Farnsworth et al. (2007) estimated absolute abundances with a modelling approach which included regression-based prediction from the initial (rising) slope of numbers at the bait, the rate of departure during the declining phases and a hidden Markov model estimate of the number of fish out of the field of view. Given the relatively shallow depth in this study and the use of stills images rather than video footage, $N_{\max}$ was chosen as the most suitable index for estimating relative abundances despite its likely conservative reflection of true abundance. It is also one of the most widely used indices and in this paper we investigated the potential effects of current speed and light level (day/night) on this index of abundance of fish attracted to a baited camera system in a shallow water environment in the North Sea. The species chosen for investigation were those most commonly observed in photographs, and displaying differing behavioural traits.

Materials and methods

Study site

The study area lies 55 km northeast of Peterhead, Scotland (57°48′N, 0°58′W) between the "Fladden" and "Bosies Bank" fishing grounds (Fig. 1). It is a flat, sandy area of approximately 100 m depth. Data were obtained on three cruises: 25th April to 2nd of May 2005 on board of *FRV Scotia* (length: 68.6 m), 23rd to 30th April 2007 on board of the fishing charter vessel *MFV Prowess* (length: 50 m) and 8th May to 4th June 2008 on board of the *VOS Lismore* (length: 54 m). Details of deployments are given in Table 1.

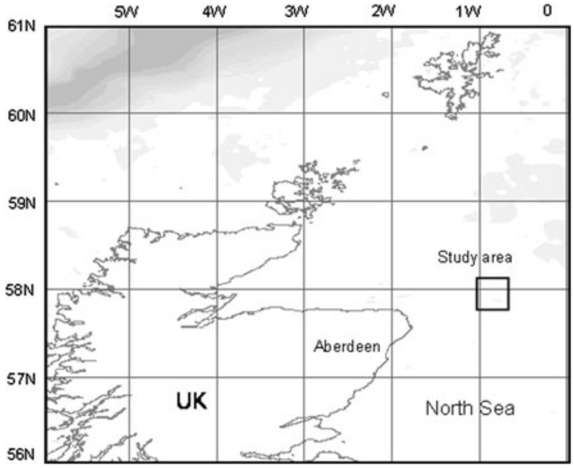


Fig. 1 Map showing sampling location, Central North Sea within ICES division IVa lower confine, 50 nautical miles NE of Aberdeen, Scotland

Baited underwater camera system

The BUC used for these experiments was based on the Robust BIOdiversity (ROBIO) lander designed for deep water research (Jamieson & Bagley, 2005). A downward facing Kongsberg 5 mega pixel underwater camera OE14-208 and flash unit OE11-242, with 24 V battery pack were mounted on an aluminium frame (height 1.8 m, base 1.3 m; Fig. 2) suspended 2.1 m above the seafloor by flotation attached to a mooring line and a 120 kg ballast weight tethered to the frame. The camera and flash were orientated to view approximately 3.2 m^2 of the seafloor. A standard bait (500 g) of fresh mackerel (*Scomber scombrus*) was attached to a reference scale fixed to the ballast weight. A current meter (Aquadopp, Nortek Ltd) was also attached to measure temperature, depth, speed and direction of the current. A scanmar depth sensor was used to provide real-time readings of the depth of the camera frame. A mooring line to a surface marker buoy and flag allowed recovery of the camera frame and ballast at the end of each deployment. An acoustic ballast release system was used as a back-up method for retrieving the BUC.

The camera was programmed to take one picture every minute and the current meter recorded measurements every 30 s. The current was averaged for the first 2 h of deployment, discarding readings where the current meter indicated excessive tilt or spin of the BUC system. All fish observed in the photos were identified (to species level where possible) and counted. Flatfish were consistently difficult to identify to species level as a direct result of the poor contrast between the seabed and the flatfish's colouration. Sub-sampling of particularly high quality images suggested that over 97% of the flatfish attracted to the baited camera were common dab (*Limanda limanda*), with occasional plaice (*Pleuronectes platessa*) and long-rough dab (*Hippoglossodes platessoides*). Deployment times varied from 49 to 423 min but only those over 2 h in duration were considered. The time period from which $N_{\max}$ (maximum number) was taken was standardised to the first 2 h. Deployments were classed as day or night as defined by rising and setting sun times for the day and location obtained from the U.S. Navy Observatory Astronomical Applications (http://www.usno.navy.mil/).

Data analysis

All statistical analyses were performed using R software version 2.4. Non-parametric univariate Kruskal–Wallis rank sum tests were performed to test for differences in water current speed and temperature with the factor year. Temperature did not vary significantly between years and was not included in any further analyses. Factors affecting $N_{\max}$ for the four most abundant species observed at the bait were analysed using generalised linear models (GLMs) and generalised additive models (GAMs). Current velocity at the seabed was treated as a continuous explanatory variable with light level (day/night) and year treated as categorical variables. $N_{\max}$ is a count data type and therefore was assumed to follow a Poisson distribution. When necessary, models were corrected for overdispersion. Models were optimised with backward selection (drop1 and step functions in R). The effects of adding interactions were also considered. Where necessary, nested models were compared and the model with the lowest AIC (Akaike Information Criteria) accepted. During the process of model selection and when the models showed very similar AIC values (differences in AIC <2), an *F* test was performed to compare and select between nested models. The influence of possible outliers or influential points in each model was evaluated by Cook's distance (values >1). Only the final optimal models are reported, provided no outliers were detected and no serious patterns remained in the residuals.

Table 1 Deployment, date, time of deployment (GMT), light level (D = day, N = night) and mean bottom current speed (cm s^{-1}) during the 2 h of deployment

Deploy	Date	Time (GMT)	Light	Current (cm s^{-1})
S0506	27/04/2005	15:58	D	8.3
S0508	28/04/2005	11:21	D	12.8
S05010	29/04/2005	23:03	N	5.8
S0509	29/04/2005	16:59	D	9.2
S05011	30/04/2005	09:13	D	8.8
S05012	30/04/2005	15:39	D	15.3
S05013	30/04/2005	23:35	N	5.7
S05014	01/05/2005	07:39	D	15.0
J07010	24/04/2007	12:31	D	16.3
J07011	24/04/2007	17:57	D	16.8
J07012	25/04/2007	23:17	N	13
J07013	25/04/2007	04:10	D	14.4
J07014	25/04/2007	09:59	D	21.1
J07015	25/04/2007	15:19	D	11.9
J07016	25/04/2007	20:28	N	16.6
J07017	26/04/2007	01:36	N	13.2
J07018	26/04/2007	06:45	D	19
J07019	26/04/2007	12:01	D	20.2
J07021	27/04/2007	22:59	N	12.1
J07022	27/04/2007	04:09	D	11.8
J07023	27/04/2007	09:18	D	15.5
J07024	27/04/2007	14:25	D	23.2
Lis08_10	17/05/2008	10:23	D	13.8
Lis08_11	19/05/2008	08:00	D	11.3
Lis08_13	20/05/2008	08:40	D	15.9
Lis08_14	21/05/2008	08:28	D	9.4
Lis08_17	22/05/2008	08:30	D	9.8

In addition to the models, the effect of light level on median $N_{\max}$ values is presented using boxplots for each of the four species.

Results

Four species of fish consistently appeared at the baits: haddock (*Melanogramus aeglefinus*), whiting (*Merlangius merlangus*), common dab (*Limanda limanda*) and hagfish (*Myxine glutinosa*). Plaice (*Pleuronectes platessa*) also co-occurred with the dabs from which they could not always be reliably distinguished so dabs and plaice are treated together in this analysis as flatfish.

A total number of 27 deployments (April 2005, $n = 8$, April 2007, $n = 14$ and May 2008, $n = 5$) were used for analysis, of which 6 deployments took place at night. Mean water current speed (cm s^{-1}) during the first 2 h of baited camera deployments ranged from 5.7 to 23.2 cm s^{-1} (Table 1) and was significantly greater in 2007 than in 2005 and 2008 (K–W, $P = 0.006$). The results of the GLMs and GAMs of $N_{\max}$ for the four species as a function of the chosen variables are presented in Table 2.

First arrival times were short, with 50% of the fish observed occurring during the first 10 min of deployment. The majority of arrival times (~90%) occurred within 40 min for whiting, 30 min for flatfish, 15 min for hagfish and 50 min for haddock. Although in general terms arrival times were shorter for higher $N_{\max}$ values, this was not true in the case of whiting and flatfish. Arrival times also were not significantly different between diurnal and nocturnal deployments regardless the magnitude of $N_{\max}$. Therefore, it was considered that $N_{\max}$ was a better reflection on the relative changes in fish population.

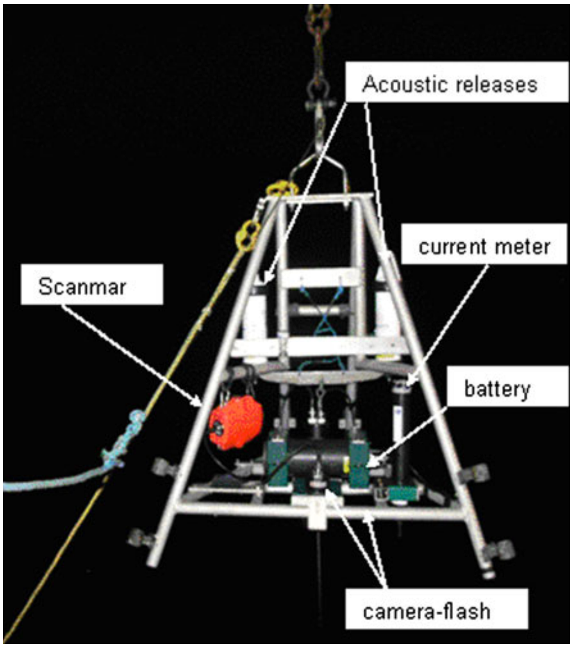


Fig. 2 Marine Scotland–Marine Laboratory baited camera system. Lander aluminium frame fitted with a Kongsberg stills camera and flash gun, battery and a Nortek Aquadopp current meter. Two acoustic releases where fitted as back up recovery system together with a scanmar unit to assess for frame tilt

Whiting were observed in 26 of the 27 deployments with maximum numbers of up to 31. When a Poission-GLM was fitted, overdispersion was detected, therefore, the standard errors were corrected using a quasi-GLM model (Zuur et al., 2009) with current speed, light level and year included as explanatory variables. The effect of current speed was not significant (Fig. 3a) and was dropped from the model. The optimal model showed that the two remaining explanatory variables (day/night and year) had a significant effect, explaining 37.2% of the deviance. *P* values for the factors were obtained by comparing nested models (Table 2). The model estimated fewer whiting during night deployments (Fig. 3b) and a higher N_{max} in 2005. The possible outlier (whiting $N_{max} = 31$) had a Cook's distance <0.5 and was therefore retained in the model.

Flatfish were observed in all 27 deployments, with the N_{max} ranging from 2 to 54 individuals. A quasi-GLM model was fitted to the data to correct for overdispersion. No relationship was found between the N_{max} of flatfish and the explanatory variables current speed and year. However, time of day had a significant influence on flatfish N_{max} (Table 2) and the model explained 30% of the deviance. Higher numbers of flatfish were observed during the daylight deployments (Fig. 4b). The possible outlier ($N_{max} = 54$) had a Cook's distance <0.5 and was therefore retained in the model.

Hagfish were present in all deployments with an N_{max} ranging from 2 to 204 individuals. A negative binomial-GAM was used to correct for overdispersion and model N_{max} of hagfish. The effect of light and year were not significant (Fig. 5b) but the model showed a significant non-linear relationship between current speed and N_{max}. The optimal model explained 36% of the deviance and the significance level of the smoother term was $P = 0.03$ (Table 2). The model predicts higher numbers of hagfish with medium current speeds (9–14 cm s^{-1}) and a decline at higher current speeds (Fig. 5a). Cook's distance for the two possible outliers (hagfish N_{max} 204 and 137) was less than 0.5 in each case, these data points were retained after being shown to have little influence on the model.

Table 2 Results of GLMs and GAMs for data on maximum number (N_{max}) of the four most abundant species and mean bottom current speed during 2 h deployments in cm s^{-1} (S_{2h}), light level (day/night) and interannual effect (2005, 2007, 2008)

Species	Model formula	*P* values			
		S_{2h}	Light	Year	Dev explained
Whiting	q-glm (whi.max ∼ light + year)	–	0.021	0.006	37.2
Hagfish	nb-gam (hag.max ∼ s(S_{2h}))	0.03	–	–	36
Flatfish	q-glm (flat.max ∼ light)	–	0.014	–	30.4
Haddock	p-glm (had.max ∼ S_{2h})	<0.001	–	–	29.2
	p-glm (had.max ∼ light + year)	–	0.03	<0.001	67

Haddock shows the two alternative models. Table gives values of probability (*P*) for each parameter in the model and the explained deviance. (p-glm = poisson-glm, q-glm = quasipoisson-glm and nb-gam = negative-binomial-gam)

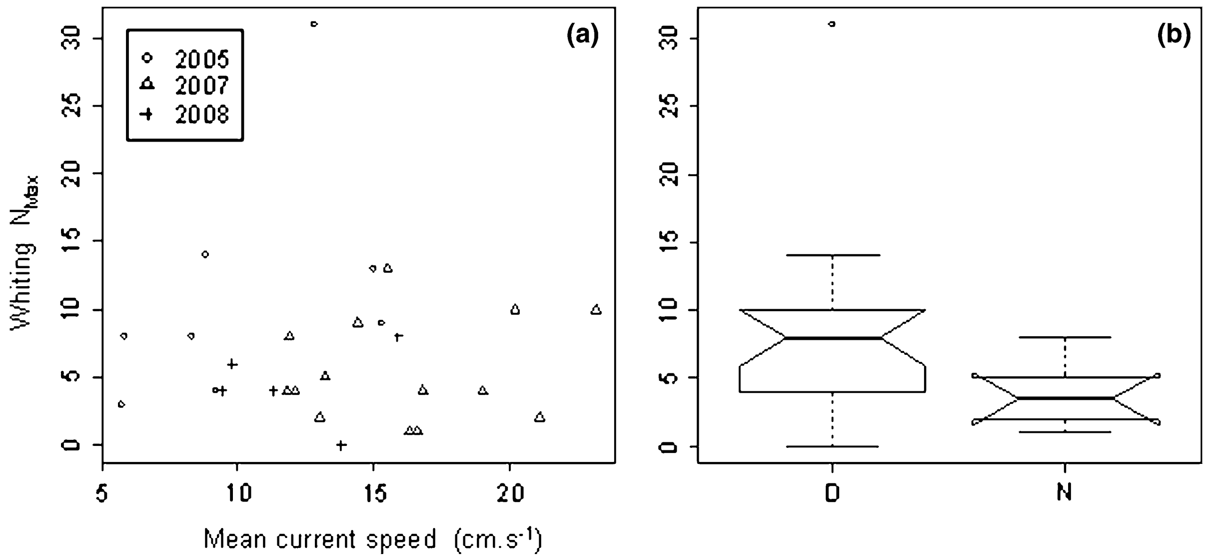


Fig. 3 **a** Whiting maximum number (N_{max}) values for BUC deployments from surveys carried out over 3 years as a function of current speed. **b** Boxplot of N_{max} of whiting during the day (D) and night (N) time sampling. The midpoint of each box represents the median N_{max} value and the 25% quartiles define the hinges. Differences between hinges show the spread of the data with whiskers representing maximum and minimum values within 1.5 times the box size. Where notches overlap the two medians do not differ

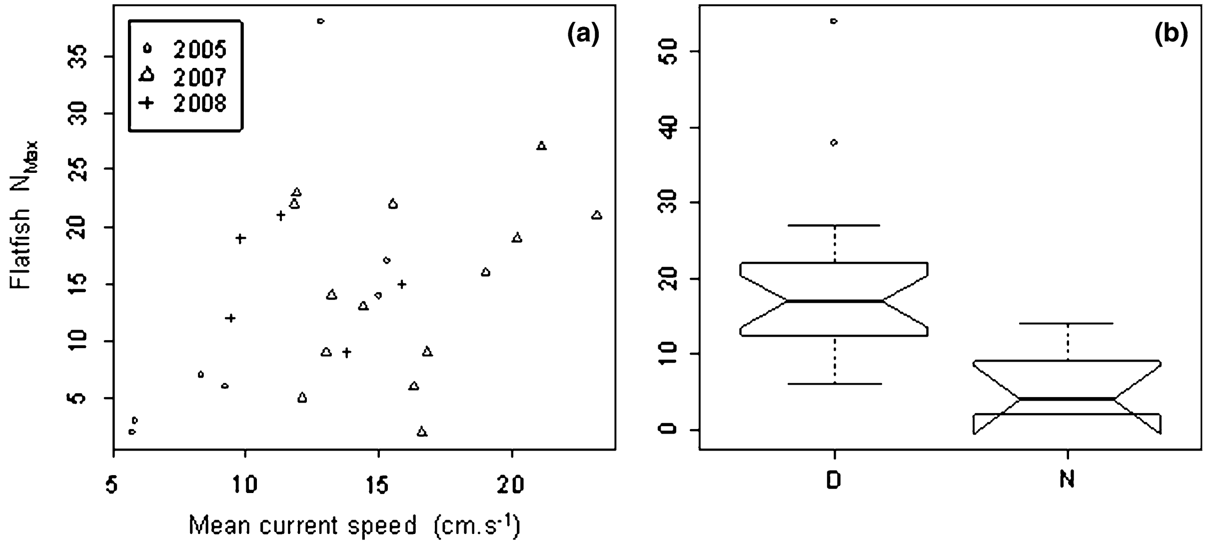


Fig. 4 **a** Flatfish maximum number (N_{max}) values for BUC deployments from surveys carried out over 3 years as a function of current speed. **b** Boxplot of N_{max} of flatfish during the day (D) and night (N) time sampling. The midpoint of each box represents the median N_{max} value and the 25% quartiles define the hinges. Differences between hinges show the spread of the data with whiskers representing maximum and minimum values within 1.5 times the box size. Where notches overlap the two medians do not differ

Haddock were observed in 25 out of 27 deployments, and were the least abundant of the four species with an N_{max} of up to 9 individuals. Both year and current speed were able to explain the variance in the N_{max}. However, the N_{max} of haddock and current speed have equivalent relationships with year, with

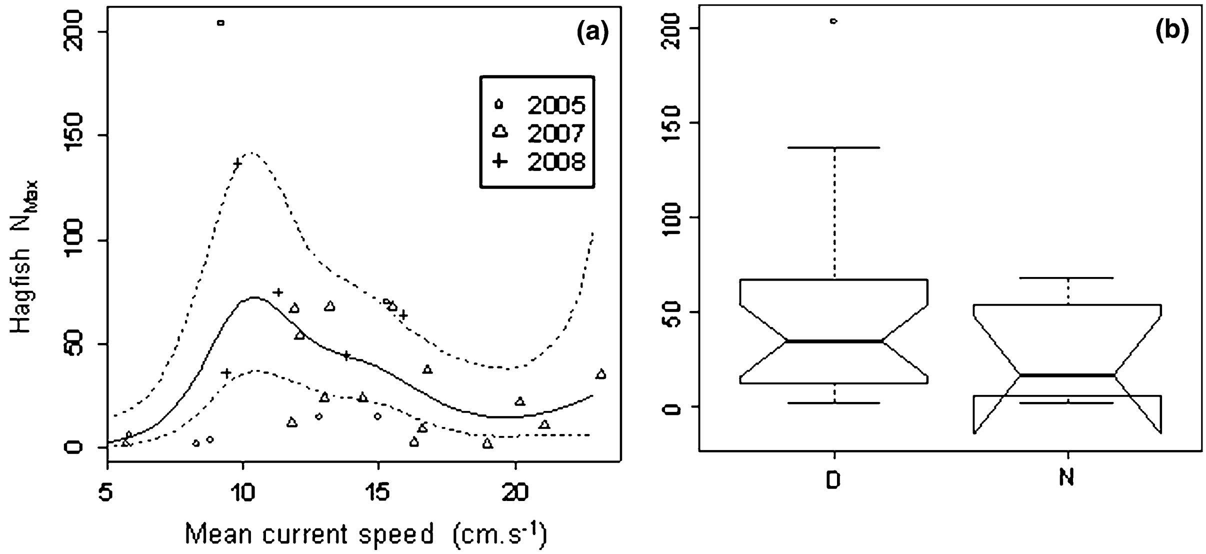


Fig. 5 **a** The effect of current speed on maximum number (N_{max}) for hagfish from three surveys with fitted values (*continuous line*) and 95% confident intervals (*dotted line*) estimated by a negative binomial-GAM. **b** Boxplot of hagfish N_{max} during the day (D) and night (N) time sampling. The midpoint of each box represents the median N_{max} value and the 25% quartiles define the hinges. Differences between hinges show the spread of the data with whiskers representing maximum and minimum values within 1.5 times the box size. Where notches overlap the two medians do not differ

more haddock and higher current on 2007 and they could not be modelled simultaneously. As both predictor variables were plausible, the model was run twice, first with current speed and then with year. In both cases a GLM with Poisson distribution was fitted. Current speed had a significant positive effect on haddock N_{max}, light (day/night) was not significant (Fig. 6; Table 2). The model explained 29.2% of the deviance. When year instead of current speed was used in the model, the light factor was borderline significant with a tendency towards higher N_{max} during the daytime (Fig. 6b) and year was highly significant with an increase in N_{max} in 2007. This model explained 67% of the deviance (Table 2).

Discussion

The hagfish *Myxine glutinosa*, the most abundant fish species attracted to the baits is known as a benthic scavenger that burrows in the sediment. The next most abundant were the flatfish, dabs *Limanda limanda* and plaice *Pleuronectes platessa* which are benthic foragers. Whiting (*Merlangius merlangus*) which are demersal and mainly piscivorous were next most abundant followed by the benthic foraging haddock (*Melanogramus aeglefinus*). These four species occurred in almost all deployments providing a basis for comparisons.

Priede et al. (1990) used 14–15 BUC deployments at each location to characterise the abyssal ichthyofauna of the Pacific Ocean, Armstrong et al. (1992) used 8–9 at each location in the abyssal Atlantic and 6–9 deployments per station enabled Priede et al. (2003) to discriminate seasonal differences. In the Mediterranean Sea Stobart et al. (2007) used 54–99 replicates per station to characterise a much more diverse fish assemblage with 21–36 species per location. Since this paper deals with only the 4 dominant species in the North Sea, 27 replicates is a sound basis for defining the differences between the species at this location. However the bias of sampling with only 6 of these deployments at night time limits the conclusions that can be drawn regarding day–night differences. All species showed lower numbers during night-time but this effect was only significant for whiting and flatfish. For future work all sampling should be standardised to occur during daylight hours, or a more balanced sampling protocol between day and night should be adopted with more deployments. Sampling was also uneven between years and different current speeds which meant that for haddock

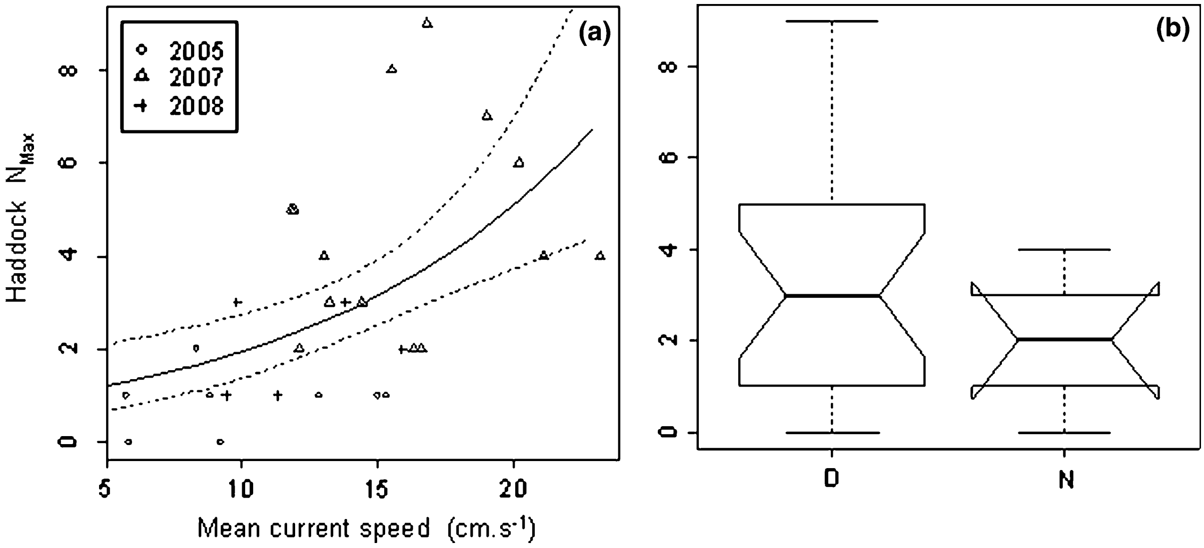


Fig. 6 **a** The effect of current speed on maximum number ($N_{\max}$) for haddock from three surveys with fitted values (*continuous line*) and 95% confident intervals (*dotted line*) estimated by a Poisson GLM. **b** Boxplot of $N_{\max}$ of haddock during the day (D) and night (N) time sampling. The midpoint of each box represents the median $N_{\max}$ value and the 25% quartiles define the hinges. Differences between hinges show the spread of the data with whiskers representing maximum and minimum values within 1.5 times the box size. Where notches overlap the two medians do not differ

it was not possible to determine whether the trend of increase in $N_{\max}$ with current speed was a real effect or the result of higher numbers of haddock in 2007. There had been a major recruitment of young haddock in 2005 resulting in high spawning stock biomass in that year. In 2007 spawning stock biomass was 25% of the biomass in 2005 suggesting the effect of current is a real effect (ICES, 2009). Many more samples over a number of years would help resolve the effect of year versus currents.

Light level had a significant effect on the $N_{\max}$ of whiting and a marginally significant effect for haddock in one of the two alternative models explored. For both species, the trend was for higher $N_{\max}$ during daytime deployments. These results fit well with what is known about their foraging behaviour. Using low light video observations, Fernö et al. (1986) also found a clear diurnal pattern for whiting attracted to baited hooks where increased activity was observed between dawn and dusk during May/June. A similar experiment with haddock and cod, noted higher numbers of haddock during the day, although the difference was not as pronounced as for cod (Løkkeborg et al., 1989). Higher day time trawl catches have also been reported for haddock in the North and Barents Sea (Engås & Soldal, 1992; Aglen et al., 1999; Petrakis et al., 2001; Adlerstein & Ehrich, 2002) and small whiting in the North Sea (Wieland et al., 1998). These activity patterns are most likely related to diel feeding migrations. Adult whiting are largely demersal, daytime foragers targeting piscivorous prey (Hislop et al., 1991; Bromley et al., 1997; Greenstreet et al., 1997) but also disperse and feed in the water column at night (Patterson, 1985; Mergardt & Temming, 1997; Pedersen, 2000; Onsrud et al., 2005). Variation in behaviour is most likely related to local environmental conditions and particular prey assemblages such as vertically migrating sand eels, the preferred prey of both haddock and whiting at certain times of the year (Temming et al., 2004). Haddock also prey on benthic species, feeding on molluscs, polychaetes, echinoderms and crustaceans and thus more closely associated with the seafloor than whiting.

Light level also had a significant effect in the flatfish model with higher $N_{\max}$ observed during daytime deployments. Common dab, the dominant species attracted to the bait, are not thought to make extensive vertical migrations (Gibson, 1973) which would make them unavailable to the baited camera. In fact, trawl catches are often much greater at night, potentially due to improved gear efficiency in low light conditions where avoidance is minimal (Petrakis et al., 2001; Adlerstein & Ehrich, 2002). Dab has a varied, benthivorous diet feeding mainly on invertebrates, in addition to small fish and fishery discards (Kaiser & Ramsay, 1997). It is generally classed as a visual daytime-feeder (Stevens, 1930; de Groot, 1969) although some studies have also observed feeding at other times (Knust, 1986; Carter et al., 1991). The diurnal differences observed in $N_{\max}$ most likely reflect a lack of nocturnal feeding activity by common dab in this area and/or at this particular time of year.

Although it is clear from $N_{\max}$ values that attraction to the bait can be influenced by the light level for some species, the first arrival time did not change significantly for the species observed in this study. This is in accordance with other studies in shallow waters (Willis et al., 2000) were the high population densities mean that the likelihood of a fish being close to the bait when it lands is high regardless of time of day.

Current speed was not found to significantly improve the performance of the models for either whiting or flatfish. However, it was the only significant explanatory variable in the haddock model when the factor year was excluded. Current speed affects baited gear in two ways; through determining the active space of the bait and through its impact on the foraging behaviour of the fish. Fernö et al. (1986) found higher foraging activity levels for whiting during periods of medium to strong current speed compared to little or no current speed. Whilst stronger water currents will disperse the odour plume over a greater area, they may also reduce the effective area through greater dilution (McQuinn et al., 1988) and require greater energy to swim against (Weihs, 1987). Løkkeborg et al. (1989) found that at current velocities between 18 and 27 cm s^{-1}, activity levels for cod and haddock around a baited hook were 30% of those at lower current speeds. Below 18 cm s^{-1} there was no clear influence on activity levels. The mean length of hooked fish in the Løkkeborg et al. study was large; nearly 50 cm for haddock and 56 cm for cod. The current speeds recorded during the present study were largely below 18 cm s^{-1} but the mean size of fish attracted to the bait was smaller with whiting and haddock maximum lengths of 45 cm and mean sizes between 20 and 30 cm (unpublished data).

Sustainable swimming speeds (U_{ms}) for haddock have been estimated at 38–60 cm s^{-1} or 3.16–1.51 BL s^{-1} (body length) for 16–42 cm fish (Breen et al., 2004). These were considered low compared to values for cod (*Gadus morhua*) and saithe (*Pollachius virens*) estimated using a similar method (He & Wardle, 1988; He, 1991). A study of whiting in situ swimming speeds in a Norwegian fjord recorded swimming speeds 14–16 cm s^{-1} during the day and 10–11 cm s^{-1} at night, thought to reflect a change from visually based daytime feeding to olfactory feeding (Onsrud et al., 2005). The length of tracked individuals in this study was not known but trawl catches gave a length frequency with modal peaks at around 10–15 and 30–35 cm. Although no experiments have compared haddock and whiting swimming ability directly, Steinhausen et al. (2005) estimated optimum swimming speed (U_{opt}) for whiting and saithe and suggested that whiting was a stronger swimmer than haddock, but not as strong as saithe. It is possible that the different results of the two models might reflect differing swimming abilities or foraging strategies of the two species; whiting is a piscivorous forager targeting active prey whilst haddock feeds predominantly on slower moving benthic prey. If we accept current speed was the main influence on haddock numbers attracted to the bait, the model suggests an exponential relationship with more fish arriving as current speed increases up to $\sim$25 cm s^{-1}. This pattern, however, does not fit that of Løkkeborg et al. (1989) although only a small number of deployments were carried out where mean current speed was above the 18 cm s^{-1}.

The alternative haddock model with factors “year” and “time of day” is also highly plausible and explains a large proportion of the variance. There is high, annual variability in recruitment of haddock within the North Sea. ICES data reported a relatively high recruitment of the 2005 cohort, and North Sea-wide trawl survey data showed a peak in haddock numbers in 2007 before a decline in 2008 (unpublished data, Marine-Scotland data). This wider population fluctuation could be reflected in the numbers of haddock observed at the study site and might override any effect that the current speed could have on the $N_{\max}$ of fish observed.

Current speed was not a significant explanatory variable in the flatfish model. No published estimates of swimming endurance for common dab were found, but this species is known to be closely associated with the seafloor and unlikely to spend large amounts of time swimming off the seabed (Gibson, 1973). It is likely that the active space for this species was much smaller than for the gadoids and less influenced by current speeds.

Hagfish, *Myxine glutinosa*, showed a pattern unlike all the other species examined. The optimal model indicated increasing numbers of hagfish with increasing bottom current up to a maximum current speed of 11 cm s^{-1}, after which the numbers declined. This primitive, jawless eel-like scavenger lives buried in muddy areas (Whitehead et al., 1984) preying on dead or disabled fish. The lack of functional eyes means that olfactory cues are the main sensory mode of attraction to bait by positive rheotaxis. The pattern of response seen in this study was as would be expected for this type of olfactory based feeder. Hagfish are unlikely to be as strong swimmers as whiting or haddock and is clearly reflected in the current speed threshold above which $N_{\max}$ declines. Straham (1963) indicate a slow swimming speed for this species, 25 cm s^{-1} although it can be increased up to 1 m s^{-1} when alarmed by divers (Foss, 1968). It is also likely that hagfish have a patchily distribution, with a level of aggregation close to a previous feeding area, which may account for some of the high numbers (>100 individuals) observed in a single picture. No inter-annual variability was found, and the effect of time of day was negligible.

Conclusion

Our analysis has shown that, depending on the species, an index derived from baited cameras can be strongly influenced by the time of deployment and/or the current speed. Such influences can confound temporal and spatial trends in relative abundance if they are not controlled for or factored into the analysis of results. Understanding these influences and accounting for them in the design of long term monitoring surveys using baited cameras is critical.

Acknowledgments The authors gratefully acknowledge the financial and logistical support of SERPENT partners NEXEN Petroleum Ltd., in particular to David Hutchinson and Karen Yorke. The assistance of the masters and crews from all vessels involved in the project is gratefully acknowledged in addition to the dedicated support of Viking Offshore (Aberdeen). Dr Dave Reid and Iain Penny, Marine Scotland-Marine Laboratory (formerly FRS) and Dr. Alan Jamieson (Oceanlab, University of Aberdeen) are thanked for their contribution and technical assistance to this project. Iñigo M. was supported during the research period through a Marie Curie Early Stage Research Grant (ECOSUMMER project. 020501-2).

References

Adlerstein, S. & S. Ehrich, 2002. Effect of deviations from target speed and of time of day on catch rates of some abundant species under North Sea International Bottom Trawl Survey protocol conditions. ICES Journal Marine Science 59: 594–603.

Aglen, A., A. Engas, I. Huse, K. Michalsen & B. K. Stensholt, 1999. How vertical fish distribution may affect survey results. ICES Journal Marine Science 56: 345–360.

Armstrong, J. D., P. M. Bagley & I. G. Priede, 1992. Photographic and acoustic tracking observations of the behaviour of the grenadier, *Coryphaenoides (Nematonurus) armatus*, the eel, *Synaphobranchus bathybius*, and other abyssal demersal fish in the North Atlantic Ocean. Marine Biology 112: 535–544.

Bossert, W. H. & E. O. Wilson, 1962. The analysis of olfactory communication among animals. Journal of Experimental Marine Biology and Ecology 5: 443–469.

Breen, M., J. Dyson, F. G. O'Neill, E. G. Jones & M. Haigh, 2004. Swimming endurance of haddock (*Melanogrammus aeglefinus* L.) at prolonged and sustained swimming speeds, and its role in their capture by towed fishing gears. ICES Journal of Marine Science 61: 1071–1079.

Bromley, P. J., T. Watson & J. R. G. Hislop, 1997. Diel feeding patterns and the development of food webs in pelagic 0-group cod (Gadus morhua L.), haddock (Melanogrammus aeglefinus L.), whiting (Merlangius merlangus L.), saithe (Pollachius virens L.), and Norway pout (Trisopterus esmarkii Nilsson) in the northern North Sea. ICES Journal Marine Science 54: 846–853.

Cappo, M., P. Speare & G. De'Ath, 2004. Comparison of baited remote underwater video stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-reefal areas of the Great Barrier Reef Marine Park. Journal of Experimental Marine Biology and Ecology 302: 123–152.

Carter, C. G., D. J. Grove & D. M. Carter, 1991. Trophic resource partitioning between two coexisting flatfish species off the north coast of Anglesey. North Wales Netherlands Journal of Sea Research 27: 325–335.

de Groot, S. J., 1969. Digestive system and sensorial factors in relation to the feeding behaviour of flatfish (Pleuronectiformes). ICES Journal Marine Science 32: 385–394.

Ellis, D. E. & E. E. Demartini, 1994. Evaluation of a video camera technique for indexing abundances of juvenile pink snapper, *Pristipomoides filamentosus*, and other Hawaiian insular shelf fishes. Fishery Bulletin 93: 67–77.

Engås, A. & S. Løkkeberg, 1994. Abundance estimation using bottom gillnet and longline—the role of fish behaviour. In Fernö, A. & S. Olsen (eds), Marine Fish Behaviour in Capture and Abundance Estimation. Fishing News Books, Oxford: 134–165.

Engås, A. & A. V. Soldal, 1992. Diurnal variations in bottom trawl catch rates of cod and haddock and their influence on abundance indices. ICES Journal of Marine Science 49: 89–95.

Farnsworth, K. D., U. H. Thygesen, S. Ditlevsen & N. J. King, 2007. How to estimate scavenger fish abundance using baited camera data. Marine Ecology Progress Series 350: 223–234.

Fernö, A., P. Solemdal & S. Tilseth, 1986. Field studies on the behaviour of whiting (*Gadus merlangus* L.) towards baited hooks. Fiskeridirektoratets Skrifter Serie Havundersøkelser 18: 83–95.

Foss, G., 1968. Behaviour of *Myxine glutinosa* L. in natural habitat: investigation of the mud biotype by a suction technique. Sarsia 31: 1–13.

Gibson, R. N., 1973. The intertidal movements and distribution of young fish on a sandy beach with special reference to the plaice (*Pleuronectes platessa* L.). Journal of Experimental Marine Biology and Ecology 12: 79–102.

Greenstreet, S. P. R., A. D. Bryant, N. Broekhuizen, S. J. Hall & M. R. Heath, 1997. Seasonal variation in the consumption of food by fish in the North Sea and implications for food web dynamics. ICES Journal Marine Science 54: 243–266.

Harvey, E. S., M. Cappo, J. J. Butler, N. Hall & G. A. Kendrick, 2007. Bait attraction affects the performance of remote underwater video stations in assessment of demersal fish community structure. Marine Ecology Progress Series 350: 245–254.

He, P. G., 1991. Swimming endurance of the atlantic cod, *Gadus morhua* L, at low-temperatures. Fisheries Research 12: 65–73.

He, P. & C. S. Wardle, 1988. Endurance at intermediate swimming speeds of Atlantic mackerel, Scomber scombrus L., herring, Clupea harengus L., and saithe, Pollachius virens L. Journal of Fish Biology 33: 255–266.

Heagney, E. C., T. P. Lynch, R. C. Babcock & I. M. Suthers, 2007. Pelagic fish assemblages assessed using mid-water baited video: Standardising fish counts using bait plume size. Marine Ecology Progress Series 350: 255–266.

Hislop, J. R. G., A. P. Robb, M. A. Bell & D. W. Armstrong, 1991. The diet and food consumption of whiting (*Merlangius merlangus*) in the North Sea. ICES Journal of Marine Science 48: 139–156.

ICES, 2009. Report of the ICES Advisory Committee 2009. ICES Advice, 2009. Book 6, 236.

Jamieson, A. J. & P. Bagley, 2005. Biodiversity survey techniques: ROBIO and DOBO landers. Sea Technology 46: 52–54.

Jones, E. G., A. Tselepides, P. M. Bagley, M. A. Collins & I. G. Priede, 2003. Bathymetric distribution of some benthic and benthopelagic species attracted to baited cameras and traps in the deep eastern Mediterranean. Marine Ecology Progress Series 251: 75–86.

Kaiser, M. & K. Ramsay, 1997. Opportunistic feeding by dabs within areas of trawl disturbance: possible implications for increased survival. Marine Ecology Progress Series 152: 307–310.

Knust, R., 1986. Food selection of the dab (*Limanda limanda* (L.)): diel and seasonal changes. ICES C.M. 1986/G: 63.

Laurel, B. J., C. H. Ryer, B. Knoth & A. W. Stoner, 2009. Temporal and ontogenetic shifts in habitat use of juvenile Pacific cod (*Gadus macrocephalus*). Journal of Experimental Marine Biology and Ecology 377: 28–35.

Løkkeborg, S., 1990. Rate of release of potential feeding attractants from natural and artificial bait. Fisheries Research 8: 253–261.

Løkkeborg, S. & T. Pina, 1997. Effects of setting time, setting direction and soak time on longline catch rates. Fisheries Research 32: 213–222.

Løkkeborg, S., Å. Bjordal & A. Fernö, 1989. Responses of cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) to baited hooks in the natural environment. Canadian Journal Fisheries and Aquatic Sciences 46: 1478–1483.

Løkkeborg, S., B. Olla, W. Pearson & M. Davis, 1995. Behavioural responses of sablefish, *Anoplopoma fimbria*, to bait odour. Journal of Fish Biology 46: 142–155.

McQuinn, I. H., L. Gendron & J. H. Himmelman, 1988. Area of attraction and effective area fished by a whelk (*Buccinum undatum*) trap under variable conditions. Canadian Journal of Fisheries and Aquatic Sciences 45: 2054–2060.

Mergardt, N. & A. Temming, 1997. Diel pattern of food intake in whiting (*Merlangius merlangus*) investigated from the weight of partly digested food particles in the stomach and laboratory determined particle decay functions. ICES Journal of Marine Science 54: 226–242.

Olsen, S. & T. Laevastu, 1983. Factors affecting catch of long lines, evaluated with a simulation model of long line fishing. Northwest and Alaska Fisheries Center, National Marine Fisheries Service, NOAA, 7600 Sand Point Way NE, Seattle, WA 98115-0070.

Onsrud, M. S. R., S. Kaartvedt & M. T. Breien, 2005. In situ swimming speed and swimming behaviour of fish feeding on the krill *Meganyctiphanes norvegica*. Canadian Journal of Fisheries and Aquatic Sciences 62: 1822–1832.

Patterson, K. R., 1985. The trophic ecology of whiting (*Merlangius merlangus*) in the Irish Sea and its significance to the Manx herring stock. ICES Journal Marine Science 42: 152–161.

Pedersen, J., 2000. Food consumption and daily feeding periodicity: comparison between pelagic and demersal whiting in the North Sea. Journal of Fish Biology 57: 402–416.

Petrakis, G., D. N. MacLennan & A. W. Newton, 2001. Day-night and depth effects on catch rates during trawl surveys in the North Sea. ICES Journal of Marine Science 58: 50–60.

Priede, I. G. & P. M. Bagley, 2001. In situ studies on deep sea demersal fishes using autonomous unmanned lander platforms. Oceanography and Marine Biology 38: 357–392.

Priede, I. G. & N. R. Merrett, 1996. Estimation of abundance of abyssal demersal fishes; a comparison of data from trawls and baited cameras. Journal of Fish Biology 49: 207–216.

Priede, I. G., K. L. Smith Jr. & J. D. Armstrong, 1990. Foraging behaviour of abyssal grenadier fish: inferences from acoustic tagging and tracking in the North Pacific Ocean. Deep-Sea Research 37: 81–101.

Priede, I. G., A. R. Deary, D. M. Bailey & K. L. Smith Jr., 2003. Low activity and seasonal change in population size structure of grenadiers in the oligotrophic central North Pacific Ocean. Journal of Fish Biology 63: 187–196.

Steinhausen, M., J. Steffensen & N. Andersen, 2005. Tail beat frequency as a predictor of swimming speed and oxygen consumption of saithe (*Pollachius virens*) and whiting (*Merlangius merlangus*) during forced swimming. Marine Biology 148: 197–204.

Stevens, G. A., 1930. Bottom fauna and the food of fishes. Journal of the Marine Biological Association of the UK 16: 677–706.

Stobart, B., J. A. Garcia-Charton, C. Espejo, E. Rochel, R. Goni, O. Renones, A. Herrero, R. Crec'hriou, S. Polti, C. Marcos, S. Planes & A. Perez-Ruzafa, 2007. A baited underwater video technique to assess shallow-water Mediterranean fish assemblages: methodological evaluation. Journal of Experimental Marine Biology and Ecology 345: 158–174.

Stoner, A. W., 2003. Hunger and light level alter response to bait by Pacific halibut: laboratory analysis of detection, location and attack. Journal of Fish Biology 62: 1176–1193.

Stoner, A. W., 2004. Effects of environmental variables on fish feeding ecology: implications for the performance of baited fishing gear and stock assessment. Journal of Fish Biology 65: 1445–1471.

Stoner, A. W., B. J. Laurel & T. P. Hurst, 2008. Using a baited camera to assess relative abundance of juvenile Pacific cod: field and laboratory trials. Journal of Experimental Marine Biology and Ecology 354: 202–211.

Straham, R., 1963. The behaviour of the myxinoids. Acta Zoologica 44: 73–102.

Temming, A., S. Gotz, N. Mergardt & S. Ehrich, 2004. Predation of whiting and haddock on sandeel: aggregative response, competition and diel periodicity. Journal of Fish Biology 64: 1351–1372.

Weihs, D., 1987. Hydromechanics of fish migration in variable environments. American Fisheries Society Symposium 1: 254–261.

Whitehead, P. J. P., M. L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese, 1984. Fishes of the North-Eastern Atlantic and the Mediterranean. UNESCO, Paris: 1473.

Wieland, K., L. Fosdager, R. Holst & A. Jarre-Teichmann, 1998. Spatial distribution of estimates of juvenile (age 1 and 2) whiting and cod in the North Sea. ICES CM 1998/J: 7.

Willis, T. J., R. B. Millar & R. C. Babcock, 2000. Detection of spatial variability in relative density of fishes: Comparison of visual census, angling, and baited underwater video. Marine Ecology Progress Series 198: 249–260.

Willis, T. J., R. B. Millar & R. C. Babcock, 2003. Protection of exploited fish in temperate regions: high density and biomass of snapper *Pagrus auratus* (Sparidae) in northern New Zealand marine reserves. Journal of Applied Ecology 40: 214–227.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev & G. M. Smith, 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.

Hydrobiologia (2011) 670:35–47
DOI 10.1007/s10750-011-0671-y

ECOSYSTEMS AND SUSTAINABILITY

Assessing *Octopus vulgaris* distribution using presence-only model methods

**Consuelo Hermosilla · Francisco Rocha ·
Vasilis D. Valavanis**

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The distribution of the common octopus (*Octopus vulgaris*) in the Mediterranean and Eastern Atlantic waters is evaluated using two presence-only analyses: The maximum entropy model (Maxent) and the ecological niche factor analysis (ENFA). Maxent predicts those geographical areas that satisfy the environmental or abiotic requirements of a species while ENFA explores the niche and habitat preferences of *O. vulgaris*. The analyses were implemented recovering the spatial information from 213 octopus presence data collected from surveys and bibliographical records. Together, these analyses provided reasonable estimates of the species distribution and the octopus habitat. Among the gathered set of explanatory environmental variables, sea bottom temperature, sea bottom salinity, surface dissolved oxygen and sea surface chlorophyll-α appear as the main variables involved in *O. vulgaris* distribution. These results were confronted with the available literature.

Keywords *Octopus vulgaris* · Species distribution modelling · Environment · Presence data · Maxent · ENFA

Introduction

The comprehension of the geographical distribution of a species is one of the most important aspects for the study of its populations, ecology and management. In marine species, the relationships between species distribution and environmental factors are essential to comprehend many aspects of their ecology towards effective conservation and management and assessment of possible impacts from anthropogenic activities (MacLeod et al., 2008; Valavanis et al., 2008). However, in many cases, the distribution limits of a species are difficult to define due to inherent difficulties in detecting marine species. As indicated by Tsoar et al. (2007) for most regions and taxa, detailed data on species distribution are usually not available and collecting such data is costly and labour intensive (Prendergast et al., 1999; Bowker, 2000; Ottaviani et al., 2004).

One solution to this lack of distributional data is the use of mathematical approaches to investigate the relationships between environmental variables and species occurrence modelling the species distribution

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

C. Hermosilla · F. Rocha (✉)
Department of Ecology and Animal Biology, Campus As Lagoas Marcosende, University of Vigo, 36310 Vigo, Spain
e-mail: frocha@uvigo.es

V. D. Valavanis
Marine GIS Laboratory, Institute of Marine Biological Resources, Hellenic Centre for Marine Research, Thalassocosmos, 71003 Heraklion Crete, Greece

and habitat. These relationships can be used to predict the areas where species most likely occur (e.g. Elith et al., 2006; Basille et al., 2008; MacLeod et al., 2008; Ready et al., 2010). However, many potential techniques require both accurate presence and absence data that not always can be available, particularly for marine species that are difficult to detect due to their mobility and habitat preferences or inadequate sampling procedures. In this sense, the alternative presence-only approaches are of special interest for modelling marine species distributions (Tsoar et al., 2007; MacLeod et al., 2008) and the use of such models to study marine species with poorly known or limited presence records facilitates the generation of distributional maps that can be used as a tool for studying in danger or protected species where sampling is limited (Hirzel et al., 2002a; Ready et al., 2010).

One of these approaches is the maximum entropy model (Maxent), developed by Phillips et al. (2006) to assess species geographic distributions in relation to environmental variables with limited presence-only data. Maxent takes as input a set of commonly georeferenced gridded layers or environmental variables as well as a set of species occurrence locations and produces a model of the species distribution (Phillips et al., 2004, 2006; Phillips & Dudík, 2008). Maxent has been used to study the distribution of terrestrial (Elith et al., 2006; Phillips et al., 2006; Basille et al., 2008; Veloz, 2009) and marine (Lefkaditou et al., 2008; Ready et al., 2010) species.

Another approach to evaluate the relationship with environmental variables using presence-only data is the ecological niche factor analysis (ENFA), developed by Hirzel et al. (2002b), based in Hutchinson's niche concept (Hutchinson, 1957). Specifically, Basille et al. (2008) have conceived and implemented ENFA in R (R Development Core Team, 2010) as an exploratory tool to identify the variables regulating the niche of the species and to distinguish between the available conditions and the habitat selected by the species.

One marine species with unresolved distribution and habitat preferences in European waters is the common octopus, *Octopus vulgaris* Cuvier, 1797, despite the fact it is the most important harvested octopus species from the Atlantic and the Mediterranean waters reaching global catches of more than 37,000 tonnes in 2007 (Josupeit, 2000; FAO, 2009). A great amount of knowledge is available on several aspects of its biology and ecology (e.g. Mangold, 1983; Guerra, 1992; Domain et al., 2000; Hernández-López et al., 2001; Otero et al., 2008, 2009; Hermosilla et al., 2010), although, some aspects like its geographical distribution are still poorly known. Furthermore, its populations show notable yearly fluctuations on abundance and catch due to the high paralarval and settlement mortality (Otero et al., 2008, 2009) as well as species' rapid response to environmental variation (Sobrino et al., 2002; González et al., 2005). Production fluctuations can be detected even in nearby ports in the same season, a fact that has been reported by Murphy et al. (2002) in Northern and Southern Sahara Bank and Mauritania.

In the past, the presence of *O. vulgaris* has been recorded in several regions of the world suggesting that this was a cosmopolitan species (Mangold, 1983; Roper et al., 1984; Warnke et al., 2004). However, this statement has been questioned by other authors (Mangold, 1998; Mangold & Hochberg, 1991) that suggest *O. vulgaris* is one member of a species complex (Norman, 2000) and its distribution is only limited to east Atlantic and Mediterranean waters (Guerra, 1992; Mangold, 1998). Remarkably, recent evidences support the previous assertions suggesting *O. vulgaris* distribution is probably cosmopolitan and extending to Mediterranean Sea, Eastern Atlantic Ocean (from South England to Southwest African waters), Azores, Canary, Cape Verde, St. Helena, Tristan da Cunha Islands, southern Brazil, Isla Margarita (Venezuela), southeastern coast of South Africa, Japanese and Taiwanese waters in the Pacific and Saint Paul and Amsterdam Islands in the Indian Ocean (Warnke et al., 2004; Pardo-Gandarillas et al., 2009; Guerra et al., 2010). In any case, the most probable situation of *O. vulgaris* could be that its distribution is wider than the Mediterranean and Eastern Atlantic and that it overlaps the distributions of other similar species forming a species complex.

In this study, we used two complementary presence-only analyses, Maxent and ENFA, to model the spatial distribution of *O. vulgaris* in the Mediterranean and Eastern Atlantic waters and to reveal its habitat preferences, respectively. Since these two analyses approach species distribution modelling in a different manner, it is interesting to determine which environmental variables are more relevant in each analysis for the octopus.

Materials and methods

Octopus data sets

Two data sets were used in this study, following the indications of Fielding & Bell (1997) and Veloz (2009). The first data set was used to develop both models and hence, was called the training data. It consists of 213 *O. vulgaris* records (Fig. 1) from different sources, including sampling data collected from 2006 to 2008 as well as bibliographical records (Table 1). An independent (non-correlated) data set was used to test and validate the Maxent and ENFA models. This data set was kindly provided by I. Sobrino, P. Pascual and L. Gil de Sola from Instituto Español de Oceanografía and consists of a set of *O. vulgaris* presence data obtained during several research surveys in the Gulf of Cadiz (survey years from 1993 to 2009), Morocco coast (survey years from 1989 to 1990), Mauritania coast (survey years from 2006 to 2007) and Mediterranean Sea (Alborán Sea and Vera Gulf, from MEDITS survey, 2005–2008). These data sets were too large and redundant. Thus, for each data set only 57 presence records were selected avoiding repetitions and bias. When more presence records were available in a data set, only 57 of them were randomly selected using R. In consequence, a total test sample size of 171 octopus presence records was used to test models. The independent test sample size is smaller than the training data set used to develop the models ($n = 213$) because the test sample represents a smaller area than that represented by the training data set.

Environmental variables

A total of 17 environmental or abiotic variables were assembled and utilized in this study, selected because they were directly or indirectly related to octopus biology and ecology. Whereas it is true that more variables are related to its biology, habitat preferences and distribution, there are not public datasets available for all variables that we would like to include. In other cases, when the data set exist, it does not cover the complete area included in the study.

From the 17 environment variables, only five of them were used in the final model development: dissolved oxygen (DO0 and DO200 in ml O_2/l, at 0 and 200 m depth, respectively), sea bottom salinity (SBS ‰), sea bottom temperature (SBT in °C) and chlorophyll-α (CHLA in mg/m^3). All the variables were assembled as climatological (long-term) averages, except from CHLA, which was assembled as annual average for 2006 (most of sampling data were collected during that year). Long-term averages were used because this is the first attempt to create a picture of the distribution of the species in a wide area utilizing the wide extent of satellite data (the use of time-stamps according to samples dates would not allow to produce a wide-area map).

The other variables examined (estuary area, euphotic depth, mean primary production, precipitation, sea

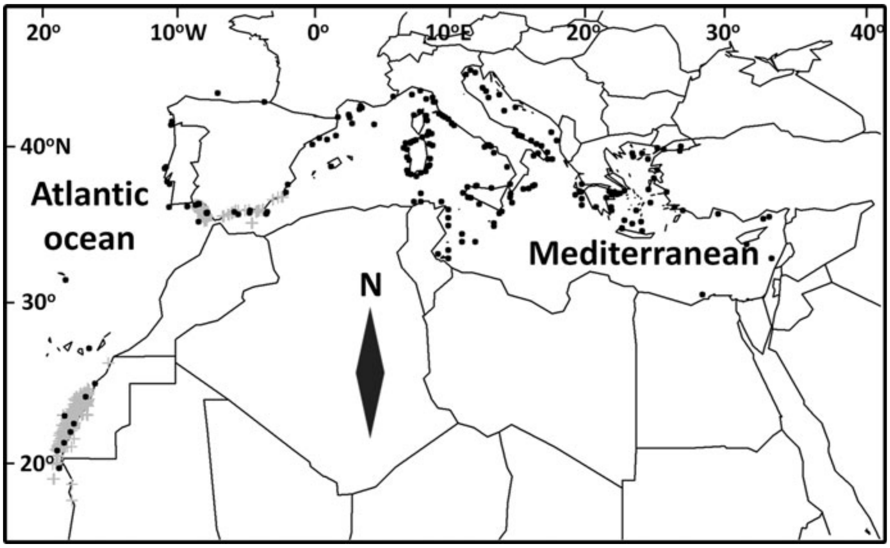


Fig. 1 Map showing the location of *O. vulgaris* presence data utilized in this study. *Black dots* represent presence data used in Maxent and ENFA models. *Grey crosses* represent test data used in Maxent model

Table 1 List of survey data and bibliographical records used in this study

Source	Area sampled
This study	A total of 12 sampling localities in: Spain (Vigo, Vilanova i la Geltrú, Málaga, Cádiz, Bilbao); Southern Portugal; Italy (Bari, Sicily, Naples); Greece (Lesbos and Crete), and; Mauritania (Atlantic)
Belcari et al. (2002)	Mediterranean region: Alborán Sea; Alicante region; Catalonian Sea; Morocco; Gulf of Lion; Corsican Sea; Ligurian Sea; Sardinian Sea; Tyrrhenian Sea; Sicilian Channel; Adriatic Sea; Ionian Sea; Argosaronikos region; Aegean Sea
Borges-Seixas et al. (2002)	Portuguese Atlantic Coast: Viana do Castelo and Cascais
Byrne et al. (2002)	Mediterranean coast of France (Corse: Stareso, Calvi) and Italy (Bay of Naples)
Cabranes et al. (2008)	Spanish Atlantic Coast (Asturias, Galicia and Portugal); Atlantic (Canary Island); Mediterranean (Murcia)
Catalán et al. (2006)	Spanish Atlantic coast: Gulf of Cadiz
Cerezo-Valverde & García-García (2005)	Spanish Mediterranean coast: coast of Murcia
Chapela et al. (2006)	Spanish Atlantic coast: Vigo
Costa et al. (2004)	Portuguese Atlantic coast: Peniche (NW) and Olhâo (South)
Ezzeddine & El-Abed (2004)	Mediterranean region: Tunisia (Gulf of Gabès)
Faraj & Bez (2007)	Atlantic Coast of Africa: Southern Morroco (26°N to 21°N)
Hernández-López et al. (2001)	Atlantic waters: Canary Islands (Spain)
Katsanevakis & Verriopoulos (2004b)	Mediterranean region: Aegean Sea
Katsanevakis & Verriopoulos (2006b)	Mediterranean region: Aegean Sea
Lefkaditou et al. (2003)	Mediterranean region: Ionian Sea
Madan & Wells (1996)	Mediterranean region: Coast of France
Maltagliati et al. (2002)	Mediterranean region: Spain (Vilanova i la Gertrú); Italy (Western Sardinia, Gulf of Olbia, Leghorn and Porto Santo Stefano in Tuscany, Gulf of Naples, Porto Palo in Sicily), Greece (Crete north of Heraklion)
Miliou et al. (2005)	Mediterranean region: Saronicos Gulf (Aegean Sea)
Quetglas et al. (1998)	Mediterranean region: Palma de Mallorca (Spain)
Rodriguez-Rúa et al. (2005)	Spanish Atlantic coast: Huelva and Bay of Cadiz
Salman et al. (2002)	Mediterranean region: Turkish coastal waters: Sea of Marmara, Aegean sea and eastern Mediterranean

surface salinity, sea surface temperature, sediment type, tidal range, wind speed, wind direction) were discarded from analysis due to low contribution to model development (data not shown). In addition, bathymetry and shelf area were also evaluated preliminary (data not shown) but since *O. vulgaris* is a species known to live in the continental shelf, i.e. in shallow waters, these variables were not included in the analysis, in order to reveal less obvious factors that affect octopus distribution and habitat preferences. Furthermore, it is known that bathymetry and shelf area are correlated with other variables selected in the final analysis, such as DO, which generally declines with depth, SBT and SBS (the deeper, the colder and saltier), therefore, since it is not recommended to incorporate highly correlated variables into Maxent we did not include them into the analysis.

Data were obtained from NASA's OceanColor website (CHLA, oceancolor.gsfc.nasa.gov), Aquamaps (SBS, SBT, Kaschner et al., 2008, http://www.aquamaps.org/) and World Ocean Atlas 2005 (DO0 and DO200, Garcia et al., 2006, http://www.nodc.noaa.gov/OC5/WOA05/pr_woa05.html). All data were converted to ESRI's ArcGIS grids and resampled under a common spatial resolution (0.05° or 5 km approximately) covering the Mediterranean and Eastern Atlantic (17°–48°N, 19°W–42°E using SAGA-GIS software (Conrad, 2006).

Maxent model

Maxent analysis was carried out using Maxent software (V. 3.3.2) and downloaded from the 'Maxent software for species habitat modelling' web page (www.cs.princeton.edu/~schapire/maxent). As previously mentioned, 171 independent data points were used to test the reliability of the model using a binomial test of omission to evaluate the statistical significance of the prediction.

The importance of each environmental variable in the model was evaluated by means of a heuristic estimation during training of the model and by means of a jackknife test. The latter test was executed initially running the model by excluding one variable in each run, then running the model with only one variable and finally, including all variables in the model. Next, the performance of the different models was evaluated. Response curves were created for each environmental variable showing the effect of every environmental variable on the logistic prediction, i.e. the marginal effect of changing one variable in the model, by keeping the remaining variables at their average value. The model was evaluated with the threshold-independent receiver operating characteristic (ROC) approach, by calculating the area under the ROC curve (AUC) as the measure of the prediction success. The ROC curve is obtained by plotting all true positive values (sensitivity fraction) against their equivalent false positive values (1-specificity fraction, Phillips et al., 2006). Analysis was performed 10 times to generate 95% confidence intervals.

ENFA model

The ENFA analysis compares the total area combinations of habitat variables available to the species with the combinations of habitat variables at the locations where the species is found by means of the terms marginality and specialization. Marginality is a measure of the separation between this optimal habitat combinations (those of the actual presence sites) and the average available environmental conditions within the study area; specialization contrasts the global distribution variance with the species habitat variance, i.e. it measures how restricted is the species niche in comparison with available habitat combinations (Hirzel et al., 2002a; Basille et al., 2008).

ENFA is implemented in the Adehabitat package (Calenge, 2006) of R software (R Development Core Team, 2010). The analysis performs a principal-component analysis-like analysis, calculating factors that have biological meaning. The first factor explains marginality (m) and the remaining ones explain the specialization (s). Larger values of marginality indicate that the species is not equally distributed in the environment and that the habitats utilized strongly differ from the average conditions in the study area (Hirzel et al., 2002a; Basille et al., 2008). Alternatively, small values of specialization represent less restricted niches on some particular environmental variable (Basille et al., 2008) and high values of specialization means the species do not tolerate variation in that dimension.

Adehabitat package also provides global marginality (M) and global tolerance (T) of the species to the habitat evaluated: the greater the marginality, the more the niche deviates from the available conditions; the smaller the tolerance, the more restricted is the niche, i.e. more specialized is the species. These global estimations, however, must be used cautiously as they only apply to the specific area covered in a specific study and assumes that the environmental variables do not change over time (Hirzel et al., 2002a; Basille, per. comm.).

Marginality, the specialization axes and tolerance were evaluated with Monte-Carlo tests to assess their significance after 999 replicates (Basille et al., 2008, 2009; Calenge & Basille, 2008). As in Maxent, the analysis was also evaluated with ROC approach. In order to perform this validation, ENFA was applied to a set of 200 random absence points created with Dismo package (Hijmans et al., 2010) of R software, avoiding land areas and areas nearby to presence points to minimize 'false' absences in these points. Then, the predicted suitability of both training and test data was contrasted with the ENFA results performed with the absence points by means of ROCR package (Sing et al., 2009) of R software.

Results

According to the heuristic estimations of Maxent, the variables that mostly contribute to the model are

Table 2 Relative contributions of the environmental variables to the Maxent and ENFA models

Variable	MAXENT	ENFA		
	Percent contribution	Marginality	Specialization 1	Specialization 2
CHLA	6.1	−0.078	0.231	−0.177
DO0	14.7	0.124	−0.575	−0.615
DO200	3.5	0.656	0.763	−0.491
SBS	12.7	1.452	−0.129	−0.319
SBT	63.0	1.700	−0.132	0.498

SBT, DO0 and SBS (63.0, 14.7 and 12.7% of contribution to the model, respectively, Table 2). The jackknife test shows that the variables that produce the greater gain in the model when considered in isolation are SBT, SBS and CHLA (longest dark grey bars in Fig. 2), which therefore appear to be more relevant for the octopus distribution; those that mostly decrease the gain of the model when they are omitted is DO0, CHLA and SBT (shortest light grey bars in Fig. 2), which consequently appear to have the most information that is not present in the other variables.

Maxent model indicates that predicted suitability of *O. vulgaris* increases in zones where SBT, DO0, SBS and CHLA range is 15–30°C, 5.0–7.5 ml/l, 30–45‰ and 0–50 mg/m^3, respectively (Fig. 3). The area under the curve (AUC) for the training data was 0.958 and for test data was 0.946 (Fig. 4A), which means that the model prediction is higher than chance (AUC = 0.5). Furthermore, the binomial test of omission was less than 0.0001 indicating the model estimations were significantly better than random predictions. The map generated by Maxent shows the areas with the best predicted conditions for octopus occurrence (Fig. 5) and it is congruent with the known distribution of *O. vulgaris*. In addition, several areas with higher probability of presence are coincident with the current fisheries areas for the species (e.g. Sahara bank, Iberian Peninsula, Italy and Greece).

Global marginality estimated by ENFA was 5.45 ($P = 0.001$), which indicates that the area used by octopus strongly differs from the average conditions in the Mediterranean Sea and Atlantic Ocean (niche centroid shown in Fig. 6) and that it requires a specific niche of habitat. In terms of variable importance, the analysis indicates that SBT and SBS are the main variables that contribute to marginality (Table 2, longest arrow projections on marginality axis in Fig. 6). SBT and SBS have large and positive values, meaning that octopus prefers areas where these variables have an average greater than those of the environment (see Fig. 7).

Two specialization axes were kept in the analysis because they accounted for the main part of specialization (data not shown). They correspond to an eigenvalue of 12.7 ($P = 0.005$) and 9.1 ($P = 0.001$), meaning that the variance of the available

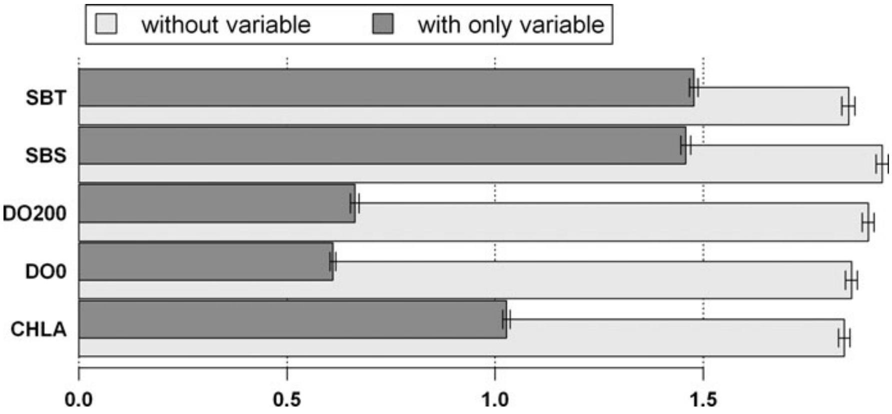


Fig. 2 Results of the jackknife test of variable importance in the Maxent model. In *dark grey*, model gain using only that variable; *light grey*, effect of removing that variable from the model. *Bars* indicate 95% CI

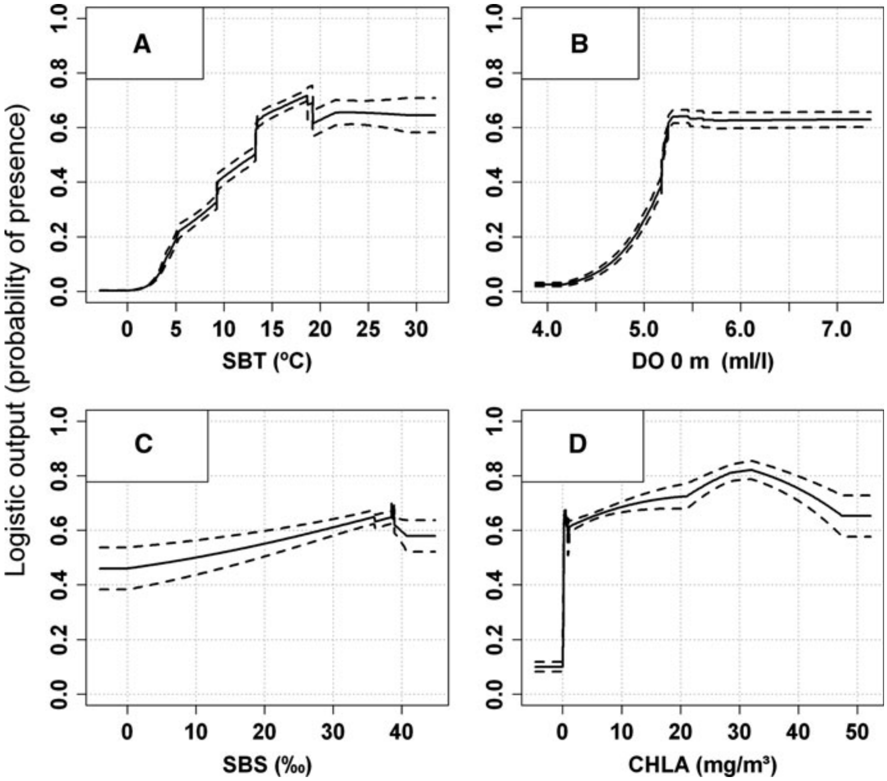


Fig. 3 Response curves for the most important variables of the Maxent model. **A** SBT; **B** DO at 0 m; **C** SBS; **D** CHLA. *Solid lines* represent the effect of the variable on the model; *dashed lines* represent 95% CI

environment is 12.7 and 9.1 times larger than the variance of the ecological niche in each of those dimensions. Hence, the niche appears rather restricted in those dimensions.

Indeed, the biplot of ENFA (Fig. 6) showed that the niche is quite restricted, since low values of tolerance indicate constrained niches (Dolédec et al., 2000; global tolerance is 1.15, $P = 0.001$). At this point, it is worth mentioning that it is not recommended to compare marginalities, specializations and tolerance from different studies, i.e., a tolerance value from a model built with three variables cannot be compared to a tolerance value of a model built with 10 variables (Basille, per. comm.).

DO200 and DO0 are the variables with higher specialization coefficients, thus they are the most critical in terms of habitat selection (longest arrow projection on specialization axis in Figs. 6 and 7, Basille et al., 2008). The AUC for the training data was 0.784 and for test data was 0.659 (Fig. 4B), meaning that the model prediction is also better than randomness (AUC = 0.5), although comparatively, the ability of ENFA to predict suitability areas is inferior to that of Maxent model.

Discussion

This study represents the first attempt to understand *O. vulgaris* habitat preferences and distribution in the Mediterranean and Eastern Atlantic waters using novel techniques such as Maxent modelling and ENFA analysis, which integrate multiple environmental variables with presence-only data. To date, studies have been focused in local abundance and/or fisheries of *O. vulgaris* in relation to some environmental variables like SST, CHLA, Season, Rainfall or Depth and the North Atlantic Oscillation (NAO) (Hernández-López, 2000; Balguerías et al., 2002; González & Sánchez, 2002; Sobrino et al., 2002; Katsanevakis & Verriopoulos, 2004a; Vargas-Yáñez et al., 2009; Caballero-Alfonso et al., 2010). Similar studies showed positive relationships between paralarval abundance and SST and upwelling (Katsanevakis & Verriopoulos, 2006a; Moreno et al., 2009; Otero et al., 2009). This study integrates octopus presence data from a wide distribution range with an extensive number of environmental variables.

Nevertheless, it would be relevant for future studies, to extend the number of training and test

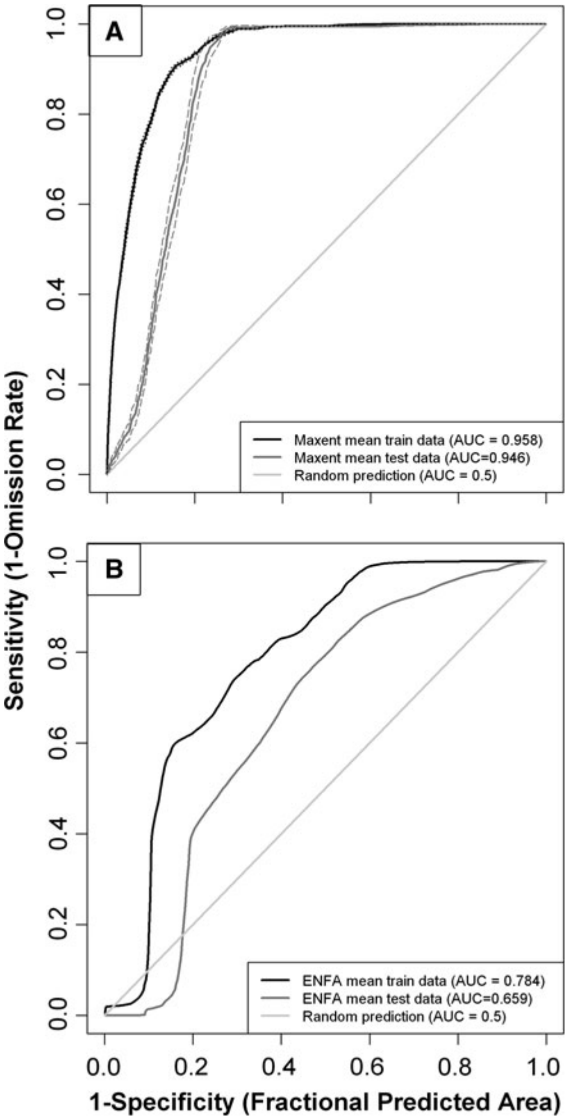


Fig. 4 Receiver operating characteristic (ROC) curve for *O. vulgaris* data using Maxent model (**A**) and ENFA (**B**). *Dashed lines* represent 95% CI

records, avoiding biases due to region misrepresentation. For instance, it is urgent to include more records from eastern and southern Mediterranean, which is clearly less studied. An effort must be made in this sense to carry out more surveys and to publish the results in peer review journals.

In terms of analysis comparison, Maxent model is more robust than ENFA as a predictive tool: both training and test AUC plots confirmed its high performance (Fig. 4A), though test AUC had a lower value because test data did not include the entire area that was incorporated in the training data. In contrast, ENFA had a lower fitness because the absence model was built using random points that covered most of the total available habitat, thus the model could not perform adequately.

Both analyses agreed that SBS and SBT are relevant variables to octopus spatial distribution and habitat preferences (Figs. 2, 6), although they showed a small discrepancy in terms of the remaining variables. For instance, to Maxent, CHLA is a decisive variable (Fig. 2) whereas to ENFA, i.e. to habitat preferences, it does not seem to be a relevant variable (low marginality and specialization value; Fig. 6). This is a reflection of the differences of both analyses. Whereas Maxent deals with spatial distribution, ENFA focuses in habitat preferences. Therefore, to Maxent model, CHLA, a measure of primary production, is essential to spatial distribution of the species, because high levels of CHLA are in direct relationship with high productivity areas and food availability, key element to cephalopod abundance, especially for octopus (Rocha et al., 1999; González et al., 2005; Otero et al., 2009). In the other hand, CHLA is not influential to its habitat preferences, which make sense because a benthic species should not select its (benthonic) habitat according to CHLA values.

Previous studies have shown the importance of SST in octopus habitat preferences, distribution and ecology; however, results have shown both positive correlations (Demarcq & Faure, 2000; Balguerías et al., 2002; Sobrino et al., 2002; Vargas-Yáñez et al., 2009; Caballero-Alfonso et al., 2010) and negative relationships (González & Sánchez, 2002) to abundance and catch. However, results of this study do not show a major effect of SST in *O. vulgaris* distribution; in fact, SST was discarded during preliminary evaluation due to its low contribution to the analyses. Instead, our study places SBT as an important factor in octopus spatial distribution and habitat preferences, in accordance to octopus nature. Most likely, the relevance of SST in regulating adult *O. vulgaris* spatial distribution could have been overestimated, due to its availability as marine environmental variable or due to the limited use of SBT in their analysis.

In this sense, SST can affect mainly octopus paralarvae abundance (Moreno et al., 2009) in their pelagic zooplanktonic habitat where their survival

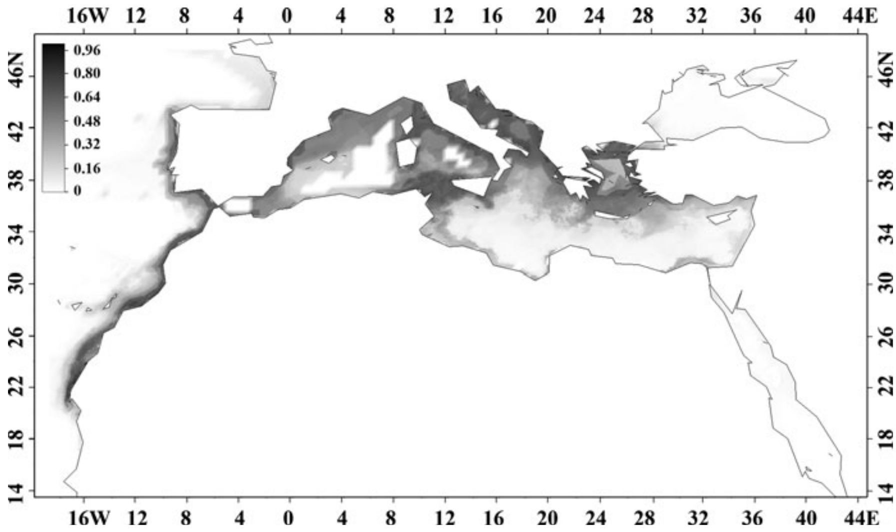


Fig. 5 Distribution representation for *O. vulgaris* using Maxent model. *Darker colours* show areas with better predicted conditions. 1 represents more suitable prediction and 0 represents not suitable prediction for octopus

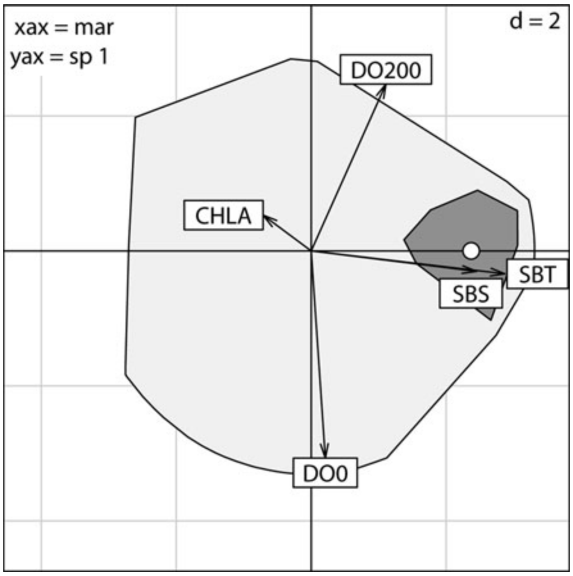


Fig. 6 *Octopus vulgaris* niche display in the study area. Axes represent marginality (*x*-axis) and specialization (*y*-axis). *Light grey* area represents total available area and *dark grey* represents used area (niche). *White dot* represents the niche centroid (centroid of the available area is located at the origin of the coordinates 0,0). *Arrows* represent the projections of the environmental variables on the marginality and specialization axes

depends on sea surface temperature and productivity (Rocha et al., 1999; González et al., 2005). However, when octopus juveniles leave zooplanktonic habitat to live as adult benthonic species (Guerra, 1992; Belcari et al., 2002; González & Sánchez, 2002), the importance of SST could be limited. In consequence, for the spatial distribution and habitat preferences of *O. vulgaris* juveniles and adults, the bottom temperature must be considered an important factor instead of sea surface temperature.

In the other hand, SBS, it is not surprising an essential variable to *O. vulgaris*. This cephalopod is known as a species that cannot tolerate low values of salinity (Vaz-Pires et al., 2004); indeed, low salinity phenomena can be highly stressful or even fatal to this species (Chapela et al., 2006). Therefore, bottom salinity can explain the absence of *O. vulgaris* in low salinity waters, like estuarine environments and Black Sea.

DO0 and DO200 were more relevant to ENFA than to Maxent (Figs. 2, 6), because they contributed the most to the specialization axis, meaning that they do not tolerate drastic changes in DO0 and DO200. This coincides with basic physiological requirements of this species (Vaz-Pires et al., 2004). Besides, DO0 levels estimated by Maxent model (Fig. 3), agreed with results from Cerezo-Valverde & García-García (2005), who report that ventilatory frequency is altered below mean oxygen levels of 4.5 ± 0.95 mg O_2/l (~3.1 ± 0.66 ml O_2/l; mean temperature 20.5°C); above that value, ventilatory frequency is optimal. In addition, mean critical oxygen saturation reported at 2.3 ± 0.57 mg O_2//l (~1.6 ± 0.40 ml O_2/l; mean temperature 20.5°C) (Cerezo-Valverde & García-García, 2005). Consequently and according to results in the present work, octopus is searching for areas with optimal oxygen levels.

Because cephalopods can be easily affected by environmental changes (Lefkaditou et al., 2008;

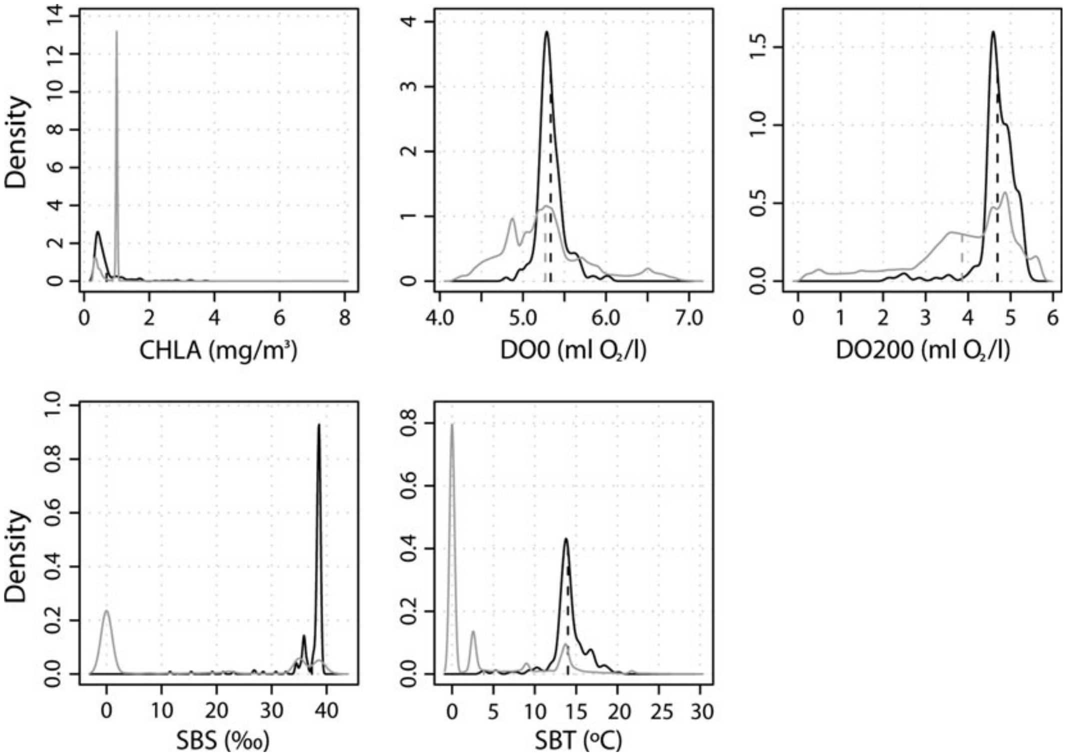


Fig. 7 Representation of the niche–environment system in the study area. *Black lines* represent the used units (the niche) and *grey lines* represent the available resource units (the environment)

Pierce et al., 2008; Tian, 2009), it could be meaningful to carry out the presented analysis on annual basis in order to evaluate variations in octopus spatial distribution and changes in habitat preferences due to environmental fluctuations caused by climate change and global warming. Furthermore, it could be interesting to include other variables in the models, such as the NAO index and sea currents data to further understand the distribution of *O. vulgaris*.

Finally, Maxent, as a tool to assess how environmental variables are related to the *Octopus vulgaris* spatial distribution in the Mediterranean and Eastern Atlantic can be used to generate valid distribution models for this species, given its geographical distribution is poorly known (Elith et al., 2006; Kaschner et al., 2006; FAO, 2010; Ready et al., 2010). In this study, we have tested part of the known distribution of the species in the Mediterranean and Eastern Atlantic producing a robust model, based on environmental variables, capable of predicting its distribution.

In the future, this model can be applied in a wider geographical area to locate other habitable areas for *O. vulgaris*, generating a distributional pattern at global scale. It can also be used to complement the current distribution map provided by FAO (FAO, 2010), adding information about more or less suitable areas for the species and helping to elucidate the possible *O. vulgaris* presence in regions where the actual identity of the species is uncertain and where no genetic and taxonomic studies are possible.

Conclusion

Together, Maxent model and ENFA identified habitat preferences and spatial distribution patterns of *Octopus vulgaris*. Main environmental variables related to them are congruent with known species life history and previous studies. To Maxent, sea bottom temperature, sea bottom salinity and chlorophyll-α are the most relevant variables to its spatial distribution; whereas to ENFA, sea bottom temperature, sea bottom salinity, dissolved oxygen level (at 0 and 200 m) are the key variables in terms of habitat preferences. Maxent can successfully predict octopus spatial distribution with the capability to evaluate which variables are more important to its distribution. This could be very useful to understanding the distributional patterns and ecology of the species. Finally, if these models are generated annually, it could be possible to evaluate variations in octopus distribution due to changes caused by climate change and global warming.

Acknowledgments This research was supported by the ECOSUMMER Project (Marie Curie Action 20501) funded by the European Commission. C. Hermosilla had an early stage researcher training through the project and F. Rocha is an Isidro Parga Pondal Researcher. The authors thank to I. Sobrino, P. Pascual and L. Gil de Sola from Instituto Español de Oceanografía for providing the *O. vulgaris* distribution data used to test the models.

References

Balguerías, E., C. Hernández-González & C. Perales-Raya, 2002. On the identity of *Octopus vulgaris* Cuvier, 1797 stocks in the Saharan bank (northwest Africa) and their spatio-temporal variations in abundance in relation to some environmental factors. Bulletin of Marine Science 71: 147–163.

Basille, M., C. Calenge, E. Marboutin, R. Andersen & J. M. Gaillard, 2008. Assessing habitat selection using multivariate statistics: some refinements of the ecological-niche factor analysis. Ecological Modelling 211: 233–240.

Basille, M., I. Herfindal, H. Santin-Janin, J. D. C. Linnell, J. Odden, R. Andersen, K. Arild Høgda & J.-M. Gaillard, 2009. What shapes Eurasian lynx distribution in human dominated landscapes: selecting prey or avoiding people? Ecography 32: 683–691.

Belcari, P., D. Cuccu, M. González, A. Srairi & P. Vidoris, 2002. Distribution and abundance of *Octopus vulgaris* Cuvier, 1797 (Cephalopoda: Octopoda) in the Mediterranean Sea. Scientia Marina 66(Suppl. 2): 157–166.

Borges-Seixas, S., T. Pinheiro & C. Sousa-Reis, 2002. Lead in octopus (*Octopus vulgaris*) in Portugal: a preliminary study. Bulletin of Marine Science 71: 1091–1093.

Bowker, G. C., 2000. Mapping biodiversity. International Journal of Geographic Information Systems 14: 739–754.

Byrne, R. A., M. Kuba & U. Griebel, 2002. Lateral asymmetry of eye use in *Octopus vulgaris*. Animal Behaviour 64: 461–468.

Caballero-Alfonso, A. M., U. Ganzedo, A. Trujillo-Santanaa, J. Polanco, A. Santana del Pino, G. Ibarra-Berastegi & J. J. Castro-Hernández, 2010. The role of climatic variability on the short-term fluctuations of octopus captures at the Canary Islands. Fisheries Research 102: 258–265.

Cabranes, C., P. Fernandez-Rueda & J. L. Martínez, 2008. Genetic structure of *Octopus vulgaris* around the Iberian Peninsula and Canary Islands as indicated by microsatellite DNA variation. ICES Journal of Marine Science 65: 12–16.

Calenge, C., 2006. The package adehabitat for the R software: a tool for the analysis of space and habitat use by animals. Ecological Modelling 197: 516–519.

Calenge, C. & M. Basille, 2008. A general framework for the statistical exploration of the ecological niche. Journal of Theoretical Biology 252: 674–685.

Catalán, I. A., M. T. Jiménez, J. I. Alconchel, L. Prieto & J. L. Muñoz, 2006. Spatial and temporal changes of coastal demersal assemblages in the Gulf of Cadiz (SW Spain) in relation to environmental conditions. Deep-Sea Research II 53: 1402–1419.

Cerezo-Valverde, J. & B. García-García, 2005. Suitable dissolved oxygen levels for common octopus (*Octopus vulgaris* Cuvier, 1797) at different weights and temperatures: analysis of respiratory behaviour. Aquaculture 244: 303–314.

Chapela, A., A. F. González, E. G. Dave, F. Rocha & A. Guerra, 2006. Growth of common octopus (*Octopus vulgaris*) in cages suspended from rafts. Scientia Marina 70: 121–129.

Conrad, O., 2006. SAGA—program structure and current state of implementation. In Böhner, J., K. R. McCloy & J. Strobl (eds), SAGA—Analysis and Modelling Applications. Göttinger Geographische Abhandlungen, Vol. 115. 130 pp.

Costa, P. R., R. Rosa & M. A. M. Sampayo, 2004. Tissue distribution of the amnesic shellfish toxin, domoic acid, in *Octopus vulgaris* from the Portuguese coast. Marine Biology 144: 971–976.

Demarcq, H. & V. Faure, 2000. Coastal upwelling and associated retention indices derived from satellite SST. Application to Octopus vulgaris recruitment. Oceanologica Acta 23: 391–408.

Dolédec, S., D. Chessel & C. Gimaret-Carpentier, 2000. Niche separation in community analysis: a new method. Ecology 81: 2914–2927.

Domain, F., D. Jouffre & A. Caverivière, 2000. Growth of *Octopus vulgaris* from tagging in Senegalese waters. Journal of the Marine Biological Association of the United Kingdom 80: 699–705.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudík, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, Overton J. McC, A. Townsend Peterson, S. J. Phillips, K. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberón, S. Williams, M. S. Wisz & N. E. Zimmermann, 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29: 129–151.

Ezzeddine, S. & A. El-Abed, 2004. Potential biological and environmental influences on the *Octopus vulgaris* population of the Gulf of Gabès (south-eastern Tunisian coast). MedSudMed Technical Documents 2: 42–49.

FAO, 2009. Global production statistics 1950–2007. United Nations Food and Agriculture Organization [available on internet at http://firms.fao.org/gfcm/topic/17105/en]. Accessed October 2009.

FAO, 2010. Species fact sheets, *Octopus vulgaris* (Lamarck, 1798). United Nations Food and Agriculture Organization [available on internet at http://www.fao.org/fishery/species/3571/en]. Accessed July 2010.

Faraj, A. & N. Bez, 2007. Spatial considerations for the Dakhla stock of *Octopus* indicators, patterns, and fisheries interactions. ICES Journal of Marine Science 64: 1820–1828.

Fielding, A. H. & J. F. Bell, 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24: 38–49.

Garcia, H. E., R. A. Locarnini, T. P. Boyer & J. I. Antonov, 2006. World Ocean Atlas 2005, Volume 3: Dissolved Oxygen, Apparent Oxygen Utilization, and Oxygen Saturation. S. Levitus, Ed. NOAA Atlas NESDIS 63, U.S. Government Printing Office, Washington, DC, 342.

González, A. F. & P. Sánchez, 2002. Cephalopod assemblages caught by trawling along the Iberian Peninsula Mediterranean coast. Scientia Marina 66: 199–208.

González, A. F., J. Otero, A. Guerra, R. Prego, F. J. Rocha & A. W. Dale, 2005. Distribution of common octopus and common squid paralarvae in a wind-driven upwelling area (Ria of Vigo, northwestern Spain). Journal of Plankton Research 27: 271–277.

Guerra, A., 1992. Mollusca, Cephalopoda. In Ramos, M. A., J. A. Tercedor, X. Bellés, J. G. Gonsálbez, A. Guerra, E. Macpherson, F. Martín, et al. (eds), Fauna Ibérica, Vol. 1. Nacional de Ciencias Naturales, CSIC, Madrid: 1–327.

Guerra, A., A. Roura, A. F. González, S. Pascual, Y. Cherel & M. Pérez-Losada, 2010. Morphological and genetic evidence that *Octopus vulgaris* Cuvier, 1797 inhabits Amsterdam and Saint Paul Islands (Southern Indian Ocean). ICES Journal of Marine Science 67: 1401–1407.

Hermosilla, C. A., F. Rocha, G. Fiorito, A. F. González & A. Guerra, 2010. Age validation in common octopus *Octopus vulgaris* using stylet increment analysis. ICES Journal of Marine Science 67: 1458–1463.

Hernández-López, J. L., 2000. Biología, ecología y pesca del pulpo común ("*Octopus vulgaris*", Cuvier 1797) en aguas de Gran Canaria. PhD thesis, Universidad de las Palmas de Gran Canaria.

Hernández-López, J. L., J. J. Castro-Hernández & V. Hernández-García, 2001. Age determined from the daily deposition of concentric rings on common octopus (*Octopus vulgaris*) beaks. Fishery Bulletin 99: 679–684.

Hijmans, R., S. Phillips, J. Leathwick & J. Elith, 2010. Dismo: Species distribution modeling. R package version 0.5-4 [available on internet at http://CRAN.R-project.org/package=dismo].

Hirzel, A. H., J. Hausser, D. Chessel & N. Perrin, 2002a. Ecological-niche factor analysis: how to compute habitat-suitability maps without absence data? Ecology 83: 2027–2036.

Hirzel, A. H., J. Hausser, N. Perrin, 2002b. Biomapper 3.1. Lab. for Conservation Biology. Department of Ecology and Evolution, University of Lausanne [available on internet at http://www.unil.ch/biomapper].

Hutchinson, G. E., 1957. Concluding remarks. Cold Spring Harbour Symposium on Quantitative Biology 22: 415–427.

Josupeit, H., 2000. Los mercados mundiales de cefalópodos. Productos del Mar Noviembre-Diciembre: 43-48.

Kaschner, K., J. S. Ready, E. Agbayani, J. Rius, K. Kesner-Reyes, P. D. Eastwood, A. B. South, S. O. Kullander, T. Rees, C. H. Close, R. Watson, D. Pauly & R. Froese, 2008. AquaMaps: predicted range maps for aquatic species. World Wide Web electronic publication, www.aquamaps.org, Version 10/2008.

Kaschner, K., R. Watson, A. W. Trites & D. Pauly, 2006. Mapping world-wide distributions of marine mammal species using a relative environmental suitability (RES) model. Marine Ecology Progress Series 316: 285–310.

Katsanevakis, S. & G. Verriopoulos, 2004a. Abundance of *Octopus vulgaris* on soft sediment. Scientia Marina 68: 553–560.

Katsanevakis, S. & G. Verriopoulos, 2004b. Den ecology of *Octopus vulgaris* Cuvier, 1797, on soft sediment: availability and types of shelter. Scientia Marina 68: 147–157.

Katsanevakis, S. & G. Verriopoulos, 2006a. Modelling the effect of temperature on hatching and settlement patterns of meroplanktonic organisms: the case of the octopus. Scientia Marina 70: 699–708.

Katsanevakis, S. & G. Verriopoulos, 2006b. Seasonal population dynamics of *Octopus vulgaris* in the eastern Mediterranean. ICES Journal of Marine Science 63: 151–160.

Lefkaditou, E., C. H. Mytilineou, P. Maiorano & G. D'onghia, 2003. Cephalopod species captured by deep-water exploratory trawling in the north-eastern Ionian Sea. Journal of Northwest Atlantic Fishery Science 31: 431–440.

Lefkaditou, E., C.-Y. Politou, A. Palialexis, J. Dokos, P. Cosmopoulos & V. D. Valavanis, 2008. Influences of environmental variability on the population structure and distribution patterns of the short-fin squid *Illex coindetii* (Cephalopoda: Ommastrephidae) in the Eastern Ionian Sea. Hydrobiologia 612: 71–90.

MacLeod, C. D., L. Mandleberg, C. Schweder, S. M. Bannon & G. J. Pierce, 2008. A comparison of approaches for modelling the occurrence of marine animals. Hydrobiologia 612: 21–32.

Madan, J. J. & M. J. Wells, 1996. Cutaneous respiration in *Octopus vulgaris*. The Journal of Experimental Biology 199: 2477–2483.

Maltagliati, F., P. Belcari, D. Casu, M. Casu, P. Sartor, G. Vargiu & A. Castelli, 2002. Allozyme genetic variability and gene flow in *Octopus vulgaris* (Cephalopoda, Octopodidae) from the Mediterranean Sea. Bulletin of Marine Science 71: 473–486.

Mangold, K., 1983. Octopus vulgaris. In Boyle, P. R. (ed.), Cephalopod Life Cycles, Vol. 1. Species Accounts. Academic Press, London, United Kingdom: 335–364.

Mangold, K., 1998. The Octopodinae from the Eastern Atlantic Ocean and the Mediterranean Sea. Smithsonian Contributions to Zoology 586: 521–528.

Mangold, K. & F. G. Hochberg, 1991. Defining the genus *Octopus*: redescription of *Octopus vulgaris*. Bulletin of Marine Science 49: 665.

Miliou, H., M. Fintikaki, T. Kountouris & G. Verriopoulos, 2005. Combined effects of temperature and body weight on growth and protein utilization of the common octopus, *Octopus vulgaris*. Aquaculture 249: 245–256.

Moreno, A., A. dos Santos, U. Piatkowski, A. M. P. Santos & H. Cabral, 2009. Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. Journal of Plankton Research 31: 73–91.

Murphy, J. M., E. Balguerías, L. N. Key & P. R. Boyle, 2002. Microsatellite DNA markers discriminate between two

Octopus vulgaris (Cephalopoda: Octopoda) fisheries along the northwest African coast. Bulletin of Marine Science 71: 545–553.

Norman, M., 2000. Cephalopods—A World Guide. ConchBooks, Hackenheim, Germany.

Otero, J., X. A. Álvarez-Salgado, A. F. González, A. Miranda, S. B. Groom, J. M. Cabanas, G. Casas, B. Wheatley & A. Guerra, 2008. Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. Marine Ecology Progress Series 362: 181–192.

Otero, J., X. A. Álvarez-Salgado, A. F. González, M. Gilcoto & A. Guerra, 2009. High frequency coastal upwelling events influence *Octopus vulgaris* larval dynamics on the NW Iberian shelf. Marine Ecology Progress Series 386: 123–132.

Ottaviani, D., G. J. Lasinio & L. Boitani, 2004. Two statistical methods to validate habitat suitability models using presence-only data. Ecological Modelling 179: 417–443.

Pardo-Gandarillas, M. C., C. M. Ibáñez, A. T. Palma & E. Poulin, 2009. Origin and molecular divergence of *Octopus crusoe*, octopus endemic to the Juan Fernandez Archipelago. In Guerra, A. & A. F. González (eds), Abstract Book, Cephalopod International Advisory Council, CIAC Symposium. Vigo, Spain: 33.

Phillips, S. J. & M. Dudík, 2008. Modelling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography 31: 161–175.

Phillips, S. J., M. Dudík & R. E. Schapire, 2004. A Maximum entropy approach to species distribution modelling. In Brodley, C. (ed), Proceedings of the Twenty-First International Conference on Machine learning, Banff, Canada: 655–662.

Phillips, S. J., R. P. Anderson & R. E. Schapire, 2006. Maximum entropy modelling of species geographic distributions. Ecological Modelling 190: 231–259.

Pierce, G. J., V. D. Valavanis, A. Guerra, P. Jereb, L. Orsi-Relini, J. M. Bellido, I. Katara, U. Piatkowski, J. Pereira, E. Balguerias, I. Sobrino, E. Lefkaditou, J. Wang, M. Santurtun, P. R. Boyle, L. C. Hastie, C. D. MacLeod, J. M. Smith, M. Viana, A. F. González & A. F. Zuur, 2008. A review of cephalopod–environment interactions in European Seas. Hydrobiologia 612: 49–70.

Prendergast, J. R., R. M. Quinn & J. H. Lawton, 1999. The gaps between theory and practice in selecting nature reserves. Conservation Biology 13: 484–492.

Quetglas, A., F. Alemany, A. Carbonell, P. Merella & P. Sánchez, 1998. Biology and fishery of *Octopus vulgaris* Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, Western Mediterranean). Fisheries Research 36: 237–249.

R Development Core Team, 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0 [available on internet at http://www.R-project.org].

Ready, J., K. Kaschner, A. B. South, P. D. Eastwood, T. Rees, J. Rius, E. Agbayani, S. Kullander & R. Froese, 2010. Predicting the distributions of marine organisms at the global scale. Ecological Modelling 221: 467–478.

Rocha, F., A. Guerra, R. Prego & U. Piatkowski, 1999. Cephalopod paralarvae and upwelling conditions off Galician waters (NW Spain). Journal of Plankton Research 21: 21–33.

Rodriguez-Rúa, A., I. Pozuelo, M. A. Prado, M. J. Gómez & M. A. Bruzón, 2005. The gametogenic cycle of *Octopus vulgaris* (Mollusca: Cephalopoda) as observed on the Atlantic coast of Andalusia (south of Spain). Marine Biology 147: 927–933.

Roper, C. F. E., M. J. Sweeney & C. E. Nauen, 1984. FAO species catalogue. Vol. 3. Cephalopods of the world. An annotated and illustrated catalogue of interest to fisheries. FAO Fisheries Synopsis 125: 1–277.

Salman, A., T. Katagan & H. A. Benli, 2002. Cephalopod fauna of the eastern Mediterranean. Turkish Journal of Zoology 26: 47–52.

Sing, T., O. Sander, N. Beerenwinkel & T. Lengauer, 2009. ROCR: Visualizing the performance of scoring classifiers. R package version 1.0-4 [available on internet at http://CRAN.R-project.org/package=ROCR].

Sobrino, I., L. Silva, J. M. Bellido & F. Ramos, 2002. Rainfall, river discharges and sea temperature as factors affecting abundance of two coastal benthic cephalopod species in the Gulf of Cadiz (SW Spain). Bulletin of Marine Science 71: 851–865.

Tian, Y., 2009. Interannual-interdecadal variations of spear squid Loligo bleekeri abundance in the southwestern Japan Sea during 1975–2006: impact of the trawl fishing and recommendations for management under the different climate regimes. Fisheries Research 100: 78–85.

Tsoar, A., O. Allouche, O. Steinitz, D. Rotem & R. Kadmon, 2007. A comparative evaluation of presence-only methods for modelling species distribution. Diversity and Distribution 13: 397–405.

Valavanis, V. D., G. J. Pierce, A. F. Zuur, A. Palialexis, A. Saveliev, I. Katara & J. Wang, 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612: 5–20.

Vargas-Yáñez, M., F. Moya, M. García-Martínez, J. Rey, M. González & P. Zunino, 2009. Relationships between *Octopus vulgaris* landings and environmental factors in the northern Alboran Sea (Southwestern Mediterranean). Fisheries Research 99: 159–167.

Vaz-Pires, P., P. Seixas & A. Barbosa, 2004. Aquaculture potential of the common octopus (*Octopus vulgaris* Cuvier, 1797): a review. Aquaculture 238: 221–238.

Veloz, S. D., 2009. Spatially autocorrelated sampling falsely inflates measures of accuracy for presence-only niche models. Journal of Biogeography 36: 2290–2299.

Warnke, K., R. Söller, D. Blohm & U. Saint-Paul, 2004. A new look at geographic and phylogenetic relationships within the species group surrounding *Octopus vulgaris* (Mollusca, Cephalopoda): indications of very wide distribution from mitochondrial DNA sequences. Journal of Zoological Systematics and Evolutionary Research 42: 306–312.

Hydrobiologia (2011) 670:49–65
DOI 10.1007/s10750-011-0693-5

ECOSYSTEMS AND SUSTAINABILITY

Environmental drivers of the anchovy/sardine complex in the Eastern Mediterranean

Isidora Katara · Graham J. Pierce ·
Janine Illian · Beth E. Scott

Published online: 3 May 2011
© Springer Science+Business Media B.V. 2011

Abstract The anchovy/sardine complex is an important fishery resource in some of the largest upwelling systems in the world. Synchronous, but out of phase, fluctuations of the two species in distant parts of the oceans have prompted a number of studies dedicated to determining the phenomena, atmospheric and oceanic, responsible for the observed synchronicity and the biological mechanisms behind the population changes of the two species. Anchovy and sardine are of high commercial value for the fishing sector in Greece; this study investigates the impact of large-scale climatic indices on the anchovy/sardine complex in the Greek seas using fishery catches as a proxy for fish productivity. Time series of catches for both species were analysed for relationships with teleconnection indices and local environmental variability. The connection between the teleconnection indices and local weather/oceanic variation was also examined in an effort to describe physical mechanisms that link large-scale atmospheric patterns with anchovy and sardine. The West African Summer Monsoon, East Atlantic Jet and Pacific–North American (PNA) pattern exhibit coherent relationships with the catches of the two species. The first two aforementioned patterns are prominent atmospheric modes of variability during the summer months when sardine is spawning and anchovy juveniles are growing. PNA is related with El Niño Southern Oscillation events. Sea Surface Temperature (SST) appears as a significant link between atmospheric and biological variability either because higher temperatures seem to be favouring sardine growth or because lower temperatures, characteristic of productivity-enhancing oceanic features, exert a positive influence on both species. However at a local scale, other parameters such as wind and mesoscale circulation describe air–sea variability affecting the anchovy/sardine complex. These relationships are non-linear and in agreement with results of previous studies

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

Electronic supplementary material The online version of this article (doi:10.1007/s10750-011-0693-5) contains supplementary material, which is available to authorized users.

I. Katara · G. J. Pierce · B. E. Scott
School of Biological Sciences (Zoology),
University of Aberdeen, Tillydrone Avenue,
AB24 2TZ Aberdeen, UK

I. Katara (✉)
Department of Biology, Dalhousie University, Halifax,
NS B3H4J1, Canada
e-mail: ikatara@mathstat.dal.ca

G. J. Pierce
Centro Oceanográfico de Vigo, Instituto Español de
Oceanografía, P.O. Box 1552, 36200 Vigo, Spain

J. Illian
School of Mathematics and Statistics, The Observatory,
University of St. Andrews, Buchanan Gardens,
KY16 9LZ St. Andrews, UK

stressing the importance of optimal environmental windows. The results also show differences in the response of the two species to environmental forcing and possible interactions between the two species. The nature of these phenomena, e.g., if the species interactions are direct through competition or indirect through the food web, remains to be examined.

Keywords Anchovy/sardine complex · Environmental effects · Teleconnections

Introduction

Global fluctuations in the abundance of anchovy and sardine, in particular the apparent alternation of high abundance phases of both species and their possible relationship with climatic cues are an area of intense scientific study. Records of fin-scale deposition in coastal upwelling systems show cycles of expansion and contraction of the sardine and anchovy populations with a periodicity of 30 years for sardine (*Sardinops sagax*), 50–60 years for anchovy (*Engraulis encrasicolus*) and 25 years for both (Lehodey et al., 2006; Valdés et al., 2008).

The regime shift from anchovy (genus *Engraulis*) dominance to sardine (genera *Sardinops* or *Sardina*) dominance during the mid 1970s in the Pacific was one of the most pronounced phenomena of synchronisation of sardine and anchovy landings in distant areas and indicative of opposite phase fluctuations between the two species (Kawasaki, 1983; Schwartzlose et al., 1999; Chavez et al., 2003; Alheit & Bakun, 2009). The movement of the Humboldt current near the coast of Peru, during El Niño events decreases the spatial extent of anchovy (*Engraulis ringens*) spawning habitat thus adversely affecting recruitment and rendering the population more susceptible to predation (Alheit & Niquen, 2004; Lett et al., 2007; Swartzman et al., 2008), while creating favourable feeding conditions for sardine (*S. sagax*). Contemporaneously in the Kuroshio current, sea surface temperature (SST) and productivity fluctuations, attributable to the dislocation of frontal structures and mixed layer depth changes co-varied with anchovy/sardine alternations (Alheit & Bakun, 2009 and references therein). Other mechanisms explaining sardine and anchovy co-variation in the north Pacific involve direct effects of temperature and different optima for sardine (*Sardinops melanostictus*) and anchovy (*Engraulis japonicus*) spawning (Takasuka et al., 2008).

Such coincident changes in oceanographic and biological parameters led to the concept of regime shifts. The complexity of the connections among the different parameters precludes unambiguous conclusions on a mechanism linking the different components, biotic and abiotic, of these ecosystems. However, there is an established agreement that synchronised shifts are forced by large-scale atmospheric and oceanic phenomena (Schwing et al., 2010). In the case of the Humboldt and Kuroshio currents, the North Pacific Gyre Oscillation has been suggested as the synchronising phenomenon between the two distant Pacific ecosystems (Di Lorenzo et al., 2008).

In some cases, synchronicity between different sites is only observed for the Pacific sardine (*S. sagax*) and not for anchovy (*E. ringens*) (Lluch-Belda et al., 1992; Schwartzlose et al., 1999), while short-term events such as the 1997–1998 El Niño do not favour sardine over anchovy (Bertrand et al., 2004). Ultimately, climate variability will cause these stocks to interact through resource competition and predation (Miller & Schneider, 2000).

The striking synchronisation phenomena of anchovy and sardine alternations observed in the Pacific are comparable to synchronous but out of phase fluctuations of another pair of small pelagic species, herring (*Clupea harengus*) and sardine (*Sardina pilchardus*) along the coast of north-east Atlantic. The North Atlantic Oscillation (NAO) is suggested as the climatic phenomenon governing these fluctuations (Alheit & Hagen, 1997; Parsons & Lear, 2001). Synchrony in the abundance of another group of short-lived, pelagic marine organisms, squids, was revealed by Waluda et al. (2004). Moreover, El Niño Southern Oscillation (ENSO) related movement of the Antarctic Circumpolar Wave is suggested as an important factor influencing recruitment strength of *Illex argentinus* in the south Atlantic (Waluda et al., 1999, 2001). The important role of regime shifts and environmental change in driving the variability of squid fisheries in different areas in the world is reviewed by Pierce et al. (2008) and Rodhouse (2009).

The alternation between anchovy-dominated and sardine-dominated ecosystems is a common observation for most upwelling areas where the two species co-exist (Lluch-Belda et al., 1992). Sardine and anchovy are the most exploited small pelagic species in the Mediterranean but causes of large-scale fluctuations in their stock sizes have been undecipherable.

The theory of 'ocean triads' (Agostini & Bakun, 2002) has been an important discussion point for the majority of studies of environmental effects on small pelagic fish in the Mediterranean. Upwelling events and mesoscale features regulate offshore transport and retention of fish eggs and larvae in various areas of the Mediterranean and can thus determine recruitment success (Santos et al., 2004; Lafuente et al., 2005).

In the Adriatic, inter-decadal variability of small pelagic fish is related with an 80-year cycle of climatic oscillations (Grbec et al., 2002). The physical mechanism suggested involves NAO-related atmospheric pressure differences over the Adriatic that modulate the inflow of Levantine Intermediate Water (LIW) into the Adriatic, inducing stock fluctuations in small pelagic fish. Possible biological mechanisms discussed by the authors include bottom-up effects, different environmental optima for the different species and reorganisation of the trophic web.

The hydrology of the Black Sea, strongly connected with the Mediterranean is associated with the NAO and the East Atlantic–Western Russian pattern (Oguz et al., 2006). Regime shifts in the Black Sea ecosystem are related with climate-induced variations in nutrient enrichment of the water column; they are speculated to involve transition from top-down to bottom-up food web structures and are occasional events (Oguz & Gilbert, 2007).

Stergiou (1991) was probably the first author to describe the sardine (*S. pilchardus*) /anchovy (*E. encrasicolus*) complex in Greek waters, influenced by an increasing interest in the mechanisms driving this complex. His findings reveal a 3-year periodicity in catches and a negative correlation between the catches of the two species. The author suggests that, along with fishing effort, environmental, biological and economic parameters drive catches. Of the environmental factors, local sea level (atmospheric) pressure and meridian winds have been associated with the ratio of anchovy/sardine in catches (Stergiou & Lascaratos, 1997). Possible mechanisms, suggested by the same authors, include changes in currents, wind-induced productivity favourable for anchovy larvae and different growth rates.

Agostini & Bakun (2002) used the Aegean as a study area to highlight the importance of 'ocean triads' for the recruitment of small pelagic fish, with anchovy as their case study species. They stress the importance of large-scale upwelling in the eastern part of the Aegean (Bakun & Agostini, 2001) and mesoscale fronts in the western part for the spawning and recruitment of the species. Moreover, Giannoulaki et al. (2005) related distribution patterns of anchovy and sardine with anticyclonic features in the north Aegean and with currents carrying water from the Black Sea. Gyres and fronts are retention areas for zooplankton (Somarakis et al., 2002), fish eggs and larvae (Heath, 1992; Somarakis & Nikolioudakis, 2007). Suitable grounds for juvenile sardines in the Aegean are inshore, semi-closed, highly productive areas near estuaries (Tsagarakis et al., 2008).

The large-scale atmospheric patterns driving oceanic circulation in the Mediterranean and the response of anchovy and sardine to environmental cues set the framework for the identification of relationships between the anchovy/sardine complex and teleconnection patterns. In this study, we explored possible mechanisms that link variability in anchovy and sardine fishery catches in the eastern Mediterranean to local and large-scale physical phenomena, mainly teleconnection patterns. Based on previous knowledge of the teleconnections related with oceanic circulation in the eastern Mediterranean and the descriptions of covariance between the anchovy/sardine complex and environmental factors, we tested for empirical relationships between the complex and teleconnection indices; thus, we identified a small number of teleconnections correlated with anchovy and sardine. The effect of local variability, mainly upwelling and frontal structures on the different life stages of the two species has been revealed in various studies mentioned above. Therefore, we hypothesised that oceanic features could modify the impact of teleconnections (Schwing et al., 2010) on anchovy and sardine at a local scale. This hypothesis can be divided into two questions: is there a relationship between the teleconnections and local environmental variability; and is there a relationship between local environmental parameters and the anchovy/sardine complex? Answering these questions will give insights into the mechanistic links connecting teleconnection patterns with anchovy and sardine production, highlighting oceanic features of importance and suggesting possible biological processes involved in these relationships.

Materials and methods

Catch data are used as the only available long-term proxy of population status in this area. The calculation of the catch ratio of the two species is a common practice to prevent fishing effort and gear changes from affecting results in catch–environment relationship studies (Stergiou, 1991). Furthermore, the extraction of temporal trends from the catch time series and their comparison with fishing effort trends and known legislative decisions concerning this fishery has allowed for the unravelling of the different sources of variation.

The methodology was designed to answer three interlinked questions (Fig. 1) in an effort to obtain a complete picture of the physical mechanisms through which teleconnection patterns influence anchovy and sardine fishing yield. With the first group of models based on dynamic factor analysis, teleconnection indices associated with anchovy and sardine catches were identified. These indices were used as explanatory variables in the second group of models that investigated the impact of these teleconnections on local environmental variability. Finally, the links between local environmental variability and anchovy–sardine fisheries production were investigated using non-linear regression models.

Catches and environmental data

Catch data on anchovy (*E. encrasicolus*) and sardine (*S. pilchardus*) were obtained through the Greek National Statistics Services (GNSS). The collection scheme of the dataset is described in Stergiou et al. (1997) and Stergiou & Lascaratos (1997); it consists of a network of the major areas around Greece where catches of all commercially important species have been recorded on a monthly basis since 1964. The data are presented in annual reports, the GNSS bulletins. For this study the annual averages of the catches of the two species in 16 areas were used (Fig. 2). The ratio of anchovy over sardine catches was also calculated for each area (Fig. 3). The data therefore consist of annual time series of catches for anchovy, sardine and their ratio for each area over the period 1964–2005. It should be noted that data from the Turkish fishing fleet that operates in some of these areas are not readily available. However, the use of multivariate time series statistics based on the extraction of trends will compensate for this lack of data.

Fishing effort data in the form of numbers of fishing vessels and their horsepower are available from the same source (GNSS bulletins) as an average for all the areas on annual basis. To avoid collinearity, when the time-series describing fishing effort are used as

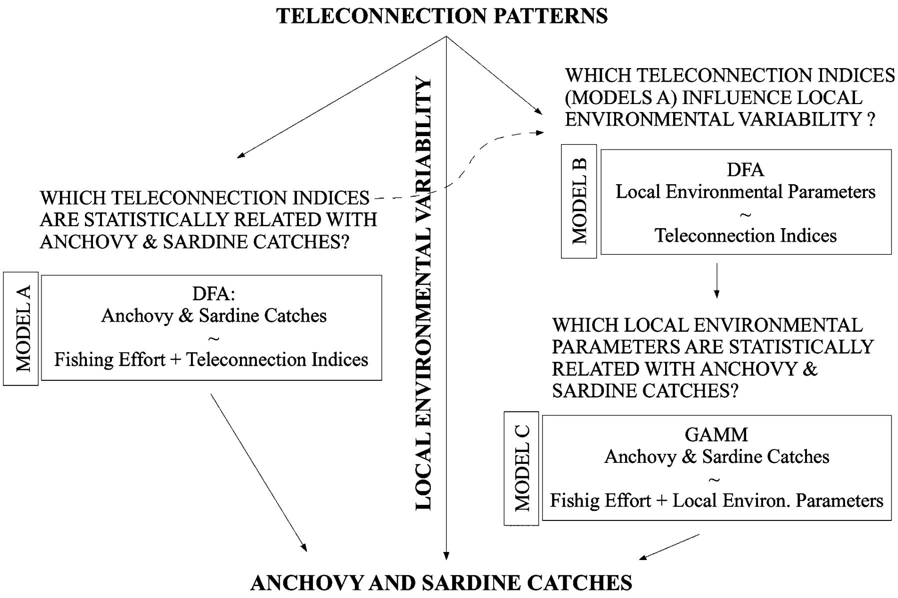


Fig. 1 Models A investigate the possible impact of teleconnection patterns on anchovy and sardine catches. The indices found to be statistically significant are used in the B Models in order to describe their effects on local environmental variability in the study area. C Models were applied to study the relationships of anchovy and sardine catches with local environmental parameters. Thus, possible physical mechanisms were derived, linking teleconnection patterns to biological variability for the two species

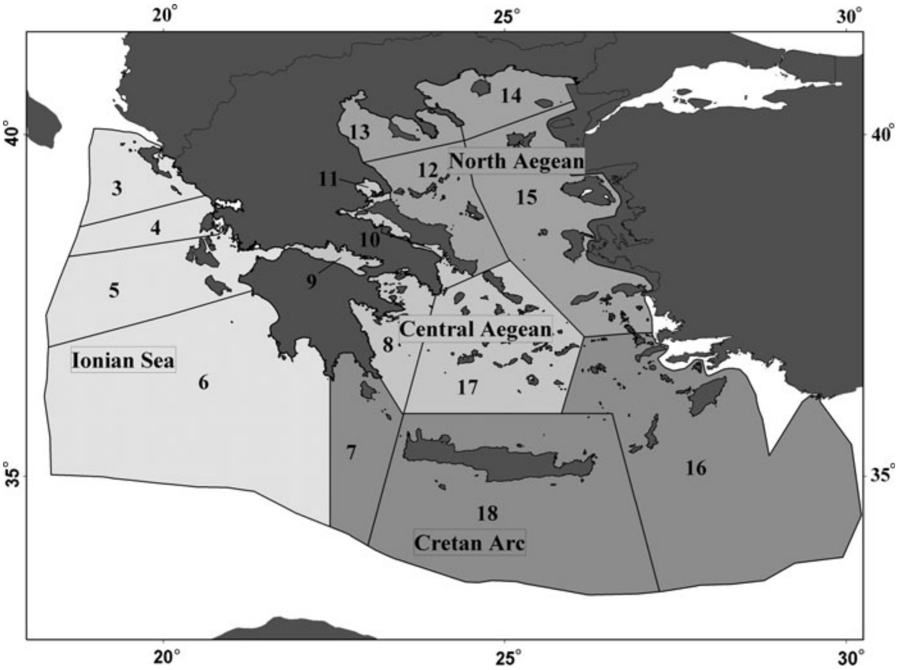


Fig. 2 The collection scheme of the data is organised in 18 areas of which the first area concerns catches from the Atlantic Ocean, the second area concerns catches from the south Levantine Sea and areas 3–18 can be seen in the map. These areas are further grouped into oceanographically coherent larger areas, for the application of GAMMs. These areas are the North Aegean (12–15) influenced by the northwest Aegean upwelling and the intrusion of Black Sea water from the Dardanelles straits; the central Aegean (8–11, 17), including the Cyclades plateau and small bays and enclosed areas around the central continental Greece; the Ionian Sea (3–6) and the Cretan Arc (7, 16, 18) characterised by a row of interconnected cyclonic and anti-cyclonic gyres. Areas 1 and 2 are outside the Greek waters and are not used in the analyses

explanatory variables, Min/Max Autocorrelation Factor Analysis (MAFA) (Solow, 1994) was applied on these time series and the basic trends in fishing effort were derived. Five trends—Fishing Effort (FE) MAFs—were statistically significant and were used to account for fishing effort variability in the subsequent statistical analyses (Supplementary material).

A series of teleconnection indices, previously found to be related to oceanic variability in the Mediterranean (Alpert et al., 2006) are used to account for large-scale atmospheric variability (Table 1). Local environmental variability is described using SST derived from NOAA AVHRR, http://eoweb.dlr.de:8080, sea surface height (SSH), and zonal and meridional wind stress (ZWS and MWS, respectively) from Carton-Giese SODA Version 2.0.2-4, http://iridl.ldeo.columbia.edu/SOURCES/.CARTON-GIESE/SODA/.v2p0p2-4/.ssh/ (Carton & Giese, 2008). The data cover the period 1960–2007. They were processed using ArcGIS modules and annual averages for each of the local environmental parameters were calculated for each of the 16 areas.

Statistical analysis

To identify which teleconnection indices might be related to the anchovy/sardine complex, a DFA model (Molenaar, 1985; Zuur et al., 2003a, b; Huang et al., 2006) was developed using the time series of the ratio of the catches in each area as response variables, fitting one common trend and using the teleconnection indices and fishing effort as explanatory variables. The analysis was also repeated when 1 and 2 (year) time lags were introduced between the explanatory and the response variables. Similar models were built using the original catches of anchovy and sardine separately (Table 2).

The teleconnection indices that were identified as being statistically significant in the above-mentioned models were used to explain the variability of local oceanic parameters for the period 1960–2007. The time series of SST were treated as response variables

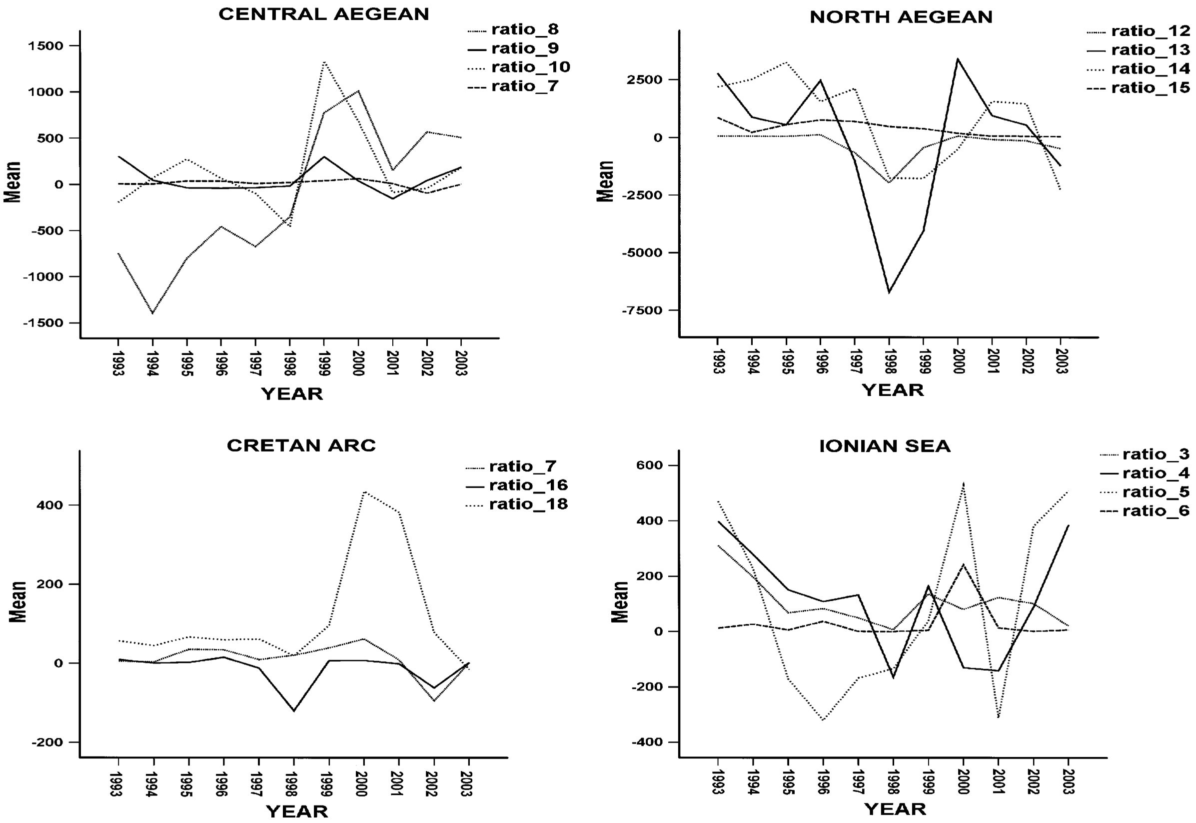


Fig. 3 Ratio of anchovy/sardine for each of the areas of the catches collection scheme. Each time series corresponds to one of the sampled areas as seen in Fig. 2 (ratio_area number)

Table 1 Teleconnection indices used to account for large-scale atmospheric variability and their sources

Teleconnection index	Source
North Atlantic Oscillation (NAO)	NOAA/Climate Prediction Centre
East Atlantic (EA)	NOAA/Climate Prediction Centre
East Atlantic/Western Russia (EA–WR)	NOAA/Climate Prediction Centre
East Atlantic Jet	NOAA/Climate Prediction Centre
Scandinavia (SCA)	NOAA/Climate Prediction Centre
Polar/Eurasia (POL)	NOAA/Climate Prediction Centre
West Pacific (WP)	NOAA/Climate Prediction Centre
East Pacific–North Pacific (EP–NP)	NOAA/Climate Prediction Centre
Pacific/North American (PNA)	NOAA/Climate Prediction Centre
Tropical/Northern Hemisphere (TNH)	NOAA/Climate Prediction Centre
Pacific Transition (PT)	NOAA/Climate Prediction Centre
Indian Monsoon (IM)	Wang & Fan (1999)
Western North Pacific Monsoon (WNPM)	Wang et al. (2001)
Webster and Yang Monsoon Index (WYM)	Webster & Yang (1992)
West African Summer Monsoon Index (WASMI)	Li & Zeng (2005)
Southern Oscillation Index (SOI)	NOAA/Climate Prediction Center

The indices derived from NOAA/Climate Prediction Centre, were calculated by applying Rotated Principal Component Analysis on 500-mb height atmospheric pressure anomalies in the region 20°N–90°N

Table 2 DFA models to identify relationships between the anchovy/sardine complex and the climatic (teleconnection) indices

Ratio ~ 1 trend + fishing effort + lag 0 teleconnection indices
Ratio ~ 1 trend + fishing effort + lag 1 teleconnection indices
Ratio ~ 1 trend + fishing effort + lag 2 teleconnection indices
Anchovy ~ 1 trend + fishing effort + lag 0 teleconnection indices
Anchovy ~ 1 trend + fishing effort + lag 1 teleconnection indices
Anchovy ~ 1 trend + fishing effort + lag 2 teleconnection indices
Sardine ~ 1 trend + fishing effort + lag 0 teleconnection indices
Sardine ~ 1 trend + fishing effort + lag 1 teleconnection indices
Sardine ~ 1 trend + fishing effort + lag 2 teleconnection indices

Table 3 DFA models to identify relationships between local oceanic parameters and climatic indices identified previously as important for the anchovy/sardine complex

SST ~ 1 trend + lag 0 teleconnection indices
SST ~ 1 trend + lag 1 teleconnection indices
SST ~ 1 trend + lag 2 teleconnection indices
SSH ~ 1 trend + lag 0 teleconnection indices
SSH ~ 1 trend + lag 1 teleconnection indices
SSH ~ 1 trend + lag 2 teleconnection indices
ZWS ~ 1 trend + lag 0 teleconnection indices
ZWS ~ 1 trend + lag 2 teleconnection indices
ZWS ~ 1 trend + lag 1 teleconnection indices
MWS ~ 1 trend + lag 0 teleconnection indices
MWS ~ 1 trend + lag 1 teleconnection indices
MWS ~ 1 trend + lag 2 teleconnection indices

in a DFA model in which this subset of teleconnection indices was used as explanatory variables. DFA models with 1 and 2 year time lags between the explanatory and the response variables were also developed. The same approach was followed for SSH, ZWS and MWS (Table 3).

The different DFA models mentioned above describe (a) relationships between atmospheric/climatic variability and oceanic circulation, and (b) relationships between anchovy–sardine and atmospheric–climatic variability. To be able to infer hypotheses on the possible mechanistic links between the teleconnection indices and the anchovy/sardine complex, we also tried to identify local environmental cues that are important for the complex. Generalised Additive Mixed Models (GAMMs) were employed to determine possible significant effects of local parameters, namely SST, SSH, ZWS and MWS on the anchovy/sardine ratio. To account for autocorrelation patterns in the data, autoregressive moving-average structures were introduced into the models. Due to failure of convergence of the maximum likelihood algorithm when a large number of time series is used as response variables in GAMMs, the time series were divided in oceanographically coherent groups corresponding to the following areas: north Aegean, central Aegean, the Ionian Sea and the Cretan Arc (Fig. 2) and a GAMM was applied for each area. The same approach was employed for the time series of sardine and the time series of anchovy catches separately.

DFA models were applied using the statistical software package Brodgar 2.6 (http://www.brodgar.com/) and following the protocols described in Zuur et al. (2003a, b) and Zuur & Pierce (2004). The GAMMs were developed in R, using the package 'mgcv' (Wood, 2006) and following the methodological approach described in Pinheiro & Bates (2000). The analyses presented involve fitting a large number of models with a large number of explanatory variables. To avoid ascribing significance to coincidental relationships, we used $P < 0.001$ to indicate significance rather than $P < 0.05$, reducing the likely frequency of type one errors from 1 in every 20 comparisons to 1 in 1,000 comparisons (Abdi, 2007).

Results

The anchovy/sardine complex and teleconnection indices

Six teleconnection indices are highlighted as statistically significant in all the DFA models: WASMI, EA-Jet, NAO, POL, PNA and EA-WR (Table 4). The importance of fishing effort decreases as the time lag increases from 0 to 2 years whereas the opposite is true for the climatic indices. WASMI is statistically significant in the DFA models for the anchovy/sardine ratio and for sardine catches, especially in the Ionian Sea but also in the north-east Aegean; in all cases the regression

Table 4 DFA models between one of the biological parameters (catch ratio, anchovy or sardine catches per area) and teleconnection indices. Statistically significant variables are indicated with an “x”

	Ratio	Anchovy	Sardine
Lag 0			
FE MAF 1	×	×	×
FE MAF 2	×	×	×
FE MAF 3			
FE MAF 4	×	×	×
FE MAF 5		×	×
EA-WR	×		
Lag 1			
FE MAF 3	×		
FE MAF 4	×		
FE MAF 5	×		
WASMI	×		×
EA-Jet	×	×	×
NAO	×		
PNA	×	×	
POL			×
Lag 2			
WASMI	×		×
EA-Jet	×		
EA-WR	×		

Fishing effort trends were also used as explanatory variables. Three models were applied for each biological parameter at time lags 0, 1 and 2 between the response and explanatory variables

coefficients are positive. EA-Jet is also statistically significant for both species and their ratio, especially in the north-east Aegean where the relationships are negative. The significant regression coefficients for the DFA models at a time lag of 1 year are shown in Fig. 4 and for a 2-year time lag in Fig. 5. EA-WR and the anchovy/sardine ratio are statistically significantly related at lag 0 in areas 6 and 18 and at lag 2 in the north part of the Aegean. PNA is statistically significant related with the anchovy/sardine ratio in the northeast Aegean at lag of 1 year.

Local oceanic/atmospheric variability and teleconnection indices

In the DFA models where parameters describing local environmental conditions were used as response variables, the relationships between WASMI and SST, and EA-Jet and SSH are significant at all time lags (Fig. 6). Statistically significant relationships include: NAO with wind stress; SST with NAO, EA-Jet and WASMI; EA-Jet and SSH at all time lags; and SSH with POL.

Anchovy/sardine complex and local oceanic/atmospheric variability

At 0 lag, the common trends in fishing effort are the significant explanatory variables present in the majority of the models of fish catches (Table 5). At lag 1, SST, SSH and MWS also show statistically significant correlations (Table 6). At lag 0, SST has a statistically significant effect for area 5 in the Ionian Sea.

The shape of the statistically significant relationships of SST at lag 1 with anchovy, sardine and their ratio are shown in Fig. 7. Although extremely high temperatures create unfavourable conditions for both species, anchovy seems to be doing better than sardine under these adverse conditions. A weak dome-shaped relationship between SST at lag 1 and sardine catches is observed in the north Aegean.

Sardine catches in areas 10 and 8 are related with MWS; sardine catches decrease when MWS increases above a level of 5 N/m^2 (Fig. 8). Anchovy catches in the central Aegean show a U-shaped relationship with SSH (Fig. 8).

Discussion

Inter-decadal cycles of the alternate dominance of anchovy and sardine have been attributed to ocean temperatures, productivity of coastal and open sea ecosystems and climatic variability (Schwartzlose et al., 1999; Chavez et al., 2003; Valdés et al., 2008). In the Mediterranean, a combination of hydrological features that enhance productivity and retain fish eggs and larvae, the ‘ocean triads’, seems to be crucial for successful recruitment, especially of small pelagic fish (Agostini & Bakun, 2002; Santos et al., 2004; Lafuente et al., 2005). In this study, we linked climate-induced oceanic variability with fluctuations of the anchovy/sardine complex in the northeastern Mediterranean.

Most of the correlations between the environmental parameters and fishery catches, observed in this study, are enhanced after hysteresis (i.e. time-lagged effects) has been introduced into the models, reinforcing the

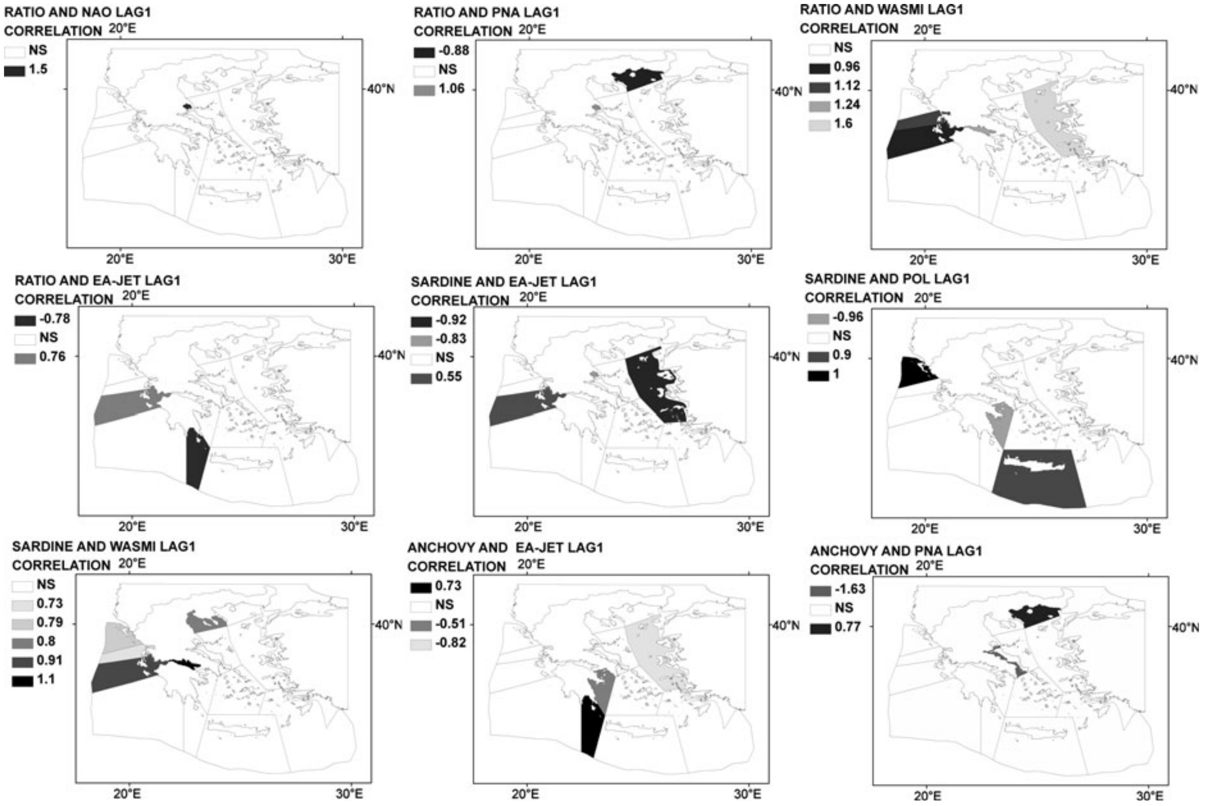
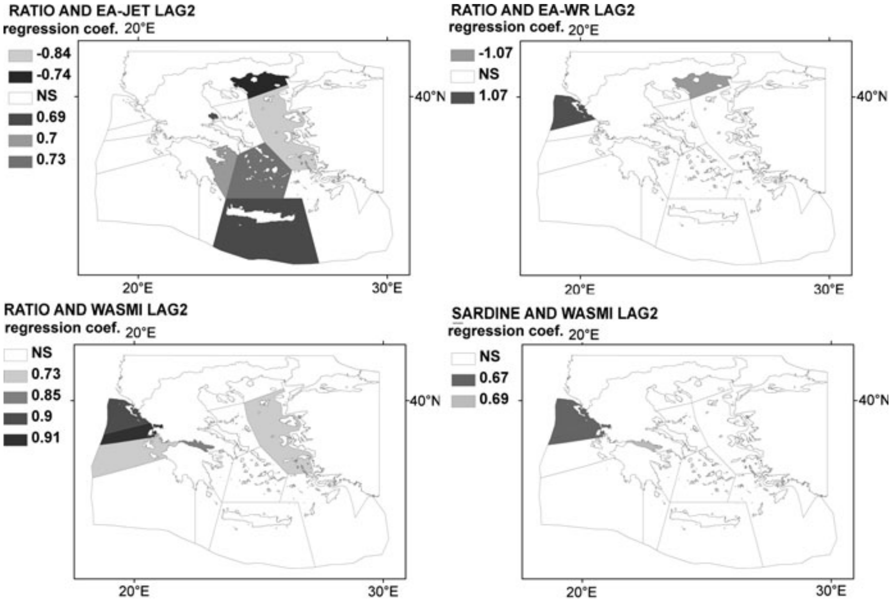


Fig. 4 Regression coefficients derived from the DFA models relating sardine and anchovy catches (response variables) with teleconnection indices at lag 1 (explanatory variables). *NS* non significant

Fig. 5 Regression coefficients derived from the DFA models relating sardine and anchovy catches (response variables) with teleconnection indices at lag 2 (explanatory variables). *NS* non significant



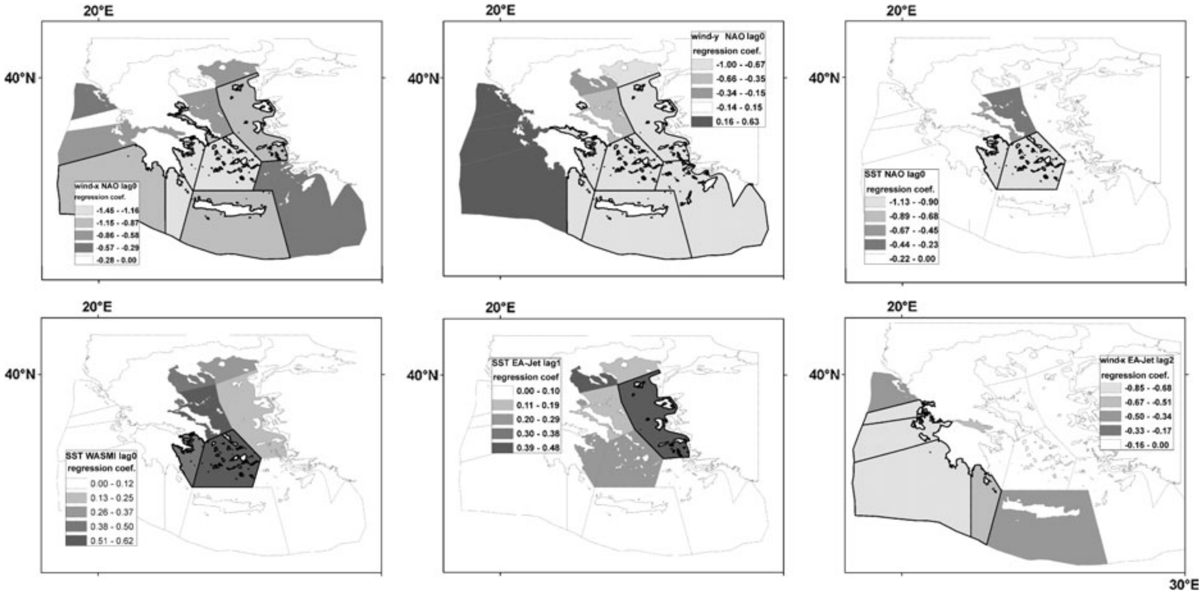


Fig. 6 Regression coefficients for relationships of local environmental parameters (response variable) with teleconnection indices (explanatory variables). The statistically significantly correlated areas (99% confidence) are highlighted with a *black border*

Table 5 Final GAMMs for the different areas at lag 0

Ionion
- Ratio (normalised) ~ FE4***
- Pilchard (normalised) ~ SST
- Anchovy ~ SST(by region)*

North Aegean
- Ratio (normalised) ~ FE3(by region)** + FE5(by region)*** + FE2*** + SST
- Pilchard (normalised) ~ FE2(by region)*** + FE5(by region)*** + FE4(by region) + MWS
- Anchovy(normalised) ~ FE2(by region)*** + SST

Central Aegean
- Ratio (normalised) ~ FE4(by region)*** + SST
- Pilchard (normalised) ~ FE4(by region)*** + SST
- Anchovy (normalised) ~ FE3(by region)***

Cretan Arc
- Ratio (normalised) ~ FE3*** + SST
- Pilchard ~ SST
- Anchovy (normalised) ~ FE2(by region)*** + FE4(by region)

FE *fishing effort* and the number for the MAF, *ZWS* zonal wind stress, *MWS* meridional wind stress. 'by region' indicates that the relationship with the explanatory variable is different for each area, i.e. an interaction between the explanatory variable and the 'area' treated as a factor. (Significance codes: 0 '***', 0.001 '**', 0.5 '*')

hypothesis that environmental impacts on small pelagic fish are mainly felt through recruitment or growth with the subsequent effects this may have on population dynamics. Some relationships become apparent with a time lag of 2 years. Effects on egg production, hatching success and growth and survival of early life stages would be expected to impact on the fished populations of sardine and anchovy with a lag of one or more years. In the northeast Atlantic, sardine (*S. pilchardus*) and anchovy (*E. encrasicolus*) typically recruit to the fishery at age one (e.g. ICES, 2010). Such effects can be additive if the same or other forcing factors persist for consecutive periods. Different theories such as of changes in the migratory behaviour of the species (*S. pilchardus*: Muzinic, 1963; Škrivanić & Zavodnik, 1973), interactions between anchovy and sardine such as the 'school trap' mechanism and trophic relations (Miller & Schneider, 2000 on *S. sagax*; Cubillos & Arcos, 2002 on *E. ringens* and *Strangomera betincki*), differences in the adaptations of the species to adverse conditions (Irigoien et al., 2007 on *E. encrasicolus*) or density-dependent effects (Shepherd & Cushing, 1980; Voulgaridou & Stergiou, 2003 on *S. pilchardus*) are discussed as possible explanations for the relationships that arise from our results.

Table 6 Final GAMMs for the different areas at lag 1

Ionion	
Ratio (normalised)	~ SST
Pilchard (normalised)	~ SST
Anchovy	~ SST*
North Aegean	
Ratio (normalised)	~ FE5(by region)** + SST*
Pilchard (normalised)	~ FE2(by region)*** + FE5(by region)*** + FE4*** + SST**
Anchovy(normalised)	~ FE2(by region)*** + SST*
Central Aegean	
Ratio (normalised)	~ FE2(by region)**
Pilchard	~ FE1(by region)*** + MWS(by region)***
Anchovy (normalised)	~ FE1 + SSH** + SST
Cretan arc	
Ratio (normalised)	~ FE3(by region)*** + SST
Pilchard (normalised)	~ SST*
Anchovy (normalised)	~ FE2(by region)*** + SST

FE *fishing effort* and the number for the MAF, *ZWS* zonal wind stress, *MWS* meridional wind stress. 'by region' indicates that the relationship with the explanatory variable is different for each area i.e. an interaction between the explanatory variable and the 'area' treated as a factor. (Significance codes: 0 '***', 0.001 '**', 0.5 '*')

Teleconnection patterns and the Anchovy/sardine complex

The role of three teleconnection patterns, namely the East Atlantic jet, West African Summer Monsoon and PNA pattern, as forcing factors for the anchovy/sardine complex was highlighted in this study. These patterns are related to a number of local phenomena describing air-sea interactions in the Mediterranean with the potential to influence anchovy and sardine population dynamics in the area.

The EA-Jet is the third mode of low frequency variability found over the North Atlantic from April to August. One of its anomaly centres is located over Northern Africa and the Mediterranean Sea (NOAA-CPC, 2005) and the EA-Jet index presents inter-decadal variability. Wind variability, cyclone tracks over the Mediterranean, precipitation and chlorophyll concentration at the northern coast of the Sea are associated with the EA-Jet (Barnston & Livezey, 1987 Alpert et al., 1990; Trigo et al., 1999; Dünkeloh & Jacobeit, 2003; Katara et al., 2008).

The African monsoon is associated with dry and hot summers over the Mediterranean (Ziv et al., 2004; Alpert et al., 2006). Intense West African Monsoon effects enhance the meridional Hadley circulation, thus strengthening the north-easterly winds over the eastern Mediterranean (Gaetani et al., 2008, 2009).

PNA is a principal mode of low-frequency variability in the Northern Hemisphere mid-latitudes. It is associated with ENSO episodes and over the western Mediterranean cold ENSO events become apparent as PNA-like variability (Alpert et al, 2006). Thus, its impact could be perceived as a strong ENSO signal over the Mediterranean.

Although the influence of the teleconnection patterns mentioned above on local weather and oceanic circulation in the Mediterranean has already been established, the combination of hypotheses tested in this study allows for a more thorough description of possible physical mechanisms modulating the influence of these teleconnections on the anchovy/sardine complex.

Physical mechanisms

Sea surface temperature, one of the most important oceanographic variables influencing biological indicators appears as a crucial factor affecting sardine and anchovy catch fluctuations and is suggested to be the mediator between the teleconnection patterns and the anchovy/sardine complex. Variability in SST has been related to various oceanic processes such as current advection, direct surface heating, upwelling and changes in mixing (Miller & Schneider, 2000).

The positive relationship between sardine and WASMI, at time lags of 1 and 2 years, might be related with the elevated SST in most of the area during the positive phase of the WASM. Sardine shows a preference for warm and shallow waters (Giannoulaki et al., 2005); it spawns during winter, and its association with warm waters during the summer confers the benefit of increased growth rate (Ursin, 1979).

The most plausible mechanism, through which teleconnection indices can influence the anchovy/sardine complex in the Mediterranean, seems to be climate-induced variability of oceanic features that interrupt the oligotrophic regime dominating this area. These features, mainly upwelling and gyres are characterised by cold nutrient-rich waters that reach the sea surface through wind-induced mixing. This

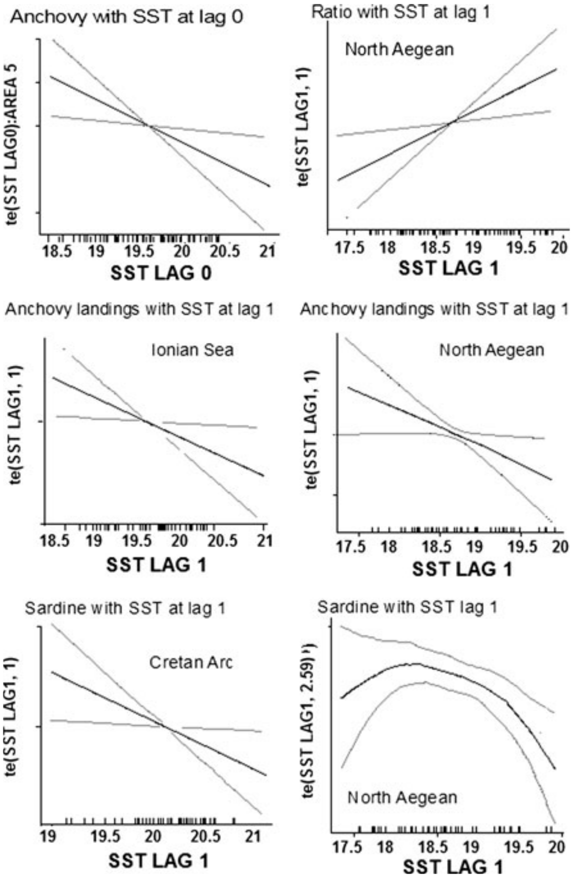


Fig. 7 A negative relationship of anchovy catches (response variable) and SST at lag 0 is observed for area 5, also between anchovy catches (response variable) and SST at lag 1 in the Ionian and north Aegean seas and between SST and sardine catches (response variable) for the Cretan arc. A weak dome-shaped relationship between sardine catches (response variable) and SST is observed for the north Aegean. The anchovy/sardine ratio time series (response variable) has a positive linear relationship with SST

relationship is manifested in various forms and areas for both study species.

Anchovy and sardine catches are both negatively correlated with SST at a time lag of 1 year. The relationship is the same for anchovy in the Aegean and the Ionian; although the two seas are inhabited by two different populations (Kristoffersen & Magoulas, 2008). In agreement with the 'oscillating control hypothesis' (Hunt et al., 2002), 'cold' climate regimes have been associated in the Black sea with systems controlled by small planktivorous fish that thrive under this regime due to the climate-induced increase in nutrient enrichment of the surface sea layers (Oguz et al., 2006; Oguz & Gilbert, 2007).

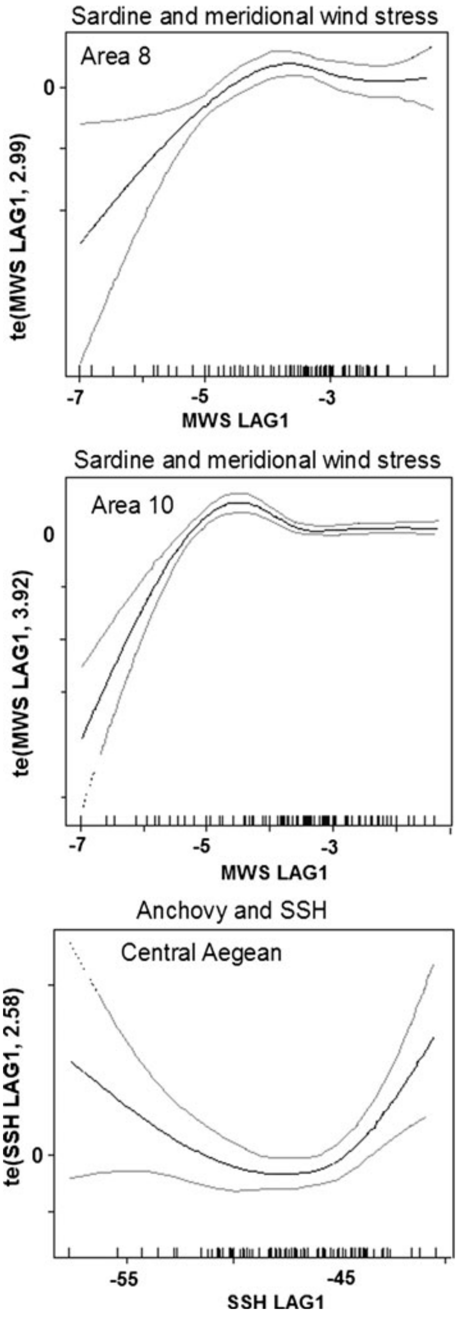


Fig. 8 Non- linear relationships are observed between sardine catches (response variable) and meridional wind stress at lag 1 in areas 8 and 10. Anchovy catches (response variable) are related to Sea Surface Height at lag 1, with a U-shaped relationship, in the central Aegean

As for a physical mechanism through which PNA influences anchovy and sardine production in the Thracian Sea, it seems to be related with gyres retaining nutrient-rich and cold Black Sea water; these oceanic features constitute auspicious spawning grounds (Somarakis et al., 2002, Giannoulaki et al., 2005). The resolution of the data used did not allow for a thorough investigation of such a link but the observed relationships of the species with PNA and SST do support this hypothesis.

Along the east coast of the Aegean, a physical mechanism linking both species with EA-Jet through SST is revealed. This area is dominated by strong upwelling that have been suggested to be a part of an 'ocean triad' affecting small pelagic fish recruitment in the Aegean (Bakun & Agostini, 2001; Schismenou et al., 2008). WASMI also appears as an important climatic forcing in the area at lags of 1 and 2 years favouring anchovy. Although a physical mechanism is not apparent from the results, the effect of WASMI on MWS and the impact of wind-induced upwelling in the area are known from previous studies and could constitute a tenable process linking atmospheric to biological variability.

The positive effects of WASMI and EA-Jet on sardine and the anchovy/sardine ratio are most profound in the Ionian Sea. The main hydrographical feature of the area is the LIW, which has been shown to influence productivity and species distribution in the Adriatic and has been related with climate oscillations (Grbec et al., 2002).

Non-linear effects of local environmental variability

The recognition of non-linear relationships between species abundance and physical characteristics of their environment has been suggested as a step forward to improve our understanding of the processes behind climatic impacts on ecosystems (Ottersen et al., 2010). Such relationships were observed in this study for a number of local parameters and provide an insight into different aspects of the impact of the environment on anchovy and sardine.

The shape of the relationship of sardine abundance with southerly winds at a time lag of a year, at two of its important spawning grounds in the central Aegean, is in agreement with other studies that have suggested that low to medium wind forcing is advantageous for recruitment of small pelagic fish (Bay of Biscay, Borja et al., 1998). A possible reason is that intense mixing could prevent the development of phytoplankton blooms (Bakun & Agostini, 2001) or hinder the feeding activity of larvae and juveniles (Mackenzie, 2000). A preference of sardine for an enriched but stable environment has also been suggested by Cury & Roy (1989, for *S. sagax*) and Bakun & Parrish (1990, for *Sardinella aurita*). On the other hand, anchovy in the central Aegean prefers extreme values for SSH (i.e. abundance is lowest around the mean value of SSH), indicative of changes in the mesoscale circulation patterns in the area, increased turbulence and nutrient enrichment of the surface layers. Tsagarakis et al. (2008) also found important relationships between sea level anomalies and distribution of juvenile sardines in the Aegean. Our results also agree with the findings of Skogen (2005), who found a positive relationship of anchovy (*S. sagax*) recruits with productivity-enhancing oceanic processes and an optimal environmental window for sardine in the Benguela upwelling. In contrast, both Allain et al. (2001) and Uriarte et al. (2002) showed a negative correlation between wind-induced stratification disruption events and anchovy (*E. encrasicolus*) recruitment levels in the Bay of Biscay. Roy et al. (1992) and Roy (1993) suggest a dome-shaped relationship between upwelling strength and anchovy (*Engraulis mordax*) recruitment. The differences in our results could be attributed to the oligotrophic nature of the Mediterranean, where oceanic processes, which increase primary productivity such as the northwest Aegean upwelling, the Rhodes Gyre, the east Aegean fronts etc, are crucial for the survival of the ecosystems. Therefore, the Mediterranean might only show features on the 'inclining' arch of relationship.

Different responses of the two species and implications

Interactions between the two study species, differences in their preferences and adaptations towards environmental change and possible migratory movements as response to climatic variability are phenomena that add to the complexity of the interactions of these fish species with their environment. Such implications also arise in this study and are interpreted with reference to integrative hypotheses that combine biological interactions and migrations with environmental forcing (Bakun, 2009).

Anchovy and sardine have a negative relationship with temperature in the North Aegean. However, the anchovy/sardine ratio in this area is positively associated with temperature indicating that anchovy might be able to find a spatial or temporal 'loophole' and outperform sardine under unfavourable conditions. A similar mechanism has been suggested for anchovy (*E. ringens*) off Peru, which is able to exploit small-scale spatiotemporal 'loopholes' during short-term El Niño events (Bertrand et al., 2004) and for the anchovy (*E. encrasicolus*) population in the Bay of Biscay, where anchovy is taking advantage of lower predation in offshore waters (Irigoien et al., 2007).

In the Thracian sea (area 14), although anchovy abundance is positively correlated with PNA at lag 1, the anchovy/sardine ratio is negatively related with the same index at lag 1 suggesting an indirect effect on sardine such as a biological mechanism of interaction between the two species (*Strangomera benthincki* and *E. ringens*: Cubillos & Arcos, 2002; Pedraza-Garcia & Cubillos, 2008). A similar mechanism can be suggested for the WASMI and EA-Jet effects on the anchovy/sardine complex in the Ionian Sea. Both sardine catches and the anchovy/sardine ratio are positively correlated to the two aforementioned teleconnection indices, indicating indirect effects on anchovy through interactions of the two species. Such mechanisms pertain to interactions between species in mixed schools; when climate favours the growth of one species, another species that schools with it might be disadvantaged.

The interpretation of the results for the northern Aegean becomes more complicated, if we consider the possibility of inflow of recruits from the Black sea as observed for anchovy (Mantzouni et al., 2007). Such enrichment in recruits could counteract negative effects of the environment on the recruitment of the resident population and obscure environmental relationships in quantitative analyses. Furthermore, this phenomenon impedes our effort to disentangle environmental effects on fisheries productivity in the area as any correlation observed can be attributed either to local oceanic variation or input from the Black Sea.

The anchovy/sardine ratio in the central Aegean is positively related with EA-Jet at a lag of 2 years whereas anchovy is negatively related with EA-Jet at a lag of 1 year in Saronikos Bay (area 8). At the same time, in the area west of Crete, an area dominated by the west Cretan gyre (Robinson & Golnaraghi, 1993), the correlation sign for anchovy and EA-Jet at lag 1 is reversed and a positive relationship between anchovy and EA-Jet at lag 1 is observed. A definite mechanism for the impact of this teleconnection pattern in the area cannot be deduced from the results. It is however obvious that both species are affected by EA-Jet-related variability in the west Cretan Gyre. The complexity of the results might be a manifestation of climate-related changes in migrations of the two species to more favourable areas when the conditions become adverse or due to higher levels of competition or predation when primary productivity increases.

Conclusions

There are a number of issues that might blur our perception of the mechanistic links between climatic variation and the anchovy/sardine complex. The over-exploited state of the stocks might not allow solid conclusions about the impact of the environment on population dynamics of the two species (Daskalov, 2003). Also the relationship between abundance and catches might be clear for anchovy but less so for sardine because anchovy is the target species for the Mediterranean fleets (Abad et al., 1998; Stergiou & Lascaratos, 1997). Long time series and better spatiotemporal resolution of biological indicators are needed for an in-depth investigation of the possible mechanisms of climate-biological relationships for the small pelagic fish studied here. However, it becomes apparent that such a relationship does exist and could potentially assist in improving our predictions, and therefore management for anchovy and sardine in the Greek Seas. Some aspects of this relationship are revealed in this study and could provide guidance for the finer-scale studies that are proving to be essential in such a variable environment as the Mediterranean. Our results highlight the role of productivity-enhancing oceanic features as the physical link between atmospheric and biological variability and stress the implications of non-linear relationships, interactions between species and migrations for our interpretation of biological-environmental relationships for the anchovy/sardine complex.

Acknowledgments I. Katara was funded by the 'ECO-SUMMER'' Marie Curie training site (MEST-CT-2005-020501).

G.J. Pierce was supported by the ANIMATE project (MEXC-CT-2006-042337). Authors thank the German Aerospace Agency for the distribution of AVHRR data, the IRI/LDEO Climate Data Library for providing databases with the Carton-Giese SODA data and NOAA/Climate Prediction Centre for maintaining a database of the major teleconnection indices. Authors would also like to thank the reviewers of this article for their valuable comments.

References

Abad, R., J. Miquel, M. Iglesias & F. Alvarez, 1998. Acoustic estimation of abundance and distribution of anchovy in the NW Mediterranean. Scientia Marina 62: 37–43.

Abdi, H., 2007. Bonferroni and Šidák corrections for multiple comparisons. In Salkind, N. J. (ed.), Encyclopedia of measurement and statistics. Sage, Thousand Oaks [available on internet at http://www.utdallas.edu/~herve/Abdi-Bonferroni2007-pretty.pdf].

Agostini, V. & A. Bakun, 2002. ''Ocean triads'' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). Fisheries Oceanography 11: 129–142.

Alheit, J. & A. Bakun, 2009. Population synchronies within and between ocean basins: apparent teleconnections and implications as to physical-biological linkage mechanisms. Journal of Marine Systems. doi:10.1016/j.jmarsys.2008.11.029.

Alheit, J. & E. Hagen, 1997. Long-term climate forcing of European herring and sardine populations. Fisheries Oceanography 6: 130–139.

Alheit, J. & M. Niquen, 2004. Regime shifts in the Humboldt Current ecosystem. Progress in Oceanography 60: 201–222.

Allain, G., P. Petitgas & P. Lazure, 2001. The influence of mesoscale ocean processes on anchovy (*Engraulis encrasicolus*) recruitment in the Bay of Biscay, estimated with a three-dimensional hydrodynamic model. Fisheries Oceanography 10: 151–163.

Alpert, P., B. U. Neeman & Y. Shay-El, 1990. Intermonthly variability of cyclone tracks in the Mediterranean. Journal of Climate 3: 1474–1478.

Alpert, P., M. Baldi, R. Ilani, S. Krichak, C. Price, X. Rodo, H. Saaroni, B. Ziv, P. Kishcha & J. Barkan, 2006. Relations between climate variability in the Mediterranean region and the tropics: ENSO, South Asian and African monsoons, hurricanes and Saharan dust, Chapt. 2 In Lionello, P., P. Malanotte-Rizzoli & R. Boscolo (eds), Mediterranean Climate Variability. Elsevier, Amsterdam: 149–177.

Bakun, A. 2009. Linking climate to population variability in marine ecosystems characterized by non-simple dynamics: conceptual templates and schematic constructs. Journal of Marine Systems. doi:10.1016/j.jmarsys.2008.12.008.

Bakun, A. & V. N. Agostini, 2001. Seasonal patterns of wind-driven upwelling/ downwelling in the Mediterranean Sea. Scientia Marina 65: 243–257.

Bakun, A. & R. H. Parrish, 1990. Comparative studies of coastal pelagic fish reproductive habitats: the Brazilian sardine (*Sardinella aurita*). ICES Journal of Marine Science 46: 269–283.

Barnston, A. G. & R. E. Livezey, 1987. Classification, seasonality, and persistence of low-frequency atmosphere circulation patterns. Monthly Weather Review 115: 1083–1123.

Bertrand, A., M. Segura, M. Gutiérrez & L. Vásquez, 2004. From small-scale habitat loopholes to decadal cycles: a habitat-based hypothesis explaining fluctuation in pelagic fish populations off Peru. Fish and Fisheries 5: 296–316.

Borja, A., A. Uriarte, J. Egaña, L. Motos & V. Valencia, 1998. Relationships between anchovy (*Engraulis encrasicolus*) recruitment and environment in the Bay of Biscay (1967–1996). Fisheries Oceanography 7: 375–380.

Carton, J. A. & B. S. Giese, 2008. A reanalysis of ocean climate using Simple Ocean Data Assimilation (SODA). Monthly Weather Review 136: 2999–3017.

Chavez, F. P., J. Ryan, S. E. Lluch-Cota & C. M. Ñiquen, 2003. From anchovies to sardines and back: multidecadal change in the Pacific Ocean. Science 299: 217–221.

Cubillos, L. A. & D. F. Arcos, 2002. Recruitment of common sardine (*Strangomera bentincki*) and anchovy (*Engraulis ringens*) in the 1990s, and impact of the 1997–98 El Niño. Aquatic Living Resources 15: 87–94.

Cury, P. & C. Roy, 1989. Optimal environmental window and pelagic fish recruitment success in upwelling areas. Canadian Journal of Fisheries and Aquatic Sciences 46: 670–680.

Daskalov, G. M., 2003. Long-term changes in fish abundance and environmental indices in the Black Sea. Marine Ecology Progress Series 255: 259–270.

Di Lorenzo, E., N. Schneider, K. M. Cobb, K. Chhak, P. J. S. Franks, A. J. Miller, J. C. McWilliams, S. J. Bograd, H. Arango, E. Curchister, T. M. Powell & P. Rivere, 2008. North Pacific Gyre Oscillation links ocean climate and ecosystem change. Geophysical Research Letters 35: L08607.

Dünkeloh, A. & J. Jacobeit, 2003. Circulation dynamics of mediterranean precipitation variability 1948–98. International Journal of Climatology 23: 1843–1866.

Gaetani, M., M. Baldi & G. A. Dalu, 2008. Influence of the West African Monsoon on the Summer Mediterranean Climate. EMS 8th Annual Meeting. Amsterdam, The Netherlands.

Gaetani, M., M. Baldi & G. A. Dalu, 2009. Mutual interaction between the West African Monsoon and the summer Mediterranean climate. Geophysical Research Abstracts 11: EGU2009-8443.

Giannoulaki, M., A. Machias, S. Somarakis & N. Tsimenides, 2005. The spatial distribution of anchovy and sardine in the northern Aegean Sea in relation to hydrographic regimes. Belgian Journal of Zoology 135: 151–156.

Grbec, B., J. Dulcic & M. Morovi, 2002. Long-term changes in landings of small pelagic fish in the eastern Adriatic-possible influence of climate oscillations over the Northern Hemisphere. Climate Research 20: 241–252.

Heath, M. R., 1992. Field investigations of the early life stages of marine fish. Advances in Marine Biology 28: 1–174.

Huang, J.-J., G.-H. Tzeng & C.-S. Ong, 2006. A novel algorithm for dynamic factor analysis. Applied Mathematics and Computation 175: 1288–1297.

Hunt, G. L. Jr., P. J. Stabeno, G. Walters, E. Sinclair, R. D. Brodeur, J. M. Napp & N. A. Bond, 2002. Climate change and control of the southeastern Bering Sea pelagic ecosystem. Deep-Sea Research II 49(26): 5821–5853.

ICES, 2010. Report of the Working Group on Anchovy and Sardine (WGANSA), 24–28 June 2010, Lisbon, Portugal: 285 pp.

Irigoien, X., Ø. Fiksen, U. Cotano, A. Uriarte, P. Alvarez, H. Arrizabalaga, G. Boyra, M. Santos, Y. Sagarminaga, P. Otheguy, E. Etxebeste, L. Zarauz, I. Artetxe & L. Motos, 2007. Could Biscay Bay Anchovy recruit through a spatial loophole? Progress in Oceanography 74: 132–148.

Katara, I., J. Illian, G. J. Pierce, B. E. Scott & J. Wang, 2008. Atmospheric forcing on chlorophyll concentration in the Mediterranean. Hydrobiologia 612: 33–48.

Kawasaki, T., 1983. Why do some pelagic fishes have wide fluctuations in their numbers? Biological basis of fluctuation from the viewpoint of evolutionary ecology. FAO Fisheries Report 291: 1065–1080.

Kristoffersen, J. B. & A. Magoulas, 2008. Population structure of anchovy *Engraulis encrasicolus* L. in the Mediterranean Sea inferred from multiple methods. Fisheries Research 91: 187–195.

Lafuente, J. G., J. M. Vargas, F. Criado, A. Garcia, J. Delgado & S. Mazzola, 2005. Assessing the variability of hydrographic processes influencing the life cycle of the Sicilian Channel anchovy, *Engraulis encrasicolus*, by satellite imagery. Fisheries Oceanography 14: 32–46.

Lehodey, P., J. Alheit, M. Barange, T. Baumgartner, G. Beaugrand, K. Drinkwater, J. M. Fromentin, S. R. Hare, G. Ottersen, R. I. Perry, C. Roy, C. D. van der Lingen & F. Werner, 2006. Climate variability, fish, and fisheries. Journal of Climate 19: 5009–5030.

Lett, C., P. Penven, P. Ayón & P. Fréon, 2007. Enrichment, concentration and retention processes in relation to anchovy (*Engraulis ringens*) eggs and larvae distributions in the northern Humboldt upwelling ecosystem. Journal of Marine Systems 64: 189–200.

Li, J. & Q. Zeng, 2005. A new monsoon index, its interannual variability and relation with monsoon precipitation. Climatic and Environmental Research 10: 351–365.

Lluch-Belda, D., R. A. Schwartzlose, R. Serra, R. Parrish, T. Kawasaki, D. Hedgecock & R. J. M. Crawford, 1992. Sardine and anchovy regime fluctuations of abundance in four regions of the world oceans: a workshop report. Fisheries Oceanography 1: 339–347.

MacKenzie, B. R., 2000. Turbulence, larval fish ecology and fisheries recruitment: a review of field studies. Oceanologica Acta 23: 357–375.

Mantzouni, I., S. Somarakis, D. K. Moutopoulos, A. Kallianiotis & C. Koutsikopoulos, 2007. Periodic, spatially structured matrix model for the study of anchovy (*Engraulis encrasicolus*) population dynamics in N Aegean Sea (E Mediterranean). Ecological Modelling 208: 367–377.

Miller, A. J. & N. Schneider, 2000. Interdecadal climate regime dynamics in the North Pacific Ocean: theories, observations and ecosystem impacts. Progress in Oceanography 4: 355–379.

Molenaar, P. C. M., 1985. A dynamic factor model for the analysis of multivariate time series. Psychometrica 50: 181–202.

Muzinic, R., 1963. Further observations on schooling and aggregating behaviour of sardines (*Sardina pilchardus* Walb.). FAO 1964. In Proc. Gen. Fish. Coun. Medit. no 7, Technical Paper no 31: 319–323.

NOAA-CPC, 2005. East Atlantic Jet Teleconnection Pattern. NOAA/NWS/CPC Indices and Forecasts. NOAA National Weather Service, Center for Climate Prediction, Camp Springs [available on internet at http://www.cpc.ncep.noaa.gov/data/teledoc/eajet.html].

Oguz, T. & D. Gilbert, 2007. Abrupt transitions of the top-down controlled Black Sea pelagic ecosystem during 1960–2000: evidence for regime-shifts under strong fishery exploitation and nutrient enrichment modulated by climate-induced variations. Deep Sea Research I 54: 220–242.

Oguz, T., J. W. Dippner & Z. Kaymaz, 2006. Climatic regulation of the Black Sea hydro-meteorological and ecological properties at interannual-to-decadal time scales. Journal of Marine Systems 60: 235–254.

Ottersen, G., S. Kim, G. Huse, J. J. Polovina & N. C. Stenseth, 2010. Major pathways by which climate may force marine fish populations. Journal of Marine Systems 79: 343–360.

Parsons, L. S. & W. H. Lear, 2001. Climate variability and marine ecosystem impacts: a north Atlantic perspective. Progress in Oceanography 49: 167–188.

Pedraza-Garcia, M. & L. A. Cubillos, 2008. Population dynamics of two small pelagic fish in the central-south area off Chile: delayed density-dependence and biological interaction. Environmental Biology of Fishes 82: 111–122.

Pierce, G. J., V. D. Valavanis, A. Guerra, P. Jereb, L. Orsi-Relini, J. M. Bellido, I. Katara, U. Piatkowski, J. Pereira, E. Balguerias, I. Sobrino, E. Lefkaditou, J. Wang, M. Santurtun, P. R. Boyle, L. C. Hastie, C. D. MacLeod, J. M. Smith, M. Viana, A. F. González & A. F. Zuur, 2008. A review of cephalopod-environment interactions in European Seas and other world areas. Hydrobiologia 612: 49–70.

Pinheiro, J. C. & D. M. Bates, 2000. Mixed Effects Models in S and S-PLUS. Springer, Berlin.

Robinson, A. R. & M. Golnaraghi, 1993. Circulation and dynamics of the Eastern Mediterranean Sea; quasi-synoptic data-driven simulations. Deep Sea Research 40: 1207–1246.

Rodhouse, P. G. 2009. Response of cephalopod populations to large-scale environmental variability. In Cephalopod International Advisory Council Symposium (CIAC09).

Roy, C., 1993. The optimal environmental window hypotheses: a non linear environmental process affecting recruitment success. ICES Journal of Marine Science 76: 1–13.

Roy, C., P. Cury & S. Kifani, 1992. Pelagic fish recruitment success and reproductive strategy in upwelling areas: environmental compromises. South African Journal of Marine Science 12: 135–146.

Santos, A. M. P., A. Peliz, J. Dubert, P. B. Oliveira, M. M. Angélic & P. Ré, 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. Continental Shelf Research 24: 149–165.

Schismenou, E., M. Giannoulaki, V. D. Valavanis & S. Somarakis, 2008. Modeling and predicting potential spawning habitat of anchovy (*Engraulis encrasicolus*) and round sardinella (*Sardinella aurita*) based on satellite environmental information. Hydrobiologia 612: 201–214.

Schwartzlose, R. A., J. Alheit, A. Bakun, T. R. Baumgartner, R. Cloete, R. J. M. Crawford, W. J. Fletcher, Y. Green-Ruiz, E. Hagen, T. Kawasaki, D. Lluch-Belda, S. E. Lluch-Cota, A. D. MacCall, Y. Matsuura, M. O. Nevárez-Martínez, R. H. Parrish, C. Roy, R. Serra, K.

V. Shust, M. N. Ward & J. Z. Zuzunaga, 1999. Worldwide large-scale fluctuations of sardine and anchovy populations. South African Journal of Marine Science 21: 289–347.

Schwing, F. B., R. Mendelssohn, S. J. Bograd, J. E. Overland, M. Wang & S. Ito, 2010. Climate change, teleconnection patterns, and regional processes forcing marine populations in the Pacific. Journal of Marine Systems 79: 245–257.

Shepherd, J. G. & D. H. Cushing, 1980. A mechanism for density-dependent survival of larval fish as the basis of a stock-recruitment relationship. ICES Journal of Marine Science 39: 160–167.

Skogen, M. D., 2005. Clupeoid larval growth and plankton production in the Benguela upwelling system. Fisheries Oceanography 14: 64–70.

Škrivanić, A. & D. Zavodnik, 1973. Migration of the sardine (*Sardina pilchardus*) in relation to hydrographical conditions in the Adriatic Sea. Netherlands Journal of Sea Research 7: 7–18.

Solow, A. R., 1994. Detecting change in the composition of a multispecies community. Biometrics 50: 556–565.

Somarakis, S. & N. Nikolioudakis, 2007. Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolous*) in the northern Aegean Sea (eastern Mediterranean). Marine Biology 152: 1143–1158.

Somarakis, S., P. Drakopoulos & V. Filippou, 2002. Distribution and abundance of larval fishes in the northern Aegean Sea-eastern Mediterranean- in relation to early summer oceanographic conditions. Journal of Plankton Research 24: 339–357.

Stergiou, K. I., 1991. Describing and forecasting the sardine-anchovy complex in the eastern Mediterranean using vector autoregressions. Fisheries Research 11: 127–141.

Stergiou, I. K. & A. Lascaratos, 1997. Climatic variability and the anchovy/sardine ratio in Hellenic waters. GeoJournal 41.3: 245–254.

Stergiou, K. I., E. D. Christou, D. Georgopoulos, A. Zenetos & C. Souvermezoglou, 1997. The Hellenic Seas: physics, chemistry, biology and fisheries. Oceanography Marine Biology Annual Review 35: 415–538.

Swartzman, G., A. Bertrand, M. Gutiırrez, S. Bertrand & L. Vasquez, 2008. The relationship of anchovy and sardine to water masses in the Peruvian Humboldt Current System from 1983 to 2005. Progress in Oceanography 79: 228–237.

Takasuka, A., Y. Oozeki, H. Kubota & S. E. Lluch-Cota, 2008. Contrasting spawning temperature optima: why are anchovy and sardine regime shifts synchronous across the North Pacific? Progress in Oceanography 77: 225–232.

Trigo, I. G., T. D. Davies & G. R. Bigg, 1999. Objective climatology of cyclones in the Mediterranean Region. Journal of Climate 12: 1685–1696.

Tsagarakis, K., A. Machias, S. Somarakis, M. Giannoulaki, A. Palialexis & V. D. Valavanis, 2008. Habitat discrimination of juvenile sardines in the Aegean Sea using remotely-sensed environmental data. Hydrobiologia 612: 215–223.

Uriarte, A., B. Roel, A. Borja, G. Allain & C. M. O'Brien, 2002. Role of environmental indices in determining the recruitment of the Bay of Biscay anchovy. ICES CM 2002/O:25.

Ursin, E. 1979. Principles of growth in fishes. In Miller, P. J. (ed.) Fish Phenology: Anabolic Adaptiveness in Teleosts. Academic Press, London. Symposia of the Zoological Society of London 44:63–87.

Valdés, J., L. Ortlieb, D. Gutierrez, L. Marinovic, G. Vargas & A. Sifeddine, 2008. 250 Years of sardine and anchovy scale deposition record in Mejillones Bay, northern Chile. Progress in Oceanography, the Northern Humboldt Current System: Ocean Dynamics, Ecosystem Processes, and Fisheries 79: 198–207.

Voulgaridou, P. & K. I. Stergiou, 2003. Long-term trends in various biological parameters of the European sardine, *Sardina pilchardus* (Walbaum, 1792), in the Eastern Mediterranean Sea. Scientia Marina 67: 269–280.

Waluda, C. M., P. N. Trathan & P. G. Rodhouse, 1999. Influence of oceanographic variability on recruitment in the genus *Illex argentinus* (Cephalopoda: Ommastrephidae) fishery in the South Atlantic. Marine Ecology Progress Series 183: 159–167.

Waluda, C. M., P. G. Rodhouse, P. N. Trathan & G. J. Pierce, 2001. Remotely sensed mesoscale oceanography and the distribution of *Illex argentinu*s in the South Atlantic. Fisheries Oceanography 10: 207–216.

Waluda, C. M., P. N. Trathan & P. G. Rodhouse, 2004. Synchronicity in southern hemisphere squid stocks and the influence of the Southern Oscillation. Fisheries Oceanography 13: 255–266.

Wang, B. & Z. Fan, 1999. Choice of South Asian summer monsoon indices. Bulletin of American Meteorological Society 80: 629–638.

Wang, B., R. Wu & K.-M. Lau, 2001. Interannual variability of Asian summer monsoon: Contrast between the Indian and western North Pacific-East Asian monsoons. Journal of Climate 14: 4073–4090.

Webster, P. J. & S. Yang, 1992. Monsoon and ENSO: selectively interactive systems. Quarterly Journal of the Royal Meteorological Society 118: 877–926.

Wood, S. N., 2006. Generalized Additive Models: An Introduction with R. Chapman and Hall/CRC, Boca Raton.

Ziv, B., H. Saaroni & P. Alpert, 2004. The factors governing the summer regime of the eastern Mediterranean. International Journal of Climatology 24: 1859–1871.

Zuur, A. F. & G. J. Pierce, 2004. Common trends in northeast Atlantic squid time series. Journal of Sea Research 52: 57–72.

Zuur, A. F., R. J. Fryer, I. T. Jolliffe, R. Dekker & J. J. Beukema, 2003a. Estimating common trends in multivariate time series using dynamic factor analysis. Environmetrics 14: 665–685.

Zuur, A. F., I. D. Tuck & N. Bailey, 2003b. Dynamic factor analysis to estimate common trends in fisheries time series. Canadian Journal Fish. Aquatic Sciences 60: 542–552.

Hydrobiologia (2011) 670:67–85
DOI 10.1007/s10750-011-0670-z

ECOSYSTEMS AND SUSTAINABILITY

Assessment of the deep water trawl fishery off the Balearic Islands (western Mediterranean): from single to multi-species approach

**Beatriz Guijarro · George Tserpes ·
Joan Moranta · Enric Massutí**

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The bottom trawl fishery developed on the slope off Balearic Islands (western Mediterranean) has been analysed from different sources of information: (i) data obtained during experimental bottom trawl surveys developed annually since 2001; (ii) daily sale bills from the bottom trawl fleet, available since 2000. Considering both hydrographical and geomorphologic conditions, the study area was divided in four geographical sectors. Multivariate techniques were applied to identify assemblages and their main species, and to investigate the influence of environmental variables in the slope communities. Fishery-independent and fishery-dependent indicators were calculated, both at specific and community level, for the assemblages identified. In all cases, they were summarised using the Traffic Light approach. Three assemblages have been identified in the slope trawl fishing grounds off the Balearic Islands: (1) the almost unexploited shelf break, where teleosts predominate; (2) the upper slope, where teleosts still predominate but crustaceans are also very important (with *Nephrops norvegicus* as target species); (3) and the middle slope, where crustaceans predominate (with *Aristeus antennatus* as target species). Depth was the main factor affecting the species composition of the assemblages, although other factors such as area, year and effort level also affect. Indicators estimated from both sets of data suggested an improvement in the state of *N. norvegicus*, although the results suggest the influence of other factors than fishing impact in the state of this resource. For *A. antennatus*, marked differences have been found depending on the data source. In the upper slope, indicators suggested an improvement in the state of this assemblage, which can be related to a decreasing trend found in the fishing effort exerted in this depth range. Indicators from the middle slope showed differences for both sets of data. The characteristics of both data sources and of the species analysed are discussed as responsible of these differences.

Keywords *Nephrops norvegicus* · *Aristeus antennatus* · Assessment · Community · Ecosystem approach · Indicators · Traffic Lights

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

B. Guijarro (✉) · J. Moranta · E. Massutí
Instituto Español de Oceanografía, Centre Oceanogràfic de les Balears, Moll de Ponent s/n, 07015 Palma, Spain
e-mail: beatriz@ba.ieo.es

B. Guijarro · G. Tserpes
Hellenic Centre for Marine Research (HCMR), Institute of Marine Biological Resources, P.O. Box 2214, 71003 Poros, Heraklion, Greece

Introduction

Mediterranean bottom trawl fisheries are multispecies as they are targeting more than 100 demersal species of fish, crustaceans and molluscs, with an important commercial value and abundance (Caddy, 1993; Lleonart & Maynou, 2003). In spite of that monospecific approaches are usually the rule in assessments (e.g. Farrugio et al., 1993; Lleonart & Maynou, 2003). In recent years, there has been a progressive change from the traditional approach of fishery assessment to a new ecosystem approach (Browman & Stergiou, 2004; Pikitch et al., 2004). This new approach is particularly important in the multispecies Mediterranean fishery, as the calculations for a single species are of limited value for management in this type of fisheries (Caddy, 1993).

Within the ecosystem-based approach, there is an increasing need for measuring the impact of fishing on ecosystems and, as a consequence, many indicators targeting various components of ecosystems have been developed and discussed (e.g. Trenkel & Rochet, 2003; Nicholson & Jennings, 2004). One of the ways of using indicators is examining whether the indicator is currently changing (Rochet & Trenkel, 2003). However, the complexity of exploited ecosystems forces to examine multiple indicators to accumulate evidence, raising the question of how to summarise them. One of the ways is the Traffic Light (TL) method. This approach was firstly proposed as a type of precautionary management framework suitable for use in fishery assessment in data-poor situations (Caddy, 1999, 2002), but it can be used to assess the status of all stocks whether rich or poor in data (Halliday et al., 2001). The TL displays time series in such a way that helps to perceive likely transitions of indicators and relationships between variables visually (Caddy et al., 2005). This methodology has been applied for single- and multi-species assessments both in the Atlantic and the Mediterranean (Caddy et al., 2005; DFO, 2005; Ceriola et al., 2007, 2008) and appears to be more precautionary than traditional stock assessment methods (Koeller et al., 2000).

Over the last times, the methods available to be used in stock assessment models have changed from those using only catch, catch-at-age and survey or CPUE data to methods that use every source of data available in a totally integrated framework (Hilborn, 2003). In this sense, TL provides a way of bringing a variety of monitoring results, results from traditional stock assessment models and methods, anecdotal observations and political/economic considerations into management decisions (Koeller et al., 2000). One of the main shortcomings in Mediterranean fishery assessments, which is related to their diversity and complexity, is the lack of suitable data because in spite of the existence of fairly reliable historical data series of landings, data on effort are almost absent (Lleonart & Maynou, 2003). In this sense, experimental bottom trawl surveys are very useful, not only because they avoid factors such as fisheries behaviour which could bias estimates on commercial data, but also because they can provide information from the entire epibenthic community. In this sense, fishery-independent data allow the study of single species (e.g. Abella et al., 1999; Lombarte et al., 2000; Tserpes & Peristeraki, 2002), taxonomic groups (Abelló et al., 2002a; Massutí & Moranta, 2003) and faunal assemblages (Moranta et al., 1998; Massutí & Reñones, 2005; Dimech et al., 2008), constituting an appropriate tool for an ecosystem-based fisheries management.

In the Balearic Islands, assessments of some of the main species exploited by the bottom trawl fishery have been made based on population dynamics (Oliver, 1993; García-Rodríguez & Esteban, 1999), production models (Carbonell & Azevedo, 2003) and regression analysis (Alemany & Alvárez, 2003), all of them have considered the single species as the basic unit of the analysis. Multi-species studies have been performed considering fish and cephalopod assemblages separately (Massutí et al., 1996; Moranta et al., 2000; Quetglas et al., 2000), as well as the entire megafaunal demersal communities (Massutí & Reñones, 2005; Moranta et al., 2008; Ordines & Massutí, 2009).

The aim of this article is to assess the deep water trawl fishery resources off the Balearic Islands from single to multi-species approach. This article has three main objectives: (i) to identify and characterise the species assemblages in the deep water trawl fishing grounds off the Balearic Islands; (ii) to analyse the spatio-temporal trends in abundance and biomass from different taxonomic groups, including the influence of environmental conditions and (iii) to estimate ecological indicators (both at single- and multi-species level) to assess the impact of fishing on these communities.

Materials and methods

Study area

The Balearic Islands delimit two sub-basins in the western Mediterranean, the Balearic sub-basin (BsB) in the north and the Algerian sub-basin (AsB) in the south (Fig. 1). The shelf in the Balearic archipelago is narrow and steep on the northern side, and wider and gentler in the south. Terrigenous-muddy sediments are not abundant due to the absence of river discharges. Sandy-muddy and detrital sediments occur at the shelf-slope break, whereas muddy bottoms of biogenic origin dominate the deeper areas (Acosta et al., 2002). The hydrographic conditions of the Islands have been studied widely, with the Balearic channels described as important passages for the exchange between the cooler, more saline waters of the BsB and the warmer, fresher waters of the AsB (Pinot et al., 2002). The temporal variability in hydrodynamic conditions of the area is mainly conditioned by the Northern Current (NC), which carries waters formed during winter in the Gulf of Lions southwards along the continental slope, and reaches the channel between the Iberian coast and the Islands (BsB) (Pinot et al., 2002; López-Jurado et al., 2008; Monserrat et al., 2008).

The demersal communities of the Balearic Island fishing grounds are exploited by a relatively small bottom trawl fleet (around 50 boats), which performs daily fishing trips. Different levels of effort have been detected around the islands, showing the higher levels in the fishing grounds sited in the south and west of Mallorca (Moranta et al., 2008; Serrano et al., 2008). Four different fishing tactics are developed by this fleet, associated with the shallow and deep continental shelf, and the upper and middle continental slope (Guijarro & Massutí, 2006; Ordines et al., 2006). They are mainly targeting striped red mullet (*Mullus surmuletus*), European hake (*Merluccius merluccius*), Norway lobster (*Nephrops norvegicus*) and red shrimp (*Aristeus antennatus*), respectively. Taking into account both environmental and fishing effort variability, four different areas have been considered during this study (Fig. 1; Table 1).

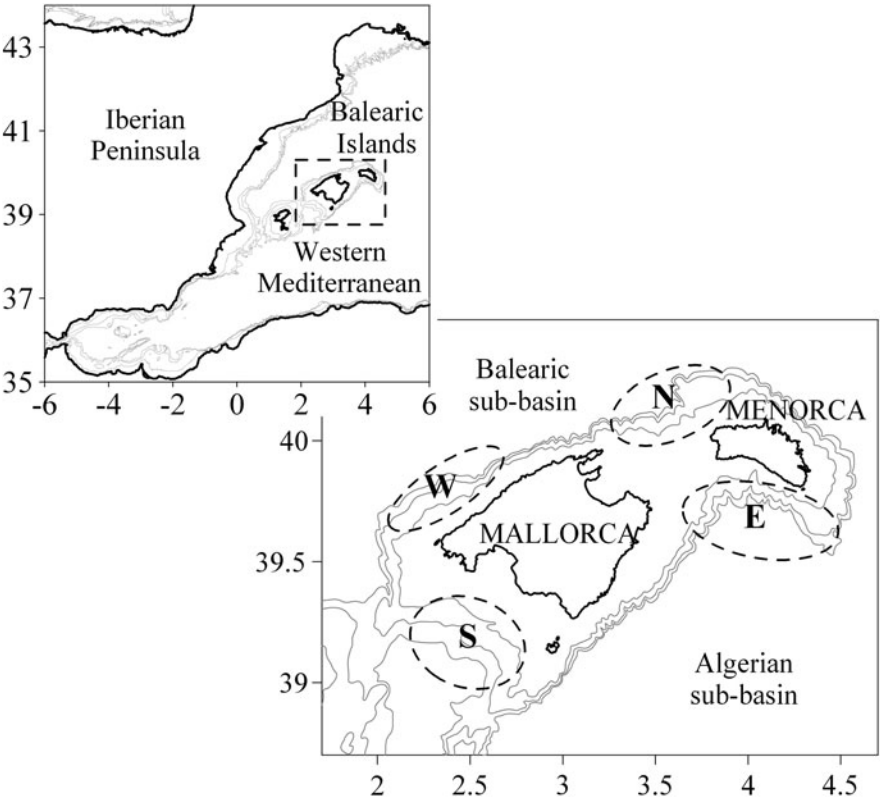


Fig. 1 Map of the study area, showing the areas considered for the analysis of data obtained during the experimental bottom trawl surveys, and the 200, 600 and 800 isobaths

Table 1 Summary of the environmental and fishery characteristics of each of the areas of the Balearic Islands analysed during the study, based on previous studies carried out in the study area (Pinot et al., 1995, 2002; Acosta et al., 2002, 2004; López-Jurado et al., 2008; Monserrat et al., 2008; Serrano et al., 2008)

Area	North	West	South	East
Sub-basin	Balearic	Balearic	Algerian	Algerian
Hydrology	Balearic front	Balearic front	No Balearic front	No Balearic front
	Cooler and more saline waters	Cooler and more saline waters	Warmer and fresher waters	Warmer and fresher waters
Bottom	Steep	Steep	Gentle, small canyons, submarine mountain	Steep, one big submarine canyon
Fishing effort	Low	Medium–High	Medium–High	Low

For fishing effort, low: <500 days/year; medium: 500–1000 days/year; high: >1000 days/year

Data source

Two different sources of data were used. Firstly, fishery-independent data was obtained from experimental bottom trawl surveys carried out in the Balearic Islands between 2001 and 2008, during late spring and early summer, following the international MEDITS bottom trawl surveys protocol (Bertrand et al., 2002). For an initial number of 452 stations sampled, 153 were considered for this study (15–20 stations by year), which correspond to those carried out in the slope (between 200 and 800 m depth). The number of stations analysed by area were 44, 35, 43 and 31 for the northern, western, southern and eastern areas, respectively. Information obtained was related to the total catch (in biomass and abundance) by species, length frequency distributions of all fish, and commercial crustaceans and cephalopods, and biological sampling (individual length, weight, sex and maturity) of the main commercial species. Secondly, fishery data was obtained from the landings of the bottom trawl fleet which operates in the island of Mallorca between 2000 and 2008. This information consisted in the biomass landed by species or commercial categories. Each of the 60,847 daily sale bills was assigned to one or more fishing tactics following the methodology described by Palmer et al. (2009) and only those trips carried out in the upper and middle slope (41,729) were used.

Community analysis

Different multivariate techniques were used in order to analyse biomass and abundance indices by station, obtained from the surveys, expressed in terms of weight (g) and number per km^2. Only species with a frequency of occurrence (FO) larger than 15% in all surveys were included in the analysis in order to reduce the variability in the species matrix due to the presence of a lot of zero values. As a first step, cluster analysis and multidimensional scaling were applied after square root transformation to identify assemblages. The Bray-Curtis similarity index was chosen as the similarity coefficient and Group Average was utilised as the clustering algorithm (Clarke & Warwick, 1994). Similarity Percentage Analysis (SIMPER) was applied to identify the species that mostly contribute to the differences among the detected station-groups (Clarke, 1993). In addition, supervised machine-learning approaches (Mitchel, 1997), and in particular the decision-tree construction method as implemented by the C4.5 algorithm (Quinlan, 1993), were employed as an alternative method to identify the power of the examined species (attributes) in classifying the stations into the pre-defined depth classes. In order to assess the power of the predicted classification scheme, the analysis was firstly applied to the 2001–2005 data set (learning data), and the decision trees built were used to predict the classification of the 2006–2008 data (test data) into the assemblages previously detected.

Redundancy Analysis (RDA, ter Braak & Smilauer, 2002) was also applied to link the species composition matrix (biomass and biomass) directly with the environmental matrix. Environmental information available was depth, hydrography (temperature, salinity and flow velocity near the bottom) obtained from a model and phytoplankton pigment concentration (ppc, mg Chl a/m^3), obtained from Kempler (2009), as indicator of the availability of trophic resources at the lowest levels of the trophic chain (Cartes et al., 2004). Data from different periods, simultaneously and before 1, 2, 3 and 4 months of the sampling, were considered. Fishing effort was also included in the model. Three levels of effort were considered: low (<500 days/year), medium (500–1000 days/year) and high (>1000 days/year). For each of the stations, an effort level was assigned taking into account the fishing tactic, the number of daily fishing trips and the nearest port. The variables included in the model were depth, area, year, effort, temperature, current velocity, simultaneous ppc and ppc 3 and months before sampling. Generalized Additive Models (GAM; Hastie & Tibshirani, 1990) were used to relate abundance of different taxonomic groups with environmental variables. The taxonomic groups considered were elasmobranchs, teleosts, crustaceans, molluscs and others. The variables included in the model were the same than for the RDA.

Fishery-independent indicators

At population level (i.e. *N. norvegicus* and *A. antennatus*), indicators used were FO, abundance (A, n/km^2), biomass (B, g/km^2), centre of gravity (COG, Daget, 1976), size-based indicators and condition index (Kn; Le Cren, 1951). FO, A and B were computed both for total population and for recruits. The COG model allows calculating and locating with precision the centre of species distributions by means of a descriptor (in this case, depth). It is determined as follows:

$$\mathrm{COG} = (x_1 + 2x_2 + 3x_3 + \cdots + nx_n)/\Sigma(x_i),$$

where x_i represents the calculated mean abundance values of the species x present in the stratum i (before analysis the sampled depth was divided into strata of 100 m). Size-based indicators calculated were mean (ML) and mean maximum length (MML) and mean (MM) and mean maximum body mass (MMBM). MML and MMBM were computed averaging the maximum length/body mass of each species in each haul. The individual weight of each specimen was calculated from bibliographic length–weight relationships (Merella et al., 1997; Quetglas et al., 1998a, b; Company & Sardà, 2000; Papaconstantinou & Kapiris, 2003; Morey et al., 2003; Mendes et al., 2004) as well as own data obtained during these surveys and other surveys carried out in the same area (Moranta et al., 2008). Kn was calculated as observed weight over expected weight, estimated from a length–weight relationship calculated considering all biological data available, by sex.

At the community level, assessment was performed using density, diversity and size-based indicators. Density indicators were abundance (n/km^2), biomass (g/km^2) and percentage of non-commercial species (both in abundance and biomass). Diversity indicators were total number of species (S), species richness (Margalef, $d = (S - 1)/\log(N)$, where N is the total number of individuals), Pielou's evenness ($J' = H'/\log(S)$), Shannon–Wiener index ($H' = -\mathrm{SUM}(P_i * \log_2(P_i))$, where P_i is the proportion of each species in each sample) and ABC plots (K-dominance curves, Warwick, 1986; Clarke, 1990). For the latter, those species only reported in a single haul or in a single survey were removed. As the results of ABC plots depend on the species included in the analysis (Jouffre & Inejih, 2005), a second analysis was performed including only those species which have appeared in all surveys. Size-based indicators were biomass, abundance and diversity spectra, and the above-described MML, ML, MMBW and MBW.

Normalised biomass size spectra (Jennings et al., 2002) were computed by dividing the biomass in a given body-mass class interval by the width of that class interval (in antilog dimensions). The relationship between body mass (as $\log_2$ classes) and total normalised biomass ($\log_2$ transformed) was described using least squared linear regressions, considering only the body mass class in which the biomass starts to decline. Size spectra (Bianchi et al., 2000) were constructed for each survey by plotting the natural logarithm of the total number of individuals caught by 5-cm length class against the natural logarithm of the middle of each length class. Diversity spectra (Gislason & Rice, 1998) were constructed by plotting the Shannon–Wiener index (calculated for each 5-cm length class) against the mid-length of each length group. For these three metrics, the slope of the spectrum for each year or area was used as an indicator of changes in the exploitation rate (Bianchi et al., 2000). The slope decreases when decreases fishing mortality (Hall et al., 2006), so it can be considered a good measure of fishing impacts (Gislason & Rice, 1998).

Fishery-dependent indicators

At population level, density and economic indicators were computed. Density indicators were annual landings by vessel (as kg/vessel), landings per day for total fleet (as kg/day), landings per vessel and day (LPUE, as kg/day/vessel), both for total population and the small-sized commercial category (as a proxy of recruits). Economic indicators (Ceriola et al., 2008) were revenue by vessel (€/vessel), revenue by day (as €/day), revenue by vessel and day (RPUE, as €/day/vessel) and average price (as €/kg). At community level, these same indicators were computed, but without taking into account size-groups. Number of boats and number of days were also calculated.

Traffic Lights

The summary of these indicators, both fishery-dependent and independent indicators and both at population and community level, was performed using TL (Caddy, 1999, 2002; Koeller et al., 2000). The TL is a system of red, yellow and green lights which categorise multiple indicators of the state of a fishery and ecosystem, considering red as bad, yellow as intermediate and green as good. A number of options are available for establishing boundaries for TL, like Limit Reference Points, a percentage of the average value or using the 33rd and 66th percentiles (Halliday et al., 2001). This last option was used in this work, adapting the rules depending of the expected effect of increasing fishing pressure in each indicator. Although TL has been usually employed for evaluating temporal variation of indicators in assessment (DFO, 2005; Ceriola et al., 2007, 2008), in this work, both spatial and temporal trends were tested using this method. Thus, a mean value for each of the indicators has been computed by year or area and a different colour was assigned if this value was under the 33rd percentile (red, bad state), between the 33rd and 66th percentile (yellow, intermediate state) or over the 66th percentile (green, good state). For those indicators that we expect to increase with high fishing pressure (like effort indicators themselves or percentage of non-commercial species), the rules were the opposite. As a summary, a single TL was created for fishery-independent and fishery-dependent data, assigning the colour of the most abundant one for each year or area.

Results

Community description and influence of environmental variables

Total catch during the 8 years of experimental bottom trawl surveys in the fishing grounds of the slope of the Balearic Islands was 11,391 kg and 725,817 individuals, of a total of 363 species (or families when it was not possible to arrive at species level) corresponding to 108 teleosts, 19 elasmobranchs, 75 crustaceans, 46 molluscs, 27 echinoderms and 94 belonging to other groups.

Cluster and MDS results showed three different groups related to depth (Fig. 2), corresponding to those hauls carried out between 200 and 299 m depth (SB, shelf break), 300 and 499 m depth (US, upper slope) and 500 and 800 m depth (MS, middle slope). The results of the SIMPER analysis (Table 2) showed high values of average dissimilarity between groups, larger than 90%, and confirmed the presence of these well-defined groups. The species that characterise the SB were mostly teleosts (like *Capros aper*, *M. merluccius* and *Micromesistius poutassou*) and the elasmobranch *Scyliorhinus canicula*. The most important species in the US were also teleosts, crustaceans (from the genus *Plesionika*, *Parapenaeus longirostris* and *N. norvegicus*), the elasmobranch *Galeus melastomus* and the cephalopod *Sepietta oweniana*. In the MS, crustaceans were predominant (like *A. antennatus*), followed by teleosts and the elasmobranchs *G. melastomus* and *Etmopterus spinax*. Decision-tree analysis confirmed these groups, as the supervised machine-learning showed a very high level of classification (Table 3). Miss-classification was higher for the stations belonging to the shallowest group in which the number of samples was the lowest.

Full models from the RDA results were found significant ($P < 0.01$) both for biomass and abundance (Table 4). Depth was the main factor affecting the species composition, while the rest of the significant variables explained low percentages of the variance. In any of the models, the variables related to primary production were found to be significant. Deviance explained from the GAM models applied to biomass of all the groups analysed varied between 25 and 79% (Table 5). In all cases, depth and year were significant factors. For total, elasmobranchs, teleosts, molluscs

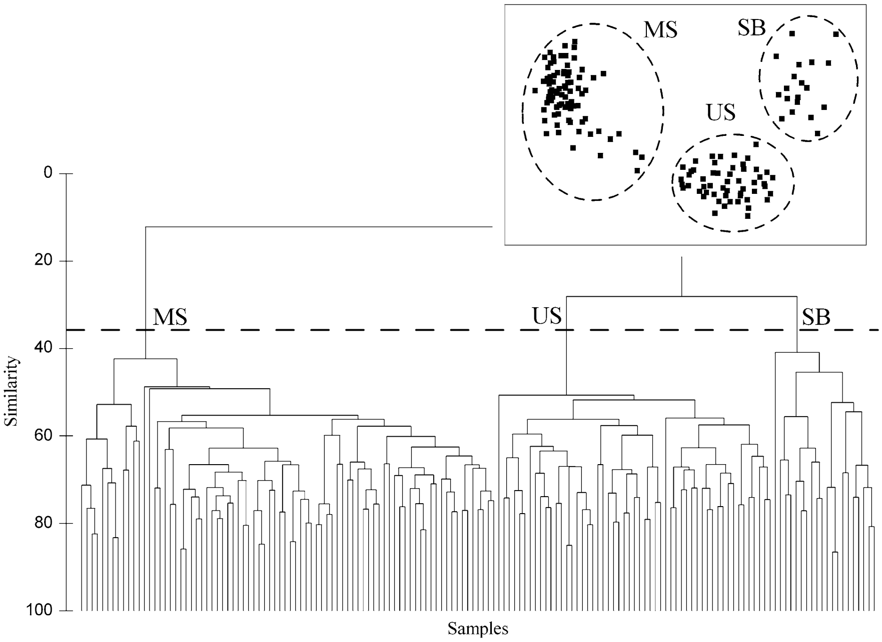


Fig. 2 Dendrogram and MDS ordination of samples made during the experimental bottom trawl surveys, showing the three different assemblages obtained from the cluster analysis: shelf break (SB, 200–299 m), upper slope (US, 300–499 m) and middle slope (MS, 500–800 m)

and others there was a non-linear decreasing trend with depth and crustaceans showed an increasing trend with depth (Fig. 3). By years, both elasmobranchs and crustaceans showed the highest values for 2002, and molluscs in 2005, although any taxonomic group showed a clear inter-annual trend. Area was a significant factor for some of the taxonomic groups as biomass showed the highest values in the north for total and teleosts and in the south for crustaceans. Fishing effort was marginally significant for elasmobranchs and crustaceans, with different trends: the highest values of biomass for elasmobranchs were found with low or medium levels of fishing effort, but for crustaceans it was higher with medium and high levels.

Fishery assessment

Traffic Light method for *N. norvegicus* showed similar results among years and areas with both set of data (Fig. 4). The highest proportion of reds was found in the first years (except for 2002 for density indicators) and of greens in the last years. Condition of both males and females did not show this trend, with the lowest values during the last year. Spatially, the highest number of greens were found in the northern and southern locations (although the northern location showed reds in the length-based indicators) and of reds in the eastern. In fact, in this location, length-based indicators could not be computed due to the low number of individuals caught. On the contrary, TL for *A. antennatus* showed very different results with both set of data (Fig. 5). Density indicators from surveys showed high numbers of reds during the last years (except for 2008). The opposite trend has been found for fleet-based indicators, with the highest numbers of reds at the beginning of the series. Condition showed differences between both sexes, with the lowest values of the series for males in the last 2 years. Spatial TL showed some differences but depending on the group of indicator. For example, in the case of fishery-independent indicators, the east showed high number of greens for density indicators but reds for size-based and condition indicators. In the case of fishery-dependent indicators, the south showed high number of reds for density indicators but of greens for economic ones. On the contrary, the west showed the highest numbers of greens in all cases.

At community level, TL for US showed similar results with both sources of data (Fig. 6). Although the summary in both cases was quite similar, without red values in the last 3 years, fishery-independent indicators showed reds, yellows and greens distributed heterogeneously along years. By contrast, number of reds was very low for the fishery-dependent indicators during the last 3 years. Spatially, fishery-independent indicators showed high number of reds

Table 2 SIMPER results for each assemblage identified from the dendogram and for the species that contributed to at least 90% of the differences between these groups: mean abundance (Av. Ab. as n/km^2), average similarity (Av. Sim.), standard deviation (SD), percentage contribution to the similarity (Contrib%), percentage contribution to the similarity accumulated (Cum.%), average dissimilarity (Av. Diss.)

Species	Tax.	Av. Ab.	Av. Sim.	Sim/SD.	Contrib%	Cum.%
Shelf break; Av. Sim. = 27.21						
Scyliorhinus canicula	EL	3545.91	9.46	0.85	34.78	34.78
Capros aper	TE	18084.62	6.32	0.65	23.24	58.02
Merluccius merluccius	TE	1714.21	3.66	0.58	13.46	71.47
Micromesistius poutassou	TE	3982.98	2.55	0.36	9.39	80.86
Synchiropus phaeton	TE	1077.83	1.55	1.05	5.68	86.54
Helicolenus dactylopterus	TE	645.19	1.35	0.76	4.96	91.50
Upper slope; Av. Sim. = 33.37						
Gadiculus argenteus	TE	13609.48	14.21	1.09	42.59	42.59
Galeus melastomus	EL	2438.46	2.18	0.70	6.53	49.13
Caelorynchus caelorhynchus	TE	1961.63	2.06	0.88	6.16	55.29
Plesionika heterocarpus	CR	4543.87	1.97	0.45	5.91	61.19
Sepietta oweniana	MO	1319.66	1.65	0.81	4.94	66.13
Chlorophtalmus agassizi	TE	2406.85	1.59	0.64	4.76	70.90
Plesionika giglioli	CR	857.83	1.50	0.61	4.51	75.40
Helicolenus dactylopterus	TE	1048.90	1.26	1.02	3.77	79.17
Phycis blennoides	TE	545.00	1.19	0.89	3.57	82.75
Micromesistius poutassou	TE	1534.96	1.16	0.63	3.47	86.21
Plesionika antigai	CR	731.23	0.59	0.62	1.77	87.99
Parapenaeus longirostris	CR	966.57	0.58	0.45	1.75	89.74
Nephrops norvegicus	CR	553.20	0.52	0.37	1.57	91.31
Middle slope; Av. Sim. = 36.17						
Aristeus antennatus	CR	2687.68	12.16	1.05	33.63	33.63
Lampanictus crocodrilus	TE	1924.95	7.80	1.12	21.57	55.19
Plesionika martia	CR	811.75	2.78	0.81	7.68	62.87
Galeus melastomus	EL	704.30	2.20	0.77	6.08	68.95
Phycis blennoides	TE	348.62	2.12	0.94	5.87	74.82
Pasiphaea multidentata	CR	554.38	2.11	0.54	5.83	80.65
Nezumia aequalis	TE	273.38	1.67	1.03	4.61	85.26
Plesionika acantonothus	CR	220.29	0.87	0.62	2.41	87.67
Sergia robusta	MO	258.48	0.80	0.46	2.22	89.89
Geryon longipes	CR	130.28	0.74	0.52	2.05	91.93

Pairwise comparisons	Av. Diss.
Shelf break vs. upper slope	89.73
Shelf break vs. middle slope	99.03
Upper slope vs. middle slope	94.39

Taxonomic group (Tax.) for each of the species is also shown (*TE* teleosts, *EL* elasmobranchs, *CR* crustaceans, *MO* molluscs)

in the south and of greens in the east and the north. For fishery-dependent indicators, number of reds was lower in the north and south. The west and south showed the highest levels of effort. TL for MS showed several differences between both sources of data, but depending on the type of indicators (Fig. 7).

Table 3 Estimated "information gain" for the 15 highest rates for the examined attributes (species) from the supervised machine-learning approach and classification success by assemblage for the training and the test data sets

Attribute (species)	Information gain
Sepietta oweniana	0.941
Aristeus antennatus	0.850
Gadiculus argenteus	0.829
Lampanictus crocodrilus	0.773
Synchiropus phaeton	0.753
Nezumia aequalis	0.751
Lepidorhombus boscii	0.722
Scyliorhinus canicula	0.692
Capros aper	0.691
Sergia robusta	0.644
Parapenaeus longirostris	0.639
Helicolenus dactylopterus	0.625
Chlorophtalmus agassizi	0.613
Trigla lyra	0.606
Geryon longipes	0.584

Class	Classification success	
	Training (%)	Test (%)
Shelf break	100	63
Upper slope	100	100
Middle slope	100	96

Density indicators from fishery-independent data showed a temporal transition from high number of greens to high number of reds. The opposite trend was found for diversity indicators. Size-based indicators did not show any clear temporal trend. In general, fishery-dependent indicators went from high number of reds to high number of greens. Spatially, results were similar between both sources of information, with the highest number of reds in the north and the lowest in the east.

Discussion

Three different assemblages have been detected in the slope off the Balearic Islands, with the bathymetric gradient as the main factor conditioning them: the shelf break (200–299 m), the upper slope (300–499 m) and the middle slope (500–800 m). The shallowest assemblage corresponds to a bathymetric zone where the fleet usually does not operate. The other two assemblages can be comparable to the fishing tactics (FT) identified in the crustacean bottom trawl fishery in the Balearic Islands, targeting the Norway lobster *N. norvegicus* and the red shrimp *A. antennatus*, in the upper and middle slope, respectively (Guijarro & Massutí, 2006; Palmer et al., 2009). These assemblages are in agreement with previous studies performed in the same area (Massutí & Reñones, 2005), where the existence of six species assemblages was described, three of them in the slope similarly to those found in this study.

Depth is generally considered as the main factor governing the faunal assemblages (see Carney, 2005 for a general review). In our study, depth was found to be significant for all the analysis performed. However, it is not a causative factor and other factors, such as temperature, high pressure and limited food availability have also been proposed as causal factors (Carney, 2005). Depth-related trends in density were different depending on the taxonomic group analysed. Teleosts showed a clear decreasing trend, similarly than elasmobranchs and others and both crustaceans and molluscs showed an increasing trend until a maximum (Labropoulou & Papaconstantinou, 2000; Colloca et al., 2003; Massutí & Reñones, 2005). The variance explained in the model by the rest of significant factors (year, area, effort and hydrographic characteristics) was quite low (less than 7%). In the case of year, the low variance explained seems to be more related to differences in temporal species-specific abundances than to assemblage species composition. The high rate of classification success for the test data set in the supervised machine-learning suggests the consistency of the observed species pattern throughout the examined years. Although there was not a clear trend by years, both elasmobranchs and crustaceans showed the highest values in 2002.

Geographical variations found can be related to the different hydrodynamic and geo-morphology conditions, which can vary between these closed geographical areas, similarly to other studies (Abelló et al., 2002b; Massutí et al., 2004; Gaertner et al., 2005). Spatial differences can also be related to differences in fishing exploitation (Gristina et al., 2006; Dimech et al., 2008; Moranta et al., 2008). Larger values of density were detected in the eastern and northern areas, where fishing

Table 4 Results of the redundancy analysis for the density (abundance in n/km^2 and biomass in g/km^2) matrix of the species

	Depth * Area * Year * Effort * Temperature * Velocity * Primary production					
	Effect	Cov	Trace	EV (%)	*F* ratio	*P* value
Abundance	Full model		0.595	59.5	10.941	0.002
	Depth	A, Y, E, T, V, PP	0.278	27.8	92.097	0.002
	Area	D, Y, E, T, V, PP	0.028	2.8	3.075	0.002
	Year	D, A, E, T, V, PP	0.033	3.3	1.539	0.016
	Effort	D, A, Y, T, V, PP	0.018	1.8	2.965	0.006
	Temperature	D, A, Y, E, V, PP	0.023	2.3	7.661	0.002
	Velocity	D, A, Y, E, T, PP	0.060	6.0	19.850	0.002
	Primary production	D, A, Y, E, T, V	0.007	0.7	0.778	0.784
Biomass	Full model		0.576	57.6	10.104	0.002
	Depth	A, Y, E, T, V, PP	0.272	27.2	85.863	0.002
	Area	D, Y, E, T, V, PP	0.026	2.6	2.708	0.002
	Year	D, A, E, T, V, PP	0.027	2.7	1.234	0.080
	Effort	D, A, Y, T, V, PP	0.016	1.6	2.546	0.006
	Temperature	D, A, Y, E, V, PP	0.019	1.9	6.157	0.002
	Velocity	D, A, Y, E, T, PP	0.046	4.6	14.679	0.002
	Primary production	D, A, Y, E, T, V	0.009	0.9	0.967	0.510

The trace, the explained variance (EV), the *F* statistic and its significance (*P* value) for both the full model, which contains all the variables included in the model and for each individual variable after extracting the effect of the covariable is also indicated. *D* depth, *A* area, *Y* year, *E* effort, *T* temperature, *V* current velocity, *PP* primary production. Only non-correlated variables were included in the model

Table 5 Results of generalised additive models (GAM) applied to the different groups of species and all the species (Total), showing the dependent variable (abundance in n/km^2 and biomass in g/km^2), the explanatory variables (*D* depth, *A* area, *Y* year, *E* effort, *T* temperature, *PP* primary production), as well as the score, *R*-squared (R^2) and deviance explained (DE) values

	D	A	Y	E	T	PP	Score	R^2	DE (%)
Abundance									
Total	***	***	***	ns	ns	ns	0.421	0.178	80.0
Elasmobranchs	***	ns	***	+	ns	ns	0.507	0.078	46.9
Teleosts	***	***	***	ns	ns	ns	0.336	−0.023	82.7
Crustaceans	***	+	***	+	ns	ns	0.269	0.047	52.1
Molluscs	***	ns	***	ns	ns	ns	0.732	0.133	63.1
Others	***	***	***	ns	ns	ns	1.598	0.103	59.8
Biomass									
Total	***	***	***	ns	ns	ns	0.429	0.191	71.5
Elasmobranchs	***	ns	***	+	ns	ns	0.894	0.143	25.5
Teleosts	***	***	**	ns	ns	ns	0.190	0.125	79.2
Crustaceans	***	+	***	+	ns	ns	0.251	0.126	32.9
Molluscs	***	*	***	ns	ns	ns	0.592	0.386	51.4
Others	***	***	***	ns	ns	ns	1.840	−0.580	42.2

The significance of each explanatory variable (*** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, $^{+}$ $P < 0.1$; ns: not significant or $P \geq 0.1$) is also indicated

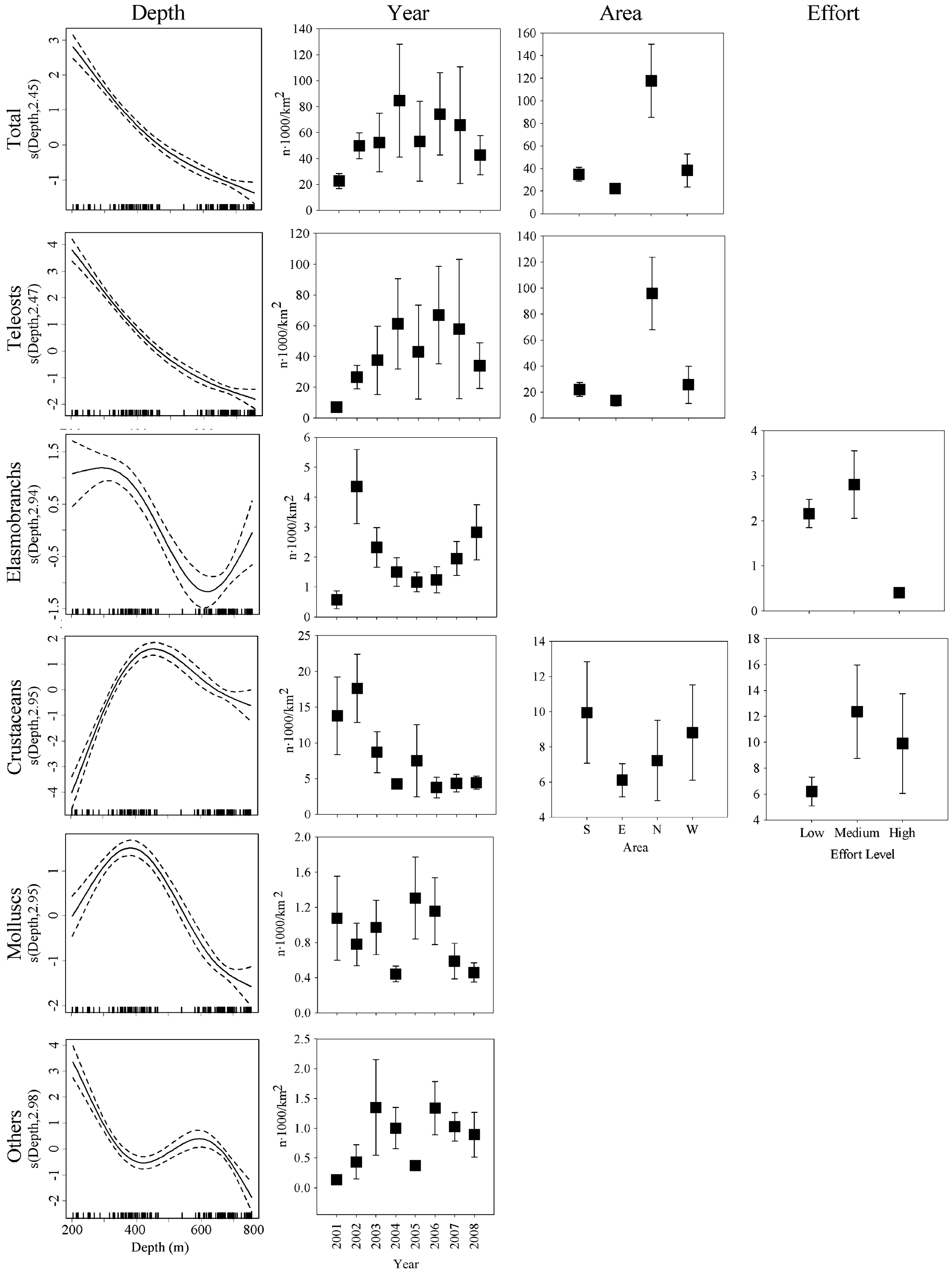


Fig. 3 Plots of the best significant generalised additive modelling (GAM) applied to the abundances of each of the taxonomic groups (total, teleosts, elasmobranchs, crustaceans, molluscs and others) for depth, and mean abundance values (n/km^2) for the significant factors year, area (*S* south, *E* east, *N* north, *W* west) and effort level (low: <500 days/year; medium: 500–1000 days/year; high: >1000 days/year). Error lines are standard errors

effort was lower. However, effort was only marginally significant for two groups, elasmobranchs and crustaceans. Larger values of elasmobranch density were found with low and middle effort. This trend can be related to the biological characteristics of these species, which made them particularly vulnerable to fishing pressure (e.g. Aldebert, 1997; Bertrand et al., 1998; Stevens et al., 2000). The Balearic Islands have been reported as an area of higher diversity of demersal elasmobranchs in comparison to the adjacent waters off the Iberian Peninsula (Massutí & Moranta, 2003). The differences found at the short spatial scale analysed in this study remarked the especially vulnerability of this species to fishing pressure. Crustaceans showed higher values of biomass with middle and high effort levels. For *A. antennatus*, small-scale temporal variability in catches has been related to the ability of fishermen to remove competitors at a differentially higher rate, leading to a higher catch of this species (Sardà & Maynou, 1998).

The use of TL for integrating different type of indicators in the assessment of the deep water fishery of the Balearic Islands has been revealed as a simple and useful tool for summarising large amount of both scientific and fishery data. By taking into consideration a wider range of factors than traditional assessment methods, the TL precautionary decision framework reduces the risk of missing important stock dynamics, environmental or ecosystem signals (Halliday et al., 2001). Its simplicity is also a strong argument for using this method and it does not require that previously used analyses be abandoned as their outputs can be incorporated into this more general framework. The method has potential not only for single species assessment, but also as a decision framework in management at the ecosystem level. In fact, indicators are needed to support the implementation of an ecosystem approach to fisheries (Jennings, 2005) and the complexity of exploited ecosystems forces to examine multiple indicators and a need to summarise them (Rochet & Trenkel, 2003).

For *N. norvegicus*, there was a quite clear improvement in the state of the population. The state of exploitation of the stocks of *N. norvegicus* in the Mediterranean, appears to be from slightly growth-overexploited to near the optimum level of exploitation depending on the area (Sardà et al., 1998). However, in the Balearic Islands there has been an overall negative trend in the landings of this species between 1986 and 1995 (Merella et al., 1998), although we should take into account that landings do not represent the state of the resources as well as CPUE does (Lleonart & Maynou, 2003). In fact, the fishing effort in the Balearic Islands has been gradually decreasing from 1994 to 2008 (personal communication). Recent results using more traditional assessment methodology (i.e. virtual population and yield per recruit analysis), showed that the species seems to be moderately exploited (Guijarro et al., 2009b). When comparing the different areas, Norway lobster population from the northern and southern areas are those in the better state, which are the two areas where the species is mainly landed by the trawl fleet (Merella et al., 1998). The worst estate was found in the eastern area, although this is the area with the lowest levels of effort. Thus, other factors than fishing pressure may influence this resource. Differences in population structure for this species have been related to exploitation levels (Abelló et al., 2002a) and environmental conditions such as sediment characteristics, related to its burrower behaviour (Maynou & Sardà, 1997). In the study area, short spatial differences on other crustaceans have been related to sediment characteristics and trophic webs (Guijarro et al., 2009a). The relative contribution of decapod crustacean feeding guilds closely related to the bottom resources are higher in the BsB (e.g., Maynou & Cartes, 2000; Cartes et al., 2008a) than in the AsB. This could explain the scarcity of this species in the eastern area, but not the presence and good state of *N. norvegicus* in the south, so probably the occurrence of both adequate sediment composition and prey availability seem to mark the short spatial differences found for this species.

For *A. antennatus*, differences between both sources of data can be explained by the high seasonal variability in the population dynamics of this resource. Large mature females aggregations in summer have been detected during the spawning period and a maximum recruitment of juveniles in the fishing grounds in the BsB have been detected during autumn–winter (Sardà et al., 1994, 1997; Tudela et al., 2003; Guijarro et al., 2008). This seasonal dynamics of the species also determines the fleet behaviour. In fact, there is an annual displacement of the trawl fleet targeting red shrimp from the southern fishing grounds to the northern ones during the spawning period, targeting the adult females

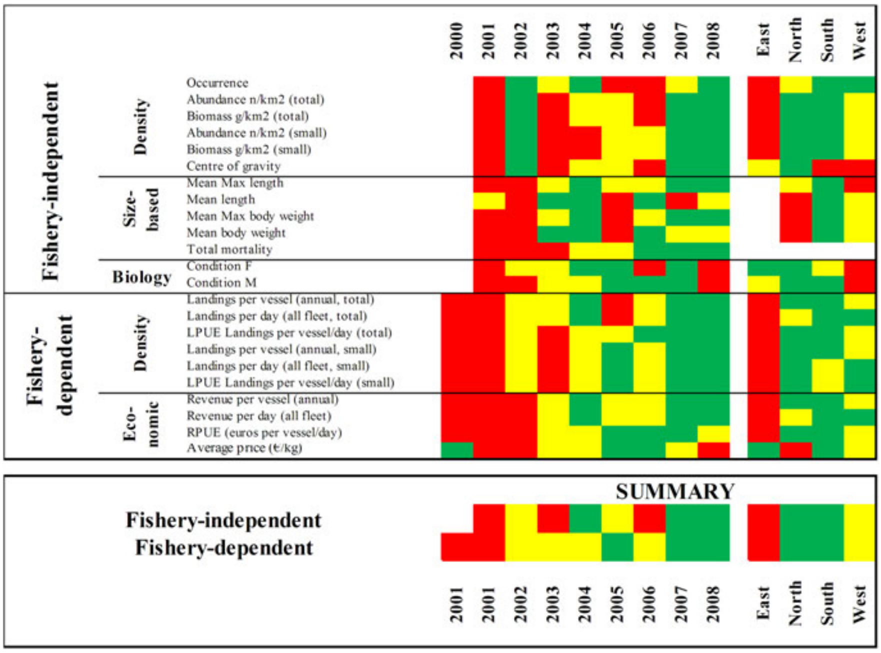


Fig. 4 Traffic Light tables displaying biological and economic indicators response for the Norway lobster *Nephrops norvegicus* in the Balearic Islands. *Red*: <33rd percentile; *yellow*: 33rd–66th percentiles; *green*: >66th percentile

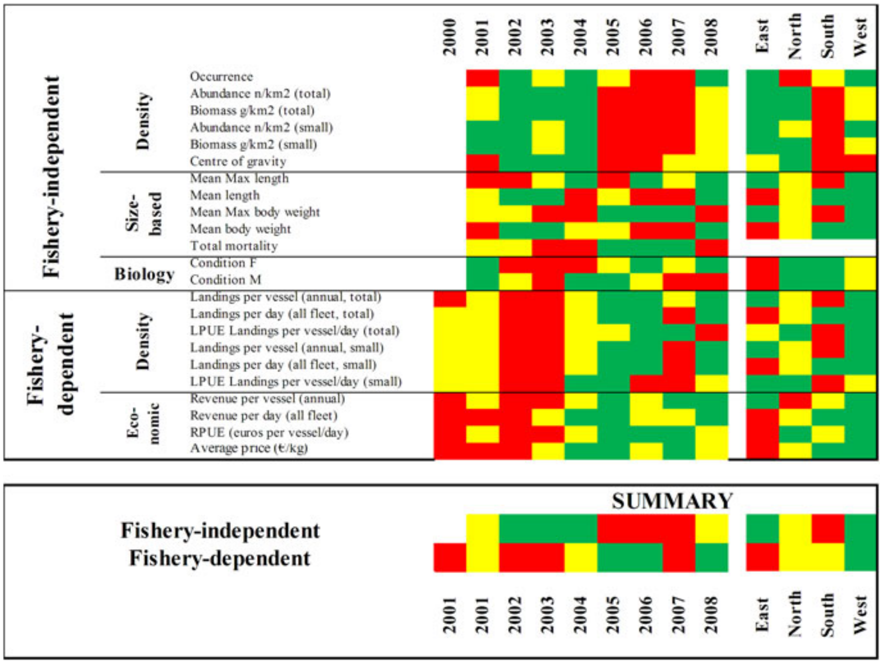
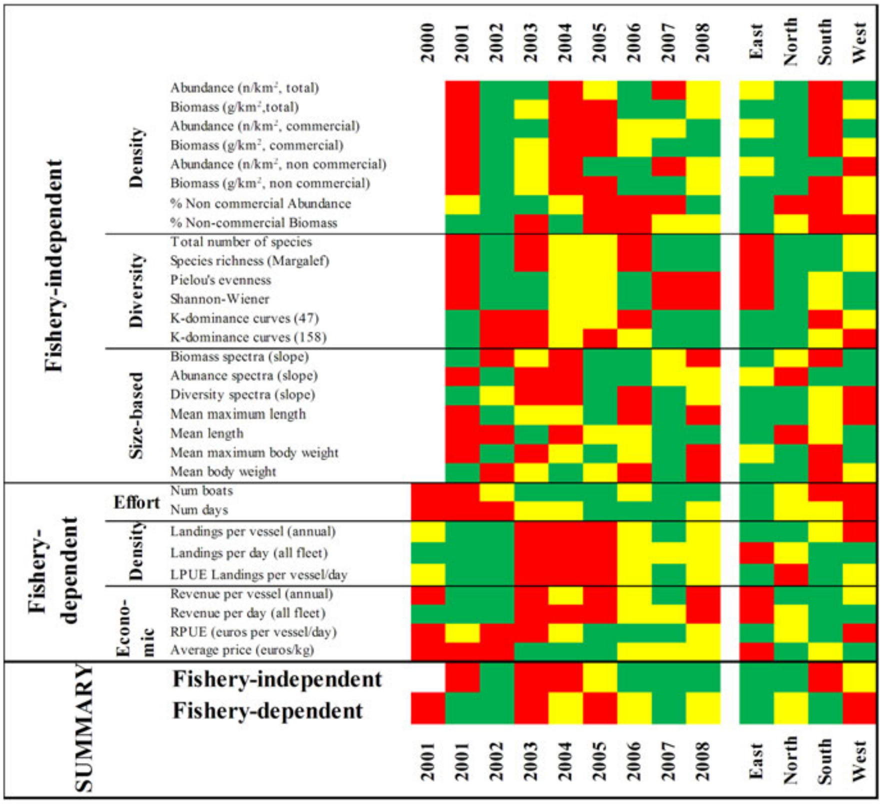


Fig. 5 Traffic Light tables displaying biological and economic indicators response for the red shrimp *Aristeus antennatus* in the Balearic Islands. *Red*: <33rd percentile; *yellow*: 33rd–66th percentiles; *green*: >66th percentile

aggregations (Guijarro et al., 2008; Moranta et al., 2008). In our case, fishery-dependent data provides better information for this resource than fishery-independent data, as the first one covers all the year while the second has been obtained during a concrete period of the year. Our survey data were obtained during spring, without spawning aggregations or recruitment processes. In this sense, fishery-dependent indicators showed an improvement in the state of this stock during the last years. In fact, the assessment of this species using non-equilibrium production models in this areas suggested that red shrimp is slightly under-exploited (Carbonell & Azevedo, 2003). It is important to remark that

Fig. 6 Traffic Light tables displaying biological and economic indicators response for the upper slope assemblage in the Balearic Islands. *Red* <33rd percentile; *yellow* 33rd–66th percentiles; *green* >66th percentile, except for percentage of non-commercial species and effort variables (number of boats and number of days) in which opposite



			2000	2001	2002	2003	2004	2005	2006	2007	2008	East	North	South	West
Fishery-independent	Density	Abundance (n/km², total)		🟥	🟩	🟩	🟥	🟨	🟩	🟥	🟨	🟨	🟩	🟥	🟩
		Biomass (g/km²,total)		🟥	🟩	🟨	🟥	🟥	🟩	🟩	🟨	🟩	🟩	🟥	🟨
		Abundance (n/km², commercial)		🟥	🟩	🟩	🟥	🟥	🟨	🟨	🟩	🟨	🟩	🟥	🟩
		Biomass (g/km², commercial)		🟥	🟩	🟨	🟥	🟥	🟨	🟩	🟩	🟩	🟩	🟥	🟨
		Abundance (n/km², non commercial)		🟥	🟩	🟨	🟥	🟩	🟩	🟥	🟨	🟨	🟩	🟩	🟥
		Biomass (g/km², non commercial)		🟥	🟩	🟨	🟥	🟥	🟩	🟩	🟨	🟩	🟩	🟥	🟨
		% Non commercial Abundance		🟨	🟩	🟩	🟨	🟥	🟥	🟥	🟩	🟩	🟥	🟥	🟨
		% Non-commercial Biomass		🟩	🟩	🟥	🟩	🟥	🟥	🟨	🟨	🟩	🟨	🟥	🟥
	Diversity	Total number of species		🟥	🟩	🟥	🟨	🟨	🟥	🟩	🟩	🟥	🟩	🟩	🟨
		Species richness (Margalef)		🟥	🟩	🟥	🟨	🟨	🟥	🟩	🟩	🟥	🟩	🟩	🟨
		Pielou's evenness		🟥	🟩	🟩	🟨	🟨	🟩	🟥	🟥	🟥	🟩	🟨	🟩
		Shannon-Wiener		🟥	🟩	🟩	🟨	🟨	🟩	🟥	🟥	🟥	🟩	🟨	🟩
		K-dominance curves (47)		🟩	🟥	🟥	🟨	🟨	🟥	🟩	🟩	🟩	🟩	🟥	🟨
		K-dominance curves (158)		🟩	🟥	🟥	🟨	🟥	🟨	🟩	🟩	🟩	🟩	🟨	🟥
	Size-based	Biomass spectra (slope)		🟩	🟥	🟨	🟥	🟩	🟩	🟨	🟥	🟩	🟨	🟥	🟩
		Abunance spectra (slope)		🟥	🟩	🟥	🟥	🟩	🟩	🟨	🟨	🟨	🟥	🟩	🟩
		Diversity spectra (slope)		🟩	🟨	🟥	🟥	🟩	🟥	🟩	🟨	🟩	🟩	🟨	🟥
		Mean maximum length		🟥	🟩	🟨	🟨	🟩	🟥	🟩	🟥	🟩	🟩	🟨	🟥
		Mean length		🟥	🟥	🟩	🟥	🟨	🟨	🟩	🟩	🟩	🟥	🟨	🟩
		Mean maximum body weight		🟥	🟩	🟥	🟨	🟩	🟨	🟩	🟥	🟨	🟩	🟥	🟩
		Mean body weight		🟩	🟥	🟨	🟩	🟨	🟥	🟩	🟥	🟩	🟩	🟥	🟨
Fishery-dependent	Effort	Num boats	🟥	🟥	🟨	🟩	🟩	🟩	🟨	🟩	🟩	🟩	🟨	🟥	🟥
		Num days	🟥	🟥	🟥	🟨	🟨	🟩	🟩	🟩	🟨	🟩	🟨	🟨	🟥
	Density	Landings per vessel (annual)	🟨	🟩	🟩	🟥	🟥	🟥	🟨	🟩	🟨	🟩	🟩	🟨	🟥
		Landings per day (all fleet)	🟩	🟩	🟩	🟥	🟥	🟥	🟨	🟨	🟨	🟥	🟨	🟩	🟩
		LPUE Landings per vessel/day	🟨	🟩	🟩	🟥	🟥	🟥	🟨	🟩	🟨	🟩	🟥	🟩	🟨
	Econo-mic	Revenue per vessel (annual)	🟥	🟩	🟩	🟥	🟨	🟥	🟨	🟩	🟥	🟥	🟩	🟩	🟨
		Revenue per day (all fleet)	🟩	🟩	🟩	🟥	🟥	🟥	🟨	🟨	🟥	🟥	🟨	🟩	🟩
		RPUE (euros per vessel/day)	🟥	🟨	🟥	🟥	🟨	🟩	🟩	🟩	🟨	🟩	🟨	🟩	🟥
		Average price (euros/kg)	🟥	🟥	🟥	🟩	🟩	🟩	🟨	🟨	🟨	🟥	🟩	🟨	🟩
SUMMARY		**Fishery-independent**		🟥	🟩	🟥	🟥	🟨	🟩	🟩	🟩	🟩	🟩	🟥	🟨
		Fishery-dependent	🟥	🟩	🟩	🟥	🟨	🟥	🟨	🟩	🟨	🟩	🟨	🟩	🟥
			2001	2001	2002	2003	2004	2005	2006	2007	2008	East	North	South	West

condition showed differences between both sexes, with the lowest values of the series for males in the last 2 years, similarly of what has been detected from commercial fleet data (Carbonell et al., 2008). Geographically, the western area presented the best state of exploitation, although the fishing effort is high. This western areas has been pointed out as highly productive area (Guijarro et al., 2009a). In this area, higher abundance of suprabenthos and zooplankton has been detected (Cartes et al., 2008a), and also it is more influenced by the more productive waters coming from the Gulf of Lions (Champalbert, 1996; Bosc et al., 2004; Canals et al., 2006), which increases the primary production in the area and thus enriches the trophic chain (Estrada, 1996; Fernández de Puelles et al., 2004). These oceanographic conditions could favour *A. antennatus* more than *N. norvegicus* due to the differences on trophic webs already mentioned, as *A. antennatus* diet is based of mesopelagic preys (Cartes et al., 2008b).

At community level, different results have been found in the two assemblages analysed with TL. In the case of the upper slope, similar results have been found using fishery-dependent and fishery-independent data. In this case, it seems to be an improvement of the state of this assemblage during last years. This can be probably due to the decreasing trend in effort found especially from 2003. Also the highest values of abundance for elasmobranchs and crustaceans detected by other methods in 2002 was reflected both from the fishery-dependent and fishery-independent data. The eastern location seemed to be in a better state, which was the area with the lowest levels of effort. On the contrary, the western area, with high levels of effort, showed the worst state for this assemblage.

In the case of the middle slope, where *A. antennatus* is the most important species in the assemblage (around 30% in abundance) and represents near 80% of landings (Moranta et al., 2000), several differences have been found between both sources of information. Fishery-depended annual indicators seem to show an improvement in the state of this assemblage, while it seems the contrary for the fishery-independent data, especially for density indicators and size-based ones. When these indicators

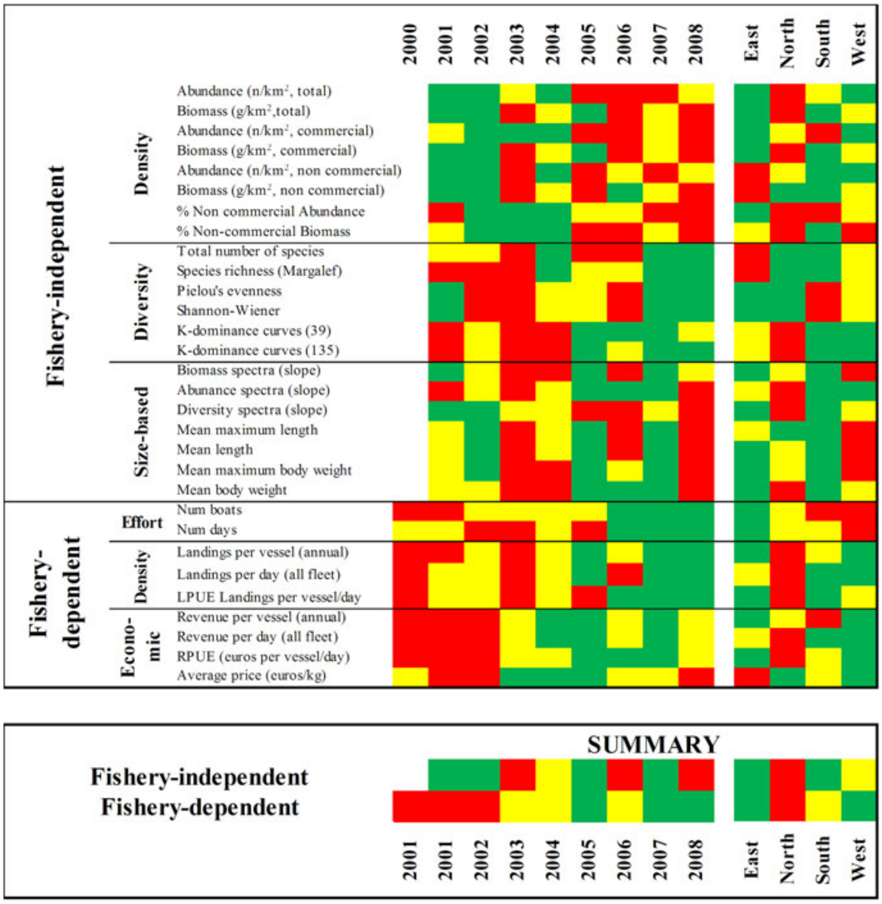


Fig. 7 Traffic Light tables displaying biological and economic indicators response for the middle slope assemblage in the Balearic Islands. *Red* <33rd percentile; *yellow* 33rd–66th percentiles; *green* >66th percentile, except for percentage of non-commercial species and effort variables (number of boats and number of days) in which opposite

were computed geographically, these differences were not so important. The better state was found in the eastern location but the worst in the northern, although this area presents intermediate levels of effort, which suggest that not only fishing pressure is the responsible of the state of this assemblage. Differences between both sources of information can also be attributed to the different species included in each case. Fishery-dependent data only included information from the landed species and not from those discarded, while fishery-independent data covered the entire nekton-benthic community. In the former set of data, discards from *A. antennatus* can be considered nil (Carbonell et al., 1999). These differences can also be attributed to the difference efficiency between the experimental and commercial gears which, in the case of commercial Italian trawl, have shown higher efficiency for benthic species when compared with the experimental and lower for some others released from the bottom as well as for the pelagic ones (Fiorentini et al., 1999). Experimental bottom trawl also showed a great variability of escapement values among species, which could affect the proportion rates of the species sampled during a standard survey as well as the size-frequency distribution as for some species the escape rate was size-dependant, like in the case of large-size classes escaping effectively from the trawl, probably because their greater swimming endurance (Dremière et al., 1999). Although this, for some species, the comparison from surveys and commercial fleet has given very acceptable results (Abella et al., 1999).

Conclusions

Although both fishing exploitation and the environment affect the population dynamics of marine resources (Hughes et al., 2003; Hsieh et al., 2006; Cury et al., 2008), their responses can be different depending on the species, taxonomic groups and their predominance in the communities. In this sense, although studies covering the entire epibenthic community are essential for implementing an ecosystem-based fishery management, species-based approaches are also necessary to understand single populations and to analyse how they contribute to the general trends of the entire community. However, analysis of indicators at single species level has provided a good response to know the status of the population, whereas the multi-species indicators have revealed some difficulties in interpretation (Ceriola et al., 2008). In fact, ecosystem considerations do not substitute for what is already known from a single species approach (Link, 2002) and the ecosystem-based approach can be implemented in systems with different levels of information and uncertainty (Pikitch et al., 2004). Finally, it is important to take into account that although fishery-independent information provides high quality and useful data, we have to take the results carefully for those species with important seasonal variations in their catchability and population dynamics and for those communities where these species predominate.

Acknowledgments The authors wish to thank to all the crew of the R/V *Francisco de Paula Navarro* and *Cornide de Saavedra* and all the participants who took part in the BALAR and MEDITS surveys. These surveys were carried out in the framework of the MEDER, DEMO, EVADEM and EVADEMED projects, financed by IEO and, from 2007, by the EU. Dr. Gabriel Jordà and Dr. Mar Flexas provided the data of the hydrodynamic model. This study was funded by an ECOSUMMER fellowship to BG from the Marine Curie Research Training Network under the EU Sixth Framework Programme.

References

Abella, A., A. Belluscio, J. Bertrand, P. L. Carbonara, D. Giordano, M. Sbrana & A. Zamboni, 1999. Use of MEDITS trawl survey data and commercial fleet information for the assessment of some Mediterranean demersal resources. Aquatic Living Resources 12: 155–166.

Abelló, P., A. Abella, A. Adamidou, S. Jukic-Peladic, P. Maiorano & M. T. Spedicato, 2002a. Geographical patterns in abundance and population structure of *Nephrops norvegicus* and *Parapenaeus longirostris* (Crustacea: Decapoda) along the European Mediterranean coasts. Scientia Marina 66: 125–141.

Abelló, P., A. Carbonell & P. Torres, 2002b. Biogeography of epibenthic crustaceans on the shelf and upper slope off the Iberian Peninsula Mediterranean coasts: implications for the establishment of natural management areas. Scientia Marina 66: 183–198.

Acosta, J., M. Canals, J. López-Martínez, A. Muñoz, P. Herranz, R. Urgeles, C. Palomo & J. L. Casamor, 2002. The Balearic Promontory geomorphology (western Mediterranean): morphostructure and active processes. Geomorphology 49: 177–204.

Acosta, J., M. Canals, A. Carbó, A. Muñoz, R. Urgeles, A. Muñoz-Martín & E. Uchupi, 2004. Sea floor morphology and plio-quaternary sedimentary cover of the Mallorca channel, Balearic islands, western Mediterranean. Marine Geology 206: 165–179.

Aldebert, Y., 1997. Demersal resources of the Gulf of Lions (Mediterranean). Impact on fish diversity. Vie et Milieu 47: 275–284.

Alemany, F. & F. Alvárez, 2003. Determination of effective fishing effort on hake *Merluccius merluccius* in a Mediterranean trawl fishery. Scientia Marina 67: 491–499.

Bertrand, J. A., Y. Aldebert & A. Souplet, 1998. Temporal variability of demersal species in the Gulf of Lions from trawl surveys (1983-1997). IFREMER Actes des Colloques 26: 153–164.

Bertrand, J. A., L. G. de Sola, C. Papaconstantinou, G. Relini & A. Souplet, 2002. The general specifications of the MEDITS surveys. Scientia Marina 66: 9–17.

Bianchi, G., H. Gislason, K. Graham, L. Hill, X. Jin, K. Koranteng, S. Manickchand-Heileman, I. Paya, K. Sainsbury, F. Sánchez & K. Zwanenburg, 2000. Impact of fishing on size composition and diversity of demersal fish communities. ICES Journal of Marine Science 57: 558–571.

Bosc, E., A. Bricaud & D. Antoine, 2004. Seasonal and interannual variability in algal biomass and primary production in the Mediterranean Sea, as derived from 4 years of SeaWiFS observations. Global Biogeochemical Cycles 18: GB1005.

Browman, H. I. & K. I. Stergiou, 2004. Perspectives on ecosystem-based approaches to the management of marine resources. Marine Ecology-Progress Series 274: 269–270.

Caddy, J. F., 1993. Some future perspectives for assessment and management of Mediterranean fisheries. Scientia Marina 57: 121–130.

Caddy, J. F., 1999. Deciding on precautionary management measures for a stock based on a suite of Limit Reference Points (LRPs) as abasis for a multi-LRP harvest law. NAFO Scientific Council Studies 32: 55–68.

Caddy, J. F., 2002. Limit reference points, traffic lights, and holistic approaches to fisheries management with minimal stock assessment input. Fisheries Research 56: 133–137.

Caddy, J. F., E. Wade, T. Surette, M. Hebert & M. Moriyasu, 2005. Using an empirical traffic light procedure for monitoring and forecasting in the Gulf of St Lawrence fishery for the snow crab, *Chionoecetes opilio*. Fisheries Research 76: 123–145.

Canals, M., P. Puig, X. D. de Madron, S. Heussner, A. Palanqués & J. Fabres, 2006. Flushing submarine canyons. Nature 444: 354–357.

Carbonell, A. & M. Azevedo, 2003. Application of non-equilibrium production models to the red shrimp (*Aristeus antennatus*, Risso, 1816) fishery in the northwestern Mediterranean. Fisheries Research 65: 323–334.

Carbonell, A., M. Carbonell, M. Demestre, A. Grau & S. Monserrat, 1999. The red shrimp *Aristeus antennatus* (Risso, 1816) fishery and biology in the Balearic Islands, Western Mediterranean. Fisheries Research 44: 1–13.

Carbonell, A., J. Lloret & M. Demestre, 2008. Relationship between condition and recruitment success of red shrimp (*Aristeus antennatus*) in the Balearic Sea (Northwestern Mediterranean). Journal of Marine Systems 71: 403–412.

Carney, R. S., 2005. Zonation of deep biota on continental margins. Oceanography and Marine Biology: An Annual Review 43: 211–278.

Cartes, J. E., J. Rey, D. Lloris & L. Gil de Sola, 2004. Influence of environmental variables on the feeding and diet of European hake (*Merluccius merluccius*) on the Mediterranean Iberian coasts. Journal of the Marine Biological Association of the United Kingdom 84: 831–835.

Cartes, J. E., T. Madurell, E. Fanelli & J. L. López-Jurado, 2008a. Dynamics of suprabenthos-zooplankton communities around the Balearic Islands (western Mediterranean): influence of environmental variables and effects on the biological cycle of *Aristeus antennatus*. Journal of Marine Systems 71: 316–335.

Cartes, J. E., V. Papiol & B. Guijarro, 2008b. The feeding and diet of the deep-sea shrimp *Aristeus antennatus* off the Balearic Islands (Western Mediterranean): influence of environmental factors and relationship with the biological cycle. Progress in Oceanography 79: 37–54.

Ceriola, L., N. Ungaro & F. Toteda, 2007. A "Traffic" Light approach for the assessment of the Broadtail shortfin squid Illex coindetii Verany, 1839 in the Southern Adriatic Sea (Central Mediterranean). Reviews in Fish Biology and Fisheries 17: 145–157.

Ceriola, L., P. Accadia, P. Mannini, F. Massa, N. Milone & N. Ungaro, 2008. A bio-economic indicators suite for the appraisal of the demersal trawl fishery in the Southern Adriatic Sea (Central Mediterranean). Fisheries Research 92: 255–267.

Champalbert, G., 1996. Characteristics of zooplankton standing stock and communities in the Western Mediterranean Sea: relations to hydrology. Scientia Marina 60: 97–113.

Clarke, K. R., 1990. Comparisons of dominance curves. Journal of Experimental Marine Biology and Ecology 138: 143–157.

Clarke, K. R., 1993. Nonparametric multivariate analyses of changes in community structure. Australian Journal of Ecology 18: 117–143.

Clarke, K. R. & R. M. Warwick, 1994. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. Plymouth Marine Laboratory, Plymouth.

Colloca, F., M. Cardinale, A. Belluscio & G. Ardizzone, 2003. Pattern of distribution and diversity of demersal assemblages in the central Mediterranean sea. Estuarine, Coastal and Shelf Science 56: 469–480.

Company, J. B. & F. Sardà, 2000. Growth parameters of deep-water decapod crustaceans in the Northwestern Mediterranean Sea: a comparative approach. Marine Biology 136: 79–90.

Cury, P. M., Y. J. Shin, B. Planque, J. M. Durant, J. M. Fromentin, S. Kramer-Schadt, N. C. Stenseth, M. Travers & V. Grimm, 2008. Ecosystem oceanography for global change in fisheries. Trends in Ecology & Evolution 23: 338–346.

Daget, J., 1976. Ordination des profils ecologiques. Naturalia Monspeliensia. Série botanique 26: 109–128.

DFO, 2005. Northern Shrimp on the Eastern Scotial Shelf (SFA 13-15). DFO Canadian Science Advisory Secretariat. Science Advisory Reports 2005/060.

Dimech, M., M. Camilleri, J. G. Hiddink, M. J. Kaiser, S. Ragonese & P. J. Schembri, 2008. Differences in demersal community structure and biomass size spectra within and outside the Maltese Fishery Management Zone (FMZ). Scientia Marina 72: 669–682.

Dremière, P. Y., L. Fiorentini, G. Cosimi, I. Leonori, A. Sala & A. Spagnolo, 1999. Escapement from the main body of the bottom trawl used for the Mediterranean international trawl survey (MEDITS). Aquatic Living Resources 12(3): 207–217.

Estrada, M., 1996. Primary production in the northwestern Mediterranean. Scientia Marina 60: 55–64.

Farrugio, H., P. Oliver & F. Biagi, 1993. An overview of the history, knowledge, recent and future research trends in Mediterranean fisheries. Scientia Marina 57: 105–119.

Fernández de Puelles, M. L., J. Valencia & L. Vicente, 2004. Zooplankton variability and climatic anomalies from 1994 to 2001 in the Balearic Sea (Western Mediterranean). ICES Journal of Marine Science 61: 492–500.

Fiorentini, L., P. Y. Dremière, I. Leonori, A. Sala & V. Palumbo, 1999. Efficiency of the bottom trawl used for the Mediterranean international trawl survey (MEDITS). Aquatic Living Resources 12(3): 187–205.

Gaertner, J. C., J. A. Bertrand, L. G. de Sola, J. P. Durbec, E. Ferrandis & A. Souplet, 2005. Large spatial scale variation of demersal fish assemblage structure on the continental shelf of the NW Mediterranean Sea. Marine Ecology Progress Series 297: 245–257.

García-Rodríguez, M. & A. Esteban, 1999. On the biology and fishery of *Aristeus antennatus* (Risso, 1816), (Decapoda, Dendrobranchiata) in the Ibiza Channel (Balearic Islands, Spain). Scientia Marina 63: 27–37.

Gislason, H. & J. Rice, 1998. Modelling the response of size and diversity spectra of fish assemblages to changes in exploitation. ICES Journal of Marine Science 55: 362–370.

Gristina, M., T. Bahri, F. Fiorentino & G. Garofalo, 2006. Comparison of demersal fish assemblages in three areas of the Strait of Sicily under different trawling pressure. Fisheries Research 81: 60–71.

Guijarro, B. & E. Massutí, 2006. Selectivity of diamond- and square-mesh codends in the deepwater crustacean trawl fishery off the Balearic Islands (western Mediterranean). ICES Journal of Marine Science 63: 52–67.

Guijarro, B., E. Massutí, J. Moranta & P. Díaz, 2008. Population dynamics of the red shrimp *Aristeus antennatus* in the Balearic Islands (western Mediterranean): short spatio-temporal differences and influence of environmental factors. Journal of Marine Systems 71: 385–402.

Guijarro, B., E. Massutí, J. Moranta & J. E. Cartes, 2009a. Short spatio-temporal variations in the population dynamics and biology of the deep-water rose shrimp *Parapenaeus longirostris* (Decapoda: Crustacea) in the western Mediterranean. Scientia Marina 73: 183–197.

Guijarro, B., M. Valls, F. Ordines & E. Massutí, 2009b. Stock assessment of Norway lobster (*Nephrops norvegicus*) from GFCM-GSA05 (Balearic Islands). General Fisheries Commission for the Mediterranean. Scientific Advisory Committee. Sub-Committee on Stock Assessment.

Hall, S. J., J. S. Collie, D. E. Duplisea, S. Jennings, M. Bravington & J. Link, 2006. A length-based multispecies model for evaluating community responses to fishing. Canadian Journal of Fisheries and Aquatic Sciences 63: 1344–1359.

Halliday, R. G., L. P. Fanning & R. K. Mohn, 2001. Use of the Traffic Light Method in Fishery Management Planning. Canadian Science Advisory Secretariat Research Document 2001/108.

Hastie, T. J. & R. J. Tibshirani, 1990. Generalized Additive Models. Chapman and Hall, New York.

Hilborn, R., 2003. The state of the art in stock assessment: where we are and where we are going. Scientia Marina 67: 15–20.

Hsieh, C. H., C. S. Reiss, J. R. Hunter, J. R. Beddington, R. M. May & G. Sugihara, 2006. Fishing elevates variability in the abundance of exploited species. Nature 443: 859–862.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. B. C. Jackson, J. Kleypas, J. M. Lough, P. Marshall, M. Nystrom, S. R. Palumbi, J. M. Pandolfi, B. Rosen & J. Roughgarden, 2003. Climate change, human impacts, and the resilience of coral reefs. Science 301: 929–933.

Jennings, S., 2005. Indicators to support an ecosystem approach to fisheries. Fish and Fisheries 6: 212–232.

Jennings, S., S. P. R. Greenstreet, L. Hill, G. J. Piet, J. K. Pinnegar & K. J. Warr, 2002. Long-term trends in the trophic structure of the North Sea fish community: evidence from stable-isotope analysis, size-spectra and community metrics. Marine Biology 141: 1085–1097.

Jouffre, D. & C. A. Inejih, 2005. Assessing the impact of fisheries on demersal fish assemblages of the Mauritanian continental shelf, 1987–1999, using dominance curves. ICES Journal of Marine Science 62: 380–383.

Kempler, S., 2009. Ocean Color Time-Series Online Visualization and Analysis [available on internet at http://reason.gsfc.nasa.gov/Giovanni/] August 2008.

Koeller, P., L. Savard, D. G. Parsons & C. Fu, 2000. A precautionary approach to assessment and management of shrimp stocks in the Northwest Atlantic. Journal of Northwest Atlantic Fishery Science 27: 235–246.

Labropoulou, M. & C. Papaconstantinou, 2000. Community structure of deep-sea demersal fish in the North Aegean Sea (northeastern Mediterranean). Hydrobiologia 440: 281–296.

Le Cren, E. D., 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology 20: 201–219.

Link, J. S., 2002. What does ecosystem-based fisheries management mean? Fisheries 27: 18–21.

Lleonart, J. & F. Maynou, 2003. Fish stock assessments in the Mediterranean: state of the art. Scientia Marina 67: 37–49.

Lombarte, A., L. Recasens, M. González & L. G. de Sola, 2000. Spatial segregation of two species of Mullidae (*Mullus surmuletus* and *M* barbatus) in relation to habitat. Marine Ecology Progress Series 206: 239–249.

López-Jurado, J. L., M. Marcos & S. Monserrat, 2008. Hydrographic conditions affecting two fishing grounds of Mallorca island (Western Mediterranean): during the IDEA Project (2003–2004). Journal of Marine Systems 71: 303–315.

Massutí, E. & J. Moranta, 2003. Demersal assemblages and depth distribution of elasmobranchs from the continental shelf and slope off the Balearic Islands (western Mediterranean). ICES Journal of Marine Science 60: 753–766.

Massutí, E. & O. Reñones, 2005. Demersal resource assemblages in the trawl fishing grounds off the Balearic Islands (western Mediterranean). Scientia Marina 69: 167–181.

Massutí, E., O. Reñones, A. Carbonell & P. Oliver, 1996. Demersal fish communities exploited on the continental shelf and slope off Majorca (Balearic Islands, NW Mediterranean). Vie et Milieu 46: 45–55.

Massutí, E., J. D. M. Gordon, J. Moranta, S. C. Swan, C. Stefanescu & N. R. Merrett, 2004. Mediterranean and Atlantic deep-sea fish assemblages: differences in biomass composition and size-related structure. Scientia Marina 68: 101–115.

Maynou, F. & J. E. Cartes, 2000. Community structure of bathyal decapod crustaceans off south-west Balearic Islands (western Mediterranean): seasonality and regional patterns in zonation. Journal of the Marine Biological Association of the United Kingdom 80: 789–798.

Maynou, F. & F. Sardà, 1997. *Nephrops norvegicus* population and morphometrical characteristics in relation to substrate heterogeneity. Fisheries Research 30: 139–149.

Mendes, B., P. Fonseca & A. Campos, 2004. Weight-length relationships for 46 fish species of the Portuguese west coast. Journal of Applied Ichthyology 20: 355–361.

Merella, P., A. Quetglas, F. Alemany & A. Carbonell, 1997. Length-weight relationship of fishes and cephalopods from the Balearic Islands (western Mediterranean). Naga, The Worldfish Center Quarterly 20: 66–68.

Merella, P., F. Alemany, A. Carbonell & A. Quetglas, 1998. Fishery and biology of Norway lobster *Nephrops norvegicus* (Decapoda: Nephropidae) in Mallorca (western Mediterranean). Journal of Natural History 32: 1631–1640.

Mitchel, T. M., 1997. Machine Learning. McGraw-Hill, New York.

Monserrat, S., J. L. López-Jurado & M. Marcos, 2008. A mesoscale index to describe the regional circulation around the Balearic Islands. Journal of Marine Systems 71: 413–420.

Moranta, J., C. Stefanescu, E. Massutí, B. Morales-Nin & D. Lloris, 1998. Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian basin, western Mediterranean). Marine Ecology Progress Series 171: 247–259.

Moranta, J., E. Massutí & B. Morales-Nin, 2000. Fish catch composition of the deep-sea decapod crustacean fisheries in the Balearic Islands (western Mediterranean). Fisheries Research 45: 253–264.

Moranta, J., A. Quetglas, E. Massutí, B. Guijarro, M. Hidalgo & P. Díaz, 2008. Spatio-temporal variations in deep-sea demersal communities off the Balearic Islands (western Mediterranean). Journal of Marine Systems 71: 346–366.

Morey, G., J. Moranta, E. Massutí, A. Grau, M. Linde, F. Riera & B. Morales-Nin, 2003. Weight-length relationships of littoral to lower slope fishes from the western Mediterranean. Fisheries Research 62: 89–96.

Nicholson, M. D. & S. Jennings, 2004. Testing candidate indicators to support ecosystem-based management: the power of monitoring surveys to detect temporal trends in fish community metrics. ICES Journal of Marine Science 61: 35–42.

Oliver, P., 1993. Analysis of fluctuations observed in the trawl fleet landings of the Baleric Islands. Scientia Marina 57: 219–227.

Ordines, F. & E. Massutí, 2009. Relationships between macro-epibenthic communities and fish on the shelf grounds of the western Mediterranean. Aquatic Conservation-Marine and Freshwater Ecosystems 19: 370–383.

Ordines, F., E. Massutí, B. Guijarro & R. Mas, 2006. Diamond vs square mesh codend in a multi-species trawl fishery of the western Mediterranean: effects on catch composition, yield, size selectivity and discards. Aquatic Living Resources 19: 329–338.

Palmer, M., A. Quetglas, B. Guijarro, J. Moranta, F. Ordines & E. Massutí, 2009. Performance of artificial neural networks and discriminant analysis in predicting fishing tactics from multispecific fisheries. Canadian Journal of Fisheries and Aquatic Sciences 66: 224–237.

Papaconstantinou, C. & K. Kapiris, 2003. The biology of the giant red shrimp (*Aristaeomorpha foliacea*) at an unexploited fishing ground in the Greek Ionian Sea. Fisheries Research 62: 37–51.

Pikitch, E. K., C. Santora, E. A. Babcock, A. Bakun, R. Bonfil, D. O. Conover, P. Dayton, P. Doukakis, D. Fluharty, B. Heneman, E. D. Houde, J. Link, P. A. Livingston, M. Mangel, M. K. McAllister, J. Pope & K. J. Sainsbury, 2004. Ecosystem-based fishery management. Science 305: 346–347.

Pinot, J. M., J. Tintoré & D. Gomis, 1995. Multivariate analysis of the surface circulation in the Balearic Sea. Progress in Oceanography 36: 343–376.

Pinot, J. M., J. L. López-Jurado & M. Riera, 2002. The CANALES experiment (1996–1998). Interannual, seasonal, and mesoscale variability of the circulation in the Balearic Channels. Progress in Oceanography 55: 335–370.

Quetglas, A., F. Alemany, A. Carbonell, P. Merella & P. Sánchez, 1998a. Biology and fishery of Octopus vulgaris Cuvier, 1797, caught by trawlers in Mallorca (Balearic Sea, western Mediterranean). Fisheries Research 36: 237–249.

Quetglas, A., F. Alemany, A. Carbonell, P. Merella & P. Sánchez, 1998b. Some aspects of the biology of *Todarodes sagittatus* (Cephalopoda: Ommastrephidae) from the Balearic sea (western Mediterranean). Scientia Marina 62: 73–82.

Quetglas, A., A. Carbonell & P. Sánchez, 2000. Demersal continental shelf and upper slope cephalopod assemblages from the balearic sea (north-western Mediterranean). Biological aspects of some deep-sea species. Estuarine, Coastal and Shelf Science 50: 739–749.

Quinlan, R. J., 1993. C4.5: Programs for Machine Learning. Morgan Kaufmann, San Mateo, CA.

Rochet, M. J. & V. M. Trenkel, 2003. Which community indicators can measure the impact of fishing? A review and proposals. Canadian Journal of Fisheries and Aquatic Sciences 60: 86–99.

Sardà, F. & F. Maynou, 1998. Assessing perceptions: do Catalan fishermen catch more shrimp on Fridays? Fisheries Research 36: 149–157.

Sardà, F., J. E. Cartes & W. Norbis, 1994. Spatiotemporal structure of the deep-water shrimp *Aristeus antennatus* (Decapoda, Aristeidae) population in the Western Mediterranean. Fishery Bulletin 92: 599–607.

Sardà, F., F. Maynou & L. Tallo, 1997. Seasonal and spatial mobility patterns of rose shrimp *Aristeus antennatus* in the western Mediterranean: results of a long-term study. Marine Ecology Progress Series 159: 133–141.

Sardà, F., J. Lleonart & J. E. Cartes, 1998. An analysis of the population dynamics of *Nephrops norvegicus* (L.) in the Mediterranean Sea. Scientia Marina 62: 135–143.

Serrano, A., M. Ramón, N. Sánchez, F. Ordines, B. Guijarro, J. Moranta, A. Quetglas & E. Massutí, 2008. Benthic assemblages of the Balearic Islands: role of environmental variables. XV Simpósio Ibérico de Estudos de Biologia Marinha.Madeira (Portugal).

Stevens, J. D., R. Bonfil, N. K. Dulvy & P. A. Walker, 2000. The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES Journal of Marine Science 57: 476–494.

ter Braak, C. J. F. & P. Smilauer, 2002. CANOCO Reference Manual and User's Guide to Canofo for Windows: Software for Canonical Community Ordination (Version 4.5). Microcomputer Power, Ithaca.

Trenkel, V. M. & M. J. Rochet, 2003. Performance of indicators derived from abundance estimates for detecting the impact of fishing on a fish community. Canadian Journal of Fisheries and Aquatic Sciences 60: 67–85.

Tserpes, G. & P. Peristeraki, 2002. Trends in the abundance of demersal species in the southern Aegean Sea. Scientia Marina 66: 243–252.

Tudela, S., F. Sarda, F. Maynou & M. Demestre, 2003. Influence of submarine canyons on the distribution of the deep-water shrimp, *Aristeus antennatus* (Risso, 1816) in the NW Mediterranean. Crustaceana 76: 217–225.

Warwick, R. M., 1986. A new method for detecting pollution effects on marine macrobenthic communities. Marine Biology 92: 557–562.

Hydrobiologia (2011) 670:87–109
DOI 10.1007/s10750-011-0722-4

ECOSYSTEMS AND SUSTAINABILITY

Spatiotemporal patterns of marine mammal distribution in coastal waters of Galicia, NW Spain

Evangelos Spyrakos · Tania C. Santos-Diniz · Gema Martinez-Iglesias · Jesus M. Torres-Palenzuela · Graham J. Pierce

Published online: 6 May 2011
© Springer Science+Business Media B.V. 2011

Abstract The spatial and seasonal distribution of cetaceans and possible links with environmental conditions were studied at the Galician continental shelf. Data were collected between February–August 2001 and June–September 2003 during opportunistic surveys onboard fishing boats. Seven species of cetaceans were identified from 250 sightings of 6,846 individuals. The common dolphin (*Delphinus delphis*) was by far the most frequently sighted and the most widely distributed species. Spatiotemporal trends in cetacean distribution and abundance, and their relationships with environmental parameters (sea depth, SST and chlorophyll-*a*) were quantified using generalised additive models (GAMs). Results for all cetaceans were essentially the same as for common dolphins alone. Modelling results indicated that the number of common dolphin sightings per unit effort was higher further south. The number of individual common dolphins seen per sighting of this species (i.e. group size) was however higher in the north and west of the study area, higher later in the year and higher in 2001 than in 2003. In contrast, the number of common dolphin calves seen (per sighting of this species) was higher in the south. Models including environmental variables indicated larger common dolphin group sizes in deeper waters and at higher chlorophyll concentrations (i.e. in more productive areas). There was also a positive relationship between survey effort and group size, which is probably an artefact of the tendency of the survey platforms (fishing boats) to spend most time in areas of high fish abundance. Numbers of common dolphin calves per sighting were found to be higher in shallower waters. The results are consistent with common dolphins foraging mainly in deeper waters of the Galician continental shelf, while more southern inshore waters may represent a nursery area.

Keywords Cetaceans sighting · GAMs · GIS · Galician waters

Introduction

Defining geographical ranges and distribution limits for highly mobile marine species such as cetaceans is

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

E. Spyrakos · T. C. Santos-Diniz · G. Martinez-Iglesias · J. M. Torres-Palenzuela (✉)
Remote Sensing and GIS Laboratory, Department of Applied Physics, Sciences Faculty, University of Vigo, Campus Lagoas Marcosende, Vigo, Spain
e-mail: jesu@uvigo.es

G. J. Pierce
School of Biological Sciences (Zoology), University of Aberdeen, Aberdeen, UK

G. J. Pierce
Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, Spain

intrinsically difficult. Nevertheless, many studies have shown that the distribution of cetaceans (especially in relation to foraging areas) is linked to environmental features, both physiographic (e.g. water depth) and oceanographic (such as temperature and chlorophyll-*a* (chl-*a*) concentrations) at various scales (e.g. Evans, 1987; Baumgartner et al., 2001; Murase et al., 2002; Tynan et al., 2005; Marubini et al., 2009; Scott et al., 2010). Such relationships may be either direct or indirect. Thus, temperature may have direct and indirect effects on cetacean distribution, for example through its effects on the energetic costs of thermoregulation (MacLeod et al., 2009) and on the distribution of fish, cephalopod and zooplankton prey (Rubín, 1994; Baumgartner, 1997; Davis et al., 1998; Murase et al., 2002; Tynan et al., 2005). As evident from recent interest in defining characteristics of Essential Fish Habitat (e.g. Valavanis, 2008), the distributions of fish and cephalopods have been found to be related to numerous oceanographic and environmental features, including depth (Gil de Sola, 1993), upwelling (Guerra, 1992; Rubín, 1997) and fronts, which create hotspots of primary and secondary production (Rubín, 1994).

The horizontal and vertical mobility of the prey of cetaceans, combined with temporal variability, make it difficult to predict habitat use of cetaceans over small spatial and temporal scales. In general, it is easier to measure environmental parameters accurately than fine-scale prey distribution. According to Torres et al. (2008), environmental parameters can generate better models of cetacean habitat preferences than models derived from prey distribution data, due to the difficulty of accurately measuring the latter at an appropriate scale.

Understanding the relationships between cetacean distribution and environmental factors is necessary to identify cetacean habitat requirements, to predict their distribution and provide insights into their feeding habits. In turn, this provides valuable information to underpin conservation measures directed at cetaceans, for example identifying areas suitable for designation as Special Areas of Conservation (as required under the EU ‘Habitats Directive’, Directive 92/43/EEC, in relation to bottlenose dolphins and harbour porpoises) and mitigating impacts of anthropogenic threats such as naval sonar trials, collisions with ships and fishery by-catch (e.g. Redfern et al., 2006). In addition, implementation of the Ecosystem Approach to Fisheries Management (EAFM) and the Marine Strategy Framework Directive (MSFD) require collection of data on the status of all ecosystem components, including top predators.

Over the last two decades, most studies on cetacean ecology and conservation in the coastal waters of Galicia (NW Spain), e.g. on interactions with fisheries (López et al., 2003), have been carried out by or based on data and samples provided by the non-governmental organisation Coordinadora para o Estudio dos Mamíferos Mariños (CEMMA, see López et al., 2002). Diets of common and bottlenose dolphins along the Galician coast have been described in several previous studies (e.g. González et al., 1994; Santos et al., 2004, 2007). The most important prey of common dolphins in Galician waters are blue whiting (*Micromesistius poutassou*) and sardine (*Sardina pilchardus*) (Santos et al., 2004) while the most important prey of bottlenose dolphin are blue whiting and hake (*Merluccius merluccius*). The majority of the main prey species of these cetaceans are of high commercial importance in Galician waters. Although there considerable overlap in the diets of the three main cetacean species in these waters (e.g. the generally high importance of blue whiting), dietary differences may reflect different habitat preferences.

Geographical Information Systems (GIS) offer a powerful tool in ecosystem studies, facilitating mapping of species occurrence and abundance in relation to a range of environmental variables, construction of empirical habitat preference models and suggesting hypotheses about mechanisms that determine species distribution (e.g. Meaden & Do Chi, 1996; Sakurai et al., 2000; Eastwood et al., 2001; Wang et al., 2003; Koubbi et al., 2006). Among the statistical tools available for constructing habitat models, Generalised Additive Models (GAMs), first proposed by Hastie & Tibshirani (1990), are particularly appropriate. GAM is a non-parametric generalisation of linear regression, allowing non-normal distributions and non-linear relationships between an independent variable and multiple predictors. In the context of variation of species abundance along ecological gradients, non-linear relationships are more common than linear relationships (Oksanen & Minchin, 2002), while the capability to use non-normal distributions permits the use of presence–absence (bionomial) or count (e.g. Poisson or negative binomial) data as response variables. GAMs have been regularly used to analyse distributions of commercially exploited marine species in relation to geographical and environmental variables (e.g. Swartzman et al., 1992; Daskalov 1999; Bellido et al., 2001; Maravelias & Papaconstantinou, 2003; Valavanis et al., 2008) and there are increasing numbers of applications to marine mammal habitat use (see Redfern et al., 2006 for a review).

A frequent problem in studies of marine mammal distribution is that dedicated surveys are time-consuming and expensive. An alternative is to use opportunistically collected sightings data, e.g. from observers place on ferries or fishing boats. Clearly, this tends to result in imperfect survey designs, with non-random distribution of survey effort, so that variation in survey effort must be taken into account in the model-building process. In addition, when data are collected by fishery observers, the efficiency of detection of marine mammals is inevitably reduced (especially when the catch is being sampled), and the reliability of absence records may therefore be doubtful.

There are few published studies about marine mammal distribution in Galician waters. López et al. (2004) summarised results on cetacean distribution and relative abundance from opportunistic boat-based surveys in Galician waters during 1998 and 1999. Pierce et al. (2010) reported on spatiotemporal and environmental trends in land-based sightings of cetaceans along the Galician coast and identified some broad-scale relationships between local cetacean occurrence and productivity. However, there have been no similar studies on relationships between at-sea cetacean occurrence and oceanographic parameters (e.g. SST and chl-*a* concentration) in this area.

The present study utilises GIS and statistical modelling to analyse data collected by fishery observers during 2001 and 2003 and aims to (a) describe spatiotemporal (geographical, seasonal, between-year) trends in distribution of different cetacean species in Galician continental shelf waters, (b) test whether relative local abundance is dependent on environmental conditions, specifically, depth, SST and chl-*a* concentration, (c) for the most common cetacean species (common dolphin), to identify potential 'nursery areas' (i.e. where calves were present) and determine their characteristics.

Methodology

Study area

Galicia (NW Spain) has a coastline of about 1,200 km (Fig. 1). It has a relatively narrow continental shelf with a total surface area of approximately 15,000 km^2 (Fariña et al., 1997). The Galician continental shelf and the Galician rías (coastal fjords according to Vidal-Romaní, 1984) lie at the northern edge of one of the major upwelling areas in the world, the eastern boundary system off NW Africa and SW Europe (Wooster et al., 1976). The frequent upwelling of cold and dense North Atlantic Central Water (NACW) results in nutrient enrichment of the area (Blanton et al., 1984) and this area is among the most productive oceanic regions of the world. Upwelling reaches its highest intensity during summer (April to September) (Fraga, 1981; Prego & Bao, 1997). Up to 300 species of fish (Solórzano et al., 1988) and around 80 species of cephalopods (Guerra, 1992) have been recorded in Galician coastal waters. The area constitutes an important nursery ground for several commercially important fish species, e.g. hake, *Merluccius merluccius* (Pereiro et al., 1980; Fariña et al., 1985). The broad-scale distribution of fish assemblages over the continental shelf area is mainly determined by depth and hydrographic structure and, in general, density, biomass and species richness all decrease with increasing depth (Fariña et al., 1997), reflecting the general phenomenon that species with more restricted depth ranges tend to occur in the shallowest waters (Smith & Brown, 2002). Galician waters are also an important area for marine mammals, including 16 cetacean and four pinniped species. Resident cetaceans in Galicia include the common dolphin (*Delphinus delphis*), the bottlenose dolphin (*Tursiops truncatus*) and the harbour porpoise (*Phocoena phocoena*). These three species are seen all along the Galician coast, although with different geographical patterns of local abundance (López et al., 2004; Pierce et al., 2010), and are listed as vulnerable in Spain's National Endangered Species Act (Cañadas et al., 2002).

Data collection and processing

Cetacean sightings data were collected from Galician coastal waters by four observers on-board a total of

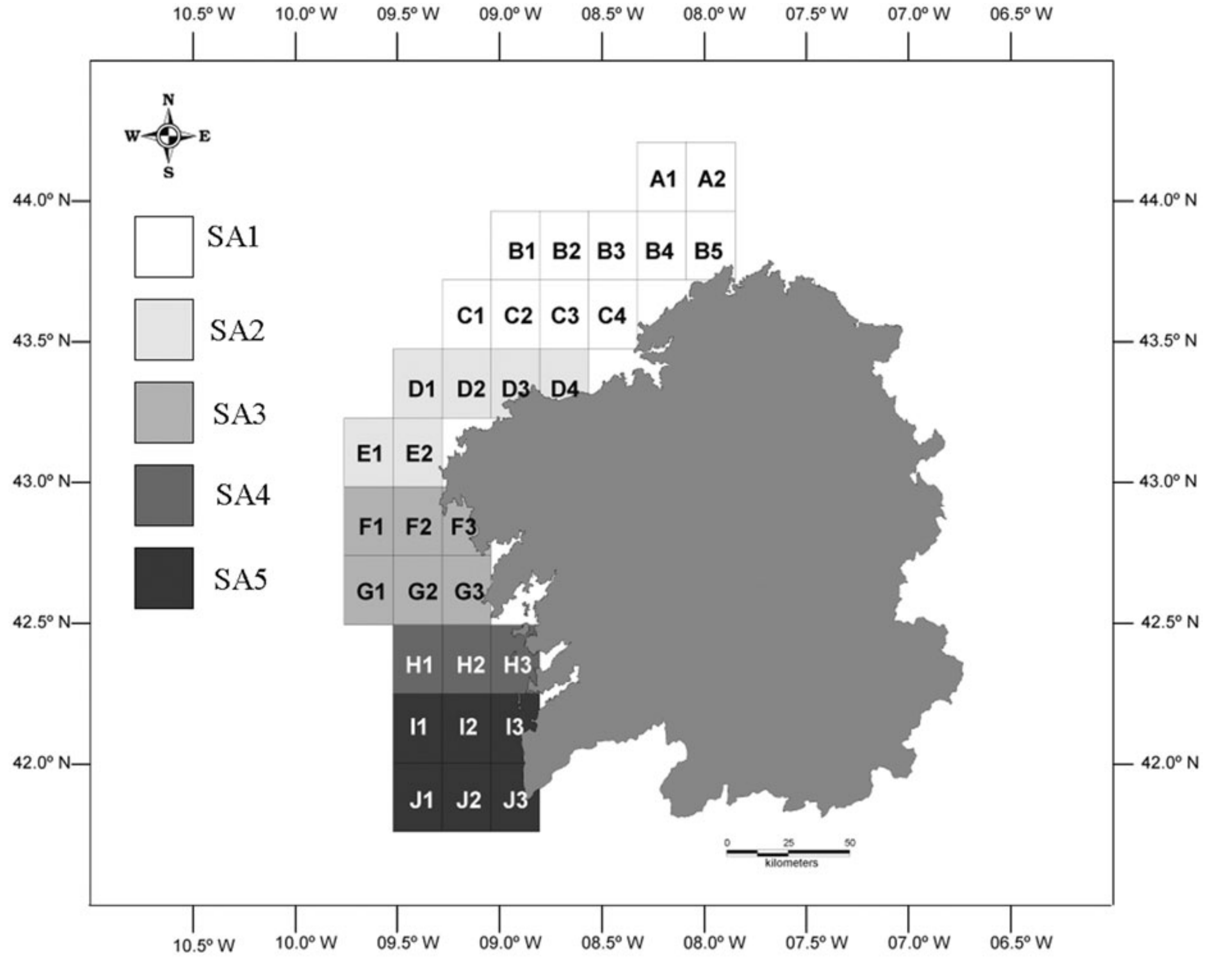


Fig. 1 Map of Galicia and its coastal waters, showing the grid system used (32 cells, from with A1 and A2 in the north to J1… J3 in the south). The five sub-areas are identified by differing shading (north to south: SA1, SA2, SA3, SA4 and SA5)

16 Galician fishing vessels working out of ports all along the Galician coast (between A Guardia in the south and A Coruña in the north), during February–August 2001 and June–September 2003. The survey routes were determined by the primary activity of the fishing vessels (i.e. fishing and travelling to and from fishing grounds).

The observers were responsible for monitoring both fishing activity and presence of cetaceans. All observers had previous experience in detecting and counting cetaceans at sea. While an observer was on-board, vessel location was recorded using a GPS system, every 15 min (providing a set of 'control points' and allowing effort to be quantified in terms of 15-min periods) and whenever the boat changed its route. Cetacean observations were carried out continuously during daylight hours except while catch compositions were being recorded. All cetacean observations involved scanning with the naked eye supplemented by occasional scans with binoculars.

The following data were recorded for each cetacean sighting: location of the fishing vessel, identity (to the lowest possible taxonomic grouping; to species where possible), number of individuals present (the average of minimum and maximum counts of individuals), presence and number of calves and a description of behaviour. Distance and bearing of the cetacean from the boat were not recorded and boat position was therefore used as a proxy for the location of sighted cetaceans. Nevertheless, most sightings are thought to have been within 1 km of the position of the boat and in any case the final analysis uses a coarser-scale (grid cell) spatial resolution.

The study area was divided into a base-grid of 32 cells of dimensions 14′12″ longitude and 14′42″ latitude (area approximately 530 km^2), which covered the area of Galician coastal waters between latitudes 41° to 45°N and longitudes 7° to 12°W. This grid size was a compromise between the aim of determining environmental relationships and the need to avoid the majority of cells having no sightings (this being a function of the amount of survey effort). A grid-based approach also reduces potential problems with autocorrelation in the data. Five sub-areas were also defined along the north–south axis (Fig. 1). Survey routes and sightings positions data were imported into GIS (MapInfo; Idrisi Taiga). The system used included detailed bathymetry data.

Satellite-derived sea surface temperature (SST) data were sourced from Plymouth Marine Laboratory (Natural Environment Research Council, UK). All level-2 images used in this study were geo-referenced and masked out manually in black for clouds, land and sun-glint. The SST images were from the AVHRR (Advanced Very High Resolution Radiometer) sensor onboard the NOAA satellite series. Satellite-derived images of chl-*a* concentration were from the SeaWIFS (Sea Viewing Wide Field-of-View Sensor) colour sensor. Treatment of SeaWIFS images included application of nearest neighbour interpolation. Raster data were extracted on a standard digital 0–255 colour or grey-scale value for each pixel. Chl-*a* concentration is calculated based to the reflectance ratio between 490 and 555 nm (McClain, 1997). Both satellite sensors provide data with a 1.1 km on-ground resolution in nadir.

The conversion from the standard Digital Number (DN) 0–255 scale integer value stored in the image, to obtain the real-world SST values (°C), used the AVHRR Oceans Pathfinder SST algorithm (Walton, 1988; Walton et al., 1990): $\mathrm{SST} = \mathrm{DN} \times 0.1 + 5.0$. Conversion from DN to real values of Chl-*a* (mg/m^3) used the following equation: $\text{Chl-}a = 10^{((0.015\times\mathrm{DN})+\log_{10}(0.01))}$.

Information on calendar day, depth (m) and associated effort was available for all cetacean observations. In addition, depth (minimum, maximum and average) and total effort were derived for each grid cell. Satellite-derived data for SST and chl-*a* were available for slightly over half of the sightings records (missing values are due to cloud cover).

Data analysis

Data were analysed at two levels of temporal resolution, by cell over the whole study period and by cell per day. The former provides a coarse-scale view of distribution without the possibility to examine temporal trends but avoids problems of temporal autocorrelation. The latter is potentially more powerful but the daily by-cell sightings data included a very high proportion of zero values, making model fitting difficult and with a high likelihood of significant temporal (or spatial) autocorrelation. In addition, at present, satellite data have not been obtained for all the absence records. Therefore, fine-scale analysis was restricted to an analysis of trends in cetacean abundance among the subset of presence records. Note that a further option for analysing the data would have been to use (15 min) survey legs as the basic unit of data. However, this suffers similar limitations to the by cell by day analysis.

Daily survey effort within a grid cell was estimated from the number of GPS positions recorded within the cell, counting only the ‘control point’ position records, i.e. those taken at 15-min intervals. To generate summary statistics we expressed total survey effort per cell as a percentage of the total number of control points over the whole study area and period ($N = 2{,}002$ within the study grid). Thus, a figure of 1% represents approximately 5 h of observation time (2,002 × 0.25 h/100).

To provide overall indices of relative abundance, totals for sightings and survey effort were extracted by grid cell, and two measures of sighting rate were derived: sightings per unit effort (SPUE, i.e. number of sightings per 15 min search effort) and individuals per unit effort (IPUE, number of individuals per 15 min search effort).

The environmental characteristics of locations at which each species was seen were summarised: although absolute values may be biased due to uneven distribution of effort, comparisons between species are potentially informative.

Generalised additive models were used to determine environmental relationships for (a) cetacean sightings rate per cell over the study period and

(b) for the subset of cetacean sightings records, variation in numbers of cetaceans (given presence). In the latter case, search effort for the relevant grid cell, day and year combination was used as one of the explanatory variables. Since common dolphins were by far the most frequently recorded species, both analyses were repeated for common dolphin sightings only. Finally, the analysis of numbers given presence was also repeated for common dolphin calves.

Between-cell variation in abundance

The overall cetacean SPUE by cell and common dolphin SPUE by cell were modeled as a function of grid cell location (as northing and easting, i.e. equivalent to latitude and longitude) and average sea depth. Since all three explanatory variables are continuous variables they were fitted as smoothers. SPUE was assumed to be normally distributed and an identity link function was used. The assumption of normality was validated by examining the distribution of model residuals. Separate GAMS were not fitted for any other cetacean species since there were insufficient non-zero records.

Abundance given presence

For this analysis, each sighting was treated as a separate data point, with the response variables being (a) number of cetaceans sighted, (b) number of common dolphins sighted and (c) number of common dolphin calves sighted. The suite of explanatory variables tested was: grid cell location (as northing and easting), year, calendar day, depth, effort (for the cell and day) and satellite image-derived values for SST and chl-*a*. Since some of the available explanatory variables potentially explain the same variation in abundance, three types of models were fitted:

(1) models with only effort, time and location used as explanatory variables, i.e. models describing spatiotemporal variation in abundance;
(2) models with environmental variables used in place of the time and location variables, i.e. models to test the proportion of spatiotemporal variation that can be ascribed to environmental conditions;
(3) models using all available explanatory variables, thus allowing both 'environmental' and 'non-environmental' components of spatiotemporal patterns to be included (although the latter may of course be a consequence of environmental variables not included in the analysis).

Since SST was significantly correlated with calendar day ($r = 0.69$), we derived residual SST from a Gaussian GAM model of SST in relation to calendar day for use in models which included both calendar day and SST. Thus, the seasonal component of SST variation will be contained within the variable 'calendar day' while residual variation in SST is included as a separate explanatory variable. Chl-*a* values showed a complex and non-linear relationship with bathymetry, in that both the highest values and the widest range of values were found in shallow waters. Data on SST and chl-*a* were not available for all sightings, mainly due to high cloud cover on some days. Therefore, for those models which included 'environmental' variables, we separately tested use of (i) depth alone and (ii) depth, SST and chl-*a*.

Initial GAM fits using a Poisson distribution for abundance data indicated substantial overdispersion of the response variable. Adult numbers were markedly more overdispersed than those for calves so a negative binomial distribution was used for the former and quasi-Poisson for the latter, in both cases using a log-link function. Abundance of other cetacean species was too low to fit separate models.

For all GAMs, the final model was selected on the basis of the AIC, individual significance of explanatory variables and examination of diagnostic plots (e.g. residual plots, hat values, etc.). To avoid overfitting, the maximum value of k (knots, i.e. a measure of the maximum complexity of the fitted curve) was set at 4 for all explanatory variables. Note that, since we used grid cells as spatial units, there were few unique values of latitude and longitude and higher k values could not have been used for these variables. F tests were used to compare the nested models (Zuur et al., 2007). Significance of smooth terms is reported along with an indication of the estimated degrees of freedom, a measure of the complexity of the curve, where edf = 1 indicates a linear fit and higher values indicate curves. Brodgar software (www.brodgar.com), a menu-based interface for R (R Core Development Team, 2006), was used for fitting GAMs.

Results

Survey effort

Surveys took place during 119 non-consecutive days over 2 years, with observers present on-board Galician fishing vessels during February–August 2001 (85 days) and June–September 2003 (34 days). A total of 136 observer-days at sea was achieved (102 and 34, in 2001 and 2003, respectively), with 2,116 control points acquired over a broad area within Galician coastal waters, 2,002 of which fell within the grid. There was considerable variation in the survey coverage within each grid-square, mainly due to the routes and preferred fishing areas of the fishing vessels. Grid cell B1 (Fig. 1) was not surveyed during the entire study period but all the other 31 grid cells were surveyed at least once. Total survey effort within the grid was 2,002 × 0.25 h = 500.5 h, with effort per grid cell ranging from 0.25 h (0.05% of the total) to 69.2 h (13.84%) (Fig. 2). Most effort (40.6% of the total) was recorded in sub-area SA5, i.e. the southernmost area. The mean number of grid cells surveyed per day was 4 (SD = 2.34, range 1–11).

Cetacean diversity, abundance and distribution

In total, there were 250 cetacean sightings, comprising 6,846 individuals belonging to at least seven species (Table 1). The common dolphin (*Delphinus*

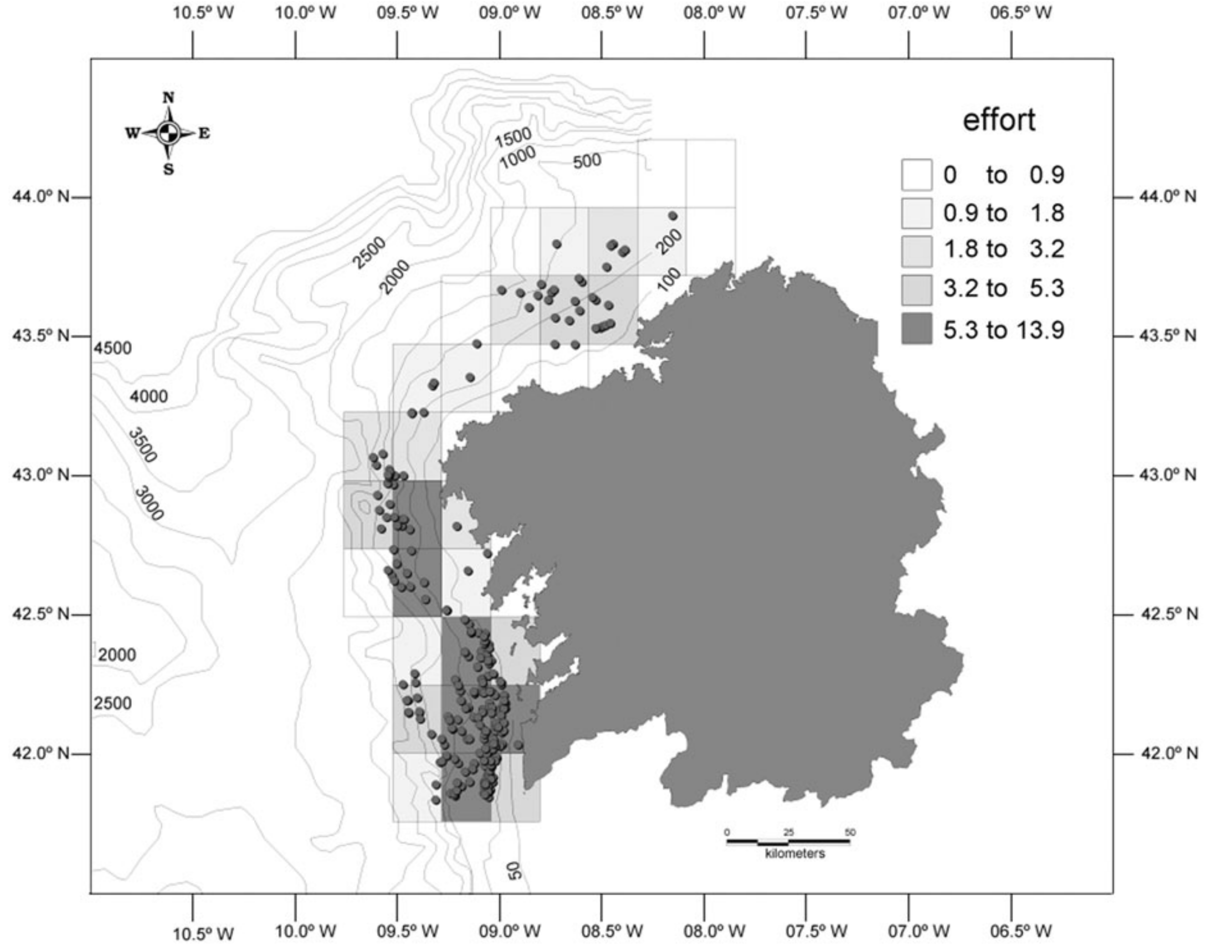


Fig. 2 Cetacean distribution (sightings locations) in relation to survey effort by grid cell. Here survey effort is expressed as a percentage of the total, with 1% point being equivalent to approximately 5 h of observation (see text). Also shown are bathymetry contours (50, 100, 200, 500, 1000,… 4500 m)

Table 1 Number of cetaceans recorded during surveys, by species: number of sightings, sums of minimum, maximum and mean counts, total number of groups seen and mean group size

Species	Sightings	Minimum	Maximum	Mean	Number of groups	Mean group size
Delphinus delphis	205	5410	7368	6389	252	25.4
Globicephala melas	13	208	265	236.5	20	11.8
Tursiops truncatus	7	69	90	79.5	9	8.8
Grampus griseus	6	61	86	73.5	6	12.3
Phocoena phocoena	5	8	8	8	5	1.6
Stenella coeruleoalba	4	25	30	27.5	4	6.9
Balaenoptera physalis	1	1	1	1	1	1
Unidentified mysticeti	5	6	6	6	5	1.2
Unidentified delphinid	2	18	25	21.5	2	10.8

delphis) was by far the most frequently sighted species (205 sightings, 82.4% of the all-species total). The other species recorded were long-finned pilot whale (*Globicephala melas*) (13 sightings), bottlenose dolphin (*Tursiops truncatus*) (9), Risso's dolphin (*Grampus griseus*) (6), harbour porpoise (*Phocoena phocoena*) (5), striped dolphin (*Stenella coeruleoalba*) (4) and fin whale (*Balaenoptera physalus*) (1). In addition, there were two sightings of unidentified Delphinidae and five sightings of unidentified mysticetes. For further analysis, the unidentified mysticetes and the fin whale were grouped as mysticetes.

Delphinus delphis was also the most abundant species in the study area, accounting for 93.3% of individual cetaceans seen. *G. melas* was the second most abundant species (3.5%), followed by *T. truncatus* (1.3%) and *G. griseus* (1.1%). Common dolphins tended to be seen in large groups while mysticetes were seen alone or in very small groups.

Calves were recorded during 60 sightings (24% of the total), with numbers ranging from 1 (21 sightings) to 18 (1 sighting) individuals. Calves of five species were recorded: *D. delphis* (158 individuals from 48 sightings), *G. melas* (18 individuals, 8 sightings); *T. truncatus* (2 individuals, 2 sightings); *G. griseus* (2 individuals, 1 sighting) *P. phocoena* (1 individual, 1 sighting).

Two sightings of *T. truncatus* were outside the predefined study area and therefore excluded from further analysis. Of the remaining 248 sightings, the highest percentages were recorded in sub-areas SA5 (40.6% of sightings) and SA3 (20.2%, Fig. 2). Most sightings in SA5 occurred between the 100 and 200 m isobaths although further north there appear to be fewer sightings in such shallow waters. Taking into account survey effort, overall the sightings rate (SPUE) per grid cell was generally higher in the south (Fig. 3) while the spatial pattern in abundance (IPUE) is less clear (Fig. 4). The highest values of both SPUE (0.24) and IPUE (10.86) were seen in SA5.

Delphinus delphis was the most widely distributed cetacean and was present in all sub-areas, although over half of the sightings (51.3%) were in SA5 (Fig. 5), *G. melas* was present all along the coast but mostly seen in SA3 and SA1 (38.5 and 30.8%, respectively) and generally not close to the shore (Fig. 6). For the other species, the small number of sightings precludes any firm conclusions about distribution, although *T. truncatus* was most often sighted in SA5 (40%) and *P. phocoena* was only sighted in SA5.

Delphinus delphis was sighted mainly in May to August, although it should be noted that the months June to August were the only months sampled in both years. The second most frequently sighted species, *G. melas* ($N = 13$) was seen most often in May.

Cetacean distribution and abundance in relation to environmental parameters

Cetacean sightings were recorded in water depths ranging from 7–1,432 m. The majority of *D. delphis* sightings were in waters of less than 200 m depth, although it was also the only species sighted in waters over 1,050 m depth (11 out of 205 sightings). Once

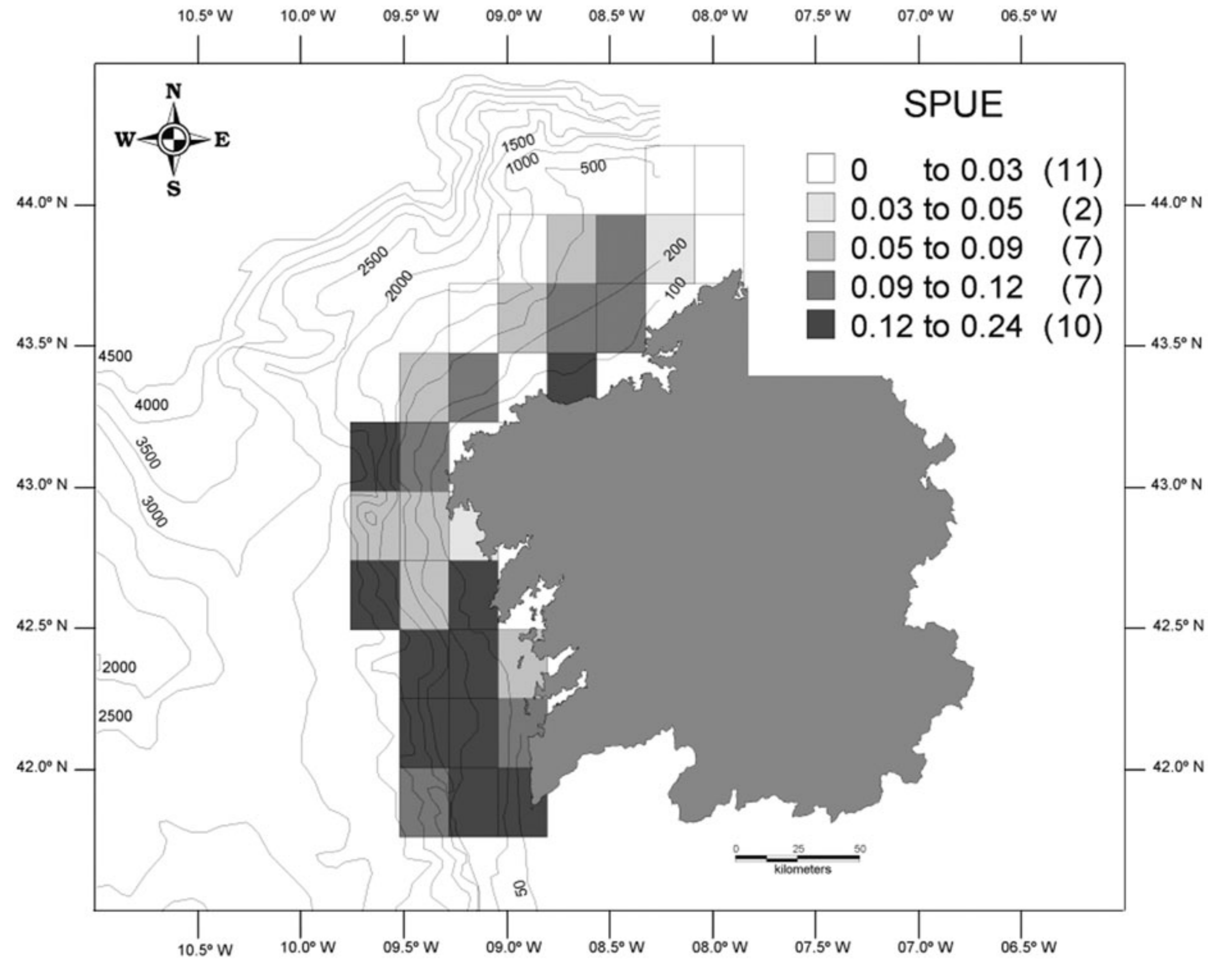


Fig. 3 SPUE per grid cell, SPUE being the number of cetacean sightings events per 15-min track segment, over the whole study period

survey effort was taken into account, it became apparent that this species was relatively more abundant (higher SPUE) in deeper waters (Table 2). *D. delphis* was seen in areas with SST ranging from 14.8–21.5°C, with most sightings in waters of 18–19°C.

Globicephala melas was seen in waters of up to approximately 900 m depth, with only 2 out of 13 sightings in waters of less than 200 m depth. Maximum SPUE was in the depth range 200–500 m (Table 2). For all other species, there were fewer than 10 sightings and results on depth distribution should thus be treated with caution. *T. truncatus* was seen in both shallow (<200 m) and deep (>800 m) waters, *S. coeruleoalba* was seen over water depths of up to 940 m and *G. griseus* over depths of up to 470 m. Both *P. phocoena* and Mysticetes were seen only in waters of <200 m depth.

Models of distribution and abundance

Distribution of sightings

Average all-species SPUE per cell was related to latitude (edf = 1, $P < 0.0001$), with a linear decline in sightings rate further north. Once latitude was included in the model, depth and longitude had no significant effects and were therefore excluded from the final model. The model explained 41.6% of deviance. The final model for common dolphin SPUE also included only latitude (edf = 1, $P = 0.0002$) and explained 38.3% of deviation ($N = 31$). It

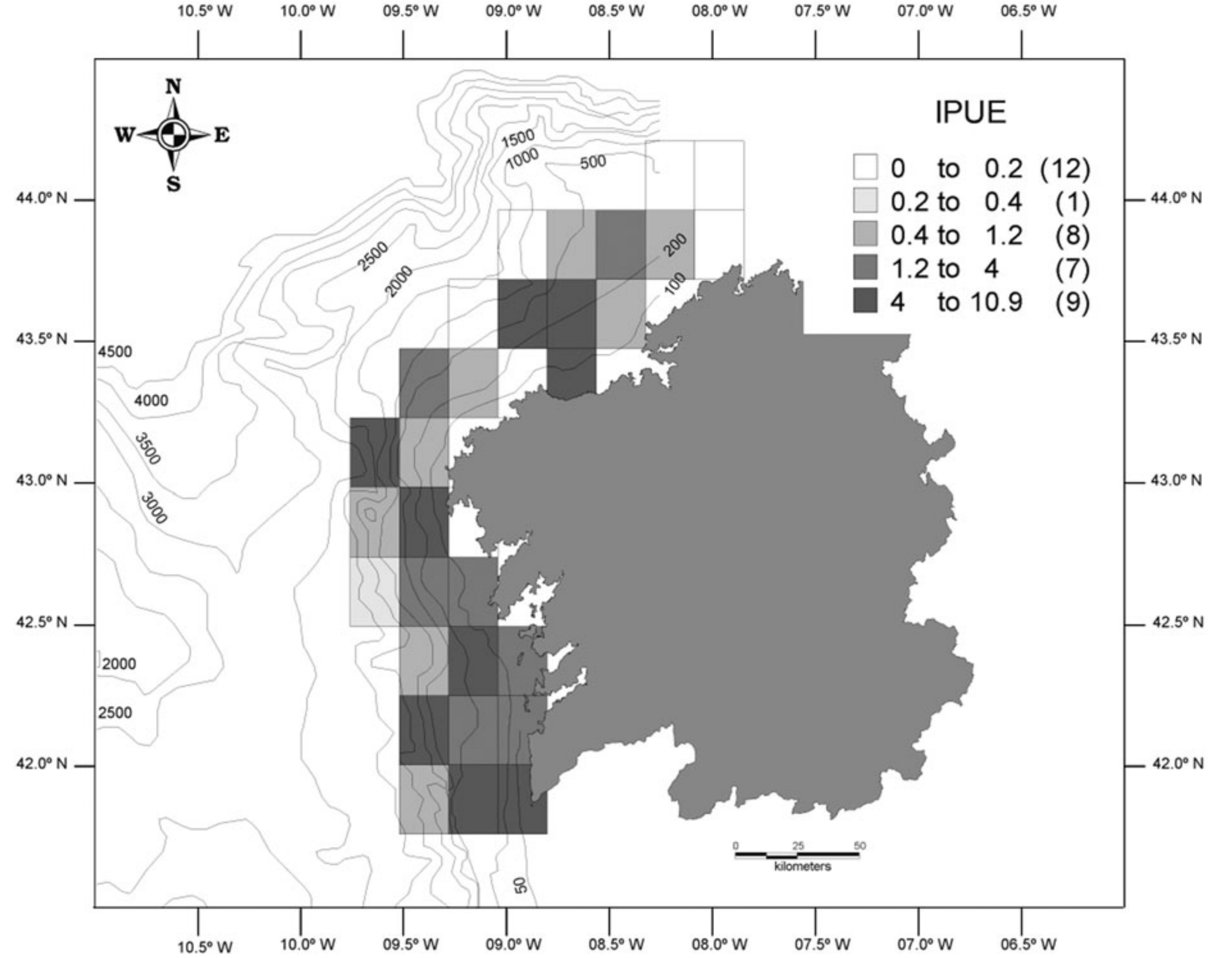


Fig. 4 IPUE per grid cell, IPUE being the number of individual cetaceans sighted per 15-min track segment, over the whole study period

indicated a similar trend of lower SPUE further north. Examination of diagnostic plots indicated no important patterns in residuals, which were approximately normally distributed.

Abundance variation in space and time and in relation to survey effort

The best (negative binomial GAM) model for spatial, temporal and effort-related patterns in the number of cetaceans sighted (when sightings took place) included all five explanatory variables. The trends were for higher numbers per sighting in the north (latitude, edf = 1.38, $P = 0.1182$) and west (longitude, edf = 2.78, $P = 0.0593$) of the study area, higher numbers later in the year (edf = 2.71, $P < 0.0001$), lower numbers where survey effort was lowest (edf = 2.46, $P = 0.0002$, Fig. 7) and higher numbers in 2001 than in 2003 ($P = 0.0022$). Although individual effects of latitude and longitude were not statistically significant, removing either of them resulted in a significantly poorer fit; the reduction in goodness of fit from removing latitude ($F = 5.21$, $P = 0.0061$) was greater than that resulting from removing longitude ($F = 3.49$, $P = 0.0374$). An apparent trend for lower numbers at the highest levels of effort cannot be confirmed due to the very wide confidence limits in this part of parameter space. Overall this model explained 19.4% of deviance ($N = 248$).

The best (negative binomial GAM) model for spatial, temporal and effort-related patterns in the number of common dolphins sighted, when sightings

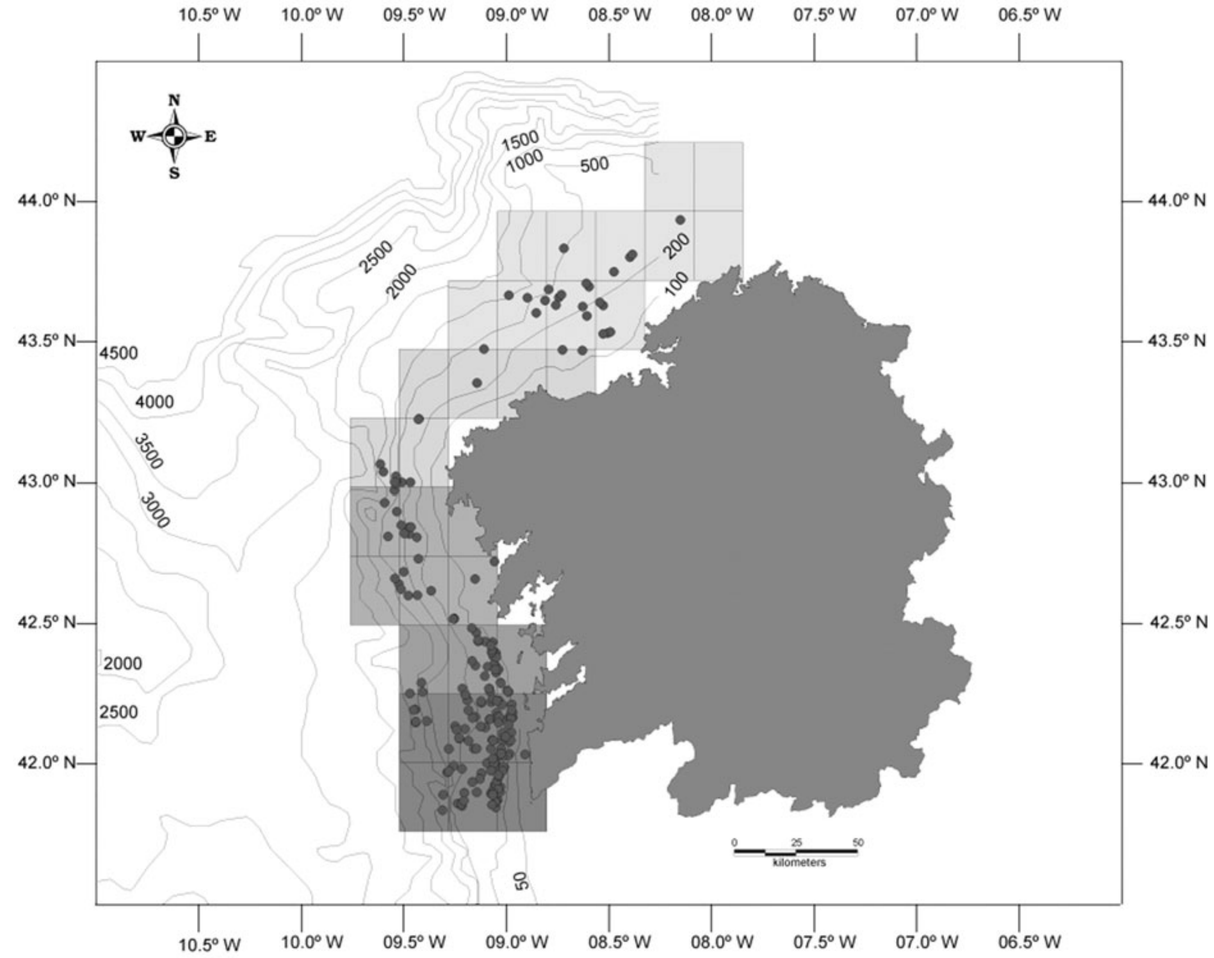


Fig. 5 Locations of *Delphinus delphis* sightings. *Shading* indicates different sub-areas

took place, also included all five explanatory variables. As seen for the all-species model, trends were for higher numbers in the north (edf = 1, $P = 0.0287$) and west (edf = 2.73, $P = 0.0576$) of the study area, higher numbers later in the year (edf = 2.71, $P = 0.0025$), lower numbers where survey effort was lowest (edf = 2.345, $P = 0.0001$; see Fig. 8) and higher numbers in 2001 than in 2003 ($P = 0.0017$). Overall the model explained 19.9% of deviance ($N = 205$).

The best (quasi-Poisson GAM) model for spatial, temporal and effort-related patterns in the number of common dolphin calves sighted, when sightings of this species took place, included effects of latitude (edf = 2.79, $P = 0.0002$), calendar day (edf = 2.71, $P = 0.0393$) and year ($P = 0.0069$). The trends were for more calves to be seen in the south of the study area (the opposite to the trend for total numbers of common dolphins), more towards the end of the year (Fig. 9) and more in 2001 than in 2003. Effects of longitude and effort were not significant and were dropped from the final model. This model explained 19.1% of deviance ($N = 205$).

Models of environmental effects on local abundance of all cetaceans combined

The best 'environmental' model (i.e. excluding direct descriptors of time and location) for numbers of cetaceans seen per sighting contained only the effect of depth (edf = 2.89, $P < 0.0001$). The trend was for numbers sighted to increase over greater water depths, at least up to around the 400 m isobath (Fig. 10a). This model explained 11.9% of deviance

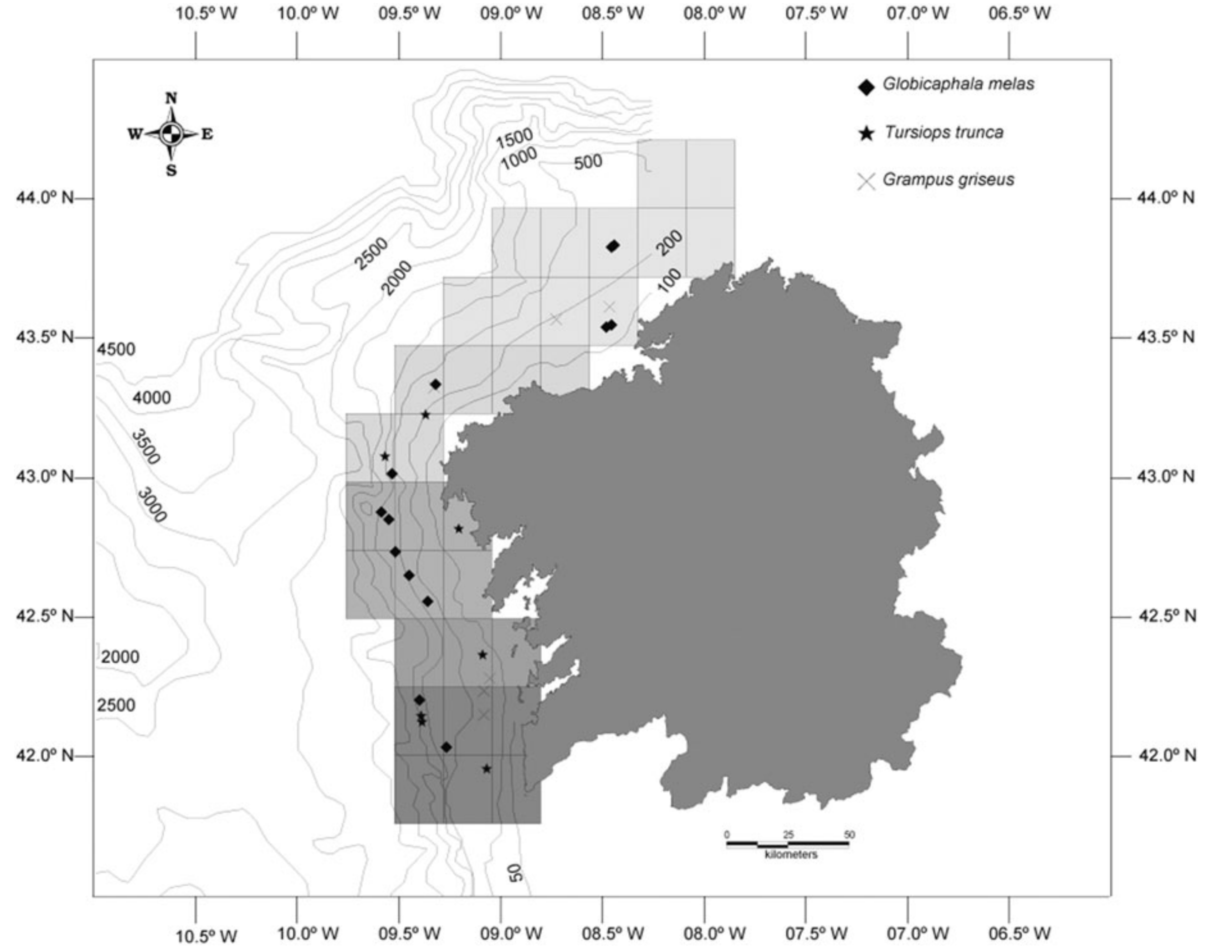


Fig. 6 Locations of sightings of *Globicephala melas, Tursiops truncatus* and *Grampus griseus*. *Shading* indicates different sub-areas

Table 2 Species IPUE per depth interval: mean ± SD (with sample size, *N*, in parentheses)

Species	<100 m	101–200 m	201–500 m	501–800 m	>800 m
Balaenoptera physalus	–	0.2 (1)	–	–	–
Delphinus delphis	3.39 ± 4.82 (21)	4.40 ± 6.61 (110)	5.87 ± 6.85 (37)	8.10 ± 12.20 (16)	6.36 ± 6.36 (21)
Globicephala melas	–	2.63 ± 3.00 (2)	3.29 ± 3.30 (6)	3.01 ± 0.96 (3)	1.93 ± 1.12 (2)
Grampus griseus	–	1.74 ± 1.41 (4)	2.14 ± 2.06 (2)	–	–
Tursiops truncatus	1.40 (1)	0.82 ± 0.96 (3)	–	–	1.59 ± 1.00 (3)
Phocoena phocoena	–	0.30 ± 0.39 (5)	–	–	–
Stenella coeruleoalba	1.25 (1)	0.90 ± 0.28 (2)	–	–	0.5 (1)
Unidentified delphinids	–	1.46 ± 0.47 (2)	–	–	–
Unidentified mysticetes	–	0.14 ± 0.13 (5)	–	–	–

($N = 248$). Addition of chl-*a* or SST as explanatory variables did not improve this model. When the effect of adding the spatiotemporal explanatory variables was explored, two alternative 'best' models were encountered, which are difficult to compare due to the great difference in sample size. However, common

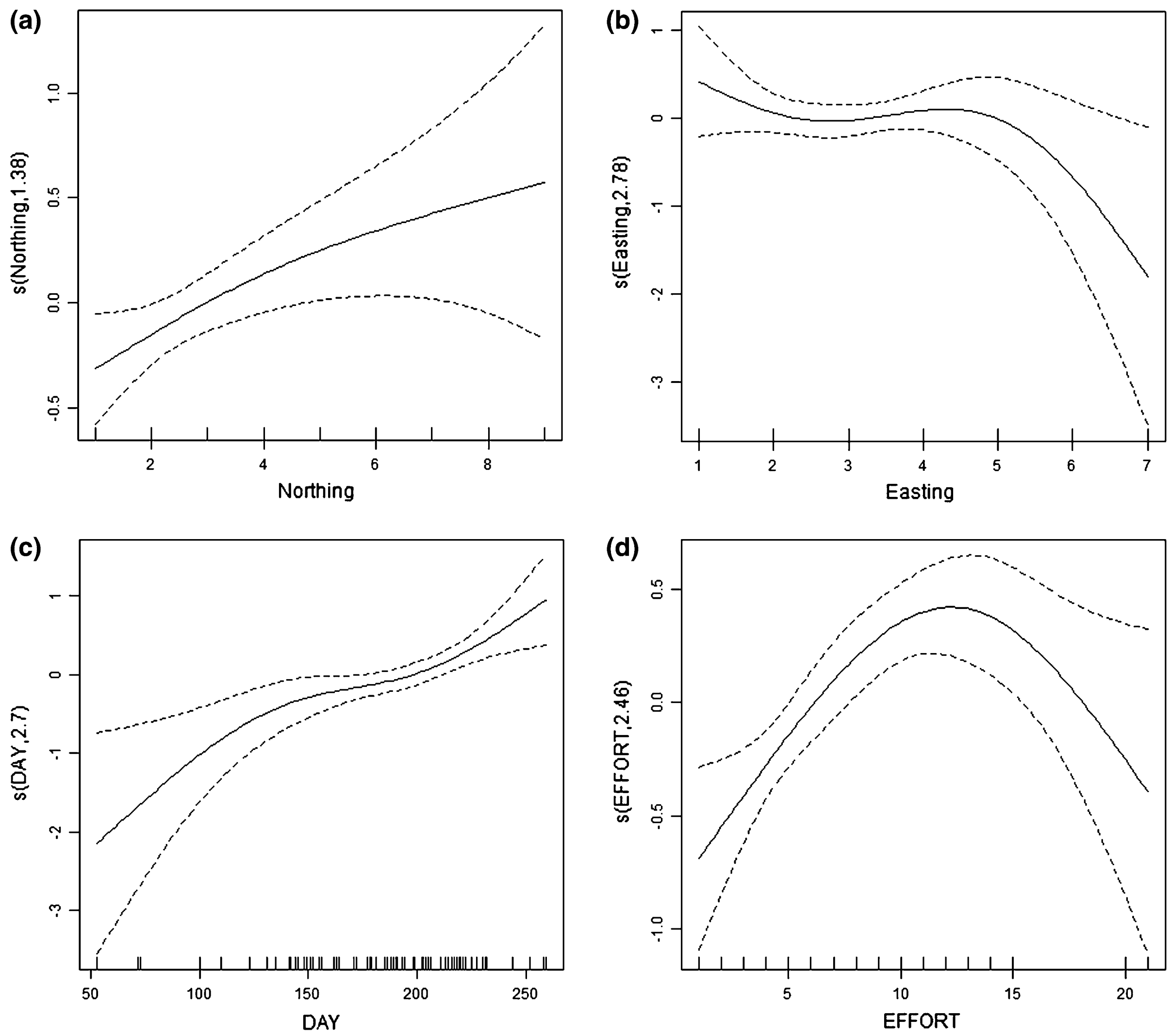


Fig. 7 GAM results: models of cetacean abundance (all spp) given presence: smoothers showing partial effects on number of cetaceans recorded during a sighting, for: **a** latitude (expressed as northing, where 1 is the southernmost grid cell row), **b** longitude (expressed as easting, where 1 is the westernmost grid cell column), **c** calendar day and **d** search effort (number of control points in the grid cell in which the sighting occurred, on that day)

patterns are apparent in both of these models. The first 'best' model (Fig. 10b–d) included positive effects of depth (edf = 1, $P < 0.0001$) and chl-*a* (edf = 1, $P = 0.0220$), higher numbers in 2001 than in 2003 ($P = 0.0114$) and a positive effect of effort (edf = 2.28, $P = 0.0039$) at low effort levels. This model explained 27.2% of deviance but, due to missing values for chl-*a*, had a relatively small sample size ($N = 138$). The second of the 'best' models (Fig. 10e–g) also included a positive effect of depth (edf = 2.67, $P = 0.0017$) and a positive effect of effort (edf = 2.28, $P = 0.0036$) at low effort levels. In addition, numbers were seen to be higher later in the year (edf = 1.93, $P = 0.0009$) and higher in 2001 than in 2003 ($P = 0.0036$). This model explained 19% of deviance ($N = 248$).

Models of environmental effects on local abundance of common dolphins

As for the all-species data, the best environmental model for common dolphin numbers contained only an effect of depth (edf = 2.84, $P < 0.0001$). This effect was positive from the coast to the 400 m

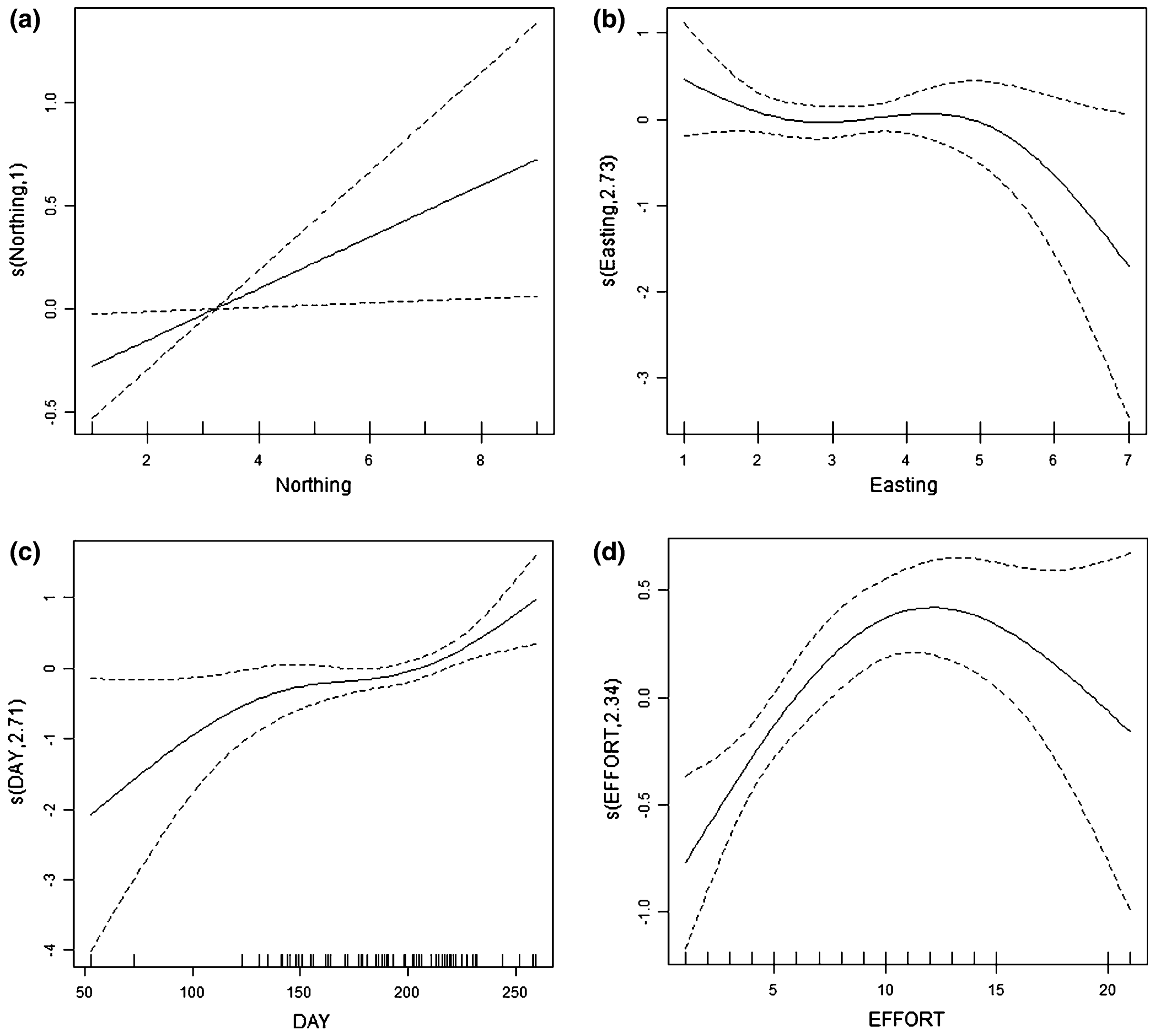


Fig. 8 GAM results: models of common dolphin abundance given presence: smoothers showing partial effects on number of common dolphins recorded during a sighting, for: **a** latitude (expressed as northing, where 1 is the southernmost grid cell row), **b** longitude (expressed as easting, where 1 is the westernmost grid cell column), **c** calendar day and **d** search effort (number of control points in the grid cell in which the sighting occurred, on that day)

isobath (Fig. 11a) and the model explained 11.9% of deviance ($N = 205$). Again, once spatiotemporal explanatory variables were also considered, two 'best' models were obtained. The first model (Fig. 11b–d) explained 18.2% of deviance ($N = 205$) and included effects of depth (edf = 1, $P = 0.0001$), effort (edf = 2.16, $P = 0.0030$), day (edf = 1, $P = 0.0436$) and year ($P = 0.0008$). All effects were essentially as previously described for the equivalent all-species model. The second model (Fig. 11e–g) explained 34.3% of deviance but, again was based on a much smaller sample size ($N = 109$). The model contained effects of depth (edf = 1, $P < 0.0001$), effort (edf = 2.20, $P = 0.0026$), chl-*a* (edf = 1, $P = 0.0298$) and year ($P = 0.0012$). Again these effects are as previously described for the equivalent all-species model.

The best pure environmental model for common dolphin calf numbers contained only the effect of depth (edf = 2.01, $P = 0.0451$), with most calves seen in the shallowest waters (Fig. 11h). This model explained only 4.13% of deviance ($N = 205$). When spatiotemporal explanatory variables were also considered, depth dropped out and the resulting best

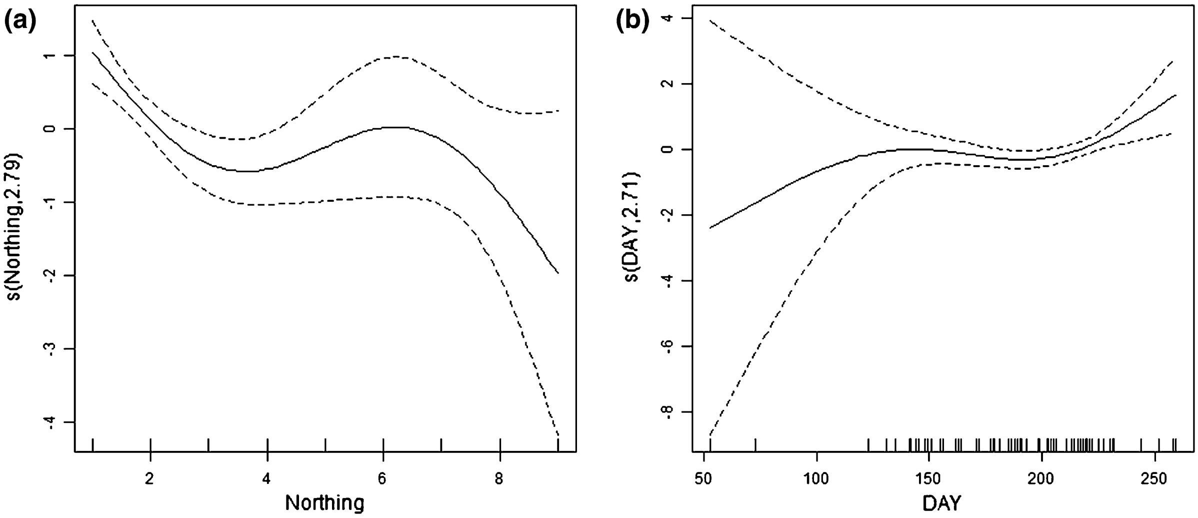


Fig. 9 GAM results: models of common dolphin calf abundance given presence of common dolphins: smoothers showing partial effects on number of calves recorded during a sighting, for: **a** latitude (expressed as northing, where 1 is the southernmost grid cell row) and **b** calendar day

model was that obtained originally for spatiotemporal variation in calf abundance (i.e. with latitude, day and year).

Discussion

Of the seven cetacean species that were identified from sightings during this study, *D. delphis* was by far the most frequently sighted. This result is in agreement with previous studies in Galician waters which suggest that common dolphin is the most abundant cetacean in the region (Aguilar, 1997; López et al., 2004). *D. delphis* were observed most frequently in the south of Galicia and in waters of <200 m depth (Fig. 5). Once survey effort was taken into account, there was no relationship between sightings frequency and depth, although a north–south gradient was confirmed, with fewer sightings in the north. However, the number of animals seen per sighting was higher in the north and in deeper waters (which is indicative of larger group sizes). López et al. (2004) previously noted higher abundance of this species in deeper waters off Galicia. This apparent preferential use of deeper waters may relate to foraging preferences. One of the two main prey species, blue whiting, is a shoaling mesopelagic fish which lives in mid-water, mainly over depths of 160–3,000 m—although immature fish are found in shallow water in summer (Wheeler, 1969; Whitehead et al., 1989). Model results also suggested a weak positive effect of chl-*a* concentration on common dolphin abundance (see below for further discussion).

Although the present surveys did not take place all year round, and the trends identified were not strong, modelling results indicate that common dolphin calves were most numerous later in the year and (opposite to the overall geographical trend in numbers of individuals sighted) higher numbers were seen in shallower waters to the south of the study area, suggesting that southern Galician coastal waters could represent a nursery area for this species.

Other cetacean species

Globicephala melas was the second most frequently recorded species in this study and was seen mainly in deeper waters (>200 m). This species is essentially oceanic and is known to dive to 600 m, enabling it to exploit a wide diversity of prey species (Mead & Brownell, 2005). However, it is known to forage occasionally in more coastal waters and coastal cephalopod species appear in its diet (González et al., 1994; M.B. Santos, unpubl. data). Long-finned pilot whales account for around 5% of cetacean strandings along the Galician coast (López et al., 2002).

The bottlenose dolphin is one of two cetacean species (the other being the harbour porpoise) for

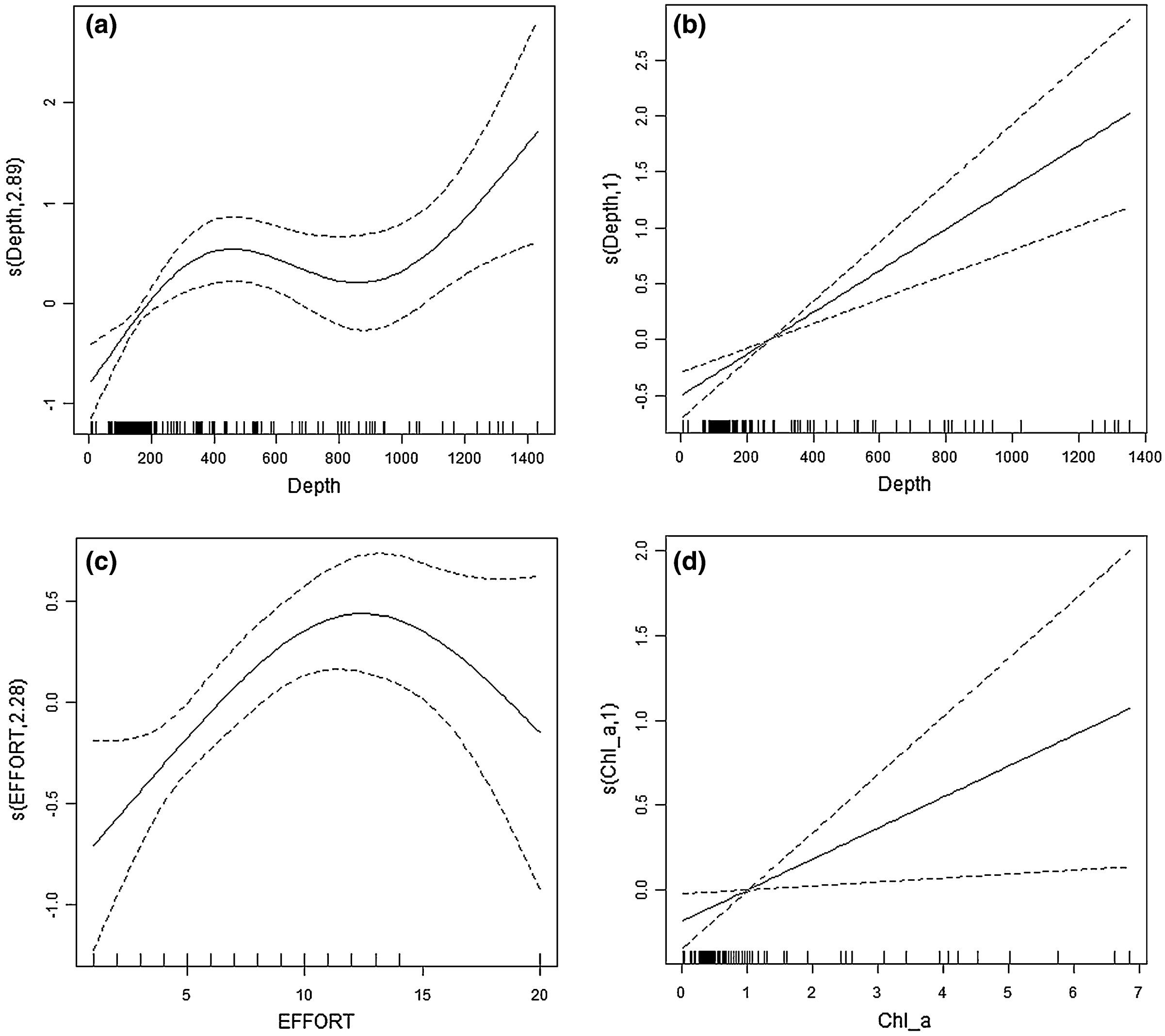


Fig. 10 GAM for numbers of individuals per sighting: environmental model for all spp., **a** effect of depth (in m). 'Combined' model 1 for all spp.: effects of **b** depth, **c** effort, **d** chl-*a*. Combined model 2 for all spp.: effects of **e** depth, **f** effort and **g** day

which the EU Habitats Directive requires designation of Special Areas of Conservation. The low frequency of sightings reflects the fact that the study did not extend into the inshore waters of the rias. It is known that *T. truncatus* is present within the platform and inshore waters of the southern part of the study area, including the Ria of Vigo (Fernádez-Cordeiro et al., 1996) and this appears to be a resident population. In the past, two hypotheses have been proposed to explain the occurrence of this species in offshore waters: there could be a separate offshore population or members of the resident population may sometimes travel offshore to forage (López et al., 2004). The most recent evidence, from studies on stable isotopes and microsatellite DNA suggest that animals from the southern rías differ both ecologically and genetically from animals occurring further north and generally in offshore waters (Fernández et al., 2011a, b).

Harbour porpoises in the NW Iberian peninsula appear to be genetically isolated from those in the rest of Europe (Fontaine et al., 2007). These findings have led to increasing interest in the conservation of the species in Galicia. Although coastal sightings and strandings suggest that this is the third most abundant cetacean in coastal waters of Galicia (López et al.,

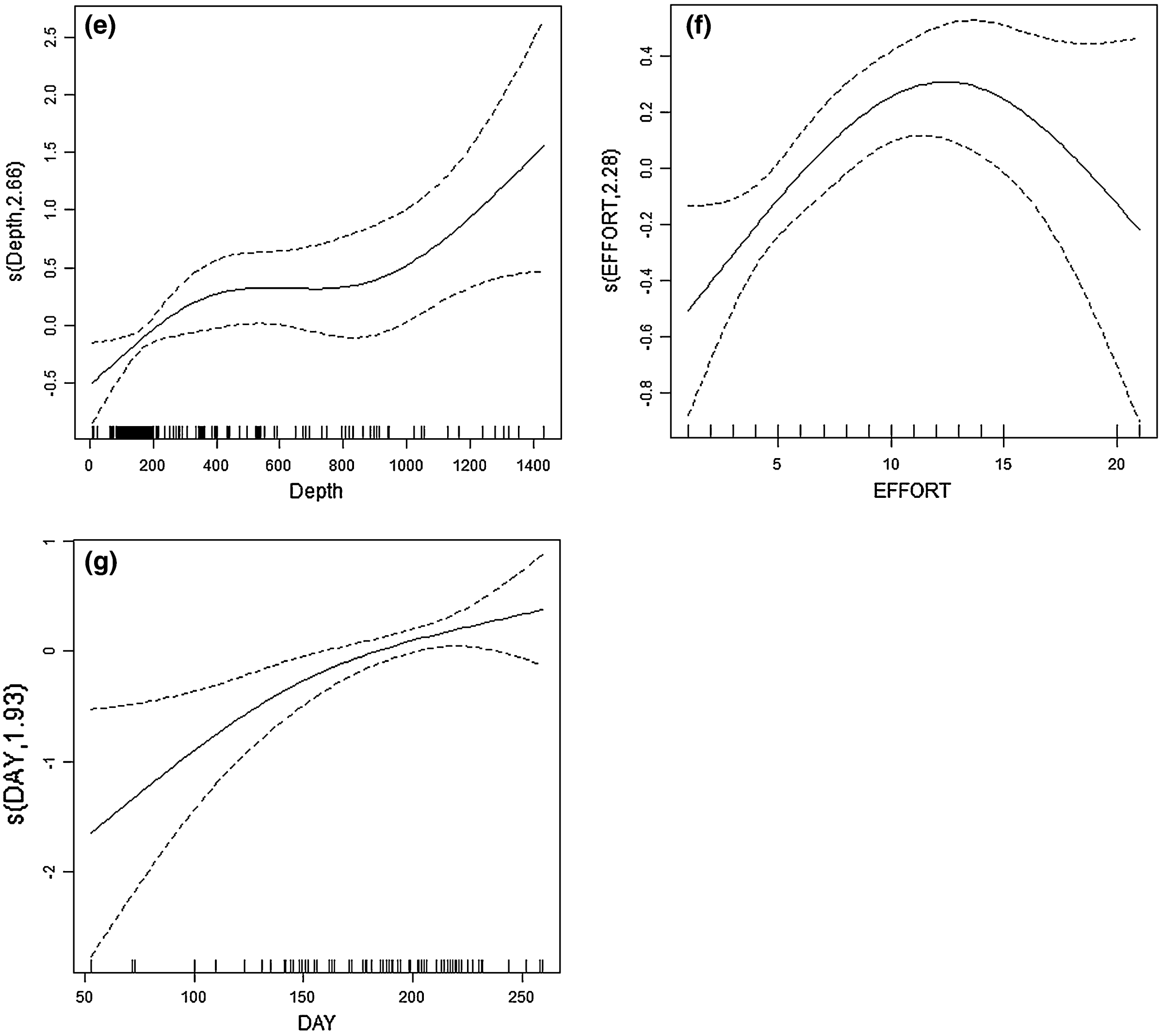


Fig. 10 continued

2002; Pierce et al., 2010), during the present study, individuals of *P. phocoena* were sighted on only five occasions, all in the southern part of the study area, within continental shelf waters (100–200 m depth). It is true that this species is difficult to observe at sea, mainly due to the small body size, the absence of large groups and the relative inconspicuous behaviour of individuals when they are at the surface. However, the infrequency of sightings in the present study is also consistent with results of the boat-based sightings study in 1998–1999 (López et al., 2004) and suggests that at least part of the reason for the contrast between results from strandings and land-based surveys on the one hand, and at-sea surveys on the other, could be that porpoises are found mainly close to the coast. The main prey species recorded in porpoise stomachs in Galicia are scad (*Trachurus trachurus*), *Trisopterus* spp., garfish (*Belone bellone*) and blue whiting (Pierce et al., 2010; Santos, unpubl. data). Scad occurs near the coast in warmer months of the year but moves to deeper water in winter, while garfish is basically an oceanic species that regularly enters coastal waters. Bib (*Trisopterus luscus*) is the most common member of its genus in Iberian peninsula waters and is a coastal species (Wheeler, 1969).

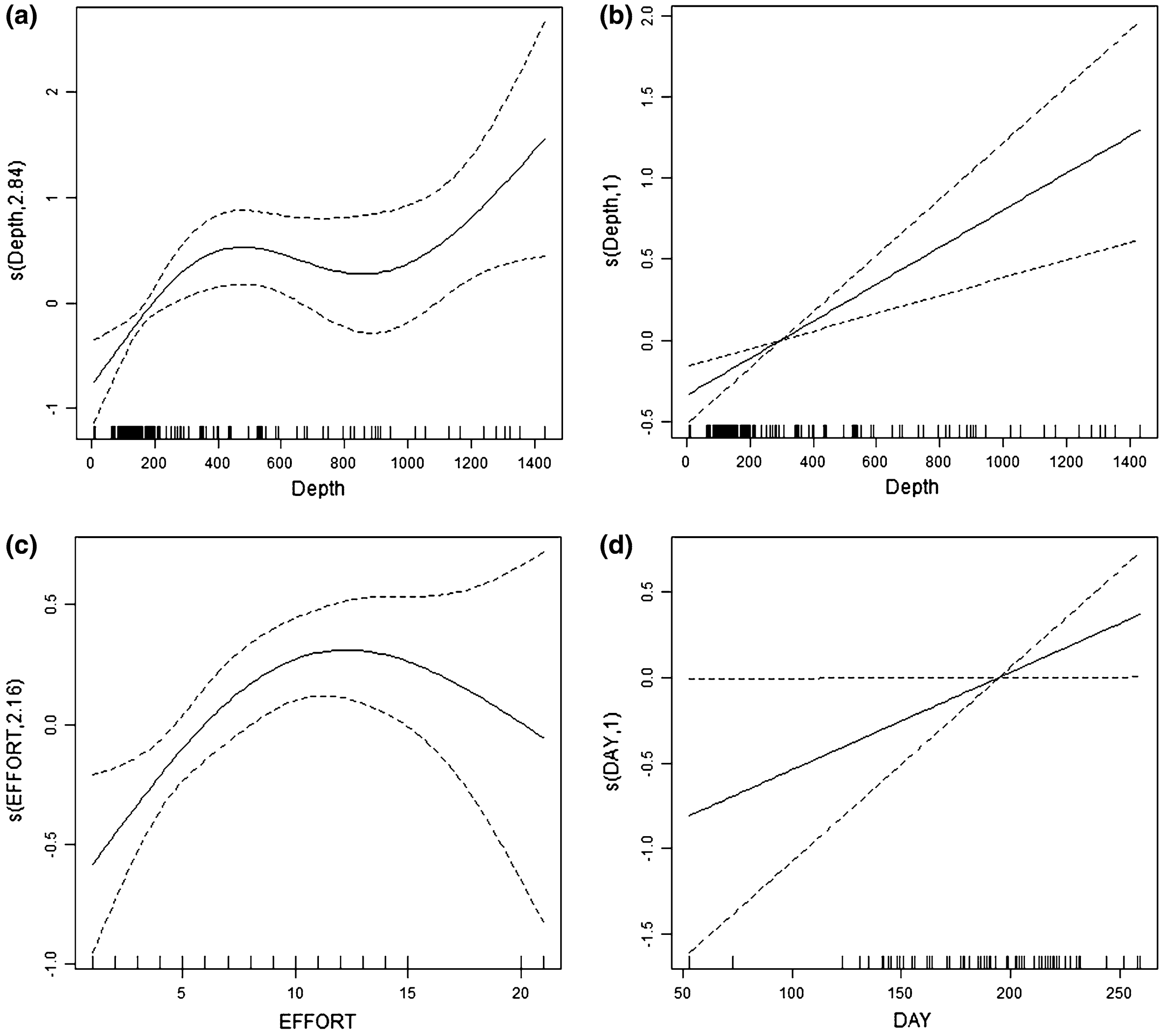


Fig. 11 GAMs for numbers of individual common dolphins per sighting of common dolphins: environmental model for common dolphins: **a** effect of depth. Combined model 1 for common dolphins: effects of **b** depth, **c** effort and **d** day. Combined model 2 for common dolphins: effects of **e** depth, **f** effort and **g** chl-*a*. Environmental model for calves: **h** effect of depth

The other cetacean species recorded during this study period were *Balaenoptera physalus* (along with several Mysticetes which were not identified to species), *Grampus griseus*, and *Stenella coeruleoalba*. None of these species was sighted frequently and they are probably not abundant in the surveyed area. Data from strandings suggest that *S. coeruleoalba* is the most numerous of the three species (López et al., 2002), although coastal sightings suggest that *G. griseus* is more common (Pierce et al., 2010). In the present study *S. coeruleoalba* was sighted in depths of 100–500 m along both the western and northern coasts of Galicia. Mysticetes were seen only towards the south of the study area (sub-areas 4 and 5) in waters less than 200 m deep. In the western North Atlantic, fin whales regularly feed over continental shelf waters less than 200 m in depth (Katona et al., 1993). Nevertheless, in Galicia, *B. physalus* probably occurs primarily in offshore waters: it was the most frequently sighted cetacean species in offshore Galician waters during the CODA survey (CODA, 2009) and indeed, historically, was on of the main species taken by Galician whalers (Valdés Hansen, 2010).

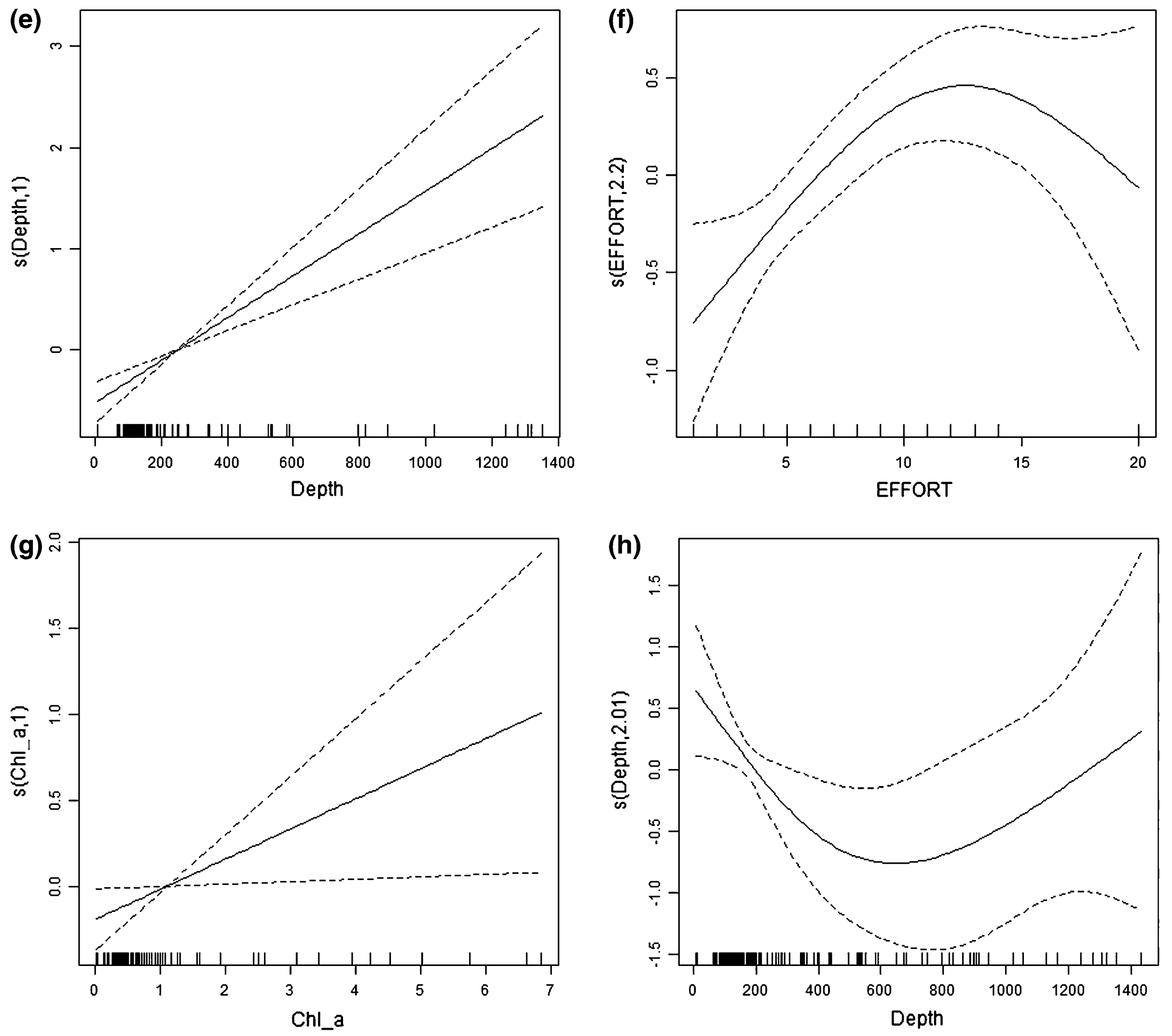


Fig. 11 continued

Environmental relationships

In quantifying environmental relationships it is important to be able to control for variable search effort, especially in opportunistic studies where this is not wholly under the investigator′s control. In the present study, there was a trend for numbers of animals sighted (given occurrence of a sighting) to be positively related to search effort in a given grid cell on a given day. The most likely explanation is that, since the study was based on opportunistic use of fishing vessels which, clearly, tend to go to areas of high fish abundance, there was more observer effort in areas where some species of cetaceans are also likely to congregate.

The environmental trends identified in the present study were higher numbers of cetaceans to be seen in deeper waters (except in the case of calves) and in areas of higher chl-*a* concentration. The majority of cetaceans seen were common dolphins and the trends observed thus essentially concern this species; we have insufficient data to determine environmental relationships for the other species. Note also that the analysis concerns the number of cetaceans present per sighting, rather than the presence of cetaceans per se.

Cetaceans are known to actively select habitats with certain measurable and consistent oceanographic qualities (Tynan et al., 2005) and the association (at various scales) of a range of pelagic predator species in areas of high productivity (including meso-scale fronts and upwelling areas) is well-documented (e.g. Jaquet & Whitehead, 1996; Zainuddin et al., 2006). Thus, the association of higher cetacean numbers with higher chlorophyll concentrations is not unexpected. Higher numbers of common dolphins were seen over deeper waters, although the survey did not extend beyond the shelf waters used by the fishing fleet. Oceanic cetaceans may undertake feeding excursions into coastal waters, congregating in areas where there is high abundance of prey, thus feeding at relatively shallow depths (Katona et al., 1993). This is likely in the Atlantic (western) area of Galician waters, where the effect of coastal upwelling (during April–September) is known to be more intense (Fraga, 1981; Blanton et al., 1984; Castro et al., 1994) and prey availability would be higher than in offshore waters (FAO, 1987; Fariña et al., 1997; Cañadas et al., 2002; Smith & Brown, 2002).

Understanding the spatiotemporal relationships linking oceanographic variables such as SST and chl-*a* to diversity and abundance of cetaceans is not straightforward, e.g. due to questions about the appropriate scale at which relationships will be seen. Hotspots of primary production resulting from oceanographic phenomena are often localised in both space and time. In addition, as pointed out by Grémillet et al. (2008), we tend to forget that top predators do not consume phytoplankton and the relationship between primary production and the presence of cetaceans may involve significant time-lags (e.g. several weeks) and/or spatial displacement (e.g. tens of kilometres) (e.g. Brown & Winn, 1989; Littaye et al., 2004; Walker, 2005). Without good knowledge of local oceanography and current systems and of the ecology of the cetaceans, such relationships can easily be missed. An additional logistical issue associated with fine-scale studies is the availability of cloud-free satellite images for the desired area and time-window.

Although the present study provides some useful preliminary indications of habitat preferences and environmental relationships in Galician cetaceans, further studies on cetacean habitat preferences in the area are needed and would benefit from use of on-board CTD, permitting measurement of additional oceanographic variables and providing the further benefit of allowing whole water column profiles to be constructed (Scott et al., 2010).

Acknowledgments We gratefully acknowledge the input of the four observers, who were funded by the European Commission's Directorate General for Fisheries under Study Project 00/027, 'Pelagic fisheries in Scotland and (UK) and Galicia (Spain): observer studies to collect fishery data and monitor by-catches of small cetaceans' (2001) and the Xunta de Galicia under project PGIDIT02MA00702CT, 2002–2005, 'Predictive system of fishing efforts for the Galician artisan fleet'). TCSD, ES and GMI would like to acknowledge funding from EU Marie Curie project 20501 'ECOsystem approach to Sustainable Management of the Marine Environment and its living Resources'—ECOSUMMER. GJP was funded by a Marie Curie excellence grant (MEXC-CT-2006-042337, 'Anthropogenic Impacts on the Atlantic marine Ecosystems of the Iberian Peninsula'—ANIMATE). We also thank Ruth Fernandez and Begoña Santos for comments on the manuscript.

References

Aguilar, A., 1997. Inventario de los cetáceos de las aguas atlánticas peninsulares: aplicación de la directiva 92/43/CEE. Memoria Final. Departamento de Biología Animal (Vert.), Facultad de Biología, Universitat de Barcelona, Spain.

Baumgartner, M. F., 1997. The distribution of Risso's dolphins (*Grampus griseus*) in relation to the physiography of the northern Gulf of Mexico. Marine Mammal Science 13: 614–638.

Baumgartner, M. F., K. D. Mullin, L. N. May & T. D. Leming, 2001. Cetacean habitats in the northern Gulf of Mexico. Fishery Bulletin 99: 219–239.

Bellido, J. M., G. J. Pierce & J. Wang, 2001. Modelling intra annual variation of squid *Loligo forbesi* in Scottish waters using generalized additive models. Fisheries Research 52: 23–39.

Blanton, J. O., L. P. Atkinson, C. F. Fernández & A. Lavin, 1984. Coastal upwelling off the Rías Baixas, Galicia Northwest Spain. I: hydrographic studies. Rapp. P.-V Reun. Const. int. Explor. Mer 183: 79–90.

Brown, C. W. & H. E. Winn, 1989. Relationship between the distribution pattern of right whales, *Eubalaena glacialis*, and satellite-derived sea surface thermal structure in the Great South Channel. Continental Shelf Research 9: 247–260.

Cañadas, A., R. Sagarminada & S. García-Tiscar, 2002. Cetacean distribution related with depth and slope in the Mediterranean waters of Southern Spain. Deep-Sea Research I 49: 2053–2073.

Castro, C. G., F. F. Pérez, X. A. Alvarez-Salgado, G. Rosón & A. F. Ríos, 1994. Hydrographic conditions associated with the relaxation process o fan upwelling event off Galicia coast (NW Spain). Journal of Geophysical Research 99: 5135–5147.

CODA, 2009. Cetacean Offshore Distribution and Abundance. Final Report. Available from SMRU, Gatty Marine Laboratory, University of St Andrews, St Andrews, Fife, KY16 8LB, UK.

Daskalov, G., 1999. Relating fish recruitment to stock biomass and physical environment in the Black Sea using generalized additive models. Fisheries Research 41: 1–23.

Davis, R. W., G. S. Fargion, N. May, T. D. Leming, M. Baumgartner, W. E. Evans, L. J. Hansen & K. Mullin, 1998. Physical habitat of cetacean along the continental slope in the north-central and western Gulf of Mexico. Marine Mammal Science 14: 490–507.

Eastwood, P. D., G. J. Meaden & A. Grioche, 2001. Modelling spatial variations in spawning habitat suitability for the sole *Solea solea* using regression quantiles and GIS procedures. Marine Ecological Progress Series 224: 251–266.

Evans, P. G. H., 1987. The natural history of whales and dolphins. Christopher Helm Beckenham, London.

FAO, 1987. Fiches FAO d'identification des especes pour les besoins de la peche. Mediterranée et Mer Noire. Zone de Peche 37. Révision 1. Vol. II: Vertebres. FAO, Rome.

Fariña, A. C., F. J. Pereiro & A. Fernández, 1985. Peces de los fondos de arrastre de la plataforma continental de Galicia. Boletín del Instituto Español de Oceanografía 2: 89–98.

Fariña, A. C., J. Freire & E. González-Gurriarán, 1997. Demersal fish assemblages in the Galician continental shelf and upper slope (NW Spain): spatial structure and long-term changes. Estuarine and Coastal Shelf Science 44: 435–454.

Fernández, R., S. García-Tiscar, M. B. Santos, A. López, J. A. Martínez-Cedeira, J. Newton & G. J. Pierce, 2011a. Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? Marine Biology 158: 1043–1055.

Fernández, R., M. B. Santos, G. J. Pierce, A. Llavona, A. López, M. A. Silva, M. Ferreira, M. Carrillo, P. Cermeño, S. Lens & S. Piertney, 2011b. Fine scale genetic structure of bottlenose dolphins (*Tursiops truncatus*) off Atlantic waters of the Iberian Peninsula. Hydrobiologia (this volume).

Fernández-Cordeiro, A. F. Torrado-Fernández, R. Pérez-Pintos, M. Garci-Blanco & A. Rodríguez-Folgar, 1996. The bottlenose dolphin, *Tursiops truncatus*, along the Galician coast, with special reference to the ría de Vigo herd. European Research on Cetaceans 10: 213–216.

Fontaine, M. C., S. J. E. Baird, S. Piry, N. Ray, K. A. Tolley, S. Duke, A. Birkun, M. Ferreira, T. Jauniaux, A. Llavona, B. Öztürk, A. A. Öztürk, V. Ridoux, E. Rogan, M. Sequeira, U. Siebert, G. A. Vikingsson, J. M. Bouquegneau & J. R. Michaux, 2007. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. BMC Biology 5: 30–46.

Fraga, F., 1981. Upwelling of the Galician coast, Northwest Spain. In Richards, F. (ed.), Coastal Upwelling. American Geophysical Union Washington, DC: 176–182.

Grémillet, D. Lewis, S. Drapeau, L. van Der, C. D. Lingen, J. A. Huggett, J. C. Coetzee, H. M. Verheye, F. Daunt, S. Wanless & P. G. Ryan, 2008. Spatial match-mismatch in the Benguela upwelling zone: should we expect chlorophyll and sea-surface temperature to predict marine predator distributions? Journal of Applied Ecology 45: 610–621.

Guerra, A., 1992. Mollusca, Cephalopoda. Fauna Ibérica, Vol. 1. Museo Nacional de Ciencias Naturales, Madrid.

Gil de Sola, L., 1993. Las pesquerías demersales del Mar de Alborán (Surmediterráneo ibérico). Evolución en los últimos decenios. Informes Tecnicos Instituto Español de Oceanografía.

González, A. F., A. López, A. Guerra & A. Barreiro, 1994. Diets of marine mammals stranded on the northwestern Spanish Atlantic coast with special reference to Cephalopoda. Fisheries Research 21(1–2): 179–191.

Hastie, T. & R. Tibshirani, 1990. Generalized Additive Models. Chapman and Hall, London.

Jaquet, N. & H. Whitehead, 1996. Scale-dependent correlation of sperm whale distribution with environmental features and productivity in the South Pacific. Marine Ecology progress Series 135: 1–9.

Katona, S. K., V. Rough & D. T. Richardson, 1993. A Field Guide to Whales, Porpoises, and Seals from Cape Cod to Newfoundland. Fourth Edition, Revised. Smithsonian Institution Press, Washington DC.

Koubbi, P., C. Loots, G. Cotonnec, X. Harlay, A. Grioche, S. Vaz, C. Martin, M. Walkey & A. Carpentier, 2006. Spatial patterns andGIS habitat modelling of *Solea solea*, *Pleuronectes flesus* and *Limanda limanda* fish larvae in the eastern English Channel during the spring. Scientia Marina 70: 147–157.

Littaye, A., A. Gannier, S. Laran & J. P. F. Wilson, 2004. The relationship between summer aggregation of fin whales and satellite-derived environmental conditions in the northwestern Mediterranean Sea. Remote Sensing of Environment 90: 44–52.

López, A., M. B. Santos, G. J. Pierce, A. F. González, X. Valeiras & A. Guerra, 2002. Trends in strandings and by-catch of marine mammals in north-west Spain during the 1990s. Journal of the Marine Biological Association of the United Kingdom 82(3): 513–521.

López, A., G. J. Pierce, M. B. Santos, J. Gracia & A. Guerra, 2003. Fishery by-catches of marine mammals in Galician waters: results from on-board observations and an interview survey of fishermen. Biological Conservation 111: 25–40.

López, A., G. J. Pierce, X. Valeiras, M. B. Santos & A. Guerra, 2004. Distribution patterns of small cetaceans in Galician waters. Journal of the Marine Biological Association of the United Kingdom 84: 283–294.

Macleod, C. D., C. R. Weir, M. B. Santos & T. E. Dunn, 2009. Temperature-based summer habitat partitioning between white-beaked and common dolphins around the United Kingdom and Republic of Ireland. Journal of the Marine Biological Association of the United Kingdom 88: 1193–1198.

Maravelias, C. & C. Papaconstantinou, 2003. Size-related habitat use, aggregation patterns and abundance of anglerfish (*Lophius budegassa*) in the Mediterranean Sea determined by generalized additive modelling. Journal of the Marine Biological Association of the United Kingdom 83: 1171–1178.

Marubini, F., A. Gimona, P. G. H. Evans, P. J. Wright & G. J. Pierce, 2009. Habitat preferences and interannual variability in occurrence of the harbour porpoise *Phocoena phocoena* off northwest Scotland. Marine Ecology Progress Series 381: 297–310.

McClain, C., 1997. SeaWiFS Bio-Optical Algorithm Mini-workshop (SeaBAM) Overview, SeaBAM Technical Memo, NASA SeaWiFS Project.

Mead, J. G. & R. L. Brownell Jr, 2005. Order Cetacea (pp. 723–743). In Wilson, D. E. & D. M. Reeder (eds), Mammal Species of the World: A Taxonomic and Geographic Reference, 2 Vols. (3rd ed.). Johns Hopkins University Press, Baltimore: 2142 pp.

Meaden, G.J. & T. Do Chi, 1996. Geographical Information Systems: Applications to Marine Fisheries. FAO Fisheries Technical Paper No. 356. FAO, Rome, Italy: 335 pp.

Murase, M., K. Matsuoka, T. Ichii & S. Nishiwaki, 2002. Relationship between the distribution of euphausiids and baleen whales in the Antarctic (35°E–145°W). Polar Biology 25(2): 135–145.

Oksanen, J. & P. R. Minchin, 2002. Continuum theory revisited: what shape are species responses along ecological gradients? Ecological Modelling 157: 119–129.

Pereiro, F. J., A. Fernández & S. Iglesias, S, 1980. Relationships between depth and age, and recruitment indexes of hake on Galicia and Portugal shelf. International Council for the Exploration of the Sea (CM Papers and Reports), CM 1980/G.32.

Pierce, G. J., M. Caldas, J. Cedeira, M. B. Santos, A. Llavona, P. Covelo, G., Martinez, J. Torres, M. Sacau & A. López, 2010. Trends in cetacean sightings along the Galician coast, north-western Spain, 2003–2007, and inferences about cetacean habitat preferences. Journal of the Marine Biological Association of the United Kingdom.

Prego, R. & R. Bao, 1997. Upwelling influence on the Galician coast: silicate in shelf water and underlying surface sediments. Continental Shelf Research 17: 307–318.

R Development Core Team, 2006. R: a language and environment for statistical computing. Vienna, Austria. Available via http://www.R-project.org.

Redfern, J., M. C. Ferguson, E. A. Becker, K. D. Hyrenbach, C. Good, J. Barlow, K. Kaschner, M. F. Baumgartner, K. A. Forney, L. T. Ballance, P. Fauchald, P. Halpin, T. Hamazaki, A. J. Pershing, S. S. Qian, A. Read, S. B. Reilly, L. F. Torres & F. Werner, 2006. Techniques for cetacean habitat modelling. Marine Ecology Progress Series 310: 271–295.

Rubín, J. P., 1994. El ictioplacton y el medio marino en los sectores norte y sur del mar de Alborán, en junio de, 1992. Informe Técnico del Instituto Español de Oceanografía 146.

Rubin, J. P., 1997. La influencia de los procesos fisico-quimicos y biológicos en la composición y distribución del ictioplacton estival en el mar de Alborán y estrecho de Gibraltar. Informe Técnico del Instituto Español de Oceanografía 24.

Sakurai, Y., H. Kiyofuji, S. Saitoh, T. Goto & Y. Hiyama, 2000. Changes in inferred spawning areas of *Todarodes pacificus* (Cephalopoda: Ommastrephidae) due to changing environmental conditions. ICES Journal of Marine Science 57: 24–30.

Santos, M. B., R. Fernández, A. López, J. A. Martínez & G. J. Pierce, 2007. Variability in the diet of bottlenose dolphin, Tursiops truncatus, in the Galician waters, north-western Spain, 1990–2005. Journal of the Marine Biological Association of the United Kingdom 87: 231–241.

Santos, M. B., G. J. Pierce, R. J. Reid, H. M. Ross, I. A. P. Patterson, D. G. Reid & K. Peach, 2004. Variability in the diet of harbour porpoises (*Phocoena phocoena*) in Scottish waters 1992-2003. Marine Mammal Science 20: 1–27.

Scott, B. E., J. Sharples, O. N. Ross, J. Wang, G. J. Pierce & C. J. Camphuysen, 2010. Sub-surface hotspots in shallow seas: fine-scale limited locations of marine top predator foraging habitat indicated by tidal mixing and sub-surface chlorophyll. Marine Ecology Progress Series 408: 207–226.

Smith, K. F. & J. H. Brown, 2002. Patterns of diversity, depth range and body size among pelagic fishes along a gradient of depth. Global Ecology and Biogeography 11(4): 313–322.

Solórzano, M. R., J. L. Rodríguez, J. Iglesias, F. X. Pereiro & F. Alvarez, 1988. Inventario dos peixes do litoral galego (Pisces: Cyclostomata, Condrichthyes, Osteichthyes). Cadernos da Area de Ciencias Biológicas. Seminarios de Estudios Galegos.

Swartzman, G., C. Huang & S. Kaluzny, 1992. Spatial analysis of Bering Sea groundfish survey data using generalized additive models. Canadian Journal of Fisheries and Aquatic Sciences 49(7): 1366–1378.

Torres, L. G., A. J. Read & P. Halpen, 2008. Fine-scale habitat modeling of a top marine predator: do prey data improve predictive capacity? Ecological Applications 18: 1702–1717.

Tynan, C. T., D. G. Ainley, J. A. Barth, T. J. Cowles, S. D. Pierce & L. B. Spear, 2005. Cetacean distributions relative to ocean processes in the northern California Current system. Deep-Sea Research II 52: 145–167.

Valavanis, V. D. (ed.), 2008. Essential fish habitat mapping in the Mediterranean. Hydrobiologia 612: 297–300.

Valavanis, V. D., G. J. Pierce, A. F. Zuur, A. Palialexis, A. Saveliev, I. Katara & J. Wang, 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612: 5–20.

Valdés Hansen, F., 2010. Los balleneros en Galicia (siglos XIII al XX). Colleción Galicia Histórica. Fundación Pedro Barrié de la Maza, A Coruña: 591 pp.

Vidal-Romaní, J. R., 1984. A orixe das rias galegas: Estado da cuestión (1886–1983). Cuadernos da Área de Ciencias Mariñas, Seminario de Estudios Galegos 1: 13–26.

Walker, D., 2005. Using Oceanographic Features to Predict Areas of High Cetacean Diversity. MSc thesis, University of Wales, Bangor, U.K.

Walton, C. C., 1988. Nonlinear multichannel algorithms for estimating sea surface temperature with AVHRR satellite data. Journal of Applied Meteorology 27: 115–124.

Walton, C. C., E. P. McClain & J. F. Sapper. 1990. Recent changes in satellite based multichannel sea surface temperature algorithms. Marine Technology Society Meeting, MTS' 90, Washington D.C, September 1990.

Wang, J., G. J. Pierce, P. B. Boyle, V. Denis, J. P. Robin & J. M. Bellido, 2003. Spatial and temporal patterns of cuttlefish (*Sepia officinalis*) abundance and environmental influences: a case study using trawl fishery data in French Atlantic coast, English Channel, and adjacent waters. ICES Journal of Marine Science 60: 1149–1158.

Wheeler, A., 1969. The Fishes of the British Isles and North-West Europe. Michigan State University Press, East Lansing: 613 pp.

Whitehead, P. J. P., M. -L. Bauchot, J.-C. Hureau, J. Nielsen & E. Tortonese (eds), 1989. Fishes of the North-eastern Atlantic and the Mediterranean. UNESCO, Paris: 1461 pp.

Wooster, W. S., A. Bakun & D. R. McLain, 1976. The seasonal upwelling cycle along the eastern boundary of the North Atlantic. Journal of Marine Research 34: 131–141.

Zainuddin, M., H. Kiyofuji, K. Saitoh & S. I. Saitoh, 2006. Using multisensor satellite remote sensing and catch data to detect ocean hot spots for albacore (*Thunnus alalunga*) in the northwestern North Pacific. Deep-Sea Research Part II 53: 419–431.

Zuur, A. F., E. N. Ieno & G. M. Smith, 2007. Analysing Ecological Data. Springer, New York.

Hydrobiologia (2011) 670:111–125
DOI 10.1007/s10750-011-0669-5

ECOSYSTEMS AND SUSTAINABILITY

Fine-scale genetic structure of bottlenose dolphins, *Tursiops truncatus*, in Atlantic coastal waters of the Iberian Peninsula

Ruth Fernández · M. Begoña Santos · Graham J. Pierce · Ángela Llavona · Alfredo López · Mónica A. Silva · Marisa Ferreira · Manuel Carrillo · Pablo Cermeño · Santiago Lens · Stuart B. Piertney

Published online: 5 April 2011
© Springer Science+Business Media B.V. 2011

Abstract In the NE Atlantic, evidence has been found of genetic discontinuities between Iberian bottlenose dolphins and those of Scotland and the Mediterranean. Here, we explored the genetic relationships between resident populations of dolphins from southern Galicia (NW Spain) and the Sado estuary (S Portugal), and their relationship with dolphins inhabiting neighbouring areas. A total of 91 skin and muscle samples were taken from stranded and biopsied animals between 1994 and 2008 in southern Galicia ($N = 29$), the Sado estuary ($N = 5$) and five other geographical locations ($N = 57$) including sites around the Iberian Peninsula, the Canary Islands and the Azores. Individuals were genotyped at 10 microsatellite loci and sequenced at the highly variable mitochondrial control region. From individual-based analyses of microsatellite data, dolphins from southern Galicia and the Sado estuary were assigned to an individual genetic population, though nine dolphins were identified as possible migrants between putative populations as their genetic makeup did not correspond with their geographical stranding location. Pairwise estimates of genetic differentiation (F_{ST}) based on mitochondrial and nuclear DNA also revealed genetic differences between populations. The existence of fine-scale population substructure should be considered in the future designation of Special Areas of Conservation

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

---

R. Fernández (✉) · G. J. Pierce · S. B. Piertney
Institute of Biological and Environmental Sciences
(IBES), University of Aberdeen, Tillydrone Avenue,
Aberdeen AB24 2TZ, UK
e-mail: r.fernandez@abdn.ac.uk

R. Fernández · Á. Llavona · A. López
CEMMA, Ap. 15, 36380 Gondomar, Spain

M. B. Santos · G. J. Pierce · S. Lens
Instituto Español de Oceanografía, Cabo Estay, Canido,
P.O. Box 1552, 36200 Vigo, Spain

M. A. Silva
Departamento de Oceanografia e Pescas, Centro do
Instituto do Mar (IMAR) da Universidade dos Açores,
9901-862 Horta, Portugal

M. A. Silva
Department of Biology, MS#33, Woods Hole
Oceanographic Institution, Woods Hole, MA 02543, USA

M. Ferreira
Departamento de Biologia, CBMA/Sociedade Portuguesa
de Vida Selvagem, Universidade do Minho, Campus de
Gualtar, 4710-057 Braga, Portugal

M. Carrillo
Canarias Conservación, C/Maya 8, 4° D, 38204 La
Laguna, Tenerife, Spain

P. Cermeño
AMBAR, Blas de Otero, n° 18 5° izq., 48014 Bilbao,
Spain

(SACs) for the species, as required by the European Habitats Directive.

Keywords Genetic differentiation · Resident populations · Cetaceans · Migrants · Conservation

Introduction

The bottlenose dolphin, *Tursiops truncatus*, has a worldwide distribution from temperate to tropical seas, displaying strong behavioural and ecological plasticity that allows it to inhabit marine and estuarine ecosystems, even ranging into rivers (Wells & Scott, 2002). Satellite tracking has demonstrated that individual dolphins can travel very large distances in short periods of time (2,000–4,000 Km in 43–47 days) (Wells et al., 1999). A high propensity for dispersal, coupled with an apparent lack of barriers to movement, suggests that high levels of gene flow over large geographic areas are possible. Such predictions have been confirmed by low levels of population structure among oceanic bottlenose dolphins (Quérouil et al., 2007). However, many studies have shown significant population genetic structure, frequently without correlation with geographical distance. There is clear genetic structure extending from the Black Sea to Scotland (UK), with discontinuities separating Iberian animals from those around Scotland and the Mediterranean (Natoli et al., 2005). Genetic differentiation is also apparent between offshore and coastal populations in the NW Atlantic (Hoelzel et al., 1998; Natoli et al., 2005), as well as within ecotypes over short geographic distances (Krützen et al., 2004; Sellas et al., 2005; Segura et al., 2006; Parsons et al., 2006; Tezanos-Pinto et al., 2009). Strong site fidelity, with resident groups inhabiting coastal inlets and estuaries, plus resource specialisation, as a consequence of different social and behavioural strategies, could be some of the factors leading to genetic structure (Hoelzel, 1998; Wiszniewski et al., 2009).

Around the Iberian Peninsula, bottlenose dolphins are present continuously along the Atlantic coast (VVAA, 2007; Brito et al., 2009) with recognised resident populations in southern Galicia (NW Spain) and in the Sado estuary (S Portugal) (dos Santos & Lacerda, 1987; López, 2003; Brito et al., 2009). The southern Galician population inhabits a series of large inlets or rías, characterised by their considerable width and a SW–NE orientation (Méndez & Vilas, 2005). The Sado estuary is located in the SW coast of Portugal and it is divided in an outer and inner areas separated by a shallow area with tidal flats (Caeiro et al., 2005). Groups of bottlenose dolphins enter the river mouth to forage, play and socialise on a year-round daily basis (dos Santos et al., 2005).

The West coast of Portugal and Galicia is influenced by the NW Africa upwelling system, and therefore upwelling–downwelling dynamics are encountered in the area enhancing productivity (Santos et al., 2001; Figueiras et al., 2002). This seasonal characteristic pattern can vary considerably: in Galician waters the spring transition (from downwelling to upwelling) can occur in February or in April, while the autumn transition shows less variability and generally takes place around October (Figueiras et al., 2002).

The resident populations of bottlenose dolphins from southern Galicia and the Sado estuary are characteristically small (a minimum figure of 123 animals in Galicia based on simultaneous counts (López, 2003), and 25 dolphins with a negative population trend in the Sado estuary (Silva, 2008)), and are likely to face greater threats than offshore dolphin populations from habitat degradation, pollution, and other anthropogenic impacts. Therefore, understanding the structure of these populations and their relationships with other groups/populations should be a pre-requisite to establishing appropriate management units and define future Special Areas of Conservation (SACs) as required by the EU Habitats Directive (92/43/CEE).

Here, the spatial distribution of microsatellite and mitochondrial DNA variation was used to characterise levels of fine-scale and regional population genetic structure among putatively resident populations from southern Galicia and the Sado estuary. Divergence is assessed in relation to differences among more distant locations such as the Canary Islands and the Azores. Both archipelagos are separated from the mainland by thousands of kilometres, including vast areas of low productive waters of depths greater than 5,000 m. As a consequence, isolation by distance (IBD) was expected to occur between insular and continental bottlenose dolphins.

Materials and methods

Study area and sample collection

Skin and muscle samples were obtained from stranded and by-caught bottlenose dolphins from four geographical locations: Galicia (GAL, $N = 48$), mainland Portugal (MPOR, $N = 21$), the Basque Country (BCO, $N = 4$) and the Canary Islands (CAN, $N = 8$). Dolphins stranded in Galicia were further classified as southern (SGAL, $N = 29$), when stranded in the area delimited by the border with Portugal and "Punta Queixal" in the Mount Louro (geographic limit between the southern inlets and the northern Galician coastline), and northern (NGAL, $N = 19$) when stranded in the area extending from "Punta Queixal" to the border with Asturias (Fig. 1). Dolphins stranded in mainland Portugal were divided in non-resident bottlenose dolphins (POR, $N = 16$) and in Sado estuary residents (SAD, $N = 5$). All stranded dolphins classified as Sado estuary residents had been previously photo-identified as locals through the individually distinct marks and nicks present on their dorsal fin by Gaspar (2003). In addition, skin samples from the Azores (AZO, $N = 10$) were collected between 2002 and 2004 using a biopsy darting system (see Quérouil et al., 2007). Sampling locations are shown in Fig. 1.

Samples were preserved in: 20% dimethyl sulfoxide (DMSO) solution saturated with NaCl with subsequent storage at −20°C; 96% ethanol with storage at ambient temperature; or frozen directly at -20°C. DNA was extracted using the DNeasy blood and tissue extraction kit (Qiagen Ltd) according to the manufacturer's instructions with dilution of DNA in sterile water to ~ 20 ng μl^{-1}. It should be noted that as the majority of the samples came from stranded dolphins, state of preservation at the time of sample collection varied considerably between animals.

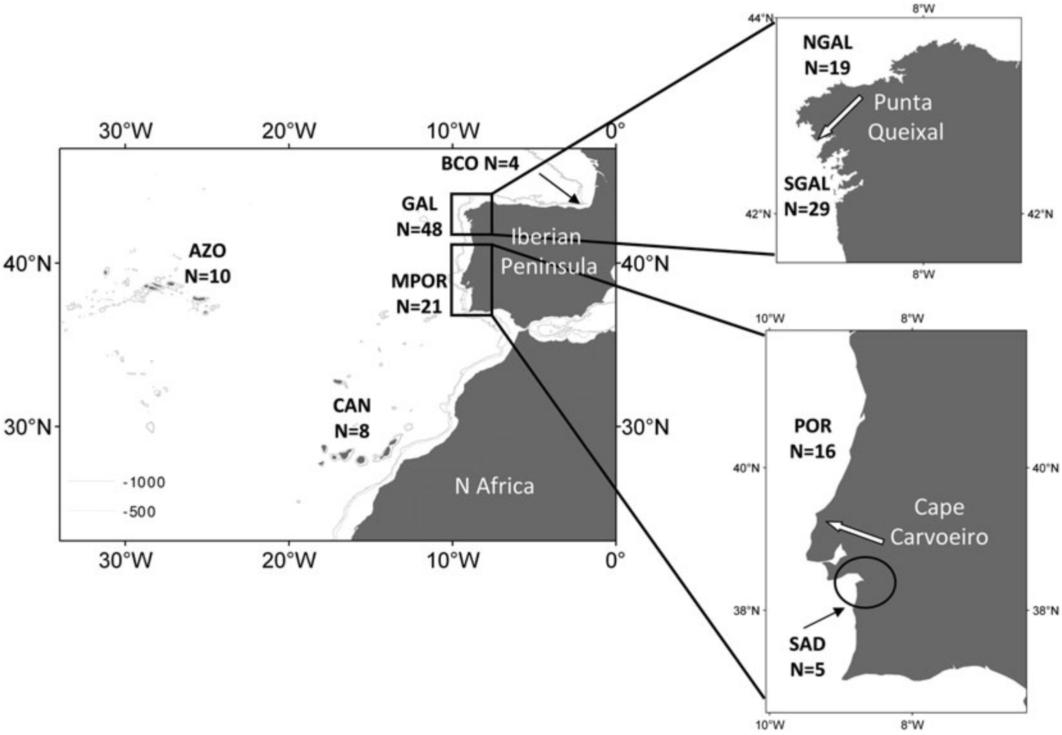


Fig. 1 Locations from which *T. truncatus* samples were obtained: The Basque Country (*BCO*), Galicia (*GAL*), mainland Portugal (*MPOR*), the Azores (*AZO*), and the Canary Islands (*CAN*). Detailed maps of sample origin for GAL, divided into northern and southern (NGAL and SGAL, respectively) and MPOR, divided into Sado Estuary (SAD) resident dolphins and other mainland Portuguese strandings (POR) are shown

Microsatellite genotyping

Individuals were genotyped at ten microsatellite loci: Dde59, Dde65, Dde72 (Coughlan et al., 2006); Ttr04, Ttr19, Ttr34, Ttr48, Ttr58, Ttr63, and TtrRC11 (Rosel et al., 2005). PCRs were carried out in a 10 µl volume containing 20 ng of DNA, 1.5 mM $MgCl_2$, 75 mM Tris–HCL pH 9.0, 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 0.2 mM dNTP's, 5 pmol of each primer and 0.5 units of Taq polymerase (Bioline Ltd). The thermal profile included an initial denaturation step at 95°C for 3 min, followed by 30 cycles of: 35 s at 95°C, 30 s at the specified annealing temperature (see Rosel et al., 2005; Coughlan et al., 2006), 30 s at 72°C and 3 min a 72°C. All loci were fluorescently tagged on the 5′ terminus, allowing alleles to be resolved on an ABI3730 automated DNA sequencer and allele sizes were determined using an internal size standard.

Microsatellite analysis

Evidence for the presence of null alleles or large allele dropout was tested using the program MICRO-CHECKER 2.2.3. (Van Oosterhout et al., 2004). Allele frequencies per locus and sample were calculated using the software FSTAT 2.9.3. (Goudet, 2001).

The program *STRUCTURE* 2.2. (Pritchard et al., 2000) was used to determine the most probable number of putative populations (K) that best explained the pattern of genetic variability. Correlated allele frequencies and an admixture model were assumed and performed with a burn-in period and number of simulations that ranged from 100,000 and 10,000 to 500,000 and 100,000 repetitions, respectively. Due to the different geographical origin of the samples, that could represent different genetic units, we used values of K between 1 and 6. Ten replicates for each proposed value of K were performed and consistency of results was assessed. *STRUCTURE* 2.2. was also used to assign individuals among the putative genetic groupings.

There is considerable debate in the population genetics literature about the relative merits of maximising numbers of loci or individuals in analysis of population genetic structure (Kalinowski, 2002; Ryman et al., 2006). This issue is especially pertinent in studies where the quality of DNA obtained amongst individuals varies such that unambiguous genotypes cannot be obtained for all individuals at all loci. This was the case in this study, and as such, parallel *STRUCTURE* analyses were undertaken on two datasets, one based on 10 loci for 51 individuals, followed by a second analysis with samples size increased to 71 after addition of 20 individuals with genotypes obtained from 5 loci. Congruent outcomes derived from both datasets were taken to indicate biologically meaningful patterns. Prior to the *STRUCTURE* analysis, a power analysis was undertaken based on 5 microsatellite loci with the software POWSIM 4.0. (Ryman & Palm, 2006). The method uses multilocus allele frequency data to assess the probability of detecting significant differentiation using Fisher's exact tests and the more conservative χ^2 test. A specified level of genetic divergence is assumed by applying the formula $1 = (1 - 1/N_e)^t$ where t is the time since divergence, and N_e is the effective population size assuming complete isolation between populations. Simulations were run assuming two (sample sizes of $N = 25$ and $N = 45$) and six subpopulations (sample sizes of $N = 25$, $N = 30$, $N = 35$, $N = 40$, $N = 45$ and $N = 50$). Various combinations of N_e and t were used, leading to F_{ST} of 0.025–0.05, which approximates the values obtained from empirical data based on 10 loci (see below). Six simulations were performed for each of the two levels of population substructure; three simulations for an F_{ST} of 0.025 using N_e/t of 500/25, 1000/40 and 2000/101 and three for an F_{ST} of 0.05 using N_e/t of 500/51, 1000/102 and 2000/205. Additional simulations were performed omitting the drift steps ($t = 0$, $N_e = 500$, $F_{ST} = 0$) to approximate the type I error (null hypothesis is incorrectly rejected). A thousand replicates were run for each simulation and power was estimated as the proportion of these 1,000 tests that indicated significant genetic differentiation ($P < 0.005$).

Principal coordinates analysis (PCoA) was undertaken using the program GENALEX 6 (Peakall & Smouse, 2006). Genetic distances were calculated between pairs of individuals and translated into ordination distances in a low-dimensional space. As such, samples with similar genetic genotypes are expected to be closer together in the ordination plot. The presence of Isolation by Distance (IBD) was tested with GENALEX 6. A matrix of geographical distances between pairs of sampled dolphins was created based on the latitudes and longitudes of stranding positions. This matrix was correlated against a genetic distance matrix between pairs of dolphins and tested using Mantel permutations (10,000 iterations).

Genetic diversity was assessed as the number of alleles, number of private alleles and allelic richness using the software FSTAT 2.9.3 (Goudet, 2001). Observed (Ho) and expected (He) heterozygosities were calculated at each loci and population and deviations from the Hardy–Weinberg equilibrium were tested using the Markov chain method (chain length; 1,000,000, dememorization steps; 100,000) with ARLEQUIN 3.11. For each population, the overall deviation from the Hardy–Weinberg equilibrium and the degree of inbreeding were estimated based on F_{IS} values (10,000 randomisations) using the software FSTAT 2.9.3. A classical estimation of between-population F_{ST} was calculated using ARLEQUIN 3.11. Sex-biased dispersal was examined by determining sex-specific F_{ST} values using FSTAT 2.9.3 and tested using 10,000 randomizations. Populations were defined based upon their geographic stranding location (Fig. 1).

Mitochondrial DNA sequencing

A 549 bp fragment of the mitochondrial control region was PCR amplified using the primers L15926 (5′-ACACCAGTCTTGTAAACC-3′; Eggert et al., 1998) and H16498 (5′-CCTGAAGTAAGAACCAG ATG-3′; Rosel et al., 1995). Amplification reactions were carried out in a 25 µl volume containing 50 ng DNA, 2 mM $MgCl_2$, 75 mM Tris–HCL (pH 9.0), 20 mM $(NH_4)_2SO_4$, 0.01% Tween-20, 0.2 mM dNTP’s, 5 pmol of each primer and 0.5 units of Taq polymerase (Bioline Ltd). The thermocycle profile followed a 10° “touchdown” procedure which included 2 min of initial denaturation at 95°C, followed by 20 cycles of: 30 s denaturation at 92°C, 30 s annealing at 60°C (which decreases 0.5°C in each of the 20 cycles) and 45 s extension at 72°C, then 20 cycles of: 30 s denaturation at 92°C, 30 s annealing at 50°C and 45 s extension at 72°C, with a final 2.5 min extension at 72°C. PCR products were purified using a Qiaquick PCR purification kit (Qiagen). Samples were sequenced in both directions on an ABI3730 automated sequencer using the L15926 and H16498 PCR primers as the sequencing primers.

The dataset was augmented with 19 published mtDNA bottlenose dolphin sequences from mainland Portugal and the Azores obtained from Genbank (accession numbers: DQ073641, DQ073644, DQ073646, DQ073647, DQ073669, DQ073688, DQ073699, DQ073700, DQ073706, DQ073710, DQ073718, DQ073720, DQ073722–DQ073725, DQ073727–DQ073729; Quérouil et al., 2007). Given these sequences were shorter than those obtained in this study, all subsequent analyses are based upon a 426 bp truncated alignment.

Mitochondrial DNA analysis

All sequences were aligned using Clustal W within MEGA 4.0 (Tamura et al., 2007). Phylogenetic relationships among the mtDNA haplotypes were inferred from a median-joining network constructed using the program NETWORK 4.5 (Bandelt et al., 1999; http://www.fluxus_engireering.com). Population differentiation was estimated as F_{ST} using the program ARLEQUIN 3.11. Haplotype diversity (Hd) and nucleotide diversity (π) were estimated using DnaSP vs5 (Librado & Rozas, 2009).

Results

Given variability in the quality of DNA obtained from samples, successful PCR amplifications were not achieved for every sample at every locus. Details of the number of animals included in microsatellite and mtDNA data analyses are given in Table 1. For 20 individuals, microsatellite genotypes were only obtained for five of the loci, so separate statistical analyses were performed including and excluding these individuals. Power analysis based on these five loci, two subpopulations and an F_{ST} of 0.025 showed that in >98% of the runs the tests detected a genetic difference between subpopulations. When F_{ST} was set to 0.05, genetic differentiation was found in 100% of the tests. Considering six subpopulations, genetic differentiation was found in 100% of the runs independent of the F_{ST} value. When F_{ST} was set to 0, between 3 and 6% of the runs returned evidence of genetic differentiation which approximates the expected Type I error rate of 5%. Among the microsatellite loci, there was no evidence for null alleles or large allele dropout.

Table 1 Composition of the set of samples

Population	SGAL	SAD	BCO	NGAL	POR	AZO	CAN	TOTAL
N	29	5	4	19	16	10	8	91
mtDNA	25	4 (2[a])	2	18	16 (7[a])	10[a]	6	81
Microstatellites	24	4	4	14	14	6	5	71

SGAL South Galicia, *SAD* Sado estuary, *BCO* Basque Country, *NGAL* North Galicia, *POR* Mainland Portugal, *AZO* The Azores, *CAN* Canary Islands

[a] Number of sequences obtained from Genbank

Microsatellite DNA

STRUCTURE analyses based on either 51 (10 loci) or 71 samples (10 and 5 loci genotypes) indicated that the most probable number of populations was two (Ln $Pr(X/K) = -1539.27 \pm 1.81$ for 51 samples and $P(X/K) = -1887.28 \pm 1.18$ for 71 samples; Fig. 2). Individuals from the different sampling locations were apportioned among these two populations according to Fig. 3a for the analysis involving 51 samples and Fig. 3b for the analysis involving 71 samples.

Genetic differences were found when 51 dolphins (10 loci) were examined. Nineteen of the 22 SGAL dolphins were identified as belonging to a single population together with four animals stranded in NGAL and the single Sado estuary (SAD) resident dolphin. All the other dolphins were classified as belonging to a second population. Thus, seven Galician animals (three from SGAL and four from NGAL) were identified as possible migrants between populations as their genetic signature did not correspond to the majority of individuals from their geographical stranding location (Fig. 3a). When 71 samples were considered, only two individuals remained poorly resolved, showing proportions of coefficients of admixture between populations higher than 0.25 (sample 54 and sample 60; Fig. 3b). In this case, the seven potential migrant individuals previously identified can still be recognised. All the AZO animals are classified as belonging to the second undifferentiated population while dolphins from the Sado estuary (SAD) appear to be genetically similar to the SGAL population. However, among the 20 new individuals included in the analysis (five loci samples), two new potential migrants are found between the SAD population and the POR groups (Fig. 3b).

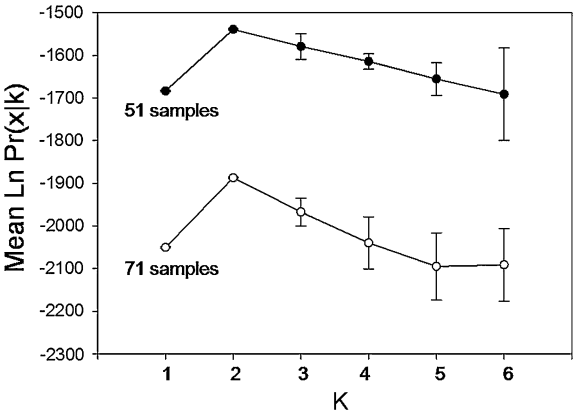


Fig. 2 Results of the *STRUCTURE* analysis, showing mean (±SD) probabilities of the data (Ln *Pr*(*x*|*k*)) based on 10 *STRUCTURE* replicated runs plotted as a function of the putative number of clusters (*K*). *Black dots* 51 samples analyzed; *white dots* 71 samples analyzed

Only those individuals from which results over 10 loci were available were used in further statistical analyses, therefore Azorean individuals and all but one SAD dolphin were excluded from additional calculations. The PCoA analysis confirmed a level of population structure among samples (Fig. 4). Analyses show most of the SGAL animals and the single SAD dolphin grouped towards one side of the ordination plot, with the two first principal coordinates explaining 50.5% of the variation. Mantel tests demonstrate the existence of IBD ($P = 0.003$) which was expected due to the inclusion of distant dolphins from the Canary Islands. However, IBD was also patent when these insular dolphins were excluded from the analysis ($P = 0.001$).

Pairwise estimates of genetic differentiation (F_{ST}) were calculated between SGAL, NGAL and POR. SGAL animals were significantly different to the other two groups although no significant difference was found between NGAL and POR dolphins (Table 2). Sex-specific F_{ST} were calculated between

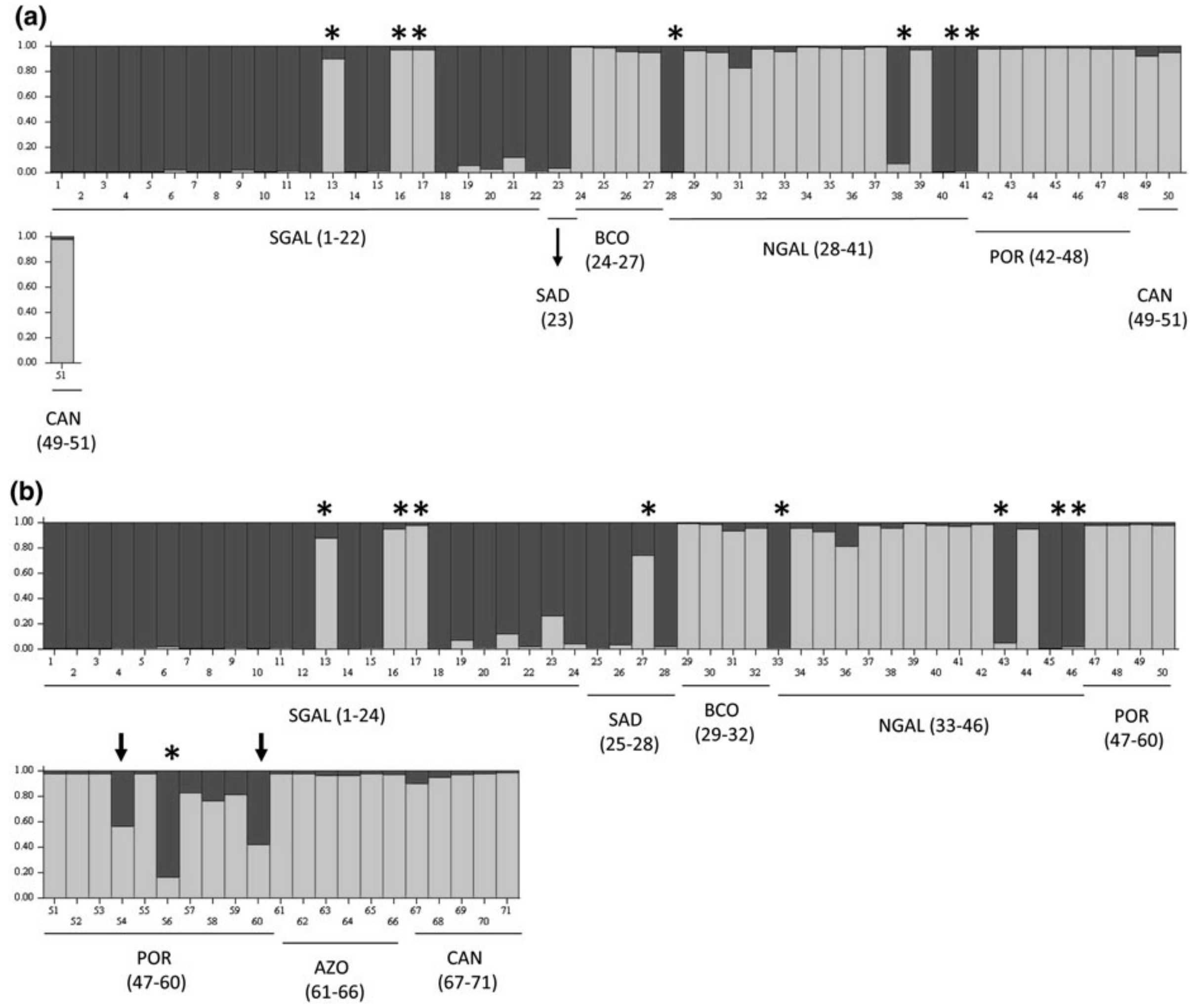


Fig. 3 Estimated proportions of the coefficient of admixture of each individual's genome that originated from population *K*, for $K = 2$. Each individual is represented by a column. *Asterisk* indicates individuals identified as possible migrants. *Down arrow symbol* indicates animals poorly resolved (proportions of coefficients of admixture between populations higher than 0.25). **a** Analysis including 51 samples. **b** Analysis including 71 samples (for 20 individuals only results over 5 loci were available). *SGAL* Southern Galicia, *SAD* Sado Estuary, *BCO* the Basque Country, *NGAL* northern Galicia, *POR* Portugal, *CAN* the Canary Islands, *AZO* the Azores

these same groups and females showed only slightly higher F_{ST} values than males (F_{ST} females $= 0.091$, F_{ST} males $= 0.089$; $P = 0.967$) suggesting no evidence of sex-biased dispersal.

The highest allelic richness was found in NGAL and POR dolphins even when sample sizes were considerably smaller than that from SGAL (Table 3). The number of private alleles found in SGAL dolphins was much lower than that found in NGAL or CAN which had much smaller sample sizes, especially the latter (Table 3).

No deviation from the Hardy–Weinberg equilibrium was found based on F_{IS} values for any of the groups considered (SGAL, NGAL, POR, BCO, and CAN). However, when dolphins stranded outside SGAL and SAD were grouped as a single population, as identified by the *STRUCTURE* analysis, F_{IS} estimates showed a significant deviation from the Hardy–Weinberg equilibrium ($F_{IS} = 0.094$, $P = 0.0004$) reflecting a deficiency of heterozygote genotypes. NGAL and MPOR populations showed deviations from the Hardy–Weinberg equilibrium at a single

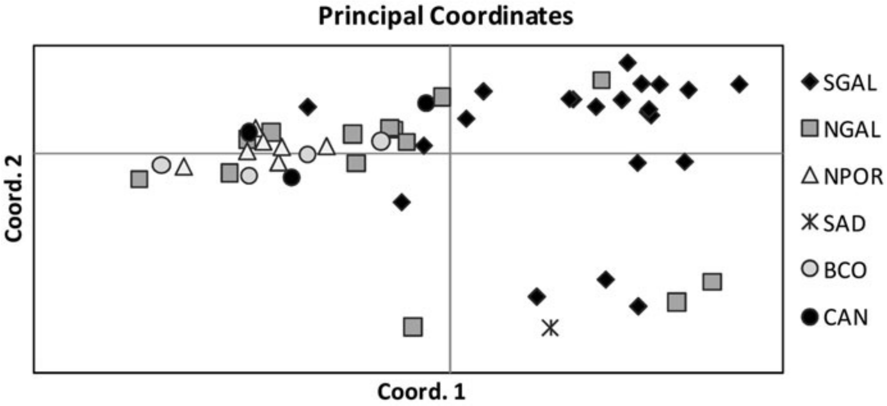


Fig. 4 Principal coordinates analysis, PCoA, based on genetic distances between individuals, showing main patters of data variation over 10 loci: 50.5% of the variability explained by principal coordinates 1 and 2. *SGAL* Southern Galicia, *SAD* Sado Estuary, *BCO* the Basque Country, *NGAL* northern Galicia, *POR* Portugal, *CAN* the Canary Islands

Table 2 Estimates of population differentiation expressed as F_{ST} based on microsatellite length polymorphism (below diagonal) and mtDNA haplotype frequencies (above diagonal) where * $P < 0.05$ (not significant after Bonferroni correction) and *** $P < 0.00001$

Population *N*:	*N*	SGAL 25	NGAL 18	POR 16	CAN 6	AZO 10
SGAL	22	–	0.124***	0.471***	0.434***	0.461***
NGAL	14	0.063***	–	0.137*	0.131*	0.098
POR	7	0.132***	0.015	–	0.144*	0.104***

Sample sizes for the microsatellite and mitochondrial analyses for each group are given in the second column and row, respectively. Only groups with a sample size higher than $N = 5$ were considered for the present analyses

SGAL South Galicia, *NGAL* North Galicia, *POR* Mainland Portugal, *CAN* Canary Islands, *AZO* The Azores

Table 3 Within population measures of nuclear DNA genetic diversity calculated over 10 loci

	SGAL	NGAL	POR	BCO	CAN
N	22	14	7	4	3
Overall F_{IS}	0.063	0.066	0.119	0.107	0.211
Private alleles	2	9	5	2	9
Average ± SD					
N alleles	5.9 ± 1.969	7.6 ± 1.647	6.3 ± 1.829	4.1 ± 1.101	3.7 ± 0.675
Allelic richness	5.648 ± 1.975	7.205 ± 1.753	5.932 ± 1.531	3.775 ± 0.690	3.160 ± 0.386
Ho	0.568 ± 0.226	0.762 ± 0.195	0.804 ± 0.148	0.733 ± 0.232	0.717 ± 0.261
He	0.604 ± 0.229	0.786 ± 0.124	0.832 ± 0.074	0.807 ± 0.077	0.850 ± 0.074

SGAL South Galicia, *NGAL* North Galicia, *POR* Mainland Portugal, *BCO* Basque Country, *CAN* Canary Islands

locus, Ttr19 ($P = 0.036$) and Dde59 ($P = 0.004$), respectively, due to heterozygote deficiency.

Mitochondrial DNA sequences

Thirty-four different mtDNA haplotypes were found among 81 individuals (Table 4); sixteen haplotypes were newly discovered (Accession numbers GU599885-GU59899 and HM236171; Table 4). From thirty-four polymorphic sites, 23 were transitions (one transition being a transversion for two animals), seven were transversions and four were insertions–deletions. Unique haplotypes were found for all geographic regions (Table 4). The most common haplotype was present in 30 dolphins from SGAL, NGAL, SAD and AZO, which matches the

Table 4 Mitochondrial control region haplotype polymorphic nucleotides and haplotype frequencies

Hapl.	Nucleotide position																																	
	1	16	25	26	51	60	64	71	72	74	75	76	80	85	89	90	103	105	109	149	150	160	205	244	256	277	310	313	321	351	361	373	379	393
H_02	G	–	G	T	C	G	T	G	G	A	G	A	G	G	T	A	G	G	G	A	G	A	G	A	G	A	G	G	A	–	G	T	A	G
H_01[a]	A	A	.	.	T	.	A	.	.	.	.	.	.	.	G	.	.	.	A	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_03	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	A	G	.	G	.	.	A	.	.	.	.	.	.	.	.	.
H_04[a]	A	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	C	.
H_05	.	.	.	.	T	.	.	.	A	.	.	G	.	A	G	.	.	.	A	.	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_06	.	.	.	.	T	.	.	.	.	.	.	G	.	.	A	.	.	.	A	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_07	.	.	.	.	.	A	.	.	.	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_08[a]	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	A	.	A	.	.	T	.	.	.	.
H_09	.	.	.	.	.	.	.	.	.	.	A	.	–	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_10[a]	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_11[a]	.	.	.	.	.	A	.	A	.	.	A	.	.	.	.	.	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_12	.	.	.	.	.	.	.	.	A	.	.	G	.	.	.	.	.	.	.	G	.	.	.	.	A	.	A	.	.	T	.	.	.	.
H_13[a]	A	A	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_14	.	.	.	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_15	.	.	.	.	.	A	.	.	A	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_16[a]	.	.	.	.	T	.	.	A	.	.	.	G	.	T	G	G	.	.	A	.	.	.	A	.	A	.	.	.	.	T	.	.	.	A
H_17	.	.	A	.	.	.	.	.	.	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_18[a]	.	.	.	.	T	.	.	.	.	.	.	G	.	.	G	.	.	.	.	G	.	.	A	G	A	.	.	.	G	T	.	.	.	.
H_19	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_20[a]	.	.	.	.	T	.	.	.	.	.	.	G	.	.	G	.	A	.	.	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_21[a]	A	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H_22[a]	.	.	.	.	T	.	.	.	A	.	.	.	.	.	G	.	.	.	A	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_23[a]	.	.	.	.	T	.	.	.	A	.	.	.	.	.	G	.	.	.	A	G	.	.	A	G	A	.	.	T	G	.	.	.	.	.
H_24	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	A	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_25	.	.	.	.	T	.	.	.	.	.	.	.	.	T	G	G	.	.	A	.	.	.	A	A	A	.	.	.	.	T	.	.	.	.
H_26	.	.	.	.	.	.	.	.	.	.	A	G	–	.	.	.	.	.	A	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_27	.	.	.	.	T	.	.	.	A	.	.	G	.	A	G	.	.	A	A	.	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_28	.	.	.	.	T	.	.	.	.	.	.	G	.	.	A	.	.	.	A	G	.	.	A	G	A	–	.	.	G	.	.	.	.	.
H_29	.	.	.	.	T	.	.	.	A	G	.	G	.	A	G	.	.	.	A	.	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_30	.	.	.	.	T	.	.	.	.	.	.	G	.	.	G	.	.	.	.	G	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_31[a]	.	.	A	.	.	A	.	.	A	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.
H_32[a]	A	A	.	.	.	A	.	.	.	.	A	G	.	.	.	.	.	.	A	G	A	.	.	.	A	.	.	.	.	.	.	.	.	.
H_33[a]	A	A	.	A	T	.	A	.	A	.	.	G	.	A	G	.	.	.	.	.	.	.	A	G	A	.	.	.	G	.	.	.	.	.
H_34[a]	A	.	.	.	.	A	.	.	.	.	A	G	.	.	.	.	.	.	A	G	.	.	.	.	A	.	.	.	.	.	.	.	.	.

Hapl.	Location						
	SGAL	SAD	BCO	NGAL	POR	AZO	CAN
H_02	20	2		7		1	
H_01[a]	1						

Table 4 continued

Hapl.	Location						
	SGAL	SAD	BCO	NGAL	POR	AZO	CAN
H_03				1	1		
H_04[a]	1						
H_05				2			
H_06				1			
H_07				1	5		
H_08[a]				1			
H_09				1	1	1	
H_10[a]				1			1
H_11[a]				1			
H_12	1			1		1	
H_13[a]	1						
H_14	1				2		
H_15					1		
H_16[a]					1		
H_17					1	1	
H_18[a]			1				
H_19			1				
H_20[a]					1		
H_21[a]							1
H_22[a]				1			
H_23[a]					1		
H_24					1	1	
H_25					1		
H_26		2					
H_27						1	
H_28						2	
H_29						1	
H_30						1	
H_31[a]							1
H_32[a]							1
H_33[a]							1
H_34[a]							1

[a] The correspondent haplotypes have been registered in Genbank with accession numbers GU599885–GU59899 and HM236171

Hapl. Haplotype, *SGAL* South Galicia, *SAD* Sado estuary, *BCO* Basque Country, *NGAL* North Galicia, *POR* Mainland Portugal, *AZO* The Azores, *CAN* Canary Islands

haplotype that Parsons et al. (2002) identified as the most common among UK bottlenose dolphins (GenBank accession no. AF268357).

Pairwise estimates of genetic differentiation (F_{ST}) were calculated between those groups with sufficient sample size ($N > 5$; SGAL, NGAL, POR, CAN and AZO). After the Bonferroni correction was applied, ssignificant mtDNA population differentiation was observed between SGAL dolphins and all the other groups and also between POR and AZO individuals

Table 5 Within population measures of mitochondrial DNA genetic diversity: nucleotide diversity, π (±SD) and haplotype diversity, Hd (±SD)

Population	SGAL	SAD	NGAL	POR	BCO	CAN	AZO
N	25	4	18	16	2	6	10
π (±SD)	0.005 ± 0.002	0.006 ± 0.002	0.013 ± 0.002	0.012 ± 0.002	0.012 ± 0.006	0.018 ± 0.004	0.015 ± 0.002
Hd (±SD)	0.367 ± 0.122	0.667 ± 0.204	0.856 ± 0.079	0.908 ± 0.063	1.000 ± 0.500	1.000 ± 0.096	0.978 ± 0.054

SGAL South Galicia, *SAD* Sado estuary, *BCO* Basque Country, *NGAL* North Galicia, *POR* Mainland Portugal, *AZO* The Azores, *CAN* Canary Islands

(Table 2). Overall haplotypic diversity (Hd) among the 81 bottlenose dolphins was estimated to be 0.855 (±0.037) although clear differences between putative populations do exist (Table 5). Lowest nucleotide (π) and haplotypic (Hd) diversities were found in the SGAL and SAD populations ($\pi = 0.005 \pm 0.002$, Hd $= 0.367 \pm 0.122$ and $\pi = 0.006 \pm 0.002$, Hd $= 0.667 \pm 0.204$, respectively).

The genetic relationships among haplotypes are given as a median-joining network in Fig. 5. Two main clusters, separated by five mutational steps, were identified, with most of the SGAL and SAD dolphins (except haplotype H_01; Genbank accession number GU599885) represented in one of the groups. The SAD population unique haplotype differs by one mutational step from haplotypes present in dolphins from other geographical areas while SGAL exclusive haplotypes were 1 to 6 mutational steps apart from sequences present in other groups (Fig. 5). The SGAL population showed very low levels of variation, with most of the individuals sharing the same haplotype (H_02) (Table 4).

Discussion

Both microsatellite and mtDNA analyses indicate genetic structure within our sample set. Individual-

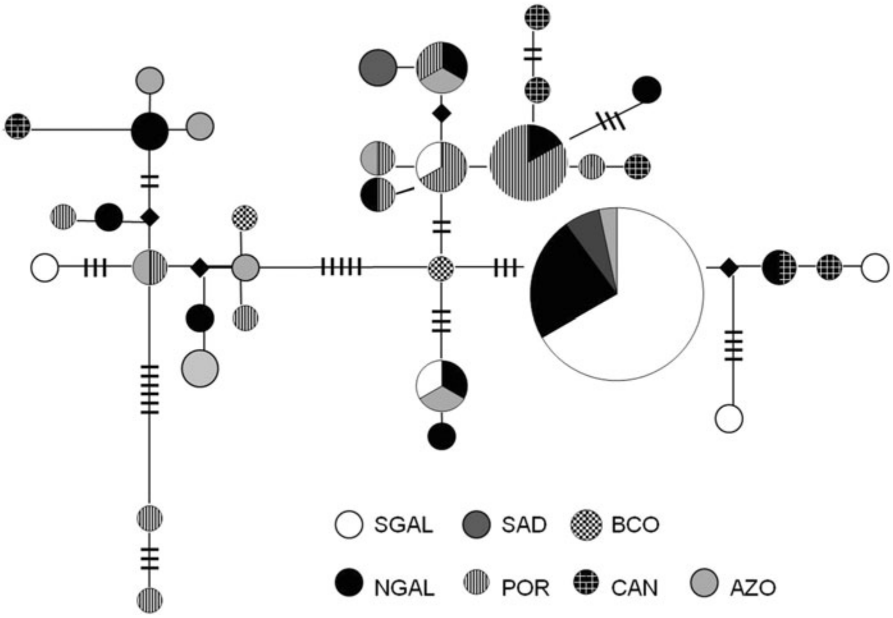


Fig. 5 Median-joining network of bottlenose dolphin mtDNA haplotypes. Higher weights were applied to insertions–deletions. *Circle size* is approximately proportional to the number of individuals exhibiting the corresponding haplotype. Connector length is proportional to the number of mutations between haplotypes. *Black diamonds* indicate potential intermediate haplotypes that were not sampled. *Hatch marks* indicate total number of mutations between haplotypes when more than one mutation is present. *Numbers* correspond to haplotypes from Table 4. *SGAL* Southern Galicia, *SAD* Sado Estuary, *BCO* the Basque Country, *NGAL* northern Galicia, *POR* Portugal, *CAN* the Canary Islands, *AZO* the Azores

based analysis of microsatellite genotypes identified southern Galician and Sado estuary animals as more similar to each other than to dolphins sampled elsewhere. However, the existence of strong gene flow between both sites is unlikely. Around 500 km of coastline with continuous presence of bottlenose dolphins separate the two resident communities and, as shown by this study, gene flow between resident and immediate neighbouring populations in our research area is limited. More likely, low sample size precluded accurate estimates of population structure. Bigger sample sizes (especially for Sado resident dolphins) and a higher number of genetic markers are needed to better understand the relationships between these resident populations. Previous genetic studies carried out with samples from the area did not find evidence of population structure among bottlenose dolphins from mainland Portugal and Galicia (Natoli et al., 2005). Differences between this article and conclusions from Natoli et al. (2005) may be also due to an uneven distribution of animals (with fewer animals coming from southern Galicia or the Sado estuary), the small sample size used in Natoli's study ($N = 35$), some inherent bias caused by the use of stranded individuals for which the true provenance of samples (i.e. where the animals lived as opposed to where the carcases were found) is unclear, or a combination of the three. It should be noted that carcases may be transported considerable distances along the coast before being stranded (Peltier et al., 2009) and some of these biases could also apply to this study.

The high mobility of marine species and the lack of obvious barriers to gene flow in the oceans should translate in low levels of population genetic structure in marine animals (Fontaine et al., 2007). However, cetaceans often show local structure at regional or fine scales that is not necessarily related to physical features or geographic separation. Frequently, these patterns are attributable to complex behaviours such as local resource specialisation, philopatry or social organization into kin groups (Hoelzel, 1998; Fontaine et al., 2007). In this study, we found evidence of IBD. However, the strong genetic differentiation found between southern Galician dolphins and animals from neighbouring locations may reflect more complex reasons such as resource partitioning. Indeed, bottlenose dolphins from northern and southern Galicia were found to display different proportions of the main prey species (e.g. blue whiting, *Micromesistius poutassou*) in their diet and dissimilar stable isotope signatures (Fernández, 2010; Fernández et al., 2011). It is also generally recognised that higher levels of genetic diversity usually occur towards the centre of a species' range while subsequent founder events could originate a stepwise loss of microsatellite diversity (Hoffman et al., 2009). The low levels of genetic diversity found in dolphins from southern Galicia suggest that, despite the lack of physical barriers, this population inhabits peripheral areas and gene flow with adjacent sites is restricted. Philopatry and kin association among individuals within resident populations can also act to reduce genetic diversity.

Bottlenose dolphins live in fission–fusion societies (Connor et al., 2000). Therefore, even within resident groups, some individuals may move frequently between schools, contributing to gene flow and influencing the genetic make-up of the community. In this study and despite the genetic substructure found, nine possible migrants between populations were identified. The existence of migrants in Galician waters is not unlikely given the high mobility of the bottlenose dolphin community in the region. Based on stomach contents analyses Santos et al. (2007) suggested that bottlenose dolphins in the area could perform foraging trips towards offshore waters, while López (2003), based on photo-identification matches, registered dolphin movements between distant inlets (rías). Two animals were also identified as potential migrants between the Sado estuary and other sites in mainland Portugal, indicating medium and/or long distance movements between locations. In this case, the Sado estuary dolphin with distinct genetic makeup was a well-known resident male named Nune. The other migrant dolphin was found stranded in the proximities to Cape Carvoeiro (Fig. 1), although it was genetically similar to southern Galician and Sado estuary dolphins. These results indicate that cases of immigration and emigration between populations currently occur.

The most common haplotype found within southern Galician dolphins matches the most frequent haplotype observed among UK bottlenose dolphins (Parsons et al., 2002; GenBank accession no. AF268357). This haplotype represents one of the only two sequences found among 15 samples from Moray Firth (Scotland, UK) resident dolphins (Parsons et al., 2002). In this study, and despite the shorter mtDNA sequence, six different haplotypes were recorded from 25 dolphins stranded in southern Galicia. However, we found an uneven distribution of haplotypes, with most of the dolphins sharing the same sequence and, as a result, levels of diversity were similar to the ones reported in the Moray Firth (Parsons et al., 2002). Measures of mtDNA diversity were low for southern Galician and Sado estuary dolphins but comparable to levels found in other studies targeting small local populations of bottlenose dolphins (Krützen et al., 2004; Sellas et al., 2005).

Inherently low sample size makes further portioning of the microsatellite data to assess sex-biased dispersal problematic. Notwithstanding, divergence estimates were comparable between males and females (though F_{ST} values were slightly higher for females than for males) suggesting limited dispersal of both sexes. Moreover, divergence estimates for mtDNA were also significant.

Previous studies found high levels of gene flow between animals from the Azores and dolphins from Madeira and mainland Portugal (Quérouil et al., 2007) which agrees with individual-based analysis from the present *STRUCTURE* analysis, in which Azorean and Canary Island animals are identified as a single genetic group together with non-resident dolphins found throughout the Iberian coastline. In this paper, one Azorean animal included in the *STRUCTURE* analysis had been identified as resident (Silva, personal communication) although no apparent genetic differences between this individual and non-resident dolphins were highlighted in our results. As suggested by Silva et al. (2008), the extensive ranging behaviour exhibited by some Azorean resident bottlenose dolphins and the apparent lack of territoriality may allow genetic interchange to occur, thus preventing genetic divergence. In addition, both Azorean and Canary Island dolphins shared one haplotype with dolphins stranded in the mainland and the median-joining network did not show any clear clustering patterns based on the geographic origin of the samples. Nonetheless, IBD was found and significant differences in F_{ST} based on mtDNA haplotype frequencies were shown between animals from mainland Portugal (excluding Sado estuary dolphins) and dolphins from the Azores. Given the remoteness of the Azorean archipelago, IBD may prevent complete panmixia between insular and continental dolphins. In addition, certain degree of heterozygosity deficit was found when dolphins from locations different than southern Galicia and the Sado estuary were grouped as a single population that could suggest cryptic local structure (Wahlund effect).

The bottlenose dolphin is recorded in the Habitats Directive as a Species of Special Interest (Directive 92/43/CEE) which protection requires the designation of special areas of conservation (SACs) by the Member States. The Sado estuary is already a Marine Protected Area (MPA) and candidate SAC (Hoyt, 2005) based on its resident population of bottlenose dolphins. On the other hand, in southern Galicia only a set of small islands located towards offshore waters is currently protected and proposed as a SAC (Hoyt, 2005). The bottlenose dolphin is also identified as a key species to be preserved in southern Galicia although the legally protected sites represent a very small fraction of the habitat used by dolphins in the region. Coastal cetaceans could face extra threats than offshore ones such as geographically restricted ranges, relatively narrow ecological niches, and dependence on resources that are also used intensively by humans (Reeves et al., 2003). Indeed, a previous extinction of an isolated, coastal population of *T. truncatus* in European waters has been reported (Nichols et al., 2007). Genetic isolation could represent an added risk for the southern Galician and Sado estuary populations and ensuring the viability of both local populations must be guaranteed in future conservation plans including the designation of SACs.

Conclusion

This study adds to the growing body of evidence that bottlenose dolphins display fine-scale genetic structure, and that detailed genetic and demographic studies are needed to accurately determine the complex patterns of gene flow within any particular area. In this case, the resident population in southern Galicia is genetically distinct which needs to be considered when defining the most appropriate scale of management, especially given that such resident individuals may be facing added threats relative to non-resident dolphins.

Acknowledgements The authors gratefully acknowledge the assistance of volunteers from the Coordinadora para o Estudio dos Mamíferos Mariños (CEMMA), Sociedade Portuguesa de Vida Selvagem (SPVS), Canarias Conservación, Instituto da Conservação da Natureza and Sociedad para el Estudio y la

Conservación de la Fauna Marina (AMBAR). We wish to thank M. Sequeira who provided tissue samples. Thanks to F. Marshall, G. Murray-Dickson, C.D. MacLeod and C. Gubili at the University of Aberdeen and to A. Centeno-Cuadros at the Estación Biológica de Doñana (CSIC) for useful comments during data analysis and discussion. R.F. was supported during the research period through Marie Curie Early-Stage Research Grant (ECOSUMMER project. 020501-2). G.J.P. was supported by the ANIMATE project (MEXC-CT-2006-042337). M.A.S. was supported by an FCT (Portuguese Science and Technology Foundation) postdoctoral grant (SFRH/BPD/29841/2006). IMAR-DOP/UAç is the R&D Unit #531 and part of the Associated Laboratory #9 (ISR) funded through the pluriannual and programmatic funding schemes of FCT-MCTES and DRCT-Azores. Sample collection in The Azores was funded by the FCT, under the CETMARH project (POCTI/BSE/38991/01).

References

Bandelt, H. J., P. Forster & A. Röhl, 1999. Median-joining networks for inferring intraspecific plylogenetics. Molecular Biology and Evolution 16: 37–48.

Brito, C., N. Vieira, E. Sá & I. Carbalho, 2009. Cetaceans' occurrence off the west central Portugal coast: a compilation of data from whaling, observations of opportunity and boat-based surveys. Journal of Marine Animals and their Ecology 2(1): 10–13.

Caeiro, S., M. H. Costa, T. B. Ramos, F. Fernandes, N. Silveira, A. Coimbra, G. Medeiros & M. Painho, 2005. Assessing heavy metal contamination in Sado Estuary sediment: an index analysis approach. Ecological indicators 5: 151–169.

Connor, R. C., R. S. Wells, J. Mann & A. J. Read, 2000. The bottlenose dolphin. In Mann, J., R. C. Connor, P. L. Tyack & H. Whitehead (eds), Cetacean Societies. University of Chicago Press, London: 19–125.

Coughlan, J., L. Mirimin, E. Dillane, E. Rogan & T. F. Cross, 2006. Isolation and characterization of novel microsatellite loci for the short-beaked common dolphin (*Delphinus delphis*) and cross-amplification in other cetacean species. Molecular Ecology Notes 6: 490–492.

dos Santos, M. E. & M. Lacerda, 1987. Preliminary observations of the bottlenose dolphin (*Tursiops truncatus*) in the Sado estuary (Portugal). Aquatic Mammals 13: 65–80.

dos Santos, M. E., S. Louro, M. Couchinho & C. Brito, 2005. Whistles of bottlenose dolphins in the Sado estuary, Portugal: characteristics, production rates and long-term contour stability. Aquatic Mammals 31(4): 453–462.

Eggert, S. L., C. A. Lux, G. M. O'Corry-Crowe & A. E. Dizon, 1998. Dried dolphin blood on fishery observer records provides DNA for genetic analyses. Marine Mammal Science 14: 136–143.

Fernández, R., 2010. Ecology of the bottlenose dolphin, *Tursiops truncatus* (Montagu 1821), in Galician waters, NW Spain. PhD thesis, Universidade d Vigo, Spain.

Fernández, R., S. García-Tiscar, M. B. Santos, A. López, J. A. Martínez-Cedeira & G. J. Pierce, 2011. Stable isotope analysis in two sympatric populations of bottlenose dolphins *Tursiops truncatus*: evidence of resource partitioning? Marine Biology. doi:10.1007/s00227-011-1629-3.

Figueiras, F. G., U. Labarta & J. M. Fernández-Reiriz, 2002. Coastal upwelling, primary production and mussel growth in the Rías Baixas of Galicia. Hydrobiologia 484: 121–131.

Fontaine, M. C., S. J. E. Baird, S. Piry, N. Ray, K. A. Tolley, S. Duke, A. Birkun, M. Ferreira, T. Jauniaux, A. Llavona, B. Öztürk, A. A. Öztürk, V. Ridoux, E. Rogan, M. Sequeira, U. Siebert, G. A. Vikingsson, J. M. Bouquegneau & J. R. Michaux, 2007. Rise of oceanographic barriers in continuous populations of a cetacean: the genetic structure of harbour porpoises in Old World waters. BMC Biology 5: 30–46.

Gaspar, R., 2003. Status of the resident bottlenose dolphin population in the Sado Estuary: past, present and future. PhD thesis, University of St Andrews, UK.

Goudet, J., 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3) [available on internet at http://www2.unil.ch/popgen/softwares/fstat.htm].

Hoelzel, R. A., 1998. Genetic structure of cetacean populations in sympatry, parapatry and mixed assemblages: Implications for conservation policy. The Journal of the Heredity 89(5): 451–457.

Hoelzel, A. R., C. W. Potter & P. B. Best, 1998. Genetic differentiation between parapatric "nearshore" and "offshore" populations of the bottlenose dolphin. Proceedings of the Royal Society Series B, Biological Sciences 265(1402): 1177–1183.

Hoffman, J. I., K. K. Dasmahapatra, W. Amos, C. D. Phillips, T. S. Gelatt & J. W. Bickham, 2009. Contrasting patterns of genetic diversity at three different genetic markers in a marine mammal metapopulation. Molecular Ecology 18: 2961–2978.

Hoyt, E., 2005. Marine Protected Areas for Whales, Dolphins and Porpoises: A Worldwide Handbook for Cetacean Habitat Conservation. Earthscan, London.

Kalinowski, S. T., 2002. How many alleles per locus should be used o estimate genetic distances? Heredity 88: 62–65.

Krützen, M., W. B. Sherwin, P. Berggren & N. Gales, 2004. Population structure in an inshore cetacean revealed by microsatellite and mtDNA analysis: bottlenose dolphins (*Tursiops* sp.) in Shark Bay, Western Australia. Marine Mammal Science 20(1): 28–47.

Librado, P. & J. Rozas, 2009. DnaSP vs.5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25: 1451–1452.

López, A., 2003. Estatus dos pequenos cetáceos da plataforma de Galicia. PhD thesis, Universidade de Santiago de Compostela, Spain.

Méndez, G. & F. Vilas, 2005. Geological antecedents of the Rias Baixas (Galicia, northwest Iberian Peninsula). Journal of Marine Systems 54: 195–207.

Natoli, A., A. Birkun, A. Aguilar, A. López & A. R. Hoelzel, 2005. Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). Proceedings of the Royal Society Series B, Biological Sciences 272: 1217–1226.

Nichols, C., J. Herman, O. E. Gaggiotti, K. M. Dobney, K. Parsons & A. R. Hoelzel, 2007. Genetic isolation of a now extinct population of bottlenose dolphins (*Tursiops* *truncatus*). Proceedings of the Royal Society Series B, Biological Sciences 274: 1611–1616.

Parsons, K. M., L. R. Noble, R. J. Reid & P. M. Thompson, 2002. Mitochondrial genetic diversity and population structuring of UK bottlenose dolphins *Tursiops truncatus*: is the NE Scotland population demographically and geographically isolated? Biological Conservation 108: 175–182.

Parsons, K. M., J. W. Durban, D. E. Claridge, D. L. Herzing, K. Balcom & L. R. Noble, 2006. Population genetic structure of coastal bottlenose dolphins (*Tursiops truncatus*) in the northern Bahamas. Marine Mammal Science 22(2): 276–298.

Peakall, R. & P. E. Smouse, 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6: 288–295.

Peltier, H., G. Certain, O. Van Canneyt, P. Daniel & V. Ridoux, 2009. How strandings can inform on cetacean at sea: an attempt to model and validate drift and discovery rates. In 23th Conference of the European Cetacean Society, 2–4 March 2009, Istambul, Turkey.

Pritchard, J. K., M. Stephens & P. Donnelly, 2000. Inference of population structure from multi-locus genotype data. Genetics 155: 945–959.

Quérouil, S., M. A. Silva, L. Freitas, R. Prieto, S. Magalhães, A. Dinis, F. Alves, J. A. Matos, D. Mendoça, P. S. Hammond & R. S. Santos, 2007. High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. Conservation Genetics 81: 1405–1419.

Reeves, R. R., B. D. Smith, E. A. Crespo & G. Notarbartolo di Sciara, 2003. Dolphins, Whales and Porpoises: 2002–2010 Conservation Plan for the World's Cetaceans. IUCN/SSC Cetacean Specialist Group. IUCN, Gland, Switzerland and Cambridge, UK: 139 pp.

Rosel, P. E., A. E. Dizon & M. G. Haygood, 1995. Variability of the mitochondrial control region in populations of the harbour porpoise, *Phocoena phocoena*, on interoceanic and regional scales. Canadian Journal of Fisheries and Aquatic Sciences 52: 1210–1219.

Rosel, P. E., V. Forgetta & K. Dewar, 2005. Isolation and characterization of twelve polymorphic microsatellite markers in bottlenose dolphins (*Tursiops truncatus*). Molecular Ecology Notes 5: 830–833.

Ryman, N. & S. Palm, 2006. POWSIM: a computer program for assessing statistical power when testing for genetic differentiation. Molecular Ecology Notes 6: 600–602.

Ryman, N., S. Palm, C. André, G. R. Carvalho, T. G. Dahlgren, P. E. Jordes, L. Laikre, L. C. Larsson & A. Palmé, 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Molecular Ecology 15: 2031–2045.

Santos, A. M. P., M. F. Borges & S. Groom, 2001. Sardine and horse mackerel recruitment and upwelling off Portugal. ICES Journal of Marine Science 58: 589–596.

Santos, M. B., R. Fernández, A. López, J. A. Martínez & G. J. Pierce, 2007. Variability in the diet of bottlenose dolphin, Tursiops truncatus, in Galician waters, northwestern Spain, 1990–2005. Journal of the Marine Biological Association of the United Kingdom 87: 231–242.

Segura, I., A. Rocha-Olivares, S. Flores-Ramírez & L. Rojas-Bracho, 2006. Conservation implications of the genetic and ecological distinction of *Tursiops truncatus* ecotypes in the Gulf of California. Biological Conservation 133: 336–346.

Sellas, A. B., R. S. Wells & P. E. Rosel, 2005. Mitochondrial and nuclear DNA analyses reveal fine scale geographic structure in bottlenose dolphins (*Tursiops truncatus*) in the Gulf of Mexico. Conservation Genetics 6: 715–728.

Silva, C., 2008. A população residente de *Tursiops truncatus* num quadro de gestão integrada do estuário do Sado: Proposta de um acordo voluntário. Master's thesis, Faculdade de Ciências da Universidade de Lisboa, Portugal.

Silva, M. A., R. Prieto, S. Magalhães, M. I. Seabra, R. S. Santos & P. S. Hammond, 2008. Ranging patterns of bottlenose dolphins living in oceanic waters: implications for population structure. Marine Biology 156: 179–192.

Tamura, K., N. Dudley, M. Nei & S. Kumar, 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Molecular Biology and Evolution 24: 1596–1599.

Tezanos-Pinto, G., C. S. Baker, K. Russell, K. Martien, R. W. Baird, A. Hutt, G. Stone, A. A. Mignucci-Giannoni, S. Caballero, T. Endo, S. Lavery, M. Oremus, C. Olavarría & C. Garrige, 2009. A worldwide perspective on the population structure and genetic diversity of bottlenose dolphins (*Tursiops truncatus*) in New Zealand. Journal of the Heredity 100(1): 11–24.

Van Oosterhout, C., W. F. Hutchinson, D. P. M. Wills & P. Shipley, 2004. MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. Molecular Ecology Notes 4: 535–538 [available on internet at http://www.microchecker.hull.ac.uk/].

VVAA, 2007. Bases para la conservación y la gestión de las especies de cetaceos amenazadas en las aguas atlánticas y cantábricas. Memoria Final Proyecto Fundación Biodiversidad. CEMMA, Gondomar, Spain: 155 pp.

Wells, R. S. & M. D. Scott, 2002. Bottlenose dolphins: *Tursiops aduncus* and *Tursiops truncatus*. In Perrin, W. F., B. Würsic & J. G. M. Thewissen (eds), Enciclopedia of Marine Mammals. Academic Press, San Diego: 122–128.

Wells, R. S., H. L. Rhinehart, P. Cunningham, J. Whaley, M. Baran, C. Koberna & D. P. Costa, 1999. Long distance offshore movements of bottlenose dolphins. Marine Mammal Science 15(4): 1098–1114.

Wiszniewski, J., L. B. Beheregaray, S. J. Allen & L. Möller, 2009. Environmental and social influences on the genetic structure of bottlenose dolphins (*Tursiops aduncus*) in Southeastern Australia. Conservation Genetics 11: 1405–1419.

Hydrobiologia (2011) 670:127–140
DOI 10.1007/s10750-011-0664-x

Survey and management of mussel *Mytilus* species in Scotland

**Patricia Joanna Dias · Stuart B. Piertney ·
Mike Snow · Ian M. Davies**

Published online: 27 April 2011
© Crown copyright 2011

Abstract The important ecological role of *Mytilus* mussels in marine ecosystems, their high abundance in coastal waters and the demand for human consumption has made them a target species for aquaculture. Mussel cultivation is the most important and rapidly growing sector of the Scottish shellfish aquaculture industry and until recently production was considered to be based exclusively on the native species *Mytilus edulis*. However, the sympatric occurrence of *M. edulis*, *M. trossulus*, *M. galloprovincialis* and their hybrids in cultivation has recently been reported and significant production losses (over 50% at some sites) have been attributed to the presence of fragile-shelled *M. trossulus*. Given the ecological and economical importance of these species, an urgent need arose for a wider understanding of *Mytilus* species distribution on Scottish coasts and its implication for the sustainability of the Scottish shellfish industry. Here we present a summary of a 3-year project established within the "ECOsystem approach to SUstainable Management of the Marine Environment and its living Resources" (ECOSUMMER) Marie Curie network to address this need. We developed DNA-based molecular assays for the detection and surveillance of the different *Mytilus* species in Scotland. Several potential management strategies have been explored, aimed at favouring *M. edulis* production at mixed-species sites, but these have so far not been found to provide the reliable efficacy necessary for adoption by the industry. Complete eradication of *M. trossulus* from economically affected areas in Scotland may be unrealistic, especially considering that its introduction and distribution mechanisms in the environment remain uncertain. Area-specific solutions to managing the problem may thus be required, which may or may not involve eradication and fallowing (clearance of mussels from production sites for a given period of time). Nevertheless, the current distribution of *M. trossulus* is limited and its spread outside its existing range is clearly undesirable. Any management solutions must also be accompanied by an industry wide strategy and awareness, for example, through the development of an industry supported code of good practice.

Keywords *Mytilus trossulus* · Hybridisation · Me 15/16 · Real-time PCR · Shellfish aquaculture

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

P. J. Dias · M. Snow · I. M. Davies (✉)
Marine Scotland, Marine Laboratory, 375 Victoria Road,
Aberdeen AB11 9DB, UK
e-mail: Ian.Davies@scotland.gsi.gov.uk

S. B. Piertney
Institute of Biological and Environmental Sciences,
University of Aberdeen, Aberdeen AB24 2TZ, UK

Introduction

Mussels of the genus *Mytilus* are among the commonest marine molluscs found in coastal ecosystems of temperate and boreal regions of both northern and southern hemispheres (Gosling, 1992; Hilbish et al., 2000). The important ecological role of these mussels in marine ecosystems, their high abundance in coastal waters and the demand for human consumption has made them a target species for aquaculture (Gosling, 1992). Most of the current mussel production in Europe (over 800,000 tonnes/year) consists of *Mytilus edulis* from the Atlantic and North Sea coasts and *Mytilus galloprovincialis* from the Atlantic and the Mediterranean Sea, and originates from the historically big producers: Spain (250,000 tonnes/year), France (60,000 tonnes/year) and the Netherlands (80,000 tonnes/year), followed by a marked increase in production in the UK, Ireland and Norway (Smaal, 2002; Kijewski et al., 2006).

In Scotland, shellfish farming is expanding, dominated by the mussel *M. edulis*. Mussel production increased from just 262 t in 1986 to 5968 t (worth £5.9 million) in 2008 (FRS, 1996, Scotland, 2009) from a total of 52 farms, distributed mainly along the west coast of the Scottish mainland and in the Shetland Islands. Mussels are mostly rope grown on longlines in sea lochs (fjordic inlets) and production depends exclusively on the settlement of natural seed in these lochs. Mussel ropes are introduced to the lochs around February/March, at the beginning of the spawning season, and larval settlement occurs throughout the summer until September. Mussels are left to grow on the ropes until they reach an acceptable harvest size, between 2 and 3 years after settlement. The great majority of cultured mussel production is sold as live in-shell product, after primary processing to declump, remove byssus, grade and depurate the harvested mussels. Thus, characteristics such as shell strength (which affects susceptibility to shell breakage during primary processing) and final product appearance are considered to be critical to successful mussel production businesses (Penney et al., 2007; Beaumont et al., 2008; Dias et al., 2011).

In 2004, *M. trossulus*, *M. galloprovincialis* and their hybrids with the native species of mussel *M. edulis* were detected in both farmed and natural populations of mussels in Loch Etive, a historically important site of the Strathclyde production area in the west of Scotland (Beaumont et al., 2008). At Loch Etive, *M. trossulus* has been associated with significant (over 50%) losses in production [Scottish shellfish farms production survey data, impact on the Strathclyde total mussel production can be seen by comparing FRS (2008) and Marine Scotland (2009)] mainly due to generally presenting poor meat contents and thin, fragile shells that were easily damaged during the harvesting and grading processes (Beaumont et al., 2008). Beaumont et al. (2008) described fragile-shelled mussels in Loch Etive as having elongated "paddle-shaped" shells that were flexible and would gape when squeezed, in contrast to normal *M. edulis*-type mussels in the same loch. By sampling mussels from ropes at different depths at two sites, Beaumont et al. (2008) found fragile mussels (genetically identified as being mostly *M. trossulus* and *M. trossulus* × *M. edulis* hybrids) to be significantly more frequent closer to the surface on the ropes, and on a farm site located in the upper region of the loch.

Due to the higher abundance of fragile mussels in the landward part of the loch and in the upper lower salinity water of the loch, this factor was suggested to be the main environmental parameter likely to influence the recruitment and settlement of fragile mussels in Loch Etive (Beaumont et al., 2008). While *M. galloprovincialis* is seen as a recent invader that has spread into the Atlantic and northwards (Beaumont et al., 2008; Gosling et al., 2008), the origins of *M. trossulus* in Scotland are unclear. Beaumont et al. (2008) suggested *M. trossulus* to be a post-glacial relict species restricted to the low salinity areas of some lochs, which had recently increased in abundance due to commercial mussel growing activity.

Given the ecological and economical importance of these species, an urgent need arose for a wider understanding of *Mytilus* species distribution on Scottish coasts and its implications for the sustainability of the Scottish shellfish industry. Here we present a summary of a 3-year (2007–2009) project established within the "ECOsystem approach to SUstainable Management of the Marine Environment and its living Resources" (ECOSUMMER) Marie Curie network to address this need. In order to enable a comprehensive surveillance and a primarily analysis of the potential impact of *Mytilus* species in both natural and artificial environments, such as aquaculture systems, we developed a novel real-time PCR assay based on the Me 15/16 marker, capable of identifying discriminatory *Mytilus* species-specific alleles (Dias et al., 2008). This assay was developed with the main objective of establishing an efficient and cost-effective tool to use in large-scale surveys (Dias et al., 2008, 2009b) aimed at clarifying the distribution in Scotland of the non-native species *M. galloprovincialis* and in particular *M. trossulus*, due to the economic impact of the presence of this species at aquaculture units.

At affected sites, we investigated and explored potential differences in genotypes distribution that could form a basis for the development of effective management strategies. At Loch Etive, and building on the work by Beaumont et al. (2008), a more detailed analysis of *Mytilus* species distribution in relation to key parameters such as depth, location and salinity was performed (Dias et al., 2009a). Also, the reproductive cycles of *M. edulis*, *M. trossulus* and *M. edulis* × *M. trossulus* hybrids were investigated in an attempt to identify possible times of the year when rope deployment could favour the settlement and overall production of *M. edulis* (Dias et al., 2009c). Finally, we investigated the relative performance of *M. edulis*, *M. trossulus* and their hybrids from three cultivation areas in order to infer on the potential influence of site factors and/or production strategies on shell and meat characteristics and advised on future management of mixed-species areas in Scotland (Dias et al., 2011).

Materials and methods

Development of a real-time PCR assay for the survey of *Mytilus* species

Three specific TaqMan®–MGB probes (one for each *Mytilus* species) and one universal set of primers were designed based on the previously described Me 15/16 primers targeting the adhesive protein gene sequence (Inoue et al., 1995). Multiplex assays were run to test the specificity of the method on DNA samples extracted from mussels of all three species and hybrids. Efficiencies of primers and probes were assessed using triplicate tenfold serial dilutions of clones of Me 15/16 PCR products specific to each of the three *Mytilus* species (for details on assay development and reaction conditions see Dias et al., 2008). A survey aimed at clarifying the distribution of *M. galloprovincialis* and *M. trossulus* and the abundance of *M. trossulus* in farmed and natural populations in Scotland was initiated (Dias et al., 2008, 2009b). A total of 85 samples (34 shore sites, 10 marinas and 41 aquaculture sites) of 30 mussels were collected. Approximately 5 mg of gill tissue from each of the 30 individuals in a sample were pooled together, resulting in a total of 85 pooled tissue samples. All pooled samples were screened for the presence/absence of *M. edulis*, *M. galloprovincialis* and *M. trossulus* alleles using the real-time PCR multiplex assay developed by Dias et al. (2008). If pooled samples tested positive for the *M. trossulus* allele, and originated from a site where this species had not previously been reported, DNA was extracted from approximately 5 mg of gill tissue from each of the individuals in the sample separately, in order to determine genotype frequencies in these samples. Genotyping of individual mussels was carried out by PCR amplification using the Me15/16 markers (Inoue et al., 1995). This methodology involves the PCR amplification of a species-specific diagnostic region of the adhesive protein gene and subsequent separation of PCR products by size through electrophoresis and visualisation in agarose gels. Individuals which give single PCR products of 180, 168 or 126 bp are identified as being *M. edulis*, *M. trossulus*, and *M. galloprovincialis* homozygotes, respectively. Individuals from which PCR products generated two products of different sizes are identified as hybrids of these species (for details on methodology, see Dias et al., 2008, 2009b).

Distribution of *Mytilus* genotypes in cultivation at Loch Etive

Mussels were collected from 10 aquaculture sites in Loch Etive (Fig. 1). One rope of mussels was sampled randomly at each site and 30 adult mussels were taken haphazardly at each of 3 depths (2, 5 and 8 m from the surface as measured on the dropper rope) where they were available. Salinity profiles were taken at each site at the time of sampling using a SAIV® CTD ST204 with Seapoint Fluorometer and turbidity meter. Mussels were dissected and gill tissue sampled and preserved in 70% ethanol and stored at −20°C. DNA was extracted from

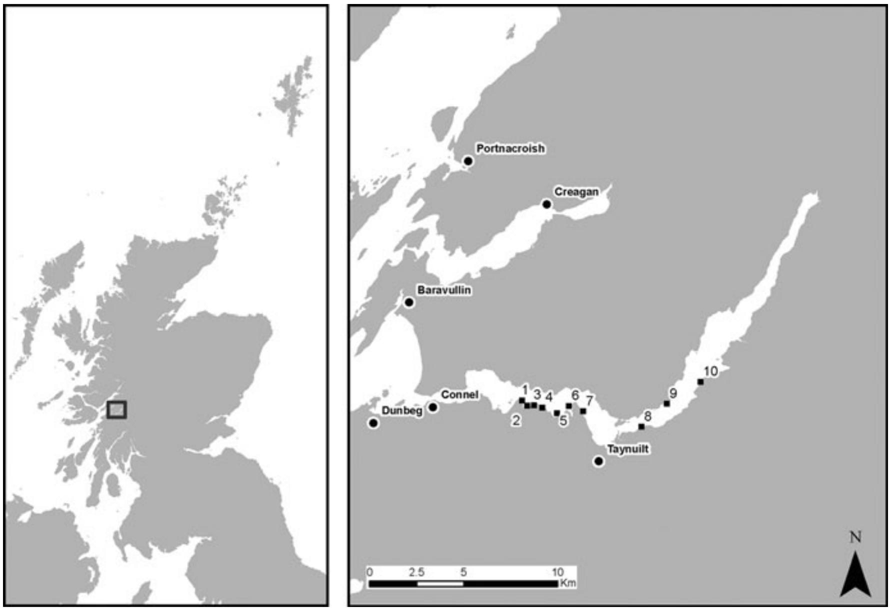


Fig. 1 Map (ArcGis©) showing sampling sites at Loch Etive (*right image*) in Scotland (*left*). Sites were numbered 1 to 10, site 1 being the closest to the Loch entrance and site 10 the most distant

approximately 0.5 mg of gill tissue from each mussel using a Qiagen BioRobot M48 and Qiagen M48 MagAttract DNA Mini Kit, following the manufacturer's instructions. Identification of individual genotypes was carried out by PCR amplification and electrophoresis using the Me15/16 primers (Inoue et al., 1995). Deviations from the Hardy–Weinberg expectations for the Me 15/16 locus in each sample were estimated from Fis values within FSTAT 2.9.3 (Goudet, 1995). Distribution of genotype frequency over sampling sites and depths, and its possible relation with salinity and year of settlement was investigated using Generalised Linear Models in GenStat© (for details on methodology, see Dias et al., 2009a).

Gametogenic asynchrony of mussels *Mytilus* at Loch Etive

We used two approaches to investigate the reproductive cycles of *M. edulis*, *M. trossulus* and *M. edulis* × *M. trossulus* hybrids in Loch Etive. First, 120 adult mussels were collected monthly by hand from aquaculture ropes at Loch Etive. Each month, samples of mantle from 20 individuals identified as *M. trossulus*, 20 *M. edulis* and 20 *M. trossulus* × *M. edulis* hybrids among the 120 individuals sampled were processed, cross-sectioned, stained with haematoxylin–eosin and permanently mounted for histological analysis (Progenix Lda.). The slides were examined for gonad development stages using an Olympus BX60 microscope equipped with a digital camera. Second, plankton samples were collected in parallel to the sampling of adult mussels using a Lund tube. Plankton samples were immediately pre-filtered through a 1 mm mesh, retained on a 40 µm filter and fixed with Lugol's iodine (Nalepa & Schloesser, 1993). DNA extraction from samples was performed using a Qiagen BioRobot M48 and Qiagen M48 MagAttract DNA Mini Kit, following the manufacturer's instructions, and stored at −20°C. Detection of mussel species-specific *M. edulis*, *M. trossulus* and *M. galloprovincialis* alleles from plankton samples was assessed using the real-time PCR assay described by Dias et al. (2008). In order to check for PCR inhibition from plankton samples, a real-time PCR assay was conducted including DNA from all plankton samples and the use of Taqman® Exogenous Internal Positive Control (IPC) reagents (Applied Biosystems). Laboratory-cultured D-stage veliger larvae were used for the establishment of a quantification curve (for details on methodology, please see Dias et al., 2009c).

Performance of *M. edulis*, *M. trossulus* and their hybrids in three lochs

We sampled 20 *M. edulis*, 20 *M. trossulus*, and 20 *M. edulis* × *M. trossulus* hybrid adult mussels at three farm sites, each from three different lochs (referred to as sites A, B and C, names are not given due to the commercial sensitivity of this problem). A piece of gill tissue was removed from each individual mussel for genetic identification and the remaining flesh removed and weighed. The flesh was freeze-dried and re-weighed and shells were weighed, measured for length, height (or depth) and width with digital callipers. Two-way analysis of variance (ANOVA) was performed for all measurements, in order to investigate differences between genotypes and sampling sites. Two-way ANOVA were also performed to investigate differences in meat yields between genotypes and sampling sites. In order to investigate the potential for assigning mussels to their true genotype group (*M. edulis*, *M. trossulus* or hybrids) within each loch, we used multivariate discriminant function analysis (DFA). During grading, perception of differences between genotypes would be mainly dictated by shell shape ("appearance") parameters, and therefore we used shell length, height, width and weight in the analysis (for details on methodology, please see Dias et al., 2011).

Results

Development of a real-time PCR assay for the survey of *Mytilus* species

The primers and probes were designed to be able to detect and differentiate between *M. edulis*, *M. trossulus* and *M. galloprovincialis*, and were specific for these species. Results obtained from amplification trials proved the developed assay to be effective, efficient and highly reproducible (for full technical details and discussion of results, see Dias et al., 2008). Alleles of the endemic species of blue mussel *M. edulis* were present in all of the samples collected during the surveys, supporting the expected dominant presence of this species in Scotland (Figs. 2, 3). Within the 44 samples taken from shores and marinas, 16 samples taken from the south west and south east of Scotland showed exclusively *M. edulis* alleles (Fig. 2). Within the 41 samples taken at farm sites, only two sites in the Dumfries and Galloway area of south west Scotland showed exclusively *M. edulis* alleles (Fig. 3). *M. galloprovincialis* allele presence was detected extensively throughout the northwest and northeast of mainland Scotland and Shetland Islands (Figs. 2, 3). *M. trossulus* alleles were identified in six samples from shore sites and marinas in the south west of Scotland (Fig. 2, named on Table 1), and five farm sites corresponding to five different farms in the west and south west of Scotland, considerably extending the recently reported evidence of *M. trossulus* presence in cultivation at Loch Etive (Beaumont et al., 2008). Two of the farms were within the four farms where the presence of *M. trossulus* had previously been observed (Dias et al., 2009c). The remaining three new cases (A, B and C, Table 1, names are not given due to the commercial sensitivity of this problem) increase the total number of farms at which *M. trossulus* has been detected in Scotland to seven.

Distribution of *Mytilus* genotypes in cultivation at Loch Etive

Of the total individuals sampled in this study (n = 810), 30% were *M. edulis*, 37% were *M. trossulus* and 23% were *M. edulis* × *M. trossulus* genotypes. The *M. galloprovincialis* genotype was very rare. *Mytilus galloprovincialis* hybrids were more frequent and were present at an average proportion of 3% for *M. galloprovincialis* × *M. trossulus* and 7% for *M. galloprovincialis* × *M. edulis* hybrids. Genotype frequencies were in Hardy–Weinberg equilibrium at all depths and sites. No consistent significant differences were observed between samples that could be related to site location, considering factors such as distance to the mouth of the loch (and hence seawater/freshwater flow influence), as suggested by Beaumont et al. (2008). Differences between the present and the previous study by Beaumont et al. (2008) are most likely influenced by the much more comprehensive sampling in the present study compared to the previous study when sampling was limited to two sites widely spaced in the loch. No significant differences between depths of 2 and 5 m, or between the distributions of *M. trossulus* × *M. edulis* hybrids with sampling depth were observed. However, within sites, *M. trossulus* appears more frequent, and *M. edulis* less frequent, in near-surface samples (2 and 5 m) than at 8 m rope depth.

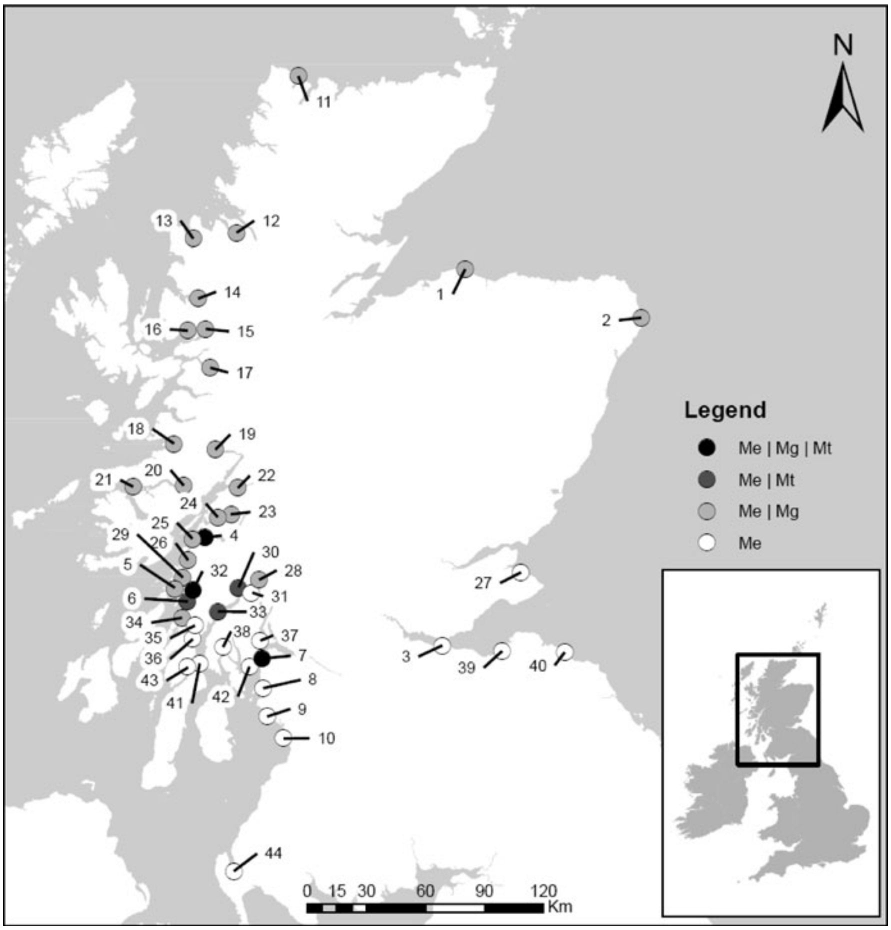


Fig. 2 Map showing sampling sites (1–44) from the intertidal zone and marinas in Scotland, and also the sites detected positive for Me (*M. edulis*), Mg (*M. galloprovincialis*) and Mt (*M. trossulus*)

Gametogenic asynchrony of mussels *Mytilus* at Loch Etive

The histological data indicated significant differences in the timing of gametogenic development in *M. trossulus* and *M. edulis*, with *M. edulis* initiating spawning earlier in the year. However, *M. trossulus* and *M. trossulus* × *M. edulis* hybrid gonads in a spawning state were observed during most of the year (Fig. 4). Also, real-time PCR detection of *Mytilus* species-specific alleles indicates that *M. trossulus* and/or hybrid larvae are present in the plankton during most months of the year (Fig. 5). Observations that the most significant spawning period for *M. trossulus* occurs later than that for *M. edulis*, and that *M. trossulus* and/or *M. trossulus* × *M. edulis* hybrid larvae are present in the plankton for most of the year, suggest there may be heavy over-settlement of *M. edulis* by *M. trossulus*.

Performance of *M. edulis*, *M. trossulus* and their hybrids at three lochs

Two-way analyses of variance of the data for each of the six shell and meat variables (shell length, height, width and weight, and meat fresh and dry weight), classified by sampling site (A, B and C) and genotype (*M. edulis*, *M. trossulus* and hybrids), showed significant differences ($P < 0.05$) in all variables measured between sampling sites and for all three genotypes. Over all species, site B mean shell length, height and width were significantly ($P < 0.05$) larger, and significantly heavier, in-shell weight, fresh meat weight and dry meat weight than samples from site A and site C which were only significantly different from one another in terms of fresh and dry meat weight with site C having the higher average values (Dias et al., 2011). Meat yields, when calculated as the ratio of dry meat weight to total weight, were significantly

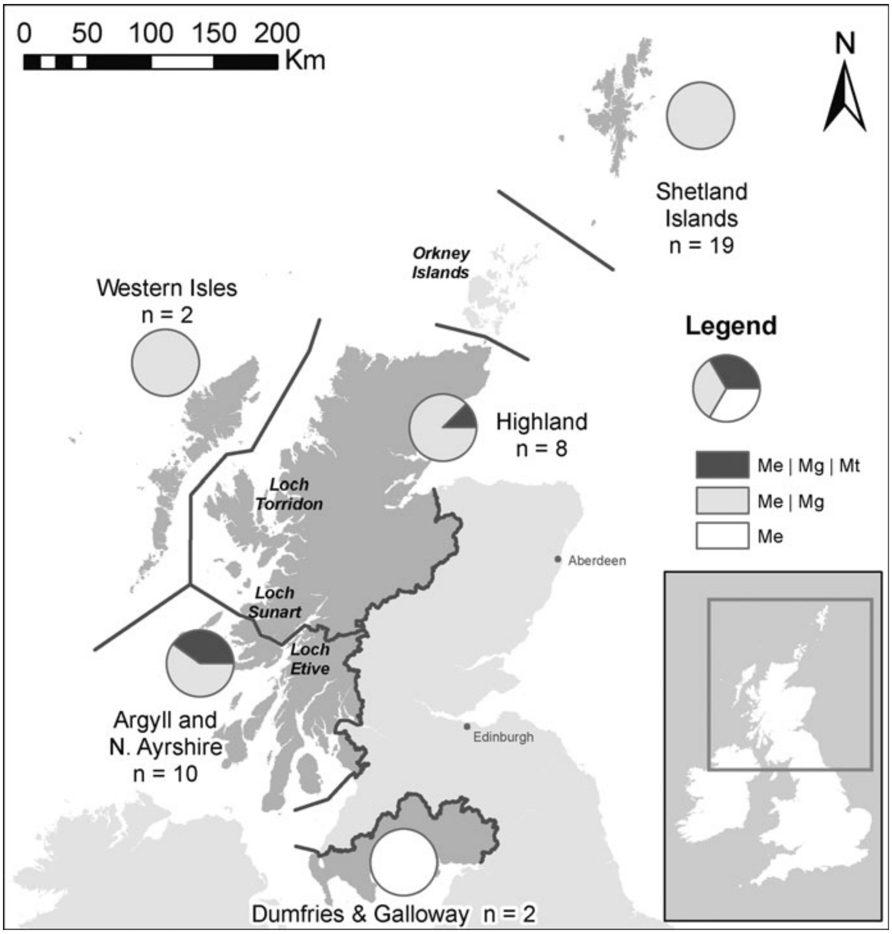


Fig. 3 Map showing the distribution of samples taken at mussel aquaculture sites in Scotland. Number of aquaculture sites sampled, and detections obtained of the *M. edulis* (Me), *M. galloprovincialis* (Mg) and *M. trossulus* (Mt) species-specific alleles at the Me 15/16 locus, are given per local authority area

Table 1 Number of individuals of each genotype, *M. edulis* (Me), *M. galloprovincialis* (Mg), *M. trossulus* (Mt), *M. edulis* × *M. trossulus* hybrids (Me × Mt), *M. edulis* × *M. galloprovincialis* hybrids (Me × Mg) *M. galloprovincialis* × *M. trossulus* (Mg × Mt) hybrids, found in the samples from the intertidal zone and marinas, and three newly discovered positive farm sites (A, B and C) positive for *M. trossulus* in Scotland

Site	Number individuals analyzed	Number individuals genotyped	Me	Mg	Mt	Me × Mt	Me × Mg	Mg × Mt
(4) Dunstaffnage Marina	30	9	4	0	2	1	2	0
(6) Ardfern Marina	30	10	5	0	2	2	0	1
(7) Inverkip Marina	30	26	4	0	13	7	0	2
(30) Loch Fyne	30	30	29	0	0	1	0	0
(32) Ardfern 2	30	30	7	0	12	10	1	0
(33) Loch Fyne Minard	30	30	29	0	0	1	0	0
A	30	30	23	0	1	5	1	0
B	30	30	26	0	0	3	1	0
C	30	30	28	0	0	1	1	0

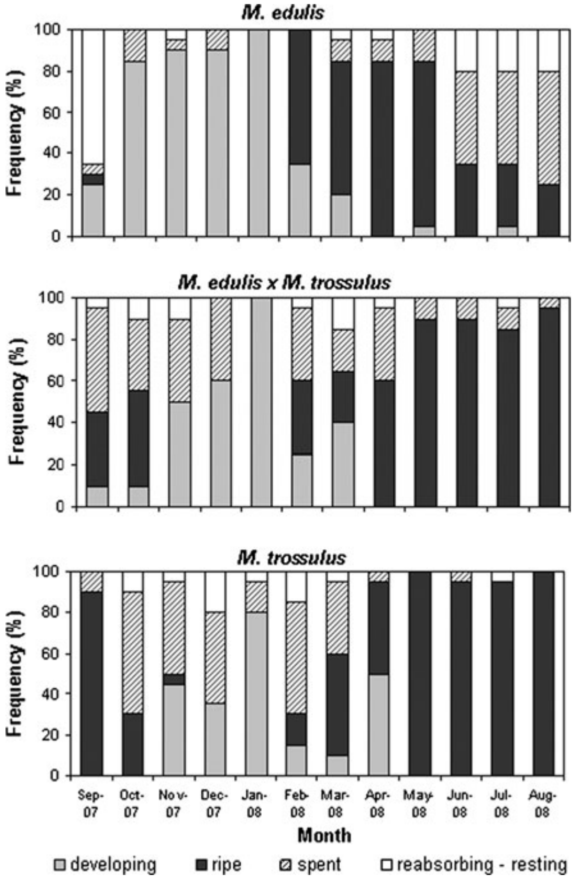


Fig. 4 *Mytilus edulis*, *M. trossulus* and *M. edulis* × *M. trossulus* frequency distribution of gonad maturation stages observed between September 2007 and August 2008

different between both genotypes and sampling sites ($P < 0.05$). *M. edulis* presented higher average dry meat yield values than *M. trossulus* and hybrids at all sampling sites. For all genotypes, sampling site B presented higher average dry meat yield values than site A. Site C presented intermediate values that were generally closer to the ones observed at site B (Dias et al., 2011). Over all, based on all four shell measurements (weight, length, height and width), DFA allowed for over half of the mussels within each sampling site to be correctly assigned to their true genotype group (58% correctly assigned at site A and B, and 68% at site C) (Table 2). However, when considering the identification of *M. edulis* and non-edulis only, that is, grouping *M. trossulus* and hybrids together, resulted in a marked improvement in the overall proportion of mussels correctly classified (82–93%) at all sites (Table 3). This was due to, at all sampling sites, misclassified *M. trossulus* individuals being generally put into the hybrid genotype group, and vice versa (Dias et al., 2011).

Discussion

One of the most important outputs of this project was undoubtedly the establishment of effective methodologies able to identify and distinguish between the three species, *M. edulis*, *M. galloprovincialis*, *M. trossulus* and their hybrids, present in Scotland. The PCR-based

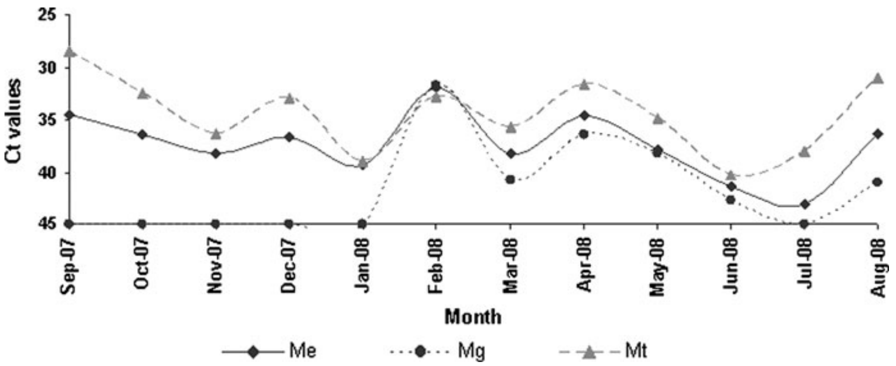


Fig. 5 Real-time PCR cycle threshold (Ct) detection values obtained for *M. edulis* (Me), *M. trossulus* (Mt) and *M. galloprovincialis* (Mg) alleles present in the plankton samples analysed by real-time PCR. Because higher Ct values correspond to initially lower template DNA quantity, Ct values in the y axis are inverted and cross the *x*-axis at the maximum value, Ct 45, which corresponds to no template DNA being detected in the plankton sample

Table 2 Summary of classification from discriminant function analysis (DFA) after cross-validation, within each sampling site (A, B and C), using the predictors shell weight (g), length (mm), height (mm) and width (mm)

Linear method for response: genotype			
Group:	Me	Me × Mt	Mt
Count:	20	20	20
Summaries of classifications with cross-validation			
Site A			
Put into group	Me	Me × Mt	Mt
Me	16	2	0
Me × Mt	3	5	6
Mt	1	13	14
Total *N*	20	20	20
N correct	16	5	14
Proportion	0.8	0.25	0.70
Summary	$N = 60$	*N* correct = 35	Proportion correct = 0.583
Site B			
Put into group	Me	Me × Mt	Mt
Me	17	6	0
Me × Mt	3	5	7
Mt	0	9	13
Total *N*	20	20	20
N correct	17	5	13
Proportion	0.85	0.25	0.65
Summary	$N = 60$	*N* correct = 35	Proportion correct = 0.583
Site C			
Put into group	Me	Me × Mt	Mt
Me	16	0	0
Me × Mt	1	12	7
Mt	3	8	13
Total *N*	20	20	20
N correct	16	12	13
Proportion	0.80	0.60	0.65
Summary	$N = 60$	*N* correct = 41	Proportion correct = 0.683

Groups are the three genotypes *M. edulis* (Me), *M. trossulus* (Mt) and *M. edulis* × *M. trossulus* hybrids (Me × Mt)

Me 15/16 nuclear marker developed by Inoue et al. (1995) was essential to this work. Basing the development of a real-time PCR assay on this marker allowed the method to be promptly established and to proceed with samples analysis within a reasonable time frame, allowing results to be effectively passed on to all interested parties. The molecular methods and research conducted within the 3 years of the ECO-SUMMER research network have led to a series of outcomes/recommendations of both scientific and practical importance.

The effective application of the real-time PCR method to the detection of *M. trossulus* alleles from bulk samples of tissue from 30 individuals represents considerable time and cost savings whenever in need to process a high number of samples in future surveys. The same assay was also successfully applied to the identification of *Mytilus* larvae and species-specific alleles in plankton samples and represents the best available tool to date for the identification of these species genetic pool from plankton samples (for details and discussion on other methodologies available, see Dias et al., 2008). Using single nuclear markers, however, comes with the inherent disadvantage of these markers inability to distinguish “pure” genotypes from backcrosses. *Mytilus* species and hybrids are fertile and produce backcrosses and therefore, if interest in investigating detailed introgression levels of *Mytilus* populations in Scotland arises in the future, other markers will necessarily have to be considered for use, or in combination with the Me 15/16 (Inoue et al., 1995).

Table 3 Summary of classification from discriminant function analysis (DFA) after cross-validation, within each sampling site (A, B and C), using the predictors shell weight (g), length (mm), height (mm) and width (mm)

Linear method for response: genotype			
Group:	Me	Non-Me	
Count:	20	40	
Summaries of classifications with cross-validation			
Site A			
Put into group	Me	Non-Me	
Me	16	2	
Non-Me	4	38	
Total *N*	20	40	
N correct	16	38	
Proportion	0.80	0.95	
Summary	$N = 60$	*N* correct = 54	Proportion correct = 0.90
Site B			
Put into group	Me	Non-Me	
Me	17	8	
Non-Me	3	32	
Total *N*	20	40	
N correct	17	32	
Proportion	0.85	0.80	
Summary	$N = 60$	*N* correct = 49	Proportion correct = 0.817
Site C			
Put into group	Me	Non-Me	
Me	16	0	
Non-Me	4	40	
Total *N*	20	40	
N correct	16	40	
Proportion	0.80	1.00	
Summary	$N = 60$	*N* correct = 56	Proportion correct = 0.933

Groups are *M. edulis* (Me) and non-edulis (non-Me) genotypes. Non-edulis are the sum of *M. trossulus* and *M. edulis* × *M. trossulus* hybrids genotypes

Before the current study, surveys of *Mytilus* species in Scotland were limited to a few samples of mussels collected over 25 years ago in the work of Skibinski et al. (1983), and the recent finding of all three species and hybrids at one location, Loch Etive (Beaumont et al., 2008). It is therefore not difficult to recognise that the survey presented, involving the collection of mussels at a total of 85 natural and farmed sites, has made a valuable contribution to the knowledge of *Mytilus* species distribution in Scottish waters. *M. edulis* is the dominant species in Scotland and its exclusive detection from samples collected from the Scottish east and Irish Sea coasts suggests these areas to be potential sources of *M. edulis* spat for mussel seed exportation and/or restocking of *M. trossulus* affected sites in the future.

Although *M. galloprovincialis* genotypes appear widespread at natural and farmed sites, the low abundance of both adults and planktonic larvae of this species and the fact that *M. galloprovincialis* is an important commercially cultivated species in other countries suggest its presence is unlikely to have a significant impact on either farmed or natural mussel populations in Scotland, in the short-term. The fact that *M. trossulus* and its hybrids can be present at high frequencies on artificial structures like marina pontoons, and especially on aquaculture ropes, suggests that these structures may act as a sheltered niche that is most likely to be contributing to the proliferation of this species. Environmental factors like salinity, together with the fact that these structures eliminate the stress of aerial exposure at low tide and reduce the accessibility to benthic predators, contribute to explain the large differences in abundance of *M. trossulus* observed between populations on aquaculture ropes and pontoons, and on nearby shores (see Dias et al., 2008, 2009a, b for more detailed discussion). The good news is that *M. trossulus* presence appears to be restricted to farms of the west and southwest Scotland and that this together with the fact that significant abundance of thinner shelled *M. trossulus* at sites was easily noticed by experienced growers, suggests that the potential for wider impact on cultivation may be controllable.

Taken together, and similarly to what has been observed in Canada (Mallet & Carver, 1995, 1999; Penney et al., 2002, 2006, 2007, 2008; Penney & Hart, 1999), our results indicate that, within mixed-species areas in Scotland, *M. edulis* is likely to outperform *M. trossulus* and hybrids in terms of commercial quality. Differences in the spawning behaviour of *M. trossulus*, *M. edulis* and hybrids appear to be too small to allow for a "rope-dropping time frame" strategy that would avoid *M. trossulus* settlement. Nevertheless, any practical interventions towards minimising the presence of *M. trossulus* on ropes (e.g. grading and resocking of mussels, fallowing or harvesting of affected sites) is likely to be more efficient if performed before the main spawning season for mussels.

Hybrids were observed to be morphometrically similar to *M. trossulus* rather than *M. edulis*, suggesting that the grading of non-edulis genotypes during mussel harvest might have the potential to identify and remove a high proportion of *M. trossulus* genotypes from the stock. Differences between the bulk and strength of these two similar types and *M. edulis* appear to be easily noticed by mussel growers, especially if they are alert to the problem. *M. trossulus* have been identified to be significantly more frequent on ropes in the upper 5 m of the water column, suggesting that changes in cultivation practices to avoid settlement in these depths are likely to reduce *M. trossulus* production and proliferation. Nevertheless, the practical costs and/or benefits of introducing such a labour-intensive and time-consuming process could only be assessed through the establishment of small-scale technical experiments, the potential feasibility of the non-edulis mussels being commercialised as an alternative meat processed "out of the shell" product, and the relative costs and benefits of implementing other potential strategies such as the fallowing of sites and transfer of unispecific *M. edulis* seed into mixed species areas.

The distinct situations offered by sites A, B and C sampled in Dias et al. (2011) represent a good example of candidate locations to further investigate the feasibility of distinct management strategies aimed at favouring *M. edulis* production. At farm site A, given its high mussel production capacity and the significantly lower levels of meat yield obtained, the simultaneous fallowing of all sites in the area and transfer of *M. edulis* unispecific seed stock is likely to provide the best long-term strategy towards the re-establishment of *M. edulis* stock and improvement of overall product quality. At farm site B, given the smaller farm size and the higher meat yields observed, grading operations and the potential use of *M. trossulus* meats for secondary processing are more likely to favour *M. edulis* production than if these strategies were implemented at site A. The farm site C represents an unusual case as it also produces other species of shellfish; all species being sold direct to restaurants. In these restaurants, mussel meats are often sold smoked or pickled; a factor that could favour the commercialisation of thin shelled *M. trossulus* that would, however, present reasonable meat contents, and that might contribute to growers at site C not feeling greatly affected by the presence of *M. trossulus* at their farm. It would be interesting to further investigate the feasibility of marketing *M. trossulus* as "out of the shell product" meat processed products at this farm.

The transfer of unispecific *M. edulis* seed into mixed-species areas has been particularly suggested by Canadian researchers as a strategy to overcome the problem of having *M. trossulus* in cultivation (Penney et al., 2007, 2008; Penney & Hart, 1999). In Scotland, the fallowing of sites in heavily economically impacted areas, coupled with the transfer of *M. edulis* unispecific seed stock is currently being considered. This approach is seen as likely to provide the best long-term strategy towards the re-establishment of *M. edulis* stock and improvement of overall product quality. However, such measures represent a radical intervention both from an economical and environmental point of view. The practical capability to collect and transfer unispecific *M. edulis* seed stock is limited and the fallowing of a significant number of sites will necessarily mean an extreme reduction in production and cash flow to growers. The disposal of a significant quantity of live mussels is costly and involves the consideration of environment impacts. Finally, the effectiveness of the strategy in greatly reducing future natural *M. trossulus* settlement and re-establishing a profitable production area, although theoretically encouraging, is uncertain.

Complete eradication of *M. trossulus* from economically affected areas in Scotland may be unrealistic, especially considering that its introduction and distribution mechanism in the environment remain uncertain. Area-specific solutions to managing the problem may thus be required, which may or may not involve eradication and fallowing (clearance of mussels from production sites for a given period of time). Nevertheless, *M. trossulus* current distribution is limited and its spread outside its existing range is clearly undesirable. Managing the impact of *M. trossulus* at both the regional and national scale is of fundamental importance in ensuring the long term sustainability of mussel production in Scotland. The different situations observed at mixed-species sites indicates management of this problem in Scotland is likely to involve the implementation of area-specific measures, and the establishment of clear guidance on good practice aiming at preventing further distribution of *M. trossulus*.

Outlook

One of the most attractive features of molecular methods like PCR and real-time PCR is the fact of being high-throughput techniques, able to process up to 96 reactions in one run, each reaction including multiple targets. In real-time PCR, although the limited number and the emission overlap of fluorophoric labels is likely to limit the quantification of multiple reaction products, significant progress is being made. Real-time PCR technology, chemistries and platforms are evolving and detection of up to five targets in one reaction is currently available. This opens up possibilities regarding further development and optimisation of the assay developed for the three *Mytilus* species. Including further target sequences of other cultured bivalve species (i.e. Pacific Oyster *Crassostrea gigas*) or harmful toxic algae in the assay could strengthen the relevance of its application to plankton samples. Such assays would potentially provide valuable support to the shellfish industry.

Managing *M. trossulus* at a wider scale, taking into account all the sites where *M. trossulus* genotypes have been detected presents a big challenge. At farms where *M. trossulus* is present but is not reported to cause an impact on profitability, growers may be unwilling to make any technical changes to production unless such changes were likely to lead to significantly increased production and profitability in the medium to long term. This could be the case for introduction of a sub-surface mussel rope culture system. In New Zealand, these ropes are suspended approximately 3 m below the surface in order to exploit the depth-related differences in settlement of two co-occurring species; the green-lipped mussel *Perna* sp. and *M. galloprovincialis* (Alfaro & Jeffs, 2003; Bownes & McQuaid, 2006), and optimise the production of the preferred species *Perna* sp. Previous observations of *M. trossulus* being significantly more abundant on the upper 2–5 m of mussel cultivation ropes (Dias et al., 2009b) suggest this technical approach could have the potential to significantly decrease the proportion of *M. trossulus* at cultivation sites. The New Zealand highly automated mussel rope culture system consists of single headlines equipped with continuously looped, pegless rope and has been reported to enable rapid harvesting and husbandry operations. It has been recently introduced in Scotland at a pilot scale and, if proven feasible in Scottish conditions, the introduction of such systems at farms within the *M. trossulus* distribution area represents a further option that could potentially provide a long-term solution to this problem.

Within the management context, it also becomes important to clarify the status of *M. trossulus* in Scotland. Although to date there is no evidence of *M. trossulus* acting as an invasive species, if *M. trossulus* proves to be an alien species to any of the affected areas in Scotland, or its apparent dispersion resulted from aquaculture practices, the industry is likely face stricter regulation and pressures regarding the establishment of new farm sites and movements from affected areas. Zbawicka et al. (2010) very recently reported the *M. trossulus* population in Loch Etive to have been established following an invasion from North America towards the end of the last glacial period. These findings confirm the comments by Beaumont et al. (2008), who suggested *M. trossulus* in Loch Etive to be a relict population, increased in recent years by aquaculture practices. It would be of interest to build on these findings by further investigating the establishment of *M. trossulus* populations at two other lochs and their potential relation with populations at Loch Etive. It would be particularly interesting to determine if *M. trossulus* presence in Scotland: (1) is a result of a single invasion from North America towards the end of the last glacial period occurred simultaneously at several sites Scotland; (2) if *M. trossulus* is a relict population at Loch Etive that has been subsequently spread by human-mediated activities; or (3) if *M. trossulus* is a relict population at Loch Etive but has been more recently introduced in other areas as a consequence of human-mediated introductions from the Baltic Sea or overseas from the Canadian Maritimes.

Acknowledgments The authors would like to thank all colleagues at Marine Scotland and at the University of Aberdeen at Aberdeen, UK, and the Hellenic Centre for Marine Research in Greece for the support provided during the 3 years of the project. Thank you to the Association of Scottish Shellfish Growers and all mussel farmers involved in the project for support, advice and interest in our work. Joana Dias was funded by the ECOSUMMER Marie Curie training site (MEST-CT-2005-020501). The years of training within the ECOSUMMER network represented a very good and rewarding experience and this could not have been achieved without Graham Pierce's dedication. Thank you so much to Graham Pierce, Begoña Santos, Alejandro Gallego, Vasilis Valavanis and all the people that dedicated their time and contributed to the organisation and success of the network. Thank you dearly to all ECOSUMMER trainees for such a good time.

References

Alfaro, A. C. & A. G. Jeffs, 2003. Variability in mussel settlement on suspended ropes placed at Ahipara Bay, Northland, New Zealand. Aquaculture 216: 115–126.

Beaumont, A. R., M. P. Hawkins, F. L. Doig, I. M. Davies & M. Snow, 2008. Three species of *Mytilus* and their hybrids identified in a Scottish Loch: natives, relicts and invaders? Journal of Experimental Marine Biology and Ecology 367(2): 100–110.

Bownes, S. J. & C. D. McQuaid, 2006. Will the invasive mussel *Mytilus galloprovincialis* Lamarck replace the indigenous *Perna perna* L. on the south coast of South Africa? Journal of Experimental Marine Biology and Ecology 338(1): 140–151.

Dias, P. J., L. Sollelis, E. Cook, S. B. Piertney, I. M. Davies & M. Snow, 2008. Survey of *Mytilus edulis* complex species in Scotland using real-time PCR. Journal of Experimental Marine Biology and Ecology 367: 253–258.

Dias, P. J., M. Bland, A. M. Shanks, A. R. Beaumont, S. B. Piertney, I. M. Davies & M. Snow, 2009a. *Mytilus* species under rope culture in Scotland: implications for management. Aquaculture International 17(5): 437–448.

Dias, P. J., A. Dordor, D. Tulett, S. Piertney, I. M. Davies & M. Snow, 2009b. Survey of mussel (*Mytilus*) species at Scottish shellfish farms. Aquaculture Research 40: 1715–1722.

Dias, P. J., F. M. Batista, A. M. Shanks, A. R. Beaumont, I. M. Davies & M. Snow, 2009c. Gametogenic asynchrony of mussels *Mytilus* in a mixed-species area: implications for management. Aquaculture 295: 175–182.

Dias, P. J., B. Malgrange, M. Snow & I. M. Davies, 2011. Performance of mussels *Mytilus edulis*, *M. trossulus* and their hybrids in cultivation at three Scottish lochs. Journal of the World Aquaculture Society 42(1): 111–121.

FRS, 1996. Scottish Shellfish Farm Production Survey. Fisheries Research Services, Aberdeen, UK.

FRS, 2008. Scottish Shellfish Farm Production Survey. Fisheries Research Services, Aberdeen, UK.

Gosling, E., 1992. The mussel *Mytilus*: ecology, physiology, genetics and culture. Elsevier Developments in Aquaculture and Fisheries Science 25: 589.

Gosling, E., S. Doherty & N. Howley, 2008. Genetic characterization of hybrid mussel (*Mytilus*) populations on Irish coasts. Journal of the Marine Biological Association of the UK 88(2): 341–346.

Goudet, J., 1995. FSTAT (version 1.2): a computer program to calculate F-statistics. Journal of Heredity 76: 111–117.

Hilbish, T. J., A. Mullinax, S. I. Dolven, A. Meyer, R. K. Koehn & P. D. Rawson, 2000. Origin of the antitropical distribution pattern in marine mussels (*Mytilus* spp.): routes and timing of transequatorial migration. Marine Biology 136: 69–77.

Inoue, K., J. H. Waite, M. Matsuoka, S. Odo & S. Harayama, 1995. Interspecific variations in adhesive protein sequences of *Mytilus edulis*, *M. galloprovincialis* and *M. trossulus*. Biological Bulletin 189: 370–375.

Kijewski, T. K., M. Zbawicka, R. Väinölä & R. Wenne, 2006. Introgression and mitochondrial DNA heteroplasmy in the Baltic populations of mussels *Mytilus trossulus* and *M. edulis*. Marine Biology 149: 1371–1385.

Mallet, A. L. & C. E. A. Carver, 1995. Comparative growth and survival patterns of *Mytilus trossulus* and *Mytilus edulis* in Atlantic Canada. Canadian Journal of Fisheries and Aquatic Sciences 52: 1873–1880.

Mallet, A. L. & C. E. A. Carver, 1999. Maritime distribution and commercial production performance of *Mytilus edulis* and *Mytilus trossulus*. Bulletin of the Aquaculture Association of Canada 99(3): 7–13.

Marine Scotland, 2009. Scottish Shellfish Farm Production Survey. Marine Scotland Marine Laboratory, Aberdeen, UK.

Nalepa, T. F. & D. W. Schloesser, 1993. Zebra Mussels: Biology, Impacts and Control. CRC Press, Boca Raton, FL: 810.

Penney, R. W. & M. J. Hart, 1999. Distribution, genetic structure, and morphometry of *Mytilus edulis* and *M. trossulus* within a mixed species zone. Journal of Shellfish Research 18: 367–374.

Penney, R. W., M. J. Hart & N. Templeman, 2002. Comparative growth of cultured blue mussels, *Mytilus edulis*, *M. trossulus* and their hybrids, in naturally occurring mixed-species stocks. Aquaculture Research 33: 693–702.

Penney, R. W., M. J. Hart & N. D. Templeman, 2006. Genotype-dependent survival, growth, and production in cultured blue mussels *Mytilus* spp.: results of a reciprocal seed transfer experiment. Journal of Shellfish Research 25(2): 515–525.

Penney, R. W., M. J. Hart & N. Templeman, 2007. Shell strength and appearance in cultured Blue Mussels *Mytilus edulis*, *M. trossulus*, and *M. edulis* × *M. trossulus* hybrids. North American Journal of Aquaculture 69: 3281–3295.

Penney, R. W., M. J. Hart & N. D. Templeman, 2008. Genotype-dependent variability in somatic tissue and shell weights and its effect on meat yield in mixed species [*Mytilus edulis* L., *M. trossulus* (Gould), and their hybrids] cultured mussel populations. Journal of Shellfish Research 27(4): 827–834.

Skibinski, D. O. F., J. A. Beardmore & T. F. Cross, 1983. Aspects of the population genetics of *Mytilus* (Mytilidae; molluscs) in the British Isles. Biological Journal of the Linnean Society 19: 137–183.

Smaal, A. C., 2002. European mussel cultivation along the Atlantic coast: production status, problems and perspectives. Hydrobiologia 484: 89–98.

Zbawicka, M., A. Burzyński, D. Skibinski & R. Wenne, 2010. Scottish *Mytilus trossulus* mussels retain ancestral mitochondrial DNA: complete sequences of male and female mtDNA genomes. Gene 456(1–2): 45–53.

Hydrobiologia (2011) 670:141–163
DOI 10.1007/s10750-011-0674-8

ECOSYSTEMS AND SUSTAINABILITY

Application of a bioenergetics growth model for European anchovy (*Engraulis encrasicolus*) linked with a lower trophic level ecosystem model

Dimitris V. Politikos · George Triantafyllou · George Petihakis · Kostas Tsiaras · Stylianos Somarakis · Shin-Ichi Ito · Bernard A. Megrey

Published online: 1 April 2011
© Springer Science+Business Media B.V. 2011

Abstract A bioenergetics model is implemented for European anchovy (*Engraulis encrasicolus*) and applied to the north-eastern Aegean Sea (eastern Mediterranean Sea). The model reproduces the growth of anchovy in a one-way linked configuration with a lower trophic level (LTL) ecosystem model. The LTL model provides densities for three zooplankton functional groups (heterotrophic flagellates, microzooplankton and mesozooplankton) which serve as available energy via consumption for the anchovy model. Our model follows the basic structure of NEMURO.FISH type models (North Pacific Ecosystem Model for Understanding Regional Oceanography for Including Saury and Herring). Several model parameters were specific for the Mediterranean or the Black Sea anchovy and some others were adopted from related species and NEMURO.FISH due to lack of biological information on *E. encrasicolus*. Simulation results showed that the fastest growth rate occurs during spring and the slowest growth rate from August to December. Zooplankton abundance during autumn was low implying that decreased prey density lead to a reduction in anchovy weight, especially for the age-3 class. Feeding parameters were adjusted to adequately fit the model growth estimates to available weight-at-age data. A detailed sensitivity analyses is conducted to evaluate the importance of the biological processes (consumption, respiration, egestion, specific dynamic action, excretion and egg production) and their parameters to fish growth. The most sensitive parameters were the intercept and exponent slope of the weight-dependent consumption and respiration process equations. Fish weight was fairly sensitive to temperature-dependent parameters.

Keywords Bioenergetics model · Anchovy (*Engraulis encrasicolus)* · Lower trophic ecosystem model · Northern Aegean Sea · NEMURO.FISH

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

---

D. V. Politikos · G. Triantafyllou (✉) ·
G. Petihakis · K. Tsiaras · S. Somarakis
Hellenic Centre for Marine Research,
19013 Anavissos, Greece
e-mail: gt@ath.hcmr.gr

D. V. Politikos
Department of Mathematics, School of Applied
Mathematical and Physical Sciences, National Technical
University of Athens, Zografou Campus,
157 80 Athens, Greece

S.-I. Ito
Tohoku National Fisheries Research Institute,
Fisheries Research Agency, 3-27-5 Shinhama-cho,
Shiogama, Miyagi 985-0001, Japan

B. A. Megrey
National Oceanic and Atmospheric Administration,
National Marine Fisheries Service, Alaska Fisheries
Science Center, 7600 Sand Point Way NE,
Seattle, WA 98115, USA

Introduction

European anchovy (*Engraulis encrasicolus*) is one of the most important pelagic species in the Mediterranean (Lleonart & Maynou, 2003). The northern Aegean Sea anchovy stock, influenced by the outflow of Black Sea waters (BSWs), is one of the largest stocks in the eastern Mediterranean constituting a key fisheries resource in this area (Somarakis et al., 2006). The integration of environmental factors with biological attributes and fishery knowledge has been recognised as a needful task in order to improve the understanding of the mechanisms that drive the pelagic fishery resources variability in the Mediterranean Sea (Giannoulaki et al., 2005). From an ecosystem perspective, small pelagic fish play a key role in connecting the lower and upper trophic levels marine ecosystems, due to their significant biomass at mid-trophic levels (Bakun, 1996).

Several fish models have been developed with different characteristics in terms of orientation, objectives and perspectives. For a detailed review of the current modelling approaches in fisheries, we propose the paper of Plagányi (2007). During the last few years, various food web models have been published emphasizing the importance of linking lower trophic levels (LTLs) with higher trophic levels (HTLs) for exploring the environment and fishing effects on ecosystems (Travers et al., 2007). For example, Bryant et al. (1995) proposed the development and analysis of a fish module within the nutrient-based ecosystem model ERSEM (Baretta et al., 1995). NEMURO.FISH (Ito et al., 2004; Megrey et al., 2007; Rose et al., 2007) has been developed for saury (*Cololabis saira*) and herring (*Clupea harengus*) through linking a LTL biogeochemical model with a HTL fish population–bioenergetics model. Oguz et al. (2008) developed a one-dimensional coupled model of LTL and anchovy population dynamics to analyse the mechanisms of sharp biomass transitions of anchovy and gelatinous zooplankton in the Black sea. Individual-based models (IBMs) offers also a promising approach for modelling population and community dynamics contributing to our understanding of recruitment processes in marine fish populations (Miller, 2007). The consequences of global warming on capelin distribution and population dynamics through an IBM model linked with a biophysical ocean model were investigated by Huse & Ellingsen (2008), whilst a two-dimensional IBM model coupled with fish bioenergetics was developed to simulate migration and growth of Japanese sardine (*Sardinops melanostictus*) in the western North Pacific (Okunishi et al., 2009).

Working in this framework, an attempt to develop a model that can efficiently represent the growth of a pelagic fish in a Greek marine system by bringing together a LTL food web model with a fish growth model is presented. More specifically, a complex LTL (Princeton Ocean Model–European Regional Seas Ecosystem Model, POM–ERSEM) was linked with a bioenergetics model for European anchovy following the NEMURO.FISH modelling approach (Ito et al., 2004; Megrey et al., 2007). The present LTL model provides concentrations of three zooplankton groups (heterotrophic flagellates, microzooplankton and mesozooplankton), which serve as the available energy intake via consumption for the anchovy model. In addition, the anchovy habitat temperature that significantly affects the fish growth is also based on the LTL model simulated hydrodynamics. The model performance is explored through validation and sensitivity tests.

Materials and methods

Study area

Although the Aegean Sea is characterized as an overall oligotrophic environment, there is significant spatial variability with the northern Aegean Sea being a more productive area compared to the southern. The water column in the northern part is highly stratified, consisting essentially of three layers. The upper layer (0–100 m) is a mixture of BSW and Levantine Intermediate Water (LIW) with a 20-m thick surface layer of modified BSW. Below this (100-400 m), a layer of warm and saline layer of Levantine origin (LIW) is found. The deeper layer is composed of very dense northern Aegean Deep water (NADW). The northern Aegean is characterized by an overall cyclonic circulation, overlaid with several transient and semi-permanent eddies, such as the anticyclone surrounding the Samothraki and Imvros islands (Zervakis & Georgopoulos, 2002). A prominent characteristic is the brackish water inputs from N. Aegean rivers (Fig. 1) and the Black Sea which significantly influence both the hydrology and ecology of the area. The depth of the euphotic zone varies between 80 and 100 m, whilst nutrient (nitrates and phosphates) concentrations in the surface layer are similar to the southern Aegean with nitrate levels (0–200 m) varying between 0.05 and 1.6 μM and phosphate 0.02–0.08 μM. The seasonal variability is low with a small increase in nutrients during March related to winter mixing.

Field studies (Lykousis et al., 2002; Siokou-Frangou et al., 2002) show that the partitioning of carbon among the different plankton compartments is not a broad-based pyramid, where autotrophic biomass overwhelms that of heterotrophs as the ratio of heterotrophs/autotrophs is 0.9–2.0 indicating the importance of microheterotrophs (Bacteria, Heterotrophic Nanoflagellates and Ciliates). Autotrophic biomass values (integrated to 100 m) range between 1,072 and 2,568 mg C m^{-2} with small size cells (<3 μm) dominating (80%). Most of the biomass (49–56%) is due to the picoplankton fraction (0.2–1.2 μm), whilst the ultraplankton fraction (1.2–3.0 μm) accounts for 18–23% and the nano + microplankton fraction (>3.0 μm) for 20–33%. Heterotrophs play a significant role in the northern Aegean Sea with biomass values ranging from 1,847 to 2,421 mg C m^{-2} with the upper 50 m layer exhibiting higher values. Bacteria constitute the largest fraction of this biomass (59–69%) whilst in terms of mesozooplankton, copepods are the most abundant. The relatively high abundance of cladocerans and appendicularians, which are organisms able to exploit the available small autotrophs and heterotrophs, indicate rather efficient trophic relationships within the food web. There are significant export fluxes of particulate organic carbon from the euphotic zone comprising a mixture of plankton cell and faecal pellets to depths of >1,500 m; which implies a rapid and close relationship between benthic/pelagic coupling (Siokou-Frangou et al., 2002).

Lower trophic level ecosystem model

The zooplankton biomass values and temperature that are used as input to the fish model are provided by a 3-D coupled physical–biogeochemical model that is implemented in the northern Aegean area (model domain shown in Fig. 1). The model simulated zooplankton and temperature 3-D fields are averaged over a representative area (indicated in the box of Fig. 1) in the Thracean Sea, which is a well-known habitat for anchovy in the N. Aegean (Somarakis et al., 2007) and therefore was chosen as the study area. The hydrodynamic model is based on the

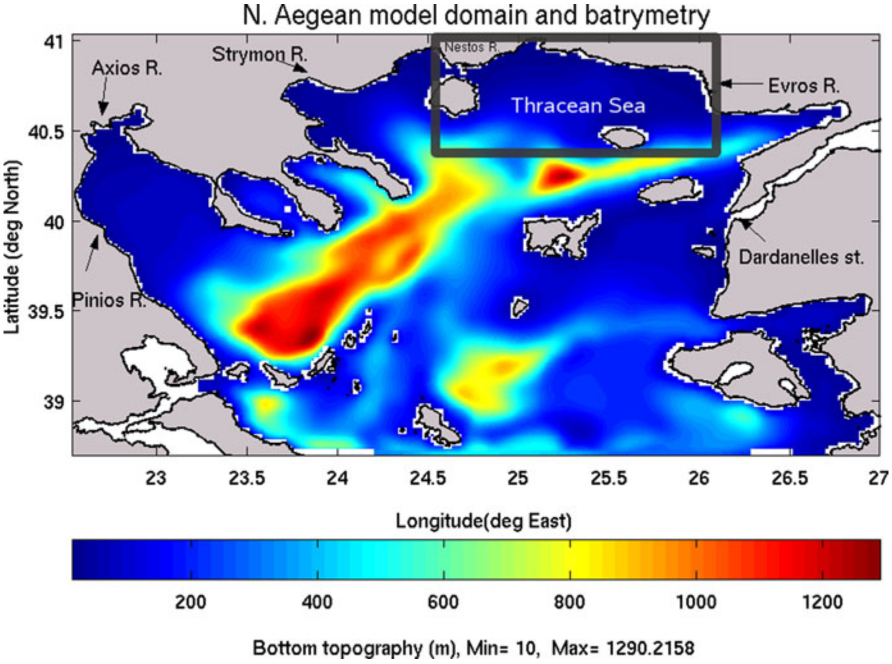


Fig. 1 Study area: northern Aegean Sea. Model domain and bathymetry. *SA* Samothraki Island, *IM* Imvros Island. The indicated *black box* represents the Thracean Sea area over which the 3D-LTL model output is averaged

Princeton Ocean Model (Blumberg & Mellor, 1983), which is a primitive equation, sigma-coordinate circulation model embedded with a Mellor-Yamada 2.5 turbulence closure sub-model (Mellor & Yamada, 1982) that is used to compute vertical mixing coefficients. POM (http://www.aos.princeton.edu/WWWPUBLIC/htdocs.pom) is a widely spread community model with numerous applications. In the N. Aegean it has been applied by Kourafalou & Barbopoulos (2003), Kourafalou & Tsiaras (2007) whilst it is also implemented in the Aegean Sea as part of the operational 'POSEIDON' forecasting system (Nittis et al., 2006; Korres et al., 2002). The biogeochemical model is based on ERSEM II (Baretta et al., 1995) and has been implemented at sub-basin (Aegean–Levantine), shelf and coastal areas of the eastern Mediterranean (Petihakis et al., 2002; Triantafyllou et al., 2007; Petihakis et al., 2009). Furthermore, a basin-scale Mediterranean coupled model (POM–ERSEM) is operational as part of the 'POSEIDON' forecasting system (www.poseidon.ncmr.gr).

The coupled physical–biogeochemical model of the N. Aegean sea has a horizontal resolution of 1/10° (~10 km), and 25 bottom following sigma levels resolved in the vertical, whilst the integration time step is set to 10 min. A logarithmic distribution allows a finer resolution at the layers close to the surface. Thus, in the area of interest (Fig. 1) the vertical resolution varies from 0.5 m near the surface to 5 m at mid-depth.

Coupled physical–biogeochemical model climatological simulations were performed using high-resolution (6 h, 1/10°) atmospheric forcing provided by the operational HCMR (Hellenic Centre for Marine Research) 'POSEIDON' system model (Papadopoulos et al., 2002).

For the biology, a complex pelagic system is used with state variables describing four autotrophic and four heterotrophic plankton groups, dissolved and particulate organic matter and the essential dissolved inorganic nutrients (nitrate, phosphate, ammonia and silicate). Biotic groups are distinguished not by species, but by their functional role in the ecosystem using size as the major characteristic. Both physiological (ingestion, respiration, excretion, egestion, etc.) and population processes (growth, migration and mortality) are included whilst biologically driven carbon dynamics are coupled to the chemical dynamics of nitrogen, phosphate, silicate and oxygen with dynamically varying ratios. Autotrophs are light limited, competing with heterotrophic pelagic bacteria for nutrients with the latter acting as remineralisers. Nutrient uptake is controlled by the difference between the internal nutrient pool and external nutrient concentration. Heterotrophs exploit phytoplankton and bacteria as well as smaller heterotrophic groups. In the absence of fish predators a sigmoid density-dependent loss function (Edwards & Yool, 2000) is adopted for zooplankton, parameterising top-predator mortality. Finally, the benthic–pelagic coupling is described by a simple first order benthic return module, which includes the settling of organic detritus into the benthos and diffusional nutrient fluxes into and out of the sediment.

Fish model

In order to describe the growth of an individual anchovy, the bioenergetics modelling approach was adopted. Bioenergetics models have been increasingly employed to analyse the mechanisms of changes in fish growth (Ursin, 1967; Stewart et al., 1983; Kitchell et al., 1997). These models are based on balanced energy equations in which growth is represented as the difference between consumption and energy losses such as respiration, specific dynamic action (SDA), egestion, excretion and egg production (EGG). Following the basic structure of NEMURO.FISH type bioenergetics models (Ito et al., 2004; Megrey et al., 2007) the specific growth of anchovy was calculated as weight increment per unit of weight per day

$$\frac{1}{W}\cdot\frac{\mathrm{d}W}{\mathrm{d}t} = [C-(R+F+\mathrm{SDA}+E+\mathrm{EGG})] \cdot\frac{\mathrm{CAL_z}}{\mathrm{CAL_f}}, \tag{1}$$

where W is the fish wet weight (g-fish), t is the time (days), C is the consumption, R is the respiration (or losses due to metabolism), F is the egestion (or losses due to faeces), SDA is the specific dynamic action (or losses due to energy costs for food digestion), E is the excretion (or losses of nitrogenous excretory wastes) and EGG is the egg production (or losses due to reproduction). Components of the energy budget (C, R, SDA, F, E and EGG) are in units of g zooplankton g fish^{-1} day^{-1}, which are converted to g fish g fish^{-1} day^{-1} by using the ratio of $\mathrm{CAL_z}$ is the caloric equivalent of zooplankton (J g zooplankton^{-1}) to $\mathrm{CAL_f}$ is the caloric equivalent of fish (J g fish^{-1}).

A fixed energy density coefficient for zooplankton was adopted, with a value of $\mathrm{CAL_z} = 2{,}580$ J g zooplankton^{-1} for marine copepods (Laurence, 1976). The scientific interest in energy density of fish has lately increased due to its use in bioenergetics models. Improper or constant values can have misleading effects on model results (Stewart & Binkowski, 1986). In this work, we used a length-based energy density coefficient $\mathrm{CAL_f}$ for anchovy based on experiments. Particularly, Tirelli et al. (2006) showed that the energy density for *E. encrasicolus* exhibits a rather wide range from 2,667 to 7,022 J g fish^{-1}, following a linearly increasing energy density pattern for fish lengths between 40 and 90 mm and a more variable pattern for lengths >90 mm. Based on these measurements, the following average values were chosen

$$\mathrm{CAL_f} = \begin{cases} 3120, & \text{if length} < 40\ \text{mm} \\ 3520, & \text{if } 40 \leq \text{length} < 60\ \text{mm} \\ 4048, & \text{if } 60 \leq \text{length} < 90\ \text{mm} \\ 5150, & \text{if length} \geq 90\ \text{mm} \end{cases} \qquad (2)$$

As fish shows notable biological differentiations (feeding preferences, habitat selection, reproduction capacity and length–weight relationship) during its growth, anchovy's life cycle was divided into four different stages according to length: embryonic (egg + yolk-sac larvae) stage (<3.5 mm), larval (3.5–38.0 mm), juvenile (38–105 mm) and adult (105–165+ mm) stages (Table 1).

The duration of the embryonic stage was estimated by temperature-dependent curves calculated from laboratory experiments in the Adriatic Sea (Regner, 1996). These curves show an inverse relationship between developmental time (days) and temperature (°C). In the northern Aegean sea, the water temperature at which anchovy eggs are released favour a rather short embryonic duration of approximately 5 days, whilst the mean growth rate of larvae was estimated to approximately 0.5 mm day^{-1} (Somarakis & Nikolioudakis, 2007). This means that anchovy larvae need almost 70 days in order to become juveniles. The juvenile stage lasts until anchovy reach maturity at approximately 105 mm total length with the completion of its first year of life (Somarakis et al., 2006).

Table 1 Life stages of anchovy adopted in the bioenergetics model

Stage	Length (mm)	Age (days)
1. Embryonic	<3.5	0–5
2. Larval	3.5–38	6–76
3. Juvenile	38–105	77–364
4. Adult	105–165+	365–1,205

The growth of anchovy reproduced by the bioenergetics model is calculated as wet weight per day. However, as anchovy's life stage definition is based on length, a length–weight relationship is required. Such relationship in fishes usually changes significantly at important ontogenetic thresholds such as metamorphosis (from larva to juvenile) and onset of sexual maturity (Fuiman & Werner, 2002). In anchovy, these thresholds occur at about 38 (Somarakis, 1999) and 105 mm (Somarakis et al., 2006), respectively. A standard length–weight relationship was calculated [log(wet weight] = $a + b$ * [log(length)] using data from 120 larvae (length range: 3.1–21.2 mm) collected during June 1995–1996 (Somarakis, 1999), 605 juveniles (43–104 mm) collected during June 2007 (unpublished data) and 1,792 adults (105–157 mm) collected during 2006 in the north-eastern Aegean Sea (Somarakis et al., 2007). Indeed, these relationships had significantly different slopes ($b = 3.34$, 3.00 and 3.79 for larvae, juveniles and adults, respectively, F test = 217.72, $P < 0.0001$).

In order to obtain a relationship that could describe all three ontogenetic stages, a piecewise allometric regression was fitted to the data using non linear regression

$$y = b_0 + b_1 x + b_2(x - d_1)(x \geq d_1) + b_3(x - d_2)(x \geq d_2), \qquad (3)$$

where y and x are the fish wet weight and length, respectively (log-transformed), b_0 is the y-intercept, b_1 is the slope of the function for the larval stage, b_2 is the slope change for the juvenile stage, d_1 is the inflexion point at which the slope changes (fixed at 38 mm, i.e., $d_1 = \log[38]$), b_3 is the subsequent slope change for the adult stage and d_2 is the corresponding length for this slope change (fixed at 105 mm, i.e., $d_2 = \log[105]$) (see also, Somarakis & Nikolioudakis, 2007). Equation 3 with estimated parameters $b_0 = -6.1158$, $b_1 = 3.576$, $b_2 = -0.616$ and $b_3 = 0.7137$ (Fig. 2), explained the data very satisfactorily in terms of the amount of variance ($r^2 = 0.998$) and the

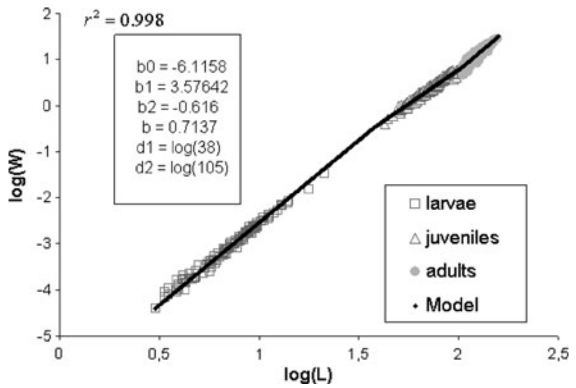


Fig. 2 Fitted piecewise allometric log-transformed relationship between weight in g (*W*) and length in mm (*L*), (3)

properties of the residuals (normally distributed, homoscedastic).

Due to the limited available data, values for *E. encrasicolus* from the Black Sea (Oguz et al., 2008) and for *E. capensis* from South Africa (James & Findlay, 1989; James & Probyn, 1989; James et al., 1989) were used for the parameterization of the growth model. However, it must be noted that according to recent studies concerning *E. capensis* (Huggett et al., 2003; Van der Lingen et al., 2006), *E. encrasicolus* and *E. capensis* must be considered as a single species (*E. encrasicolus*). Further data gaps were filled with parameter values from related species (Vasquez, 1989; Luo & Brandt, 1993; Rose et al., 1999) and NEMURO.FISH model (Ito et al., 2004; Megrey et al., 2007).

Consumption

Daily consumption rate (C) is described as the proportion of a maximum consumption rate $C_{\max}$ modelled as a function of fish weight and water temperature:

$$C_{\max} = a_c W^{b_c} f_C(T), \tag{4}$$

where a_c is the maximum consumption rate of 1 g anchovy at the optimal temperature for consumption (g prey g fish^{-1} day^{-1}), b_c is the exponent for the weight dependence of the consumption and $f_C(T)$ is a temperature-dependent function for consumption.

The adopted temperature-dependent function of maximum consumption, often used for warm water species, was taken from Kitchell et al. (1997) using the auxiliary terms V, S, Y, X:

$$f_C(T) = V^X e^{X(1-V)}, \tag{5}$$

$$V = \frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \tag{5a}$$

$$S = (\ln Q_c)(T_{\max} - T_{\text{opt}}) \tag{5b}$$

$$Y = (\ln Q_c)(T_{\max} - T_{\text{opt}} + 2) \tag{5c}$$

$$X = \frac{S^2(1 + (1 + 40/Y)^{1/2})^2}{400}, \tag{5d}$$

where $T_{\max}$ is the maximum temperature for consumption (approximated by the upper lethal temperature), T_{opt} is the optimum temperature for consumption, Q_c is the slope estimate and T is the ambient temperature. Although this equation was originally applied to yellow perch and walleye, the function has been also applied to other warm water species (Luo & Brandt, 1993; Rose et al., 1999). The values of a_c and b_c in (4) are provided by Luo & Brandt (1993) and Oguz et al. (2008), respectively, (Table 2). The value Q_c in (5b–5c) was set to 2.2 for the larval stage (Luo & Brandt, 1993) and slightly increased to 2.4 for juvenile–adult stage in order to avoid underestimation of temperature limitation at low temperature values.

Studies in the northern Aegean Sea (Somarakis & Nikolioudakis, 2007) have shown that larval anchovies significantly prefer waters of high temperature (21.5–23.5°C at 10 m depth). Although we recognize that these observations are not directly associated with optimal consumption rates, it is acceptable to assume that optimal temperature T_{opt} for larval consumption lies within this range (set to 23°C, Table 2). Following the same argument as in Ito et al. (2004), for the determination of maximum temperature $T_{\max}$ for larval stage (set 32°C in this study), a value higher than the maximum sea surface temperature reported in the northern Aegean Sea (29°C in Giannoulaki et al., 2008) was set. This is important in order to avoid underestimating consumption at higher temperatures.

Juvenile anchovies in the northern Aegean sea are generally found in shallow waters inhabiting depths around the thermocline (ANREC, 2006) with reported mean temperature for these depths, 17–18°C (Isari et al., 2008). Adult anchovy exhibit high feeding activity below the thermocline during

Table 2 Symbols, parameter identification, and parameters values used to implement the bioenergetics model (1) for anchovy (*Engraulis encrasicolus*) in the eastern Mediterranean

Symbol	Parameter identification	Parameter value
Maximum consumption ($C_{\max}$) (Eqs. 4, 5)		
a_c	Intercept for consumption	0.41
b_c	Exponent for consumption	−0.31
Q_c	Slope for temperature dependence	2.22^{a}, $2.4^{b,c}$
T_{opt}	Optimum temperature (°C)	23^{a}, $17^{b,c}$
$T_{\max}$	Maximum temperature (°C)	32^{a}, $32^{b,c}$
Respiration (R) (Eq. 7)		
a_r	Intercept for respiration	0.024
b_r	Exponent for respiration	−0.34
Q_{10}	Control parameter	2.22
T_m	Mean annual mean temperature	18^{a}, $15^{b,c}$
Activity (A) (Eqs. 8, 9)		
d_r	Coefficient for R for swimming speed U	0.022
a_A	Intercept U (<12.0°C)	2.0
a_A	Intercept U (≥12.0°C)	12.25^{a}, 11.98^{b}, 14.21^{c}
a_A	Intercept U (≥12.0°C) (during low feeding activity)	9.97^{c}
b_A	Coefficient U for weight	0.27^{a}, 0.33^{b}, 0.27^{c}
c_A	Coefficient U vs. temperature (<12.0°C)	0.149
c_A	Coefficient U vs. temperature (≥12.0°C)	0.0
Egestion (F) (Eq. 10)		
a_F	Proportion of food egested	0.15^{a}, $0.126^{b,c}$
Specific dynamic action (S) (Eq. 11)		
a_{sda}	Specific dynamic action coefficient	0.10
Excretion (E) (Eq. 12)		
a_e	Excretion coefficient	0.415
b_e	Proportion of food excreted	0.01
Egg production (EGG) (Eq. 13)		
a_{egg}	Proportion of consumption for reproduction	0.19
Multispecies functional response (Eq. 6b)		
v_{11}	Vulnerability of z6 to larval stage	0.5
v_{12}	Vulnerability of z6 to juvenile stage	0.0
v_{13}	Vulnerability of z6 to adult stage	0.0
v_{21}	Vulnerability of z5 to larval stage	1.0
v_{22}	Vulnerability of z5 to juvenile stage	0.5
v_{23}	Vulnerability of z5 to adult stage	0.5
v'_{23}	Vulnerability of z5 to adult stage (during low feeding activity)	1.0
v_{31}	Vulnerability of z4 to larval stage	0.0
v_{32}	Vulnerability of z4 to juvenile stage	0.5
v_{33}	Vulnerability of z4 to adult stage	1.0
K_{11}	Half-saturation constant for z6 to larval stage	0.035
K_{12}	Half-saturation constant for z6 to juvenile stage	0.4
K_{13}	Half-saturation constant for z6 to adult stage	0.3
K_{21}	Half-saturation constant for z5 to larval stage	0.035

Table 2 continued

Symbol	Parameter identification	Parameter value
K_{22}	Half-saturation constant for z5 to juvenile stage	0.15
K_{23}	Half-saturation constant for z5 to adult stage	0.082
K'_{23}	Half-saturation constant for z5 to adult stage (during low feeding activity)	0.082
K_{31}	Half-saturation constant for z4 to larval stage	0.3
K_{32}	Half-saturation constant for z4 to juvenile stage	0.025
K_{33}	Half-saturation constant for z4 to adult stage	0.072

[a] Egg–larval stage

[b] Juvenile stage

[c] Adult stage

daytime, migrating to the upper layers (above the base of the thermocline) at dusk, just after the daily afternoon feeding peak, and remain in the warmer surface waters during the night (ANREC, 2006; Palomera et al., 2007). Giannoulaki et al. (2005) reported that during summer adult anchovy is positively associated with bottom depth temperatures of 14–16°C in the North Aegean Sea. Based on these observations, the optimum temperature for consumption of both juveniles and adults was set to 17°C, whereas the corresponding maximum temperature was set to 32°C. All related values are summarized in Table 2.

The curve of temperature dependence of $C_{\max}$for larval, juvenile and adult stages is a rising function up to 1.0 at optimal temperature T_{opt}, then dropping to zero at a maximum temperature $T_{\max}$ (Fig. 3).

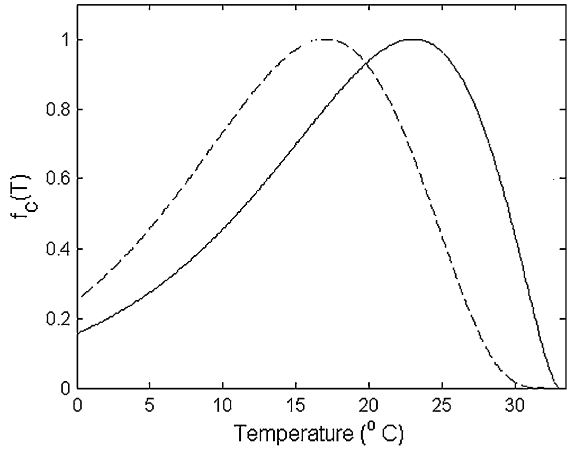


Fig. 3 Effect of temperature function (5) on consumption; larval stage (*continuous line*), juvenile–adult stages (*dashed line*)

Realized consumption of an organism is modelled by a functional response equation which describes the relationship between available prey densities and those consumed by a predator. In the fish model, the daily consumption C of each life stage of anchovy (C_i, g prey g fish^{-1} day^{-1}) is determined as the sum of its consumption rates for each prey type j through a type II functional response equation for multiple prey types (Rose et al., 1999; Megrey et al., 2007)

$$C_i = \sum_{j=1}^{3} C_{ij}, \tag{6a}$$

$$C_{ij} = \frac{C_{\max}(\mathrm{PD}_{ij} v_{ij}/K_{ij})}{1 + \sum_{k=1}^{3}(\mathrm{PD}_{ik} v_{ik}/K_{ik})}, \tag{6b}$$

where $C_{\max}$ is the maximum consumption rate (g prey g fish^{-1} day^{-1}), PD_{ij} the density of prey type j ($j = 1$ for heterotrophic flagellates, $j = 2$ for microzooplankton and $j = 3$ for mesozooplankton) (g prey m^{-3}) to life stage i, v_{ij} the vulnerability of prey type j to stage i (dimensionless) and K_{ij} is a half-saturation constant (g prey m^{-3}) for life stage i feeding on prey type j. Keeping to the terminology of ERSEM the three prey types are denoted in the model as z6 = heterotrophic flagellates, z5 = microzooplankton and z4 = mesozooplankton.

Although the abovementioned prey types are characterized as heterotrophic flagellates, microzooplankton and mesozooplankton in reality each type comprises a much wider range of organisms which have the same functional role defined mainly by the corresponding prey–predator relations. Thus, the smaller prey size organisms ($j = 1$) includes not only heterotrophic flagellates, but also small ciliates and early life stages of bigger animals such as micro and mesozooplankton that forage on the same food source and are themselves preyed upon by the same predator. The second prey type the microzooplankton ($j = 2$) includes ciliates and metazoan zooplankton. The last prey type ($j = 3$) includes mainly mesozooplankton.

Apportioning the prey types, anchovy follows a size-selected pattern as it grows, selecting predominantly the largest prey particles through a biting feeding mode, whilst it uses a filter feeding mode on smaller prey sizes (Plounevez & Champalbert, 2000). Similar feeding behaviour has been shown for other *Engraulis* species (Hunter & Dorr, 1982). Although fish foraging can be rather complex it is reasonable to assume that there is a preference for particular organisms related to their size and shape and that under starvation fish will exploit other less preferred food sources.

Stomach content analysis in fish from various regions (Adriatic Sea, northwestern Mediterranean) (Conway et al., 1998; Tudela et al., 2002) shows that anchovy larvae target mainly copepod eggs, copepodites, and particularly nauplii. A recent study by Rossi et al. (2006) using fatty acids as trophic markers, indicated that small anchovy larvae also consume ciliates and flagellates that would not be detected in gut contents analysis. Hence in this application, anchovy larvae were assumed to feed mainly on prey type $j = 2$ ($v_{21} = 1.0$) and partially on the smaller $j = 1$ ($v_{11} = 0.5$).

With regard to juvenile stage, there is a lack of information on diet composition, so it is assumed that juvenile anchovy initially prey on microzooplankton and afterwards they can also exploit mesozooplankton. Thus, a partial vulnerability scheme (preference factor) has been adopted with juveniles exploiting mainly mesozooplankton ($j = 3$) ($v_{32} = 0.5$) and microzooplankton ($j = 2$) ($v_{22} = 0.5$).

Although the diet of adult fish is composed of large mesozooplankton prey items such as big copepods, other organisms such as mysids decapod larvae, fish-eggs and fish-larvae can be also found in their stomachs (Tudela & Palomera, 1997, 1999). In an attempt to represent the feeding behaviour of the adult anchovy, both biting and filter feeding modes were used. Thus, it is assumed that adults exploit big zooplankton ($j = 3$) using biting feeding, and at the same time exploit smaller animals ($j = 2$) through filter feeding with a partial vulnerability ($v = 0.5$).

James & Findlay (1989) noted that filtering may be of importance for adult anchovy whenever there are no large particles or when feeding activity is low. Since Tudela & Palomera (1997) have noted that the feeding behaviour of *E. encrasicolus* is similar to the feeding behaviour of *E. capensis*, it is assumed that below a zooplankton threshold concentration of $z4_{\mathrm{th}} < 0.1\ \mu\mathrm{molNl}^{-1}$, anchovy switches its feeding mode to filter feeding with complete vulnerability (1.0) on smaller microzooplankton. The choice of the threshold mesozooplankton value is chosen in way to avoid severe weight loss (more than 50%).

During the approximately 5 days embryonic stage the anchovy does not feed externally as it is nourished solely by its yolk reserves. As the increase in weight during the embryonic period is trivial, a fixed weight is considered.

Feeding half-saturation constants were calibrated during the implementation of the model in order to acquire correspondence between modelled and observed fish growth. Vulnerability constants were assigned according to the feeding preferences of anchovy's life stages described above. Values of vulnerabilities and half-saturation constants are presented in Table 2.

Since zooplankton densities in the LTL model are calculated in $\mathrm{mol\ Nl}^{-1}$ they were converted to $\mathrm{g\ wet\ weight\ m}^{-3}$ (in order to be compatible with the fish model energy rates) by multiplying with the term:

$$\frac{14\mathrm{g\ N}}{\mathrm{mol\ N}}\,\frac{1\ \mathrm{g\ dry\ weight}}{0.07\ \mathrm{gN\ dry\ weight}}\,\frac{1\ \mathrm{g\ wet\ weight}}{0.2\ \mathrm{g\ dry\ weight}}\,\frac{10^3\ \mathrm{l}}{\mathrm{m}^3} = 10^{-6}\ \mathrm{g\ wet\ weight\ m}^{-3}.$$

Respiration

Respiration rate is modelled as an allometric function of body weight, water temperature and fish activity level:

$$R = a_{\mathrm{r}} W^{b_{\mathrm{r}}} f_{\mathrm{R}}(T) A, \tag{7}$$

where a_{r} is the standard (with no motion) respiration rate of a 1 g anchovy at the optimal temperature for respiration and b_{r} is the exponent for the weight dependence of respiration. Since there are no laboratory data for respiration of *E. encrasicolus* the parameters a_{r} and b_{r} used by Oguz et al. (2008) in the

Black Sea were adopted. The f_R (t) is a Q_{10} temperature relationship, $f_R(T) = e^{\frac{\log_e(Q_{10})\,(T-T_m)}{10}}$ with $Q_{10} = 2.22$ for all life stages (Rose et al., 1999) and annual mean depth-based water temperature T_m (set to 18°C for the larval stage at 0–40 m and to 16°C for other stages at 0–100 m of the water column) (Isari et al., 2008).

Term A is an activity parameter accounting for respiration due to increased metabolic costs and in particular swimming. The energetic cost of activity is an exponential function of swimming speed described by

$$A = e^{d_r U}, \tag{8}$$

where U is the swimming speed and d_r is an exponent for swimming speed given by James & Probyn (1989). Swimming speed (U) expressed in units of cm s^{-1}, depends on anchovy weight and temperature by the following equation:

$$U = a_A W^{b_A} e^{(c_A T)}. \tag{9}$$

Taking into account that the mean swimming speed whilst feeding in biting mode is 2.41 times the body length (James & Findlay, 1989) and using the weight–length relationship (3), values a_A, b_A for each life stage are estimated considering that the coefficient for temperature dependence of the swimming speed is zero ($c_A = 0$). When water temperature drops below 12.0°C a single value for a_A and b_A and c_A is adopted for all stages (Table 2).

In addition, *E. encrasicolus* regulates swimming speed according to prey sizes (Van der Lingen et al., 2006). The swimming speed is significantly lower in filter feeding mode (1.69 ± 0.591 body length s^{-1}) than in biting feeding mode since filter feeding energetic costs increase at rising swimming speeds (James & Findlay, 1989). In the model, an average value of 1.69 body length s^{-1} was chosen for the filter feeding mode below the previously defined zooplankton threshold. Sensitivity analysis tests were performed to evaluate the importance of swimming speed assumptions on fish weight.

Egestion

Egestion accounts for the portion of the energy of ingested material that is not assimilated and is released back to the environment as unused faeces (Valiela, 1995). In the model, egestion is modelled as a direct proportion of consumption:

$$F = a_f C, \tag{10}$$

where C is the consumption, a_f is the proportion coefficient of egestion adopted from James et al. (1989). The latter authors estimated that 12.62% of the nitrogen (N) ingested from a zooplanktonic diet is not assimilated. In general, the assimilation efficiency is lower in larval clupeoids than in juveniles and adults due to immaturity of the digestive system (Blaxter & Hunter, 1982). For this reason, a slightly increased value for the larval stage was used, $a_f = 0.15$.

Specific dynamic action

SDA is a component of the total respiration, defined as the energy cost of assimilation and storage of products of digestion, in the deamination of amino acids and for the synthesis of nitrogenous excretory products (Tytler & Calow, 1985). SDA is considered as a constant proportion of assimilated energy

$$\mathrm{SDA} = a_{sda}(C - F), \tag{11}$$

where a_{sda} is the SDA coefficient, C is the consumption rate and F is the egestion rate. A single value for all stages was adopted from Luo & Brandt (1993).

Excretion

Excretion (E) is a part of the energy budget that represents the elimination of nitrogenous products such as urea and uric acid (Valiela, 1995) and is described by a linear relationship related to the assimilated ration (James et al., 1989)

$$E = a_e(C - F) + b_e \tag{12}$$

The units of E adopted by James et al. (1989) are mg N g-fish^{-1} dry wt^{-1} day^{-1} with parameter values $a_e = 0.415$, $b_e = 0.475$. Assuming that 1 mg wet weight of zooplankton contains 0.015 mg N (Gorsky et al., 1988) and considering that dry weight of anchovy is 32.01% of wet weight (Tudela & Palomera, 1999), excretion (E) can be converted to the current model units (g zooplankton g fish^{-1} day^{-1}) with parameters: $a_e = 0.415$, $b_e = 0.01$. For the conversion the following term is applied:

$$\frac{\text{mg N}}{\text{g fish dry wt} \cdot \text{day}} = \frac{66.66\,\text{mg} - \text{zooplankton}}{3.124\,\text{g -fish wet wt} \cdot \text{day}} \cdot \frac{1\,\text{g}}{1,000\,\text{mg}} = 0.021\,\text{g} - \text{zooplankton g} - \text{fish}^{-1}\text{wet weight day}^{-1}.$$

Egg production

The spawning of anchovy in the northern Aegean Sea starts in May, peaks in June and gradually decreases to September (Somarakis et al., 2006). Estimates of spawning frequency (the ratio of spawning females to all mature females) in June ranged from 0.34 to 0.44 (Somarakis et al., 2007), implying that reproduction occurs every 2–3 days, on average. According to the monthly evolution of the gonadosomatic index of anchovy in the Aegean Sea (Somarakis et al., 2006), it is assumed that spawning frequency is normally distributed, with spawning taking place every 4–5 days in July and almost every 5–6 days in May and August. This gives an estimated total number of 28 annual egg batches. It is also assumed that age 1 fish group starts to spawn later than older fish as they are not fully mature in May. Thus, 22 was set as the number of annual egg batches for age-1 class and 28 for age-2 and age-3 classes. Anchovy is considered to be an income breeder, which means that energy allocated to reproduction comes primarily from food intake, rather than from energy reserves (Somarakis et al., 2004). Therefore, in the bioenergetics model, EGG is assumed to be proportional to the consumption

$$\text{EGG} = a_{\text{egg}}C. \tag{13}$$

The mean wet weight of an anchovy egg was considered 27.7 μg (Somarakis, 1999). The batch fecundity Fec (mean number of eggs per mature female) generally depends on weight by a relationship of the form Fec $= f \cdot W$, where f is the numbers of eggs g female^{-1}. Setting $f = 450$, (Somarakis, 2005), the total daily number of eggs produced by each female becomes, Fec $= 450 \cdot W$.

The total daily weight of eggs, $\text{EGG}_{\text{weight}}$ (g egg day^{-1}) released per day, assuming an average spawning interval of 3.5 days, is

$$\text{EGG}_{\text{weight}} = 7.91\,\mu\text{g} \cdot 450 \cdot W \tag{14}$$

Using the calorie content of egg, $\text{CAL}_{\text{egg}} = 5{,}600$ J g egg^{-1} (Valdés, 1993) and zooplankton CAL_{z}, the EGG is calculated

$$\text{EGG} = 3.56 \cdot 10^{-3}\ \text{g}\frac{\text{CAL}_{\text{egg}}}{\text{CAL}_{\text{z}}} = 0.0077\,\text{g zooplankton g fish}^{-1}\ \text{day}^{-1}$$

Since the daily food ingestion (g-prey day^{-1}) of anchovy in the Mediterranean is estimated to approximately 4% of its wet weight (Plounevez & Champalbert, 2000), the daily consumption is $C = 0.04$ g-zooplankton g-fish^{-1} day^{-1}. Evaluating the ratio EGG to C, the coefficient, $a_{\text{egg}} = 0.19$ is estimated.

Sensitivity analysis

A number of sensitivity analysis experiments involving most of the bioenergetics model parameters were performed whilst the relative importance of input parameters to the model's performance was evaluated by using a traditional one-parameter-at-a-time analysis. Each parameter was separately varied ±10% and the simulation was rerun with one of the parameters changed whilst the others were kept at their nominal values. Parameters, such as optimum and maximum temperatures for consumption and respiration were varied by ±1°C. Following, the change in model output between the standard simulation and the sensitivity outcome indicated the importance of each parameter.

More precisely, as a sensitivity measure, a sensitivity index $S_{\text{A}}(p_{\text{s}})$ developed by Kitchell et al. (1997) and subsequently applied in several models (Stewart et al., 1983; Luo & Brandt, 1993) was used. The aim was to relate a change in an input parameter to a change in the growth output, as calculated by the model, subject to the new parameter value:

$$S_{\text{A}}(p_{\text{s}}) = \frac{\Delta A}{A_{\text{s}}} \cdot \frac{p_{\text{s}}}{\Delta p},$$

where p_{s} and Δp are the nominal and input deviation of parameter values, whilst A_{s} and ΔA are the nominal and output deviation response measure due to Δp. $S_A(p_s)$ was calculated as the normalized change in the output of the response measure for each change made in a model input parameter. If S is zero, A does not change with parameter p; if the parameter index is $S(A, p) = 1$ it means that a 10% change in parameter p_s causes a 10% increase in the output response, whilst if $S(A, p) = -1$, then a 10% change in p_s causes a 10% decrease in A. Simulated weight with $|S_A(p_s)| > 1$ demonstrates a high sensitivity to the parameter change, whereas values of $|S_A(p_s)| < 1$ indicate moderate to low sensitivities.

Design and analysis of simulations

In this application, the HTL model was one-way linked to the LTL. The averaged over the study area of Thracean Sea zooplankton biomasses simulated by the 3D POM–ERSEM model were used as forcing prey biomass for the fish model. The absence of high frequency temporal and spatial in situ data on biotic parameters does not allow a detailed validation of the model. However, measured values of key parameters during MATER (Mass Transfer and Ecosystem Response) and ANREC (Association of Physical and Biological processes acting on Recruitment and post-Recruitment of Anchovy) projects were compared against model results (Table 3). Considering the significant model uncertainties related to river and BSW nutrient inputs (climatological inputs are adopted) as well as the important horizontal variability characterizing the North Aegean area, results are reasonably close to measured values, particularly for lower heterotrophs and mesozooplankton.

LTL model results exhibit a phytoplankton spring bloom (not shown) with chlorophyll values around 0.27–0.3 mg m^{-3}. Since the ERSEM model does not directly parameterize migration of zooplankton, the vertical distribution of the zooplanktonic functional groups is related to food availability (Fig. 4). Thus, at the surface layers during spring when nutrients start becoming available small phytoplankton due to their favourable size, are the first to bloom initiating a parallel response from their predators (Fig. 5). As nutrients become more and more available and with small phytoplankton being controlled by predation, larger cells take over. Considering the wide difference between prey–predator growth rates for large phytoplankton–zooplankton there is a significant time lag and as a result the bloom continues. Eventually, the large zooplanktonic predators benefiting from the available food sources start to control both autotrophic and heterotrophic production.

As nutrients are used up and the system moves into summer with a stratified water column, primary production is confined close to the thermocline (50 m, see Fig. 5) where available light is combined with deep water nutrients. A similar pattern is exhibited by bacteria which are rather strong competitors to phytoplankton over nutrients.

As mentioned before, since top down control is not taken into account by this modelling effort, the phytoplankton downward transport is also followed by all zooplankton groups. Considering that anchovy filter-feeds at the surface layers and exhibits particulate

Table 3 N. Aegean in situ parameters against model simulated monthly mean values, averaged over the sampling areas

MATER Siokou-Frangou et al. (2002)	March 1997		September 1997	
	In situ	Model	In situ	Model
Phyto (mg C/m^2)	1,621 ± 426	2,605	1,072 ± 227	853
Bacteria (mg C/m^2)	1,406 ± 327	984	1,471 ± 273	810
Mesozoo (mg C/m^2)	424 ± 165	508	207 ± 38	266
Heterotrophs (mg C/m^2)	1,847 ± 364	1,931	2,127 ± 225	2,054
PP (mg C/m^2/day)	1,406 ± 362	816	253 ± 70	190
BP (mg C/m^2/day)	48 ± 31	206	60 ± 11	90

ANREC Isari et al. (2006, 2007)	July 2003		September 2003		July 2004	
	In situ	Model	In situ	Model	In situ	Model
Mesozooplankton (mg C/m^3)	3.44	4.50	1.90	3.06	5.60	3.80

The ANREC (Isari et al., 2006, 2007) sampling area almost matches the Thracean Sea area, indicated in Fig. 1, whilst MATER sampling stations (indicated as NA in Siokou-Frangou et al., 2002, Fig. 1) cover a much wider area

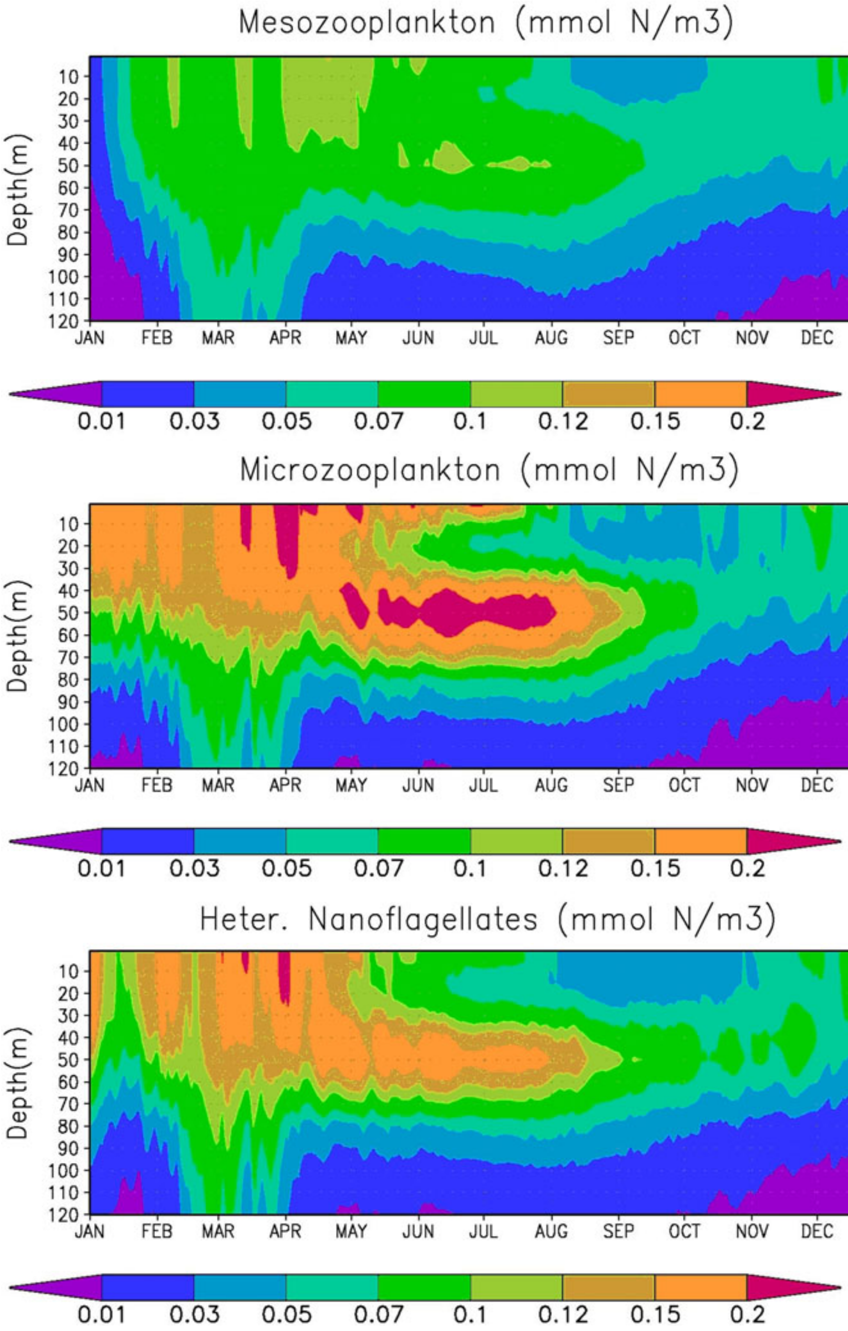


Fig. 4 Seasonal yearly variation of the zooplankton group biomasses derived from the 3D-LTL ecosystem model for depths 0–120 m, averaged over the Thracean Sea box area (as indicated in Fig. 1); mesozooplankton concentration, microzooplankton concentration and heterotrophic flagellates concentration

feeding at the thermocline, one would probably expect a vertical differentiation of zooplankton. As large animals cannot be taken up by filter feeding and smaller ones exhibit particulate feeding, they should be distributed at the surface layer and thermocline, respectively.

LTL-fish model linking assumptions

Anchovy experiences different depth histories throughout its life time according to its diurnal vertical migration pattern, the prey availability and the surrounding environment. Different fixed depth

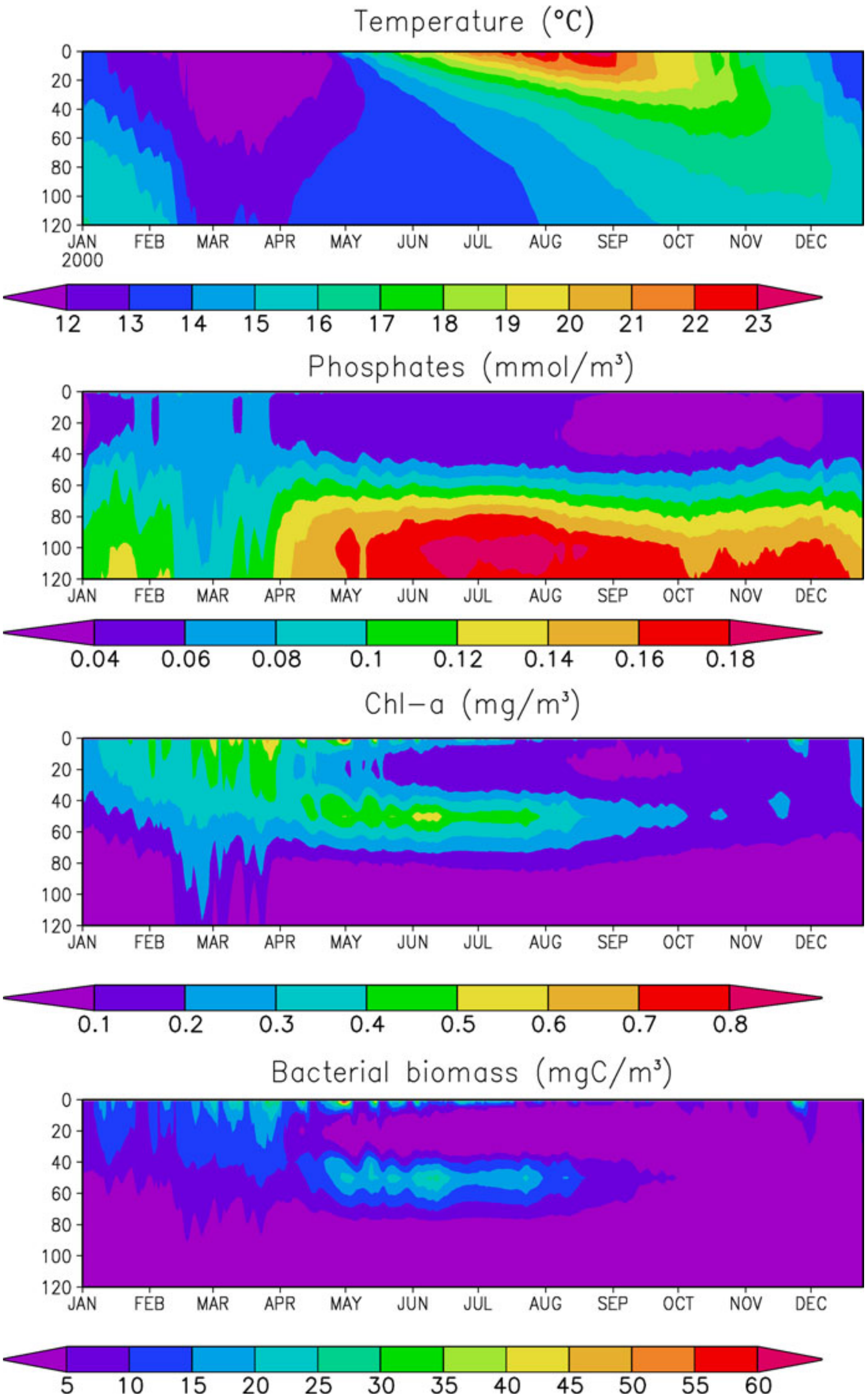


Fig. 5 Seasonal yearly variation of selected variables derived from the LTL ecosystem model for depths 0–120 m; water temperature (°C), phosphate concentrations, Chl-*a* concentrations and bacterial concentrations

intervals in the water column were adopted for each life stage, following known anchovy habitat conditions. The maximum zooplankton concentration within this depth interval and the corresponding mean temperature is then taken from the LTL model, assuming that anchovy prefer areas with maximum food availability. Since, the maximum value of each zooplankton type can be found in different depths during the day, fish may experience varying temperatures. Thus, a mean daily temperature value of the water layers that the fish can visit according to its life stage was considered.

Analysing the habitat conditions for each life stage, as spawning takes place during night at the upper layers, anchovy larvae are generally surface distributed and restricted above the thermocline (0–20 m) (Palomera et al., 2007; Somarakis & Nikolioudakis, 2007). Thus, for the larval stage, zooplankton and water temperature values from the 0 to 20 m water layer are provided following the prescribed rationale. The available food from 20 to 40 m has been supplied to the juvenile stage based on the hypothesis that juveniles usually concentrate in shallow waters (mostly <50 m) inhabiting depths around the thermocline when waters are thermally stratified (ANREC, 2006; Tirelli et al., 2006). Adult anchovies accomplish diurnal vertical migrations, staying below the thermocline during daytime whereas during the night are scattered in the upper water column layers (Plounevez & Champalbert, 2000; ANREC, 2006). Therefore, for the adult stage, prey availability and water temperature values are used from the 35 to 80 m layer during the day and from the 20 to 40 m layer during night to force the fish model. At the sensitivity analysis section, multiple simulations with different depth histories have been conducted to investigate a possible optimal trajectory for fish weight.

Model results

The simulated anchovy weight and length as fit to the mean weight and length estimates (○, ×) of age-1, age-2 and age-3 classes are shown in Fig. 6. Starting the model run at the 1st of June, model results show a moderate increase for age-1 class wet weight from June to December where anchovy passes through larval and juvenile stage (Fig. 6, upper panel). Pre-adult anchovy after a continuous growth from January to June reaches about 7.63 g weight and 107 mm length with the completion of the first year of life. At the start of age-2 class, fish manages to maintain its weight during the 3 month spawning season from June to September. After that, anchovy

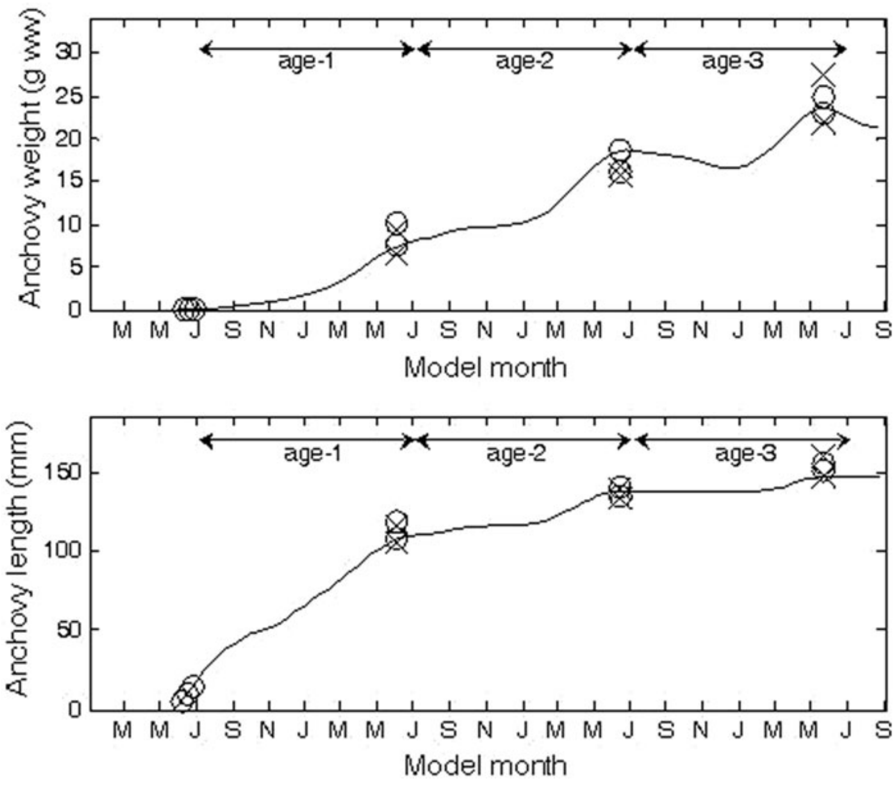


Fig. 6 Simulated anchovy weight (*upper panel*) and length (*lower panel*) of anchovy, as fit to available weight-at-age and length-at-age estimates (O) (Machias et al., 2000; ANREC, 2006; Somarakis & Nikolioudakis, 2007). For further comparisons, observations (X) from other Mediterranean areas (western and central Mediterranean) are also shown (Pertierra et al. 1997; Basilone, 2004, 2006)

Fig. 7 Monthly variation of the mean growth rates (g ww/day)

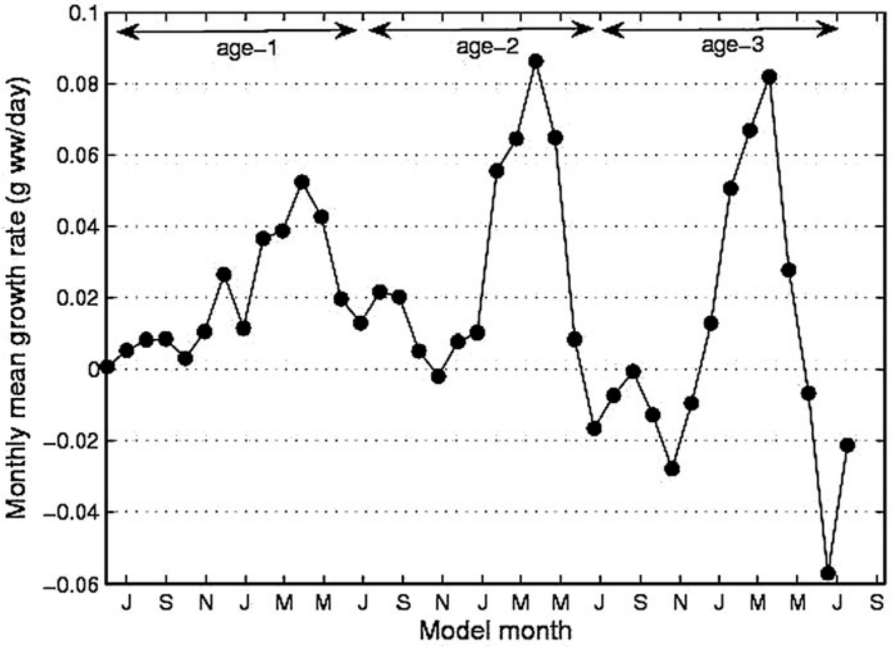


Table 4 Mean values for two seasonal periods for zooplankton groups as calculated from the zooplankton profile of Fig. 4

	Mean values for March–July	Mean values for August–December
Mesozooplankton	0.084	0.049
Microzooplankton	0.138	0.068
Heter. flagellates	0.118	0.054

The averaged values were estimated from depth 0 to 80 m

exhibits a slow growth rate (0.0062 g ww/day^{-1}) from August to December (Fig. 7). In spring bloom season, anchovy grow again rapidly. At the end of the second year, anchovy weight is 18.6 g and its corresponding length is 137.4 mm. The age-3 class is characterized by a negative average growth rates (Fig. 7) which occurs mainly after the end of the second spawning activity period from August to December causing a 7.62% reduction on weight. On the contrary, anchovy weight follows a rapid average growth rate (0.05073 g ww/day^{-1}, Fig. 7) in spring where fish is reaching the weight of 23.84 g and length 147.3 mm at the end of third year. Model run indicates that anchovy weight for age-3 fish increased 25.83% for the period of March–June. The corresponding mean monthly growth rates for this period are increased 0.05–0.08 (Fig. 7).

The variation of mean values for the three zooplankton groups during March–July and September–January periods are shown at Table 4. We point out the 41.7% reduction of mesozooplankton and 50.7% of microzooplankton values during August–December period compared to March–July period.

Maximum growth rates are realized during spring when zooplankton densities are highest. On the other hand, the decrease of zooplankton levels from August to December (Fig. 4, Table 4) induces a similar decrease in the anchovy growth rate that is followed by a decrease in the fish weight. Juvenile weight remains almost static during September–October, whereas in older fish (especially in age-2+ and age-3+ class), weight starts to decrease immediately after the end of the summer spawning season. These results can be attributed to the low zooplankton abundance levels influencing anchovy consumption rates. Activity respiration is an important component of the model, increasing the energy demands for the older ages especially during summer–autumn warmer periods.

When analysing model results, a prominent characteristic is that the most significant term in the bioenergetics model is consumption which is closely coupled to zooplankton density, following its variation. Thus, highest consumption levels are reached

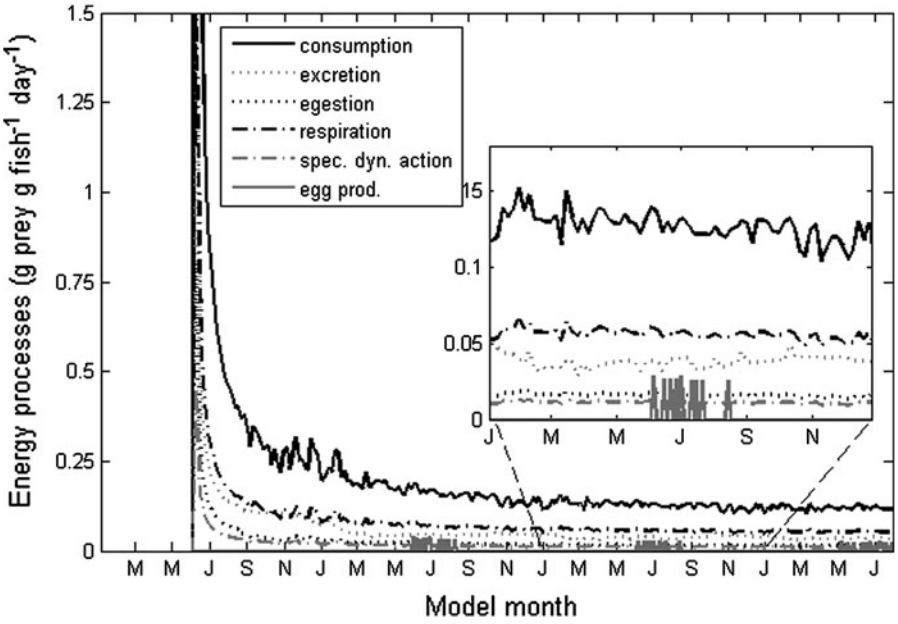


Fig. 8 Simulated energy processes: consumption (*black-solid*), respiration (*black, dash-dot*), excretion (*grey-dot*), egestion (*black-dot*), specific dynamic action (*grey, dash-dot*) and egg production (*grey-solid*)

during spring and are reduced from August to December (Fig. 8).

In addition, temperature also plays an important role in consumption especially during winter where anchovy growth rates were often negative at the highest water temperatures, increasing both consumption and respiration processes (there is a greater effect on the latter).

The model consumption rate (Fig. 8, black line) can be expressed as percentage of anchovy weight increase per day by multiplying to CAL_z/CAL_f ratio (Eq. 2). This gives an annual mean daily consumption rate of 6% for the adult anchovy which lies in reasonable agreement with the estimates (3.6–3.9%) of Plounevez & Champalbert (2000) for daytime consumption.

Sensitivity results

The sensitivity measure $S_A(p_s)$ at 1st of June for the yearly weight outputs age-1, age-2 and age-3 classes were calculated by varying each parameter with ±10%, as mentioned above (Table 4). Temperature inputs of ±1°C for consumption and respiration were changed separately for each life stage.

The intercept for maximum consumption (a_c), the standard respiration (a_r), the exponents for weight dependence of consumption and respiration (b_c) and (b_r) and the proportion of excretion (a_e) had the highest overall sensitivities. As shown in Table 5, variations of the above parameters result in a net increase of ~5 g fish weight. On the other hand, the proportion of flagellates ($z6_{var}$), the intercept a_A (larval) and proportion of energy allocated to reproduction (a_{egg}) displayed the lowest sensitivities (Table 5). A general feature common to the majority of simulations is that the maximum sensitivities for various parameters were noted for older ages. High priority should be given to better estimates of the most sensitive parameter for future improvements in the model.

Moderate sensitivities of the $z4_{var}$ and $z5_{var}$ parameters indicate that microzooplankton and mesozooplankton changes of 10% are low and do not have a significant effect on anchovy weight. However, the sensitivities of prey density depend on the choice of the value of the half-saturation constants. Sensitivity of zooplankton caloric content affected the annual growth by 12% for young age-1. Also, it is important to note that the adjusted half-saturation parameters exhibited a high sensitivity measure on yearly anchovy growth.

Evaluation of the temperature parameters T_{opt}, T_{max} and T_r sensitivities on fish growth, showed that T_{opt} and T_r in the adult stage exhibited the highest sensitivity on age-2 and age-3 (Table 6). In general,

Table 5 Summary of sensitivity measures $S_A(p_s)$ for model parameters used to simulate yearly weight (1st of June) of anchovy for age-1, age-2 and age-3 classes

Parameters	Weight (age-1)		Weight (age-2)		Weight (age-3)	
	+10%	−10%	+10%	−10%	+10%	−10%
a_c	5.78	−4.31	7.33	−5.68	7.49	−6.02
b_c	−0.48	0.41	−3.10	5.53	−3.96	7.86
Q_c	−0.60	0.67	−0.49	0.65	−0.47	0.64
a_r	−2.91	7.71	−3.96	10.91	−4.26	11.40
b_r	−0.09	−0.33	2.53	−3.04	3.81	−3.96
Q_r	0.18	−0.25	0.26	0.90	0.28	−0.34
a_f	−0.06	0.09	0.01	0.05	0.03	0.03
d_r	−0.90	1.07	−1.77	2.25	−2.11	2.83
c_A	−0.12	0.12	−0.13	0.16	−0.15	0.17
$z4_{var}$	0.67	−0.69	0.09	−0.17	−0.10	0.01
$z5_{var}$	0.33	−0.23	0.56	−0.48	0.60	−0.55
$z6_{var}$	0.15	−0.11	0.06	0.00	0.04	0.02
a_{sda}	−0.93	1.16	−1.22	1.41	−1.28	1.46
a_e	−3.00	4.11	−3.87	5.30	−4.07	5.54
b_e	−0.49	0.52	−0.93	1.05	−1.11	1.27
a_{egg}	0.00	0.00	−0.09	0.09	−0.16	0.16
a_A (larval)	−0.05	0.03	−0.01	0.01	0.00	0.00
a_A (juvenile)	−0.84	0.92	−0.20	0.22	−0.06	0.06
a_A (adult)	0.00	0.00	−0.87	0.93	−1.15	1.28
CAL_z	1.14	−1.13	0.66	−0.73	0.30	−0.37

The model was run and parameter values were changed by ±10% of their nominal values. Parameter values are defined in Table 2

$z4_{var}$ proportion of mesozooplankton variation z4, $z5_{var}$ proportion of microzooplankton variation z5, $z6_{var}$ proportion of flagellates variation z6, a_A *(larval)* intercept of swimming speed for larval stage (particulate feeding), a_A *(juvenile)* intercept of swimming speed for juvenile stage particulate feeding), a_A *(adult)* intercept of swimming speed for adult stage (particulate feeding), CAL_z energy density of zooplankton

Table 6 Estimation of sensitivity measure $S_A(p_s)$ for temperature-related parameters on yearly end-point of anchovy weight (1st June) for age-1, age-2 and age-3 classes

Parameter	Weight (age-1)		Weight (age-2)		Weight (age-3)	
	+1°C	−1°C	+1°C	−1°C	+1°C	−1°C
T_{opt} (larval)	−0.24	0.30	−0.05	0.09	−0.02	0.02
T_{opt} (juvenile)	−2.24	1.56	−0.56	0.37	−0.15	0.11
T_{opt} (adult)	0.00	0.00	−2.19	1.37	−2.65	1.67
T_r (larval)	0.37	−0.24	0.10	−0.04	0.03	−0.01
T_r (juvenile)	4.20	−3.67	0.96	−0.95	0.25	−0.27
T_r (adult)	0.00	0.00	5.09	−4.34	5.63	−5.29
T_{max} (larval)	0.02	−0.03	0.03	0.01	0.01	0.01
T_{max} (juvenile)	0.02	−0.03	0.02	0.01	0.01	0.01
T_{max} (adult)	0.02	−0.03	0.02	0.01	0.01	0.01

The model was run with parameter values changed by ±1°C of the nominal value. The optimal and maximum temperature sensitivities of consumption and respiration processes are combined with larval, juvenile and adult stage

temperature parameters (T_{max}) had a low sensitivity measure for changes of ±1°C.

Sensitivity results not only provided significant information on the relative importance of the various parameters on the models performance, but also illustrated the need for experimental data, especially focusing on the optimal temperature parameter for juveniles and adults.

The importance of swimming speed and feeding behaviour alterations of adult stage during the low feeding period were examined by performing two more experiments. In the first experiment, it was assumed that the vulnerability on microzooplankton z5 remained partial ($v_{23} = 0.5$) for all periods of growth. This assumption caused an extra reduction on anchovy weight up to 22% for older age classes. In the second experiment, holding the filter feeding related swimming speed to levels associated with the biting feeding mode resulted in an annual fish growth decrease of up to ~6 g fish (37%).

Finally, several simulations were conducted with different depth histories for the adult stage only, as larval and juvenile stage depth histories indicated no significant sensitivities. In this sensitivity test, the aim is to investigate how the depth-based habitat selection of anchovy affects its weight. Thus, prey availability and water temperature values were provided to the adult stage from selected depth intervals following the diurnal movements. The multiple weight trajectories for the several depth ranges are shown in Fig. 9. The simulations indicate no significant differentiations for the various trajectories during the period March–July. On the contrary, during the summer–autumn stratified period, weight decreases more rapidly as anchovy shifts to shallower layers (Fig. 9: 5–25 m during night; dashed dotted line). The specific depth selection is associated with food availability but also with warmer temperature values. As primary production and consequently

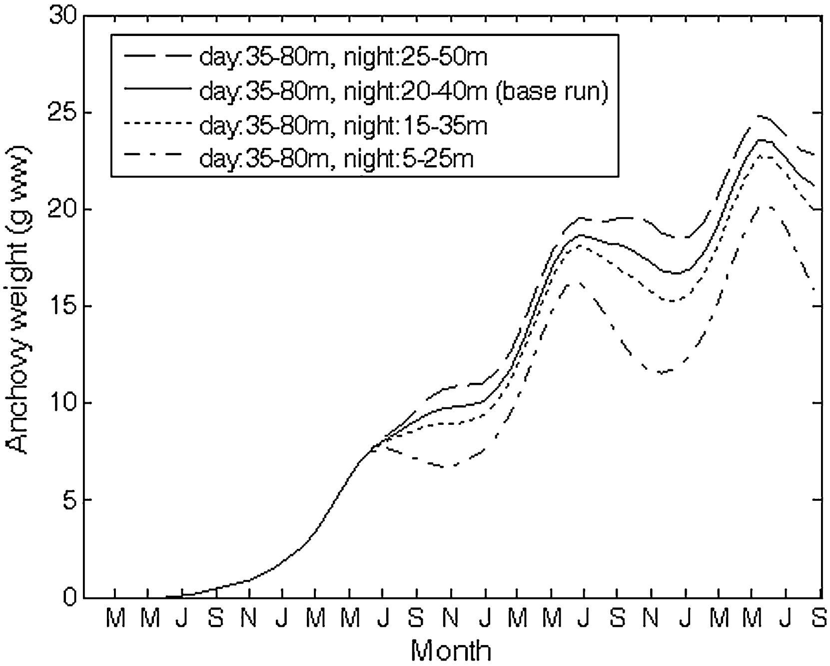


Fig. 9 Weight simulations for selected depth histories during daytime. Following the adult's diurnal movements within its habitat conditions, the different lines represent the ensemble of weight trajectories in association with the base run (*solid line*) after providing the adult stage with zooplankton and water temperature values from the depths indicated in the legend

zooplankton shift to deeper layers (Figs. 4, 5), the trajectory with the deeper depth range (Fig. 9: 25–50 m during night; dashed line) appears as the most favourable concerning anchovy weight. The seasonal variability of temperature also has an impact on fish growth as lower temperature values noticed for 25–50 m during summer–autumn (Fig. 5) have a positive effect on anchovy weight. Regarding the relation of anchovy distribution with hydrographic regimes, Giannoulaki et al. (2005) indicated a higher probability of occurrence for anchovy in deeper surface and upper mixed layers showing no especial selection for warm waters. This argument seems to coincide with our model behaviour. However, more experiments are required to support these assumptions.

Discussion

The northern Aegean Sea is an important area with great scientific interest attracting studies on oceanography, ecology and fisheries. In this article, a bioenergetic model was one-way linked with the LTL ecosystem model (POM–ERSEM) integrating biological information of anchovy with environmental factors for the northern Aegean area. The bioenergetics model has successfully simulated the weight of European anchovy indicating a seasonal evolution with stationary or negative variation mainly during autumn. Seasonality in weight and length has also been noticed for anchovy in an effort to link the fish resources with environmental conditions in the Bay of Biscay (Pecquerie et al., 2009) and Black sea (Oguz et al., 2008). The spawning activity of anchovy in Aegean Sea did not cause a negative growth rate on anchovy weight, implying that the increased zooplankton levels from March to August in the Aegean sea, which coincide with the spawning period, are adequate to support the reproduction energy demands. In case of age-2 and age-3 classes, fish weight continued to increase during reproduction period although the growth rates were smaller (Fig. 4). This seems to be in consistence with anchovy's reproduction strategy to spawn during the period of maximum prey production (Somarakis et al., 2004). In addition, Wang & Houde (1994) showed that *Anchoa mitcilli* mean weight increased during the spawning season by 26%. Another notable feature from the model's sensitivity performance is the importance of the two feeding modes. The alternations from bite to particulate feeding with a parallel reduction in swimming speed during low prey levels, possibly is an adjustive behaviour of anchovy in order to maximize energy gains and avoid starvation. However, direct observations of such fish behaviour are particularly difficult. James & Findlay (1989) indicated a lower swimming speed of Cape anchovy during filter feeding compared to bite-feeding, which is consistent with our model results. However, similar studies on other species show either lower (Batty et al., 1990) or higher (Pepin et al., 1988; Gibson & Ezzi, 1992) swimming speed during filter feeding.

From the overall study of the model, we note that the biomass of zooplankton prey is a dominant factor in the anchovy bioenergetics' response. Simulated weight showed fastest growth rates during spring and slower growth during autumn following the variation of zooplankton prey availability. In addition, growth was fairly sensitive to temperature-dependent parameters. In the model, the caloric content of zooplankton was assumed to be fixed and the caloric content of anchovy was assumed to depend only on the life stage. However, in natural conditions, seasonal variation of the caloric content of both fish and zooplankton may play an important role in influencing fish weight (Stewart & Binkowski, 1986).

It must be noted that estimates of mean size-at-age in the Mediterranean (including the western and central Mediterranean, see Fig. 6) are relatively higher than similar values available from the Black Sea (Oguz et al., 2008 and references therein). This can be explained in terms of genetic differences (the Black Sea anchovy stock is genetically distinct from Mediterranean stocks, e.g. Magoulas et al., 1996) and lower sizes-at-maturity of the Black Sea anchovy. It has been reported that young-of-the year, 55–65 cm in length, and 1.2–2.4 g in weight, i.e. two–three months old anchovy, may spawn towards the end of the spawning period in the Black Sea (Lisovenko & Andrianov, 1996). In contrast, in most areas of the Mediterranean, anchovy matures at around 11 cm, with the completion of the first year of life (Somarakis et al., 2004).

Due to the lack of adequate field observations and laboratory experiments for the European anchovy in the Mediterranean, a number of model parameters concerning energy processes were adopted from related species. Although significant effort was directed to minimize this, it is possible that uncertainties were introduced into the model results by these assumptions. New research is needed on those parameters that have major effects on fish growth as indicated from the sensitivity analysis. This will help to increase the reliability of the existing models and to the development of more complex simulation tools. Another important consideration in this study is that the half-saturation constants were adjusted in order to fit model results to observed values. Therefore, independent data is needed to completely validate the model without reliance on the observed wet weight values. Particularly, data on observed consumption rate would significantly add to the model consistency. As mentioned above pinpointing knowledge gaps, hopefully will contribute towards targeted future research (field and laboratory) which will ultimately help the development of more robust models.

Model set up was also exposed to the uncertainty concerning the linkage of the LTL to the fish model. Due to its one-way link, the zooplankton biomasses are not reduced by anchovy predation, so there was no feedback into the dynamics of ERSEM. The importance of two-way linkage has been emphasized as a necessary step towards a dynamic interaction of the ecosystem components (Travers et al., 2007). For this reason, a study to include the perspective of a two-way coupling with the LTL model is the main priority for future work.

The implementation of the anchovy model demonstrates that NEMURO.FISH type bioenergetic models are sufficiently general to be considered as useful tools for the understanding fish growth in relation to environmental parameters and LTL forcing. The present work has to be considered as the beginning for the development of a broader model scheme that describes the full life cycle of anchovy in the three-dimensional space. The incorporation of a population dynamic model by including reproduction and mortality (natural and fishing) processes and the link with an IBM model in the framework of a spatially explicit 3D scheme will allow simulations of the entire anchovy stock providing a useful tool to examine the impact of climate change on the HTLs of the marine ecosystems (Megrey et al., 2007; Travers et al., 2007). This work is in progress and will be published elsewhere.

Acknowledgments We would like to express our appreciation to Dr. A. Machias and Dr. M. Giannoulaki for providing important information on the biology of anchovy. The authors are thankful to two anonymous reviewers for their careful reading of our article. This research was supported by the SESAME project, EC Contract No GOCE-036949, funded by the European Commission's Sixth Framework Programme under the priority 'Sustainable Development, Global Change and Ecosystems'. S. Ito's participation in this research was supported by SUPRFISH Project and Global Warming Project of Ministry of Agriculture, Forestry and Fisheries Japan. B. A. Megrey's participation in this research is noted as contribution FOCI-0658 to NOAA's Ecosystem and Fisheries-Oceanography Coordinated Investigations.

References

ANREC, 2006. Association of Physical and Biological Processes Acting on Recruitment and Post-Recruitment Stages of Anchovy. Report No. QLRT-2001-01216.

Bakun, A., 1996. Patterns in the Ocean: Ocean Processes and Marine Population Dynamics. California Sea Grant College System, Mexico.

Baretta, J. W., W. Ebenhoh & P. Ruardij, 1995. The European Regional Seas Ecosystem Model, a complex marine ecosystem model. Netherlands Journal of Sea Research 33: 233–246.

Basilone, G., 2004. Linking habitat conditions and growth in the European anchovy (*Engraulis encrasicolus*). Fisheries Research 68: 9–19.

Basilone, G., 2006. Effect of habitat conditions on reproduction of the European anchovy (*Engraulis encrasicolus*) in the Strait of Sicily. Fisheries Oceangraphy 15: 271–280.

Batty, R. S., J. H. S. Blaxter & J. M. Richard, 1990. Light intensity and the feeding behaviour of herring, *Clupea harengus*. Marine Biology 107: 383–388.

Blaxter, J. H. S. & J. R. Hunter, 1982. The biology of the clupeoid fishes. Advances in Marine Biology 20: 1–223.

Blumberg, A. F. & G. L. Mellor, 1983. Diagnostic and prognostic numerical circulation studies of the South Atlantic Bight. Journal of Geophysical Research 88: 4579–4592.

Bryant, A. D., M. R. Heath, N. Broekhuizen, J. G. Ollason, W. S. C. Gurney & S. P. R. Greenstreet, 1995. Modelling the predation, growth and population dynamics of fish within a spatially-resolved shelf-sea ecosystem model. Netherlands Journal of Sea Research 33(3/4): 407–421.

Conway, D. V. P., S. H. Coombs & C. Smith, 1998. Feeding of anchovy *Engraulis encrasicolus* larvae in the North Western Adriatic Sea in response to changing hydrobiological conditions. Marine Ecology Progress Series 175: 35–49.

Edwards, A. M. & A. Yool, 2000. The role of higher predation in plankton population models. Journal of Plankton Research 22: 1085–1112.

Fuiman, L. A. & R. G. Werner, 2002. Fishery Science: The Unique Contributions of Early Life Stages. Blackwell Science Ltd, Oxford.

Giannoulaki, M., A. Machias, S. Somarakis & N. Tsimenides, 2005. The spatial distribution of anchovy and sardine in the northern Aegean Sea in relation to hydrographic regimes. Belgian Journal of Zoology 135: 151–156.

Giannoulaki, M., V. D. Valavanis, A. Palialexis, K. Tsagarakis, A. Machias, S. Somarakis & C. Papaconstantinou, 2008. Modelling the presence of anchovy *Engraulis encrasicolus* in the Aegean Sea during early summer, based on satellite environmental data. Hydrobiologia 612: 225–240.

Gibson, R. N. & I. A. Ezzi, 1992. The relative profitability of particulate- and filter-feeding in the herring, *Clupea harengus* L. Journal of Fish Biology 40: 577–590.

Gorsky, G., S. Dallot, J. Sardou, R. Fenaux, C. Carre & I. Palazzoli, 1988. C and N composition of some northwestern Mediterranean zooplankton and micronekton species. Journal of Experimental Marine Biology and Ecology 124: 133–144.

Huggett, J., P. Fréon, C. Mullon & P. Penven, 2003. Modelling the transport success of anchovy *Engraulis encrasicolus* eggs and larvae in the southern Benguela: the effect of spatio-temporal spawning patterns. Marine Ecology Progress Series 250: 247–262.

Hunter, J. R. & H. Dorr, 1982. Thresholds for Filter Feeding Northern Anchovy, *Engraulis mordax*. CalCOFI Report No. 23:198-204.

Huse, G. & I. Ellingsen, 2008. Capelin migrations and climate change—a modelling analysis. Climatic Change 87: 177–197.

Isari, S., A. Ramfos, S. Somarakis, C. Koutsikopoulos, A. Kallianiotis & N. Fragopoulu, 2006. Mesozooplankton distribution in relation to hydrology of the Northeastern Aegean Sea, Eastern Mediterranean. Journal of Plankton Research 28: 241–255.

Isari, S., S. Psarra, P. Pitta, P. Mara, M. O. Tomprou, A. Ramfos, S. Somarakis, A. Tsepelides, C. Koutsikopoulos & N. Fragopoulou, 2007. Differential patterns of mesozooplankters' distribution in relation to physical and biological variables of the northeastern Aegean Sea (eastern Mediterranean). Marine Biology 151: 1035–1050.

Isari, S., N. Fragopoulou & S. Somarakis, 2008. Interranual variability in horizontal patterns of larval fish assemblages in the northeastern Aegean Sea (eastern Mediterranean) during early summer. Estuarine, Coastal and Shelf Science 79: 607–619.

Ito, S. I., M. J. Kishi, Y. Kurita, Y. Oozeki, Y. Yamanaka, B. A. Megrey & F. E. Werner, 2004. Initial design for a fish bioenergetics model of Pacific saury coupled to a lower trophic ecosystem model. Fisheries Oceanography 13: 111–124.

James, A. G. & K. P. Findlay, 1989. Effect of particle size and concentration on feeding behaviour, selectivity and rates of food ingestion by the cape anchovy *Engraulis capensis*. Marine Ecology Progress Series 50: 275–294.

James, A. G. & T. Probyn, 1989. The relationship between respiration rate, swimming speed and feeding behaviour in the Cape anchovy *Engraulis capensis* Gilchrist. Journal of Experimental Marine Biology and Ecology 131: 81–100.

James, A. G., T. Probyn & L. J. Seiderer, 1989. Nitrogen excretion and absorption efficiencies of the Cape anchovy *Engraulis capensis* Gilchrist fed upon a variety of plankton diets. Journal of Experimental Marine Biology and Ecology 131: 101–124.

Kitchell, J. F., D. J. Stewart & D. Weininger, 1997. Applications of a bioenergetics model to yellow perch (*Perca flavescens*) and walleye (*Stizostedion vitreum vitreum*).

Journal of the Fisheries Research Board of Canada 34: 1922–1935.

Korres, G., A. Lascaratos, E. Hatziapostolou & P. Katsafados, 2002. Towards an ocean forecasting system for the Aegean Sea. The Global Atmosphere and Ocean System 8(2–3): 191–218.

Kourafalou, V. H. & K. Barbopoulos, 2003. High resolution simulations on the North Aegean Sea seasonal circulation. Annales Geophysicae 21: 251–265.

Kourafalou, V. H. & K. Tsiaras, 2007. A nested circulation model for the North Aegean Sea. Ocean Science 3: 1–16.

Laurence, G. C., 1976. Caloric content of some North Atlantic calanoid copepods. Fisheries Bulletin US 78: 218–220.

Lisovenko, L. A. & D. P. Andrianov, 1996. Reproductive biology of anchovy (*Engraulis encrasicolus ponticus Alexandrov* 1927) in Black Sea. Scientia Marina 60: 209–218.

Lleonart, J. & F. Maynou, 2003. Fish stock assessment in the Mediterranean: state of the art. Scienta Marina 67: 37–49.

Luo, J. & B. S. Brandt, 1993. Bay anchovy *Anchoa mitchilli* production and consumption in mid-Chesapeake Bay based on a bioenergetics model and acoustic measures of fish abundance. Marine Ecology Progress Series 98: 223–236.

Lykousis, V., G. Chronis, A. Tselepides, N. B. Price, A. Theocharis, I. Siokou-Frangou, F. Van Wambeke, R. Donavaro, S. Stavrakakis, G. Duineveld, D. Georgopoulos, L. Ignatiades, E. Souvermezoglou & F. Voutsinou-Taliadourou, 2002. Major outputs of the recent multidisciplinary biogeochemical researches undertaken in the Aegean Sea. Journal of Marine Systems 33–34: 313–334.

Machias, A., S. Somarakis, P. Drakopoulos, A. Magoulas & C. Koutsikopoulos, 2000. Evaluation of the Southern Greek Anchovy Stocks Project 97-0048. Report No. Final Report, Institute of Marine Biology of Crete.

Magoulas, A., N. Tsimenides & E. Zouros, 1996. Mitochondrial DNA phylogeny and the reconstruction of the population history of a species: the case of the European Anchovy (*Engraulis encrasicolus*). Molecular Biology and Evolution 13: 178–190.

Megrey, B. A., K. A. Rose, R. A. Klumb, D. E. Hay, F. E. Werner, D. L. Eslinger & S. L. Smith, 2007. A bioenergetics-based population dynamics model of Pacific herring (*Clupea harengus pallasi*) coupled to a lower trophic level nutrient-phytoplankton-zooplankton model: description, calibration, and sensitivity analysis. Ecological Modelling 202: 144–164.

Mellor, G. L. & T. Yamada, 1982. Development of a turbulence closure model for geophysical fluid problems. Review Geophysics and Space Physics 20: 851–875.

Miller, T. J., 2007. Contribution of individual-based coupled physical–biological models to understanding recruitment in marine fish populations. Marine Ecology Progress Series 347: 127–138.

Nittis, K., L. Perivoliotis, G. Korres, C. Tziavos & I. Thanos, 2006. Operational monitoring and forecasting for marine environmental applications in the Aegean Sea. Environmental Modelling and Software 21: 243–257.

Oguz, T., B. Salihoglu & B. Fach, 2008. A coupled plankton-anchovy population dynamics model assessing nonlinear controls of anchovy and gelatinous biomass in the Black Sea. Marine Ecology Progress Series 369: 229–256.

Okunishi, T., Y. Yamanaka & S. Ito, 2009. A simulation model for Japanese sardine (*Sardinops melanostictus*) migrations in the western North Pacific. Ecological Modelling 220: 462–479.

Palomera, I., M. P. Olivar, J. Salat, A. Sabatis, M. Coll, A. García & B. Morales-Nin, 2007. Small pelagic fish in the NW Mediterranean Sea: an ecological review. Progress in Oceanography 74: 377–396.

Papadopoulos, A., P. Katsafados, G. Kallos & S. Nickovic, 2002. The weather forecasting system for Poseidon—an overview. The Global Atmosphere and Ocean System 8: 219–237.

Pecquerie, L., P. Petitgas & S. Kooijman, 2009. Modelling fish growth and reproduction in the context of the dynamic energy budget theory to predict environmental impact on anchovy spawning duration. Journal of Sea Research 62: 93–105.

Pepin, P., J. A. Koslow & S. Pearre, 1988. Laboratory study of foraging by Atlantic mackerel, *Scomber scombrus*, on natural zooplankton assemblages. Canadian Journal of Fisheries and Aquatic Sciences 45: 879–887.

Pertierra, J. P., J. Lleonart & N. C. H. Lo, 1997. Application of a stage-specific matrix model and length-cohort based analysis to assess the anchovy fishery in Catalan coastal waters (NW Mediterranean Sea). Fisheries Research 30: 127–137.

Petihakis, G., G. Triantafyllou, I. J. Allen, I. Hoteit & C. Dounas, 2002. Modelling the spatial and temporal variability of the Cretan Sea ecosystem. Journal of Marine Systems 36: 173–196.

Petihakis, G., G. Triantafyllou, K. Tsiaras, G. Korres, A. Pollani & I. Hoteit, 2009. Eastern Mediterranean biogeochemical flux model—simulations of the pelagic ecosystem. Ocean Science 5: 29–46.

Plagányi E. E., 2007. Models for an Ecosystem Approach to Fisheries FAO Fisheries Technical Paper. Report No. 477, FAO, Rome.

Plounevez, S. & G. Champalbert, 2000. Diet, feeding behaviour and trophic activity of the anchovy (*Engraulis encrasicolus* L) in the Gulf of Lions (Mediterranean Sea). Oceanologica Acta 23: 175–192.

Regner, S., 1996. Effects of environmental changes on early stages and reproduction of anchovy in the Adriatic Sea. Scientia Marina 60: 167–177.

Rose, K. A., E. S. Rutherford, D. S. McDermot, J. L. Forney & E. L. Miles, 1999. Individual-based model of yellow perch and walleye populations in Oneida Lake. Ecological Monographs 69: 127–154.

Rose, K. A., F. Werner, B. A. Megrey, M. N. Aita, Y. Yamanaka & D. Hay, 2007. Simulated herring growth responses in the Northeastern Pacific to historic temperature and zooplankton conditions generated by the 3-dimensional NEMURO nutrient-phytoplankton-zooplankton model. Ecological Modelling 202: 184–195.

Rossi, S., A. Sabatés, M. Latasa & E. Reyes, 2006. Lipid biomarkers and trophic linkages between phytoplankton, zooplankton and anchovy (*Engraulis encrasicolus*) larvae in the NW Mediterranean. Journal of Plankton Research 28: 551–562.

Siokou-Frangou, I., M. Bianchi, U. Christaki, E. D. Christou, A. Giannakourou, O. Gotsis, L. Ignatiades, K. Pagou, P.

Pitta, S. Psarra, E. Souvermezoglou, F. Van Wambeke & V. Zervakis, 2002. Carbon flow in the planktonic food web along a gradient of oligotrophy in the Aegean Sea (Mediterranean Sea). Journal of Marine Systems 33–34: 335–353.

Somarakis, S., 1999. Ichthyoplankton of the Northeastern Aegean Sea with Emphasis on Anchovy E*ngraulis encrasicolus* (Linnaeus, 1758) (June 1993, 1994, 1995, 1996). University of Crete (in Greek with English Abstract).

Somarakis, S., 2005. Marked interannual differences in reproductive parameters and daily egg production of anchovy in the northern Aegean Sea. Belgian Journal of Zoology 135: 247–252.

Somarakis, S. & N. Nikolioudakis, 2007. Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolus*) in the northern Aegean Sea (eastern Mediterranean). Marine Biology 152: 1143–1158.

Somarakis, S., I. Palomera, A. Garcia, L. Quintanilla, C. Koutsikopoulos, U. Andrés & L. Motos, 2004. Daily egg production of anchovy in European waters. ICES Journal of Marine Science 61: 944–958.

Somarakis, S., D. E. Tsianis, A. Machias & K. I. Stergiou, 2006. An overview of biological data related to anchovy and sardine stocks in Greek waters. In Palomares, M. L. D., K. I. Stergiou & D. Pauly (eds), Fishes in Database and Ecosystems Fisheries Centre Research Reports 14. Fisheries Centre, University of British Columbia: 56–64.

Somarakis, S., A. Machias, M. Giannoulaki, A. Siapatis, M. Torre, K. Anastasopoulou, V. Vassilopoulou, A. Kalianiotis & C. Papaconstantinou, 2007. Icthyoplanktonic and acoustic biomass estimates of anchovy in the Aegean Sea (June 2003, 2004, 2005, 2006). Working Document Presented in the Working group on Small Pelagic Species Sub-Committee on Stock Assessment, GMCM Athens, Greece (http://wwwicmcsices/rec/projects/scsa/).

Stewart, D. J. & F. P. Binkowski, 1986. Dynamics of consumption and food conversion by Lake Michigan alewives: an energetics-modeling synthesis. Transactions of the American Fisheries Society 115: 643–651.

Stewart, D. J., D. Weininger, D. V. Rottiers & T. A. Edsall, 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. Canadian Journal of Fish and Aquatic Science 40: 681–698.

Tirelli, V., D. Borme, F. Tulli, M. Cigar, S. F. Umani & S. B. Brandt, 2006. Energy density of anchovy *Engraulis encrasicolus* L in the Adriatic Sea. Journal of Fish Biology 68: 982–989.

Travers, M., Y.-J. Shin, S. Jennings & P. Cury, 2007. Towards end-to-end models for investigating the effects of climate and fishing in marine ecosystems. Progress in Oceanography 75: 751–770.

Triantafyllou, G., G. Korres, I. Hoteit, G. Petihakis & A. C. Banks, 2007. Assimilation of ocean colour data into a biochemical flux model of the Eastern Mediterranean Sea. Ocean Science 3(3): 397–410.

Tudela, S. & I. Palomera, 1997. Trophic ecology of the European anchovy *Engraulis encrasicolus* in the Catalan Sea (northwest Mediterranean). Marine Ecology Progress Series 160: 121–134.

Tudela, S. & I. Palomera, 1999. Potential effect of an anchovy-mediated pump on the vertical availability of nitrogen for primary production in the Catalan Sea (northwest Mediterranean). Journal of Sea Research 42: 83–92.

Tudela, S., I. Palomera & G. Quilez, 2002. Feeding of anchovy *Engraulis encrasicolus* larvae in the northwest Mediterranean. Journal of Marine Biological Association of the UK 82: 349–350.

Tytler, P. & P. Calow, 1985. Fish Energetics: New Perspectives. Johns Hopkins University Press, Baltimore.

Ursin, E., 1967. A mathematical model of some aspects of fish growth, respiration and mortality. Journal of the Fisheries Research Board of Canada 24: 2355–2453.

Valdés, E. S., 1993. The energetics and evolution of intraspecific predation (egg cannibalism) in the anchovy *Engraulis capensis*. Marine Biology 115: 301–308.

Valiela, I., 1995. Marine Ecological Processes, 2nd ed. Springer, New York: 686.

Van der Lingen, C. D., L. Hutchings & J. G. Field, 2006. Comparative trophodynamics of anchovy *Engraulis encrasicolus* and sardine *Sardinops sagax* in the southern Benguela: are species alternations between small pelagic fish trophodynamically mediated? African Journal of Marine Science 28: 465–477.

Vasquez, A. V., 1989. Energetics, trophic relationships and chemical composition of bay anchovy, *Anchoa mitchilli*, in the Chesapeake Bay. University of Maryland, College Park.

Wang, S. B. & E. D. Houde, 1994. Energy storage and dynamics in bay anchovy *Anchoa mitchilli*. Marine Biology 121: 219–227.

Zervakis, V. & D. Georgopoulos, 2002. Hydrology and circulation in the North Aegean (Eastern Mediterranean) throughout 1997–1998. Mediterranean Marine Science 3: 5–19.

Hydrobiologia (2011) 670:165–188
DOI 10.1007/s10750-011-0676-6

ECOSYSTEMS AND SUSTAINABILITY

Fish distribution predictions from different points of view: comparing associative neural networks, geostatistics and regression models

A. Palialexis · S. Georgakarakos · I. Karakassis · K. Lika · V. D. Valavanis

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Accurate prediction of species distributions based on sampling and environmental data is essential for further scientific analysis, such as stock assessment, detection of abundance fluctuation due to climate change or overexploitation, and to underpin management and legislation processes. The evolution of computer science and statistics has allowed the development of sophisticated and well-established modelling techniques as well as a variety of promising innovative approaches for modelling species distribution. The appropriate selection of modelling approach is crucial to the quality of predictions about species distribution. In this study, modelling techniques based on different approaches are compared and evaluated in relation to their predictive performance, utilizing fish density acoustic data. Generalized additive models and mixed models amongst the regression models, associative neural networks (ANNs) and artificial neural networks ensemble amongst the artificial neural networks and ordinary kriging amongst the geostatistical techniques are applied and evaluated. A verification dataset is used for estimating the predictive performance of these models. A combination of outputs from the different models is applied for prediction optimization to exploit the ability of each model to explain certain aspects of variation in species acoustic density. Neural networks and especially ANNs appear to provide more accurate results in fitting the training dataset while generalized additive models appear more flexible in predicting the verification dataset. The efficiency of each technique in relation to certain sampling and output strategies is also discussed.

Keywords Species distribution predictions · Habitat modelling · Models comparison · Geostatistics

Introduction

The need for scientifically documented studies, regarding characterization of marine ecosystems, fluctuation in species biodiversity and abundance and climate change effects on marine ecosystems, has led to an increase in studies focusing on prediction of species distribution, utilizing different approaches to

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

---

A. Palialexis (✉) · I. Karakassis · K. Lika
Department of Biology, University of Crete, Vassilika Vouton, P.O. Box 2208, 71 409 Heraklion, Crete, Greece
e-mail: andreaspal@her.hcmr.gr

A. Palialexis · V. D. Valavanis
Marine GIS Lab, Hellenic Centre for Marine Research, Thalassocosmos, 71 003 Heraklion, Crete, Greece

S. Georgakarakos
Department of Marine Sciences, University of the Aegean, University Hill, 81 100 Mytilini, Lesvos, Greece

habitat modelling and identification of habitat heterogeneity (Guisan et al., 2002; Elith et al., 2006; Valavanis et al., 2008). Such tools should be easily accessible to managers and stakeholders involved in decision-making and management processes while the development of powerful analytical tools and the evolution of geographic information system (GIS) and remote sensing, combined with more accurate and extensive time series data sets, constitute a promising background for the evolution of statistical modelling techniques and the extraction of applicable results. Modelling approaches, such as resource selection functions (RSF) (Valavanis et al., 2008), quantitative structure–activity relationships (QSAR) (Tetko et al., 1995), or geostatistics (Petitgas, 2001) are commonly used for habitat modelling or species distribution predictions. Extensive reviews on species distribution models are available (Guisan & Zimmermann, 2000; Redfern et al., 2006; Richards et al., 2007; Schröder, 2008; Valavanis et al., 2008; Elith & Leathwick, 2009). These approaches could additionally be used for identifying species–environment relationships or inter- and intra-species interactions by utilizing species life history data.

Knowledge on species life-history data becomes crucial during model development and model evaluation. There is always a trade-off among model complexity–overfitting and simplicity–generality of prediction efficiency. According to Guisan & Zimmermann (2000), nature is too complex and heterogeneous to be predicted accurately in every aspect of time and space from a single, although complex, model. Levins (1966) formulated the principle that only two out of three preferable model properties (generality, reality, precision) can be improved simultaneously while the third property has to be sacrificed. The selection of the appropriate modelling technique should be based on the aim of each modelling application.

Accurate estimation of species distribution, based on sampling data, is essential for further scientific analysis, such as stock assessment, understanding of abundance fluctuation due to climate change or overexploitation, and application to management and legislation processes. This study aims to compare several fish distribution prediction techniques based on sampling data with respect to their statistical performance. Advantages and disadvantages of each modelling approach are demonstrated. Furthermore, an attempt is made to optimize the prediction of species distribution by combining the output of the most informative techniques. Species distribution predictions are applied on a training dataset, on an independent verification dataset and on a dataset derived from a wider area (compared to the sampling area). The accuracy and applicability of each modelling technique, the comparisons amongst them and the biological interpretation of small pelagic species are discussed.

Materials and methods

Study area

The study area (Fig. 1) is Thermaikos Gulf, located in the North Aegean Sea (Northeastern Mediterranean). Thermaikos Gulf is a semi-enclosed basin, and relatively productive due to run-off from four major rivers. Bottom relief is smooth due to the continuous sediment input. Thermaikos Gulf forms a wide continental shelf, which extends to the south into the 1,400 m deep Sporades Basin. Water mass circulation is predominantly cyclonic (Poulos et al., 2000). Aegean water masses enter the gulf from deeper layers (Kourafalou & Tsiaras, 2007) along the eastern coast and move counter-clockwise towards the Gulf of Thessaloniki. Riverine waters usually move to the south along the western coast.

Acoustic data

Acoustic data have been collected during April–May 1998 in Thermaikos Gulf using a calibrated 38 kHz SIMRAD EK 500/BI 500 system (Bodholt et al., 1989). The echograms were scrutinized, allocating the nautical area scattering coefficient (s_A, m^2 n mi^{-2}, NASC, MacLennan et al., 2002) to the target pelagic species, such as sardine and anchovy. The integration values, with a horizontal resolution of 1 nautical mile (nm), have been transformed to the presence–absence data (Fig. 1). Acoustic data have not been converted to biomass to avoid the propagation of uncertainty in species composition and length distribution from the trawl sampling in the response variable (Walline, 2007). Species identification based on biological sampling, as well as concurrent catch data, indicated that the majority of the target species were *Sardina*

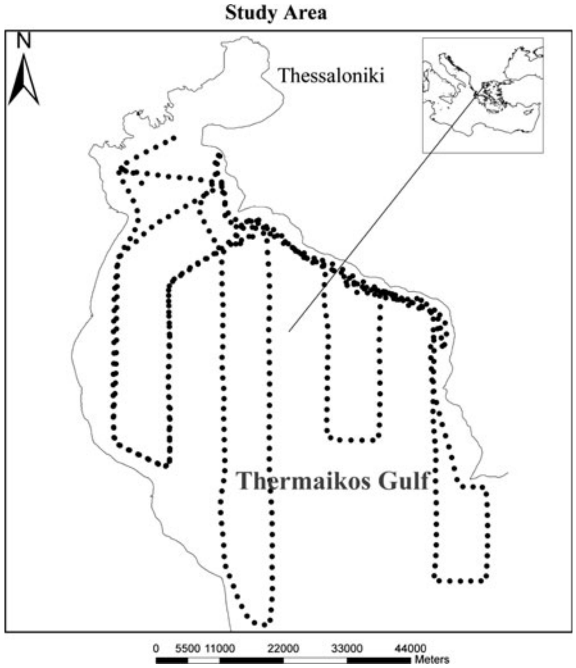


Fig. 1 Study area and sampling transects

pilchardus ($\sim$55%), *Engraulis encrasicolus* ($\sim$25%) and *Trachurus* spp. (<10%). Measurements were carried out along predetermined sampling transects, until the isobath of 100 m, near Cape Kassandra, in an area of about 1,600 nm^2 (Tsimenides et al., 1992). The acoustic data records allowed the extraction of a well-structured verification data set that was used for model evaluation. This set was constructed following an optimal dataset partitioning process (Tetko et al., 1995) where two adjacent points are separated and one included in the control and the other in the learning set. In our study, the validation dataset was formulated using one value per four sequential sampling values used for the training set. The distance between the points of the verification set (5 nm) was selected based on the fact that at this distance no spatial autocorrelation was detected. The specific selection of the validation set overcomes the underestimation of predicted errors that could caused by cross-validation approaches, especially when acoustic data are spatially autocorrelated (Hastie et al., 2009). In addition, there is no risk of comparing different sampling strategies, since the verification set is a proportion of the raw data. Semi-variograms (Matheron, 1971), calculated using ESRI's ArcGIS geostatistical analyst software (GAS) and auto-correlation function estimation plots in R statistical software (R Development Core Team, 2005), were used for identifying the spatial patterns of the raw data, the training and the verification sets and model residuals. Moran's *I* spatial autocorrelation statistic (Moran, 1950) was also used for estimate the spatial pattern of the two data sets. Furthermore, the homoscedasticity of the residuals was tested by plotting response and explanatory variables against the residuals. By these processes, the suitability of the verification set was tested. In addition, the use of this particular verification set in the validation process overcomes several assumptions that accrued from the use of cross-validation approaches. These differences between the training and the selected verification set, in addition to the fact that the verification set was not introduced to the models, makes the verification set spatially independent.

Environmental and geographic data

Models presented here were developed taking into account the ability of the available environmental parameters to explain species distribution, based on species life history characteristics. Three data sets were extracted for each explanatory variable. The first (DS1) represents the sampling points across transects, the second (DS2) represents each point of the grid that covers the sampling area at a resolution of 0.01 decimal degrees ($\sim$1 km) and the third (DS3) corresponds to the verification set. These variables as well as their sources are shown in Table 1. The spatial resolution of the explanatory variables varied from 0.01 to 0.04 decimal degrees according to spatial resolution of the raw data. For modelling purposes, all data sets were re-sampled to the highest available resolution of 0.01 decimal degrees.

A detailed exploration process was performed on the assembled data sets to identify potential spatial patterns, outliers, correlations and interactions. This process is essential for parameter selection during model development to avoid violation of model assumptions, apply any required variable transformations and gain a better understanding of the explanatory data sets. The exploration process included: (a) pair-plots, to identify collinearity, which could lead to biased parameter selection during model development (Zuur et al., 2007), (b) dot-plots and box-plots, for identification of extreme values and outliers, (c) Quantile–Quantile plots (Q–Q plots)

Table 1 Remote sensed data, metadata and their sources

Data variable	Abbreviation	Data type/sensor	Archive source
Acoustic data	s_A	Total acoustic integration (area backscattering coefficient s_A per ESDU = 1 nm)	SIMRAD EK500/BI500 system on April/May 1998 in Thermaikos Gulf
Sea surface temperature	SST	Grid/Aqua MODIS	German Aerospace Agency (DLR)
Chlorophyll-*a* concentration	CHL	Grid/Aqua MODIS	Distributed Active Archive Center (NASA)
Photosynthetically available radiation	PAR	Grid/SeaWiFS	Distributed Active Archive Center (NASA)
Sea level anomaly	SLA	Grid/Merged Jason-1, Envisat, ERS-2, GFO, T/P	AVISO
Precipitation	PRE	Grid	Mediterranean Oceanic Database (MODB)
Sea surface salinity	SSS	Grid/CARTON-GIESE SODA and CMA BCC GODAS models	Mercator operational oceanography
Bathymetry	DEP	Grid/Processed ERS-1, Geostat and historical depth soundings	Laboratory for Satellite Altimetry (NOAA)
Wind stress and direction	WS & WD	Grid & cover	Mercator operational oceanography
Coastline	Coast	Cover/Digitisation of nautical charts and aerial photography	Hellenic Ministry of Environment
Distance to coast	DCoast	Grid and cover	Extracted from coastline
Depth slope	DEPsl	Grid	Extracted from bathymetry grid
Temperature slope (thermal fronts)	SSTsl	Grid	Extracted from SST grid
Marine productivity hotspots	MPH	Grid	According to Valavanis et al. (2004)
Mesoscale thermal fronts	MTF	Cover	According to Valavanis et al. (2005)
Longitude and latitude of stations	LON, LAT	Cover in decimal degrees and metres	SIMRAD EK500/BI500 system on April/May 1998 in Thermaikos Gulf
Current speed and direction	CURSP & CURDR	Grid & cover/NEMO (OPA9 + LIM)	Mercator operational oceanography
Day-dark-night-dawn categorical factor	DDND	Cover and grid	Based on sampling date and hour
Date	DT	Cover	Based on sampling date

and histograms, for an overview of data distribution and (d) conditional box-plots, for displaying potential relationships and interactions (Zuur et al., 2010). Finally, nine variables were selected for model development, according to data exploration results, as descriptors of species distribution. These are sea surface chlorophyll-*a* concentration (CHL), sea surface temperature (SST), sea level anomaly (SLA), bathymetry (DEP), photosynthetically available radiation (PAR), distance-to-coast (DCoast), sea surface temperature slope (SSTsl), depth slope (DEPsl), and day-dark-night-dawn categorical factor (DDND) which is used for explaining the variance of the data that corresponds to behavioural variation of small pelagic species between day and night (Giannoulaki et al., 1999). Dark and dawn represent the times of onset of migration. During the night, small pelagic fish are more scattered and close to the surface while during the day, they form schools in deeper layers. SSTsl and DEPsl were generated by the SST and

DEP grids, respectively, and corresponded to the maximum rate of change in value from each grid-cell to its neighbours. Variables that were excluded after the exploration process (i.e. not used in model development) included date of sampling, precipitation, sea surface salinity, currents speed and direction, wind stress and direction, marine productivity hotspots (Valavanis et al., 2004), mesoscale thermal fronts and hour when each point was sampled. Most of these variables were excluded because they were highly correlated with variables that were actually used in the modelling process. The remotely sensed data were used in the finest temporal resolution available to describe the real-time environmental conditions during sampling. GIS routines (ESRI ArcInfo, version 9.1) were utilized for the conversion of satellite images into grids (Valavanis, 2002) and for the extraction of the environmental values at each sampling point.

Regression models

Regression-based modelling techniques are widely used for predicting species distribution. The most commonly used are generalized additive models (GAMs), generalized linear models (GLM), classification and regression trees (CART) and multivariate adaptive regression splines (MARS). An extensive description of these particular methods can be found in Elith et al. (2006) and Valavanis et al. (2008). In this study, GAMs and generalized additive mixed models (GAMMs) were selected amongst the regression models for species distribution predictions and method comparison purposes, based on the fact that GAMs are widely applied in fisheries science (e.g. Zuur et al., 2007; Palialexis et al., 2009), and they are the most common and well-developed tools for habitat modelling (Valavanis et al., 2008). GAMs are generalized models, involving a sum of smooth functions of covariates (Hastie & Tibshirani, 1990; Wood, 2006). The main advantage of GAMs over traditional regression methods is their ability to model non-linearity using non-parametric smoothers (Hastie & Tibshirani, 1990; Wood, 2006). In addition, according to Moisen & Frescino (2002) and Elith et al. (2006), GAMs perform marginally better than other regression techniques (CART and GLM). They are able to identify species–environment relationships and interactions and to provide biologically interpretable relationships between the response and explanatory variables. The advantage of GAMMs compared to GAMs is that they can deal with spatial autocorrelation, which could lead to biased models and predictions.

The total acoustic integration, natural log-transformed, was used as a response variable. The appropriate transformation method was selected by using Q–Q plots (Cleveland, 1994). Transformation permitted a Gaussian distribution to be assumed for the response variable. The identity link function was used. Depending on the corresponding Q–Q plots, some of the explanatory variables were transformed. The selection of GAM smoothing predictors was based on the method proposed by Wood & Augustin (2002), using the ‘mgcv’ library in R statistical software (R Development Core Team, 2005). The degree of smoothing was selected based on the observed data and the generalized cross validation method (Wood, 2006). The best-fitting model was selected by using Akaike’s Information Criterion (AIC) (Akaike, 1974) and a stepwise forward selection was applied to restrict collinearity amongst the explanatory variables. The GAMMs were developed based on the final GAM model, assuming that a specific correlation structure exists amongst all points of the study area. This structure was modelled using the Gaussian distribution and Gaussian spatial correlation. The GAM and GAMM that better fitted the training dataset were finally selected to predict values in the DS1, DS2 and DS3 data sets.

Associative neural networks (ANNs)

ANNs combine the complexity of some of the statistical techniques with the machine learning objective at a more ‘unconscious’ level, non-transparent to the user (Michie & Spiegelhalter, 1994). According to Haykin (1994), a neural network is a massively parallel distributed processor that has a natural propensity for storing experiential knowledge and making it available for use. It resembles the brain in two respects: Knowledge is acquired by the network through a learning process and, then, interneuron connection strengths known as synaptic weights are used for storing the stored knowledge. ANNs comprise algorithms for mapping the input vector (predictors) to an output vector (responses) through processing elements called ‘neurons’ via a training process. The most common type of ANN is a three-layer back-propagation network, which consists of three basic groups of neurons (Ripley, 1996): (a) the input neurons collect the external information and send it to the hidden neurons in the middle layer; (b) the hidden neurons accumulate the previous signals, adjusting each input by certain weights and applying certain threshold functions; and (c) the output neurons accumulate in a second step the input from the hidden neurons, applying again a set of weighting parameters and threshold functions.

The ASNN is a method with improved predictive abilities including combination of feed-forward neural networks and a *k*-nearest neighbour technique, which is a classification method based on distances amongst input samples in space. ASNNs were initially applied in chemistry (Tetko et al., 1995) providing more accurate predictions than artificial neural network ensembles (ANNEs). More detailed information on ASNN development can be found in Tetko (2002a, b). Since this is the first application of ASNN in marine science, both ANNE and ASNN were developed to compare ASNN performance to traditional ANNE and to other modelling approaches.

In this study, an ANNE, which is an averaging neural network prediction process over several independent networks, has been developed using one hidden layer with three neurons. The ASNN model was developed on the ANNE by including the number of the nearest neighbour, *k*, and parameter σ for the Parzen-window regression, which is a non-parametric way of estimating the probability density function of a random variable. These factors represent smoothing parameters of ASNN to minimize the ASNN error for the training set (Tetko et al., 1995).

The ANNE and ASNN models were selected based on selection processes that include the algorithm, the number of neurons and hidden layers, and the iterations and number of ensembles. The early stopping over ensemble (ESE) method was used for training the neural networks (Bishop, 1995; Tetko & Tanchuk, 2002). In ESE, initial training sets were randomly constructed with equal size learning and validation sets for each neural network in the ensemble. Thus, each neural network had its own learning and validation sets. The learning set was used for adjusting neural network weights. The training was stopped when a minimum error for the validation set was calculated (‘early stopping’ point). Following ensemble learning, a simple average of all networks was used for predicting the test patterns. Networks ensembles presenting the minor root mean squared error (RMSE) and mean absolute error (MAE) were finally selected (Tetko et al., 2008). These were then processed by using the second-order Levenberg–Marquardt optimization algorithm, i.e. both first- and second-order derivatives of the error function are required for the weight optimization. The Levenberg–Marquardt algorithm usually does not fall into local minima and provides the smallest errors for a fixed number of hidden neurons (Shepherd, 1997; Tetko et al., 2008). Both ASNN and ANNE were developed using three neurons in one hidden layer, 600 networks in each ensemble and 80 iterations in neural network training. These setting were optimized in a modelling selection process according to the lower RMSE and MAE. ANNE and ASNN were developed using the ASNN software VCCLAB (Virtual Computational Chemistry Laboratory, http://www.vcclab.org, 2005), while the final selected model was used for predicting the DS1, DS2 and DS3 data sets.

Kriging

The inverse distance weighted (IDW) and spline methods (i.e. GAMs) are referred to as deterministic interpolation methods because they are directly based on the surrounding measured values or on specified mathematical formulae that determine the smoothness of the resulting surface. A second family of interpolation methods consists of geostatistical methods, such as kriging, which are based on statistical models that include autocorrelation (the statistical relationships amongst the measured points). Thus geostatistical techniques have the capability of producing a prediction surface as well as provide a measure of the certainty or accuracy of the predictions (Georgakarakos & Kitsiou, 2008). The purpose of geostatistics (Matheron, 1971) is to model the spatial variability of a given variable and then utilize the model to estimate the value of the variable at given locations, with extensive applicability to estimating abundance of fish populations from scientific surveys (Petitgas, 2001).

Kriging assumes that the distance or direction between sample points reflects a spatial correlation that can be used for explaining variation in the surface. Kriging fits a mathematical function to a specified number of points or all points within a specified radius to determine the output value for each location. It is a multistep process including exploratory statistical analysis of the data, variogram modelling, creation of the surface, and (optionally) exploring a variance surface. Kriging is most appropriate in the presence of a spatially correlated distance or directional bias in the data. Ordinary kriging is the most general and widely used amongst the kriging methods. It assumes that the constant mean of the data is unknown. This is a reasonable assumption unless there is a scientific reason to reject it.

In this study, kriging results are based on geostatistical analysis using ESRI's ArcGIS GAS, which provides an extensive set of tools for performing different kriging methods. Kriging selects weights so that estimations are unbiased and the estimation variance is minimized. The best-fitted variogram is selected after running and evaluating a large number of models, a procedure supported by GAS (Georgakarakos & Kitsiou, 2008).

Finally, the developed kriging models were evaluated by analysing the leave-one-out cross-validation residuals and their statistics were compared and tested in relation to modelling assumptions and whether standard errors estimated by the model are accurate (Isaaks & Srivastava, 1989). The comparison amongst the different developed models was carried out based on the calculation of the RMSE, the average standard error (ASE) and the coefficient of determination (R^2). An ASE close to the root-mean-squared prediction error indicates a correctly assessed variability in prediction. If the ASE is above/below the root-mean-squared prediction error, the variability of the predictions is over/under estimated.

For the purposes of this study, two ordinary kriging models were finally selected. The first one (DefKrig) was developed to minimize errors by using the default settings provided by GAS. The second one (Krig) was parameterized according to data information, regarding trends, data transformations, near neighbour selection and variogram optimization. The above features were incorporated in Krig model to optimize the prediction based on data characteristics that derived through the exploration process. Both models were used in the comparison process because of their differences in explaining the training data variation. While the first model (DefKrig) is error minimization-oriented, the second one (Krig) integrates the results of the exploration process on the training data-set and thus could be characterized as data-oriented.

Prediction optimization

Each modelling technique explains the variance of the observed data from a different point of view. Additive models use a smoothing factor; ANNs are trained based on the observed data, estimating weights and subsequently retrained, and kriging estimates similarities based on distances. A combination of these predictions was applied by creating a GAM model, where the response value is the observed data and explanatory variables correspond to the predictions of each method. This GAM was developed using forward selection and AIC, as described above. The aim of this process is to utilize the predictive capacity of each technique by creating a totally new model, which explains a larger proportion of the variance of the sampling data. This could be characterized as a model averaging approach. The use of a GAM exploits predictions of different techniques in an additive way. This model is referred to as AverMod.

Method comparison

Different aspects of models' predictive performance could be compared by applying a range of evaluation statistics (Potts & Elith, 2006). Pearson's correlation coefficient, r, was used amongst the observed and the predicted values as an index of relative similarity, though a perfect correlation does not necessarily imply exact prediction. In case of perfect correlation, all predictions might be biased in a consistent direction. Spearman's rank correlation coefficient, r_s, was used for indicating the similarity between the ranks of observed and predicted values. The use of ranks means that, as long as the order of predictions is correct, the value of the statistic will be high. Kendall's rank correlation coefficient, τ, is similar to Spearman's r_s and was used for measuring the degree of correspondence between observed and predicted rankings.

The model calibration process used in this study is analogous to Pearce & Ferrier (2000) calibration analysis for binomial data (see also Potts & Elith, 2006) and is applied to complement the previously mentioned correlation indices. A simple linear regression between observed and predicted values (e.g. observed $= m$ (predicted) $+ b$) was applied, providing information for the bias and consistency of the prediction. In this equation, b provides information regarding the bias, while m provides information about the spread of predictions compared to the spread of the observations. A perfectly calibrated model would be a 1:1 regression line. A parallel regression line ($m = 1$, $b \neq 0$) to the 1:1 line indicates consistent bias. When $b \neq 0$ and $m \neq 1$, then the predicted values are both biased and distributed in a broader or narrower range than the observed values (Fig. 2).

Several summary statistics were estimated by comparing the predicted to the observed values. The objective of these statistics is to make an informative decision on which model provides more accurate prediction. Mean error (ME) should be close to 0, if the predictions are unbiased, and the root-mean-square standardized prediction error (RMSqSt) should be close to 1, if the standard errors are accurate. The root-mean-square prediction error (RMSE) should be relatively low, if the predictions are close to the measured values. Mean square error (MSqE) and mean standardized error (MStE) should be close to zero for accurate predictions while lower values of MSqE are generally interpreted as best explaining the variability in the observations. MStE is used as an assessment of uncertainty. MAE and ASE are quantities used for measuring how close predictions are to the eventual outcomes (the lower the ASE, the better the predictions). All the above statistics were estimated from the prediction errors of the final models selected.

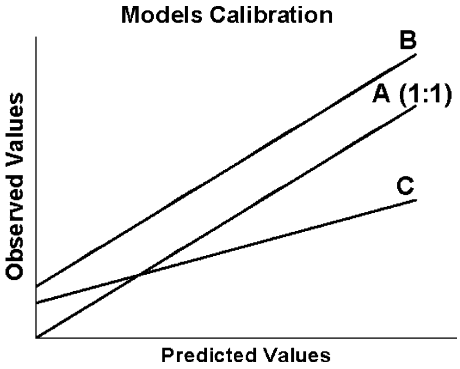


Fig. 2 Model calibration: *A* a perfectly calibrated model, *B* a model with consistent bias, *C* predicted values derived from this model are both biased and distributed in a broader or narrower range than the observed values

In addition, some more summary statistics that describe the observed and the predicted values have been estimated, like the average value (Ave) and the standard deviation (StDev).

Results

Species distribution predictions

Regression models

After data exploration, parameter selection and model optimization, a final GAM model (GAM1) was selected. Another model (GAM2), nested within GAM1, was generated by including a temporal categorical variable as a factor. The quality characteristics of GAM2 were slightly improved compared to GAM1 (AIC and deviance explained, Table 2), however, GAM2 used more degrees of freedom than GAM1, which indicates a more complex model. Both GAM1 and GAM2 were used in the comparison process to examine if the increase in complexity of GAM2 improves the predictive efficiency of the model without decreasing model generality. Together with the final selected mixed model (GAMM1) these two additive models, were used for predicting species distribution and were compared based on characteristics related to their predictive efficiency, generality and biological interpretability (Table 2).

All models were statistically significant (Table 2). According to the AIC criterion (the lower the better), GAM2 is better than GAM1. In addition, the deviance explained (not available in GAMM1) and the adjusted R^2 suggest that GAM2 explains a higher proportion of the response variance than other models. GAM2 performs better in describing the relationship between acoustic data and environmental factors.

Predictions on DS1 (Fig. 3) indicate that in certain areas models failed to accurate predict the observed data (in the north and northwest). This is also apparent in predictions for the DS3 set. Other areas were predicted relatively well in both data sets. Bar charts indicate that GAM2 and GAMM1 perform almost equally efficiently while there are visible differences between these two and GAM1.

Table 2 Final generalized additive models and their characteristics

Model's code	Explanatory variables	Dev. exp.	Res. d.f.	R^2_a	AIC	*P*-value
GAM1	s(SLA) + s(DEP:PAR)	38.9%	23.687	0.345	570.35	$\ll 0.05$
	s(SLA)		5.105			$\ll 0.05$
	s(DEP:PAR)		18.682			$\ll 0.05$
GAM2	s(SLA) + s(DEP:PAR) + as.factor(DDND)	47.9%	29.678	0.458	510.76	$\ll 0.05$
	s(SLA)		7.051			$\ll 0.05$
	s(DEP:PAR)		22.627			$\ll 0.05$
	as.factor(DDND)					$\ll 0.05$
GAMM1	s(SLA) + s(DEP:PAR) + correlation factor	NA	24.597	0.444	NA	$\ll 0.05$
	s(SLA)		5.561			$\ll 0.05$
	s(DEP:PAR)		19.036			$\ll 0.05$
	Correlation factor					$\ll 0.05$

Level of significance was set at 0.05. The ':' sign denotes interaction

Dev. Exp. deviance explained, *Res. d.f.* residual d.f., R^2_a adjusted R^2, *AIC* Akaike Information Criterion value, *P-value (chi-square)* significance values, *s* denotes smooth function of predictors

ANNs

Both ANNE and ASNN were developed using the following explanatory variables: DDND, SLA, CHL, PAR, SST, DEP, DCoast, DEPsl and SSTsl. The performances of ANNE and ASNN models for the training set are presented in Table 3.

For DS1 (Fig. 4, right), both models performed almost equally well. Visually, they seem to provide very accurate predictions in the whole area, while the local variation of the sampling data is also accurately predicted. Predictions for DS3 (Fig. 4, left) indicate that both models describe the global trend of the data accurately, although they under- or over-estimate the relatively high or low observed values of the independent set, respectively.

Kriging

Characteristics of the ordinary kriging models are presented in Table 4. The two final selected models differ significantly. The Krig model could be characterized as a locally oriented model compared to DefKrig, which is more globally oriented. This observation is also depicted in Fig. 5 where predictions differ significantly. The right map (Fig. 5) confirms that the Krig model where predictions depicted high and low observed values more accurately at a local scale, compared to DefKrig, which under-estimates the observed values. This is also obvious in predictions on the DS3 (Fig. 5, left).

Prediction optimization

The final selected model and its characteristics are presented in Table 5. The AverMod is a relatively simple GAM with two explanatory variables and relatively low degrees of freedom. However, the deviance explained is relatively high (45.2%). GAM2 was also tested, as an explanatory variable, since it performs statistically better than the other additive models but it was not significant ($P > 0.05$) and it was dropped during the selection process. Figure 6 indicates that predictions derived by AverMod for the DS1 set are relatively accurate over the whole extent of the study area (Fig. 6, left). Predictions for the independent set DS3 showed a tendency to under-predict high observed values and over-predict low values (Fig. 6, right).

Comparison

Model fit comparison

Model fit comparison was achieved by applying the previously mentioned estimates to DS1 and comparing predicted values from each model to the sampling observed values. In order to identify the most accurate fit to the training data, several model

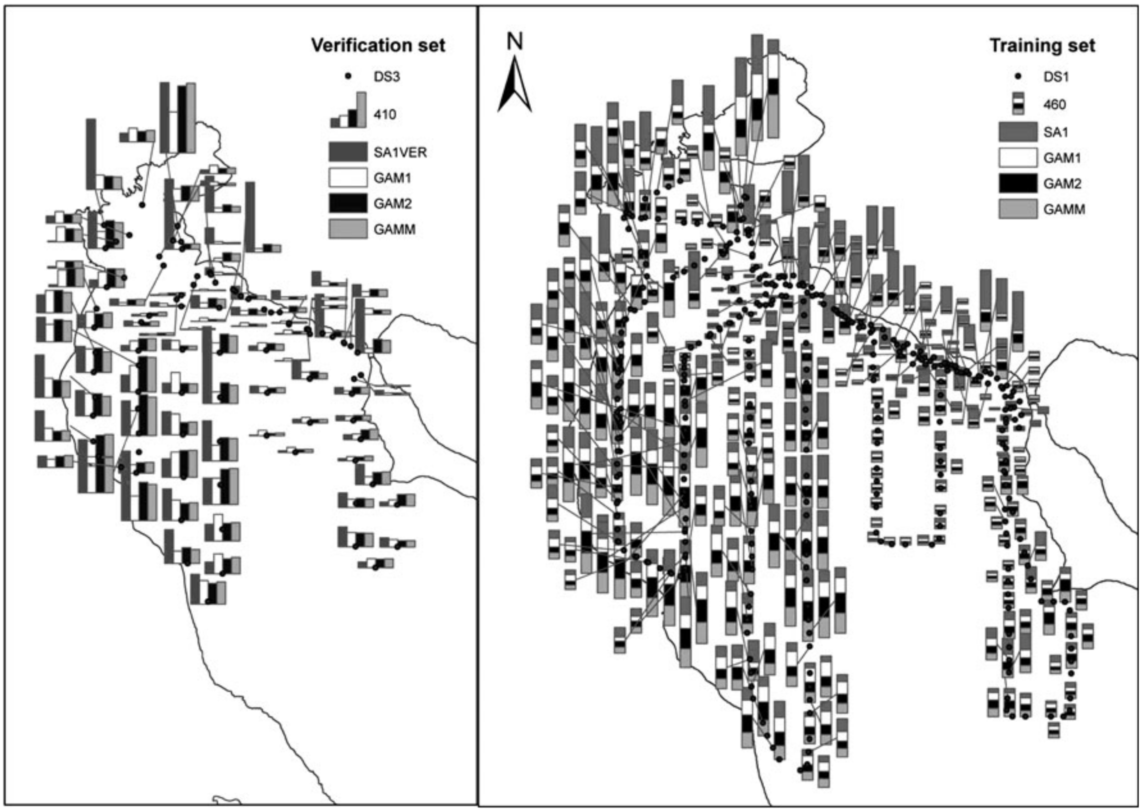


Fig. 3 A comparative representation of the observed (SA1VER for the verification set and s_A1 for the training set) and predicted values (GAM1, GAM2, GAMM) derived from generalized additive models and mixed model. Hued bar's height represents the observed and predicted fish density in each sampling point. *Left map* corresponds to the verification set (DS3-black points) and *right map* corresponds to the training set (DS1-black points). The *scale of the bar* which is shown in legend is 410 and 460, respectively

Table 3 Performance characteristics of ANNE and ASNN

Network, k, (σ)	LOO results		
	RSME	MAE	r^2
ANNE	191.10	136.64	0.219
ASNN, 54, (0.01)	176.77	116.25	0.325

RMSE root mean square error, *MAE* mean absolute error, r^2 square of correlation coefficient, *LOO* leave one out, *k* k near neighbour, σ sigma

comparison indices were used and presented in Table 6.

According to Pearson's correlation coefficient (Table 6), the similarities amongst observed and predicted values are better described by Krig while ASNN and ANNE are also efficient in their predictions. GAM2 performs better than the other additive models. On the other hand, Spearman and Kendall correlations indicate that the rank correlation between observed and predicted values was higher for Krig and GAM2 and lower for neural network techniques.

The model calibration process (Fig. 7; Table 6) indicates that neural networks are almost perfectly calibrated by minimizing the bias compared to the other approaches and by predicting values in the same range as observed values, as shown by the overlap with the 1:1 regression line in Fig. 7. There are great similarities amongst the calibrations of additive models where they present an almost constant bias and the ranges of their predicted values are quite close. The regression lines of kriging models vary greatly and seem to produce under-calibrated models.

Error analysis of predictions on DS1

Summary statistics (Ave, StDev) of the observed (s_A1-DS1) and the predicted sets as well as some

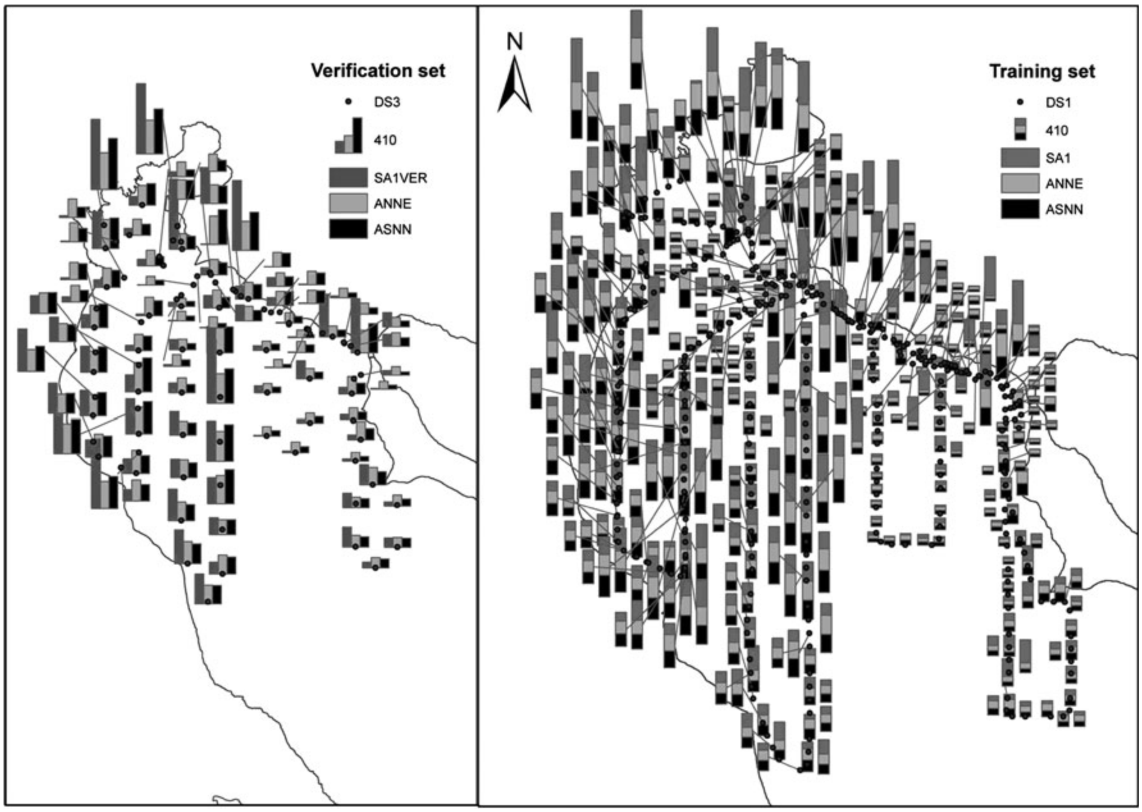


Fig. 4 A comparative representation of the observed (SA1VER for the verification set and s_A1 for the training set) and predicted values (ASNN and ANNE) derived from associative neural networks and artificial neural networks ensemble. Hued bar's height represents the observed and predicted fish density in each sampling point. *Left map* corresponds to the verification set (DS3-black points) and *right map* corresponds to the training set (DS1-black points). The *scale of the bar* which is shown in legend is 410

Table 4 Ordinary kriging models' characteristics

Krig	DefKrig
First order trend removal	No trend removal
Variogram	Variogram
15 number of lags	12 number of lags
Lag size 1852 m	Lag size 6965.1
Nugget 1.184	Nugget 46257.18
Model spherical	Model spherical
Range 26571.13 m	Range 79065.67
Anisotropy	No anisotropy
Minor range 5100.6	
Direction 3.35°	

error indices of the predictions are presented in Table 7. AverMod and DefKrig average predictions are close to the average of observed values. The ASNN and ANNE averages are also close to s_A1-DS1. The average predictions of additive models are lower than the observed while Krig over-predicts the observed average. The StDev of the observed data is relatively high, which generally characterizes the nature of acoustic data. Only the Krig model generates a similar StDev value while other models all predict lower StDev.

Error indices show that generally AverMod, DefKrig, ASNN and ANNE (in decreasing order) provide more accurate, unbiased predictions and better explain the variability of observed values, according to ME, MSqE, MStE, RMSqSt and RMSE. MAE and AverMod show that GAM2 also predicts values close to the observed. According to ME and the sum of errors, only Krig over-estimates the observed data while all other models either predict accurately or under-estimate acoustic data. In conclusion, error analysis indicates that AverMod, ASNN, ANNE and DefKrig fit the data on which

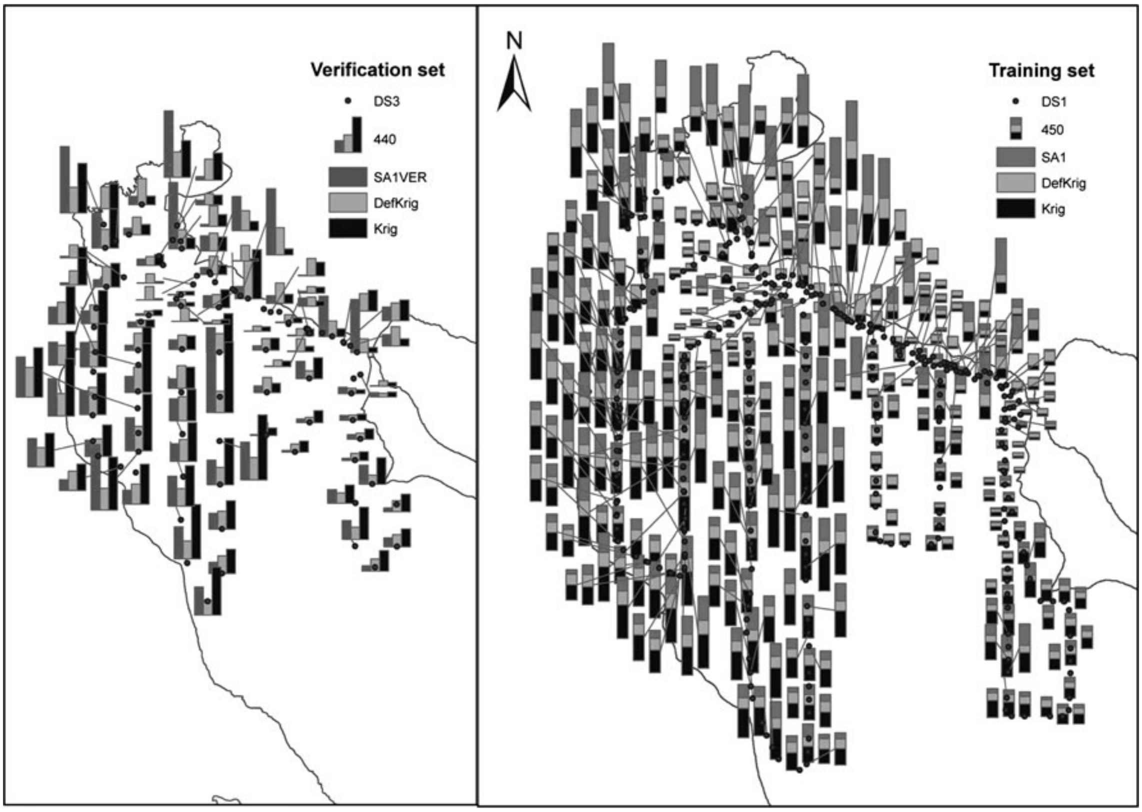


Fig. 5 A comparative representation of the observed (SA1VER for the verification set and s_A1 for the training set) and predicted values (DefKrig and Krig) derived from ordinary kriging approaches. Hued bar's height represents the observed and predicted fish density in each sampling point. *Left map* corresponds to the verification set (DS3-black points) and *right map* corresponds to the training set (DS1-black points). The *scale of the bar* which is shown in legend is 440 and 450, respectively

Table 5 Characteristics of the GAM model used for the optimization of predictions

Model's code	Explanatory variables	Dev. exp.	Res. d.f.	R_a^2	*P*-value
AverMod	s(Krig predictions) + s(ASNN predictions)	45.2%	11.093	0.436	$\ll 0.05$

Level of significance was set at 0.05

Dev. Exp. deviance explained, *Res. d.f.* residual degrees of freedom, R_a^2 adjusted R^2, *P-value (chi-square)* significance values, *s* denotes smooth function of predictors

they are trained more accurately than did the other models.

Comparing predictions on the verification dataset

Evaluation of model predictions on the verification dataset was achieved by applying the above-mentioned estimates to DS3. The best performing models would be characterized by generality and could predict species distribution along extended spatial and temporal axes more efficiently. Table 8 presents several model comparison indices to identify the most accurate fit to the independent verification set.

According to Pearson's correlation coefficient (Table 8), the similarities amongst observed and predicted values are better described by neural networks and especially by ASNN. GAM2 performs better than the other additive models while Krig performs better than DefKrig. On the other hand, Spearman and Kendall correlation analyses indicate that the correlation between the ranks of observed and predicted values is higher for Krig while there is

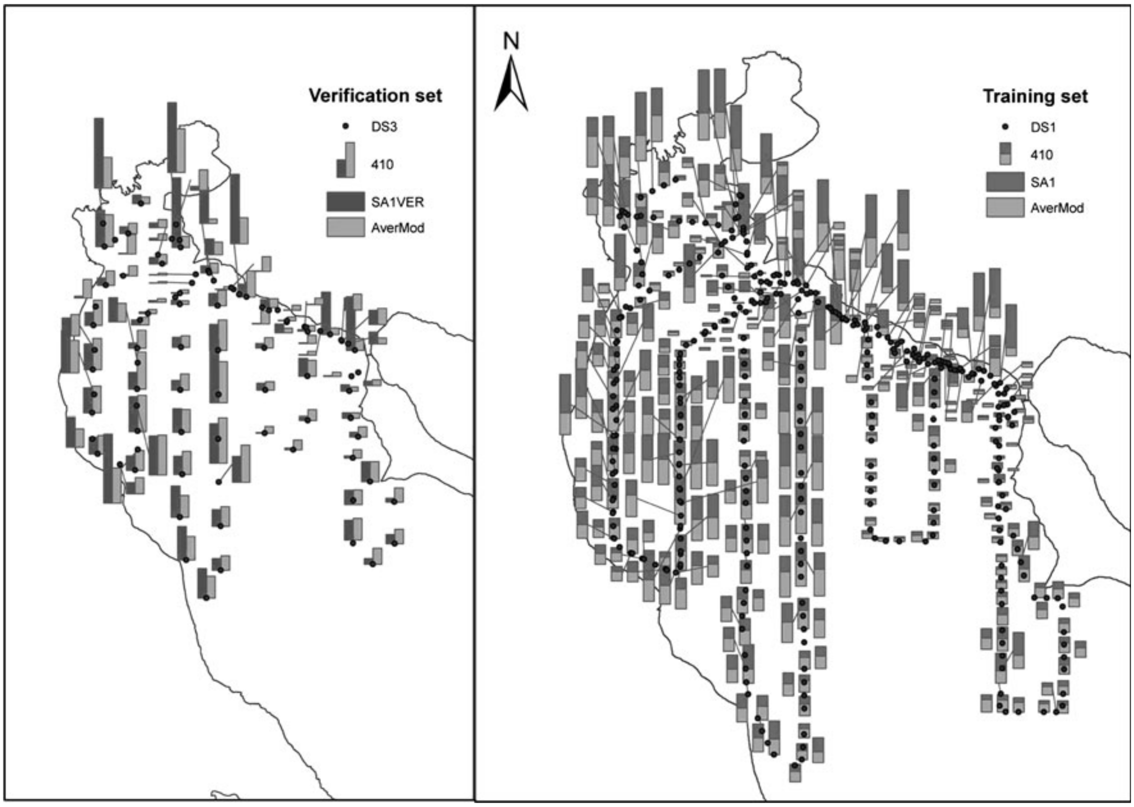


Fig. 6 A comparative representation of the observed (SA1VER for the verification set and s_A1 for the training set) and predicted values (AverMod) derived from prediction optimized model. Hued bar's height represents the observed and predicted fish density in each sampling point. *Left map* corresponds to the verification set (DS3-black points) and *right map* corresponds to the training set (DS1-black points). The *scale of the bar* which is shown in legend is 410

Table 6 Model comparison indices used for identifying the most accurate fit on training data

	GAM1	GAM2	GAMM1	DefKrig	Krig	ANNE	ASNN	AverMod
Correlation								
Pearson's r	0.38	0.49	0.45	0.50	0.59	0.57	0.56	0.67
Spearman's r_s	0.62	0.71	0.69	0.54	0.79	0.55	0.56	0.81
Kendall's τ	0.45	0.53	0.51	0.39	0.62	0.39	0.40	0.64
Calibration								
Intercept, m	0.821	0.819	0.823	1.160	0.592	0.980	0.967	1.019
Slope, b	92.89	82.65	85.51	−30.22	37.07	12.31	15.42	−3.67
R^2	0.145	0.224	0.206	0.250	0.351	0.327	0.316	0.453

little difference amongst the other techniques. The results of the model calibration process (Fig. 8; Table 8) indicate that additive model predictions are characterized by a constant bias, although the spread of the predicted values is similar to the spread of the observed values. The predictions obtained by means of neural networks for the verification set are generally less accurate than in training set, presenting bias and wider range of values, compared to the observed. The predictions derived from Krig showed a significant divergence from the 1:1 regression line while DefKrig predicts more accurately than Krig according to the evaluation process in the DS3.

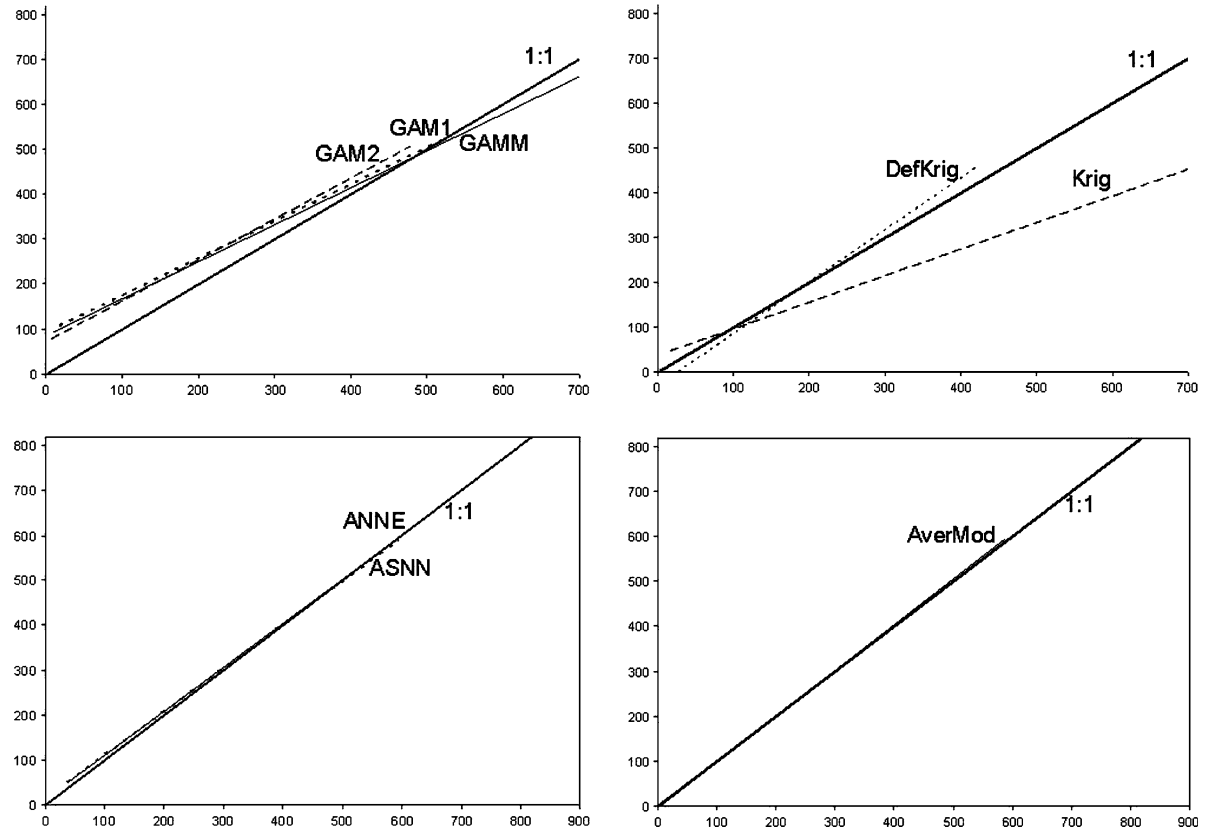


Fig. 7 Models' calibration indicated by regression lines amongst observed (y-axis) and predicted (x-axis) values on training dataset (DS1). The 1:1 regression line corresponds to a perfect calibrated model

Table 7 Models' comparison error indices used for identifying the best fit on training data

DS1	s_A1-DS1	GAM1	GAM2	GAMM1	DefKrig	Krig	ANNE	ASNN	AverMod
Ave	193.414	122.348	133.445	131.089	192.869	263.935	184.856	184.104	193.414
StDev	215.192	99.740	117.158	118.775	92.748	215.353	125.587	125.078	142.058
RMSE		1335.194	1126.701	1170.972	10.232	1324.978	160.789	174.904	~0.000
MSqE		1782742.121	1269454.155	1371174.907	104.696	1755565.923	25853.085	30591.264	~0.000
MAE		117.781	107.436	110.484	130.249	152.782	116.248	116.187	97.469
ASE		0.752	0.728	0.739	0.728	0.742	0.707	0.710	0.672
MStE		−0.356	−0.320	−0.323	−0.003	0.363	−0.048	−0.052	~0.000
RMSqSt		6.684	6.016	6.072	0.055	6.820	0.911	0.982	~0.000
ME		−71.065	−59.968	−62.325	−0.545	70.521	−8.558	−9.309	~0.000

Error analysis of predictions on DS3

Summary statistics (Ave, StDev) for the observed (s_A-DS3) and the predicted sets as well as error indices for the predictions are presented in Table 9. The error analysis of the predictions on the verification set DS3 generated different results to those obtained from the error analysis of the predictions on the training set (DS1). In this case, DefKrig and ANNE predicted the average observed values more accurately while ASNN and AverMod are also similar. Again, Krig is the only model that provides

Table 8 Models' comparison indices used for identifying the most accurate fit on the verification dataset

	GAM1	GAM2	GAMM1	DefKrig	Krig	ANNE	ASNN	AverMod
Correlation								
Pearson's r	0.48	0.58	0.56	0.53	0.58	0.67	0.73	0.67
Spearman's r_s	0.62	0.68	0.67	0.62	0.71	0.62	0.65	0.73
Kendall's τ	0.44	0.50	0.48	0.43	0.56	0.45	0.50	0.57
Calibration								
Intercept, m	1.090	0.981	0.954	1.317	0.586	1.871	1.317	1.154
Slope, b	62.92	61.83	67.97	60.09	37.33	175.28	40.79	22.14
R^2	0.233	0.338	0.315	0.281	0.332	0.454	0.536	0.445

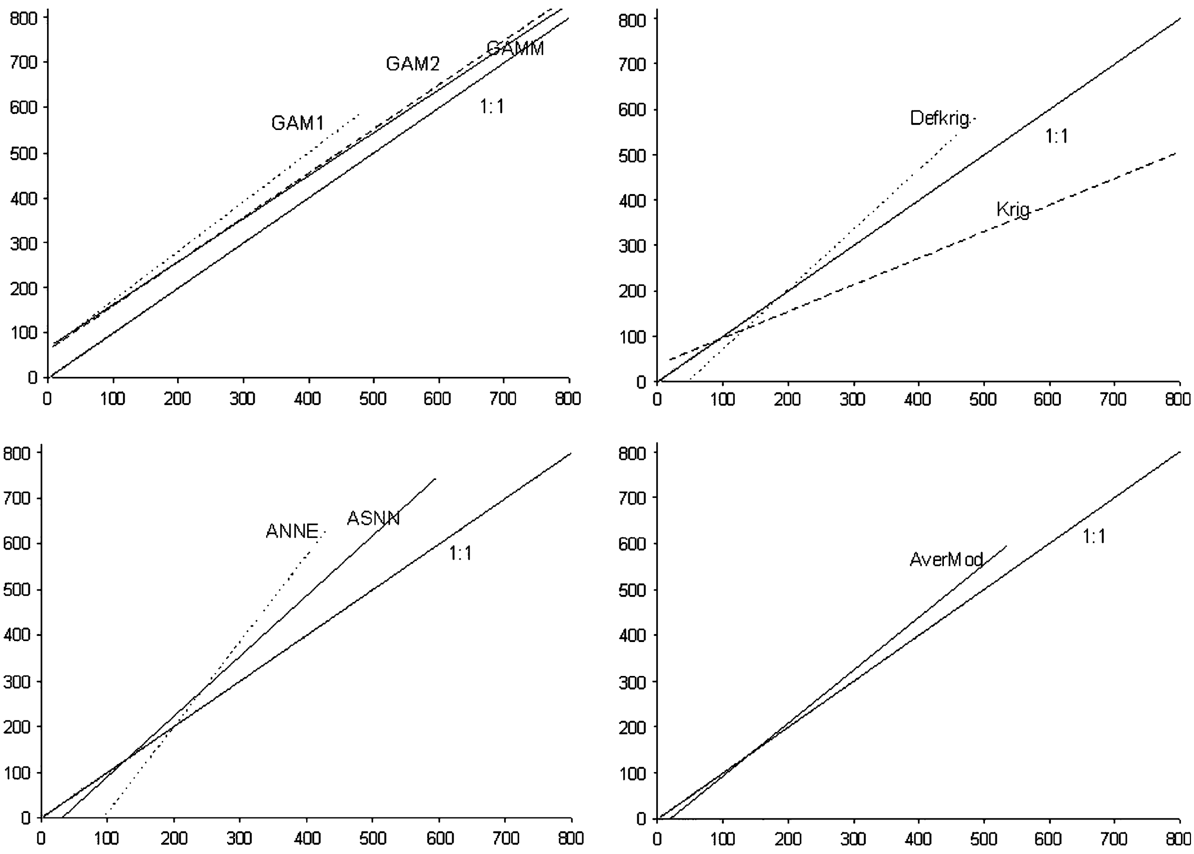


Fig. 8 Models' calibration indicated by regression lines amongst observed (y-axis) and predicted (x-axis) values on the independent verification dataset (DS3). The 1:1 regression line corresponds to a perfect calibrated model

predictions with StDev near to that of the observed values. Error indices show that DefKrig and ANNE provide the most accurate and unbiased predictions while AverMod and ASNN are also efficient. According to MAE, ASNN and GAM2 predict values similar to the observed values (AverMod, as well). ME and the sum of errors show that Krig over-estimates the observed data. Predicted values from DefKrig and ANNE are very similar to the observed values while all other models are under-estimating the observed acoustic data. The analysis of the errors derived from the predictions on DS3 indicates that ANNE outperforms ASNN. ANNE and DefKrig provide the most unbiased and accurate predictions on an independent dataset, even if AverMod and ASNN fit the training data more effectively. In both cases, GAM2 performs best amongst the additive models.

Comparing predicted grids

A global trend in acoustic density, which is described by low values to the South–Southeast and high values to the North–Northwest and in the centre of the study area, is observed in Figs. 4, 5 and 6. Locally, there is significant variation of observed values, especially in the northeast. The predicted grids (Figs. 9, 10, 11 and 12) were generated to evaluate model predictions on an extended spatial scale and depict species potential distribution in a biologically and oceanographically distinct region, such as the Thermaikos Gulf. The crucial question is which model prediction describes species habitats and distribution more accurately, both globally and locally. Values in grids (Figs. 9, 10, 11 and 12) represent acoustic fish density (black: high values, white: low values). In addition, circles represent the sampled acoustic fish density using an opposite colour-scale to that used for the grids (black circles: low values, white circles: high values).

Predicted grids generated by GAMs are shown in Fig. 9. According to model calibration, correlations, summary statistics and error analysis, GAM2 performs better than the others techniques while GAMM1 performs better than GAM1. Figure 9 provides the possibility of a visual comparison amongst predicted grids and observed values (dots). The three models could be characterized by a common pattern in predicting potential species distribution. However in GAM2 and GAMM1, there is significant evidence of the DDND factor, which causes sudden changes in the predicted acoustic density. This observation reflects the effect of day-dark-dawn-night pattern in the species distribution. Since the environmental data were selected to approximate the real sampling time, the predicted grids correspond to species distribution at the temporal scale of the sampling effort. Model generality was tested mostly in spatial mode, rather than in temporal mode, by using DS2 and DS3. GAM1 depicts a more smooth acoustic density distribution, which is in agreement with the observed global trend but fails to accurately depict the local variation and heterogeneity of the potential predicted species habitats. There are no significant differences between GAMM1 and GAM2 ($r = 0.98$).

DefKrig and Krig generated grids (Fig. 10) differ significantly ($r = 0.36$), showing the different way that these models were developed. Error analysis provides evidence of this particular difference, which is also noticeable in the scale of the predicted density in each map (Fig. 10). DefKrig does not clearly present the global trend of the acoustic density distribution, which is more obvious in Krig. On the other hand, Krig seems to over-fit the training data especially in the centre of the sampling area where the predicted high acoustic density areas mostly overlap the observed high density sampling points.

The generated predicted grids (Fig. 11) derived from ASNN and ANNE are very similar ($r = 0.98$). The DDND pattern is less obvious than in GAMM or GAM2. In general, both grids provide a smooth and un-clustered potential density distribution and they preserve model generality, which is shown by the efficient predictions in DS3. The expected global

Table 9 Models' comparison error indices and summary statistics used for identifying the best fit on the verification dataset

DS3	s_A1-DS3	GAM1	GAM2	GAMM1	DefKrig	Krig	ANNE	ASNN	AverMod
Ave	195.011	121.167	135.830	133.212	193.649	269.270	197.925	179.003	188.242
StDev	218.712	96.831	129.724	128.699	87.956	215.041	78.762	121.495	126.426
RMSE		696.642	558.315	583.008	12.852	700.551	27.485	151.023	63.861
MSqE		485310.678	311715.763	339898.565	165.183	490772.162	755.408	22808.006	4078.247
MAE		119.579	106.025	109.288	134.417	154.808	130.066	104.537	101.902
ASE		1.568	1.468	1.414	1.647	1.452	1.498	1.325	1.315
MStE		−0.338	−0.309	−0.347	−0.006	0.396	0.015	−0.102	−0.044
RMSqSt		3.185	2.912	3.276	0.053	3.735	0.138	0.967	0.415
ME		−73.844	−59.181	−61.799	−1.362	74.258	2.913	−16.008	−6.769

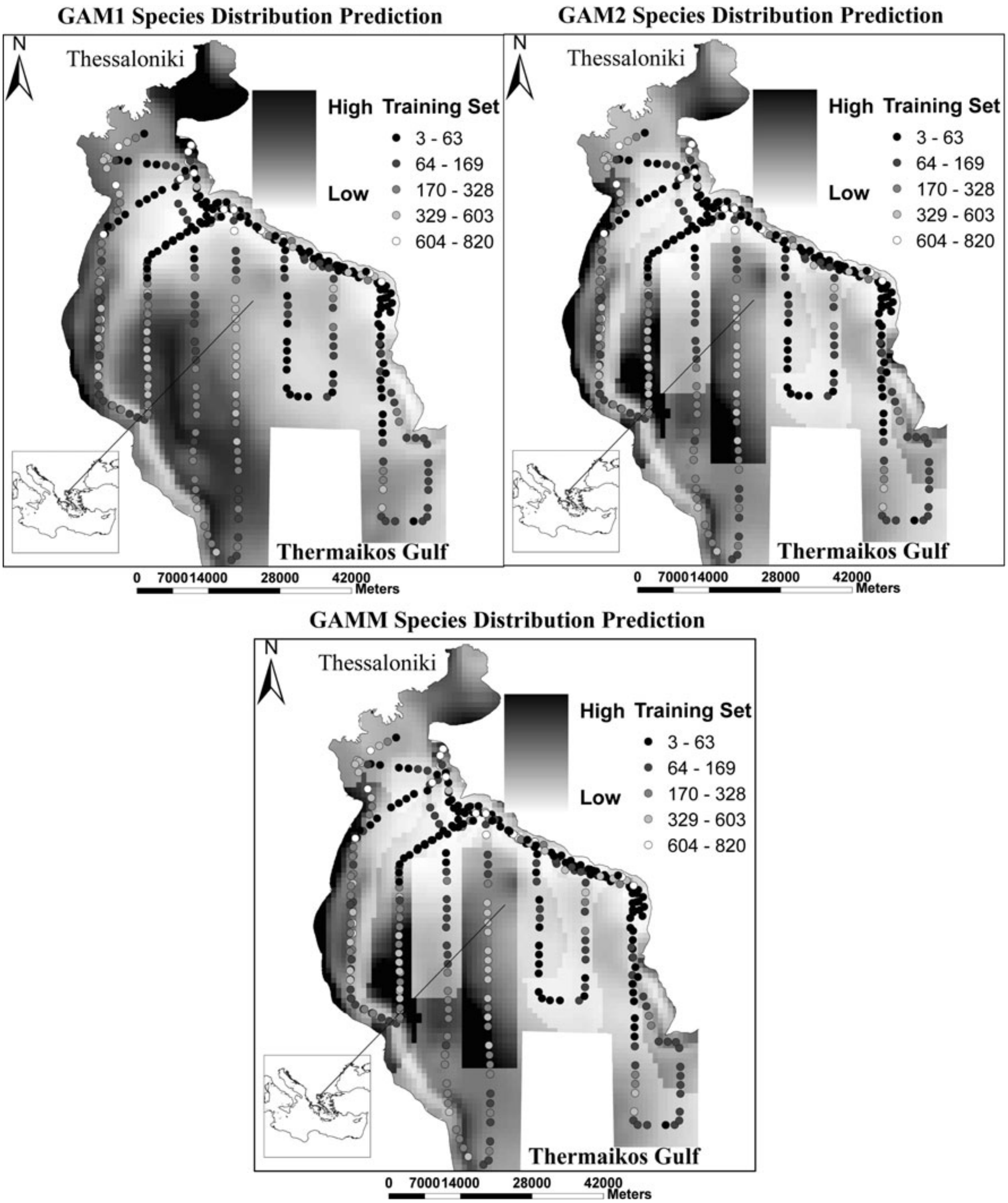


Fig. 9 Predicted grids from GAMs and GAMM that represent acoustic fish density (*black* high values, *white* low values). *Circles* represent the sampled acoustic fish density s_A1 in an opposite colour-scale than grids (*black circles* low values, *white circles* high values). *White dots* on black grid indicates accurate prediction and vice versa

trend is depicted in both grids, especially in coastal areas, but it is not very clear in the centre of the sampling area probably because of the smoother gradient of predictions.

The grid derived by AverMod prediction is shown in Fig. 12. The model calibration process, the error analysis and the correlations provide evidence that this model is characterized by a better predictive

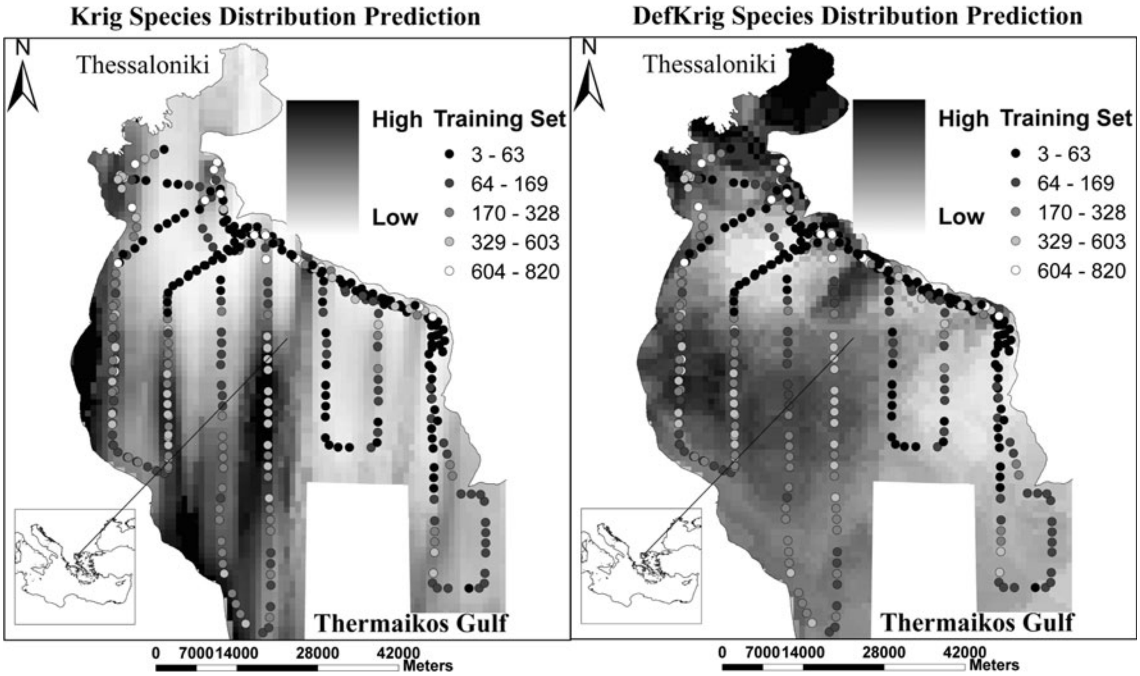


Fig. 10 Predicted grids from Krig and DefKrig that represent acoustic fish density (*black* high values, *white* low values). *Circles* represent the sampled acoustic fish density s_A1 in an opposite colour-scale than grids (*black circles* low values, *white circles* high values). *White dots* on black grid indicates accurate prediction and vice versa

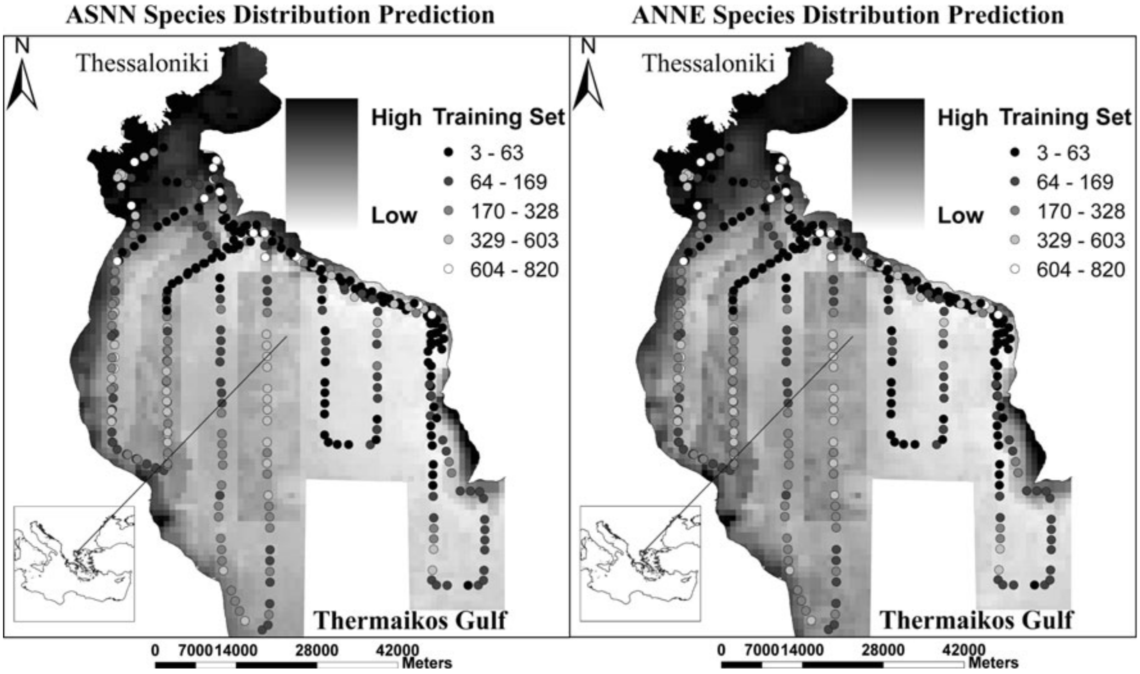


Fig. 11 Predicted grids from ASNN and ANNE that represent acoustic fish density (*black* high values, *white* low values). *Circles* represent the sampled acoustic fish density s_A1 in an opposite colour-scale than grids (*black circles* low values, *white circles* high values). *White dots* on black grid indicates accurate prediction and vice versa

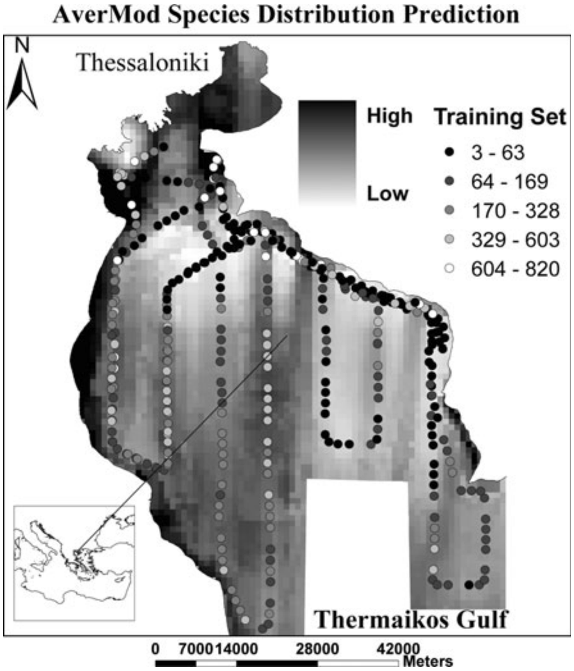


Fig. 12 Predicted grid from AverMod that represents acoustic fish density (*black* high values, *white* low values). *Circles* represent the sampled acoustic fish density $s_{\mathrm{A}}1$ in an opposite colour-scale than grids (*black circles* low values, *white circles* high values). *White dots* on black grid indicates accurate prediction and vice versa

capacity than the other techniques. This grid combines the generality of the ASNN with the local nature of Krig in an additive way, which results in a more efficient potential density distribution. The global trend of the data is preserved while several patches across the study area indicate heterogeneity in species distribution.

Discussion

Regression models

The comparison of GAMs showed that GAM2 performs better than GAM1 and GAMM1 in all aspects. During the selection process, GAM2 showed the best quality characteristics (i.e. lowest AIC, highest deviance explained) and this is reflected in the predictive capacity of GAM2 for all three data sets used for the predictions. Thus GAM2 was the most appropriate model amongst GAMs in fitting the data, predicting an independent dataset and predicting a dataset that covered a larger area than the one used for model training. GAMM1 performs almost equally to GAM2 but the inclusion of autocorrelation did not succeed in improving the predictive ability of the mixed model. GAM1 was formulated to be the simpler model to preserve generality but predictions on the independent set did not meet the original expectations.

Generality, reality and precision are the features that group modelling techniques and only two out of the three can be achieved by a model each time (Levins, 1966). Although GAM1 is a more general model and could be used in a wide range of spatial and temporal predictions, GAM2 is the model that describes the variance of the acoustic data more accurately and precisely. On the other hand, GAMM1 is the only model that deals with spatial autocorrelation issues (Keitt et al., 2002), which can cause bias when modelling acoustic data, even if it does not perform equally to GAM2 in respect of the fitting efficiency and predictive capacity.

In general, GAMs and GAMMs are able to identify specific relationships between the response and the explanatory variables. In this case, high acoustic backscattering is related to areas with high or low values of SLA and either a combination of low DEP in a wide range of PAR or a combination of high DEP and high PAR. According to SLA, which is affected by winds and surface currents in the study area, the above conditions are met along the west and east coasts of the study area. These areas are generally nutrient-rich due to upwelling and river outflows, maintaining high concentrations of small pelagic species. The same areas are pinpointed by the interaction between effects of PAR and DEP. Additional areas in the open sea of the study area, where gyres and other oceanographic features are generated, are indicated by the latter interaction. These features are related to the life history of small pelagic species (Valavanis et al., 2005).

Kriging

The kriging models used in the present study were significantly different. DefKrig is error minimizing oriented, performing much better in the calibration process and error analysis. The predicted grid, though, provides evidence that it is a relatively conservative model. On the other hand, Krig, which is the only model that preserves the variance and the StDev of the training set in its predictions, generates a more realistic potential distribution map, including important distribution heterogeneity but it tends to over-fit the training dataset.

Neural networks

ASNN and ANNE perform almost equally. However, ASNN is slightly better in fitting the observed data while ANNE provides better predictions in the independent dataset. In this study, contrary to the original expectation, ASNN's increased predictive ability did not overcome that of ANNE.

Model characteristics indicate that ASNN outperforms ANNE. A crucial feature of ASNN is the ability to interpret ANNE results by analysing the correlations between data cases in model space (Tetko, 2002b). This innovative approach provides a more complicated simulation of species–environment relationships since it is able to model local and global trends in the data (Tetko, 2002a). If new data become available, ASNN further improves its predictive ability and provides a reasonable approximation of the unknown function without the need to retrain the neural network (Tetko, 2002a). In this study, since there is no evidence of over-estimation or under-estimation of the training set, ASNN could be characterized by generality while conserving the ability to identify local variation in data. This is a result of the combination of an artificial feed-forward neural network, which is a memory-less approach, and *k*-nearest neighbours and the Parzen-window regression that represent the memory-based approaches (Tetko, 2002a). The neural networks can be considered as global models while the other two approaches are usually regarded as local models (Lawrence et al., 1996).

ANNs are generally characterized as a 'black box' approach. The output of ASNN, and the use of approaches like evaluation strip proposed by Elith et al. (2006), which enable the inspection of responses and the effects on the explanatory variables make ASNN more informative and less of a 'black box' approach.

Prediction optimization

Model optimization was performed by combining the predictions of the above techniques in an additive way, by training a new GAM. AverMod is the best calibrated model with increased similarities to the observed data without over-fitting the data and with the greatest predictive capacity. Since, each modelling approach aims to explain a portion of observed data's variation, the combination of two models could provide a model with increased ability to explain data's deviance. In addition, AverMod indicates that the optimal model provides an analogous increase in the efficacy of predictions. The potential species distribution on the predicted grids was justified by other published approaches that include oceanographic processes that are related to species occurrence and species habitat preferences derived from their life-history characteristics.

Elith & Leathwick (2009) stated that the realized species distribution is placed in both environmental and geographic space. In this study the Krig model explains the variability of the sampling data by utilizing exclusively geographic information. On the other hand, ASNN was developed by using mainly environmental data. As a result, the increased accuracy of the AverMod could partially be explained by the fact that it combines a 'geographic' model (Krig) with an 'environmental' model (ASNN) to generate a hybrid model that refers to a parameter space with both environmental and geographic dimensions.

Small pelagic species distribution, biology and comparison to other studies in the area

Small pelagic fish species are highly exploited species, characterized by large fluctuations in abundance and spatial distribution that mainly depend on fluctuations in environmental factors. Oceanographic features such as temperature fronts, eddies, rings and upwelling areas have been related to fish biomass concentration, at least at certain spatial scales (Laurs et al., 1984; Fiedler & Bernard, 1987; Chen et al., 2005). In general, warm, nutrient-depleted water has low chlorophyll-*a* content and cold, nutrient-rich water has high chlorophyll-*a* levels (Georgakarakos & Kitsiou, 2008). Some species, such as anchovy and sardine, which prefer phytoplankton-rich waters during some periods of their life cycle may exhibit a stronger linkage to chlorophyll-*a* concentrations (Ware & Thomson, 2005) than other species at higher trophic levels. European anchovy is also related to the influence of river outflow as shown in the Bay of Biscay, the Adriatic Sea and the Bay of Tunis (Motos et al., 1996; Agostini & Bakun, 2002), in the Catalan Sea and the Gulf of Lions (Palomera et al., 2007; Sabates et al., 2007).

The grids predicted for Thermaikos Gulf identify two distinct areas where fish biomass is concentrated: the west coastline from north to south, which is characterized by the presence of riverine waters, and the central study area, which is related to gyre formation (Somarakis et al., 2002). Both areas could be characterized as nutrient-rich.

Several modelling approaches have been used in Thermaikos Gulf for small pelagic species. Georgakarakos & Kitsiou (2008) applied kriging and co-kriging methods on acoustic fish density data and the conclusions are in agreement with the findings of the present study, especially in the identification of the central part of the study area with high acoustic density. Schismenou et al. (2008) have characterized Thermaikos Gulf as a known fishing ground for anchovy, sardine and sardinela. However, the output of their study was in lower resolution and could not directly be compared to data presented here, since there is no distinguishable distribution fluctuation. This is the case in Tsagarakis et al. (2008) where discriminant function analysis (DFA) is applied on sardine. Despite the low resolution mapping, which is not suitable for habitat heterogeneity identification, the output of DFA is generally in agreement with the outputs of the present study. Giannoulaki et al. (2008) developed GAMs for habitat identification of anchovy for a different time frame to the one used in this study, and they resulted to similar fish distribution trend as the one that identified in this study by the most efficient models. This agreement confirms the long-term persistence of oceanographic features, such as gyres and upwelling that favour species concentration in specific areas.

Comparison and evaluation of the modelling techniques

One step towards improving evaluation of model performance in predicting species distributions is to use independent, well-structured data sets for validation (Elith et al., 2006). For presence data, the best model evaluation is achieved by withholding data (*k*-fold partitioning) for testing model predictions or by comparing RSF predictions using models developed for different periods and study areas (prospective sampling) (Boyce et al., 2002). According to Lehmann et al. (2002), cross-validation or bootstrapping is generally more practical because it creates relatively independent random data subsets and allows the use of all available data in the modelling process. By using entirely independent data sets, there is a risk of comparing different sampling strategies instead of evaluating a model (Lehmann et al., 2002). In this study, the verification set was derived from the initial sampling strategy. On the other hand, the use of such data as a verification set presents similarities to cross-validation techniques. The extensive exploration of the raw data resulted in the construction of a verification set that is unknown to models. The selected validation dataset contains measurements omitted at each step h units (h equals at least 5 nm), whilst h is chosen according to the empirical variogram of both validation set and prediction residuals. The empirical variograms revealed a low autocorrelation, even at distances below the h limit. A similar spatial structure has been encountered in previous surveys (October 1996, May 1997) even in different seasonal conditions (Georgakarakos & Kitsiou, 2008). Results from a comparative study using series of acoustic survey data from five different locations in Europe suggested that the spatial organization of the stock would be more dependent on environmental parameters than on fish abundance (Petitgas, 2001). Thus, DS3 represents an unknown set for the training process of modelling techniques. However, from a biological point of view it maintains the sampling information. The predictive performance of species potential distribution is presented for each separate dataset.

In general, neural networks and especially ASNN are more accurate than the other techniques in fitting the training dataset, while GAMs and especially GAM2 are more flexible in predicting the independent dataset. Kriging on the other hand, is a useful tool for species distribution predictions. However, the approach of predicting distribution by utilizing the spatial correlation of sampling might not be as strong as utilizing a large number of environmental variables that are biologically related to species distribution. In any case, better quality and resolution of sampling data would provide better results since all the above modelling approaches are data sensitive. Amongst biological data sets, acoustic density abundance represents a challenging dataset for modelling studies since it is characterized by important variation. Most modelling techniques use smoothers for fitting the data (spline for GAMs, Parzen-window for ASNN) and, thus, they tend to under-predict high observed values and over-predict low values. As a result, the global error is minimized but the accuracy of predictions regarding local variation of acoustic data declines. The use of high resolution informative explanatory variables partially overcomes the above effect and reveals a more accurate variance for species distribution and thus the potential habitat heterogeneity.

ASNN and ANNE are suggested as the appropriate methods to model sampling data efficiently. Both methods represent the original data more realistically than the other techniques and they are able to identify local and global variation of the data without over-fitting the sampling/training dataset. These characteristics make ASNN and ANNE optimal approaches for presenting the realistic species distribution derived from sampling data and thus, for management purposes, marine protected areas designation, and sampling strategy selection. This study also indicates the predictive ability of GAMs, especially when predictions are required on an expanded spatial scale beyond the sampling area. Thus, GAMs could be used in studies that are oriented towards identifying potential species habitats at a larger spatial scale than in high resolution habitat heterogeneity identification. In addition, GAMs output are able to exhibit species–environment relationships that are easier to interpret than other techniques' outputs.

The overall results indicate that AverMod is the most accurate approach for predicting species distribution and thus could be characterized as the most suitable for the purposes mentioned above. The drawback of this approach is the requirement of developing several different models before combining their predictions. In addition, the combination of different model predictions should be always tested against the initial predictions since it is not a straightforward process. In this study, a GAM was used for combining different models' predictions in an additive way. Instead of GAMs, other techniques could also be used, e.g. training an ASNN by using several predictions as input. In any case, the optimization of species distribution prediction by the approach proposed in this study seems to be very promising.

Conclusions

GAMs are a very flexible approach to model fish density acoustic data and are able to identify specific relationships between the response and the explanatory variables. Kriging on the other hand, is a useful tool for species distribution predictions. However, the approach of predicting species distribution by utilizing the spatial correlation of sampling might not be as strong as utilizing a large number of environmental variables that are biologically related to species distribution. ASNN and ANNE are suggested as the appropriate methods to model sampling data efficiently. Both methods represent the original data more realistically than the other techniques and they are able to identify local and global variation of the data without over-fitting the sampling/training dataset. The prediction optimization approach over-performed the other methods, since it combines a 'geographic' model (kriging) with an 'environmental' model (ASNN) to generate a hybrid model that refers to a parameter space with both environmental and geographic dimensions.

References

Agostini, V. N. & A. Bakun, 2002. 'Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). Fisheries Oceanography 11: 129–142.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723.

Bishop, M., 1995. Neural Networks for Pattern Recognition. Oxford University Press, Oxford.

Bodholt, H., H. Nes & H. Solli, 1989. A new echo sounder system. Proceedings of the Institute of Acoustics (UK) 11(3): 123–130.

Boyce, M. S., P. R. Vernier, S. E. Nielsen & F. K. A. Schmiegelow, 2002. Evaluating resource selection functions. Ecological Modelling 157: 281–300.

Chen, I. C., P. F. Lee & W. N. Tzeng, 2005. Distribution of albacore (*Thunnus alalunga*) in the Indian Ocean and its relation to environmental factors. Fisheries Oceanography 14: 71–80.

Cleveland, W. S., 1994. The Elements of Graphing Data. Hobart Press, Summit. ISBN:0-9634884-1-4.

Elith, J. & J. R. Leathwick, 2009. Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40: 677–697.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. M. C. Overton, A. T. Peterson, S. J. Phillips, K. S. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz & N. E. Zimmermann, 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29: 129–151.

Fiedler, P. C. & H. J. Bernard, 1987. Tuna aggregation and feeding near fronts observed in satellite imagery. Continental Shelf Research 7: 871–881.

Georgakarakos, S. & D. Kitsiou, 2008. Mapping abundance distribution of small pelagic species applying hydroacoustics and co-kriging techniques. Hydrobiologia 612(1): 155–169.

Giannoulaki, M., A. Machias & N. Tsimenides, 1999. Ambient luminance and vertical migration of the sardine *Sardina pilchardus*. Marine Ecology Progress Series 178: 29–38.

Giannoulaki, M., V. D. Valavanis, A. Palialexis, K. Tsagarakis, A. Machias, S. Somarakis & C. Papaconstantinou, 2008. Modelling the presence of anchovy *Engraulis encrasicolus* in the Aegean Sea during early summer, based on satellite environmental data. Hydrobiologia 612(1): 225–240.

Guisan, A. & N. E. Zimmermann, 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135: 147–186.

Guisan, A., J. Edwards, C. Thomas & T. Hastie, 2002. Generalized linear and generalized additive models in studies of species distributions: setting the scene. Ecological Modelling 157: 89–100.

Hastie, T. & R. Tibshirani, 1990. Generalized Additive Models. Chapman & Hall, London.

Hastie, T., R. Tibshirani & J. Friedman, 2009. The Elements of Statistical Learning: Data Mining, Inference, and Prediction, 2nd ed. Springer, Berlin.

Haykin, S., 1994. Neural Networks: A Comprehensive Foundation. Macmillan, New York.

Isaaks, E. H. & R. M. Srivastava, 1989. Applied Geostatistics. Oxford University Press, New York.

Keitt, T. H., O. N. Bjornstad, P. M. Dixon & S. Citron-Pousty, 2002. Accounting for spatial pattern when modelling organism–environment interactions. Ecography 25: 616–625.

Kourafalou, V. & K. Tsiaras, 2007. A nested circulation model for the North Aegean Sea. Ocean Science 3: 1–16.

Laurs, R. M., P. C. Fiedler & D. R. Montgomery, 1984. Albacore tuna catch distributions relative to environmental features observed from satellites. Deep-Sea Research 31: 1085–1099.

Lawrence, S., A. C. Tsoi & A. D. Back, 1996. Function approximation with neural networks and local methods: bias, variance and smoothness. Australian Conference on Neural Networks. Australian National University: 16–21.

Lehmann, A., C. Overton & J. R. Leathwick, 2002. GRASP: generalized regression analysis and spatial prediction. Ecological Modelling 157: 189–207.

Levins, R., 1966. The strategy of model building in population ecology. American Scientist 54: 421–431.

MacLennan, D. N., P. G. Fernandes & J. Dalen, 2002. A consistent approach to definitions and symbols in fisheries acoustics. ICES Journal of Marine Science 59: 365–369.

Matheron, G., 1971. The Theory of Regionalized Variables and its Applications. Ecole Nationale Supérieure des Mines de Paris, Fontainebleau.

Michie, D., D. J. Spiegelhalter & C. Taylor, 1994. Machine Learning, Neural and Statistical Classification. Prentice Hall, Englewood Cliffs.

Moisen, G. G. & T. S. Frescino, 2002. Comparing five modelling techniques for predicting forest characteristics. Ecological Modelling 157: 209–225.

Moran, P. A. P., 1950. Notes on continuous stochastic phenomena. Biometrika 37: 17–23.

Motos, L., A. Uriarte & V. Valéncia, 1996. The spawning environment of the Bay Biscay anchovy (*Engraulis encrasicolus* L.). Scientia Marina 60: 117–140.

Palialexis, A., S. Georgakarakos, K. Lika & V. D. Valavanis, 2009. Use of GIS, remote sensing and regression models for the identification and forecast of small pelagic fish distribution. Proceedings of the Second International Conference on Environmental Management, Engineering, Planning and Economics (CEMEPE 09), June 21–26, Mykonos, Greece.

Palomera, I., M. P. Olivar, J. Salat, A. Sabates, M. Coll, A. Garcia & B. Morales-Nin, 2007. Small pelagic in the NW Mediterranean Sea: an ecological review. Progress in Oceanography 74: 377–396.

Pearce, J. & S. Ferrier, 2000. Evaluating the predictive performance of habitat models developed using logistic regression. Ecological Modelling 133: 225–245.

Petitgas, P., 2001. Geostatistics in fisheries survey design and stock assessment: models, variances and applications. Fish and Fisheries 2: 231–249.

Potts, J. M. & J. Elith, 2006. Comparing species abundance models. Ecological Modelling 199: 153–163.

Poulos, S. E., G. T. Chronis, M. B. Collins & V. Lykousis, 2000. Thermaikos Gulf Coastal System, NW Aegean Sea: an overview of water/sediment fluxes in relation to air-land-ocean interactions and human activities. Journal of Marine Systems 25: 47–76.

R Development Core Team, 2005. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria [available on internet at http://www.Rproject.org].

Redfern, J. V., M. C. Ferguson, E. A. Becker, K. D. Hyrenbach, C. Good, J. Barlow, K. Kaschner, M. F. Baumgartner, K. A. Forney, L. T. Ballance, P. Fauchald, P. Halpin, T. Hamazaki, A. J. Pershing, S. S. Qian, A. Read, S. B. Reilly, L. Torres & F. Werner, 2006. Techniques for cetacean–habitat modeling: a review. Marine Ecology Progress Series 310: 271–295.

Richards, C. L., B. C. Carstens & L. Knowles, 2007. Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. Journal of Biogeography 34: 1833–1845.

Ripley, B. D., 1996. Pattern Recognition and Neural Networks. Cambridge University Press, Cambridge.

Sabates, A., M. P. Olivar, J. Salat, I. Palomera & F. Alemany, 2007. Physical and biological processes controlling the distribution of fish larvae in the NW Mediterranean. Progress in Oceanography 74: 355–376.

Schismenou, E., M. Giannoulaki, V. D. Valavanis & S. Somarakis, 2008. Modeling and predicting potential spawning habitat of anchovy (*Engraulis encrasicolus*) and round sardinella (*Sardinella aurita*) based on satellite environmental information. Hydrobiologia 612(1): 201–214.

Schröder, B., 2008. Challenges of species distribution modeling belowground. Journal of Plant Nutrition and Soil Science 171: 325–337.

Shepherd, A. J., 1997. Second-Order Methods for Neural Networks. Springer-Verlag, London: 145.

Somarakis, S., P. Drakopoulos & V. Filippou, 2002. Distribution and abundance of larval fishes in the northern Aegean Sea-Eastern Mediterranean—in relation to early summer oceanographic conditions. Journal of Plankton Research 24: 339–357.

Tetko, I. V., 2002a. Associative neural network. Neural Processing Letters 16: 187–199.

Tetko, I. V., 2002b. Neural network studies. 4. Introduction to associative neural networks. Journal of Chemical Information in Computer Science 42: 717–728.

Tetko, I. V. & V. Y. Tanchuk, 2002. Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. Journal of Chemical Information in Computer Science 42: 1136–1145.

Tetko, I. V., D. J. Livingstone & A. I. Luik, 1995. Neural network studies. 1. Comparison of overfitting and overtraining. Journal of Chemical Information in Computer Science 35: 826–833.

Tetko, I. V., I. Sushko, A. K. Pandey, H. Zhu, A. Tropsha, E. Papa, T. Oberg, R. Todeschini, D. Fourches & A. Varnek, 2008. Critical assessment of QSAR models of environmental toxicity against *Tetrahymena pyriformis*: focusing on applicability domain and overfitting by variable selection. Journal of Chemical Information and Modeling 48(9): 1733–1746.

Tsagarakis, K., A. Machias, S. Somarakis, M. Giannoulaki, A. Palialexis & V. D. Valavanis, 2008. Habitat discrimination of juvenile sardines in the Aegean Sea using remotely sensed environmental data. Hydrobiologia 612(1): 215–223.

Tsimenides, N., G. Bazigos, S. Georgakarakos & A. Kapantagakis, 1992. Distribution of acoustic pelagic fish populations in the northern Aegean Sea. Proceedings of the 1st World Fisheries Congress 5: 33–42.

Valavanis, V. D., 2002. Geographic Information Systems in Oceanography and Fisheries. Taylor & Francis, London: 240.

Valavanis, V. D., Kapantagakis, A., Katara, I., Palialexis, A. 2004. Critical regions: A GIS-based model of marine productivity hotspots. Aquatic Sciences 66(1): 139–148.

Valavanis, V. D., Katara, I., Palialexis, A. 2005. Marine GIS: Identification of mesoscale oceanic thermal fronts. International Journal of Geographical Information Science 19(10): 1131–1147.

Valavanis, V. D., G. J. Pierce, A. F. Zuur, A. Palialexis, A. Saveliev, I. Katara & J. Wang, 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612(1): 5–20.

Walline, P. D., 2007. Geostatistical simulations of eastern Bering Sea walleye pollock spatial distributions, to estimate sampling precision. ICES Journal of Marine Science 64: 559–569.

Ware, D. M. & R. E. Thomson, 2005. Bottom-up ecosystem trophic dynamics determine fish production in the Northeast Pacific. Science 308: 1280–1285.

Wood, S. N., 2006. Generalized Additive Models: An Introduction with R. CRC Press, London.

Wood, S. N. & N. H. Augustin, 2002. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. Ecological Modelling 157: 157–177.

Zuur, A. F., E. N. Ieno & G. M. Smith, 2007. Analysing Ecological Data. Springer Series: Statistics for Biology and Health. Springer, New York.

Zuur, A. F., E. N. Ieno & C. S. Elphick, 2010. A protocol for data exploration to avoiding common statistical problems. Methods in Ecology and Evolution 1: 3–14.

Hydrobiologia (2011) 670:189–199
DOI 10.1007/s10750-011-0663-y

ECOSYSTEMS AND SUSTAINABILITY

Identification of subpopulations in pelagic marine fish species using amino acid composition

Isabel Riveiro · Cástor Guisande · Paula Iglesias · Gualtiero Basilone · Angela Cuttitta · Ana Giráldez · Bernardo Patti · Salvatore Mazzola · Angelo Bonanno · Alba-Ruth Vergara · Isabel Maneiro

Published online: 7 April 2011
© Springer Science+Business Media B.V. 2011

Abstract The spatial stock complexity of marine fish species requires that population structure is taken into account in fisheries management. The aim of this study was to determine whether the amino acid composition (AAC) of the adult fish allows the identification of subpopulations within the stock. During a cruise in November 2003 along the entire Mediterranean coast of Spain, individuals were collected of the following pelagic species: *Sardina pilchardus*, *Sardinella aurita*, *Engraulis encrasicolus*, *Trachurus trachurus*, *Trachurus mediterraneus*, *Scomber scombrus* and *Scomber colias*. Individuals of *S. pilchardus* and *E. encrasicolus* were also collected from the waters of the Strait of Sicily in 2002 and 2003. The AAC of the fish eyes was seen to be species specific, and therefore, the differences in AAC among species may be based on inherited characters. Moreover, a clear differentiation was seen between the Spanish and Sicilian populations of *S. pilchardus* and *E. encrasicolus*. Furthermore, in the Spanish waters of the Mediterranean Sea, discriminant analysis revealed a substantial separation between the northern and southern subpopulations of *S. pilchardus*, *S. aurita* and *E. encrasicolus*. Temporal variations in AAC within species in each area were lower than the spatial variations observed

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

---

I. Riveiro (✉)
Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Subida a Radio Faro 50, 36390 Vigo, Spain
e-mail: isabel.riveiro@vi.ieo.es

C. Guisande
Facultad de Ciencias del Mar, Universidad de Vigo, Lagoas-Marcosende, 36200 Vigo, Spain

P. Iglesias
Instituto Español de Oceanografía, Centro Oceanográfico de La Coruña, Paseo Marítimo Alcalde Francisco Vázquez n° 10, Apdo. 130, 15001 La Coruña, Spain

G. Basilone · A. Cuttitta · B. Patti · S. Mazzola · A. Bonanno
Consiglio Nazionale delle Ricerche, Istituto per l'Ambiente Marino Costiero, Sede di Mazara del Vallo, via L. Vaccara 61, Mazara del Vallo, Trapani, Italy

A. Giráldez
Instituto Español de Oceanografía, Centro Oceanográfico de Málaga, Puerto Pesquero s/n, Apdo. 285, Fuengirola, 29640 Málaga, Spain

A.-R. Vergara
Instituto de Investigaciones Marinas (CSIC), Calle Eduardo Cabello 6, 36208 Vigo, Spain

I. Maneiro
Facultad de Ciencias, Universidad de A Coruña, Campus da Zapateira, 15008 La Coruña, Spain

among areas for each species, probably reflecting the influence on the AAC of the contrasting environmental characteristics of each area. Our results indicate that the ACC of the eyes in adult fish is a good tool for discriminating among subpopulations in pelagic marine fish species.

Keywords Pelagic fishes · Amino acid composition · Population discrimination

Introduction

Fisheries management is usually based upon stock units. A fish stock can be defined as a population adapted to a particular environment, having genetic differences from other stocks as a consequence of this adaptation (MacLean & Evans, 1981).

Fish stock discrimination has been measured in a number of ways (Pawson & Jennings, 1996; Cadrin et al., 2005). Some of these stock identification techniques have been questioned, as they reflect environmental distinctiveness rather than reproductive isolation (see Swain & Foote, 1999). Genetic studies often fail to support the stock differentiation suggested by morphologic, meristic, physiologic and/or ecologic variability (Ryman et al., 1984; Kinsey et al., 1994; Turan et al., 1998). However, these methods reveal that not all individuals within the stock are affected by the same environmental conditions and, therefore, failure to recognize or to account for the spatial complexity of subpopulations within a stock may lead to the erosion of subpopulation units, with unknown consequences on stock viability (Stephenson, 1999).

The recognition of this complex spatial structure within areas, traditionally assumed to contain a single stock, led to the 'dynamic population structure concept' (Smith & Jamieson, 1986), which suggests that most fish species exist as semi-independent but not completely isolated breeding units. This concept gained support among biologists against that of a pure or discrete stock (Spanakis et al., 1989). This dynamic population model is the equivalent to considering the dynamic of marine fish populations from a metapopulation perspective (MacQuinn, 1997; Bailey et al., 1999; Stephenson, 1999; Thorrold et al., 2001; Smedbol & Wroblewski, 2002).

Andrewartha & Birch (1954) suggested that populations are structured in 'local populations' connected by migration. Levins (1970) developed this concept by coining the term metapopulation to describe a population consisting of many local populations.

In some marine fish species, such as herring (MacQuinn, 1997) and sardine (Carrera & Porteiro, 2003), a new perspective of metapopulation, different from the classical concept of Levins (1970), has been proposed to explain population dynamics. Metapopulation is a system of discrete local populations each of which, to a large extent, determines its own internal dynamics, but with a degree of identifiable and nontrivial demographic influence from other local populations through the dispersal of individuals, according to the definition of Kritzer & Sale (2004). These authors put less emphasis on the extinction dynamics and defined metapopulation according to spatial structure (and the role that interpopulation exchange plays in local population size and stability).

A necessary condition for metapopulation persistence in the face of unstable subpopulations is asynchronous local dynamics (Hanski, 1999), which means that subpopulations may experience different environmental conditions. This indicates that the factors structuring habitat suitability may vary among subpopulations. Therefore, for a correct stock management it is necessary to identify subpopulations within the stock that are affected by different environmental conditions, and to manage these as discrete groups.

The problem is to find a methodology that allows the discrimination of subpopulations within the stock (Waldman, 1999) and, for that reason, some ecologists may remain unimpressed by the metapopulation concept (Hanski, 1999). Fish subpopulations are difficult to define from traditional fisheries data, or to discriminate by conventional stock identification techniques (Stephenson, 1999). The methodology must be based on inherited characters, which also must reflect environmental distinctiveness and must be species specific (Booke, 1999). To discriminate stocks or subpopulations of fishes, the signal from among-stock variation must exceed the noise of within-stock variation (Waldman, 1999).

The amino acid composition (AAC) of the eggs and larvae of marine fishes have been successfully used to discriminate among species and spawning areas within species (Riveiro et al., 2003). One of the main problems of the study of biochemical composition is the finding of correct part of the fish body to analyse, as many fish change their biochemical composition rapidly, depending on the characteristics of the geographic area.

The aim of this study was to determine whether the AAC of some of the tissues of adult fish allows the identification of subpopulations in several pelagic fish species in the Mediterranean Sea.

Materials and methods

Sampling collection

To determine the best part of the fish body for discriminating among species, individuals of the species *Sardina pilchardus* and *Engraulis encrasicolus* were collected in the Sicilian Channel on-board fishing vessels in March 2002 (Fig. 1). The liver, blood, heart and eyes of males and females were extracted from the fishes and transferred to ultracentrifuge plastic vials and immediately frozen at −32°C. The number of individuals analysed is shown in Table 1.

For the identification of subpopulations, individuals of the species *Sardina pilchardus*, *Trachurus trachurus*, *Trachurus mediterraneus*, *Scomber scombrus*, *Scomber colias*, *Engraulis encrasicolus* and *Sardinella aurita* were collected in November–December 2003 during the ECOMED survey along the Spanish Mediterranean coast (Fig. 1, Table 1). Two areas were considered for collecting samples: the northern and southern areas separated by San Antonio Cape. Both areas have been shown to be potentially favourable habitats for fish reproduction, but with contrasting conditions (Agostini & Bakun, 2002).

Individuals of the species *E. encrasicolus* and *S. pilchardus* were collected in December 2003, again on-board fishing vessels in the same areas as in the previous sampling in the Sicilian Channel (Fig. 1, Table 1). The eyes of males and females were extracted from the fishes and transferred to ultracentrifuge plastic vials and immediately frozen at −32°C. The number of individuals analysed by species and group origins is shown in Table 1.

Analyses of amino acids

Total amino acids were analysed by high-performance liquid chromatography (HPLC) using a Waters Alliance System, a Waters 474 scanning fluorescence detector and a Waters 15 X 3.9 Nova-Pack C18 column following the method described by Van Wandelen & Cohen (1997). The AAC was analysed using the Waters AccQ-Tag® Chemistry Package. For the hydrolysis procedure for AAC, eyes were introduced in HCl 6 N at 114°C for 24 h. Afterwards, vials were introduced in a desiccator with NaOH at 55°C in order to dry the samples. For the derivatization procedure,

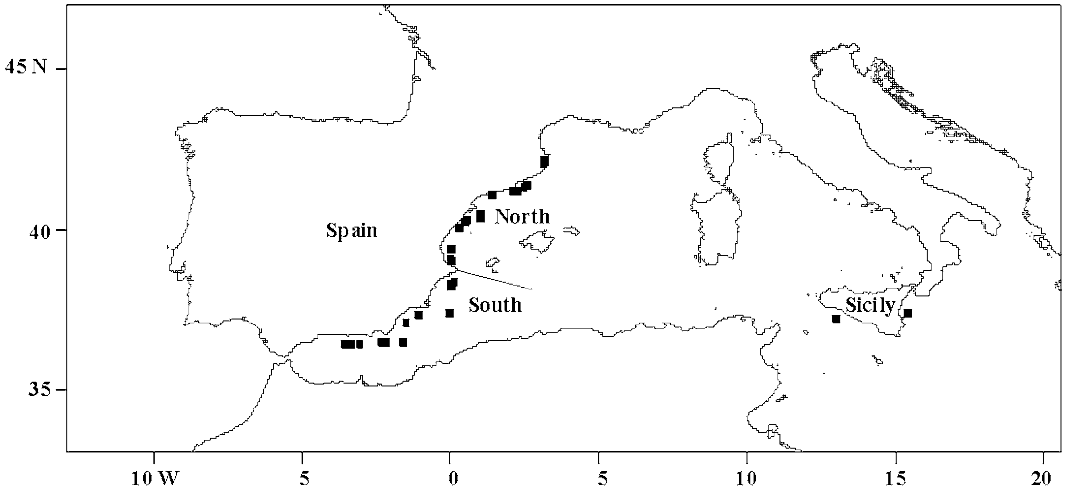


Fig. 1 Mediterranean Sea divided in arbitrary areas for this study and the sampling locations of each area (*squares*)

Table 1 Number of individuals collected for the analysis of the AAC of the different fish tissues in Sicily and Spanish Mediterranean Coast in 2002 and 2003

Year:	2002				2003		
Tissue:	Blood	Heart	Liver	Eye	Eye		
Area:	Sicily				Spain		Sicily
					North	South	
Sardina pilchardus	15	10	9	10	45	21	4
Engraulis encrasicolus	26	19	19	20	47	27	
Sardinella aurita					49	25	12
Trachurus trachurus					30	17	
Trachurus mediterraneus					12	17	
Scomber scombrus					8		
Scomber colias					22		

samples were redissolved in HCl 0.1 N and ACCQ-Fluor® Borate Buffer and AccQ-Fluor® Reagent were added and samples were placed for 10 min at 55°C. Conditions for amino acid separation for the mobile phase were as follows: A (Aqueous buffer, AccQ-Tag Eluent), B (acetonitrile), C (bidistilled water). A total of 15 amino acids were analysed: aspartic acid (ASP), serine (SER), glutamic acid (GLU), glycine (GLY), histidine (HIS), arginine (ARG), threonine (THR), alanine (ALA), proline (PRO), tyrosine (TYR), valine (VAL), lysine (LYS), isoleucine (ILE), leucine (LEU) and phenylalanine (PHE). Cysteine and methionine were destroyed during hydrolysis with 6 N HCl while amino acid standard H NCI0180 Pierce H was used for the identification and quantification of amino acids.

Discrimination among subpopulations

To determine whether it is possible to discriminate among subpopulations within species according to the AAC of the adult tissues, a standard multivariate discriminant analysis was applied to the amino acid percentages (Guisande et al., 2006). The variables that contribute most to sample differentiation were identified by their correlation to the discriminant functions and by a one-way ANOVA. The resultant discriminant functions were used to classify the individuals into samples, and the percentage of individuals correctly classified into the original sample was used to evaluate the classification success rate. All calculations were performed using SPSS software (SPSS Inc.).

Discriminant analysis has become a powerful tool in biological research and stock identification and has been successfully used to discriminate among species, stocks or spawning areas in pelagic fishes according to the AAC of the larvae (Riveiro et al., 2003; Cuttitta et al., 2006), elemental composition in fish otoliths (Geffen et al., 2003), fish parasites (Timi et al., 2005; Marques et al., 2006), etc.

Results

Table 2 shows the results of the discriminant analysis applied to the AAC of the different parts of the body

Table 2 Results of a discriminant analysis showing the percentage of individuals correctly classified according to their AAC

	Real group	Predicted groups	
		S. pilchardus	*E. encrasicolus*
Blood (97.4%)	*S. pilchardus*	100	0
	E. encrasicolus	3.8	96.2
Heart (100%)	*S. pilchardus*	100	0
	E. encrasicolus	0	100
Liver (100%)	*S. pilchardus*	100	0
	E. encrasicolus	0	100
Eye (100%)	*S. pilchardus*	100	0
	E. encrasicolus	0	100

of the adult fish from the Sicilian Channel in March 2002. It can be seen that the AAC of the blood was not the best fish tissue for discriminating among species, as the percentage of individuals correctly classified for the sardine and the anchovy was not 100%. Nevertheless, the AAC of the liver, heart an eyes were good indicators of the species, as they allowed a perfect separation of the sardine and the anchovy (Table 2).

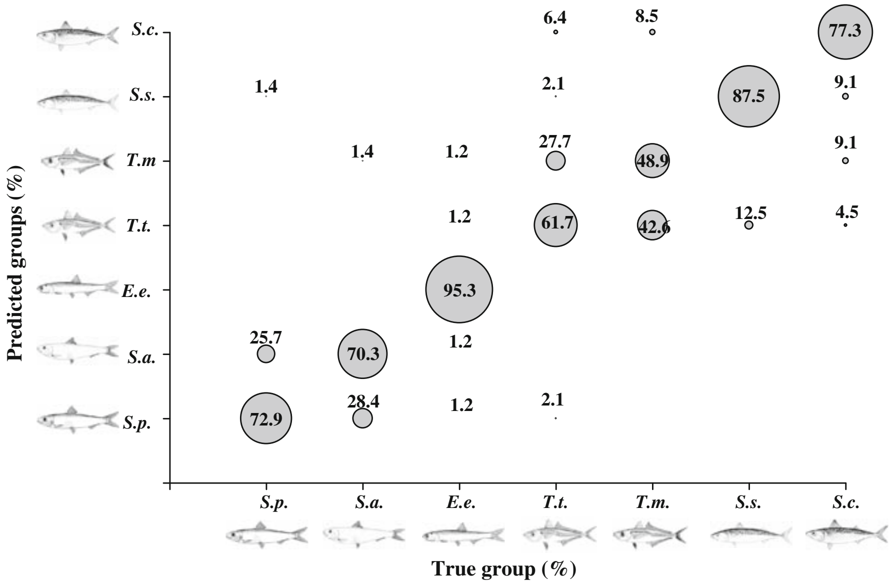
Following these preliminary results, it was decided that only fish eyes would be sampled, as this was the easiest way to obtain samples without having to dissect fish.

A discriminant analysis performed on the data of the AAC of eyes collected in 2003 of *S. pilchardus* (*S.p.*), *S. aurita* (*S.a.*), *E. encrasicolus* (*E.e.*), *T. trachurus* (*T.t.*), *T. mediterraneus* (*T.m.*), *S. scombrus* (*S.s.*) and *S. colias* (*S.c.*) from the Sicilian and Spanish coasts, showed that it is possible to identify each fish species according to their AAC (Fig. 2). The significant functions were from 1 to 5 (Wilk's lambda, $P <$ 0.005). The first two components of the discriminant analysis explained 62.4 and 29.1% of variance, respectively (Fig. 3). The percentage of fish correctly classified according to their AAC represented 73.7% of cases with cross validation (Fig. 3). Among the different species studied, the major similarities were found between *T. trachurus* and *T. mediterraneus* and between *S. pilchardus* and *S. aurita* (Fig. 3). The AAC of eyes mainly differs among species in the proportion of histidine, glycine and valine.

In a discriminant analysis performed on the AAC of the eyes of adults collected in 2003 from different areas of the Mediterranean coast of Spain (north and south) and from the Sicilian coast (only *S. pilchardus* and *E. encrasicolus*) (Table 3), the percentage of groups correctly classified was 64.3% for *S. pilchardus*, 75.7% for *S. aurita*, 74.4% for *E. encrasicolus*, 85% for *T. trachurus* and 86.9% for *T. mediterraneus* (Fig. 4). It was not possible to perform discriminant analysis for *S. scombrus* and *S. colias*, because the samples of these species were only found in the northern area of the Mediterranean coast of Spain. The AAC of eyes mainly differs among species in the proportion of glycine, proline and threonine for *S. pilchardus*, serine, threonine and arginine for *S. aurita*, tyrosine, serine and proline for *E. encrasicolus*, glycine, isoleucine and proline for *T. trachurus* and tyrosine, serine and histidine for *T. mediterraneus*.

The use of AAC allows discriminating among species and among areas within species: between the species of the Sicilian and Spanish coasts (*S. pilchardus* and *E. encrasicolus*), and also between those of the northern and southern areas of the Mediterranean Sea (Fig. 4). It is important to point out that the results of the discrimination between the Spanish and Sicilian coasts have to be interpreted with caution, because of the differences in sample

Fig. 2 Plot of the results of a discriminant analysis showing the percentage of fish species correctly classified from the original data according to the amino acid composition of the eyes. Species abbreviations as shown in Materials and Methods



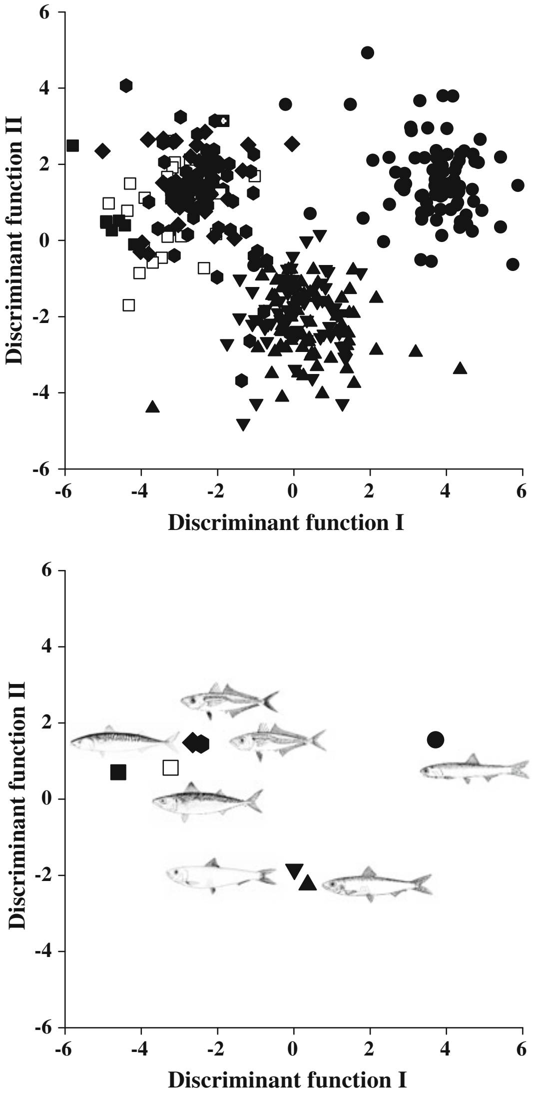


Fig. 3 Plot of the first two discriminant function scores obtained from the discriminant analysis performed on the amino acid composition of fish eyes. *Sardina pilchardus* (*triangle*), *Sardinella aurita* (*inverted triangle*), *Engraulis encrasicolus* (*circle*), *Trachurus trachurus* (*hexagon*), *Trachurus mediterraneus* (*diamond*), *Scomber scombrus* (*filled square*), *Scomber colias* (*open square*). **a** Plot of the individuals. **b** Plot of the species centroids

size between the two areas, especially given the small amount of data from the Sicilian coast (Table 1).

Furthermore, it was necessary to test whether there is temporal variation in the AAC within species in the same area. A discriminant analysis was performed on the AAC of the eyes of adult fish collected in 2003 for the different distribution areas of the Mediterranean coast of Spain (north and south) and for *S. pilchardus* and *E. encrasicolus* on the Sicilian coast. However, for Sicily, data from individuals collected in 2002 (without assigned group) were also included. Eventually, all data from Sicily in 2002 were assigned to the group for Sicily in 2003 (Table 4).

Discussion

The starting point for using the AAC for stock separation applications is that the habitat characteristics from separate geographical areas could influence AAC for fish living in those areas. Although, to our knowledge, it had never been used before, our results reveal that the AAC of the eyes provides a method for practical discrimination of fish subpopulations, where the amount of exchanges between subpopulations is low, but sufficient to prevent genetic differentiation (see Lleonart & Maynou, 2003). Therefore, this method is useful for studying the fine-scale population structure of marine fishes.

Our results show that it is possible to discriminate pelagic fish species and fish distribution based on the AAC of the eyes. A good differentiation was seen between the Spanish and Sicilian populations of *S. pilchardus* and *E. encrasicolus,* which concur with previous studies that have shown the existence of several subpopulations of both species in the Mediterranean Sea (Larrañeta, 1968; Spanakis et al., 1989; Bembo et al., 1996a, b; Tudela, 1999; Borsa, 2002). These observed differences could be a result of the contrasting environmental conditions experienced by fish in the studied areas (Agostini & Bakun, 2002).

In the Spanish Mediterranean Sea, discriminant analysis also produced a good separation between northern and southern subpopulations of *S. pilchardus*, *S. aurita* and *E. encrasicolus*. Differences in AAC are in agreement with genetic differences (Ramon & Castro, 1997), with differences in growth rates (Alemany & Alvárez, 1993) and with differences in biometric, physiologic and fisheries characteristics (Larrañeta, 1968), reported for northern and southern subpopulations of *S. pilchardus.*

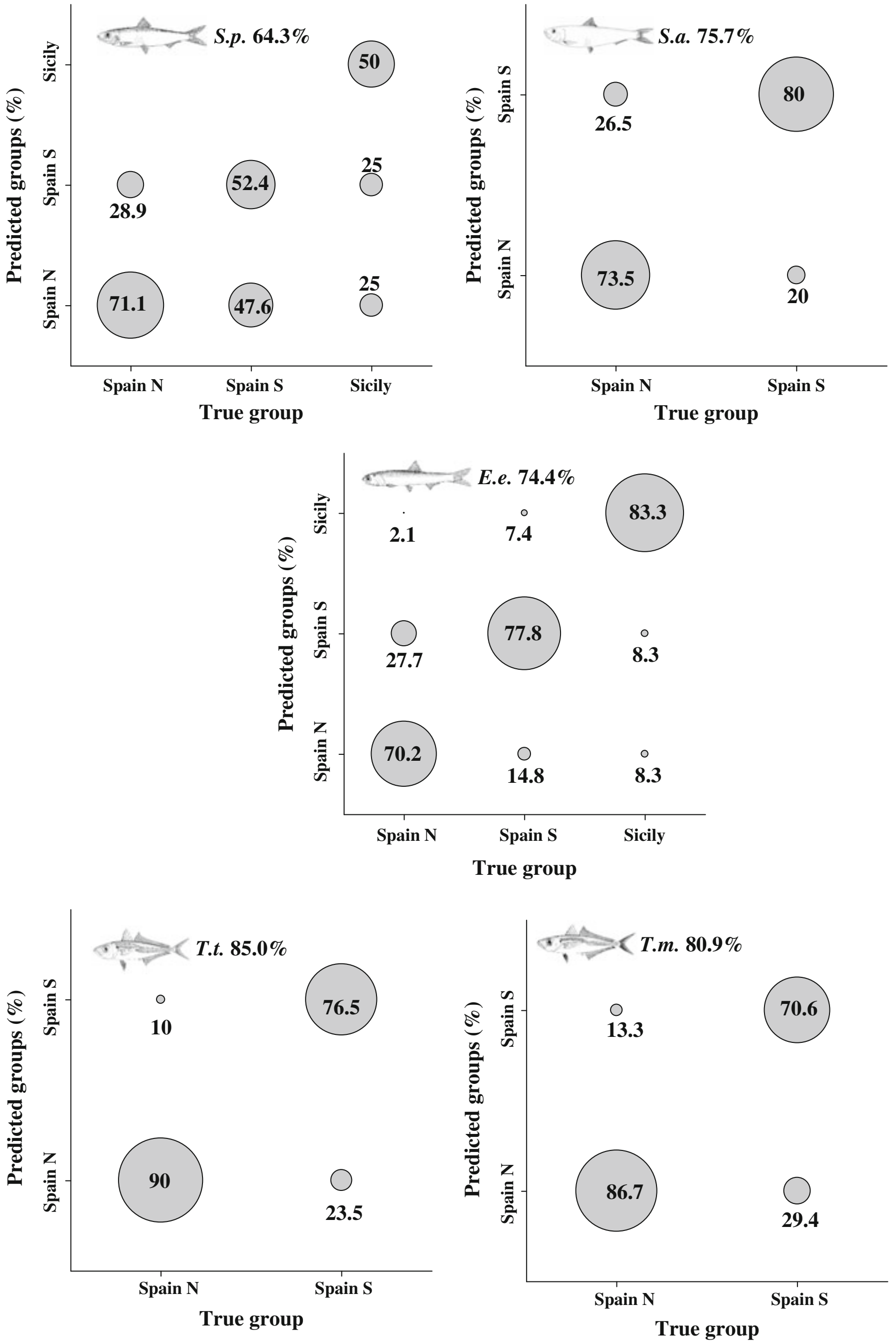
Genetic techniques cannot detect differences when there are low levels of larval or adult mixing between subpopulations (Edmonds et al., 1989; Hartl & Clark, 1989; Swan et al., 2006) and, therefore, are not useful

Table 3 Amino acid composition (mean ± SD weight percentage of yields of total amino acids) of fish eyes

	S. pilchardus			*S. aurita*		*E encrasicolus*			*T. trachurus*		*T. mediterraneus*		*S. scomber*	*S. colias*
	Spain N	Spain S	Sicily	Spain N	Spain S	Spain N	Spain S	Sicily	Spain N	Spain S	Spain N	Spain S	Spain N	Spain N
ASP	11.3 ± 0.9	11.3 ± 1.5	10.8 ± 0.5	11.5 ± 1.6	12.6 ± 1.8	11.1 ± 13	12.2 ± 1.5	10.3 ± 0.9	11.4 ± 1.5	11.6 ± 1.7	11.9 ± 1.6	11.3 ± 1.7	10.8 ± 1.0	11.8 ± 1.6
SER	5.7 ± 0.6	6.1 ± 0.9	6.2 ± 0.9	6.1 ± 0.8	5.5 ± 0.5	5.7 ± 0.9	5.2 ± 0.6	6.6 ± 0.8	6.3 ± 0.8	6.3 ± 0.8	5.9 ± 1.0	6.7 ± 0.8	6.9 ± 0.8	6.0 ± 0.7
GLU	14.4 ± 3.9	12.4 ± 3.6	10.4 ± 3.0	12.8 ± 3.4	14.7 ± 3.6	13.3 ± 3.6	15.5 ± 3.8	10.3 ± 2.3	14.0 ± 3.8	15.1 ± 3.8	13.8 ± 3.3	12.9 ± 3.7	12.3 ± 3.7	15.2 ± 3.6
GLY	10.0 ± 0.9	10.0 ± 0.7	11.8 ± 1.4	10.1 ± 1.0	9.6 ± 0.7	9.2 ± 0.7	8.4 ± 0.5	9.9 ± 0.7	10.4 ± 0.9	9.1 ± 1.0	9.8 ± 0.8	9.9 ± 1.0	12.3 ± 0.5	11.2 ± 1.0
HIS	3.4 ± 0.4	3.4 ± 0.2	3.0 ± 0.3	3.3 ± 0.4	3.3 ± 0.3	4.3 ± 0.3	3.9 ± 0.3	4.1 ± 0.4	3.4 ± 0.3	3.2 ± 0.3	3.4 ± 0.2	3.2 ± 0.4	3.3 ± 0.2	3.7 ± 0.6
ARG	5.7 ± 0.4	5.9 ± 0.5	5.7 ± 0.6	6.3 ± 0.6	5.8 ± 0.7	6.7 ± 0.6	6.2 ± 0.6	6.6 ± 0.5	7.0 ± 0.8	6.6 ± 0.8	6.9 ± 1.0	6.8 ± 0.9	6.2 ± 1.0	5.9 ± 1.0
THR	4.5 ± 0.4	4.6 ± 0.4	5.0 ± 0.8	4.6 ± 0.4	4.2 ± 0.4	4.4 ± 0.3	4.2 ± 0.9	4.3 ± 0.5	4.8 ± 0.4	4.6 ± 0.6	4.8 ± 0.4	4.8 ± 0.5	4.7 ± 0.3	4.7 ± 0.5
ALA	6.3 ± 0.8	6.5 ± 0.8	6.7 ± 1.3	6.0 ± 0.7	6.0 ± 0.4	5.7 ± 0.5	5.9 ± 0.8	6.1 ± 1.1	5.6 ± 0.5	5.8 ± 1.9	5.6 ± 0.7	6.1 ± 0.8	6.3 ± 0.4	5.6 ± 0.8
PRO	5.2 ± 0.4	5.3 ± 0.4	5.8 ± 0.6	5.2 ± 0.6	5.0 ± 0.4	4.9 ± 0.4	4.9 ± 0.4	5.4 ± 0.4	5.0 ± 0.4	4.6 ± 0.5	4.9 ± 0.4	5.2 ± 0.4	5.8 ± 0.3	5.0 ± 0.7
TYR	6.6 ± 0.8	6.6 ± 0.8	6.4 ± 1.7	6.8 ± 0.8	6.4 ± 0.7	7.6 ± 0.7	6.8 ± 0.8	9.4 ± 1.5	7.1 ± 0.8	6.5 ± 0.8	7.0 ± 0.6	5.9 ± 0.5	6.5 ± 0.6	7.2 ± 1.4
VAL	5.5 ± 0.7	5.8 ± 0.6	6.0 ± 0.5	5.7 ± 0.5	5.7 ± 0.4	5.7 ± 0.5	5.8 ± 0.4	5.4 ± 0.6	4.9 ± 0.5	5.4 ± 0.8	5.2 ± 0.5	5.5 ± 0.5	4.7 ± 0.3	4.4 ± 0.6
LYS	5.4 ± 0.7	5.6 ± 0.8	5.1 ± 0.7	5.2 ± 0.9	5.4 ± 0.4	5.0 ± 0.6	5.2 ± 0.9	4.6 ± 0.6	4.8 ± 0.6	5.4 ± 1.8	4.8 ± 0.8	5.3 ± 0.7	5.0 ± 0.4	4.4 ± 1.0
ILE	3.8 ± 0.5	4.0 ± 0.4	4.1 ± 0.2	4.0 ± 0.4	4.0 ± 0.3	4.3 ± 0.5	4.3 ± 0.3	3.9 ± 0.5	3.7 ± 0.4	4.3 ± 0.6	4.1 ± 0.5	4.3 ± 0.4	3.6 ± 0.3	3.3 ± 0.6
LEU	6.3 ± 0.7	6.5 ± 0.6	6.9 ± 1.0	6.3 ± 0.6	6.1 ± 0.5	6.0 ± 0.5	5.9 ± 0.6	6.1 ± 0.6	5.9 ± 0.5	6.0 ± 0.9	6.1 ± 0.5	6.4 ± 0.6	6.1 ± 0.2	5.7 ± 0.7
PHE	5.9 ± 0.6	6.1 ± 0.5	6.1 ± 0.8	6.0 ± 0.7	5.8 ± 0.6	6.2 ± 0.6	5.7 ± 0.6	7.0 ± 0.6	5.6 ± 0.5	5.6 ± 0.9	5.8 ± 0.5	5.5 ± 0.5	5.5 ± 0.4	5.8 ± 1.1

Amino acid abbreviations as in text

Fig. 4 Plot of the results of a discriminant analysis showing the percentage of fish species from different areas correctly classified from the original data according to the amino acid composition of the eyes. Species abbreviations as shown in Materials and Methods



for resolving the fine-scale population structure of marine fishes (to discriminate among subpopulations). Relatively low levels of exchange between stocks, negligible from a management perspective, may be sufficient to ensure genetic homogeneity (Ward & Grewe, 1994). Moreover, even if a larger number of loci are used, because the percentage of DNA expressed as proteins is low compared to total DNA available, it is uncertain whether the DNA sequence analysed is important in terms of the adaptation of the species to the habitat (Kocher, 2003). Hence, although stock discrimination derived from DNA sequences accurately represents the history of genes, it does not necessarily reflect the

Table 4 Classification results of the discriminant analysis showing percentage of cases assigned to each group for *S. pilchardus* and *E. encrasicolus*

	Predicted group		
	Spain N (%)	Spain S (%)	Sicily (%)
Real group			
S. pilchardus			
Spain N	77.8	22.2	0
Spain S	19.0	81.0	0
Sicily 2003	0	0	100
Sicily 2002 (without assigned group)	0	0	100
E. encrasicolus			
Spain N	78.7	24.3	0
Spain S	7.4	88.9	3.7
Sicily 2003	0	0	100
Sicily 2002 (without assigned group)	0	0	100

history of the population in which the variants are found.

Differences in habitat conditions, due to adaptation to different photic environments (spectral composition of shallow bodies of water is highly variable due largely to differences in the quantity and identity of the substances dissolved), may have consequences upon eye pigments, tuned by the AAC within the opsin (the protein that determines the spectral absorption characteristics of the light) (see Douglas et al., 1998).

Other methods, such as the elemental composition of otoliths, have been used as a means for resolving the fine-scale stock and population structures of marine fishes (Thresher, 1999; Thorrold et al., 2001; Geffen et al., 2003; Rooker et al., 2003; Swan et al., 2006). One of the problems of otolith chemistry is that it shows strong year-to-year variability and must be used as a seasonally stable biological tracer (Campana et al., 2000), as nursery fingerprints have been shown to vary between years (Gillanders et al., 2001; Gillanders, 2002).

Although intensive sampling is necessary for all species and areas over a long period (more than 3–5 years), our preliminary results comparing different sampling years for *S. pilchardus* and *E. encrasicolus* revealed that AAC may be used as a stable tag in pelagic fishes.

It is not suggested that the definition of the population structure of marine fishes should rely upon this amino acid technique. There is still a need for a holistic (multiple technique) approach for the study of the population structure of marine fishes (Waldman et al., 1997; Begg & Waldman, 1999; Murta, 2000), as different methods may produce different results. The information obtained from the analysis of amino acids would be complementary information to that obtained from other techniques.

Acknowledgments The authors would like to express their gratitude to the Instituto Español de Oceanografía for the ECOMED cruise and to the officers and crew of the R/V Cornide de Saavedra.

References

Agostini, V. N. & A. Bakun, 2002. 'Ocean triads' in the Mediterranean Sea: physical mechanisms potentially structuring reproductive habitat suitability (with example application to European anchovy, *Engraulis encrasicolus*). Fisheries Oceanography 11: 129–142.

Alemany, F. & F. Alvárez, 1993. Growth differences among sardine (*Sardina pilchardus* Walb.) populations in western Mediterranean. Scientia Marina 57: 229–234.

Andrewartha, H. & L. C. Birch, 1954. The Distribution and Abundance of Animals. The University Chicago Press, Chicago.

Bailey, K. M., T. J. Quinn, P. Bentzen & W. S. Grant, 1999. Population structure and dynamics of walleye Pollock: *Theragra chalcogramma*. Advances in Marine Biology 37: 179–255.

Begg, G. A. & J. R. Waldman, 1999. An holistic approach to fish stock identification. Fisheries Research 43: 35–44.

Bembo, D. G., G. R. Carvalho, N. Cingolani & T. J. Pitcher, 1996a. Electrophoretic analysis of stock structure in Northern Mediterranean anchovies, *Engraulis encrasicolus*. ICES Journal of Marine Science 53: 115–128.

Bembo, D. G., G. R. Carvalho, N. Cingolani, E. Arneri, G. Giannetti & T. J. Pitcher, 1996b. Allozymic and morphometric evidence for two stocks of the European anchovy *Engraulis encrasicolus* in Adriatic waters. Marine Biology 126: 529–538.

Booke, H. E., 1999. The stock concept revisited: perspectives on its history in fisheries. Fisheries Research 43: 9–11.

Borsa, P., 2002. Allozyme, mitochondrial-DNA, and morphometric variability indicate cryptic species of anchovy (*Engraulis encrasicolus*). Biological Journal of the Linnean Society 75: 261–269.

Cadrin, S. X., K. D. Friedland & J. R. Waldman, 2005. Stock Identification Methods. Applications in Fishery Science. Elsevier Academic Press, London.

Campana, S. E., G. A. Chouinard, J. M. Hanson, A. Fréchet & J. Brattey, 2000. Otolith elemental fingerprints as biological tracers of fish stocks. Fisheries Research 46: 343–357.

Carrera, P. & C. Porteiro, 2003. Stock dynamic of the Iberian sardine (*Sardina pilchardus*, W) and its implication on the fishery off Galicia (NW Spain). Scientia Marina 67: 245–258.

Cuttitta, A., C. Guisande, I. Riveiro, I. Maneiro, B. Patti, A. R. Vergara, G. Basilone, A. Bonanno & S. Mazzola, 2006. Factors structuring reproductive habitat suitability of *Engraulis encrasicolus* in the south coast of Sicily. Journal of Fish Biology 68: 264–275.

Douglas, R. H., J. C. Partridge & N. J. Marshall, 1998. The eyes of deep-sea fish I: lens pigmentation, tapeta and visual pigments. Progress in Retinal and Eye Research 17: 597–636.

Edmonds, J. S., M. Moran, N. Caputi & M. Morita, 1989. Trace element analysis of fish sagittae as an aid to stock identification: Pink Snapper (*Chrysophrys auratus*) in western Australian waters. Canadian Journal of Fisheries and Aquatic Sciences 46: 50–54.

Geffen, A. J., K. Jarvis, J. P. Thorpe, R. T. Leah & R. D. M. Nash, 2003. Spatial differences in the trace element concentrations of Iris Sea plaice *Pleuronectes platessa* and whiting *Merlagius merlagus* otoliths. Journal of Sea Research 50: 245–254.

Gillanders, B. M., 2002. Temporal and spatial variability in elemental composition of otoliths: implications for determining stock identity and connectivity of populations. Canadian Journal of Fisheries and Aquatic Sciences 59: 669–679.

Gillanders, B. M., P. Sanchez-Jerez, J. Bayle-Sempere & A. Ramos-Espla, 2001. Trace elements in otoliths of the two-banded bream from a coastal region in the south-west Mediterranean: are there differences among locations? Journal of Fish Biology 59: 350–363.

Guisande, C., A. Barreiro, I. Maneiro, I. Riveiro, A. R. Vergara & A. Vaamonde, 2006. Tratamiento de datos. Diaz de Santos, Spain.

Hanski, I., 1999. Metapopulation Ecology. Oxford University Press, Oxford.

Hartl, D. L. & A. G. Clark, 1989. Principles of Population Genetics. Sinauer Associates, Sunderland, MA.

Kinsey, S. T., T. Orsoy, T. M. Bert & B. Mahmoudi, 1994. Population structure of the Spanish sardine *Sardinella aurita*: natural morphological variation in a genetically homogeneous population. Marine Biology 118: 309–317.

Kocher, T. D., 2003. Evolutionary biology: fractious phylogenies. Nature 423: 489–491.

Kritzer, J. P. & P. F. Sale, 2004. Metapopulation ecology in the sea: from Levins' model to marine ecology and fisheries science. Fish and Fisheries 5: 131–140.

Larrañeta, M. G., 1968. Unités de stock de la sardine de la Mediterranée Occidentale et de l'Adriatic. Etudes et Revues CGPM 33: 1–54.

Levins, R., 1970. Extinction. Lectures on Mathematics 2: 75–107.

Lleonart, J. & F. Maynou, 2003. Fish stock assessment in the Mediterranean: state of the art. Scientia Marina 67: 37–49.

MacLean, J. A. & D. O. Evans, 1981. The stock concept, discreteness of fish stocks, and fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 38: 1889–1898.

MacQuinn, I. H., 1997. Metapopulations and the Atlantic herring. Reviews in Fish Biology and Fisheries 7: 297–329.

Marques, J. F., C. M. Teixeira & H. N. Cabral, 2006. Differentiation of commercially important flatfish populations along the Portuguese coast: evidence from morphology and parasitology. Fisheries Research 81: 293–305.

Murta, A. G., 2000. Morphological variation of horse mackerel (*Trachurus trachurus*) in the Iberian and North African Atlantic: Implications for stock identification. ICES Journal of Marine Science 57: 1240–1248.

Pawson, M. G. & S. Jennings, 1996. A critique of methods for stock identification in marine capture fisheries. Fisheries Research 25: 203–217.

Ramon, M. M. & J. A. Castro, 1997. Genetic variation in natural stocks of *Sardina pilchardus* (sardines) from the western Mediterranean Sea. Heredity 78: 520–528.

Riveiro, I., C. Guisande, C. Franco, A. Lago deLanzós, A. Solá, I. Maneiro & A. R. Vergara, 2003. Egg and larval amino acid composition as indicators of niche resource partitioning in pelagic fish species. Marine Ecology Progress Series 260: 252–262.

Rooker, J. R., D. H. Secor, V. S. Zdanowicz, G. De Metrio & L. O. Relini, 2003. Identification of Atlantic bluefin tuna (*Thunnus thynnus*) stocks from putative nurseries using otolith chemistry. Fisheries Oceanography 12: 75–84.

Ryman, N., U. Lagercrantz, L. Anderson, R. Chakraborty & R. Rosenberg, 1984. Lack of correspondence between genetic and morphologic variability patterns in Atlantic herring (*Clupea harengus*). Heredity 53: 687–704.

Smedbol, R. K. & R. K. Wroblewski, 2002. Metapopulation theory and northern cod population structure: interdependency of subpopulations in recovery of a groundfish population. Fisheries Research 55: 161–174.

Smith, P. J. & A. Jamieson, 1986. Stock discreteness in herrings: a conceptual revolution. Fisheries Research 4: 223–234.

Spanakis, E., N. Tsimedines & E. Zouros, 1989. Genetic differences between populations of sardine, *Sardina pilchardus*, and anchovy, *Engraulis encrasicolus*, in the Aegean and Ionian seas. Journal of Fish Biology 35: 417–437.

Stephenson, R. L., 1999. Stock complexity in fisheries management: a perspective of emerging issues related to population sub-units. Fisheries Research 43: 247–249.

Swain, D. P. & C. J. Foote, 1999. Stocks and chameleons: the use of phenotypic variation in stock identification. Fisheries Research 43: 113–128.

Swan, S. C., A. J. Geen, B. Morales-Nin, J. D. M. Gordon, T. Shimmield, T. Sawyer & E. Massutý, 2006. Otolith chemistry: an aid to stock separation of *Helicolenus dactylopterus* (bluemouth) and *Merluccius merluccius* (European hake) in the Northeast Atlantic and Mediterranean. ICES Journal of Marine Science 63: 504–513.

Thorrold, S. R., C. Latkoczy, P. K. Swart & C. M. Jones, 2001. Natal homing in a marine fish metapopulation. Science 291: 297–299.

Thresher, R. E., 1999. Elemental composition of otoliths as a stock delineator in fishes. Fisheries Research 43: 165–204.

Timi, J. T., J. L. Luque & N. H. Sardella, 2005. Parasites of *Cynoscion guatucupa* along South American Atlantic coasts: evidence for stock discrimination. Journal of Fish Biology 67: 1603–1618.

Tudela, S., 1999. Morphological variability in a Mediterranean, genetically homogeneous population of the European anchovy, *Engraulis encrasicolus*. Fisheries Research 42: 229–243.

Turan, C., G. R. Carvalho & J. Mork, 1998. Molecular genetic analysis of Atlanto-Scandian herring (*Clupea harengus*) populations using allozymes and mitochondrial DNA markers. Journal of Marine Biology Association UK 78: 269–283.

Van Wandelen, C. H. & S. A. Cohen, 1997. Using quaternary high-performance liquid chromatography eluent systems for separating 6-aminoquinolyl-*N*-hydroxysuccinimidyl carbamate-derivatized amino acid mixtures. Journal of Chromatography 763: 11–22.

Waldman, J. R., 1999. The importance of comparative studies in stock analysis. Fisheries Research 43: 237–246.

Waldman, J. R., R. A. Richards, W. B. Schill, I. Wirgin & M. C. Fabrizio, 1997. An empirical comparison of stock identification techniques applied to striped bass. Transactions of the American Fisheries Society 126: 369–385.

Ward, R. D. & P. W. Grewe, 1994. Appraisal of molecular genetic techniques in fisheries. Reviews of Fish Biology and Fisheries 4: 300–325.

Hydrobiologia (2011) 670:201–221
DOI 10.1007/s10750-011-0666-8

ECOSYSTEMS AND SUSTAINABILITY

Patterns of investment in reproductive and somatic tissues in the loliginid squid *Loligo forbesii* and *Loligo vulgaris* in Iberian and Azorean waters

**Jennifer M. Smith · Graham J. Pierce ·
Alain F. Zuur · Helen Martins · M. Clara Martins ·
Filipe Porteiro · Francisco Rocha**

Published online: 5 April 2011
© Springer Science+Business Media B.V. 2011

Abstract The veined squid, *Loligo forbesii*, is found throughout the northeast Atlantic, including the waters off the Iberian Peninsula, and is a socio-economically important cephalopod species, sustaining several small-scale commercial and local artisanal fisheries. This study uses Iberian and Azorean trawl survey and fishery landings data from 1990 to 1992 and employs additive modelling to examine the relationships amongst somatic growth, season and gonad growth, in an attempt to determine the relative importance of intrinsic (e.g. nutritional state and body size) and extrinsic (temperature and daylight) factors which contribute to maturation in *L. forbesii*. We compare the results with those from a comparative analysis of contemporaneous data on *Loligo vulgaris* from the Iberian coast, and with a re-analysis of previously published results for *L. forbesii* in Scottish waters. Reproductive organ weight in both sexes of *L. forbesii* from all ports shows seasonal patterns with a fall/winter peak in maturation, as is expected with the animals' year-long life cycle. Two maturity peaks (spring and winter) were found for both Azores *L. forbesii* males and females, possibly evidence of two breeding cohorts, with males maturing approximately 1 month earlier than females in both seasons. For *L. vulgaris*, three annual peaks in reproductive weight were identified. With the exception of *L. forbesii* males from Scottish waters, in all study animals, the partial effect of mantle length and mantle weight on reproductive weight is positive up to large body sizes. The relationship between nutritional status and reproductive weight is also generally positive. Geographic variation in the relationships, as seen between Scottish males and samples from the other study areas, is hypothesised to be largely attributable to local differences in environmental conditions (productivity, food availability, temperature, upwelling),

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

J. M. Smith (✉) · G. J. Pierce
School of Biological Sciences (Zoology), University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK
e-mail: jennifer.smith@abdn.ac.uk

G. J. Pierce
Centro Oceanográfico de Vigo, Instituto Español de Oceanografía, P.O. Box 1552, 36200 Vigo, Spain

A. F. Zuur
Highland Statistics Ltd., 6 Laverock Road, Newburgh, Aberdeenshire AB41 6FN, UK

H. Martins · F. Porteiro
Departmento de Oceanografia e Pescas, Universidade dos Açores, 9901-862 Horta, Portugal

M. Clara Martins
Sternaserv, Lda. Consultoria e Serviços Técnicos, 1150-215 Lisbon, Portugal

F. Rocha
Departamento de Ecología y Biología Animal, Campus as Lagoas-Marcosende, Universidad de Vigo, 36310 Vigo, Spain

although differences between *L. forbesii* from the Azores, Scotland and mainland Europe may also relate to previously documented genetic differences.

Keywords Squid · Life history · Maturation · Reproductive investment · Somatic growth · Iberian Peninsula

Introduction

Information on the timing of growth and maturation and on the factors which cause underlying variation in reproductive processes is important to underpin sustainable fishing. Fishery managers need to avoid both growth overfishing (i.e. loss of yield caused by taking animals too early in the life cycle) and recruitment overfishing (negatively impacting recruitment by removing too many animals before they can spawn). In annual, seasonally breeding species such as the squid *Loligo forbesii*, the timing of peak yield from the fishery is necessarily linked to the timing of the life cycle (see Pierce et al., 1994a, b).

The process of maturation is driven in part by intrinsic factors such as age, size and nutritional status, and extrinsic factors such as season, which affects length of daylight and water temperature. In the natural environment, these factors may very well interact. For example, water temperature will influence food availability, which will in turn affect nutritional status and growth rate. Favourable feeding and temperature conditions after hatching have been found to affect growth rates and the onset of maturation in *Loligo vulgaris* in Portuguese waters (Moreno et al., 2007), and *Loligo forbesii* from the Azores exhibited temperature-sensitive growth rates when reared in laboratory conditions (Forsythe & Hanlon, 1989). In addition, it is also debated as to whether somatic tissues are remobilised to contribute stored energy reserves to gonad growth. Although experimental laboratory studies and long time-series of data would be most beneficial in revealing responses of squid to both internal and external signals for the onset and time course of maturation, useful insights can still be extracted from cross-comparisons of biological data collected from market samples of different species and from across different geographical regions.

This article makes use of a data set collected during a European collaborative project in the early 1990s, when detailed biological data were collected over a 2-year period, to examine the intrinsic and extrinsic factors determining the timing of maturation of the squid *L. forbesii* (Cephalopoda: Loliginidae) in waters off of the Iberian Peninsula, the Azores, and Scotland, and includes a similar analysis of an additional loliginid squid, *L. vulgaris*, in an attempt to identify any differences in growth patterns between sexes and the two species, particularly in Iberian waters, where the species' ranges overlap.

The distribution of *L. forbesii* includes coastal waters of the northeast Atlantic from as far north as the Faroe Islands to the upper west coast of Africa, as well as the Azores and the Mediterranean (Roper et al., 1984; Salman & Katağan, 2002). *L. vulgaris* shares the southern part of this range (Guerra & Rocha, 1994), being almost completely absent from Scottish waters (Pierce et al., 1994b, 1998) whilst present throughout the Mediterranean (Guerra et al., 1994). During the 1990s, catches of *L. forbesii* off Iberia began to decline and, by the mid-2000s, it appeared that the species was no longer prevalent across the southern part of its range (Chen et al., 2006), although landings in northern areas have increased in recent years [e.g. northeast Scotland during the years of 2003–2005 (ICES, 2008; authors' unpublished data)], and it continues to be found in commercial and research catches in Spain and Portugal. It was hypothesised that the decrease of *L. forbesii* in these areas, and in turn, the increase in abundance in the northern area of the species' distribution range, may have been related to an increase in SST in southern French and Portuguese waters after 1993 (Chen et al., 2006). At the time when the present samples were collected, the two *Loligo* species were sympatric along the coast of the Iberian Peninsula, although results from research cruises suggested that *L. vulgaris* tended to be found closer to the coast (Cunha et al., 1995; Hastie et al., 2009) than *L. forbesii*. As such, both as a mechanism to reduce competition and/or due to genetic differences, it might be expected that growth and maturation patterns of *L. vulgaris* would differ from those of *L. forbesii*. Rocha & Guerra (1999) found differences in both body size and statoliths size (as a percentage of mantle length) between the two species in Galician waters, suggesting that their growth patterns may differ.

In Galician waters, loliginid squid are caught both as a by-catch in commercial trawling and by artisanal hand-jigging (Guerra & Rocha, 1994; Rocha & Guerra, 1999). Males and females landed in this region have been shown to mature at two different modal sizes, with males maturing earlier than females (Guerra & Rocha, 1994). *Loligo* fisheries in Portuguese waters are mainly carried out by the commercial trawl fishery, but also artisanally with hand jigs, with trawling gear accounting for the majority of the annual recorded catch (Cunha & Moreno, 1994). Two different size groups at maturity in males and females of both *L. forbesii* and *L. vulgaris* have also been documented amongst Portuguese samples (Coelho et al., 1994; Moreno et al., 1994), with males maturing at a smaller size than females for *L. vulgaris* (Moreno et al., 2005). In the Azores, a yearly, two-seasoned artisanal jig fishery (using homemade double or longlined jig gear) exists with no fishing activity during the summer months (Martins, 1982; Porteiro, 1994). The highest maturity values for *L. forbesii* landed have been found to be in winter and spring (Porteiro & Martins, 1994), corresponding with the peak landing months. Animals have been found to be of larger body size than those that landed elsewhere in Europe (Martins, 1982) and have been shown to be genetically distinct from other northeastern Atlantic *L. forbesii* populations (Norman et al., 1994; Brierley et al., 1995; Shaw et al., 1999).

This research follows a previous study by the authors on maturation of *L. forbesii* in Scottish (UK) waters, which found gonad weight in both sexes to be affected by both month (i.e. a seasonal trigger) and nutritional state (proxied by digestive gland weight), and indicated a negative relationship between somatic and ovary growth in females, whilst providing evidence of more complex relationships between growth variables in males (Smith et al., 2005), the latter finding being possibly attributable to the two to three sizes at maturity which have been documented in males of this species in Scotland (Boyle et al., 1995; Collins et al., 1999).

In the current investigation, a similar analysis is applied to data from southern European Atlantic waters collected contemporaneously with the data used in the Smith et al. (2005) study, using additive modelling to describe relationships between maturity (e.g. gonad and accessory gland weight), somatic investment (body weight), nutritional status (digestive gland weight), size (body length) and season in samples of *L. forbesii* and *L. vulgaris*, and compares results with those found in squid from Scottish waters. The data were previously used to describe the annual life cycles (Boyle & Pierce, 1994; Guerra & Rocha, 1994; Moreno et al., 1994; Porteiro & Martins, 1994) but there has been no previous attempt to investigate the links between maturation, growth, nutritional state and season. As a result of the short time-series available, it is possible to look for differences in growth and maturation between years, but not to ascribe them to specific differences in environmental conditions. It is hypothesised that differences between regions and species might arise due to the plasticity of the life cycle biology in response to extant environmental conditions and to direct competition in areas where the species overlap. Likely factors contributing to possible between-area differences in relationships between reproductive weight and the explanatory variables in both species involve differences in environmentally driven oceanographic regimes, including factors such as seasonal variation in upwelling and currents, water temperature, productivity and food supply regimes. Intrinsic factors might also contribute, e.g. stemming from genetic differences in these animals from the Azores, as *L. forbesii* from this area have previously been shown to differ morphometrically and genetically from those on the continental shelf (Norman et al., 1994; Pierce et al., 1994c; Brierley et al., 1995; Shaw et al., 1999).

It is common in studies of maturation to use simple indices such as the gonado-somatic index (GSI), in which gonad weight is expressed as a proportion of body weight (Guerra & Rocha, 1994; Porteiro & Martins, 1994; Boyle et al., 1995; Collins et al., 1995; Rocha & Guerra, 1996). However, analogous to Thorpe's (1976) objections to the use of ratio estimators in studies of morphometric variation, we argue that this makes an implicit assumption of isometric growth of body components (in relation to body size) which may not be justified. We therefore treat raw reproductive weight (gonad plus accessory organ weights) as the response variable in our models and use a generalised additive modelling (GAM) framework to avoid the necessity of assuming linear relationships.

Materials and methods

Data

The main data sets used here derive from sampling of *L. forbesii* and *L. vulgaris* and include catches taken in three localities, off the Azores islands, Portuguese mainland and NW Spanish coasts, during 1990–1992 as a part of a collaborative European project. Samples from Spanish commercial fisheries were all caught and landed from March 1991 to August 1992 for *L. forbesii* and from April 1991 to June 1992 for *L. vulgaris* (Vigo data set). Samples from Azorean waters (all *L. forbesii*) were caught in the artisanal jig fishery from November 1990 to January 1992 and landed in several ports (Azores data set). Samples of *L. vulgaris* from commercial trawling and hand-jigging in southern Portugal were landed in the ports of Portimão, Albufeira, Quarteira and Olhã from January 1991 to March 1992 (Faro data set). The sampling strategy involved random[1] sampling of around 200 individuals per month from the fish market for length, weight, sex and maturity (following a 5-point scale, see Pierce et al., 1994a), and then stratified sub-sampling 30 males and 30 females from across the available size range for collection of detailed morphometric and reproductive data. This sampling was project-based, and although some sampling continued during subsequent projects, equivalent data are not available from more recent years since recording of several variables (notably digestive gland weight and mantle weight) was not carried out after 1992. Age data were not routinely collected.

Information was extracted from the database for individual squid with values for all of the following variables: capture date, dorsal mantle length (DML, mm), sex, gonad and accessory reproductive organ weight (g) (for males: spermatophoric complex weight and testis weight; for females: ovary weight, nidamental gland weight and oviducal complex weight), mantle weight (including fins) (g) and digestive gland weight (g). The term 'reproductive weight' is used for the combined weights of gonads and accessory reproductive organs. Data were separated according to area, species and sex and separate analyses carried out for each group.

Analysis

To quantify factors affecting reproductive investment, the response variable used was 'reproductive weight' (as defined above) in both males and females. The putative explanatory variables were month, mantle length, mantle weight and digestive gland weight. Statistical analyses were carried out using the Minitab 15.1 (Ryan et al., 1985) and Brodgar v2.6.5 (www.brodgar.com). For all ports, the variables mantle length, mantle weight and digestive gland weight showed a seasonal pattern, as would be expected given the animals' suspected annual life cycle. To remove the seasonal (month) effect from examined variables, the three 'somatic' variables (mantle length, mantle weight, digestive gland weight) were 'de-seasonalised' by regressing them against month (treating month as a categorical variable) and substituted by the resulting residuals, becoming 'de-seasonalised' mantle length (DESEADML), 'de-seasonalised' mantle weight, and 'de-seasonalised' digestive gland weight. The response variables were not de-seasonalised, as time (month) was included as an explanatory variable in the analysis.

Preliminary analysis indicated strong colinearity between (de-seasonalised) mantle length, mantle weight, and digestive gland weight. Therefore, prior to fitting the final models to reproductive weights, to remove the effect of de-seasonalised mantle length on de-seasonalised mantle weight and de-seasonalised digestive gland weight, size-adjusted values for these variables were used, with the size-adjusted variable taking positive values when (seasonally adjusted) mantle or digestive gland weight was higher than expected for a given (seasonally adjusted) mantle length. Additive models (AM) were therefore fitted to the (de-seasonalised) mantle weight-mantle length and digestive gland weight-mantle length relationships, and residuals from both AMs subsequently used in place of the residual (de-seasonalised) values for mantle weight and digestive gland weight, becoming a 'double residual' (RR) or de-seasonalised, size-adjusted variables (e.g. 'RR'MTLWT and 'RR'DIGGLWT). Bivariate scatterplots, boxplots and dotplots (Cleveland, 1993) were utilised to

---

[1] Samples were bought at the fish market. There was no selection of specimens according to size and normally these species of squid are not sorted by size in the market.

identify outliers. Suspect points were tested against established length-weight relationships, and any clearly erroneous points were deleted.

To facilitate the comparison of the analysis of samples from Iberia and the Azores with previous results from Scottish waters, this double residual technique was applied to the original *L. forbesii* data from Smith et al. (2005) in which the effect of DML was previously removed from the other variables, but not the effect of month.

To help clarify the underlying relationships between reproductive weight and both month and DML, scatterplots of raw (untransformed) data with smoothing curves were also created.

Optimal additive models (GAMs with a Gaussian distribution) were determined by backwards selection, starting with full models and removing non-significant explanatory variables as well as comparing the AIC values (Akaike's Information Criterion, Akaike, 1973) for models with and without particular variables included (Hastie & Tibshirani, 1990; Wood, 2006; Zuur et al., 2007, 2009). Given the known flexibility of growth patterns in this species (e.g. multiple breeding seasons, multiple size modes at maturity), the modelled relationships may be more complex than allowed if restricting degrees of freedom for some of the original smoothers as otherwise carried out to avoid over-fitting. Thus, for the explanatory variables of month and DESEADML, cross validation (Fox, 2000, 2002; Wood, 2000, 2004) was used, with the software selecting the optimal degrees of freedom for the smoothers. For the variables RRMTLWT and RRDIGGLWT, the degrees of freedom were restricted to 3 (by setting $k = 4$) to avoid over-fitting and to make the representation of the relationships between the response and explanatory variables more clear and interpretable.

Results

Summary of relationships between variables

Explanatory variables included in the final GAMs for reproductive weight for each species, sex and area combination, along with sample size numbers, degrees of freedom, F-statistic and associated probabilities, dispersion, deviance explained and AIC values for the individual models can be found in Tables 1 and 2. A summary of the relationships (e.g. negative, positive) for the partial effect of the significant explanatory variables against reproductive weight can be found in Table 3.

Month

For animals from the Azores (all *L. forbesii*), samples which included measured values for all of the required variables were available only from November 1990 to January 1992, and as such, we treated the data as a single, continuous time series, with the variable 'time' taking values from 1 to 15, beginning with month November 1990 (time = 1) and ending with January 1992 (time = 15). Due to the summer closure of the Azores squid fishery, i.e. during June, July or August 1991, no samples were taken.

It was not possible to check for separate year and month effects for any of the combinations of species, sex, or port, as data were not available for all calendar months of all sample years, but month was used as an explanatory variable in the analyses.

For *L. forbesii*, scatterplots of raw reproductive weight values versus month (Fig. 1) indicate that the months with the lowest maturity were generally during the summer: June for males from Vigo (Fig. 1a), August for females from Vigo (Fig. 1b), September for both sexes from the Azores and for females from Scotland (Fig. 1c–d, f) and July for males from Scotland (Fig. 1e). Peaks in maturity occurred in December for males from Vigo (Fig. 1a) and in January for females from Vigo and for both sexes from all other areas [Fig. 1b–d (time = 15), e–f]. Males from the Azores also exhibited a peak in reproductive weight in March–April [Fig. 1d (time = 5–6)].

For *L. vulgaris*, scatterplots indicate that the months with lowest reproductive weight were either August (Fig. 2a–c) or July (Fig. 2d), with a peak in maturity in either the first or last month of the year (and in some cases, both), with the exception of males from Faro, which show an additional peak in reproductive weight in March–April (Fig. 2c).

In the GAMs, for the samples of *L. forbesii* from all ports, either one or two yearly peaks in maturity were identified. The smoother for the partial effect of month in *L. forbesii* males from Vigo (Fig. 3a) indicates that the most strongly negative effect occurred May through the end of August and the

Table 1 Summary of optimum GAMs employed for *L. forbesii* reproductive weight

Sample (N)	Predictor variables (df; F; P) for:				D	DE (%)	AIC
	Month	DESEADML	RRMTLWT	RRDIGGLWT			
Vigo—males (140)	8.9; 88.4; $P < 2e-16$	8.6; 52.4; $P < 2e-16$	1.0; 74.3; $P = 3.64e-14$	2.7; 8.8; $P = 2.61e-05$	5.0	94.7	642.3
Azores—males (296)	8.2; 32.7; $P < 2e-16$	5.0; 52.8; $P < 2e-16$	2.4; 3.3; $P = 0.0196$	2.9; 13.2; $P = 4.51e-08$	113.4	77.2	2261.2
Azores—females (212)	8.8; 53.2; $P < 2e-16$	3.1; 51.7; $P < 2e-16$	2.7; 2.9; $P = 0.0362$	1.0; 9.6; $P = 0.002$	1291.4	81.5	2138.2
Scotland—males (606)	8.9; 92.6; $P < 2e-16$	4.9; 139.6; $P < 2e-16$	2.8; 4.8; $P = 0.0027$	1.0; 64.4; $P = 5.58e-15$	8.3	81.1	3024.9
Scotland—females (538)	7.4; 181.7; $P < 2e-16$	7.7; 109.3; $P < 2e-16$	1.0; 7.7; $P = 0.0058$	–	216.7	83.2	4439.2

Explanatory variables: month (or ‘time’), de-seasonalised dorsal mantle length (DESEADML), residuals of mantle weight (RRMTLWT), residuals of digestive gland weight (RRDIGGLWT)

N sample size; *df* degrees of freedom; *F* *F*-statistic values; *P* associated probabilities; *D* dispersion; *DE* deviance explained; *AIC* Akaike Information Criterion value

Table 2 Summary of optimum GAMs employed for *L. vulgaris* reproductive weight

Sample (N)	Predictor variables (df; F; P) for:				D	DE (%)	AIC
	Month	DESEADML	RRMTLWT	RRDIGGLWT			
Vigo—males (210)	8.6; 28.5; $P < 2e-16$	5.8; 76.3; $P < 2e-16$	2.6; 8.5; $P = 2.42e-05$	–	5.8	86.3	985.0
Vigo—females (200)	7.7; 11.0; $P = 1.22e-12$	3.2; 51.0; $P < 2e-16$	2.2; 3.3; $P = 0.0228$	2.2; 5.5; $P = 0.0012$	251.0	74.8	1690.2
Faro—males (408)	8.8; 58.3; $P < 2e-16$	4.7; 377.3; $P < 2e-16$	2.4; 46.1; $P < 2e-16$	–	4.7	92.5	1811.3
Faro—females (367)	8.0; 3.5; $P = 0.0004$	5.1; 104.1; $P < 2e-16$	1.1; 6.7; $P = 0.0002$	1.0; 70.2; $P = 1.29e-15$	136.9	81.1	2864.8

Explanatory variables: month, de-seasonalised dorsal mantle length (DESEADML), residuals of mantle weight (RRMTLWT), residuals of digestive gland weight (RRDIGGLWT)

N sample size; *df* degrees of freedom; *F* *F*-statistic values; *P* associated probabilities; *D* dispersion; *DE* deviance explained; *AIC* Akaike Information Criterion value

Table 3 Summary of relationships for scatterplots of original values of explanatory variables against reproductive weight (denoted by *), and GAM smoothing curves for the partial effect of significant explanatory variables against reproductive weight for *L. forbesii* and *L. vulgaris*

Sample	Month*	Month	DML*	DESEADML	RRMTLWT	RRDIGGLWT
L. forbesii						
Vigo—males	Peak in Nov.–Dec.	Peak in Oct.	Positive	Positive	Positive, linear	Negative/positive
Vigo—females	Peak in Jan.	–	Positive	Positive, linear	–	–
Azores—males	Peak in Nov. 1990, Jan. 1992[a]	Peak in Dec. 1990, Mar. 1991, Jan. 1992[a]	Positive	Positive	Positive, generally	Positive, generally
Azores—females	Peak in Mar.–Apr. 1991, Jan. 1992[a]	Peak in Nov. 1990, Apr. and Dec. 1991[a]	Positive	Positive	Positive, generally	Positive, linear
Scotland—males	Peak in Jan.	Peak in Jan., Mar.	Positive	Positive	Negative, generally	Positive, linear
Scotland—females	Peak in Jan.	Peak in Jan.	Positive	Positive	Negative, linear	–
L. vulgaris						
Vigo—males	Peak in Jan., Dec.	Peak in Mar., June, Nov.	Positive	Positive	Positive, generally	–
Vigo—females	Peak in Jan. & Dec.	Peak in Jan., May, Nov.	Positive	Positive	Negative/positive	Positive
Faro—males	Peak in Mar.–Apr.	Peak in Mar., Aug., Dec.	Positive	Positive	Positive	–
Faro—females	Peak in Dec.	Peak in Mar., July, Dec.	Positive	Positive	Positive, linear	Positive, linear

Explanatory variables: month, dorsal mantle length (DML), de-seasonalised dorsal mantle length (DESEADML), residuals of mantle weight (RRMTLWT), residuals of digestive gland weight (RRDIGGLWT)

[a] Variable used was 'time'

most strongly positive effect on reproductive investment occurred in October and in January, i.e. results confirm a seasonal peak in gonad size in winter, which largely coincides with the scatterplot of original data. Month was not a significant explanatory variable for reproductive weight of *L. forbesii* females from Vigo, likely due to the low sample size as opposed to a lack of seasonality in females from this area.

The smoother for the partial effect of month (denoted here as 'time') on reproductive weight in *L. forbesii* males from the Azores (Fig. 3b) indicates that the most strongly negative effect occurred in September 1991 (time = 11) and the most strongly positive effects in late November-early December 1990 (time = 1–2), March 1991 (time = 5) and January 1992 (time = 15). Note that no samples were available for time periods 8, 9 and 10 (June to August). For females from the Azores, the smoothing graph for month ('time') (Fig. 3c) is rather similar to that of Azores males, indicating that the most strongly negative effect occurred in late October 1991 (time = 12) (as with the males, no samples were available for time periods 8–10) and the most strongly positive effect in possibly early November 1990 (time = 1), April 1991 (time = 6) and mid-December 1991 (time = 14). These negative effects correspond with the raw data scatterplots, whilst the most positive effects seen in the GAMs are stronger and more pronounced than what is found in the raw data relationships.

The smoother for the partial effect of month in *L. forbesii* males from Scotland (Fig. 3d) indicates that the most negative effect occurred in July, and the most strongly positive effect on reproductive investment in occurred in January (coinciding with the scatterplots of original raw data), with an additional, smaller peak in gonad weight in March. For females from Scotland, the partial effect of month on reproductive weight (Fig. 3e) indicates that the most strongly negative effects occurred in August and September and the most strongly positive effects in January (as with the scatterplots).

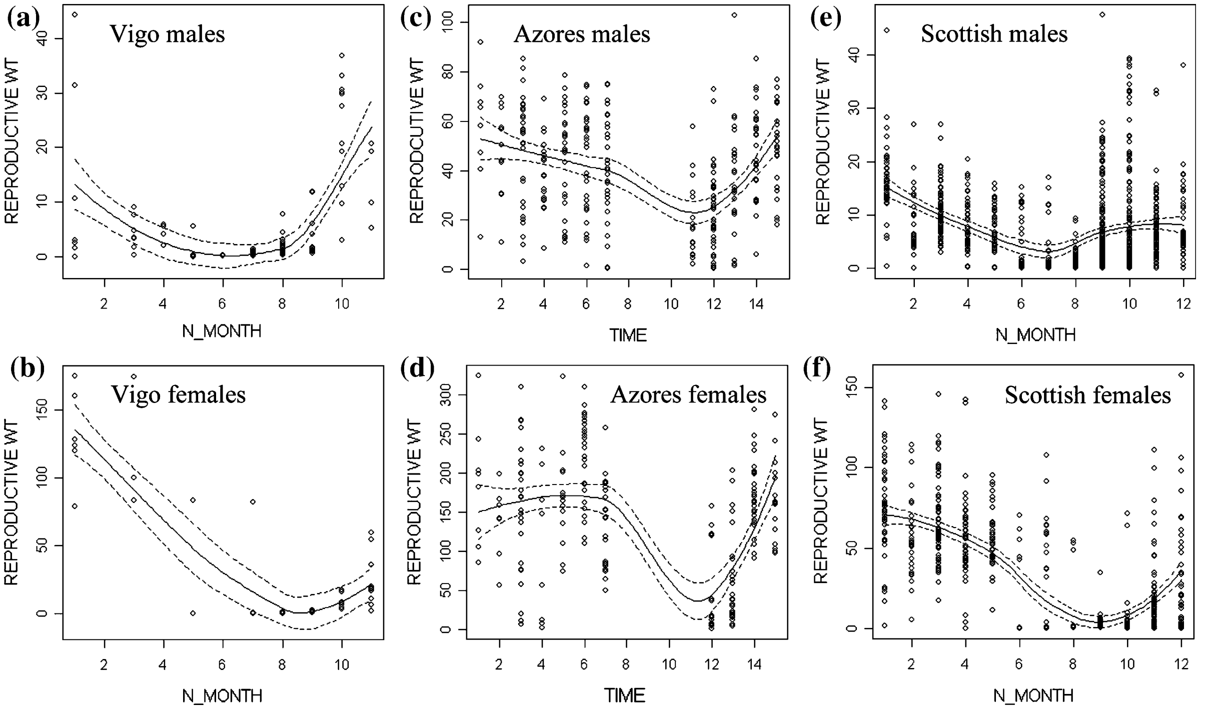
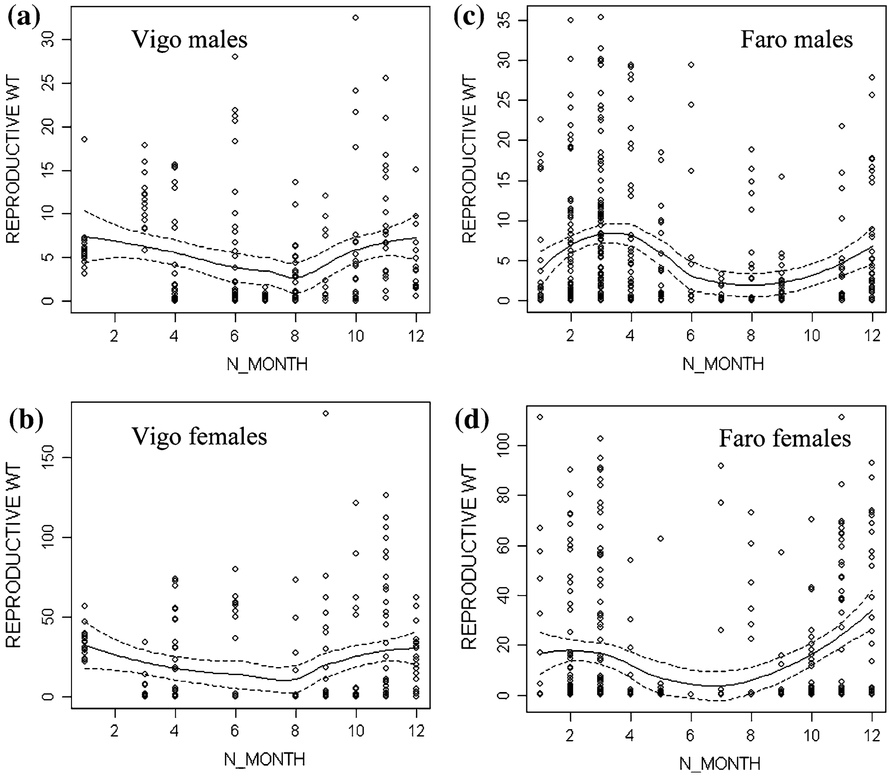


Fig. 1 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and month (or 'time') in *L. forbesii*. **a** Vigo males, **b** Vigo females, **c** Azores males, **d** Azores females, **e** Scottish males and **f** Scottish females

Fig. 2 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and month in *L. vulgaris*. **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females



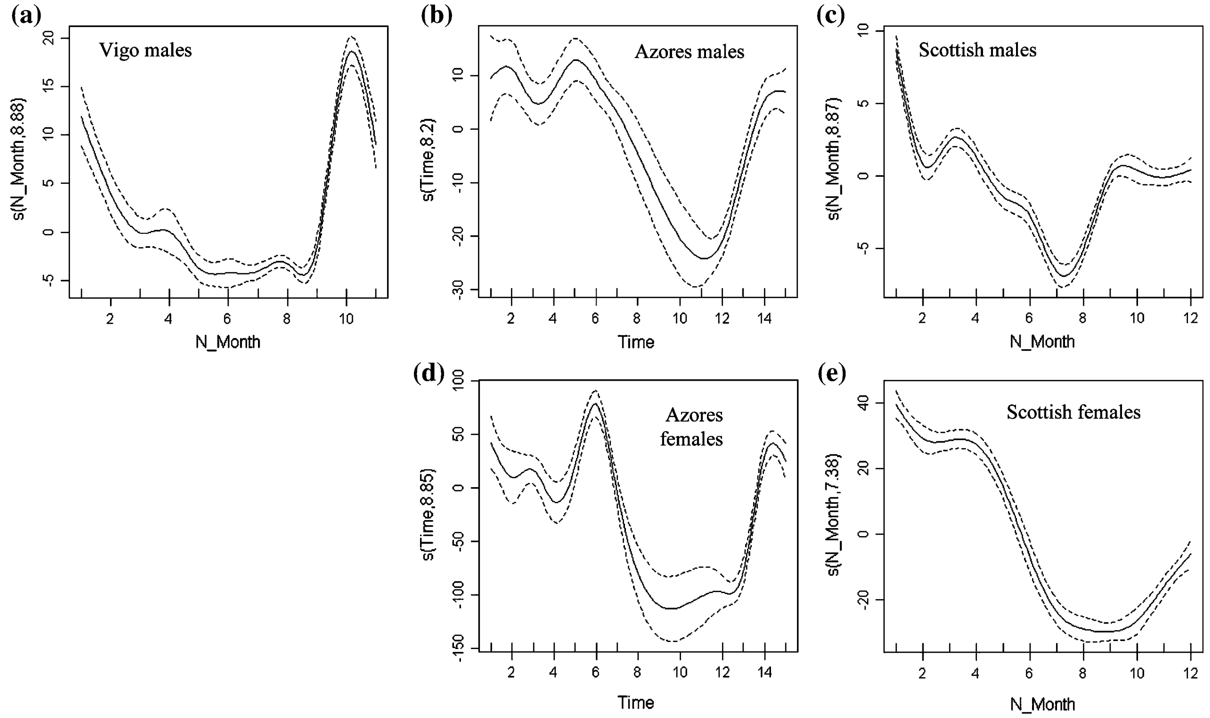


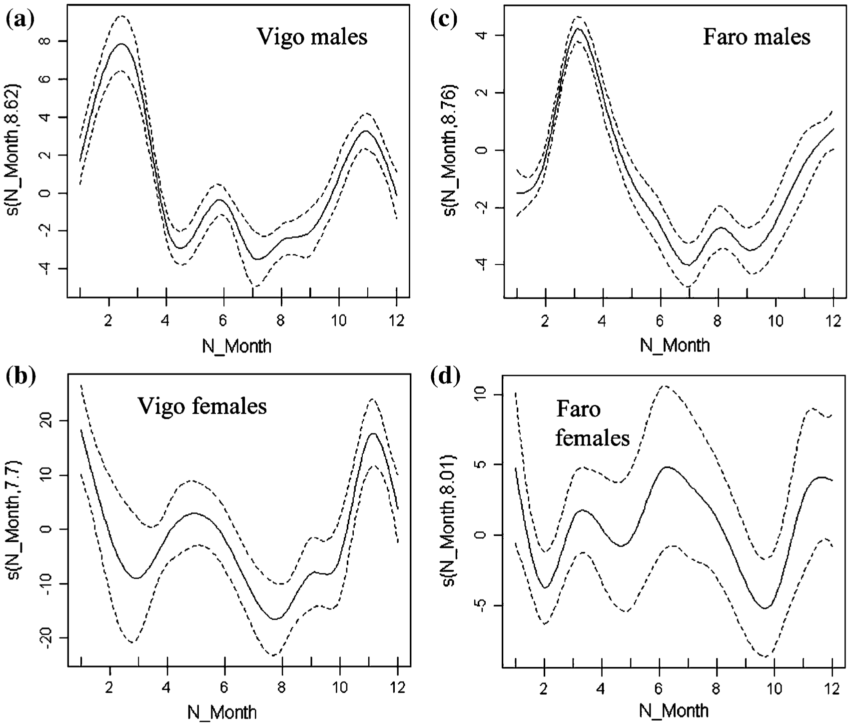
Fig. 3 GAM smoothing curves fitted to partial effects of the explanatory variable month (or ‘time’) on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of month in **a** Vigo males, **a** Azores males, **c** Azores females, **d** Scottish males and **e** Scottish females. *Dashed lines* represent 95% confidence intervals around the main effects

In all samples of *L. vulgaris* from all ports, three peaks in maturity were found, which differs from the scatterplots of raw data for this species, which identified 1–2 peaks. The smoother for the partial effect of month on reproductive weight in males from Vigo (Fig. 4a) indicates that the most strongly negative effects occurred in late April and July (at least 1 month earlier than in the raw data scatterplots) and the most strongly positive effects in early March and in November. The smoother for the partial effect of month in females from the same port (Fig. 4b) indicates that the most strongly negative effects occurred in March and late July—beginning of August, and the most strongly positive effects in January and November, with a smaller positive peak in May. Note that no samples were available for months February, May and July, although it is inferred from the smoothing curve that the highest peak in gonad maturation probably occurs late February–early March.

The smoother for the partial effect of month in *L. vulgaris* males from Faro (Fig. 4c) indicates that the most strongly negative effect occurred July through the end of September and the most strongly positive effect on reproductive investment occurred in March (primarily) and in December. Note that no samples were available for the month of October. For females from the same area, the smoother for the partial effect of month (Fig. 4d) oscillates around zero with high uncertainty due to large confidence limits, with the most strongly negative effect on reproductive investment occurring end of September to early October.

To summarise, 1–2 yearly peaks in maturity were found for *L. forbesii* and three peaks for *L. vulgaris* across all study ports. Positive peaks in maturity for *L. forbesii* samples were seen in January for Scottish animals (with the addition of March in males), later in the year for Vigo males (October), and in March or April and again at the end/beginning of the year (November–December and/or January) for the Azores (as 15 consecutive months of data, drawing from three individual calendar years, were used). For *L. vulgaris*, maturation peaks were seen in (1) winter (in either January or March), (2) mid-spring or summer, and (3) autumn (November or December).

Fig. 4 GAM smoothing curves fitted to partial effects of the explanatory variable month on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of month in **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females. *Dashed lines* represent 95% confidence intervals around the main effects



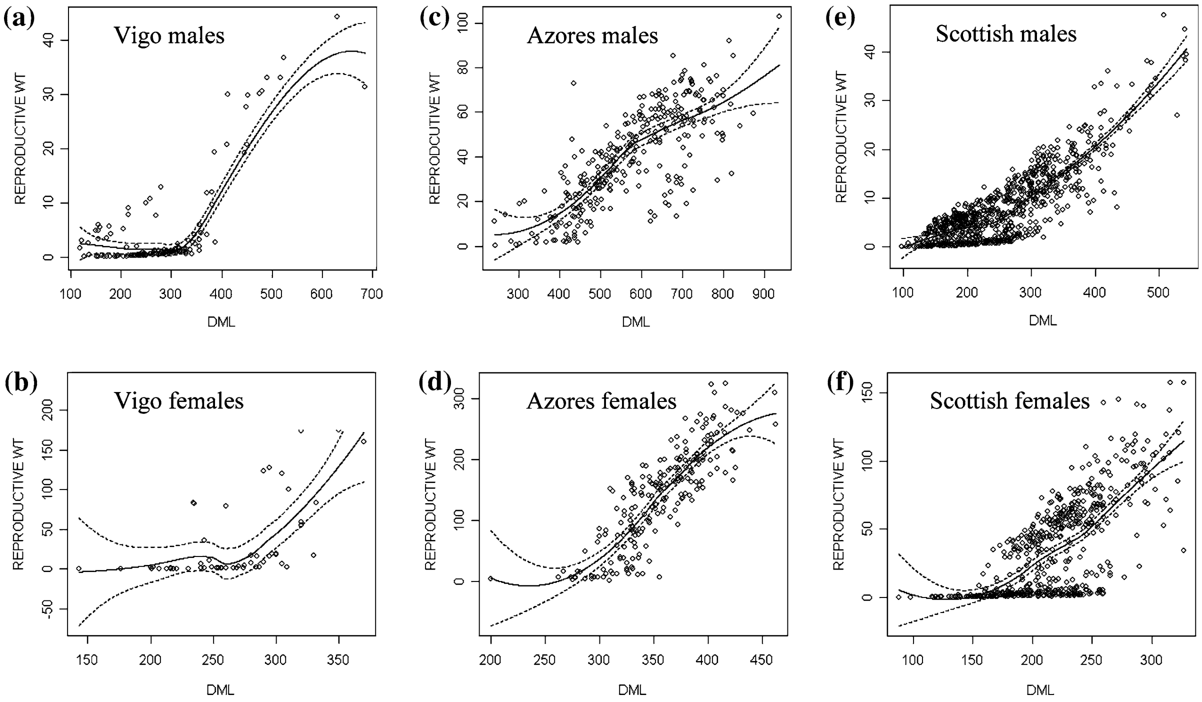
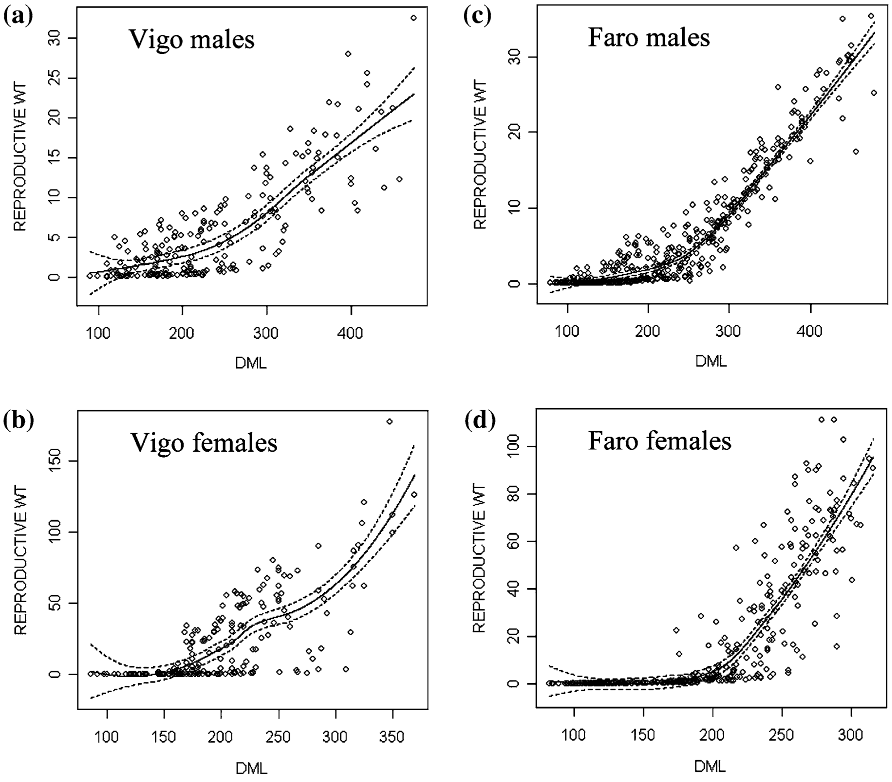


Fig. 5 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and DML in *L. forbesii*. **a** Vigo males, **b** Vigo females, **c** Azores males, **d** Azores females, **e** Scottish males and **f** Scottish females

Fig. 6 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and DML in *L. vulgaris*. **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females



DML

All scatterplots of raw data values for reproductive weight against DML for samples from the Iberian ports, the Azores and Scotland show a generally positive relationship between the two variables (Figs. 5, 6), indicating that larger animals have heavier gonads.

Similar to the scatterplots of untreated data, across both squid species, sexes and across all ports samples in the GAMs, the partial effect of de-seasonalised DML (Figs. 7, 8) indicates that once seasonality is removed, the relationship between reproductive weight and mantle length becomes positive (and nearly linear in several models). The optimal model for reproductive weight in *L. forbesii* females from Vigo was obtained by linear regression (Fig. 7b), as the degrees of freedom value obtained in the optimal additive model for the only significant continuous explanatory variable (DESEADML) was close to 1, indicating a linear relationship between residuals of DML and reproductive weight. Thus, in a given month, larger-sized animals are more likely to have heavier gonads. The smoother for the effect of reproductive weight on de-seasonalised DML for male *L. forbesii* from Vigo shows several inflection points (Fig. 7a). By analogy with a standard maturity ogive, this possibly indicates different sizes at maturity, with the first point of inflection occurring around −60 and a second occurring at around 75 (residual values). A possible third inflection point occurs at about 340, however, this effect is due to two large animals.

Mantle weight

Reproductive weight had a positive, linear relationship with de-seasonalised and size-corrected mantle weight in both *L. forbesii* males from Vigo and *L. vulgaris* females from Faro (Figs. 9a, 10d), a strongly positive relationship in *L. vulgaris* males from Faro (Fig. 10c), and a generally positive relationship in both sexes of *L. forbesii* from the Azores (Fig. 9b, c) and in *L. vulgaris* males from Vigo (Fig. 10a), indicating that animals with heavier mantles were associated with larger gonad weights, i.e. a positive association of somatic and reproductive growth.

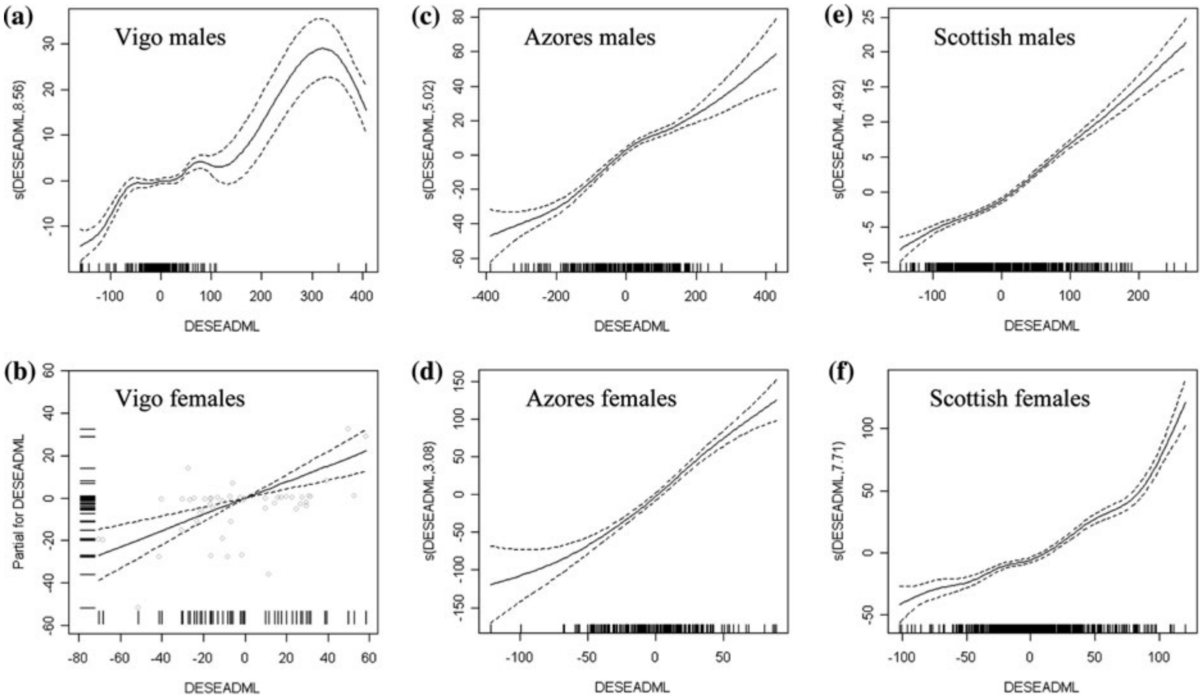


Fig. 7 GAM smoothing curves fitted to partial effects of the explanatory variable de-seasonalised DML on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of de-seasonalised DML in **a** Vigo males, **b** Vigo females (linear regression model), **c** Azores males, **d** Azores females, **e** Scottish males and **f** Scottish females. *Dashed lines* represent 95% confidence intervals around the main effects

In sharp contrast, reproductive weight had a negative effect on residuals of mantle weight in *L. forbesii* males from Scotland up until close to 100 (residual value) (Fig. 9d), above which the relationship becomes positive, i.e. for animals with the heaviest mantle weights. Moreover, in females from the same location, de-seasonalised and size-corrected mantle weight had a negative, linear relationship with reproductive weight (Fig. 9e), i.e. high gonad weights were associated with relatively low mantle weights.

In *L. vulgaris* females from Vigo, for the greater part of the size range, the smoother for reproductive weight shows a negative relationship with residuals of mantle weight (Fig. 10b), but a slightly positive trend can be seen at mantle weight (residual) values of approximately >15. Reproductive weight did not have a significant effect on the residuals of mantle weight in *L. forbesii* females from Vigo.

Digestive gland weight

In *L. forbesii* males from Vigo, reproductive weight appears to have had a negative effect on de-seasonalised digestive gland weight (i.e. heavier digestive gland, smaller gonads) for the animals with the lowest digestive gland weights (residual values), and a slightly positive effect on animals with the highest digestive gland weight residual values (Fig. 11a). Thus, animals with the poorest nutritional condition tended to have the biggest (heaviest) gonads. This may indicate cessation of feeding in mature animals rather than an effect of feeding on gonad growth.

In contrast, for all other samples across species, port and sex, where residual digestive gland weight was found to be a significant variable in the analyses, reproductive weight had a positive effect on the variable, although to differing degrees. In *L. forbesii* females from the Azores and males from Scotland, and in *L. vulgaris* females from Faro, reproductive weight had a positive, linear relationship with de-seasonalised, size-adjusted digestive gland weight (Figs. 11c, d, 12b), indicating that animals with heavier digestive gland weights had heavier gonads.

The partial effect of reproductive weight on residual digestive gland weight in *L. forbesii* males

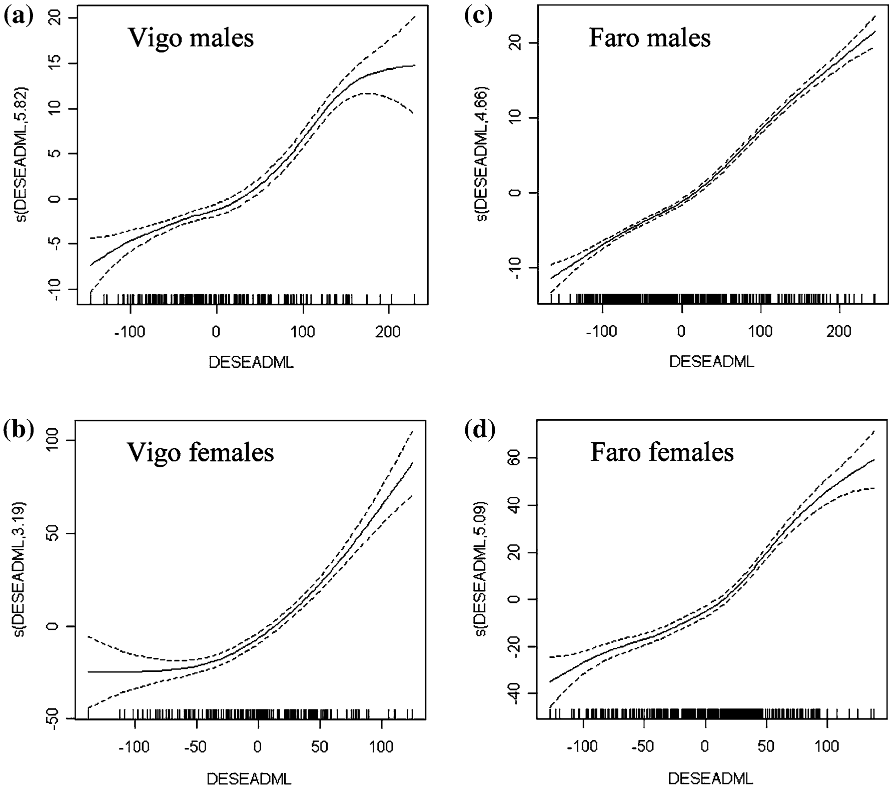


Fig. 8 GAM smoothing curves fitted to partial effects of the explanatory variable de-seasonalised DML on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of de-seasonalised DML in **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females. *Dashed lines* represent 95% confidence intervals around the main effects

from the Azores was also generally positive (Fig. 11b), although the relationship is asymptotic, with the smoothing curve levelling off at around −15 (residual value). This might suggest that once gonads have reached a certain size, increased nutritional state ceases to be important for increased maturation. Reproductive weight had a positive relationship with residuals of digestive gland weight in *L. vulgaris* females from Vigo, with the exception of two animals with the heaviest digestive gland weight (Fig. 12a).

Reproductive weight did not have a significant effect on residuals of digestive gland weight in *L. forbesii* females from Vigo and Scotland and *L. vulgaris* males from Vigo and Faro.

Discussion

Seasonality and maturation

Reproductive weight for *L. forbesii* in both males and females in all areas studied showed consistent seasonal patterns, as was found in previous research on animals from Scottish waters (e.g. Lum-Kong et al., 1992; Smith et al., 2005), and as is expected with the species' apparent nearly annual life cycle (Holme, 1974; Ngoile, 1987; Boyle & Pierce, 1994; Guerra & Rocha, 1994; Pierce et al., 1994a; Rocha & Guerra, 1999). The Azores fishery showed two apparent seasonal maturity peaks—spring and winter—for both males and females, with males maturing approximately 1 month earlier than females in both cases. The absence of data during the three month summer break in the directed fishery makes it difficult to be certain, but results are consistent with the existence of two breeding cohorts for both sexes in the Azores fishery (Porteiro & Martins, 1994), as has also been found for males in the UK waters, both in this study and elsewhere (Holme, 1974; Pierce et al., 1994a; Zuur & Pierce, 2004; Pierce et al., 2005; Smith et al., 2005). The two seasons in the Azores fishery, spring and winter, thus correspond to when squid are more mature and larger, although these samples came from a jig fishery, and as a result, might be effectively size-selected if larger animals tend to attack jigs. Likewise, *L. vulgaris* samples from both Vigo and Faro showed seasonal patterns [as was previously found by Coelho et al. (1994) and

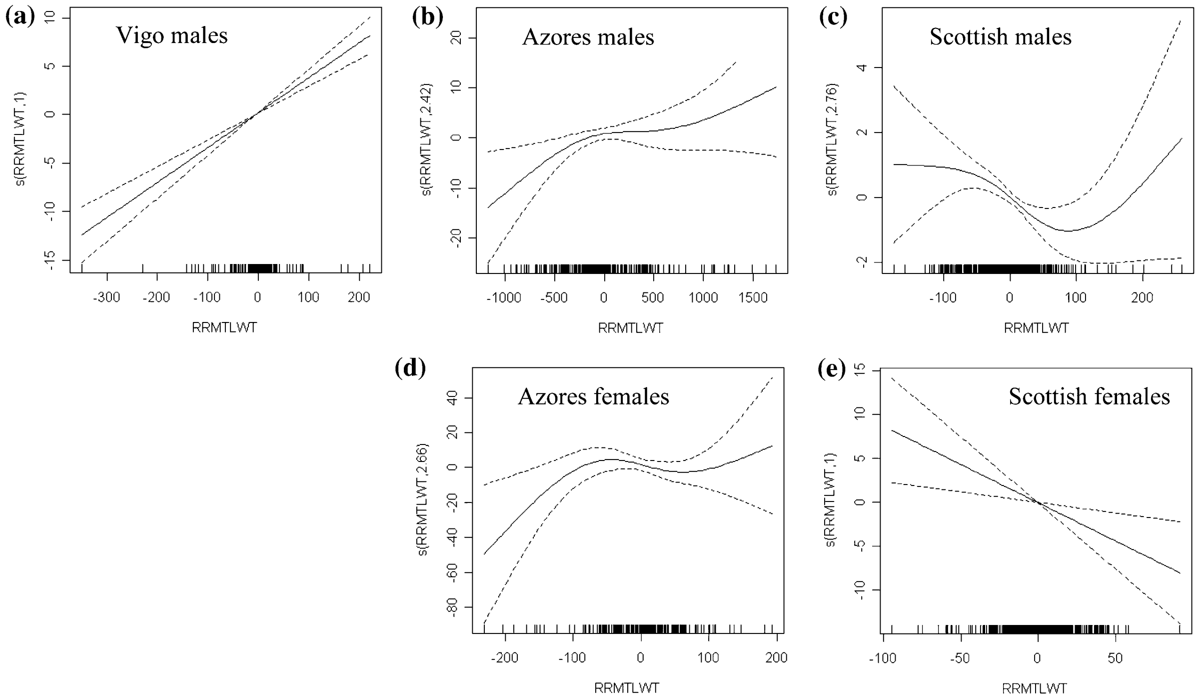


Fig. 9 GAM smoothing curves fitted to partial effects of the explanatory variable mantle weight (residuals) on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of residuals of mantle weight in **a** Vigo males, **b** Azores males, **c** Azores females, **d** Scottish males and **e** Scottish females. *Dashed lines* represent 95% confidence intervals around the main effects

Moreno et al. (1994) in samples from southern Portugal] with multiple maturity peaks, with the strongest in the winter (late February–early March) followed by two weaker peaks later in the year, possibly an indication of three cohorts.

A seasonal maturation trigger points to extrinsic factors driving maturation, (e.g. length of daylight hours, water temperature), particularly since *L. forbesii* from Vigo experience a maturity peak up to 2–3 months before that of the animals in the Azores. However, the evidence of males typically maturing earlier than females points towards a likely intrinsic component to maturation, as well.

Body length

In both sexes and both species from all ports, reproductive weight increased with increasing (seasonally adjusted) DML up to large body sizes, an indication that, in a given month, large animals have larger gonads, which implies that somatic and reproductive growth tend to occur in tandem and, assuming that large gonads lead to higher productive output, could imply a selective advantage to large body size.

The results provide no evidence for either continued somatic growth after maturation is complete or for cessation of somatic growth prior to completion of maturation, both of which phenomena would tend to generate non-linear relationships. However, it is worth noting that one constraint of this type of growth analysis is that without the availability of age data on the study samples, and because the smoothing graphs are composites of a collection of animals at different developmental points in the life cycle, it is impossible to follow the individual growth trajectories of single animals, and so inferences must be made from the population regarding growth processes (as has been done in previous research, e.g. Challier et al., 2006). As with previous results for male *L. forbesii* (and to a lesser extent, females) in Scotland and Spain (Guerra & Rocha, 1994; Collins et al., 1999; Smith et al., 2005), for *L. vulgaris* from the Iberian Peninsula (Coelho et al., 1994; Guerra & Rocha, 1994) and *Loligo reynaudii* off South Africa (Hanlon et al., 2002; Olyott et al., 2006), the

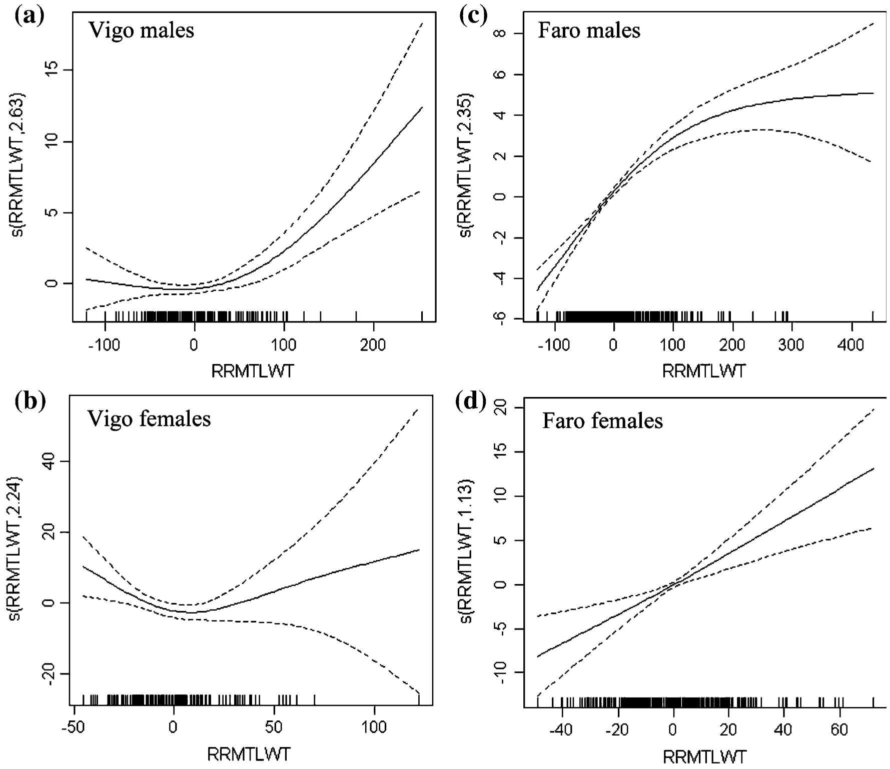


Fig. 10 GAM smoothing curves fitted to partial effects of the explanatory variable mantle weight (residuals) on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of residuals of mantle weight in **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females. *Dashed lines* represent 95% confidence intervals around the main effects

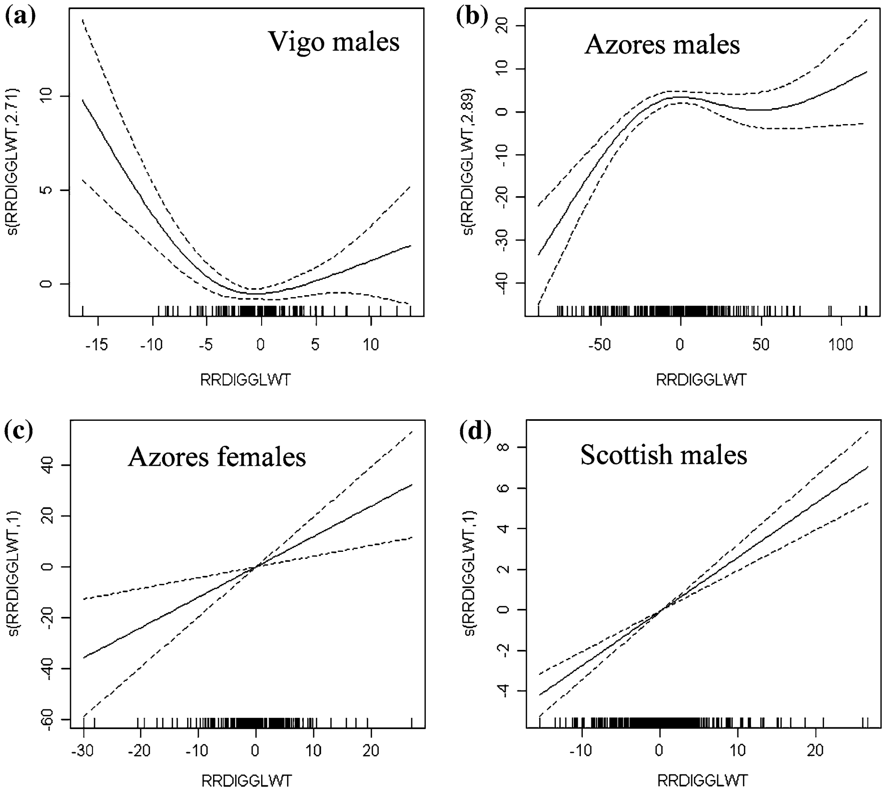


Fig. 11 GAM smoothing curves fitted to partial effects of the explanatory variable digestive gland weight (residuals) on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of residuals of digestive gland weight in **a** Vigo males, **b** Azores males, **c** Azores females and **d** Scottish males. *Dashed lines* represent 95% confidence intervals around the main effects

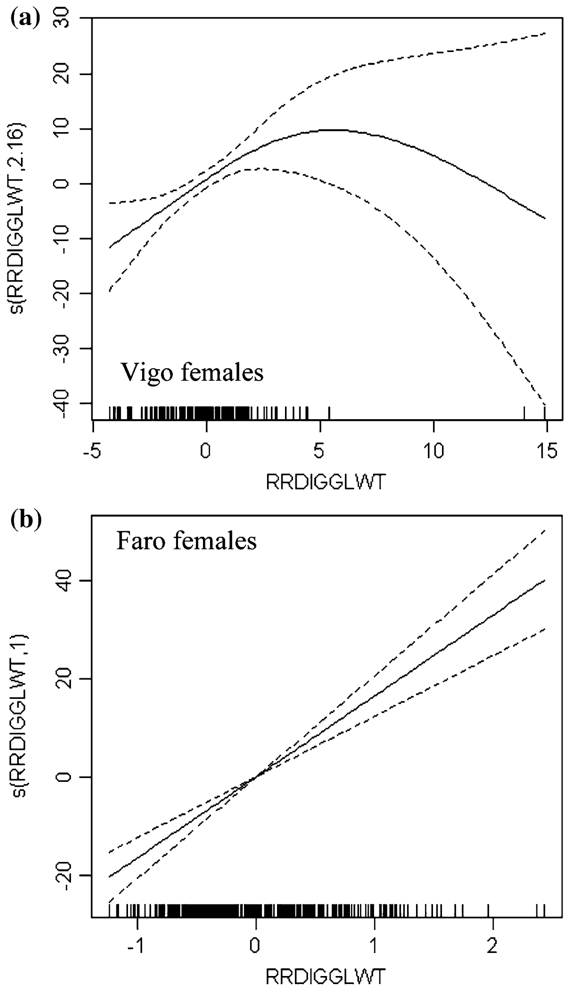


Fig. 12 GAM smoothing curves fitted to partial effects of the explanatory variable digestive gland weight (residuals) on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of residuals of digestive gland weight in **a** Vigo females and **b** Faro females. *Dashed lines* represent 95% confidence intervals around the main effects

existence of two to three size-at-maturity categories within the animals sampled, each potentially having a characteristic gonad weight-body length relationship, would tend to give the appearance of a weaker positive relationship between the variables than exists within each group alone.

Somatic versus reproductive growth

Although previous research has implied that energy for gonad growth is derived primarily from feeding (e.g. in *Illex argentinus*) (Hatfield et al., 1992), at least in some squid, for example, *Photololigo* spp., somatic growth has been shown to slow during periods of gametic growth (Moltschaniwskyj, 1995a), and a lack of large muscle fibres present in mantle tissue of mature females suggests a mobilisation of somatic energy for sexual maturation (Moltschaniwskyj, 1995b). In addition, amongst *Sepioteuthis australis* females, muscle instantaneous growth rates were negatively correlated with gonad size, an indication that energy is diverted from somatic growth to be used for reproductive growth (Ho et al., 2004).

The relationship between reproductive weight and seasonally and size-adjusted mantle weight is generally positive in *L. forbesii* Vigo males and *L. vulgaris* Faro males and females, with animals with the heaviest gonads also having the heaviest mantles. However, the relationship between reproductive and somatic growth is more complex for both sexes of Azores animals and for Scottish males, possibly attributable to the existence of multiple maturity cohorts. It has previously been argued by Rocha & Guerra (1996) that two size groups at maturity in *L. forbesii* males is more plausible than somatic growth after maturation in Galician waters. The relationship in Scottish females and for the majority of the samples for Scottish males differed from that found in all other models across the different ports and species, showing a negative, linear pattern, and as such, lower reproductive weight in the animals with the largest de-seasonalised, size-adjusted mantle weight could be an indication of energy re-mobilisation from somatic growth to reproductive growth, perhaps as a result of slower growth and maturation rates due to low SST, or due to poorer food resources in Scottish waters as compared to the Iberian Peninsula and the Azores.

Nutritional status

In the Azores, reproductive weight of male *L. forbesii* showed an asymptotic relationship with increasing (residual) digestive gland weight, whilst in females there was an almost linear, positive relationship between the two variables. The latter trend was also seen in male *L. forbesii* from Scotland and in female *L. vulgaris* from Faro, and in all the animals except those with the largest residual digestive gland weight values in females *L. vulgaris* from Vigo. These trends imply that (relatively) low nutritional status is associated with low gonad growth, and, at least in females, the highest nutritional status is associated with greatest gonad growth. In male *L. forbesii* from the Azores males, the asymptotic relationship suggests that, once a certain minimum nutritional state is achieved in these animals, full gonad growth is possible. It should be noted that Azores males reach much larger sizes than males of either studied species elsewhere.

In male *L. forbesii* from Vigo, the negative relationship seen between reproductive weight and digestive gland weight would imply that animals with larger (heavier) gonads have a poorer nutritional state (although this result is sensitive to a few animals with extremely low residual digestive gland weight values included in the sample). These results could be interpreted as indirect evidence of mobilisation of somatic tissue to build gonads, in that this would permit (whilst not explaining the advantage of) a reduction of feeding rate and hence reduced nutritional status. This is clearly dissimilar to the results obtained in males of this species in the other two study areas (Azores, Scotland).

Geographic and species variation

Aside from possible sample size issues, caution clearly is needed in inferring the existence or otherwise of geographical, sex or species differences from our results since data refer to only 1 or 2 years. Thus, food availability in a particular area and year might determine the balance between feeding and remobilisation of somatic reserves as a means of growing gonads.

Biological variability in adult size, gonadosomatic indices, size-at-maturity and length–weight relationships, has been previously demonstrated in loliginid squid (e.g. *L. vulgaris*, Moreno et al., 2002; *Alloteuthis subulata*, Hastie et al., 2009) across different geographical areas. It is suspected that environmental factors play an important role in determining regional differences in squid growth (see Pierce et al., 2008 for review of cephalopod–environment interactions), as cephalopods have been shown to be highly sensitive to the effects of temperature (e.g. loliginid squid, see Forsythe, 2004 for review), productivity (Argüelles & Tafur, 2009) and food availability (Moltschaniwskyj & Martinez, 1998; Argüelles et al., 2008). Positive phases of the North Atlantic Oscillation (NAO) have been shown to correlate with higher *L. forbesii* abundance and earlier migrations in the English Channel (Sims et al., 2001), and with higher abundance and precocious maturation in Scottish waters (Pierce et al., 2005). In addition, increased size and growth rate of *Loligo opalescens* off the coast of California has been linked to the increased productivity and food availability associated with the La Niña upwelling (Jackson & Domeier, 2003).

The Iberian Peninsula is affected by an oceanographic upwelling regime which leads to high local productivity and likely contributes to internal differences in abundance, growth rates and maturation of cephalopods landed from these waters. For example, the western coasts of Spain and Portugal are affected by the Iberian-Canary current upwelling system, in which coastal winds induce seasonal upwelling, driving annual cycles of production, nutrient levels and distribution of early life stage animals. The wind stress structure of the Galician (northwest Spain) upwelling system has been shown to influence the abundance and distribution of loliginid (Rocha et al., 1999; González et al., 2005) and octopod (González et al., 2005; Otero et al., 2008) paralarvae, as has that of the western Iberian upwelling system (Moreno et al., 2009), and has been found to largely explain variance in adult catch of *Octopus vulgaris* (Otero et al., 2008). The occurrence of upwelling might be responsible for the earlier maturation in Vigo animals. Size differences have been identified amongst *L. vulgaris* hatched in different seasons,being attributed to feeding changes due to seasonal upwelling in Galician waters, as well as suggesting that the favourable conditions produced during upwelling events would be more beneficial to *L. vulgaris* than to *L. forbesii*, as the former is more closely related to coastal waters whilst the later comparatively spends more time during its life cycle in offshore waters (Rocha & Guerra, 1999).

In contrast to the western coasts of Galicia and Portugal, the islands which comprise the Azores sit on a small continental shelf and are primarily surrounded by deep oceanic waters. Southeast of the Azores archipelago, the Azores Current (AC) transports warm and saline water high in chlorophyll *a* (Fernández & Pingree, 1996) eastward and extending near to the Gulf of Cadiz (Martins et al., 2002). A weak frontal system also exists to the southwest of the islands, known as the Azores Front (AF), which produces localised, enhanced productivity near the islands (Angel, 1989; Santos et al., 1995). It is possible that the Azores represents something of a productivity 'hotspot' for some marine species, as the timing of peak abundance of baleen whales, for example, has recently been shown to be predictable from the timing of the onset of the distinct phytoplankton spring bloom in this area (Visser et al., 2010). These conditions hypothetically could be responsible for the increased number of yearly maturity peaks in both sexes of *L. forbesii* from the Azores versus males of the same species from Vigo and females from Scotland.

The negative relationship between mantle weight and gonad growth in *L. forbesii* found only in Scottish waters (the most northern part of the species' range) is a possible indication that extrinsic factors in the environment may play a role in maturation, in this case, most likely the presence of colder water temperatures and shorter daylight during periods of reproductive growth. These conditions, possibly resulting in poorer feeding and slower maturation as compared to warmer environments, may hypothetically force the animals to use stored energy to contribute to gonad growth. Ideally, additional years of data are needed to test this hypothesis.

Between the two species of loliginid squid examined, the three seasonal maturity peaks identified amongst samples of *L. vulgaris* are in contrast to the single and double peaks found in *L. forbesii* in Vigo and the Azores, respectively. The relatively high productivity in inshore Galician waters may permit spawning over an extended time-period and hence generate numerous micro-cohorts, and a comparison of life cycles across both squid species in good and poor upwelling years would be instructive. It is also possible that a higher number of breeding micro-cohorts amongst *L. vulgaris* might serve as a buffer for the population from the effects of overfishing and environmental variation and perturbations such as increased SST and fluctuations in food availability. Other than this difference in number of yearly maturity peaks, the results from this research show no marked differences in the relationships between somatic and reproductive growth between *L. forbesii* and *L. vulgaris* from Iberian and Azorean waters. Thus, the results do not help in explaining the apparent disappearance of *L. forbesii* from the south of its geographical range in the early 1990s, whilst *L. vulgaris* continued to thrive in these waters (c.f., Chen et al., 2006).

This study, which made opportunistic use of an existing data set, has identified some variation in the relationships between size, season, nutritional state, somatic growth and reproductive growth in these two species. It would be interesting to carry out similar studies over several years, particularly years with different levels of productivity. In addition, larger sample sizes and availability of information on age would help interpretation of the effect of environmental conditions on growth and maturation.

Methodology

The technique of using residuals in place of original measured values to model patterns in somatic and reproductive growth as used in this research is useful in assisting in the interpretation of relationships between multiple co-varying explanatory variables. By separating out effects of seasonal and size-related variability (e.g. removing seasonal patterns and effects of body size), which may mask the 'true' relationships between variables, it is possible to examine the underlying effects of nutritional status and somatic weight on maturation, separating their seasonal and length-related components from the effects of variation from the seasonal norm whilst standardising for body length. However, difficulties do arise from aspects of the model fitting procedure, such as the potential subjectivity in selecting the maximum allowed degrees of freedom for smoothing functions, which can result in either under- or over-smoothing. It is therefore important to focus on general, overall trends and relationships between variables, which are likely to be biological in origin, as opposed to micro-trends in the smoothing functions, which can be model-dependent.

Acknowledgments Statistical consultation for this analysis was provided by the EC-funded CEPHSTOCK project (QOL-2001-5.1.2.). Data were collected mainly during the collaborative European project 'Fishery Potential of Northeast Atlantic Squid Stocks' (FAR MA 1.146, 1991–92) and were provided to the authors by the following: (1) Vigo: A. Guerra and team; (2) Azores: J. Goncalves and H. Martins; and (3) Faro: V. Bettencourt, M.L. Coelho, G. Olavo, J. Quintela and H. Villa.

Authors G.J.P. and J.M.S. were also supported by the ANIMATE project (MEXC-CT-2006-042337).

References

Akaike, H., 1973. Information theory as an extension of the maximum likelihood principle. In Petrov, B. N. & F. Csaksi (eds), 2nd International Symposium on Information Theory. Akademiai Kiado, Budapest: 267–281.

Angel, M. V., 1989. Vertical profiles on the pelagic communities in the vicinity of the Azores Front and their implications to deep ocean ecology. Progressive Oceanography 22: 1–46.

Argüelles, J. & R. Tafur, 2009. Life history of *Dosidicus gigas* in the Humboldt Current System: size at maturity, somatic and reproductive investment. Cephalopod International Advisory Council (CIAC) Symposium, Vigo, Spain, 3–11 September.

Argüelles, J., R. Tafur, A. Taipe, P. Villegas, F. Keyl, N. Dominguez & M. Salazar, 2008. Size increment of jumbo flying squid *Dosidicus gigas* mature females in Peruvian waters, 1989–2004. Progressive Oceanography 79: 308–312.

Boyle, P. R. & G. J. Pierce, 1994. Fishery biology of north east Atlantic squid: an overview. Fisheries Research 21: 1–15.

Boyle, P. R., G. J. Pierce & L. C. Hastie, 1995. Flexible reproductive strategies in the squid *Loligo forbesi*. Marine Biology 121: 501–508.

Brierley, A. S., J. P. Thorpe, G. J. Pierce, M. R. Clarke & P. R. Boyle, 1995. Genetic variation in the neritic squid *Loligo forbesi* (Myopsida: Loliginidae) in the northeast Atlantic. Marine Biology 122: 79–86.

Challier, L., P. Orr & J.-P. Robin, 2006. Introducing inter-individual growth variability in the assessment of a cephalopod population: application to the English Channel squid *Loligo forbesi*. Oecologia 150: 17–28.

Chen, C. S., G. J. Pierce, J. Wang, J.-P. Robin, J. C. Poulard, J. Pereira, A. F. Zuur, P. R. Boyle, N. Bailey, D. J. Beare, P. Jereb, S. Ragonese, A. Mannini & L. Orsi-Relini, 2006. The apparent disappearance of *Loligo forbesi* from the south of its range in the 1990s: trends in *Loligo* spp. abundance in the northeast Atlantic and possible environmental influences. Fisheries Research 78: 44–54.

Cleveland, W. S., 1993. Visualizing Data. AT&T Bell Laboratories, Murray Hill, NJ.

Coelho, M. L., J. Quintela, G. Bettencourt, G. Olavo & H. Villa, 1994. Population structure, patterns and fecundity of the squid *Loligo vulgaris* from southern Portugal. Fisheries Research 21: 87–102.

Collins, M. A., G. M. Burnell & P. G. Rodhouse, 1995. Reproductive strategies of male and female *Loligo forbesi* (Cephalopoda: Loliginidae). Journal of the Marine Biological Association of the UK 75: 621–634.

Collins, M. A., P. R. Boyle, G. J. Pierce, L. N. Key, S. E. Hughes & J. Murphy, 1999. Resolution of multiple cohorts in the *Loligo forbesi* population from the west of Scotland. ICES Journal of Marine Science 56: 500–509.

Cunha, M. M. & A. Moreno, 1994. Recent trends in the Portuguese squid fishery. Fisheries Research 21: 231–241.

Cunha M.M., A. Moreno, & J.M.F. Pereira, 1995. Spatial and temporal occurrences of *Loligo* spp. in Portuguese waters. International Council for the Exploration of the Sea, ICES CM 1995/K:33.

Fernández, E. & R. D. Pingree, 1996. Coupling between physical and biological fields in the North Atlantic subtropical front southeast of the Azores. Deep-Sea Research 43: 1369–1393.

Forsythe, J. W., 2004. Accounting for the effect of temperature on squid growth in nature: from hypothesis to practice. Marine and Freshwater Research 55: 331–339.

Forsythe, J. W. & R. T. Hanlon, 1989. Growth of the Eastern Atlantic squid, *Loligo forbesi* Steenstrup (Mollusca:Cephalopoda). Aquaculture 20: 1–14.

Fox, J., 2000. Multiple and Generalized Nonparametric Regression. Sage Publications, Inc., Ontario.

Fox, J., 2002. An R and S-Plus Companion to Applied Regression. Sage Publications, Inc., Ontario.

González, A. F., J. Otero, A. Guerra, R. Prego, F. J. Rocha & A. W. Dale, 2005. Distribution of common octopus and common squid paralarvae in a wind-driven upwelling area (Ria of Vigo, northwestern Spain). Journal of Plankton Research 27: 271–277.

Guerra, A. & F. Rocha, 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in Galician waters (NW Spain). Fisheries Research 21: 43–69.

Guerra, A., P. Sánchez & F. Rocha, 1994. The Spanish fishery for *Loligo*: recent trends. Fisheries Research 21: 217–230.

Hanlon, R. T., M. J. Smale & W. H. H. Sauer, 2002. The mating system of the squid *Loligo vulgaris reynaudii* (Cephalopod, Mollusca) off South Africa: fighting, sneaking, mating and egg laying behavior. Bulletin of Marine Science 71: 331–345.

Hastie, T. & R. J. Tibshirani, 1990. Generalized Additive Models. Chapman and Hall/CRC, London.

Hastie, L. C., M. Nyegaard, M. A. Collins, A. Moreno, J. M. F. Pereira, U. Piatkowski & G. J. Pierce, 2009. Reproductive biology of the loliginid squid, *Alloteuthis subulata*, in the north-east Atlantic and adjacent waters. Aquatic Living Resources 22: 35–44.

Hatfield, E. M. C., P. G. Rodhouse & D. L. Barber, 1992. Production of soma and gonad in maturing female *Illex argentinus* (Mollusca: Cephalopoda). Journal of the Marine Biological Association of the UK 72: 281–291.

Ho, J. D., N. A. Moltschaniwskyj & C. G. Carter, 2004. The effect of variability in growth on somatic condition and reproductive status in the southern calamary *Sepioteuthis australis*. Marine and Freshwater Research 55: 423–428.

Holme, N. A., 1974. The biology of *Loligo forbesi* (Steenstrup: Mollusca, Cephalopoda) in the Plymouth area. Journal of the Marine Biological Association of the UK 54: 481–503.

ICES, 2008. Report of the Working Group on Cephalopod Fisheries and Life History (WGCEPH), By Correspondence. ICES CM 2008/LRC:14: 53 pp.

Jackson, G. D. & M. L. Domeier, 2003. The effects of an extraordinary El Niño/La Niña event on the size and growth of the squid *Loligo opalescens* off Southern California. Marine Biology 142: 925–935.

Lum-Kong, A., G. J. Pierce & C. Yau, 1992. Timing of spawning and recruitment in *Loligo forbesi* (Cephalopoda:

Loliginidae) in Scottish waters. Journal of the Marine Biological Association of the UK 72: 301–311.

Martins, H. R., 1982. Biological studies of the exploited stock *Loligo forbesi* (Mollusca: Cephalopoda) in the Azores. Journal of the Marine Biological Association of the UK 62: 799–808.

Martins, C. S., M. Hamann & A. F. G. Fiúza, 2002. Surface circulation in the eastern North Atlantic, from drifters and altimetry. Journal of Geophysical Research 107: 3217–3244.

Moltschaniwskyj, N. A., 1995a. Changes in shape associated with growth in the loliginid squid *Photololigo* sp.: a morphometric approach. Canadian Journal of Zoology 73: 1335–1343.

Moltschaniwskyj, N. A., 1995b. Multiple spawning in the tropical squid *Photololigo* sp.: what is the cost in somatic growth? Marine Biology 124: 127–135.

Moltschaniwskyj, N. A. & P. Martinez, 1998. Effect of temperature and food levels on the growth and condition of juvenile *Sepia elliptica* (Hoyle 1885): an experimental approach. Journal of Experimental Marine Biology and Ecology 229: 289–302.

Moreno, A., M. M. Cunha & J. M. F. Pereira, 1994. Population biology of veined squid (*Loligo forbesi*) and European squid (*Loligo vulgaris*) from the Portuguese coast. Fisheries Research 21: 71–86.

Moreno, A., J. Pereira, C. Arvanitidis, J.-P. Robin, D. Koutsoubas, C. Perales-Raya, M. M. Cunha, E. Balguerias & V. Denis, 2002. Biological variation of *Loligo vulgaris* (Cephalopoda: Loliginidae) in the Eastern Atlantic and Mediterranean. Bulletin of Marine Science 71: 515–534.

Moreno, A., J. Pereira & M. Cunha, 2005. Environmental influences on age and size at maturity of *Loligo vulgaris*. Aquatic Living Resources 18: 377–384.

Moreno, A., M. Azevedo, J. Pereira & G. J. Pierce, 2007. Growth strategies in the squid *Loligo vulgaris* from Portuguese waters. Marine Biological Research 3: 49–59.

Moreno, A., A. Dos Santos, U. Piatkowski, A. M. P. Santos & H. C. Cabral, 2009. Distribution of cephalopod paralarvae in relation to the regional oceanography of the western Iberia. Journal of Plankton Research 31: 73–91.

Ngoile, M. A. K., 1987. Fishery biology of the squid *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. Ph.D. Thesis, University of Aberdeen.

Norman, J., J. Murphy, G. J. Pierce & P. R. Boyle, 1994. Preliminary molecular genetic analysis of stock structure in the squid *Loligo forbesi* (Steenstrup, 1856). ICES C.M. 1994/K: 23.

Olyott, L. J. H., W. H. H. Sauer & A. J. Booth, 2006. Spatio-temporal patterns in maturation of the chokka squid (*Loligo vulgaris reynaudii*) off the coast of South Africa. ICES Journal of Marine Science 63: 1649–1664.

Otero, J., X. A. Álvarez-Salgado, A. F. González, A. Miranda, S. B. Groom, J. M. Cabanas, G. Casas, B. Wheatley & A. Guerra, 2008. Bottom-up control of common octopus *Octopus vulgaris* in the Galician upwelling system, northeast Atlantic Ocean. Marine Ecology Progress Series 362: 181–192.

Pierce, G. J., P. R. Boyle, L. C. Hastie & L. Key, 1994a. The life history of *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. Fisheries Research 21: 17–41.

Pierce, G. J., P. R. Boyle, L. C. Hastie & A. Shanks, 1994b. Distribution and abundance of the fished population of *Loligo forbesi* in UK waters: analysis of fishery data. Fisheries Research 21: 193–216.

Pierce, G. J., R. S. Thorpe, L. C. Hastie, A. S. Brierley, A. Guerra, P. R. Boyle, R. Jamieson & P. Avila, 1994c. Geographic variation in *Loligo forbesi* in the Northeast Atlantic Ocean: analysis of morphometric data and tests of casual hypotheses. Marine Biology 119: 541–547.

Pierce, G. J., N. Bailey, Y. Stratoudakis & A. Newton, 1998. Distribution and abundance of the fished population of *Loligo forbesi* in Scottish waters: analysis of research cruise data. ICES Journal of Marine Science 55: 14–33.

Pierce, G. J., A. F. Zuur, J. M. Smith, M. B. Santos, N. Bailey, C. Chen & P. R. Boyle, 2005. Interannual variation in life cycle characteristics of the veined squid (*Loligo forbesi*) in Scottish (UK) waters. Aquatic Living Resources 18: 327–340.

Pierce, G. J., V. D. Valavanis, A. Guerra, P. Jereb, L. Orsi-Relini, J. M. Bellido, I. Katara, U. Piatkowski, J. Pereria, E. Balguerias, I. Sobrino, E. Lefkaditou, J. Wang, S. Santurtum, P. R. Boyle, L. C. Hastie, C. D. MacLeod, J. M. Smith, M. Viana, A. F. Gonzáles & A. F. Zuur, 2008. A review of cephalopod–environment interactions in European Seas. Hydrobiologia 612: 49–70.

Porteiro, F. M., 1994. The present status of the squid fishery (*Loligo forbesi*) in the Azores archipelago. Fisheries Research 21: 243–253.

Porteiro, F. M. & H. R. Martins, 1994. Biology of *Loligo forbesi* Steenstrup, 1856 (Mollusca: Cephalopoda) in the Azores: sample composition and maturation of squid caught by jigging. Fisheries Research 21: 103–114.

Rocha, F. & A. Guerra, 1996. Signs of an extended and intermittent terminal spawning in the squids *Loligo vulgaris* Lamarck and *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae). Journal of Experimental Marine Biology and Ecology 207: 177–189.

Rocha, F. & A. Guerra, 1999. Age and growth of two sympatric squid *Loligo vulgaris* and *Loligo forbesi*, in Galician waters (north-west Spain). Journal of the Marine Biological Association of the UK 79: 697–707.

Rocha, F., A. Guerra, R. Prego & U. Piatkowski, 1999. Cephalopod paralarvae and upwelling conditions off Galician waters (NW Spain). Journal of Plankton Research 21: 21–33.

Roper, C. F. E., M. J. Sweeny & C. E. Nauen, 1984. Cephalopods of the World: An Annotated and Illustrated Catalogue of Species of Interest to Fisheries. FAO, Rome.

Ryan, B. F., B. L. Joiner & T. A. Ryan Jr., 1985. Minitab Handbook, 2nd edn. Duxbury Press, Boston.

Salman, A. & T. Katağan, 2002. Cephalopod fauna of the eastern Mediterranean. Turkish Journal of Zoology 26: 47–52.

Santos, R. S., S. Hawkins, L. R. Monteiro, M. Alves & E. J. Isidro, 1995. Case studies and reviews: marine research, resources and conservation in the Azores. Aquatic Conservation – Marine and Freshwater Ecosystems 5: 311–354.

Shaw, P. W., G. J. Pierce & P. R. Boyle, 1999. Subtle population structuring within a highly vagile marine invertebrate, the veined squid *Loligo forbesi*, demonstrated with microsatellite DNA markers. Molecular Ecology 8: 407–417.

Sims, D. W., M. J. Genner, A. J. Southward & S. J. Hawkins, 2001. Timing of squid migration reflects North Atlantic climate variability. Proceedings of the Royal Society of London 268: 2607–2611.

Smith, J. M., G. J. Pierce & A. F. Zuur, 2005. Seasonal patterns of investment in reproductive and somatic tissues in the squid *Loligo forbesi*. Aquatic Living Resources 18: 341–351.

Thorpe R.S, 1976. Biometric analysis of geographic variation and racial affinities. Biological Reviews 51:407–452.

Visser, F., K. L. Hartman, G. J. Pierce, V. D. Valavanis & J. Huisman, 2011. Spring migration of baleen whales in relation to the timing of the North Atlantic spring bloom: Baleen whale migration and the North Atlantic spring bloom. Marine Ecology Progress Series (in review).

Wood, S. N., 2000. Modelling and smoothing parameter estimation with multiple quadratic penalties. Journal of the Royal Statistical Society Series B-Statistical Methodology 62: 413–428.

Wood, S. N., 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. Journal of the American Statistical Association 99: 637–686.

Wood, S. N., 2006. Generalized Additive Models: An Introduction with R. Chapman and Hall/CRC, London.

Zuur, A. F. & G. J. Pierce, 2004. Common trends in Northeast Atlantic squid time series. Netherlands Journal of Sea Research 52: 57–72.

Zuur, A. F., E. N. Ieno & G. M. Smith, 2007. Analysing Ecological Data. Springer, New York.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev & G. M. Smith, 2009. Mixed Effects Models and Extensions in Ecology with R. Springer, New York.

Hydrobiologia (2011) 670:223–240
DOI 10.1007/s10750-011-0723-3

ECOSYSTEMS AND SUSTAINABILITY

Temporal and ontogenetic variation in the diet of squid (*Loligo forbesii* Streenstrup) in Scottish waters

Sansanee Wangvoralak · Lee C. Hastie · Graham J. Pierce

Published online: 6 May 2011
© Springer Science+Business Media B.V. 2011

Abstract The squid *Loligo forbesii* is the only cephalopod species currently targeted by fisheries in the northern NE Atlantic. An active predator, it feeds primarily on fish, crustaceans and cephalopods. During 15 years since the only previous large-scale study of the diet of this species in Scottish waters, there have been substantial changes in marine fish abundances. The present study evaluates sources of variation (temporal, ontogenetic) in diet composition and prey size preferences of *L. forbesii*, including a comparison of contemporary (July 2006–June 2007) and historical (1990–1992) dietary datasets. Results revealed significant size-related and seasonal variation in diet composition and prey size. Teleost fish of the families Ammodytidae and Gobiidae were eaten by squid of all sampled sizes, although occurrence of gobies was generally more frequent in smaller squids, while Gadidae were eaten more frequently by larger squids. Cannibalism was also more frequent in larger squids. Compared to the 1990–1992 dataset, clupeid fish were less important in the diet of squid in 2006–2007, while the importance of gobies increased, and the size of gobies eaten also increased. The trend in gadoids differed according to the index used: their frequency of occurrence was considerably higher in 2006–2007 than in 1990–1992, but their numerical importance was slightly lower. In general, results provided little evidence that changes in the diet of *L. forbesii* correspond with changes in fish abundance, at least at the scales at which these are measured.

Keywords Diet · *Loligo forbesii* · Trophic interactions · Long-term trends

Introduction

Cephalopods play key roles as both predators and prey in marine ecosystems (e.g. Boyle, 1990; Hastie et al., 2009a). Cephalopods are usually short-lived and their populations typically display a high production to biomass ratio (Boyle & Boletzky, 1996; Caddy & Rodhouse, 1998). They provide important food resources for a range of predators, including toothed whales, seabirds and demersal fish (e.g. Furness, 1994; Daly et al., 2001; Piatkowski et al., 2001). Most cephalopod species are active predators, whose high metabolic rates result in high energy demands. Their prey includes fish, crustaceans, other molluscs, ophiuroids, polychaetes, chaetognaths and siphonophores (Martins, 1982; Nixon, 1987; Rodhouse, 1996; Boyle

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

S. Wangvoralak (✉) · L. C. Hastie · G. J. Pierce
School of Biological Sciences, University of Aberdeen,
Zoology Building, Tillydrone Avenue,
Aberdeen AB25 2TZ, UK
e-mail: s.wangvoralak@abdn.ac.uk

& Rodhouse, 2005). Since the cephalopod oesophagus passes through the brain, prey are usually cut into small pieces by the chitinous mandibles prior to ingestion and some prey had parts may be discarded rather than ingested (Bidder, 1950; Boycott, 1961; Porteiro et al., 1990). Consequently, stomach contents may provide a rather incomplete record of feeding. Nevertheless, this remains the main source of information and can still provide useful information.

The factors influencing prey selection in cephalopods are not well understood. In principle, several types of explanations are possible: (a) functional explanations related to evolutionary advantage (e.g. optimal foraging theory, Pyke, 1984); (b) mechanistic explanations dealing with the process by which prey selection occurs (e.g. morphological constraints, behavioural mechanisms); (c) explanation related to empirical relationships (e.g. dietary variation with season) for which the mechanism and/or function may or may not be apparent. Most dietary studies provide only empirical evidence of diet selection. Captive studies, however, have provided some insights into both selectivity and potential biases in stomach contents, e.g. squids may not ingest the heads of large fish (Porteiro et al., 1990).

Loligo forbesii is the only cephalopod currently targeted by fisheries in the northern NE Atlantic, although several other species are landed as by-catches (Pierce et al., 2010). Like most loliginid squids, it has a short life cycle of 1–2 years, the seasonal timing of which varies across its geographical range (Holme, 1974; Ngoile, 1987; Guerra et al., 1994; Pierce et al., 1994a; Collins et al., 1995). The main prey groups are fish, crustaceans and other cephalopods (e.g. Martins, 1982; Ngoile, 1987; Collins et al., 1994; Guerra & Rocha, 1994; Pierce et al., 1994b; Stowasser, 2004). Cannibalism is recorded most frequently in larger individuals (Collins & Pierce, 1996). The only previous large-scale study of the diet of this species in Scottish waters (1990–1992) demonstrated both size-related and seasonal variation in diet: Pierce et al. (1994b) reported that the main prey groups were fishes of the families Gadidae, Clupeidae and Ammodytidae, the first two of these families being more important in Winter, while the latter were more common prey in Summer.

It is a general feature of predator–prey relationships that prey size increases with predator size (e.g. Preciado et al., 2008, in relation to demersal fish). While there have been few studies on predator size–prey size relationship in cephalopods, increases in prey size with predator size have been previously reported in the squids *Todarodes sagittatus* (Quetglas et al., 1999) and *L. forbesii* (Collins & Pierce, 1996).

Over long (e.g. decadal) time-scales and due to changes in marine community composition, shifts in dominant prey species may be expected to occur. Over the last two decades, stocks of some of the important prey of *L. forbesii*, especially sandeels and gadoids, have declined (ICES, 2009, 2010). The North Sea has experienced a rapid increase of sea temperature in comparison with other large marine ecosystems (Belkin, 2009), which is likely to have impacted on resident marine species. Dulvy et al. (2008) and Perry et al. (2005) showed that there were changes in fish distribution according to depth (moving towards deeper water) and latitude (moving towards the north for large gadoids and the south for small gadoids) in the North Sea.

Comparison of results on diet of *L. forbesii* from 1990–1992 in Scottish waters with two other smaller-scale studies suggests that there have been changes in diet composition, although it is difficult to completely rule out sampling biases. The main prey species found during 1984–1986 by Ngoile (1987) were Argentine (*Argentina sphryaena*), Norway pout (*Trisopterus esmarkii),* whiting (*Merlangius merlangus*) and sandeel (*Ammodytes* sp.). In a more recent study by Stowasser (2004), the most frequently identified fish were *Trisopterus* sp., silvery pout (*Gadiculus argenteus*) and transparent goby (*Aphia minuta*).

The objectives of this study are:

(a) to describe the diet of *L. forbesii* in Scottish waters during 2006–2007, evaluating the importance of seasonal and ontogenetic variation in diet composition and prey size
(b) to determine whether there has been a shift in feeding patterns of *L. forbesii* since the last major study in 1990–1992 and whether any such changes can be related to changes in prey availability

Materials and methods

Diet data for 2006–2007

Monthly samples of *L. forbesii* from the Moray Firth, northern North Sea, were collected from commercial

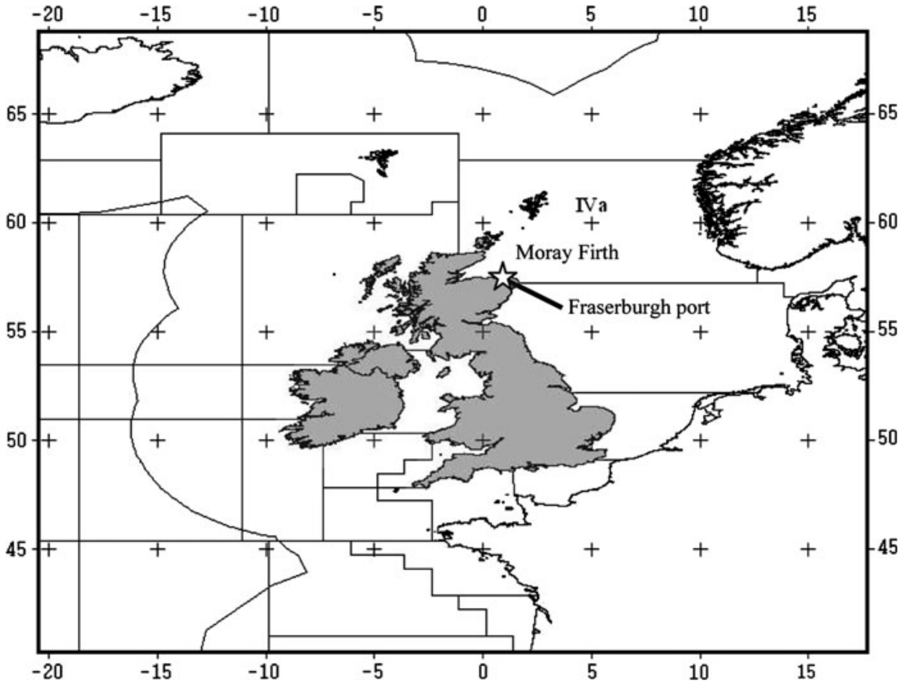


Fig. 1 Location of the port of Fraserburgh in the Moray Firth, ICES fishery subdivision IVa

trawler landings in the Scottish port of Fraserburgh (Fig. 1) from July 2006 until June 2007. In total, 360 stomachs (typically 30 per month, selected at random) were sampled. Biological data were also recorded as follows: dorsal mantle length (ML), body weight (BW), sex, maturity stage (a scale from I to V, following Boyle & Ngoile, 1993; Pierce et al., 1994a), and mantle weight (MW). Samples were stored frozen (−20°C) prior to analysis.

Stomach contents were thawed at room temperature and sorted by using a low-power, stereo microscope. Contents were initially sorted into major groups: fish, cephalopods, crustaceans, and others, and then classified to family, genus, and species, as far as possible, based mainly on identification of hard parts. The presence of fish was determined from bones (including vertebrae), otoliths, lenses, scales, fin rays, and flesh. Reference material and published guides (Härkönen, 1986; Watt et al., 1997; Scharf et al., 1998) were used to support identifications. Cephalopods were recognised from beaks, sucker rings, statoliths, gladius, lenses, and flesh. Squid beaks were identified to the lowest possible taxonomic level using Clarke's (1986) guide. Crustaceans were recognised and identified from exoskeletons, eyes, and flesh, while zooplankton were identified using the guide of Conway et al. (2003).

Dietary information was quantified using standard indices. Percent frequency of occurrence (%*F*) refers to the percentage of stomachs containing a particular prey type (and when summed across all prey types, often exceeds 100% due to stomachs containing prey more than one type (e.g., Hyslop, 1980). For graphical presentations, we used modified percentage frequency of occurrence, in which %*F* values were rescaled to sum to 100% across all prey types. We also calculated percentage numerical importance (%*N*) for each prey type, this being the total number of individuals of a particular prey type, summed across all stomachs, expressed as a percentage of the total number of individuals of all prey types (Breiby & Jobling, 1985). All such indices have limitations (see Hyslop, 1980; Tollit et al., 2010). Both %*F* and %*N* may underestimate the importance of rapidly digested prey and of large fish prey (since squids do not always consume the heads; Porteiro et al., 1990) while %*N* will tend to overestimate the dietary importance of small prey.

Fish size was estimated from measurement of otolith size, using published relationships between otolith length (OL) and width (OW) and fish length (FL) (Härkönen, 1986). Because fish otoliths and cephalopod beaks (from which prey size can also be estimated) were encountered in stomachs relatively rarely, we did not attempt to estimate overall diet composition in terms of % weight.

Historical data

In order to assess possible changes in diet over the last 15 years, raw data on *L. forbesii* diet collected during 1990–1992 (see Pierce et al., 1994b; Collins & Pierce, 1996) were re-analyzed. These data were obtained from squid caught in Scottish waters using various gears, by both commercial and research vessels. Sources of variation were assumed to be similar to those for 2006–2007, with the addition of region, since the 1990s data came from three different regions: North Sea (including the Moray Firth), West coast (i.e. ICES subdivision VIa), and Rockall (ICES subdivision VIb). We also compared dietary results in the North Sea from the two time periods (1990–1992 and 2006–2007).

Data exploration and preliminary analyses

Data exploration was undertaken to identify unusual data points, determine appropriate distributions for response variables, to indicate collinearity between explanatory variables and to display evidence of interactions between effects of explanatory variables. This involved use of Cleveland dotplots, pair plots and co-plots and calculation of the variance inflation factor (VIF). Subsequent analyses were carried out for data from 1990–1992 and 2006–2007 both separately and (for the sub-set of North Sea data) together. Results on diet composition are summarised in graphical and tabular form to identify the main size-related, seasonal, regional and between-study period patterns. Preliminary analysis of dietary preferences was carried out using simple non-parametric statistics: Spearman Rank correlations (for relationships between prey size and predator size, Kruskal–Wallis tests (for size difference among prey species) and Mann–Whitney *U* tests (for prey size differences between the two study periods). These tests were carried out using SPSS 16.

Statistical modelling

Generalized additive modelling (GAM) was applied to investigate sources of variation in dietary preferences. Binomial GAMs with logit link functions were fitted to presence-absence data for three important categories of fish prey, namely sandeels, gadoids, and gobies. There were insufficient records to do GAMs for any gadid species on their own. GAMs with Gaussian distribution were applied for analysis of prey size. The latter analyses used prey length data (for single prey species and combined across prey species), either fish length (FL) or otolith length (OL). OL could be used for analyses of size in individual prey species while for analyses in which data from several species were combined, FL was used. For most prey species, a better fit to a normal distribution was achieved by log transformation ($\log_{10}(x)$) of length data.

For all these analyses the set of putative explanatory variables comprised month (or season), individual (predator) dorsal mantle length (ML), region, predator sex and (when datasets from both time periods were combined) time period. Interactions between explanatory variables were also considered. For those analyses of prey size in which data from several prey species were combined, species identity was also included as an explanatory variable.

Mantle length was log-transformed to reduce the influence of a small number of high values. ML was fitted as a smoother with the constraint $k = 4$ (i.e. maximum of 3 degrees of freedom) to avoid "overfitting" (i.e. in this context, to avoid fitting overcomplex relationships that would be biologically unrealistic). Coplots indicated a strong interaction between effects of ML and season on response variables. Therefore smoothers for the effect of ML were fitted separately for data from each season and results compared with those from the equivalent model in which a single smoother for ML was fitted, using an *F* test. A significant *F* value confirmed the need to include the interaction. Such a procedure was also applied for other nominal variables (region, sex and year) to determine if their effects interacted with that of ML.

For all model fitting, a combined forwards and backwards selection procedure was used to find the optimal model. Comparisons of models were usually based on values of the Akaike Information Criterion (AIC) (the lower AIC is the better model) and significance of individual explanatory variables. If optimal models contained non-significant terms, an *F* test was used to confirm that the non-significant term significantly improved the overall fit Model validation included checking for influential data points (high "hat" values) and patterns in the distribution of residuals (Zuur et al., 2007). All data exploration and GAM modelling were carried out using Brodgar software V.2.6.5 (Highland Statistics Ltd.), which is a menu-driven interface for *R*.

Results

General characteristics of squid diet in 2006–2007

The set of samples from 2006–2007 consisted of stomach contents of 192 females, 158 males, and 10 individuals that could not be sexed. Observed mantle lengths (ML) ranged from 56–500 mm, and body weight (BW) ranged from 10–1845 g.

The most commonly occurring general prey category was fish (95.6% occurrence), followed by crustaceans (29.2%) and cephalopods (10.6%). Small numbers of bivalve molluscs and gastropods were also recorded, in 0.6 and 4.5% of stomachs, respectively. Among the fish remains, 10 families and 21 species of fish prey were identified, of which Gadidae (43.9%), Ammodytidae (23.1%), and Gobiidae (19.7%) were the most frequently occurring prey families. The most frequently occurring fish prey species observed were Norway pout (*T. esmarkii*), silvery pout (*G. argenteus*), and lesser sandeel (*Ammodytes marinus*). Most crustacean remains were completely macerated and difficult to identify. However, decapods and copepods (mainly *Temora turbinata*) were identified. Copepods were present during April–September, with most frequent occurrence in June. Cephalopod remains were identified in 10.6% of samples, including 1.4% of stomachs which contained *Loligo* sp. Further details of diet composition appear in Table 1.

Variation in importance of different prey categories

Generalized additive modelling results (Table 2) showed that, in general, squid (predator) size, season and region were the most important factors explaining variability in the occurrence of sandeels, gobies and gadoids in squid diet. There were also some differences in male and female diet. There were

Table 1 Prey species of *Loligo forbesii*, 2006–2007 (*%F* = percent frequency of occurrence)

Prey type	*%F* ($n = 360$)
Fish (total)	95.6
Ammodytidae (Sandeels)	
Ammodytes spp.	13.6
Ammodytes marinus	6.7
Hyperoplus lanceolatus	2.8
Argentinidae	
Argentina silus (Greater argentine)	0.3
Argentina sphyraena (Argentine)	1.1
Bothidae	
Arnoglossus laterna (Scaldfish)	0.8
Callionymidae	
Callionymus spp.	1.4
Callionymus lyra (Dragonet)	0.3
Carangidae	
Trachurus trachurus (Scad)	2.2
Clupeidae	
Unidentified Clupeidae	4.4
Gadidae (Cods and haddocks etc.)	
Micromesistius poutassou (Blue whiting)	0.8
Merlangius merlangus (Whiting)	3.6
Trisopterus spp.	9.2
Trisopterus esmarkii (Norway pout)	11.1
Trisopterus minutus (Poor cod)	0.6
Melanogrammus aeglefinus (Haddock)	2.2
Gadus morhua (Cod)	1.4
Gadiculus argenteus (Silver pout)	8.3
Enchelyopus cimbrius (Four-bearded rockling)	0.8
Unidentified Gadidae	5.8
Gobiidae	
Gobiusculus flavescens (Two-spot goby)	0.3
Aphia minuta (Transparent goby)	5.3
Pomatoschistus spp.	10.3
Unidentified Gobiidae	3.9
Pleuronectidae	
Hippoglossoides platessoides (Long rough dab)	0.8
Pholidae	
Pholis gunnellus (Butterfish)	0.8
Unidentified fish	11.9
Crustaceans (total)	29.2
Order Decapoda	
Unidentified Decapoda	4.7

Table 1 continued

Prey type	%F ($n = 360$)
Order Calanoida	
Temoridae	
Temora turbinate	0.6
Unidentified Calanoida	2.8
Unidentified crustaceans	21.1
Cephalopods (total)	10.6
Loliginidae	
Loligo sp.	1.4
Unidentified cephalopods	9.2
Bivalves (total)	0.6
Gastropods (total)	4.5

significant interactions between effects of size and season in several of the final models, notably those for presence of sandeel in the diet, and there was a significant size–sex-study period interaction in the case of gobies.

Seasonal patterns

Fish, crustaceans and cephalopods were found in the diet of *L. forbesii* all the year round during 2006–2007. The percentage occurrence of fish prey decreased during August–October, whilst that of crustacean prey increased in that period (Fig. 2). Cephalopod remains were most frequently found in stomachs during February and October. Three main families of fish prey, Ammodytidae, Gadidae and Gobiidae were present in the diet of *L. forbesii* in every season. Gadidae were the most commonly occurring fish prey in the Winter, Spring and Autumn diet, occurring in 52.1, 32.9, and 28.6% of the stomachs, respectively, while Ammodytidae were the most frequently occurring in the Summer diet (present in 36.2% of stomachs).

Generalized additive modelling results confirmed significant seasonal differences in the occurrence of sandeels, gadoids and gobies in squid diet. From the combined data for the two study periods (North Sea only), sandeels were eaten most frequently in Summer, followed by Autumn, Spring and Winter, respectively. Gobies were found in the diet most frequently in Summer, followed by Autumn, Spring and Winter, respectively. For gadoids, the separate GAM analyses of occurrence data for 1990–1992 and 2006–2007 indicated that gadoids tended to be eaten more frequently in Autumn, and less frequently in Spring (Table 2).

Size-related dietary variation

Fish were the most frequently occurring broad prey category in all size-classes of squid (with %$F > 50$% in all size classes) but their occurrence tended to increase in larger size classes of squid. Otoliths of *Callionymus lyra* and *Pholis gunnellus* were found only in smaller squids (<100 mm ML), while those of *Trachurus trachurus* were present only in larger squids (242–352 mm ML). Fish in family Ammodytidae and Gobiidae were found in a wide range of squid sizes (75–354 mm ML) but were most common in the diet of small *L. forbesii* (Fig. 3). On the other hand, gadoids trended to be found more frequently in large squids. A Kruskal–Wallis test indicated significant variation ($P = 0.032$) in the median mantle length of *L. forbesii* that had eaten different fish prey groups (Ammodytidae, Gadidae, Gobiidae, Argentinidae, and other fish prey), confirming that at least some of the trends described above are statistically significant. The percentage occurrence of crustacean prey declined from 30–40% in squid 56–145 mm ML to 6–19% in squid >145 mm ML. Cephalopod remains were found in squid from 70 mm ML upwards but tended to be found mainly in larger squids (60% occurrence in squid > 150 mm ML).

GAM results confirmed significant effects of squid size on the incidence of sandeels, gadoids and gobies in squid diet (Fig. 4) but present a less clear picture than suggested by examination of the raw data. Size-related trends in diet are illustrated in Fig. 4. Results were not completely consistent but gobies tended to be eaten more frequently by smaller squids (except for female squid in 2006–2007) and gadoids tended to be eaten more frequently by larger squid. Trends for sandeel consumption varied between seasons, with occurrence of sandeels in the 1990–1992 study peaking in larger squid in Spring but the opposite trend being apparent in Autumn.

Differences between the two study periods

Diet data collected in North Sea during 1990–1992 by Pierce et al. (1994b) were compared with the present dataset. It should be noted that the squid in the 1990–1992 sample from the North Sea were on

Table 2 Results of binomial GAM models for the presence of various fish prey in the diet of *Loligo forbesii*

Prey group	Final model	DE (%)
2006–2007		
Sandeel	Y1 $\sim$ 1 + as.factor(season) + s(ML, $k = 4$, by = as.factor(season))	21.7
($n = 345$)	Season, $P < 0.001$ (Q3 > Q2 > Q4 > Q1); s(ML):Winter, $P = 0.605$, df = 1; s(ML):Spring, $P = 0.364$, df = 2; s(ML):Summer, $P = 0.095$, df = 1; s(ML):Autumn, $P = 0.033$, df = 1	
Gadoid	Y1 $\sim$ 1 + as.factor(season) + s(ML, $k = 4$)	16.0
($n = 340$)	s(ML), $P < 0.001$, df = 3; Season, $P = 0.012$ (Q4 > Q1 > Q3 > Q2)	
Gobies	Y1 $\sim$ 1 + as.factor(season) + s(ML, $k = 4$, by = as.factor(season))	29.7
($n = 338$)	Season, $P = 0.037$ (Q2 > Q4 > Q1 > Q3); s(ML):Winter, $P = 0.051$, df = 1; s(ML):Spring, $P = 0.109$, df = 1; s(ML):Summer, $P = 0.009$, df = 2.4; s(ML):Autumn, $P = 0.060$, df = 1	
1990–1992		
Sandeel	Y1 $\sim$ 1 + as.factor(season) + as.factor(region) + s(ML, $k = 4$, by = as.factor(season))	9.7
($n = 1311$)	Season, $P < 0.001$ (Q2 > Q3 > Q4 > Q1); Region, $P < 0.001$ (RA > NS > WC); s(ML):Winter, $P = 0.412$, df = 1.4; s(ML):Spring, $P = 0.008$, df = 1; s(ML):Summer, $P = 0.132$, df = 1; s(ML):Autumn, $P = 0.009$, df = 1	
Gadoid	Y1 $\sim$ 1 + as.factor(sex) + as.factor(season) + as.factor(region) + s(ML, $k = 4$)	8.0
($n = 1282$)	s(ML), $P < 0.001$, df = 2; Season, $P < 0.001$ (Q4 > Q3 > Q1 > Q2); Sex, $P = 0.03$ (F > M); Region, $P < 0.001$ (WC > NS > RA)	
Gobies	Y1 $\sim$ 1 + as.factor(region) + s(ML, $k = 4$)	19.9
($n = 1311$)	s(ML), $P = 0.012$, df = 1.6; Region, $P = 1$ (NS > WC > RA)	
1990–2007		
Sandeel	Y1 $\sim$ 1 + as.factor(season) + s(ML, $k = 4$, by = as.factor(season))	14.2
($n = 716$)	Season, $P < 0.001$ (Q3 > Q4 > Q2 > Q1); s(ML):Winter, $P = 0.002$, df = 2.5; s(ML):Spring, $P = 0.147$, df = 1; s(ML):Summer, $P = 0.003$, df = 3; s(ML):Autumn, $P = 0.058$, df = 2	
Gadoid	Y1 $\sim$ 1 + as.factor(year) + s(ML, $k = 4$, by = as.factor(season))	10.9
($n = 701$)	Year, $P < 0.001$ (Y2 > Y1); s(ML):Winter, $P = 0.872$, df = 1; s(ML):Spring, $P = 0.037$, df = 2.9; s(ML):Summer, $P < 0.001$, df = 2.4; s(ML):Autumn, $P = 0.004$, df = 1	
Gobies	Y1 $\sim$ 1 + as.factor(sex) + as.factor(year) + as.factor(season) + s(ML, k = 4, by = as.factor(year:sex))	13.3
($n = 701$)	Season, P = 0.188 (Q3 > Q4 > Q2 > Q1); Year, P = 0.038 (Y2 > Y1); Sex, $P = 0.158$ (F > M); s(ML):Y1 and female, P = 0.683, df = 1; s(ML):Y1 and male, P = 0.019, df = 1; s(ML):Y2 and female, $P = 0.002$, df = 1; s(ML):Y2 and male, $P = 0.285$, df = 1.5	

For explanatory variables remaining in the final models, the table gives associated P values and direction of differences is indicated in parentheses. For fitted smoothers, both P values and degrees of freedom (df, a value of 1 indicates a linear fit) are given. Significant smoothers are illustrated in Fig. 4

ML dorsal mantle length, *DE* deviance explained, *Y1* dataset in 1990–1992, *Y2* Dataset in 2006–2007, *Q1* Winter, *Q2* Spring, *Q3* Summer, *Q4* Autumn, *F* female, *M* Male, *NS* North Sea, *WC* West Coast, *RC* Rockall

average larger (in terms of median mantle length) than those sampled in 2006–2007 (Mann–Whitney U test, $P < 0.001$). In both datasets, fish were the most important food source of *L. forbesii*, followed by crustaceans and cephalopods. Differences were apparent in the types of fish prey eaten (see Table 3). Clupeids, sandeels and gadoids were the most important prey groups in 1990–1992, while gobies, sandeels and gadoids were the most important prey groups in 2006–2007. Clupeids and, to a lesser extent, gadoids declined in numerical importance in 2006–2007 compared to 1990–1992, although the frequency of occurrence of gadoids was higher in 2006–2007. Among the Gadidae, smaller species (*Trisopterus* spp.) increased in terms of both percent frequency and numerical importance in 2006–2007 compared to 1990–1992. Conversely, percent frequency and numerical importance for large gadoid species (e.g. whiting) tended to be lower in 2006–2007 compared to 1990–1992. Sandeels were

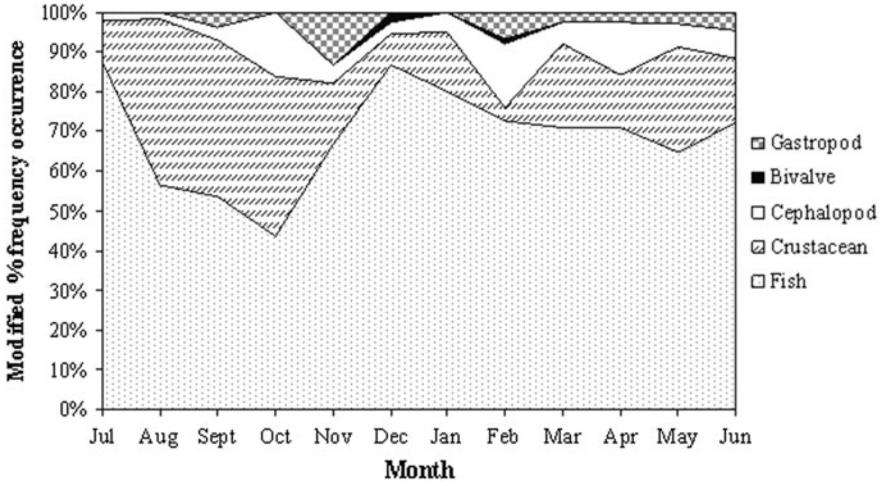


Fig. 2 Monthly variation in the diet of *Loligo forbesii* (2006–2007). The *graph* displays the modified percent frequency of occurrence of the main prey groups in non-empty stomachs

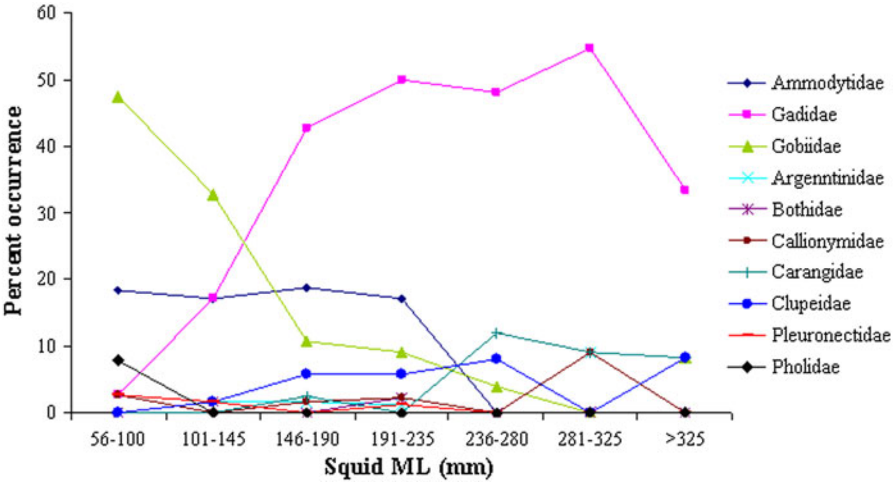


Fig. 3 Percent frequency of occurrence of identified fish prey in non-empty stomachs ($n = 360$, sampled from July 2006 to June 2007) of *Loligo forbesii* for different size classes of squid. Samples sizes of each size class were 38, 64,122, 88, 25, 11, and 12

more important, in terms of both frequency of occurrence and numbers, in the 2006–2007 dataset. Three fish prey groups were recorded in 2006–2007 but not in 1990–1992: Argentinidae (*A. silus* and *A. sphyraena*), Bothidae (*Arnoglossus laterna*) and Pholidae (*P. gunnellus*).

Comparing cephalopod remains in the stomachs between the two periods, it seems that cephalopod species identified in 1990–1992 were more diverse than in 2006–2007. Both *Alloteuthis subulata* and *Loligo* species were found in 1990–1992, while only *Loligo* species were recorded in 2006–2007. Crustacean remains identified in both periods consisted of decapods and copepods. Gastropod and bivalves were present only in 2006–2007.

Generalized additive modelling results from analysis the combined (North Sea) data set for 1990–1992 and 2006–2007 indicated that the frequency of occurrence of both gobies and gadoids was significantly higher in 2006–2007 than in 1990–1992. However, once seasonal and size-related dietary variation had been taken into account, the frequency of occurrence of sandeels did not differ between the two time periods.

Variation in prey size

In total, 329 otoliths were found in squid stomach contents in the 2006–2007 samples. However, only the 233 identifiable and intact otoliths were used to estimate the size of fish prey. The lengths of otoliths ranged from 0.33 to 6.29 mm, while the estimated fish prey sizes were 15.2–123.2 mm, in squid sizes of 74–354 mm ML (Table 4). Otoliths found in squid

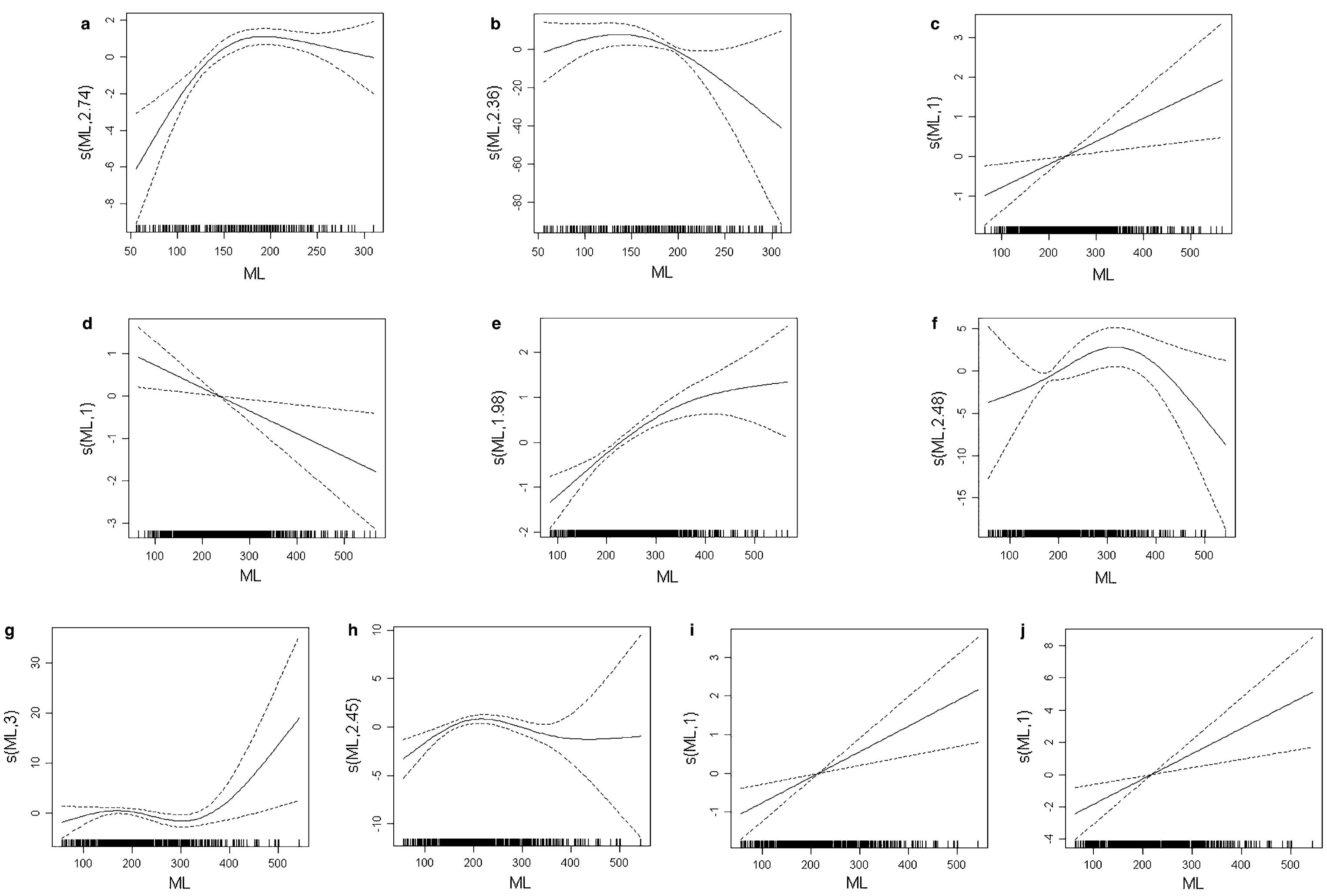


Fig. 4 GAM Smoothing curves for the effect of squid size on the presence of various fish prey in the diet of *Loligo forbesii*. The *x* axis represents the dorsal mantle length (ML) and *dotted lines* represent the 95% confidence intervals of smoothing curve. Where from both periods, i.e., 1990–2007, are used, results refer to the North Sea (NS) only. Only statistically significant relationships are illustrated: **a** Gadoids eaten in NS 2006–2007 ($P < 0.001$), **b** Gobies in Summer diet in *NS* 2006–2007 ($P = 0.009$), **c** Sandeels in Spring diet 1990–1992 ($P = 0.008$), **d** Sandeels in Autumn diet 1990–1992 ($P = 0.009$), **e** Gadoids eaten in 1990–1992 ($P < 0.001$), **f** Sandeels in Winter diet in *NS* 1990–2007 ($P = 0.002$), **g** Sandeels in Summer diet in NS 1990–2007 ($P = 0.003$), **h** Gadoids in Summer diet in NS 1990–2007 ($P < 0.001$), **i** Gadoids in Autumn diet in NS 1990–2007 ($P = 0.004$), **j** Gobies eaten in female squids in NS 2006–2007 ($P = 0.002$)

stomachs mostly belonged to Ammodytidae (mainly *A. marinus*), Gobiidae (mainly *A. minuta*), and Gadidae (mainly *T. esmarkii*). Otolith occurrence appeared to vary with season, with the highest occurrence of otoliths being found in Summer. Only transparent goby (*A. minuta*) otoliths could be found in the diet of *L. forbesii* in every season (Table 4).

Overall, there was a weak positive relationship between prey size and predator size (Spearman's $r = 0.156$, $P = 0.017$). Correlations between squid ML and the estimated size of three of the six commonest prey types were significant (transparent goby: $r = 0.454$, $P = 0.002$; other gobies: $r = 0.762$, $P = 0.002$; whiting: $r = 0.810$, $P = 0.004$). However, no significant correlation was observed for *A. marinus*, other sandeels or *T. esmarkii*.

GAM results on prey size

Generalized additive modelling results indicated that predator size, season, and prey type were the major factors influencing (fish) prey size, although some sex-related and regional differences were also apparent. For some prey groups there were also sex, region, and study period differences (Table 5). In the 1990–1992 sample, size of gadoids eaten varied with region and sex, with larger sizes eaten in the North Sea and by female squids. GAM results from the combined data for the two periods (North Sea only) indicated that the smallest fish eaten were gobies, followed by gadoids, sandeels and other groups.

Regarding the effect of season on prey size, larger fish tended to be found in stomachs in Winter compared to other seasons. For all fish prey combined there was evidence of a positive relationship between squid size and prey size eaten in the North Sea in squid up to around 300 mm ML in 1990–1992 (Fig. 5d). Although there were differences between different prey types, study periods and seasons, the majority of statistically significant prey size-predator size relationships were positive over at least part of the squid size range (Fig. 5).

Generalized additive modelling results indicated that the size of gobies eaten (in the North Sea) differed significantly between the two study periods (with gobies eaten in 2006–2007 being larger). There was, however, a significant interaction between the effects of predator size and study period on the size of sandeels eaten.

Selecting data on prey size from the same area (ICES subdivision IV, North Sea) and period (Summer and Autumn), the median length of sandeels taken by *L. forbesii* in 1990–1992 appeared to be slightly

Table 3 Numerical importance of different prey categories (as absolute numbers, *N*, and as a percentage of all prey individuals, *%N*), and frequency of occurrence (*F*, and as a percentage of stomachs contained food, *%F*) in stomachs of *Loligo forbesii* in the North Sea, 1990–1992 and 2006–2007

Fish prey	1990–1992				2006–2007			
	N	*%N* ($n = 130$)	*F*	*%F* ($n = 391$)	*N*	*%N* ($n = 391$)	*F*	*%F* ($n = 360$)
Ammodytidae	29	22.4	32	8.2	122	31.2	83	23.1
Argentinidae	–	–	–	–	6	1.5	5	1.4
Bothidae	–	–	–	–	8	2.0	3	0.8
Callionymidae	1	0.8	1	0.3	1	0.3	6	1.7
Carangidae	1	0.8	1	0.3	2	0.5	8	2.2
Clupeidae	43	33.2	43	11.0	2	0.5	16	4.4
Gadidae	25	19.3	71	18.2	62	15.9	158	43.9
Whiting	9	6.9	12	3.1	11	2.8	13	3.6
Trisopterus spp.	9	6.9	10	2.6	23	5.9	75	20.8
Gobiidae	24	18.5	10	2.6	182	46.5	71	19.7
Pleuronectidae	2	1.5	2	0.5	2	0.5	3	0.8
Pholidae	–	–	–	–	4	1.0	3	0.8
Carangidae/Scombridae	5	3.9	5	1.3	–	–	–	–

Separate information is presented for the most common small (*Trisopterus* spp.) and large (whiting) gadids in the diet

Table 4 Sizes of identified otoliths and estimated length of fish prey in the diet of *Loligo forbesii*, 2006–2007, along with biological characteristics of the squid

Prey	Size of identified otoliths		Estimated length of prey	Biological data on squid which had eaten these prey types	
	N	Mean ± SD	Mean ± SD (mm)	ML (mm) Mean ± SD	Season
Family Ammodytidae					
Ammodytidae	7	0.96 ± 0.25 (0.45-1.18)	58.7 ± 13.2 (32.1–69.8)	212 ± 31 (145-233)	Sum
Ammodytes spp.	14	1.13 ± 0.36 (0.45-1.60)	67.6 ± 18.7 (32.1–91.8)	177 ± 25 (130–213)	Sum
Ammodytes marinus	58	1.28 ± 0.43 (0.48–2.15)	75.3 ± 22.3 (33.4–120.4)	175 ± 26 (75–219)	Sum and Spr
Hyperoplus lanceolatus	32	0.83 ± 0.41 (0.38–1.78)	43.3 ± 23.2 (17.3–96.9)	194 ± 35 (155–354)	Sum, Win and Spr
Family Argentinidae					
Argentina silus	1	1.48	69.6	160	Aut
Argentina sphyraena	5	2.08 ± 0.10 (1.90–2.13)	93.7 ± 4 (86.5–95.5)	162 ± 41 (123–204)	Aut and Win
Family Callionymidae					
Callionymus lyra	1	0.68	25.4	74	Aut
Family Carangidae					
Trachurus trachurus	2	4.25 ± 0.07 (4.20–4.30)	121.5 ± 2.5 (119.7–123.2)	279 ± 64 (242–352)	Win
Family Gadidae					
Micromesistius poutassou	5	5.34 ± 1.60 (2.50–6.26)	94.7 ± 40.7 (22.6–118)	179 ± 13 (162–197)	Sum
Gadus morhua	10	3.79 ± 1.09 (2.35–6.29)	60.9 ± 16.7 (33.8–91.6)	190 ± 12 (171–202)	Sum
Trisopterus esmarkii	23	2.89 ± 0.75 (2.15–5.40)	42.9 ± 22 (20.9–116.8)	181 ± 23 (147–227)	Sum and Win
Merlangius merlangus	10	4.05 ± 1.07 (2.27–5.00)	67.6 ± 21 (32.7–86.6)	161 ± 21 (122–202)	Sum
Family Gobiidae					
Aphia minuta	44	0.65 ± 0.27 (0.30–1.13)	36.8 ± 14.6 (18.1–62.3)	160 ± 32 (74–214)	All the year
Gobiusculus flavescens	4	0.56 ± 0.09 (0.45–0.65)	18.3 ± 3.1 (15.2–21.9)	91	Aut
Pomatoschistus minutus	9	0.83 ± 0.11 (0.63–0.98)	23.8 ± 4.4 (16.5–30.7)	211 ± 88 (116–354)	Sum and and Win
Family Pholidae					
Pholis gunnellus	6	0.55 ± 0.07 (0.48–0.63)	59.9 ± 5.3 (53.4–66.5)	91 ± 1 (89–92)	Aut
Family Pleuronectidae					
Hippoglossoides platessoides	2	1.33 ± 0.64 (0.88–1.78)	39.5 ± 30.8 (17.8–61.3)	157 ± 72 (74–198)	Aut and Win
Total	233	1.55 ± 1.28 (0.30–6.29)	55.9 ± 27.5 (15.2–123.2)	175 ± 40 (74–354)	

Minimum and maximum are provided in parentheses

SD Standard deviation, *Sum* Summer, *Aut* Autumn, *Win* Winter, *Spr* Spring

Table 5 Results of Gaussian GAM models for estimating fish length in the diet of *Loligo forbesii*

Prey group	Final model	DE (%)
2006–2007		
All prey size (FL[a], $n = 207$)	Y1 ~ 1 + as.factor(Prey type) + as.factor(season) + s(ML, $k = 4$) s(ML), $P = 0.040$, df = 2; Prey type, $P < 0.001$ (OT > SA > GA > GO); Season, $P < 0.001$ (Q1 > Q3 > Q4 > Q2)	46.6
Sandseel (OL[a], $n = 109$)	Y1 ~ 1 + as.factor(season) + s(ML, $k = 4$) + ML:as.factor(season) s(ML), $P = 0.025$, df = 1; Season, $P = 0.006$ (Q3 > Q4 > Q1 and Q2); ML:season, $P < 0.001$, df = 3	52.1
Gadoid (FL[a], $n = 47$)	Y1 ~ 1 + as.factor(season) + s(ML, $k = 4$, by = as.factor(season)) Season, $P = 0.121$ (Q1 > Q4 > Q3); s(ML):Winter, $P = 0.002$, df = 0.2; s(ML):Summer, $P = 0.693$, df = 0.9; s(ML):Autumn, $P = 0.656$, df = 0.8	13.5
Gobies (OL[a], $n = 57$)	Y1 ~ 1 + as.factor(season) + s(ML[a], $k = 4$, by = as.factor(season)) Season, $P = 0.015$ (Q1 > Q4 > Q3 > Q2); s(ML):Winter, $P = 0.003$, df = 1; s(ML):Spring, $P = 0.972$, df = 1; s(ML):Summer, $P < 0.001$, df = 1; s(ML):Autumn, $P = 0.250$, df = 1	61.6
1990–1992		
All prey size (FL[a], $n = 315$)	Y1 ~ 1 + as.factor(prey type) + as.factor(sex) + as.factor(season) + s(ML, $k = 4$, by = as.factor(region)) Prey type, $P < 0.001$ (SA > GA > OT > GO); Season, P < 0.001 (Q1 > Q2 > Q4 > Q3); Sex, $P = 0.002$ (F > M); s(ML):North Sea, $P = 0.005$, df = 2.2; s(ML):West coast, $P = 0.160$, df = 2.7; s(ML):Rockall, P = 0.051, df = 3	55
Sandseel (OL[a], $n = 153$)	Y1 ~ 1 + as.factor(seasom) + s(ML[a], $k = 4$, by = as.factor(season)) + ML[a]:as.factor(sex) Season, $P < 0.001$ (Q2 > Q3 > Q1 > Q4); s(ML):Winter, $P = 0.961$, df = 1; s(ML):Spring, $P = 0.169$, df = 1; s(ML):Summer, $P = 0.003$, df = 2.7; s(ML):Autumn, $P = 0.746$, df = 1; ML:sex, $P = 0.002$, df = 2	49.7
Gadoid (FL[a], $n = 116$)	Y1 ~ 1 + as.factor(region) + as.factor(sex) + as.factor(season) + s(ML[a], $k = 4$, by = as.factor(season)) Season, $P < 0.001$ (Q1 and Q2 > Q4 > Q3); Sex, $P = 0.037$ (F > M); Region, $P = 0.007$ (NS > RA > WC); s(ML):Winter, $P = 0.591$, df = 0.9; s(ML):Spring, $P = 0.631$, df = 0.2; s(ML):Summer, $P = 0.002$, df = 2.4; s(ML):Autumn, $P = 0.079$, df = 2.6	48.5
1990–2007		
All prey size (FL[a], $n = 361$)	Y1 ~ 1 + as.factor(prey type) + as.factor(season) + s(ML[a], $k = 4$, by = as.factor(season)) Prey type, $P < 0.001$ (OT > SA > GA > GO); Season, $P < 0.001$ (Q1 > Q3 > Q4 > Q2); s(ML):Winter, $P = 0.568$, df = 1; s(ML):Spring, $P = 0.007$, df = 1; s(ML):Summer, $P = 0.012$, df = 1.9; s(ML):Autumn, $P < 0.001$, df = 1	61
Sandseel (OL[a], $n = 171$)	Y1 ~ 1 + as.factor(season) + s(ML, $k = 4$, by = as.factor(year)) Season, $P < 0.001$ (Q1 > Q4 > Q3 > Q2); s(ML):Y1, $P < 0.001$, df = 1; s(ML):Y2, $P = 0.020$, df = 2	48.5
Gadoid (FL[a], $n = 84$)	Y1 ~ 1 + as.factor(season) + s(ML[a], $k = 4$) s(ML), $P = 0.016$, df = 2; Season, $P = 0.019$ (Q1 and Q2 > Q4 > Q3)	22.1
Gobies (OL[a], $n = 86$)	Y1 ~ 1 + as.factor(year) + as.factor(season) + s(ML[a], $k = 4$) s(ML), $P < 0.001$, df = 3; Season, $P < 0.001$ (Q1 > Q4 > Q3 > Q2); Year, $P < 0.001$ (Y2 > Y1)	66.3

For explanatory variables remaining in the final models, the table gives associated *P* values, and the direction of differences is indicated in parentheses. For fitted smoothers, both *P* values and degrees of freedom (df, value of 1 indicates a linear fit) are given. Significant smoothers are illustrated in Fig. 5 ([a]Log-transformations: fish length (FL), otolith length (OL), and dorsal mantle length (ML))

DE deviance explained, *Y1* dataset in 1990–1992, *Y2* dataset in 2006–2007, *Q1* Winter, *Q2* Spring, *Q*, Summer, *Q4* Autumn, *F* female, *M* male, *NS* North Sea, *WC* West Coast, *RC* Rockall, *SA* Sandeels, *GO* Gobies, *GA* Gadoids, *OT* other fish prey

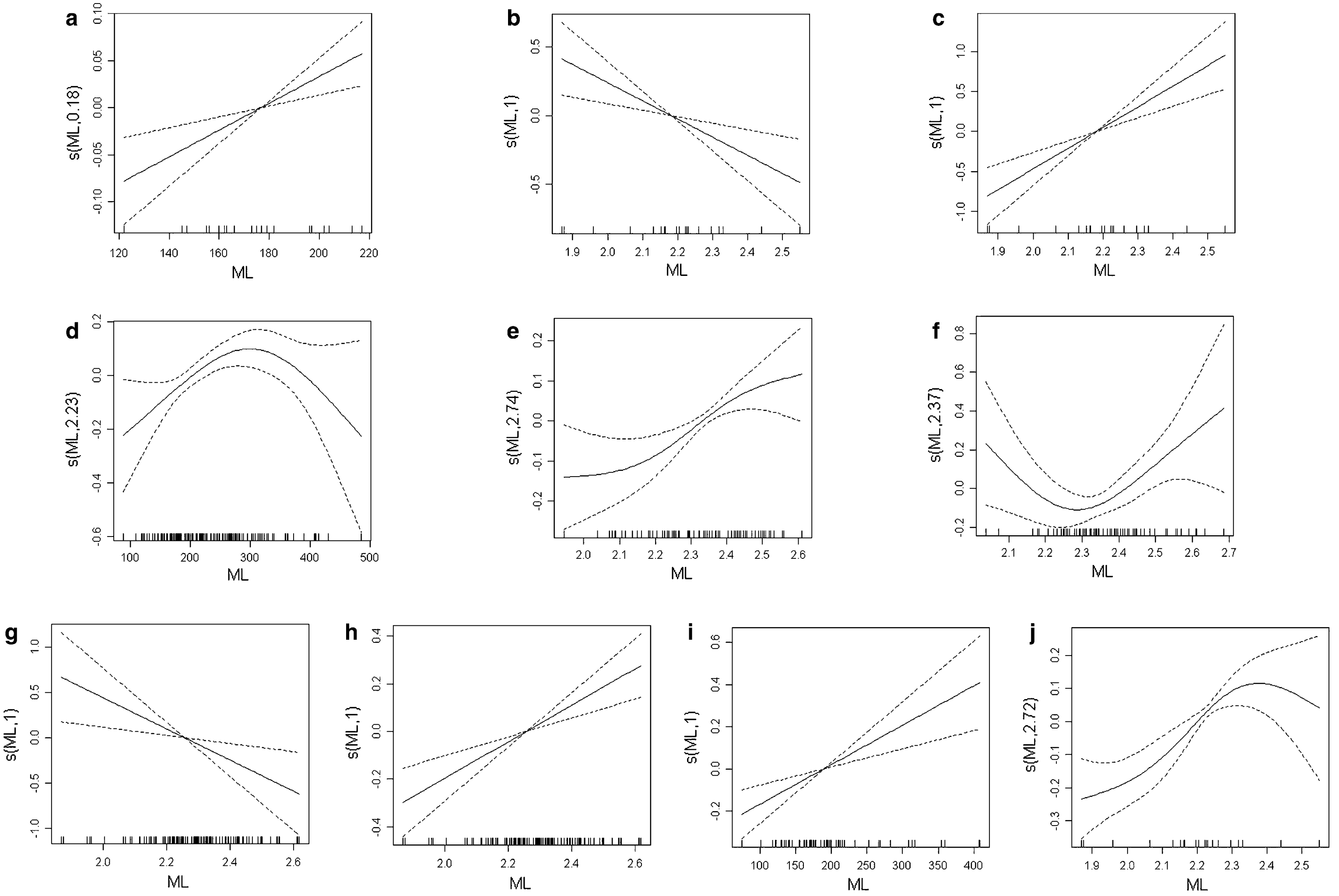


Fig. 5 GAM Smoothing curves for the effect of squid (predator) size on fish prey length in the diet of *Loligo forbesii*. The x axis represents the dorsal mantle length (ML) and dotted lines represent the 95% confidence intervals of smoothing curve. Where from both periods, i.e. 1990–2007, are used, results refer to the North Sea (NS) only. Only statistically significant relationships are illustrated: **a** Gadoids in Winter diet in NS 2006–2007 ($P = 0$ 002), **b** Gobies in Winter diet in NS 2006–2007 ($P = 0.003$), **c** Gobies in Summer diet in NS 2006–2007 ($P < 0.001$), **d** Fish prey in NS 1990–1992 ($P = 0.005$), **e** Sandeels in Summer diet in NS 1990–1992 ($P = 0.003$), **f** Gadoids in Summer diet 1990–1992 ($P = 0.002$), **g** Fish prey in Spring diet in NS 1990–2007 ($P = 0.007$), **h** Fish prey in Autumn diet in NS 1990–2007 ($P < 0.001$), **i** Sandeels eaten in 1990–1992 ($P < 0.001$), **j** Gobies eaten in NS 1990–2007 ($P < 0.001$)

greater than in 2006–2007. However, Mann–Whitney *U* test showed no significant difference ($P = 0.089$). There was no difference in median squid size between the two periods ($P = 0.692$).

Discussion

It is apparent from this study that fish were the most important prey for *L. forbesii* in Scottish waters in 2006–2007, followed by crustaceans and cephalopods, as previously reported for this area (Ngoile, 1987; Pierce et al., 1994b; Collins & Pierce, 1996; Stowasser, 2004), as well as in Irish waters (Collins et al., 1994).

Regional variation in diet

The dominant prey species in the diet of *L. forbesii* differ between regions, presumably influenced by local abundances (i.e. availability) of potential prey species. The dominant fish species in the Azores was horse mackerel (*T. picturatus*) (Martins, 1982), while sprat (*S. sprattus*), poor cod (*T. minutus*) and transparent goby (*A. minuta*) dominated the diet in Irish waters (Collins et al., 1994). In Scottish waters, gadoids and sandeels were the most frequently occurring groups as also found in the previous study by Pierce et al. (1994b). It should be noted that Gadidae (mainly, *Trisopterus* spp., and *M. merlangus*), Ammodytidae (mainly *Ammodytes* spp.), Argentinidae (mainly *Argentina* spp.) and flatfish, which were identified by both Ngoile (1987) and Pierce et al. (1994b) in the diet of this species, were also found in the present study.

Seasonal variation in diet

Seasonal variation was one of the important sources of variation in diet composition and size selectivity of *L. forbesii* in Scottish waters, which it is in agreement with previous studies by Pierce et al. (1994b) and Collins & Pierce (1996), and suggests that seasonal changes in prey availability may be important in determining squid diet. Moreover, in the present study copepods occurred more often in June. This is consistent with the peak period of copepod abundance on the eastern coast of Scotland (Broekhuizen & McKenzie, 1995).

Size-related variation

The development of the prey capture, ingestion and digestion systems (e.g. tentacles, beaks, digestive tract) during the cephalopod lifespan seems likely to be a crucial process influencing the prey selection (Boucher-Rodoni et al., 1987), defining the morphological constraints on foraging, feeding and digestion. In *L. forbesii*, it is apparent that most juveniles fed on small crustaceans or smaller fish, and then switched to eating mainly fish and cephalopods as they grew. Again, this corroborates the findings of previous studies by Collins et al. (1994) and Pierce et al. (1994b).

As seen in this study, Collins et al. (1994) and Collins & Pierce (1996) also noted that cannibalism in *L. forbesii* occurs more frequently in larger squids rather than in smaller squids. This is consistent with studies on other squid species including *L. pealei* (Macy, 1982), *T. sagittatus* (Lordan et al., 2001), *Illex argentinus* (Mouat et al., 2001) and *Dosidicus gigas* (Markaida & Sosa-Nishizaki, 2003). Cannibalism in squid may occur because of food shortage (Macy, 1982; Lordan et al., 2001). However, cannibalism in larger *L. forbesii* may be a function of abundance, for example occurring often when abundance is high (Pierce et al., 1998; Hastie et al., 2009b), as suggested for *Loligo vulgaris* in the Saharan Bank (Coelho et al., 1997).

Long-term trends in diet: species eaten

The increases in percent occurrence and numerical importance of gobies in the 2006–2007 diet of *L. forbesii* in the North Sea, compared to 1990–1992, may indicate that gobies have effectively replaced clupeids as a prey source. Among the clupeid prey, sprat was the most frequently found species (see also Collins et al., 1994). Although ICES advice indicates that the state of the North Sea Survey data showed that sprat abundance was lower (although increasing) in 1990–1992 than in 2006–2007 (although its abundance was then decreasing, see ICES, 2009). Abundance (spawning stock biomass, SSB) of herring in the North Sea seems to have been rather similar in both periods. Thus, we have no clear evidence that consumption of sprat by squid is related to sprat abundance.

Change in diet composition of *L. forbesii* in Scottish waters over time may have occurred due to changes in prey and predator abundances in the marine ecosystem. Rogers & Ellis (2000) found that there was a decrease in abundance of large species of demersal fish in British waters, while smaller and non-target fish species increased, due to the long-term impact of commercial fisheries. Jennings et al. (1999) noted that Norway pout has markedly increased in abundance in recent years. Additional evidence is apparent in stock status reports prepared by ICES (2009). During 2005–2007, SSB of sandeel and Norway pout tended to increase at the same time as SSB of herring, whiting and haddock tended to decrease (Fig. 6). Declining abundance of large fish, whiting and haddock, could be beneficial for large squids because their food in the North Sea is similar in prey species composition (Hislop et al., 1996; Greenstreet et al., 1998).

However, as for clupeids, a detailed examination of dietary importance and abundance of gadids again provides little evidence that squid predation tracks stock abundance. In the case of gadoids in the diet, although there was a slight decline in numerical importance, this was not consistent with the trend in percentage occurrence in the diet, which was upwards (Table 3). Among the gadids, whiting decreased in importance in the diet while *Trisopterus* spp. increased. Whiting abundance (SSB) in the North Sea declined substantially between 1990–1992 and 2006–2007 (consistent with dietary results) but Norway pout abundance was fairly similar in both time-periods (although it had reached a historic low point in 2005).

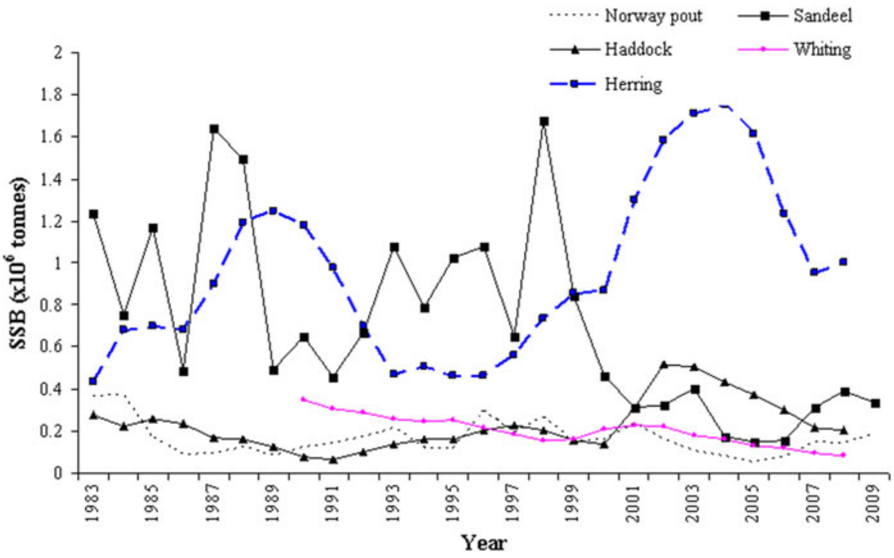
Long-terms trends in diet: prey size

In addition to changes in species composition, the size composition of the fish community in the North Sea ecosystem has been be affected by fishing pressure and climate change (Jennings et al., 2002; Ottersen et al., 2006). Aside from the decline of larger species and truncation of the age structure in many species, there are numerous reports of decreases in size-at-age and age at maturity in fished stocks, e.g. cod in the Barents sea (Ottersen et al., 2006) and in the North sea (Yoneda & Wright, 2004), and sandeels in the northwestern North Sea (Wanless et al., 2004).

Given the above-mentioned changes in the relative abundance of large and small fish, it might have been predicted that a shift towards smaller prey would be seen in squid diet. However, the only statistically significant difference in the size of prey eaten in 1990–1992 and 2006–2007 was that squid took bigger gobies in 2006–2007 compared to 1990–1992. In the case of gobies, since they are not commercially important, there is little available information on trends in stock abundance or size structure.

The findings from the present study suggest that there have been significant changes in the diet of *L. forbesii* over the last 15 years. While seasonal

Fig. 6 Spawning stock biomass (SSB, millions of tones) of herring, sandeel, Norway pout, whiting and haddock in the North Sea (data from ICES, 2009)



shifts in diet were previously attributed to changes in prey abundance (or availability, see Pierce et al., 1994b), we have been able to find little or no evidence that long-term interannual trends in squid diet are related to fish abundance. This may be a sampling issue (e.g. the relatively small numbers of stomachs examined) and it is also possible that the available abundance measures for commercially exploited fish stocks are simply at the wrong spatial scale to be relevant to predation by *L. forbesii*. In addition, it may be necessary to derive abundance indices specifically related to the size classes of fish taken by *L. forbesii.*

Any long-term shift in the diet of apparently opportunistic predators such as cephalopods is a very important consideration in the construction of large-scale, trophic network models. Therefore, it would be worthwhile to monitor future changes in squid diet in the North Sea. To ensure comparable results, sampling protocol should consider the possible influence of fishing gear, study area, and seasonal pattern on the results. In principle, predators such as *Loligo forbesii* may represent a useful biological indicator of changes in the size- and species-composition of fish communities. However, results of this study caution against uncritical use of such indicators.

Acknowledgments Sample collection during 2006–2007 was funded by Sea Fish Industry Authority (SEAFISH). We would like to thank J. Wang and M. Viana, G. Hernandez-Milian N. Serpetti and B. Thongdonphum for assistance with data collection and sample processing. S. Wangvoralak was funded by Royal Thai Government. G. J. Pierce was funded by the EU (project MEXC-CT-2006-042337). We would also like to thank to the reviewers for comments and suggestions on the manuscript.

References

Belkin, I. M., 2009. Rapid warming of large marine ecosystems. Progress in Oceanography 81: 207–213.

Bidder, A. M., 1950. The digestive mechanism of the European squids *Loligo vulgaris*, *Loligo forbesii*, *Alloteuthis media* and *Alloteutihis subulata*. Quarterly Journal of Microscopical Science S3-91(13): 1–43.

Boucher-Rodoni, R., E. Boucaud-Camou & K. Mangold, 1987. Comparative reviews. In Boyle, P. R. (ed.), Cephalopd Life Cycles. Academic Press, London: 85–108.

Boycott, B. B., 1961. The functional organization of the brain of the cuttlefish *Sepia officinalis*. Proceedings of the Royal Society of London. Series B: Biological Sciences 153(953): 503–534.

Boyle, P. R., 1990. Cephalopod biology in the fisheries context. Fisheries Research 8: 313–321.

Boyle, P. R. & S. V. Boletzky, 1996. Cephalopod Populations: Definition and Dynamics. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 351(1343): 985–1002.

Boyle, P. R. & M. A. K. Ngoile, 1993. Assessment of maturity state and seasonality of reproduction in *Loligo forbesi* (Cephalopoda: Loliginidae) from Scottish waters. In Okutani, T., R. K. O'Dor & T. Kubodera (eds), Recent Advances in Cephalopod Fisheries Biology. Tokai University Press, Tokyo: 37–48.

Boyle, P. R. & P. Rodhouse, 2005. Cephalopods: Ecology and Fisheries. Blackwell Science, Oxford.

Breiby, A. & M. Jobling, 1985. Predatory role of the flying squid (*Todarodes sagittatus*) in north Norwegion waters. NAFO Scientific Council Studies 9: 125–132.

Broekhuizen, N. & E. McKenzie, 1995. Patterns of abundance for *Calanus* and smaller copepods in the North Sea: time series decomposition of two CPR data sets. Marine Ecology Progress Series 118: 103–120.

Caddy, J. F. & P. G. Rodhouse, 1998. Cephalopod and groundfish landings: Evidence for ecological change in global fisheries? Review in Fish Biology and Fisheries 8(4): 431–444.

Clarke, M. R. (ed.), 1986. A Handbook for the Identification of Cephalopod Beaks. Clarendon Press, Oxford.

Coelho, M., P. Domingues, E. Balguerias, M. Fernandez & J. P. Andrade, 1997. A comparative study of the diet of *Loligo vulgaris* (Lamarck, 1799) (Mollusca: Cephalopoda) from the south coast of Portugal and the Saharan Bank (Central-East Atlantic). Fisheries Research 29: 245–255.

Collins, M. A. & G. J. Pierce, 1996. Size selectivity in the diet of *Loligo forbesi* (Cephalopoda: Loliginidae). Journal of the Marine Biological Association of the United Kingdom 76: 1081–1090.

Collins, M. A., S. De Grave, C. Lordan, G. M. Burnell & P. G. Rodhouse, 1994. Diet of the squid *Loligo forbesi* Steenstrup (Cephalopoda: Loliginidae) in Irish waters. ICES Journal of Marine Science 51: 337–344.

Collins, M. A., G. M. Burnell & P. G. Rodhouse, 1995. Distribution and demography of *Loligo forbesi* in the Irish Sea. Biology and Environment 95B: 49–57.

Conway, D. V. P., R. G. White, J. Hugues-Dit-Ciles, C. P. Gallienne & D. B. Robins, 2003. Guide to the Coastal and Surface Zooplankton of the South-Western Indian Ocean. Marine Biological Association of the United Kingdom, Occasional Publication No 15.

Daly, H. I., G. J. Pierce, M. B. Santos, J. Royer, S. K. Cho, G. Stowasser, J.-P. Robin & S. M. Henderson, 2001. Cephalopod consumption by trawl caught fish in Scottish and English Channel waters. Fisheries Research 52(1–2): 51–64.

Dulvy, N. K., S. I. Rogers, S. Jennings, V. Stelzenmuller, S. R. Dye & H. R. Skjoldal, 2008. Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. Journal of Applied Ecology 45: 1029–1039.

Furness, R. W., 1994. An estimate of the quantity of squid consumed by seabirds in the eastern North Atlantic and adjoining seas. Fisheries Research 21: 165–177.

Greenstreet, S. P. R., J. A. McMillan & E. Armstrong, 1998. Seasonal variation in the importance of pelagic fish in the diet of piscivorous fish in the Moray Firth, NE Scotland: a response to variation in prey abundance? ICES Journal of Marine Science 55: 121–133.

Guerra, A. & F. Rocha, 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in Galician waters (NW Spain). Fisheries Research 21: 43–69.

Guerra, A., P. Sánchez & F. Rocha, 1994. The Spanish fishery for *Loligo*: recent trends. Fisheries Research 21: 217–230.

Härkönen, T., 1986. Guide to the Otoliths of the Bony Fishes of the Northeast Atlantic. Danbiu Aps, Biological Consultants, Sweden.

Hastie, L. C., G. J. Pierce, J. Wang, I. Bruno, A. Moreno, U. Piatkowski & J.-P. Robin, 2009a. Cephalopods in the North-Eastern Atlantic: species, biogeography, ecology, exploitation and conservation. Oceanography and Marine Biology 47: 111–190.

Hastie, L., G. Pierce, C. Pita, M. Viana, J. Smith & S. Wangvoralak, 2009b. Squid Fishing in UK Waters: A Report to SEAFISH Industry Authority, SFIA, Grimsby: 84 pp [available on internet at http://www.seafish.org/pdf.pl?file=seafish/Documents/AberdeenUni_SquidFishinginUKWaters.pdf].

Hislop, J. R. G., 1996. Changes in North Sea gadoid stocks. ICES Journal of Marine Science 53: 1146–1156.

Holme, N. A., 1974. The biology of *Loligo forbesi* Steenstrup (Mollusca: Cephalopoda) in the Plymouth area. Journal of the Marine Biological Association of the United Kingdom 54: 481–503.

Hyslop, E. J., 1980. Stomach contents analysis: a review of methods and their application. Journal of Fish Biology 17: 411–429.

ICES, 2009. Report of the ICES Advisory Committee 2009. ICES Advice, 2009. Book 6. ICES, Copenhagen.

ICES, 2010. Report of the ICES Advisory Committee 2010. ICES Advice, 2010. Book 6. ICES, Copenhagen.

Jennings, S., S. P. R. Greenstreet & J. D. Reynolds, 1999. Structural change in an exploited fish community: A consequence of differential fishing effects on species with contrasting life histories. Journal of Animal Ecology 68: 617–627.

Jennings, S., S. P. R. Greenstreet, L. Hill, G. J. Piet, J. K. Pinnegar & K. J. Warr, 2002. Long-term trends in the trophic structure of the North Sea fish community: evidence from stable-isotope analysis, size-spectra and community metrics. Marine Biology 141: 1085–1097.

Lordan, C., M. A. Collins, L. N. Key & E. D. Browne, 2001. The biology of the ommastrephid squid, *Todarodes sagittatus*, in the north-east Atlantic. Journal of the Marine Biological Association of the United Kingdom 81: 299–306.

Macy, W. K., 1982. Feeding Patterns of the Long-Finned Squid, *Loligo pealei*, in New England Waters. Biological Bulletin 162: 28–38.

Markaida, U. & O. Sosa-Nishizaki, 2003. Food and feeding habits of jumbo squid *Dosidicus gigas* (Cephalopoda: Ommastrephidae) from the Gulf of California, Mexico. Journal of the Marine Biological Association of the United Kingdom 83: 507–522.

Martins, H. R., 1982. Biological studies of the exploited stock of *Loligo forbesi* (Mollusca: Cephalopoda) in the Azores. Journal of the Marine Biological Association 62: 799–808.

Mouat, B., M. A. Collins & J. Pompert, 2001. Patterns in the diet of *Illex argentinus* (Cephalopoda: Ommastrephidae) from the Falkland Islands jigging fishery. Fisheries Research 52: 41–49.

Ngoile, M. A. K., 1987. Fishery biology of the squid *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. PhD Thesis, University of Aberdeen, UK.

Nixon, M., 1987. Comparative reviews. In Boyle, P. R. (ed.), Cephalopod Life Cycles. Academic Press, London: 201–219.

Ottersen, G., D. O. Hjermann & N. C. Stenseth, 2006. Changes in spawning stock structure strengthen the link between climate and recruitment in a heavily fished cod (*Gadus morhua*) stock. Fisheries Oceanography 15: 230–243.

Perry, A. L., P. J. Low, J. R. Ellis & J. D. Reynolds, 2005. Ecology: climate change and distribution shifts in marine fishes. Science 308: 1912–1915.

Piatkowski, U., G. J. Pierce & M. Morais da Cunha, 2001. Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. Fisheries Research 52: 5–10.

Pierce, G., P. Belcari, P. Bustamante, L. Challier, Y. Cherel, Á. González, Á. Guerra, P. Jereb, N. Koueta, E. Lefkaditou, A. Moreno, J. Pereira, U. Piatkowski, C. Pita, J.-P. Robin, B. Roel, M. B. Santos, M. Santurtun, S. Seixas, P. Shaw, J. Smith, G. Stowasser, V. Valavanis, R. Villanueva, J. Wang, S. Wangvoralak, M. Weis, & K. Zumholz, 2010. The future of cephalopod populations, fisheries, culture, and research in Europe. In Pierce, G. et al. (eds), Cephalopod Biology and Fisheries in Europe. ICES Cooperative Research Report 303: 86–118.

Pierce, G. J., P. R. Boyle, L. C. Hastie & L. Key, 1994a. The life history of *Loligo forbesi* (Cephalopoda: Loliginidae) in Scottish waters. Fisheries Research 21: 17–41.

Pierce, G. J., P. R. Boyle, L. C. Hastie & M. B. Santos, 1994b. Diets of squid *Loligo forbesi* and *Loligo vulgaris* in the northeast Atlantic. Fisheries Research 21: 149–163.

Pierce, G. J., N. Bailey, Y. Stratoudakis & A. Newton, 1998. Distribution and abundance of the fished population of *Loligo forbesi* in Scottish waters: analysis of research cruise data. ICES Journal of Marine Science 55: 14–33.

Porteiro, F. M., H. R. Martins & R. T. Hanlon, 1990. Some observations on the behaviour of adult squids, *Loligo forbesi*, in captivity. Journal of the Marine Biological Association of the United Kingdom 70: 459–472.

Preciado, I., F. Velasco & I. Olaso, 2008. The role of pelagic fish as forage for the demersal fish community in the southern Bay of Biscay. Journal of Marine Systems 72: 407–417.

Pyke, G. H., 1984. Optimal foraging theory: a critical review. Annual review of ecology and systematics 15: 523–575.

Quetglas, A., F. Alemany, A. Carbonell, P. Merella & P. Sánchez, 1999. Diet of the European flying squid *Todarodes sagittatus* (Cephalopoda: Ommastrephidae) in the Balearic Sea (western Mediterranean). Journal of the Marine Biological Association of the United Kingdom 79: 479–486.

Rodhouse, P. O., 1996. Role as consumers. Philosophical Transactions of the Royal Society B: Biological Sciences 351: 1003–1022.

Rogers, S. I. & J. R. Ellis, 2000. Changes in the demersal fish assemblages of British coastal waters during the 20th century. ICES Journal of Marine Science 57: 866–881.

Scharf, F. S., R. M. Yetter, A. P. Summers & F. Juanes, 1998. Enhancing diet analyses of piscivorous fishes in the Northwest Atlantic through identification and reconstruction of original prey sizes from ingested remains. Fishery Bulletin 96: 575–588.

Stowasser, G., 2004. Squid and Their Prey: Insights from Fatty Acid and Stable Isotope Analysis. Ph.D. Thesis, University of Aberdeen, UK.

Tollit, D. L., G. J. Pierce, K. A. Hobson, W. Don Bowen & S. J. Iverson, 2010. Diet. In Boyd, I. L., W. Don Bowen & S. J. Iverson (eds), Marine Mammal Ecology and Conservation: A Handbook of Techniques. Oxford University Press, New York: 191–221.

Wanless, S., P. J. Wright, M. P. Harris & D. A. Elston, 2004. Evidence for decrease in size of lesser sandeels A*mmodytes marinus* in a North Sea aggregation over a 30-yr period. Marine Ecology Progress Series 279: 237–246.

Watt, J., G. J. Pierce & P. R. Boyle, 1997. Guide to the Identification of North Sea Fish Using Premaxillae and Vertebrae. ICES Cooperation Research Report No. 220. International Council for the Exploration of the Sea, Denmark: 231.

Yoneda, M. & P. J. Wright, 2004. Temporal and spatial variation in reproductive investment of Atlantic cod *Gadus morhua* in the northern North Sea and Scottish west coast. Marine Ecology Progress Series 276: 237–248.

Zuur, A. F., E. N. Leno & G. M. Smith, 2007. Analysing Ecological Data. Springer, New York.

Hydrobiologia (2011) 670:241–266
DOI 10.1007/s10750-011-0673-9

ECOSYSTEMS AND SUSTAINABILITY

Prediction of marine species distribution from presence–absence acoustic data: comparing the fitting efficiency and the predictive capacity of conventional and novel distribution models

**A. Palialexis · S. Georgakarakos · I. Karakassis ·
K. Lika · V. D. Valavanis**

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The accurate representation of species distribution derived from sampled data is essential for management purposes and to underpin population modelling. Additionally, the prediction of species distribution for an expanded area, beyond the sampling area can reduce sampling costs. Here, several well-established and recently developed habitat modelling techniques are investigated in order to identify the most suitable approach to use with presence–absence acoustic data. The fitting efficiency of the modelling techniques are initially tested on the training dataset while their predictive capacity is evaluated using a verification set. For the comparison among models, Receiver Operating Characteristics (ROC), Kappa statistics, correlation and confusion matrices are used. Boosted Regression Trees (BRT) and Associative Neural Networks (ASNN), which are both within the machine learning category, outperformed the other modelling approaches tested.

Keywords Species distribution models · Species distribution predictions · Habitat modelling · Models comparison · Geostatistics · Spatial autocorrelation

Introduction

Knowledge of species ecological and geographical distribution is essential for conservation planning and forecasting (Ferrier et al., 2002) as well as for assessing evolutionary determinants of spatial patterns of biodiversity (Graham et al., 2006). Several techniques have been developed for the identification of species distribution using sampling data. These may be categorized as species distribution models (SDM), which are statistical models that relate surveyed data on species distribution with the associated environmental and geographical characteristics of the surveyed locations (Elith & Leathwick, 2009). In the literature, SDMs are variously described as resource selection functions (RSFs), habitat models and ecological niche models (see Elith & Leathwick, 2009). Approaches based on SDMs have only relatively recently been applied to marine species and several novel modelling methods have been proposed (Leathwick et al., 2005, 2006a; Phillips et al., 2006; Palialexis et al., this issue). SDMs have

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

A. Palialexis (✉) · I. Karakassis · K. Lika
Department of Biology, University of Crete, Vassilika Vouton, P.O. Box 2208, 71 409 Heraklion, Crete, Greece
e-mail: andreaspal@her.hcmr.gr

S. Georgakarakos
Department of Marine Sciences, University of the Aegean, University Hill, 81 100 Mytilini, Lesvos, Greece

A. Palialexis · V. D. Valavanis
Marine GIS Lab, Hellenic Centre for Marine Research, P.O. Box 2214, 71 003 Heraklion, Crete, Greece

been also used to study relationships between environmental variables and species presence (Amara et al., 2004; Giannoulaki et al., 2008; Lefkaditou et al., 2008; Martin et al., 2008), identifying species essential habitats (Planque et al., 2007) and forecasting how species distribution may be affected by climate changes (Siapatis et al., 2008). Easy access to satellite data which cover extended geographical areas is one reason for the increasingly wide use of SDMs. Presence–absence data derived from several sampling strategies are commonly used with SDMs. Zaniewski et al. (2002) argued that modelling based on presence–absence data is more likely to reflect the present natural distribution of a species, i.e. the realized niche, whereas presence-only methods are more likely to predict potential distributions, more closely resembling the fundamental niche.

The evolution of computer science and statistics is reflected in the novel methods proposed to model species distribution. Presence-only models [e.g. Bioclim, Envelope Score, Ecological Niche Factor Analysis (ENFA)] were initially applied to terrestrial species, taking advantage of data from natural history museums (Ready et al., 2010). Environmental envelope-based models are related to niche theory, where a suitable environmental 'envelope' that favours species occurrence is estimated. Compared to presence-only models, presence–absence approaches to SDM perform more efficiently in terms of prediction (Brotons et al., 2004) since they exploit the additional information about unsuitable environmental conditions for species occurrence. Regression models, such as Generalized Linear Models (GLMs) and Generalized Additive Models (GAMs) are widely used to model presence–absence data (Olivier & Wotherspoon, 2005; Leathwick et al., 2006b). Recently, several modelling techniques were developed utilizing the evolution of methods in computer science, like Boosted Regression Trees (BRT; Leathwick et al., 2006a) and Associative Neural Networks (ASNN; Tetko, 2002a, b) combining different algorithms in order to optimize the predictive capacity of the models. Additionally, the most widely used models, like GAMs, have been further developed (e.g. BRUTO, Hastie & Tibshirani, 1990) or modified (e.g. MARS, Leathwick et al., 2006a) to meet additional requirements identified from experience with model building.

Presence–absence models are generally easier to develop since the training data have a binomial distribution while abundance models require more complicated distributions (e.g. Poisson, Gaussian) and thus, several assumptions are necessary. The validation process and error assessment for presence–absence models is correspondingly more straightforward than is the case for abundance models. Several methods have been developed to assess the quality of model predictions (Fielding & Bell, 1997; Boyce et al., 2002). Receiver Operating Characteristics (ROC), Kappa statistics and confusion matrices (Fielding & Bell, 1997) combined with omission and commission errors are widely used to estimate model performance and to compare different methods (Elith et al., 2006).

Modelling the distribution of marine species is now common component of scientific research projects and applied management. The generality of the developed models is essential for accurate predictions of species distribution over extended spatial and temporal scales. On the other hand, precision and accuracy are essential for realistic representation of species distribution and essential fish habitat identification. There is a plethora of modelling techniques suitable for fisheries and acoustic data. However, in the Mediterranean Sea, most of the studies predicting marine species distribution have utilized GAMs (Giannoulaki et al., 2008; Martin et al., 2008; Siapatis et al., 2008), Maximum Entropy Models (MAXENT) (Lefkaditou et al., 2008) and Discriminant Function Analysis (DFA) (Tsagarakis et al., 2008).

The SDMs literature is rapidly expanding, reflecting the rapid evolution of SDMs and their contribution to ecological studies. During the last decade several reviews on SDMs were published (e.g. Guisan & Zimmermann, 2000; Redfern et al., 2006; Richards et al., 2007; Schröder, 2008; Valavanis et al., 2008; Elith & Leathwick, 2009). Other studies addressing essential issues of the development of SDMs include topics such as methods of assessment of prediction errors (Fielding & Bell, 1997; Boyce et al., 2002), effects of spatial autocorrelation in SDMs (Dormann et al., 2007), SDMs and ecological theory (Guisan & Thuiller, 2005), new approaches to SDMs (Leathwick et al., 2005, 2006a) and extended SDMs comparisons to identify their efficiency and applicability for use with several data types (Caruana & Niculescu-Mizil, 2006; Elith et al., 2006; Leathwick et al., 2006a; MacLeod et al., 2008; Palialexis et al., 2009; Aertsen et al., 2010).

This study contributes to the latter two issues by comparing several well-established and recently developed techniques and by introducing the use of ASNN. We compare SDMs that are developed under different statistical principles (machine learning, regression models and others) and represent the most commonly used techniques in order to develop advice for the selection of suitable modelling approaches based on presence–absence acoustic data. The performance of models in terms of goodness of fit to a training dataset is initially tested in order to identify the ability of each approach to accurately represent species distribution. Second, a verification dataset is used to evaluate the predictive performance of each method and to contribute to a better understanding of SDM performance with an independent dataset. Finally, SDMs were applied to high resolution predictors in order to generate distribution maps for small pelagic species. Several criteria were used in order to compare the efficiency of SDMs.

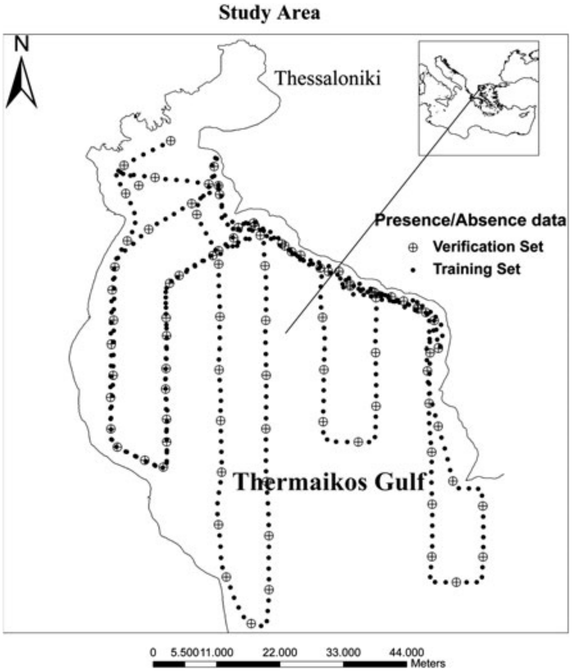


Fig. 1 Study area and sampling transects. *Black dots* represent the training dataset; *circles* represent the verification dataset

Materials and methods

Study area and data

The study area (Fig. 1) is Thermaikos Gulf in the North Aegean, Northeastern Mediterranean Sea. Thermaikos Gulf is a semi-enclosed basin, relatively productive, because of the influence of four major rivers. As a result, bottom relief is smooth due to the continuous sediment input. Thermaikos Gulf forms a wide continental shelf, which smoothly extends to the south into the 1,400 m deep Sporades Basin. Water mass circulation is predominantly cyclonic (Poulos et al., 2000). Aegean water masses enter the gulf from deeper layers along the eastern coast and move counterclockwise towards the gulf of Thessaloniki. This circulation produces a gyre in the area, which is obvious in satellite imagery and affects the life-history of pelagic marine species consisting an identified recruitment habitat (Somarakis et al., 2002). Riverine waters usually move to the south along the western coast forming nutrient-rich water masses.

Acoustic data were collected during April–May 1998 in Thermaikos Gulf using a calibrated 38 kHz SIMRAD EK 500/BI 500 system (Bodholt et al., 1989). The echograms were scrutinized, allocating the nautical area-scattering coefficient (s_A, m^2 n mi^{-2}, NASC, MacLennan et al., 2002) to the target pelagic species. The integration values, with a horizontal resolution of 1 nautical mile, have been transformed to presence–absence data (Fig. 1). Acoustic data have been not converted to biomass in order to avoid the propagation of uncertainty in species composition and length distribution from the trawl sampling in the response variable (Walline, 2007). Species identification based on biological sampling as well as concurrent catch data indicated that the majority of the target species were *Sardina pilchardus* (~55%), *Engraulis encrasicolus* (~25%) and *Trachurus* spp. (<10%). Thus, the SDMs will essentially depict the distribution of sardine and anchovy in the study area. Life-history information on these species was used to inform several parts of this study, such as variable selection, explanation of the distribution maps and comparison of the SDMs with other related studies.

The acoustic dataset has been divided into two parts. The first one is the training set, including 80% of the initial data (black dots in Fig. 1). The second one is used as the verification set (20% of the sampling data—circles with crosses in Fig. 1) in order to justify the predictive capacity of the SDMs.

Table 1 Data used in models and their sources

Data variable	Abbreviation	Data type/sensor	Archive source
Acoustic data	s_A	Total pelagic NASC (nautical area-scattering coefficient), ESDU = 1 n mi	SIMRAD EK500/BI500 system on April/May 1998 in Thermaikos Gulf
Sea surface temperature	SST	Grid/Aqua MODIS	German Aerospace Agency (DLR)
Chlorophyll-*a* concentration	CHL	Grid/Aqua MODIS	Distributed Active Archive Center (NASA)
Photosynthetically available radiation	PAR	Grid/SeaWiFS	Distributed Active Archive Center (NASA)
Sea level anomaly	SLA	Grid/Merged Jason-1, Envisat, ERS-2, GFO, T/P	AVISO
Bathymetry	DEP	Grid/Processed ERS-1, Geostat and historical depth soundings	Laboratory for Satellite Altimetry (NOAA)
Coastline	Coast	Cover/digitisation of nautical charts and aerial photography	Hellenic Ministry of Environment
Distance to coast	DCoast	Grid and cover	Extracted from coastline
Temperature slope (thermal fronts)	SSTsl	Grid	Extracted from SST grid
Longitude and latitude of stations	LON, LAT	Cover in decimal degrees and metres	SIMRAD EK500/BI500 system on April/May 1998 in Thermaikos Gulf
Day-dark-night-dawn categorical factor	DDND	Cover and grid	Based on sampling date and hour
Depth slope	DEPsl	Grid	Extracted from bathymetry grid

The partitioning of data was based on Tetko et al. (1995) whereby successive points are separated to construct the training and the verification sets. In this case, of every five sequential points, the first four are included in the training set and the fifth assigned to the verification set. The latter set thus consists of sampling points that cover the whole sampling area and are spaced ~5 Nm apart. The selection of this distance, which determines the proportion of the training and verification datasets was based on the fact that, at this distance, no spatial autocorrelation is detected. Semi-variograms (Matheron, 1971) using ESRI's ArcGIS Geostatistical Analyst Software (GAS) and Auto-correlation Function Estimation plots in R statistical software (R Development Core Team, 2005) were used to identify spatial patterns in the raw data, the training and the verification sets and model residuals. Moran's *I* spatial autocorrelation statistics (Moran, 1950) was also used to estimate the spatial pattern of the two datasets. Furthermore, the homoscedasticity of the residuals was tested by plotting response and explanatory variables against the residuals. Since the training and the verification dataset were not normally distributed non parametric test namely Mann–Whitney and Kolmogorov–Smirnov were used to compare the two datasets. By these processes, the suitability of the verification set for testing SDM predictions was verified.

The remotely sensed and topographical data that have been used for SDM development are listed in Table 1. Initially, a large number of explanatory variables were collected that could potentially be related to species distribution. These could be classified as environmental variables (i.e. sea surface salinity, current speed and direction), spatial data (i.e. distance to coast), temporal data (i.e. date and hour of sampling) and oceanographic features (i.e. productivity hotspots, Valavanis et al., 2004). After an extensive exploration process (see Palialexis et al., this issue), only the non-correlated explanatory variables were used in order to avoid any biased estimations. Small pelagic species life-history indicates that most of the variables used do greatly influence species distribution (Daskalov et al., 2003; Santos et al., 2004; Ruiz et al., 2006; Planque et al., 2007). Additionally, certain oceanographic features like upwelling, gyres and river outflows (Garcìa & Palomera, 1996; Bakun, 2001) affect small pelagic species distribution and are easily identified in remotely sensed data (sea surface temperature, chlorophyll-*a* concentration) (Valavanis et al., 2004). Spatial resolution of the explanatory variables varied from 0.01 to 0.04 decimal degrees. For modelling purposes, all datasets were interpolated to the lowest resolution. A dataset with the final selected explanatory variables that covers the study area at a resolution of 0.01 decimal degrees was applied in the SDMs in order to produce high resolution prediction maps of small pelagic species. Since the response variable is well explored, functionally relevant predictors were selected in order to cover both the environmental and geographical space (Elith & Leathwick, 2009). The multi-dimensional nature of the environmental predictors raises spatial autocorrelation issues in the SDMs, which are discussed later.

SDM

The SDM methods that were used are listed in Table 2. The selection of the explanatory variables used in each model was based on each method's parameter selection process or on the statistically meaningful contribution of the parameters to models. Approaches that were developed by the same method were compared regarding their predictive capacity and the trade-off between explained variation and model complexity. The one that performed better was finally selected. Less complex nested models developed by the same approach reduced predictive capacity and increased prediction errors compared to those finally selected. Additionally, an increase in model complexity beyond a certain threshold lead to an increase in prediction errors and was penalized. Akaike's Information Criteria (AIC) (Akaike, 1974), Cross-validation, Root-Mean-Square-Errors and Mean-Absolute-Error were used to assess the trade-off between model complexity and model predictive capacity, depending on the technique. In this way, it was confirmed that no unnecessary complexity was added in the models. The documentations and software used for each SDM are also listed in Table 2. All SDMs were developed as proposed by the authors mentioned in Table 2. A number of SDMs were implemented using several combinations of variables but only the model with the best fit and predictive capacity has been used in the comparison process.

GAMs, Generalized Additive Mixed Models (GAMMs) and Multivariate Analysis and Regression Splines (MARS) belong to the family of regression methods while MAXENT, BRT, ASNN, Artificial Neural Networks Ensemble (ANNE) and Support Vector Machines (SVM) are machine learning models. Bioclim Envelope Model (BIOCLIM) and Envelope Score (EnvScore) are envelope style methods using environmental data to define bioclimatic envelopes. Environmental Distance is a two-distance based method that makes use of a generic algorithm based on environmental dissimilarity matrices. Finally, Genetic Algorithm for Rule-set Prediction (GARP) uses a genetic algorithm that creates ecological niche models for species.

GAMs are generalized models involving a sum of smooth functions of covariates (Hastie & Tibshirani, 1990; Wood, 2006). GAMMs are also used, complementary to GAMs, in order to deal with spatial autocorrelation, which could lead to biased models and predictions. GAMs are the most frequently used approach in habitat modelling field (Valavanis et al., 2008) and several recent modifications and applications have increased their utilization (Leathwick et al., 2006b; Wood, 2006). The selection of the GAMs' smoothing predictors followed the method proposed by Wood & Augustin (2002), using the 'mgcv' library (Wood, 2008) in the R statistical software (R Development Core Team, 2005). The degree of smoothing was selected based on the observed data and the Generalized Cross Validation (GCV) method (Wood, 2006). First order interactions among the explanatory variables were also added in several GAMs. The best-fitting model was selected by using AIC and a stepwise forward selection was applied to restrict collinearity among the explanatory variables. The binomial family was applied using a logistic link function. GAMMs were developed based on the final GAM model with the assumption that a specific correlation structure exists among all sampled points in the study area. This structure was modelled by using the binomial distribution.

Multivariate Adaptive Regression Splines (MARS) (Leathwick et al., 2005) is an alternative regression-based method used for fitting non-linear responses but it differs from GAM because it utilizes piecewise linear fits instead of smoothers. In particular, MARS is a technique in which non-linear responses between a species and an environmental predictor are described

Table 2 Resource selection functions applied, variables and software used

Model	Explanatory variables	Software	Reference
Generalized Additive Models (GAM)	SST, SLA, DEP, DDND	R [18], library: mgcv	Wood (2006), Hastie & Tibshirani (1990)
Generalized Additive Mixed Models (GAMM)	SST, SLA, DEP, DDND	R, library: mgcv, geoR, spatstat, spdep	Wood (2006), Hastie & Tibshirani (1990)
Boosted Regression Trees (BRT)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	R, library: gbm	Leathwick et al. (2006a)
Multivariate Analysis and Regression Splines (MARS)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	R, library: mda	Leathwick et al. (2005)
Maximum Entropy (MAXENT)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	Maxent software for species habitat modelling	Phillips et al. (2004)
Support Vector Machines (SVM and SNM-Nu)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	openModeller Desktop	Cristianini & Shawe-Taylor (2000)
Genetic Algorithm for Rule-set Prediction (GARP)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	openModeller Desktop	Stockwell (1999)
Envelope Score (EnvScore)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	openModeller Desktop	Nix (1986), Piñeiro et al. (2007)
Bioclim Envelope Model (BIOCLIM)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	openModeller Desktop	Nix (1986)
Environmental Distance (EnvDist and EnvDistChe)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	openModeller Desktop	Carpenter et al. (1993)
Associative Neural Networks (ASNN)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	Associative Neural Network software by http://www.vcclab.org	Tetko (2002a, b)
Artificial Neural Network Ensemble (ANNE)	SST, CHL, PAR, SLA, DEP, SSTsl, DCoast, DEPsl, DDND	Associative Neural Network software by http://www.vcclab.org	Tetko (2002a, b)

by a series of linear segments of differing slope, each of which is fitted using a basis function as was described by Friedman (1991). Breaks between segments are defined by a knot in a model that initially over-fits the data and is then simplified using a backwards/forwards stepwise cross-validation procedure to identify terms to be retained in the final model. MARS is capable of fitting complex, nonlinear relationships between species and predictors and in one of its implementations can be used to fit a model describing relationships between multiple species and their environment (Leathwick et al., 2005). MARS is much faster than GAMs in model development and is easily utilized with GIS applications to generate species distribution maps. In this study, MARS was developed using the 'mda' library (Hastie et al., 1994) in R statistical software (R Development Core Team, 2005) and the 'MARS public function 3.1' by Leathwick & Elith (per. comm.). The selection of the explanatory variables was based on their contribution to model goodness of fit. Several models were developed including models with interactions among the explanatory variables. The final model was selected by comparing the predictive performance using Receiver Operating Characteristics and the Area Under Curve (ROC–AUC).

MAXENT estimates a target probability distribution by finding the probability distribution of maximum entropy (i.e. the most spread out or closest to uniform), subject to a set of constraints that represent incomplete information about the target distribution (Phillips et al., 2006). MAXENT is a general-purpose machine learning method with a simple and precise mathematical formulation and it has a number of characteristics that make it well-suited for species distribution modelling. It is based on the maximum-entropy principle developed by Jaynes (1957). Maximum Entropy Species Distribution Modelling software version 3.3.1 was used for model development. Several models were developed and the one with the lowest ROC–AUC and containing highly contributing variables was finally selected. MAXENT is user-friendly software, which provides outputs containing all the essential information about the models developed. Additionally, MAXENT's data output can be easily inserted in GIS for further analysis and generation of distribution probability maps.

In BRT (Leathwick et al., 2006a), each of the individual models consists of a simple Classification and Regression Tree (CART). The boosting algorithm uses an iterative method for developing a final model in a forward stage-wise way, progressively adding trees to the model by re-weighting the data in order to emphasize cases that are poorly predicted by the previous trees. Advantages offered by a BRT model include its ability to accommodate different types of predictor variables and missing values, its immunity to the effects of extreme outliers and the inclusion of irrelevant predictors and its facility for fitting interactions between predictors (Friedman & Meulman, 2003). BRT models were constructed using the BRT functions version 2.8, as developed by Leathwick & Elith (pers. comm.) for R statistical software (R Development Core Team, 2005), and the 'mda' library. The best performing model was selected according to the area under the Receiver Operating Characteristic curve.

Associative Neural Networks (ASNN) is a method with improved predictive abilities compared to traditional neural networks techniques, including combination of feed-forward neural networks and a *k*-nearest neighbour technique. This method uses the correlation between ensemble responses as a measure of distance of the analyzed cases for the nearest neighbour technique. This provides an improved prediction ability by correcting the bias of the neural network ensemble. An ASNN has a memory that can coincide with the training set. If new data become available, the network further improves its predictive ability and provides a reasonable approximation of the unknown function without the need to retrain the neural network ensemble. This feature of the method dramatically improves its predictive ability over traditional neural networks and *k*-nearest neighbour techniques. Here, an Artificial Neural Network Ensemble (ANNE) was developed using one hidden layer with three neurons. The number of the nearest neighbour, k, and parameter σ for the Parzen-window regression represent smoothing parameters of ASNN in order to minimize the ASNN error for the training set. ASNNs were initially applied in chemistry (Tetko et al., 1995), providing more accurate predictions than ANNE. More detailed information on ASNN development can be found in Tetko (2002a, b). Both ANNE and ASNN were developed in order to compare ASNN performance to traditional ANNE and to other modelling approaches. ANNE and ASNN models were selected based on processes including the training algorithm, the number of neurons and hidden layers and the iterations and number of ensembles. The Early Stopping over Ensemble (ESE) method was used to train the neural networks (Bishop, 1995; Tetko & Tanchuk, 2002). Models presenting the lowest Root Mean Squared Error (RMSE) and Mean Absolute Error (MAE) were finally selected (Tetko et al., 2008).

BIOCLIM is an 'envelope' method that implements a bioclimatic envelope algorithm (Nix, 1986; Busby, 1991). Environmental envelopes are conceptually closely related to niche theory as they strive to delineate the hyper-surface (or envelope) that best circumscribes suitable conditions within the niche hyper-space defined by the environmental variables. The algorithm finds the mean and standard deviation for each environmental variable (assuming normal distribution) associated with the occurrence of surveyed species presence points. Besides the envelope, each environmental variable has additional upper and lower limits taken from the maximum and minimum values related to the set of occurrence points. In this model, any point can be classified as: Suitable (when all associated environmental values fall within the calculated envelopes), Marginal (when one or more associated environmental value falls outside the calculated envelope but still within the upper and lower limits) or Unsuitable (when one or more associated environmental value falls outside the upper and lower limits). BIOCLIM's categorical output is mapped to probabilities of 1.0, 0.5 and 0.0, respectively. OpenModeller software (Muñoz et al., 2009) was used for BIOCLIM development.

Envelope Score (EnvScore) is analogous to the BIOCLIM approach and implements a Bioclimatic Envelope Algorithm. For each given environmental variable, the algorithm finds the minimum and maximum at all occurrence sites. The Envelope Score algorithm is equivalent to the inclusive 'OR' implementation of Bioclim described in Piñeiro et al. (2007). EnvScore models were developed using OpenModeller software (Muñoz et al., 2009).

Climate Space is a principle components-based algorithm developed by Neil Caithness (http://openModeller.sf.net). The component selection process in this algorithm implementation is based on the Broken-Stick cut-off, whereby any component with an eigenvalue less than *n* standard deviations above a randomised sample is discarded (see also Muñoz et al., 2009). The original Climate Space Model was written as series of Matlab functions.

Environmental Distance (EnvDist) uses a generic algorithm based on environmental dissimilarity metrics. When combined with the Gower metric (Gower & Legendre, 1986) and maximum distance is set to one, this algorithm should produce the same result as the algorithm known as DOMAIN (Carpenter et al., 1993). DOMAIN is a distance-based method that assesses new sites in terms of their environmental similarity to sites of known presence by transforming the known occurrences into an environmental space and computing the minimum distance in environmental space from any cell to a known presence of the species. EnvDistChe was developed using Chebyshev distance instead of the Gower metric. Chebyshev distance is a metric defined in a vector space such that the distance between two vectors is the greatest of their differences along any coordinate dimension. Both models were developed using OpenModeller software (Muñoz et al., 2009).

Support Vector Machines (SVMs) consists of a set of related supervised learning methods that belong to the family of generalized linear classifiers. They could be considered as a special case of Tikhonov regularization (Tychonoff & Arsenin, 1977). SVMs simultaneously minimize the empirical classification error and maximize the geometric margins. The model produced by support vector classification depends only on a subset of the training data because the cost function for building the model does not take into account training points that lie beyond the margin (Vapnik, 1995; Schölkopf et al., 2000). SVMs are able to represent nonlinear effects and interactions between variables by projecting the explanatory variables into a higher dimensional feature space where the prediction problem has a linear solution (Moguerza & Muñoz, 2006). Two SVM were developed (SVM-Nu and SVM) because of their different performances in relation to fitting efficiency and predictive capacity. SVM-Nu differs to SVM in the degrees used in Kernel function. Both approaches were developed using openModeller software.

The GARP uses a genetic algorithm to select a set of rules (e.g. adaptations of regression and range specifications) that best predicts the species distribution (Stockwell & Peters, 1999). The Genetic Algorithm used in GARP is based on the basic concept developed by Holland (1975). GARP creates ecological niche models for species, identifying where the environmental conditions could maintain populations. For input, GARP uses a set of point localities where the species are known to occur and a set of geographic layers representing the environmental parameters that might limit the species' capabilities to survive. In this study, based on openModeller software (Muñoz et al., 2009), the algorithm applies the Best Subsets procedure using the new openModeller implementation in each GARP.

Comparison

There are several techniques to validate a model or to compare the accuracy of prediction among different models. Kappa statistics, ROC–AUC, *k*-Fold cross validation, confusion matrices and classification tables are well described by Boyce et al. (2002) for presence–absence data.

Models that predict the presence or absence of a species are normally judged by the number of prediction errors. There are two types of prediction errors: false positive (FP) and false negative (FN). The performance of a presence–absence model is normally summarized in a confusion or error matrix (Table 3) that cross-tabulates the observed and predicted presence–absence patterns. Morrison et al. (1992) refer to FP errors as type I and FN errors as type II errors. FP or commission error leads to an over-prediction while FN or omission error leads to an under-prediction. Generally, omission error could be characterized as 'hard'—true error while commission might or might not be a true error. Commission error can relate to unsuitable areas (true error), suitable areas with no sampling effort (species may be there), or suitable areas where historical (barriers, dispersal capability) or biotic (competition, predation) factors have impeded occupation by the species or caused it to go extinct. An accurate presence–absence model should be characterized by low omission error. On the other hand, low commission error indicates that the model over-fits the training data while high commission error indicates that the model over-predicts the training set. Specificity and sensitivity are terms analogous to omission and commission errors, although they refer to correctly predicted presence and absence instead of the errors. Specificity is the proportion of observed negatives correctly predicted and reflects a model's ability to predict an absence given that a species actually does not occur at a location. Sensitivity is the proportion of observed positives correctly predicted and reflects a model's ability to predict a presence given that a species actually occurs at a location.

Table 3 Confusion matrix summarizes observed and predicted presence/absence values

Confusion matrix	Predicted present	Predicted absent
Actually present	True positive	False positive (error type I)
Actually absent	False negative (error type II)	True negative

In this study, SDM comparison was achieved using the best representative models derived using each function. ROC–AUC (Fielding & Bell, 1997) was used because in contrast to other model evaluation methods (Kappa statistics, confusion matrices and classification tables, see Boyce et al., 2002), it avoids the problem of threshold value selection (Lehmann et al., 2002). ROC-plots and the Area Under the Receiver Operating Characteristic Curve measure the ability of a model to discriminate between those sites where a species is present and those where it is absent, and they have been widely used in the species distribution modelling literature (Elith et al., 2006). ROC–AUC values range from 0 to 1, with 1 standing for perfect discrimination, 0.5 for predictive discrimination close to a random guess and values <0.5 indicating performance worse than random (Boyce et al., 2002; Elith et al., 2006).

The correlation (COR) between the observation in the presence–absence dataset (a dichotomous variable) and the prediction is known as the point biserial correlation, and it can be calculated as a Pearson correlation coefficient (Zheng & Agresti, 2000). It is similar to ROC–AUC but carries extra information: instead of being rank based, it takes into account the difference between the prediction and the observation. This gives further insight into the distribution of the predictions and provides information on the model's discrimination (Murphy & Winkler, 1992).

The Kappa statistic (Cohen, 1960) summarizes all the available information in the confusion matrix. Kappa measures the proportion of correctly classified units after accounting for the probability of chance agreement. Kappa, which is a chance-corrected measure of agreement, is commonly used in ecological studies with presence–absence data (Boyce et al., 2002). It requires a threshold to be applied to the predictions in order to convert them to presence–absence predictions. Kappa provides an index that considers both omission and commission errors. In this study, a maxKappa is used for each model generated by using the 'PresenceAbsence' library of the R statistical software (R Development Core Team, 2005).

Confusion matrices for the modelling approaches were formulated for both predictions on the training and the verification set. Omission and commission errors, sensitivity and specificity as well as Kappa statistics were estimated from the confusion matrices. Since Kappa is threshold-dependent, in order to avoid threshold selection, the maxKappa was used (Liu et al., 2005). The correlation coefficient between predicted and observed values in both datasets was also estimated. ROC–AUC was also used to classify the accuracy of the predictions. Finally, the probability maps/grids that were generated from each modelling technique were compared for their spatial similarities, using ESRI's ArcInfo correlation function for grids.

The Akaike Information Criterion (AIC) was also used for model selection. However, for many adaptive, nonlinear techniques, estimation of the effective number of parameters and consequently the AIC calculation is very difficult (Hastie et al., 2009). For this reason, all critical comparisons were mainly based on cross-validation techniques and ROC–AUC, whilst the AIC was used for the best candidate model within a given model family, taking into account the trade-off between model complexity and predictive capacity. Cross-validation is also preferable for theoretical reasons. Hastie et al. (2009) found, in simulation experiments, that the AIC can greatly overestimate the prediction error (>30%) compared to the cross-validation procedure. Nevertheless, the ordinary cross-validation procedure does not work well when the data are autocorrelated, resulting in underestimation in error prediction and consequentially in biased model selection (Hastie et al., 2009). This is the case in the observed spatio-temporal autocorrelation in hydroacoustic abundance records, which is a property of the biomass structure, not of the measurement processes (Simmonds & MacLennan, 2005). Their spatial characteristics, estimated for instance in geostatistics as nugget and range parameters, are affected by the selected acoustic integration unit (ESDU). Presence of a spatial structure in the errors, causes, among other things, underestimated standard errors of the slopes in the regression model and it represents a serious shortcoming for hypothesis testing and prediction (Ostrom, 1990). In this study, the spatial autocorrelation of the training and the verification dataset was estimated and the residuals of each modelling technique were checked for potential spatial patterns. In GAMMs the spatial autocorrelation pattern was inserted in the model, while in other methods several covariates were used to absorb the autocorrelated errors (Elith & Leathwick, 2009).

Initially, the predictive efficiency of each method was tested on the training set. In this case, the best performing techniques are considered to model the sampling data more accurately and thus, they describe species distribution more accurately. On the other hand, this does not necessarily reflect the predictive capacity of the methods, which is better presented by the predictions on the verification set, which is an unknown dataset to the training process of SDMs. The process of model evaluation is crucial in the SDM field, though there are diverse opinions on what properties of a model are important and how to test them appropriately (Elith & Leathwick, 2009). During SDM development to explain patterns or biological relationships, statistical tests of model fit and comparison with existing knowledge are generally used. In the case where an SDM is developed to predict species distribution in time or space, the predictive capacity is evaluated using either resampling techniques (cross-validation, bootstrapping) or an independent dataset. In this study, both the fitting efficiency and the predictive capacity of different SDMs were compared. For these processes, ROC–AUC was used as a threshold-independent index that quantifies the predictive performance of the models while omission and commission errors were used as prediction quality indices with respect to over- and under-prediction and over-fitting. MaxKappa was also used to complement ROC–AUC, as a chance-corrected measure of agreement, and COR was used to estimate the similarities between observed and predicted values.

Results

Spatial patterns and comparison among training and verification datasets

The selected validation dataset contains measurements omitting, in each step, h units (h equals at least 5 n mi), where h is chosen according to the empirical variogram of both validation set and prediction residuals. The empirical variograms (Fig. 2) and the autocorrelation function plots revealed a low autocorrelation, even in distances below the h limit, because the nugget was at least 65% of the sill and the range was larger than 10 km (5.4 n mi). The verification set presents no autocorrelation, as shown in Fig. 2. The selected distance among the points in the verification set reduces the spatial autocorrelation (Moran's I Index $= 0.03$), while in the training set the index was equal to 0.14. The index indicates that the verification set presents some clusters that might be due to random chance; however, there is less than 1% likelihood that the clusters in the training are due to chance alone. Residuals of the SDMs were also tested for spatial patterns, but no significant patterns were observed, presumably due to the adequate set of predictors used and the appropriately specified model fit (Elith & Leathwick, 2009). These results confirm that the verification set is not spatially autocorrelated, while there is an amount of autocorrelation in the training set (Fig. 2).

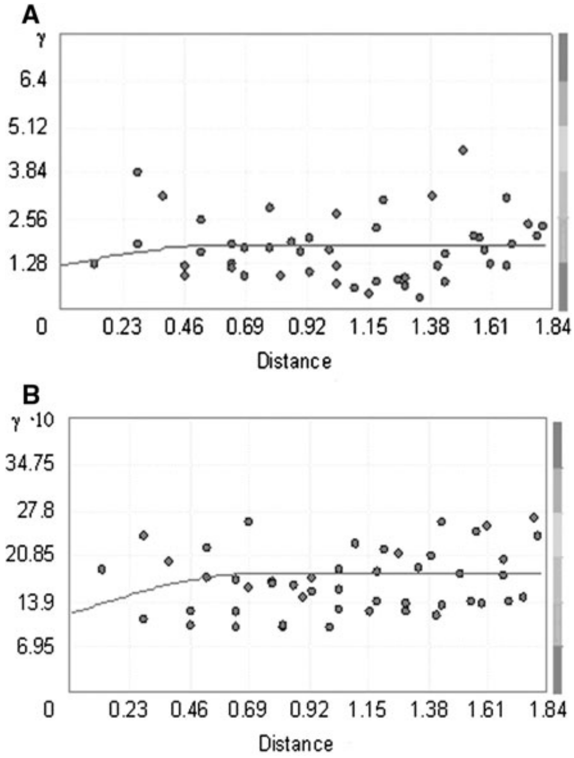


Fig. 2 Empirical variograms and autocorrelation function plots of the acoustic density that correspond to the validation dataset (**A**) and the training dataset (**B**)

Although the training and the validation datasets differed in their autocorrelation pattern, it was verified using the Mann–Whitney test that the null hypothesis was not rejected ($U = 1550$, P-value $= 0.48$) and both dataset refer to the same 'population' as derived by the definition of the test. Additionally, the Two-sample Kolmogorov–Smirnov test indicated that both dataset have the same distribution since the null hypothesis was also rejected ($Z = 0.56$, P-value $= 0.87$). The mean and standard deviation of the validation set (196 s_A and 220) was relatively higher than the training set (193 s_A and 215, respectively). Despite the similarities among the two datasets and the fact that they correspond to the same 'population' they could not be characterized as identical since their vector of values were not significantly correlated (Pearson's correlated coefficient $= -0.202$).

Fitting efficiency

ROC–AUCs and the associated standard deviations of all models are presented in Fig. 3A. Models with the highest ROC–AUC and the lowest standard deviation provide the best fitted SDMs. This is depicted in Fig. 3A (upper right). BRTs, EnvDist, EnvDistChe and SVM-Nu out-perform the other approaches achieving ROC–AUC greater than 0.9. Regression models (GAM, GAMM, MARS) as well as ASNN and SVM also acheived a high ROC–AUC (0.86–0.9). ANNE, GARP and MAXENT had AUCs in the range of 0.81–0.76. BIOCLIM, EnvScore and ClimSpace did not perform so well, achieving ROC–AUCs less than 0.64 while ClimSpace's AUC was 0.52.

The COR, which indicates the similarities between observed and predicted values, and the maxKappa are presented in Fig. 3B. Generally, the clusters (in relation to performance) of the modelling techniques are analogous to those indicated by the ROC–AUC,

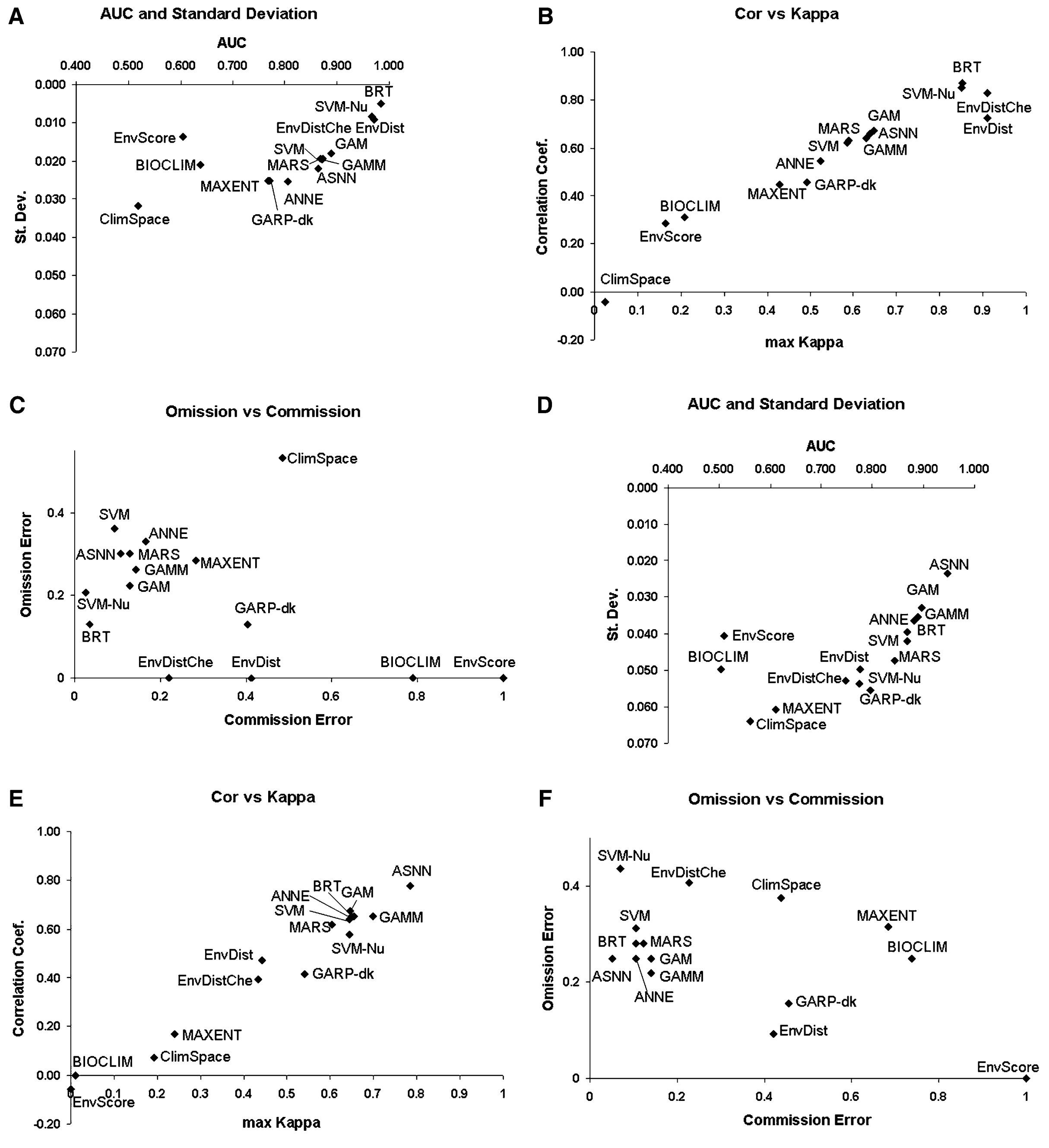


Fig. 3 Comparison of fitting efficiency (**A**, **B**, **C**) and predictive capacity (**D**, **E**, **F**) between the SDMs. ROC–AUC and the associated standard deviation scored by modelling approaches applied on the training set (**A**) and verification set (**D**). Correlation Coefficient and maxKappa scored by modelling approaches applied on the training (**B**) and verification set (**E**). Omission and Commission errors of modelling approaches applied on the training (**C**) and verification set (**F**)

though some differences in the classification are apparent for maxKappa and COR. BRTs and SVM-Nu model predictions were most highly correlated with the training dataset (0.87 and 0.85, respectively). However, EvnDist and EnvDistChe achieved the highest maxKappa (0.91). ASNN, GAM and GAMM performed almost equally (COR: 0.67–0.64 and maxKappa: 0.65–0.62). MARS performs equally to

SVM (COR: 0.63–0.62 and maxKappa: 0.59–0.58). ANNE achieved COR of 0.55 and maxKappa 0.52 while MAXENT and GARP present similar COR (0.45 and 0.46, respectively) but maxKappa is higher for GARP than for MAXENT (0.49 over 0.43). Bioclim, EvnScore and especially ClimSpace obviously failed to fit the training dataset presenting COR 0.31, 0.29 and 0.04, respectively, and maxKappa 0.2, 0.16 and 0.02, respectively.

As mentioned above, omission and commission errors reflect the quality of the predicted values with respect to over- and under-prediction and over-fitting. Figure 3C depicts the omission and commission errors of the different modelling techniques applied. High omission values indicate poor fitting efficiency (e.g. ClimSpace). Zero omission error combined with high commission error indicates over-prediction of the potential species distribution (mainly EnvScore and BIOCLIM). Zero omission error combined with no commission error indicates that predicted values over-fit the training values. So, it is expected that EnvDistChe tends to over-fit the training set more than EnvDist. The probability maps (are discussed later) that were generated by relatively high omission and commission errors SDMs provide a visual interpretation of the biased predicted patterns.

Predictive capacity

Comparison of the observed values with predicted values, derived by a dataset 'unknown' to SDMs, indicates the predictive capacity of the techniques applied and provides additional evidence of the performance of SDMs (i.e. additional to information derived from the fitting process and its diagnostics). ROC–AUC and the associated standard deviation from the application of SDMs to the validation set are presented in Fig. 3D. Models with the highest ROC–AUC and the lowest standard deviation could be characterized as those with the highest predictive capacity (Fig. 3D, upper right). Additionally, techniques that performed relatively efficiently in predicting the training dataset, but failed to accurately predict the verification set, probably tend to over-fit the training data and thus suffer decreased generality. According to the ranking in Fig. 3D, ASNN clearly out-performs the other approaches, achieving ROC–AUC close to 0.96. Regression models (GAM, GAMM, MARS) as well as BRT, SVM and ANNE also achieved high ROC–AUC (0.84–0.9). SVM-Nu, EnvDist, EnvDistChe and GARP scored ROC–AUC from 0.75 to 0.8. MAXENT and ClimSpace had ROC–AUC values of 0.61 and 0.56, respectively. BIOCLIM and EnvScore did not perform well, achieving AUC 0.50 and 0.51, respectively.

The COR values, which indicates the similarities between observed and predicted values on the verification set, as well as the maxKappa values are presented in Fig. 3E. Generally, the resulting groups of the modelling techniques are analogous to those arising from the ROC–AUC, though some differences are seen between maxKappa and COR. ASNN shows the highest predictive capacity (0.78 COR, 0.78 maxKappa). A distinct cluster described by the COR range of 0.58–0.67 and maxKappa of 0.6–0.7 includes GAM, GAMM, ANNE, BRT, SVM, SVM-Nu and MARS. GARP presents a COR of 0.42 and maxKappa of 0.54. EvnDist and EnvDistChe scored almost equally according to maxKappa (0.44) but differ in the correlation of their predictions with the verification set (0.47 and 0.39, respectively). MAXENT, ClimSpace, BIOCLIM and EvnScore present lower predictive capacities, achieving COR from 0.17–0.00 and maxKappa 0.24–0.00.

In contrast to Fig. 3C (which refers to the trained models), Fig. 3F summarizes the predictive ability of the models in relation to the verification set in terms of the associated omission and commission errors. High omission error indicated model weakness in terms of identifying species occurrence, while high commission error indicates a model's inability to distinguish unsuitable habitats. ASNN, GAM, GAMM, ANNE, BRT and SVM provide the less erroneous predictions regarding the independent verification set. MAXENT, BIOCLIM and EvnScore are the models with the highest commission error while SVM-Nu, EnvDistChe and ClimSpace have highest omission error.

Probability maps

Models developed by each approach were applied to grids of predictor variables in order to generate the corresponding species distributions maps. When presence–absence data are used, the maps generated are actually probability maps that denote the probability of species occurrence. Acoustic data used in this study were converted to presence–absence data. Presence corresponds to high acoustic density, indicating suitable species habitats, while absence corresponds to low acoustic density indicating species absence or low fish density. Environmental variable grids were used for generating maps at a spatial resolution of 0.01 decimal degrees. In addition to predicting the fine scale distribution of small pelagic species, these maps should be helpful to identify potential habitat heterogeneity. Figure 4 depicts the probability maps derived from

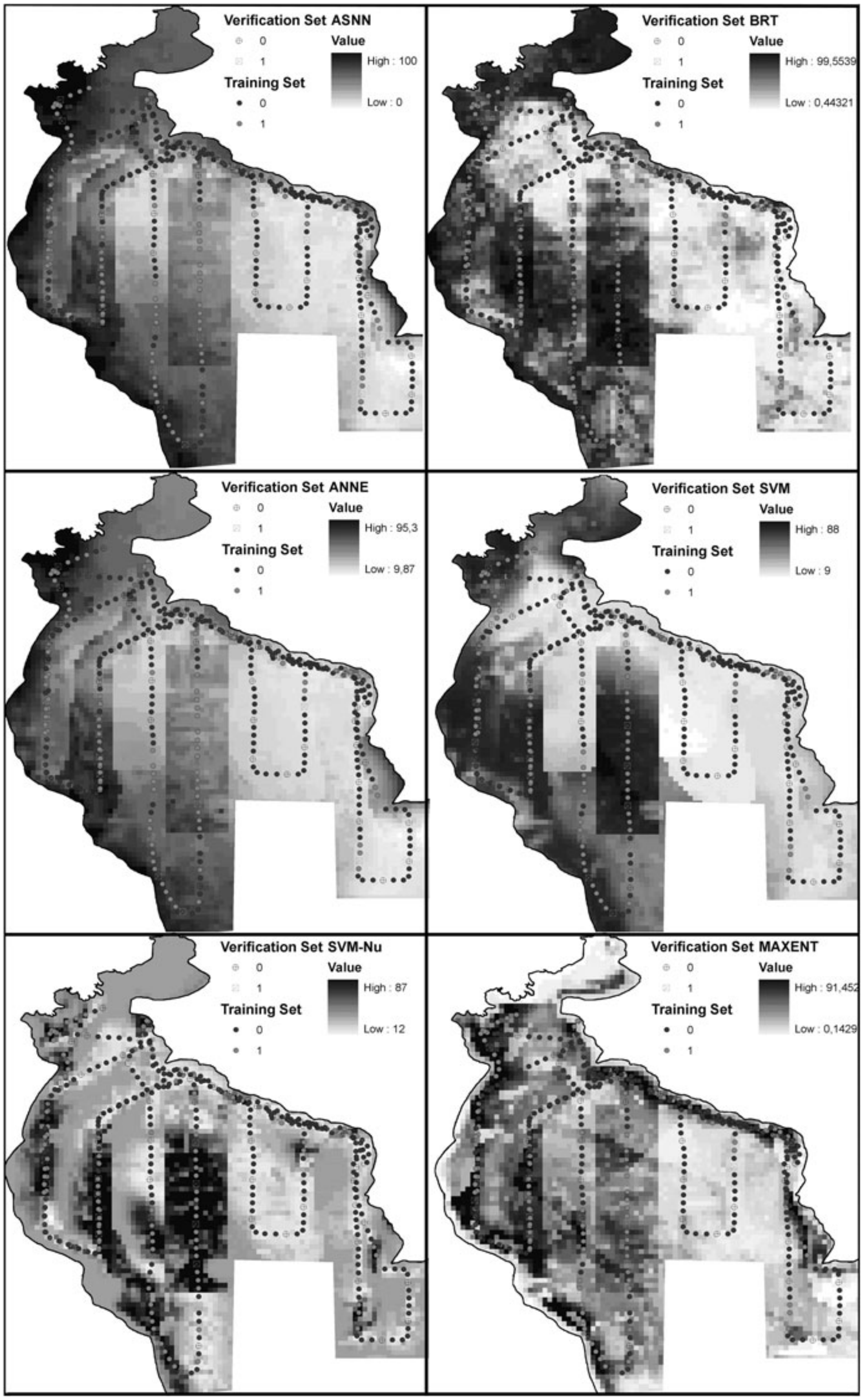


Fig. 4 Predicted probability distribution maps generated by machine learning approaches

machine learning approaches (BRT, ASNN, ANNE, MAXENT, SVM). The probability maps of the regression approaches are presented in Fig. 5, while the probability maps that arise from the envelope style approaches are presented in Fig. 6. Neither EnvScore nor BIOCLIM supports absence data. Finally, probability maps generated by EnvDist, EnvDistChe, ClimSpace and GARP are depicted in Fig. 7. Among the latter methods, only GARP supports the use of absence data.

Table 4 presents the Pearson correlation coefficients among SDMs. The upper-right part corresponds to the correlation among predictions on the validation set. The lower-left part corresponds to the correlation among the predicted grids as estimated using ESRI's ArcInfo correlation function for grids.

Discussion

Spatial structure in data

Patterns of spatial autocorrelation are common in species and biomass abundance or other ecological records (Legendre, 1993). Consequently, standard statistical models based on such data may violate the basic assumption that residuals are independent. Possible causes of spatial autocorrelation are categorized in three groups: the nature of the biological processes involved, the absence of important explanatory variables in the model and the linear modelling of a process that in reality is non-linear (Legendre & Legendre, 1998).

Commonly used methods to deal with the problem of spatial structure in the errors, are based on:

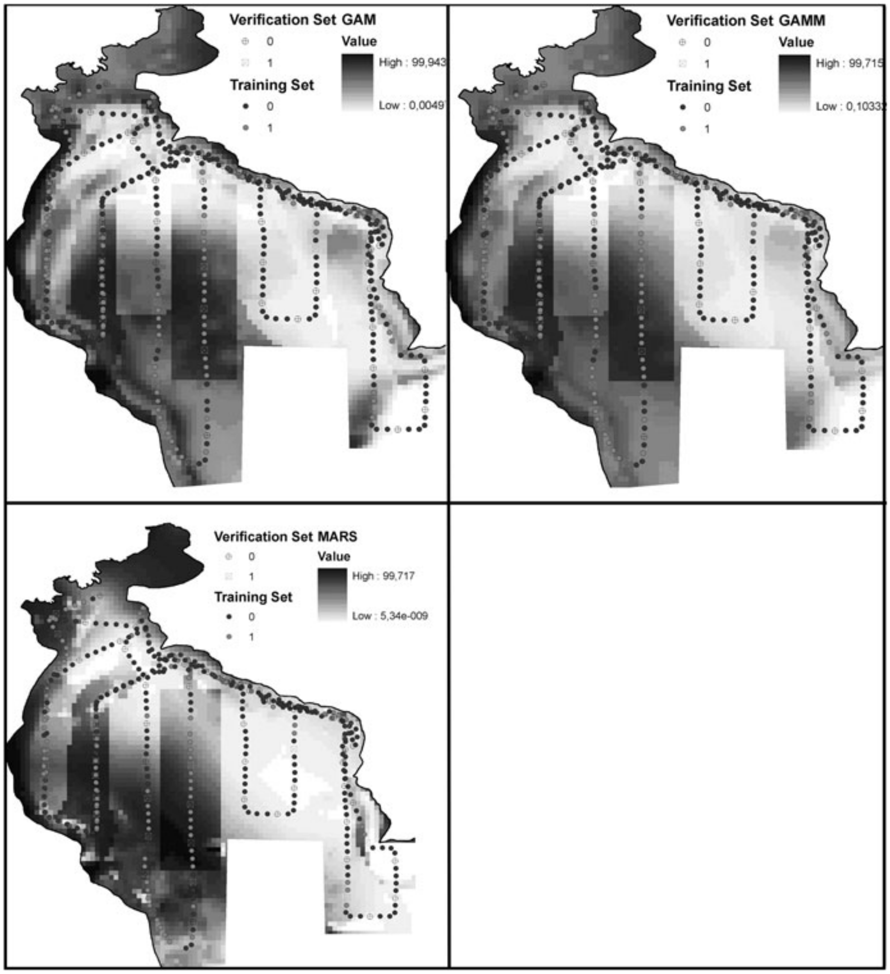


Fig. 5 Predicted probability distribution maps generated by regression approaches

Fig. 6 Predicted probability distribution maps generated by envelope style approaches

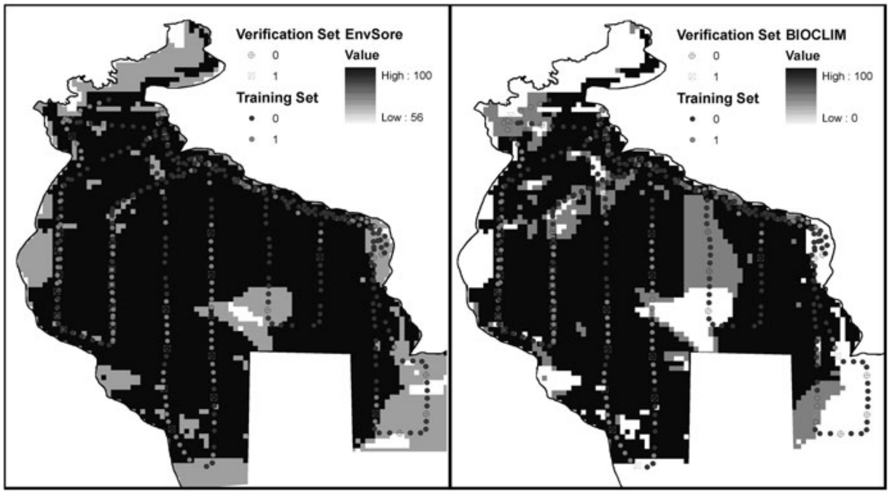


Fig. 7 Predicted probability distribution maps generated by Environmental Distance, GARP and climate space approaches

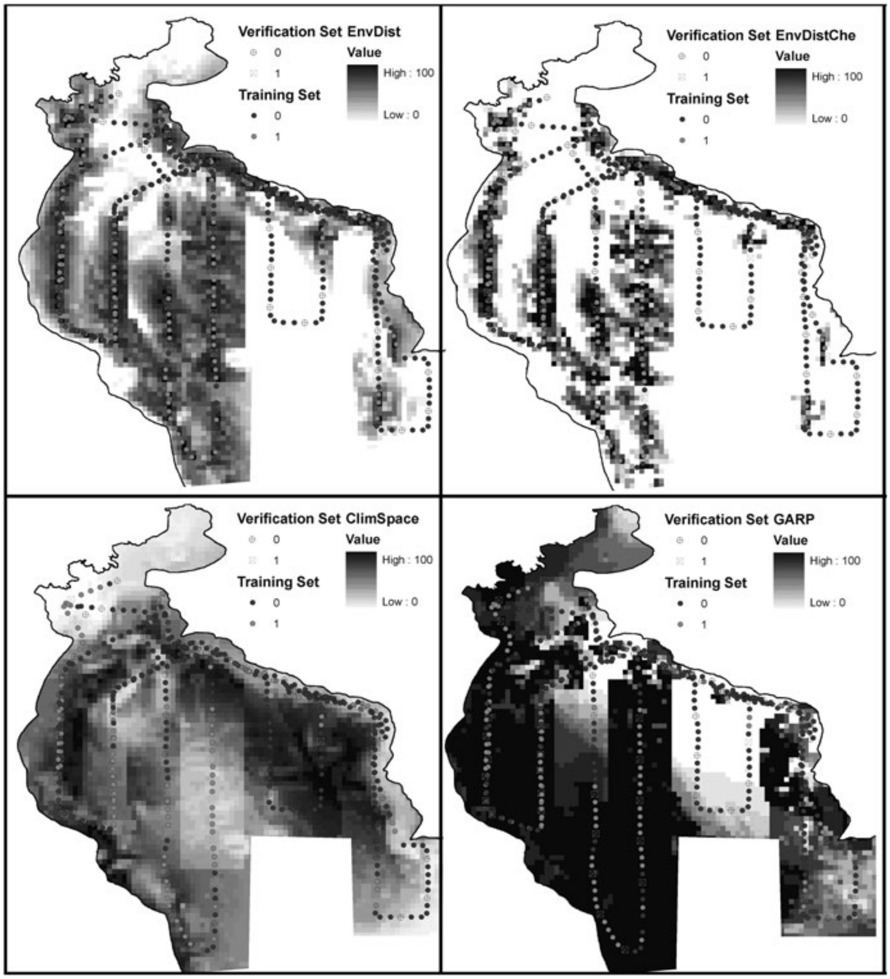


Table 4 Pearson's correlation coefficient among SDMs' predictions on the validation set (upper right) and among predicted grids (lower left)

Pearson R	GAM	GAMM	BRT	MARS	BIOCLIM	ClimSpace	EnvDist	EnvDistChe	EnvScore	GARP-dk	SVM	SVM-Nu	MAXENT	ANNE	ASNN
GAM		0.98	0.87	0.90	0.17	−0.04	0.64	0.53	0.10	0.63	0.82	0.70	0.49	0.90	0.85
GAMM	0.97		0.87	0.93	0.19	−0.02	0.61	0.49	0.12	0.69	0.85	0.70	0.48	0.93	0.85
BRT	0.84	0.87		0.85	0.18	−0.03	0.66	0.60	0.14	0.63	0.84	0.83	0.48	0.85	0.88
MARS	0.87	0.91	0.86		0.21	−0.04	0.60	0.47	0.14	0.65	0.80	0.64	0.47	0.89	0.80
BIOCLIM	−0.05	−0.02	−0.01	0.00		0.38	0.40	0.32	0.87	0.14	0.10	0.07	0.46	0.17	0.10
ClimSpace	−0.23	−0.20	−0.24	−0.26	0.41		−0.08	−0.07	0.30	0.17	0.04	0.08	−0.08	0.01	0.00
EnvDist	0.41	0.43	0.43	0.42	0.34	−0.06		0.87	0.24	0.28	0.49	0.54	0.69	0.60	0.69
EnvDistChe	0.30	0.29	0.32	0.28	0.26	−0.05	0.80		0.21	0.10	0.40	0.57	0.57	0.45	0.60
EnvScore	−0.11	−0.10	−0.10	−0.11	0.85	0.40	0.26	0.21		0.11	0.10	0.09	0.30	0.13	0.02
GARP-dk	0.55	0.60	0.56	0.55	0.16	−0.05	0.39	0.24	0.05		0.69	0.58	0.30	0.73	0.56
SVM	0.78	0.82	0.82	0.80	0.00	−0.23	0.50	0.34	−0.10	0.65		0.80	0.33	0.91	0.83
SVM-Nu	0.57	0.55	0.59	0.51	0.15	−0.14	0.63	0.65	0.09	0.46	0.67		0.20	0.75	0.82
MAXENT	0.31	0.34	0.36	0.33	0.43	−0.06	0.65	0.55	0.38	0.39	0.38	0.44		0.43	0.41
ANNE	0.73	0.79	0.71	0.76	−0.05	−0.16	0.39	0.23	−0.13	0.72	0.78	0.39	0.35		0.89
ASNN	0.73	0.78	0.72	0.76	−0.93	−0.20	0.36	0.21	−0.16	0.66	0.75	0.35	0.33	0.97	

(a) adding covariates, which can absorb the autocorrelated errors (see review by Elith & Leathwick, 2009), (b) choosing an appropriate ESDU, which reduces the autocorrelation for a given range, (c) applying wavelet-based methods for removing autocorrelation effects (Gudrun & Kühn, 2008), (d) extending the model in order to include the autocorrelation (e.g. extending GAM as GAMM) (Dray et al., 2006) or using the autocorrelation itself for interpolation purposes (Rossi et al., 1992; Simmonds & MacLennan, 2005).

Several modifications of ordinary cross-validation have been published to address the training-verification data dependence issue (Burman et al., 1994; Racine, 2000), omitting some data from the point of prediction and its neighbours within *h* units and using the remaining data for both model estimation and prediction. In this study, the selected validation dataset contains measurements omitting, in each step, *h* units (*h* equals at least 5 n mi), where *h* is chosen according to the empirical variogram of both validation set and prediction residuals. The empirical variograms and the autocorrelation function plots revealed a low autocorrelation, even in distances below the *h* limit. The verification set presents no autocorrelation, as shown in Fig. 2. A similar spatial structure has been encountered in previous surveys (October 1996, May 1997) even in different seasonal conditions (Georgakarakos & Kitsiou, 2008). Results from a comparative study using series of acoustic survey data from five different locations in Europe suggested that the spatial organization of the stock would be more dependent on environmental parameters than on fish abundance (Petitgas et al., 2001). This result disagrees with the general notion which relates stock size to spatial organization, at least for higher values of fish abundance (MacCall, 1990). In a similar study, school cluster characteristics (e.g. dimension, nb of schools) were correlated with total population school number but not with total population biomass (Muiño et al., 2003).

The autocorrelation characteristics of biomass are in agreement with the school clustering tendency of the biomass in previous surveys (1996, 1997) as this is estimated by the distance between two schools in a cluster (Petitgas et al., 2001). The estimated school and cluster descriptors from these surveys (average school number per km in the clusters, average ratio for summed school lengths/cluster length and maximum distance between two schools in a cluster) indicate a small aggregative scale in the biomass spatial structure.

Furthermore, the observed high representation in the species composition of sardine and the strong mixed aggregation with anchovy in the biological sampling did not allow development of alternative models utilizing the species composition as regressor variables. For the same reason the authors decided to work on acoustic density data without any transformation into biomass in order to avoid the propagation of the variability from the trawl sampling in the response variable. As a result, the distribution maps reflect the distribution of small pelagic species in the study area. In most SDM studies distribution patterns refer to a single species. In this case, the distribution maps correspond to small pelagic species in general (but mostly sardine and anchovy). The variable selection, the development of the models and the generation of the distribution maps were all carried out bearing in mind that the models refer to multispecies distribution; thus common features of their life-history were utilized. According to Stergiou & Lascaratos (1997), the distributions of these species are affected by environmental parameters, fishing activity, inter- and intra-specific competition. The use of small pelagic species as a group instead of a specific species includes the between-species competition and thus reduces the biotic parameters that affect species distribution. On the other hand, the distribution maps that are derived from this study are unsuitable to identify specific species–environment relations.

Verification process

The use of an independent well-structured presence–absence verification set is proposed as the optimal method to verify the predictive performance of the SDMs (Elith et al., 2006). On the other hand, the use of entirely independent datasets carries the comparing of different sampling strategies instead of evaluating a model (Lehmann et al., 2002). Alternatively, cross-validation (Jaberg & Guisan, 2001) and jackknife (Lehmann et al., 2002) are also proposed for model validation, especially where there are not sufficient data to be partitioned into a training and a validation set. According to Lehmann et al. (2002) and Jaberg & Guisan (2001), cross-validation, bootstrapping and jackknife validation approaches are generally more practical, because they create relatively independent random data subsets and allow the use of all available data in the modelling process. These approaches are very useful in cases where insufficient data are available to be partitioned in a training set and a validation set that is not used during development of SDMs. However, Fu et al. (2005) and Simon et al. (2003) observed that cross-validation and, especially, leave-one-out cross-validation could lead to underestimation of prediction errors. In this study, a validation set was selected, as described earlier, since there were sufficient sampling records to formulate both datasets. The specific selection of the validation set overcomes the underestimation of predicted errors that could caused by cross-validation approaches, especially when acoustic data are spatially autocorrelated (Hastie et al., 2009). Additionally, there is no risk of comparing different sampling strategies, since the verification set is a sub-set of the raw data. These were also verified by the comparison of the training and the verification dataset. Generally, the SDM validation process is of great concern among species distribution modellers (Elith & Leathwick, 2009), while SDM evaluation would benefit from identifying useful techniques in other fields.

SDMs comparison

Among the machine learning techniques, SVM and MAXENT do use only presence data. Brotons et al. (2004) showed that predictions based on presence–absence data generally perform better than those based on presence-only data. Presence-only models can perform almost as well as presence–absence approaches, especially when survey coverage is evenly and widely distributed (MacLeod et al., 2008) but they contain no mechanism to control for biased sampling. In the present study, MAXENT under-performed compared to other machine learning approaches whereas SVM performed equally well with approaches that use presence–absence data.

The probability maps corresponding to machine learning models present notable similarities, identifying high probabilities of species occurrence near the coast, especially the west coast, and in the centre-to-south of the study area. In SVM-Nu and MAXENT, however, the predicted probability of occurrence along the west coast is lower than was the case for the other models. Additionally, high probabilities seem to overlap with the sampling transects, which could be an indication of over-fitting. Both approaches are characterized by relatively high omission error and thus do not predict the observed data accurately. SVM-Nu shows remarkable fitting efficiency for a presence-only model, having the second best ROC–AUC and COR scores (after BRT). However, its predictive capacity is relatively poor (as was indicated by the moderate ROC–AUC in total, and the highest omission error) and SVM-Nu over-fits the training dataset. This was not the case for SVM, making it the best modelling approach among those that do not support use of absence data. SVM performs at a similar level to the regression models.

BRTs, ASNN and ANNE were among the best performing models. In particular, BRT presents the best-fitting efficiency while its predictive capacity is relatively high compared to the other models. ASNN presents the best predictive capacity, and is characterized by satisfactory fitting efficiency (ROC–AUC 0.86). ANNE performs relatively well compared to other approaches, especially regarding its predictive capacity. However, performs less well than ASNN, as might be expected given its relationship to ASNN (Tetko, 2002a, b). ASNN achieves ROC–AUC, COR and maxKappa values that are markedly higher than the second best approach (GAM).

GAM, GAMM and MARS generate very similar probability maps, which confirms the similarities in fitting efficiency and predictive capacity. The comparison among the regression models indicates that GAM performs slightly better than GAMM while GAMM performs slightly better than MARS. The similarity in their performance was expected due to their common statistical origin. Even if there are other approaches that out-perform the regression models, either in fitting efficiency or in the predictive capacity, GAM, GAMM and MARS achieve relatively high values in the criteria used for both comparisons. Thus, the widespread use of regression models compared to that of other traditional approaches, like envelope style methods, GARP and MAXENT to predict species distributions is justified by their stability and performance.

The envelope style models failed to predict species distribution, achieving the worst ROC–AUC, COR and maxKappa values. They were characterized by high commission error (1 for EnvScore and 0.79 for

BIOCLIM) and, as shown in the probability maps, both approaches over-predict the training set. Envelope style approaches were initially developed to model data on terrestrial species from natural history museums and are probably unsuitable to model high resolution and density species occurrence data and to predict any habitat heterogeneity.

EnvDist and EnvDistChe performed relatively accurately in fitting the training data (ROC–AUC 0.97 for both). Only BRT and SVM-Nu achieved higher ROC–AUC, while EnvDist and EnvDistChe achieved the highest maxKappa (0.91) and relatively high COR values (0.73 and 0.83, respectively). Both models are characterized by zero omission error and EnvDistChe has almost half of the commission error of EnvDist (0.22 compared to 0.41). The zero omission error combined to the low commission error for EnvDistChe indicate that the model over-fits the training dataset and this fact is confirmed by the probability map where high probability regions are concentrated around the sampling transects. EnvDist shows a tendency to over-fit the training set, although not as much as EnvDistChe. This is also confirmed by the predictive capacity of EnvDist and EnvDistChe. Since the latter over-fits the training set, it is unable to accurately predict the independent set, presenting lower ROC–AUC, COR and maxKappa values than EnvDist. Compared to the other approaches, both models seem less effective in their predictive capacity than regression models and most of the machine learning techniques. Among methods that do not support absence data, EnvDist and EnvDistChe perform relatively well, but not as well as SVM.

ClimSpace failed to fit the training data or to predict the independent dataset. It had the worst ROC–AUC, COR and maxKappa values. Even if several ClimSpace models were developed (the best performing is presented here), none would succeed in modelling the training set. Thus, ClimSpace seems inappropriate to predict species distribution using acoustic data.

GARP shows moderate performance in both fitting efficiency and predictive capacity. Results and errors indicate that GARP corresponds well to the variables used, though the output, which reflects the environmental conditions where species could maintain populations is relatively coarse compared to the other approaches. The inability to generate more detailed species distribution maps makes GARP less efficient than the approaches that support presence–absence data, even if generally GARP's output grid is in agreement with the high probability spatial pattern that is identified by the most accurate SDMs.

The predicted grids for Thermaikos Gulf identify two distinct areas where small pelagic species are concentrated: first the west coastline from north to south and the east coastline of the Gulf, which are characterized by the presence of riverine waters, and, second, the central study area, which is related to gyre formation (Somarakis et al., 2002). These areas are characterized as nutrient-rich resulting in aggregations of small pelagic species. The areas identified are in agreement with other studies on small pelagic species (Somarakis et al., 2002; Giannoulaki et al., 2008; Tsagarakis et al., 2008). Correlations among grids are generally in agreement to correlations among predictions of SDMs on the validation dataset. Generally, equally performing SDMs generate grids that are significantly correlated, such as MARS and GAMM. Additionally, grids that generated by SDMs resulting from similar approaches are also highly correlated, such as regression models and neural networks. In order to evaluate the predicted spatial patterns of small pelagic species distribution, we refer to both these correlations among grids and to previous studies in the area.

Evaluation of models

It is well known that species distribution modelling is only as good as the data used (Hirzel & Guisan, 2002); in addition, SDM performance depends on the number of samples that is used to train the model. Different data types (e.g. abundance, presence-only data and richness) could produce different SDM rankings (Elith et al., 2006). Generally, predictions based on presence–absence data perform better than those based on presence-only data (Brotons et al., 2004), while presence–absence models generally perform better than abundance models (Francis et al., 2005). Presence-only models can perform equally well when survey coverage is evenly and widely distributed (MacLeod et al., 2008). In principle, abundance models should be more informative, however, their poor performance in practice is related to the fact that real abundance data rarely conform to standard distributions thus, violating model assumptions. The assumptions associated with presence–absence data (binary distribution) are more easily met. Additionally, the validation process for presence–absence models (ROC–AUC, Kappa, Confusion matrix) is well developed and more informative compared to the validation techniques used in abundance models (*k*-fold cross-validation, models calibration, correlation), since it is easier to interpret presence–absence (binary distribution) models than abundance models (other distributions, e.g. Gaussian, Poisson). Finally, presence–absence models make less bold predictions about species distribution and are thus less likely to be proved wrong.

Studies of presence–absence modelling methods suggest that several non-linear techniques (e.g. GAM, ANN and MARS) are comparable in terms of predictive ability and they are often superior to methods such as traditional single decision trees (Ferrier & Watson, 1997; Elith & Burgman, 2002; Moisen & Frescino, 2002; Muñoz & Fellicisimo, 2004; Segurado & Araujo, 2004). Here, the similar performances of GAM and MARS is confirmed but ANNE and, especially, ASNN show higher predictive capacity, not only compared to traditional ANN but also compared to other widely used approaches (e.g. GAMs). Elith et al. (2006) evaluated the predictions of 11 distinct models and 16 approaches that use presence-only data. They classified the models into three performance categories. The first, highest performing, group includes MARS, BRT, generalized dissimilarity (GDM and GDM-SS) and maximum entropy (MAXENT and MAXENT-T) models. A second group of methods includes most of the standard regression methods (GAM/BRUTO, GLM, MARS and GARP). A third group includes the methods that use presence-only data (BIOCLIM, DOMAIN and LIVES). This study supports the high predictive ability of BRTs and the low predictive ability of Bioclim. EnvDist performed better in the present study, compared to the study by Elith et al. (2006), probably due to the fact that this function over-fits the training data (especially EnvDistChe), according to omission and commission errors. The small difference in ROC–AUC between MARS and GAMs that was observed in this study has also been observed in other studies. In particular, Leathwick et al. (2006a, b) fitted GAM and MARS models to the distributions of fifteen freshwater fish species in relation to their environment and, based on ROC values, they found little difference in the performances of both models. The higher predictive capacity of ASNN and ANNE in models trained with abundance data is also shown in Palialexis et al. (this issue).

The uncertainty associated with SDM predictions requires attention, especially when models are developed for decision-making and management purposes. Uncertainty in SDMs results both from data deficiencies and from errors in specification of the models (Elith & Leathwick, 2009). Problems related to uncertainty are often ignored because they are difficult to deal with. However, uncertainty can be minimized by the selection of (a) functionally relevant predictors that could explain the variance of the response variable both in environmental and geographical space and (b) SDMs that incorporate complex species–environment relations and variable interactions. The 'black-box' nature of the machine learning techniques cannot be very informative of such interactions, although results indicated their high predictive capacity. There is, however, a trade-off between variation explained and model complexity.

The use of biotic interactions, related to species life history, as explanatory variables in SDMs, e.g. prey–predator relations and fishing activity could increase the variance explained of the response variable. As mentioned by Guisan & Thuiller (2005), very few studies include variables that describe biological interactions. Elith & Leathwick (2009) indicate the difficulties of utilizing biological interactions as predictors. Such variables though could complement the variation explained in environmental space and identify more complex relationships in ecological space. In practice, the variable selection process depends on (a) the availability and quality of data, (b) the ability of data to explain a quantity of the variance of the response variable, based on biological knowledge or data exploration processes and (c) the assumptions of the SDMs. The latter point could exclude use of explanatory variables that are crucial from a biological point of view. In this case other modelling approaches could be useful in order to exploit the available biological inferences. Since the aim of this study was the comparative performance of SDMs, only well known and explored data were used. Additionally, the selection of explanatory variables was contingent on the requirements of available modelling software with grid generation capabilities. Fishing activity, interspecific competition and predator–prey relationships are all likely to affect small pelagic species distribution (e.g. Ramzi et al., 2006; Sabatés et al., 2006). Such information could potentially explain a part of the variance that is not explained by the use of solely abiotic variables in cases of identification of species interactions or of the characterization of species distribution that approaches the realized habitat (Planque et al., 2007).

Most of the SDMs were able to depict the basic species distribution pattern, which is also confirmed by other studies in the area. The relatively novel SDMs provided more detailed outputs and, potentially, can indicate habitat heterogeneity with a high spatial resolution. Among SDMs that performed equally, the different explanatory variables used varied in terms of the importance of their contribution. Each modelling technique is able to explain a quantity of the variance of the response variable. Even if the proportion of the variance explained is equal for two SDMs, the part of the variance explained might differ and this is reflected by the different weights of the explanatory variables in the SDMs. Issues as the above should be considered carefully, especially when SDMs are used to improve ecological understanding, or for conservation planning and management.

Conclusions

The comparison of 13 species distribution models incorporating 15 different statistical approaches indicated that approaches belonging to Machine Learning Techniques are generally more accurate in predicting species distribution, utilizing presence–absence data, derived from predetermined sampling transects and a sufficient number of high resolution explanatory variables. In particular, BRTs outperformed the other techniques in fitting the training data, while ASNN showed remarkable predictive capacity in comparison with the other methods. SVM was the best performing technique among the approaches that do not support absence data. The aforementioned approaches did not over-fit the training dataset. Machine learning is a scientific discipline that is concerned with the design and development of algorithms that allow computers to change behaviour based on data. The evolution in computer science supports more complex data simulations and models as well as combinations of techniques that are more accurate and efficient in their performance. That is the case in ASNN, which is a combination of an ANNE and a *k*-nearest neighbour algorithm, and in BRTs, which combine the boosting algorithm and regression trees to create a regression trees ensemble. The use and the evolution of such techniques in species distribution prediction generate a new perspective of more realistic and applicable outputs, while their performance may exceed that of more conventional techniques (Elith & Leathwick, 2009).

Regression models are ranked relatively highly compared to other techniques, with respect to their fitting efficiency and predictive capacity, and flexibility in modelling several types of data. GAMs, MARS and GAMMs performed almost similarly, though GAM output was slightly better. GAMM is able to model spatial autocorrelation, which is certainly present in the training dataset used, but did not exceed the predictive capacity of GAM. This could be due to the restricted spatial autocorrelation as shown in the variogram of the training dataset and/or because the autocorrelation in the response variable was adequately explained (statistically at least) by autocorrelation in the geographical and environmental predictors (Elith & Leathwick, 2009). As a note of caution, it should not be assumed that this will always be the case. Nevertheless, regarding regression models, it is suggested to use GAM for species distribution predictions or MARS as a more user-friendly approach.

When absence data are available, the loss of information in presence-only models affects their fitting efficiency and predictive capacity. In this study, approaches like BIOCLIM, EnvScore, ClimSpace and MAXENT failed to generate competitive outputs as compared to the other approaches. EnvDist was the only exception, although there are indications that this approach tends to over-fit the training data.

Generally, the fitting efficiency and the predictive capacity that characterize a model are strongly depended on the quality of the training data. In presence–absence data, derived from predetermined sampling transects that were modelled with high resolution environmental satellite and geographic data, BRTs and ASNN are suggested as the most appropriate techniques. Machine learning approaches, with their extensive analytical capabilities, could be useful tools for species distribution predictions. However, different study cases and datasets might require different approaches.

References

Aertsen, W., V. Kint, J. van Orshoven, K. Őzkan & B. Muys, 2010. Comparison and ranking of different modelling techniques for prediction of site index in Mediterranean mountain forests. Ecological Modelling 221: 1119–1130.

Akaike, H., 1974. A new look at the statistical model identification. IEEE Transactions on Automatic Control 19: 716–723.

Amara, R., K. Mahé, O. LePape & N. Desroy, 2004. Growth, feeding and distribution of the solenette *Buglossidium luteum* with particular reference to its habitat preference. Journal of Sea Research 51: 211–217.

Bakun, A., 2001. 'School-mix feedback': a different way to think about low frequency variability in large mobile fish populations. Progress in Oceanography 49: 485–511.

Bishop, M., 1995. Neural Networks for Pattern Recognition. Oxford University Press, Oxford.

Bodholt, H., H. Nes & H. Solli, 1989. A new echo sounder system. Proceedings of the Institute of Acoustics 11(3): 123–130.

Boyce, M. S., P. R. Vernier, S. E. Nielsen & F. K. A. Schmiegelow, 2002. Evaluating resource selection functions. Ecological Modelling 157: 281–300.

Brotons, L., W. Thuiller, M. B. Araujo & A. H. Hirzel, 2004. Presence–absence versus presence-only modelling methods for predicting bird habitat suitability. Ecography 27: 437–448.

Burman, P., E. Chow & D. Nolan, 1994. A cross-validatory method for dependent data. Biometrika 81(2): 351–358.

Busby, J. R., 1991. BIOCLIM—a bioclimate analysis and prediction system. In Margules, C. R. & M. P. Austin (eds), Nature Conservation: Cost Effective Biological Surveys and Data Analysis. CSIRO, Australia: 64–68.

Carpenter, G., A. N. Gillison & J. Winter, 1993. DOMAIN: a flexible modeling procedure for mapping potential distributions of animals and plants. Biodiversity and Conservation 2: 667–680.

Caruana, R. & A. Niculescu-Mizil, 2006. An empirical comparison of supervised learning algorithms. Proceedings of International Conference on Machine Learning, 23rd, Pittsburgh, PA.

Cohen, J., 1960. A coefficient of agreement for nominal scales. Educational and Psychological Measurement 20: 37–46.

Cristianini, N. & J. Shawe-Taylor, 2000. An Introduction to Support Vector Machines and Other Kernel-based Learning Methods. Cambridge University Press, London.

Daskalov, G. M., D. C. Boyer & J. P. Roux, 2003. Relating sardine *Sardinops sagax* abundance to environmental indices in northern Benguela. Progress in Oceanography 59: 257–274.

Dormann, C. F., J. M. McPherson, M. B. Araujo, R. Bivand & J. Bolliger, 2007. Methods to account for spatial autocorrelation in the analysis of species distributional data: a review. Ecography 30: 609–628.

Dray, S., P. Legendre & P. R. Peres-Neto, 2006. Spatial modeling: a comprehensive framework for principal coordinate analysis of neighbor matrices (PCNM). Ecological Modelling 196: 483–493.

Elith, J. & M. A. Burgman, 2002. Predictions and their validation: rare plants in the Central Highlands, Victoria, Australia. In Scott, J. M. (ed.), Predicting Species Occurrences: Issues of Accuracy and Scale. Island Press, Washington, DC: 303–314.

Elith, J. & J. R. Leathwick, 2009. Species distribution models: ecological explanation and prediction across space and time. Annual Review of Ecology, Evolution, and Systematics 40: 677–697.

Elith, J., C. H. Graham, R. P. Anderson, M. Dudik, S. Ferrier, A. Guisan, R. J. Hijmans, F. Huettmann, J. R. Leathwick, A. Lehmann, J. Li, L. G. Lohmann, B. A. Loiselle, G. Manion, C. Moritz, M. Nakamura, Y. Nakazawa, J. Mc, C. Overton, A. T. Peterson, S. J. Phillips, K. S. Richardson, R. Scachetti-Pereira, R. E. Schapire, J. Soberon, S. Williams, M. S. Wisz & N. E. Zimmermann, 2006. Novel methods improve prediction of species' distributions from occurrence data. Ecography 29: 129–151.

Ferrier, S. & G. Watson, 1997. An evaluation of the effectiveness of environmental surrogates and modelling techniques in predicting the distribution of biological diversity. Environment Australia, Canberra [avialable on internet at http://www.deh.gov.au/biodiversity/publications/technical/surrogates/].

Ferrier, M. D., G. Manion & G. Watson, 2002. Extended statistical approaches to modelling spatial pattern in biodiversity: the north-east New SouthWales experience. I. Species-level modelling. Biodiversity and Conservation 11: 2275–2307.

Fielding, A. H. & J. F. Bell, 1997. A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation 24: 38–49.

Francis, M. P., M. A. Morrison, J. Leathwick, C. Walsh & C. Middleton, 2005. Predictive models of small fish presence and abundance in northern New Zealand harbours. Estuarine, Coastal and Shelf Science 64: 419–435.

Friedman, J. H., 1991. Multivariate adaptive regression splines. Annals of Statistics 19: 1–141.

Friedman, J. H. & J. J. Meulman, 2003. Multiple adaptive regression trees with application in epidemiology. Statistics in Medicine 22: 1365–1381.

Fu, J. W., R. J. Carroll & S. Wang, 2005. Estimating misclassification error with small samples via bootstrap cross-validation. Bioinformatics 21: 1979–1986.

Garcìa, A. & I. Palomera, 1996. Anchovy early life history and its relation to its surrounding environment in the Western Mediterranean basin. Scientia Marina 60(2): 155–166.

Georgakarakos, S. & D. Kitsiou, 2008. Mapping abundance distribution of small pelagic species applying hydroacoustics and co-kriging techniques. Hydrobiologia 612(1): 155–169.

Giannoulaki, M., V. D. Valavanis, A. Palialexis, K. Tsagarakis, A. Machias, S. Somarakis & C. Papaconstantinou, 2008. Modelling the presence of anchovy *Engraulis encrasicolus* in the Aegean Sea during early summer, based on satellite environmental data. Hydrobiologia 612(1): 225–240.

Gower, J. C. & P. Legendre, 1986. Metric and Euclidean properties of dissimilarity coefficients. Journal of Classification 3(1): 5–48.

Graham, C. H., C. Moritz & S. E. Williams, 2006. Habitat history improves prediction of biodiversity in a rainforest fauna. Proceedings of the National Academy of Sciences USA 103: 632–636.

Gudrun, C. & I. Kühn, 2008. Analyzing spatial ecological data using linear regression and wavelet analysis. Stochastic Environmental Research and Risk Assessment 22: 315–324.

Guisan, A. & W. Thuiller, 2005. Predicting species distribution: offering more than simple habitat models. Ecological Letters 8: 993–1009.

Guisan, A. & N. E. Zimmermann, 2000. Predictive habitat distribution models in ecology. Ecological Modelling 135(2–3): 147–186.

Hastie, T. & R. Tibshirani, 1990. Generalized Additive Models. Chapman and Hall, London.

Hastie, T. J., R. Tibshirani & A. Buja, 1994. Flexible discriminant analysis by optimal scoring. JASA 89: 1255–1270.

Hastie, T., R. Tibshirani & J. Friedman, 2009. The Elements of Statistical Learning: Data Mining, Inference, and Prediction (Springer Series in Statistics), 2nd ed. Springer, Berlin.

Hirzel, A. H. & A. Guisan, 2002. Which is the optimal sampling strategy for habitat suitability modelling. Ecological Modelling 157: 331–341.

Holland, J. H., 1975. Adaptation in Natural and Artificial Systems. University of Michigan Press, Ann Arbor.

Jaberg, C. & A. Guisan, 2001. Modelling the distribution of bats in relation to landscape structure in a temperate mountain environment. Journal of Applied Ecology 38: 1169–1181.

Jaynes, E. T., 1957. Information theory and statistical mechanics. Physics Revisions 106: 620–630.

Leathwick, J. R., D. Rowe, J. Richardson, J. Elith & T. Hastie, 2005. Using multivariate adaptive regression splines to predict the distributions of New Zealand's freshwater diadromous fish. Freshwater Biology 50: 2034–2052.

Leathwick, J. R., J. Elith, M. P. Francis, T. Hastie & P. Taylor, 2006a. Variation in demersal fish species richness in the oceans surrounding New Zealand: an analysis using boosted regression trees. Marine Ecology Progress Series 321: 267–281.

Leathwick, J. R., J. Elith & T. Hastie, 2006b. Comparative performance of generalized additive models and multivariate adaptive regression splines for statistical modelling of species distributions. Ecological Modelling 199: 188–196.

Lefkaditou, E., C.-Y. Politou, A. Palialexis, J. Dokos, P. Cosmopoulos & V. D. Valavanis, 2008. Influences of environmental variability on the population structure and distribution patterns of the short-fin squid *Illex coindetii* (Cephalopoda: Ommastrephidae) in the Eastern Ionian Sea. Hydrobiologia 612(1): 71–90.

Legendre, P., 1993. Spatial autocorrelation: trouble or new paradigm? Ecology 74: 1659–1673.

Legendre, P. & L. Legendre, 1998. Numerical Ecology, 2nd English Edition. Elsevier Science BV, Amsterdam.

Lehmann, A., J. M. C. Overton & J. R. Leathwick, 2002. GRASP: generalized regression analysis and spatial prediction. Ecological Modelling 157: 189–207.

Liu, C., P. M. Berry, T. P. Dawson & R. G. Pearson, 2005. Selecting thresholds of occurrence in the prediction of species distributions. Ecography 28: 385–393.

MacCall, A. D., 1990. Dynamic Geography of Marine Fish Populations. University of Washington Press, Seattle: 153.

MacLennan, D. N., P. G. Fernandes & J. Dalen, 2002. A consistent approach to definitions and symbols in fisheries acoustics. ICES Journal of Marine Science 59: 365–369.

MacLeod, D., C. L. Mandleberg, C. Schweder, S. M. Bannon & G. J. Pierce, 2008. A comparison of approaches for modelling the occurrence of marine animals. Hydrobiologia 612(1): 21–32.

Martin, P., N. Bahamon, A. Sabates, F. Maynou, P. Sanchez & M. Demestre, 2008. European anchovy (*Engraulis encrasicolus*) landings and environmental conditions on the Catalan Coast (NW Mediterranean) during 2000–2005. Hydrobiologia 612(1): 185–199.

Matheron, G., 1971. The Theory of Regionalized Variables and its Applications. Ecole Nationale Supe′rieure des Mines de Paris, Fontainebleau.

Moguerza, J. & A. Muñoz, 2006. Support vector machines with applications. Statistical Science 21(3): 322–336.

Moisen, G. G. & T. S. Frescino, 2002. Comparing five modelling techniques for predicting forest characteristics. Ecological Modelling 157: 209–225.

Moran, P. A. P., 1950. Notes on continuous stochastic phenomena. Biometrika 37: 17–23.

Morrison, M. L., B. G. Marcot & R. W. Mannan, 1992. Wildlife Habitat Relationships: Concepts and Applications. University Wisconsin Press, Madison, WI: 341.

Muiño, R., P. Carrera, P. Petitgas, D. J. Beare, S. Georgakarakos, J. Haralambous, M. Iglesias, B. Liorzou, J. Masse & D. G. Reid, 2003. Consistency in the correlation of school parameters across years and stocks. ICES Journal of Marine Science 60: 164–175.

Muñoz, M. E. S., R. Giovanni, M. F. Siqueira, T. Sutton, P. Brewer, R. S. Pereira, D. A. L. Canhos & V. P. Canhos, 2009. openModeller: a generic approach to species' potential distribution modelling. GeoInformatica. doi: 10.1007/s10707-009-0090-7.

Muñoz, J. & A. M. Fellicisimo, 2004. Comparison of statistical methods commonly used in predictive modeling. Journal of Vegetation Science 15: 285–292.

Murphy, A. H. & R. L. Winkler, 1992. Diagnostic verification of probability forecasts. International Journal of Forecasting 7: 435–455.

Nix, H. A., 1986. A biogeographic analysis of Australian elapid snakes. In Longmore, R. (ed.), Atlas of Elapid Snakes of Australia (Australian Flora and Fauna Series 7). Australian Government Publishing Service, Canberra: 4–15.

Olivier, F. & S. J. Wotherspoon, 2005. GIS-based application of resource selection functions to the prediction of snow petrel distribution and abundance in East Antarctica: comparing models at multiple scales. Ecological Modelling 189: 105–129.

Ostrom, E., 1990. Governing the Commons: The Evolution of Institutions for Collective Action. Cambridge University Press, New York.

Palialexis, A., S. Georgakarakos, K. Lika & V. D. Valavanis, 2009. Comparing novel approaches used for prediction of species distribution from presence/absence acoustic data. Proceedings of the Second International Conference on Environmental Management, Engineering, Planning and

Economics (CEMEPE 09), June 21–26, 2009, Mykonos, Greece.

Palialexis, A., S. Georgakarakos, I. Karakassis, K. Lika & V. D. Valavanis, this issue. Fish distribution predictions from different points of view: comparing associative neural networks, geostatistics and regression models. doi: 10.1007/s10750-011-0676-6.

Petitgas, P., D. Reid, P. Carrera, M. Iglesias, S. Georgakarakos, B. Liorzou & J. Masse, 2001. On the relation between schools, clusters of schools, and abundance in pelagic fish stocks. ICES Journal of Marine Research 58: 1150–1160.

Phillips, S. J., M. Dudik & R. E. Schapire, 2004. A maximum entropy approach to species distribution modeling. Proceedings of the Twenty-First International Conference on Machine Learning: 655–662.

Phillips, S. J., R. P. Anderson & R. E. Schapire, 2006. Maximum entropy modelling of species geographic distributions. Ecological Modelling 190: 231–259.

Piñeiro, R., J. F. Aguilar, D. D. Munt & G. N. Feliner, 2007. Ecology matters: Atlantic-Mediterranean disjunction in the sand-dune shrub *Armeria pungens* (Plumbaginaceae). Molecular Ecology 16: 2155–2171.

Planque, B., E. Bellier & P. Lazure, 2007. Modelling potential spawning habitat of sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) in the Bay of Biscay. Fisheries Oceanography 16(1): 16–30.

Poulos, S. E., G. T. Chronis, M. B. Collins & V. Lykousis, 2000. Thermaikos Gulf Coastal System, NW Aegean Sea: an overview of water/sediment fluxes in relation to air-land-ocean interactions and human activities. Journal of Marine Systems 25: 47–76.

R Development Core Team, 2005. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria [available on internet at http://www.Rproject.org].

Racine, J., 2000. Consistent cross-validatory model-selection for dependent data: hv-block cross-validation. Journal of Econometrics 99: 39–61.

Ramzi, A., My. L. Hbid & O. Ettahiri, 2006. Larval dynamics and recruitment modelling of the Moroccan Atlantic coast sardine (*Sardina pilchardus*). Ecological Modelling 197: 296–302.

Ready, J., K. Kaschner, A. B. South, P. D. Eastwood, T. Rees, J. Rius, E. Agbayani, S. Kullander & R. Froese, 2010. Predicting the distributions of marine organisms at the global scale. Ecological Modelling 221(3): 467–478.

Redfern, J. V., M. C. Ferguson, E. A. Becker, K. D. Hyrenbach, C. Good, J. Barlow, K. Kaschner, M. F. Baumgartner, K. A. Forney, L. T. Ballance, P. Fauchald, P. Halpin, T. Hamazaki, A. J. Pershing, S. S. Qian, A. Read, S. B. Reilly, L. Torres & F. Werner, 2006. Techniques for cetacean-habitat modelling. Marine Ecology Progress Series 310: 271–295.

Richards, C. L., B. C. Carstens & L. Knowles, 2007. Distribution modelling and statistical phylogeography: an integrative framework for generating and testing alternative biogeographical hypotheses. Journal of Biogeography 34: 1833–1845.

Rossi, R. E., D. J. Mula, A. G. Journel & E. H. Franz, 1992. Geostatistical tools for modeling and interpreting ecological spatial dependence. Ecological Monographs 2: 277–314.

Ruiz, J., E. Garcia-Isarch, I. E. Huertas, L. Prieto, A. Juárez, J. L. Munõz, A. Sánchez-Lamadrid, S. Rodrìguez-Gálvez, J. M. Naranjo & F. Baldó, 2006. Meteorological and oceanographic factors influencing *Engraulis encrasicolus* early life stages and catches in the Gulf of Cádiz. Deep-Sea Research II 53: 1363–1376.

Sabatés, A., P. Martìn, J. Lloret & V. Raya, 2006. Sea warming and fish distribution: the case of the small pelagic fish, *Sardinella aurita*, in the western Mediterranean. Global Change Biology 12: 2209–2219.

Santos, A. M. P., A. Peliz, J. Dubert, P. B. Oliveira, M. M. Angélico & P. Ré, 2004. Impact of a winter upwelling event on the distribution and transport of sardine (*Sardina pilchardus*) eggs and larvae off western Iberia: a retention mechanism. Continental Shelf Research 24: 149–165.

Schölkopf, B., A. Smola, R. Williamson & P. L. Bartlett, 2000. New support vector algorithms. Neural Computation 12: 1207–1245.

Schröder, B., 2008. Challenges of species distribution modeling belowground. Journal of Plant Nutrition and Soil Science 171: 325–337.

Segurado, P. & M. B. Araujo, 2004. An evaluation of methods for modelling species distributions. Journal of Biogeography 31: 1555–1568.

Siapatis, A., M. Giannoulaki, V. D. Valavanis, A. Palialexis, E. Schismenou, A. Machias & S. Somarakis, 2008. Modelling potential habitat of the invasive ctenophore *Mnemiopsis leidyi* in Aegean Sea. Hydrobiologia 612(1): 281–295.

Simmonds, E. J. & D. N. MacLennan, 2005. Fisheries Acoustics: Theory and Practice. Blackwell Science Ltd, Oxford.

Simon, R., M. D. Radmacher, K. Dobbin & L. M. McShane, 2003. Pitfalls in the use of DNA microarray data for diagnostic and prognostic classification. Journal of the National Cancer Institute 95(1): 14–18.

Somarakis, S., P. Drakopoulos & V. Filippou, 2002. Distribution and abundance of larval fishes in the northern Aegean Sea—eastern Mediterranean—in relation to early summer oceanographic conditions. Journal of Plankton Research 24: 339–357.

Stergiou, I. K. & A. Lascaratos, 1997. Climatic variability and the anchovy/sardine ratio in Hellenic waters. GeoJournal 41(3): 245–254.

Stockwell, D. R. B., 1999. Genetic algorithms II. In Fielding, A. H. (ed.), Machine Learning Methods for Ecological Applications. Kluwer Academic Publishers, Boston: 123–144.

Stockwell, D. & D. Peters, 1999. The GARP modelling system: problems and solutions to automated spatial prediction. International Journal of Geographical Information Science 13: 143–158.

Tetko, I. V., 2002a. Associative neural network. Neural Processing Letters 16: 187–199.

Tetko, I. V., 2002b. Neural network studies. Introduction to associative neural networks. Journal of Chemical Information and Modeling 42: 717–728.

Tetko, I. V. & V. Y. Tanchuk, 2002. Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. Journal of Chemical Information and Modeling 42: 1136–1145.

Tetko, I. V., D. J. Livingstone & A. I. Luik, 1995. Neural network studies. Comparison of overfitting and overtraining. Journal of Chemical Information and Modeling 5: 826–833.

Tetko, I. V., I. Sushko, A. K. Pandey, H. Zhu, A. Tropsha, E. Papa, T. Oberg, R. Todeschini, D. Fourches & A. Varnek, 2008. Critical assessment of QSAR models of environmental toxicity against *Tetrahymena pyriformis*: focusing on applicability domain and overfitting by variable selection. Journal of Chemical Information and Modeling 48(9): 1733–1746.

Tsagarakis, K., A. Machias, S. Somarakis, M. Giannoulaki, A. Palialexis & V. D. Valavanis, 2008. Habitat discrimination of juvenile sardines in the Aegean Sea using remotely sensed environmental data. Hydrobiologia 612(1): 215–223.

Tychonoff, A. N. & V. Y. Arsenin, 1977. Solution of Ill-posed Problems. Winston and Sons, Washington. ISBN:0-470-99124-0.Valavanis, V. D., S. Georgakarakos, A. Kapantagakis, A. Palialexis & I. Katara, 2004. A GIS environmental modelling approach to Essential Fish Habitat Designation. Ecological Modelling 178: 417–427.

Valavanis V. D., A. Kapantagakis, I. Katara & A. Palialexis, 2004. Critical regions: A GIS-based model of marine productivity hotspots. Aquatic Sciences 66(1): 139–148.

Valavanis, V. D., G. J. Pierce, A. F. Zuur, A. Palialexis, A. Saveliev, I. Katara & J. Wang, 2008. Modelling of essential fish habitat based on remote sensing, spatial analysis and GIS. Hydrobiologia 612(1): 5–20.

Vapnik, V., 1995. The Nature of Statistical Learning Theory. SpringerVerlag, New York.

Walline, P. D., 2007. Geostatistical simulations of eastern Bering Sea walleye pollock spatial distributions, to estimate sampling precision. ICES Journal of Marine Science 64: 559–569.

Wood, S. N., 2006. Generalized Additive Models: An Introduction with R. Chapman & Hall/CRC Press, Boca Raton.

Wood, S. N., 2008. Fast stable direct fitting and smoothness selection for generalized additive models. Journal of the Royal Statistical Society: Series B 70(3): 495–518.

Wood, S. N. & N. H. Augustin, 2002. GAMs with integrated model selection using penalized regression splines and applications to environmental modelling. Ecological Modelling 157: 157–177.

Zaniewski, A. E., A. Lehman & J. Overton, 2002. Predicting species spatial distributions using presence-only data: a case study of native New Zealand ferns. Ecological Modelling 157: 261–280.

Zheng, B. & A. Agresti, 2000. Summarizing the predictive power of a generalized linear model. Statistics in Medicine 19: 1771–1781.

Hydrobiologia (2011) 670:267–274
DOI 10.1007/s10750-011-0667-7

ECOSYSTEMS AND SUSTAINABILITY

Spatial management of the Mediterranean bottom-trawl fisheries: the case of the southern Aegean Sea

George Tserpes · Evangelos Tzanatos ·
Panagiota Peristeraki

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract A time series of survey abundance indices for commercially important demersal fish and cephalopod species, inhabiting the narrow continental shelf of the southern Aegean Sea, is analyzed in relation to the topography of the area in order to evaluate the impact of different spatial fishery bans on the bottom-trawl fishery. With reference to the current situation, results suggested that implementation of the 1967/2006 EC Regulation, which bans bottom-trawl activities within 1.5 NM off the coast, will significantly increase (20–80%, depending on the species) the proportion of the populations that are inaccessible to the bottom-trawl fishery. It might also result in shifting of fishing activities toward deeper waters, adding fishing pressure onto slope resources inhabiting the slope. As depth determines, to a large extent, the distribution pattern of the species, it constitutes a variable of crucial importance for the spatial management of marine fisheries and should be taken into account when adopting relevant management regimes.

Keywords Mediterranean · Demersal fish · Bottom-trawl fisheries · Management · Distribution

Introduction

Management of all Mediterranean stocks, apart from bluefin tuna, is exclusively based on various effort control regimes. Direct regulation of effort is achieved through a licensing system allowing fishing in certain areas and seasons for particular vessels and gears, as well as through restrictions on the fishing capacity of licensed vessels (vessel tonnage and engine power or gear size restrictions). Direct effort regulation is typically accompanied by methods of indirect effort control, including various technical measures and management actions. Examples of such methods are closed areas and seasons, which indirectly restrict fishery input, gear restrictions such as mesh size, and minimum landing size regulations. In fact, indirect methods control the catch that can be achieved for a given effort (Nielsen et al., 2006).

In the case of bottom-trawl fisheries in Mediterranean EU waters effort control management is implemented through national and international regulations, the latter established in the frames of the Common Fisheries Policy (CFP). Apart from capacity restriction rules, i.e., limitation on national fleets horsepower and gross tonnage, the CFP controls effort through a series of technical measures and

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

G. Tserpes (✉) · E. Tzanatos · P. Peristeraki
Hellenic Centre for Marine Research, PO Box 2214,
71003 Heraklion, Greece
e-mail: gtserpes@her.hcmr.gr

management actions including: (a) prohibition of bottom trawling either within three miles of the coast or in depths less than 50 m (whatever comes first), as well as, on sensitive habitats (e.g. *Posidonia* and maerl beds), (b) a minimum mesh size of 40 mm for bottom-trawls cod-end, and (c) minimum landing sizes for a series of commercial species.

Bottom-trawling limitations in depths less than 50 m aim to protect the most shallow part of the continental shelf, which is an area under the direct influence of many physical processes and also much affected by human activities (Kaiser et al., 2005; Halpern et al., 2008). Due to its high productivity, most of the world fisheries production originates from the continental shelf zone, despite its relatively low global percentage (~8%), with reference to the total marine area (Pauly & Christensen, 1995). In the Mediterranean Sea, the vast majority of the fishing activities are concentrated along the continental shelf, although the extent of this zone on the basin is relatively narrow (Caddy, 1993; Lleonart & Maynou, 2003). This results in serious concerns about overfishing of the shelf-associated resources and destruction of their habitats (Caddy, 1993; Caddy et al., 1998). It is obvious that regulations controlling fishing activities on the continental shelf zone incorporate various elements of the marine environment and have a broader effect than managing single commercial stocks, encompassing the context of the ecosystem approach to fisheries.

A recent EU Regulation (1967/2006) attempts to strengthen the protection of shelf areas, and, apart from banning fishing activities above coralligenous habitats and maerl beds, is calling for an additional measure that includes prohibition of bottom trawling to a distance of 1.5 NM from the coast, independently of depth. It is, however, recognized that this prohibition may be harmful for fisheries operating in areas with a narrow continental shelf, as it would restrict their fishing activities to deeper waters prohibiting access to important fishery resources inhabiting more shallow continental areas; i.e., depths less than 150–200 m. For this reason, the EU countries have been asked to justify local exceptions from the banning measure, based on the amount of resources and income that will be lost if the regulation is applied.

In the case of Greece, bottom trawling within one mile off the coast is already prohibited by national legislation making a large part of the coastal fisheries resources inaccessible to the gear. It is expected that the 1.5 NM ban will significantly restrict bottom-trawl activities, especially in areas with a particularly narrow continental shelf such as the southern part of the Aegean Sea.

In the present study, based on a time series of survey data, we attempt to evaluate the effects on the volume and composition of bottom-trawl catches, resulting from the application of the new EU regulation on the southern Aegean Sea. We focus on a list of commercially important continental shelf species and, based on their depth distribution pattern and the topography of the area, try to evaluate the amount of the resource that is/will be inaccessible to the trawl fishery under different management scenarios.

Materials and methods

The depth distribution pattern of the main target species of the bottom-trawl fishery operating along the continental shelf of the southern Aegean Sea (Fig. 1) was identified through the analysis of a time series of survey abundance indices. The list of species included: *Mullus surmuletus*, *Diplodus annularis*, *Mullus barbatus*, *Eledone moschata*, *Loligo vulgaris*, *Octopus vulgaris*, *Pagellus erythrinus*, *Spicara flexuosa*, *Spicara smaris*, *Sepia officinalis*, and *Boops boops*. These species comprise the main bulk of trawl catches in the examined area (Tsimenides et al., 1991; Tserpes et al., 1999; Tserpes & Peristeraki, 2002) and they are also the main target species for most Mediterranean coastal trawl fisheries (Papaconstantinou & Farrugio, 2000).

Abundance indices were obtained from the “MEDITS” experimental bottom-trawl surveys carried out during the 1996–2006 period in the southern Aegean Sea. The survey covers a large part of the Mediterranean basin mainly aiming to monitor abundance fluctuations of demersal species and includes annual sampling at pre-defined stations, accomplished from late spring to middle summer. Further details on the sampling protocol can be found elsewhere (Bertrand et al., 2000, 2002). Since 1996 (with the exception of 2002, when the survey was not accomplished), the sampling scheme of the Greek “MEDITS” in the southern Aegean Sea covers a total

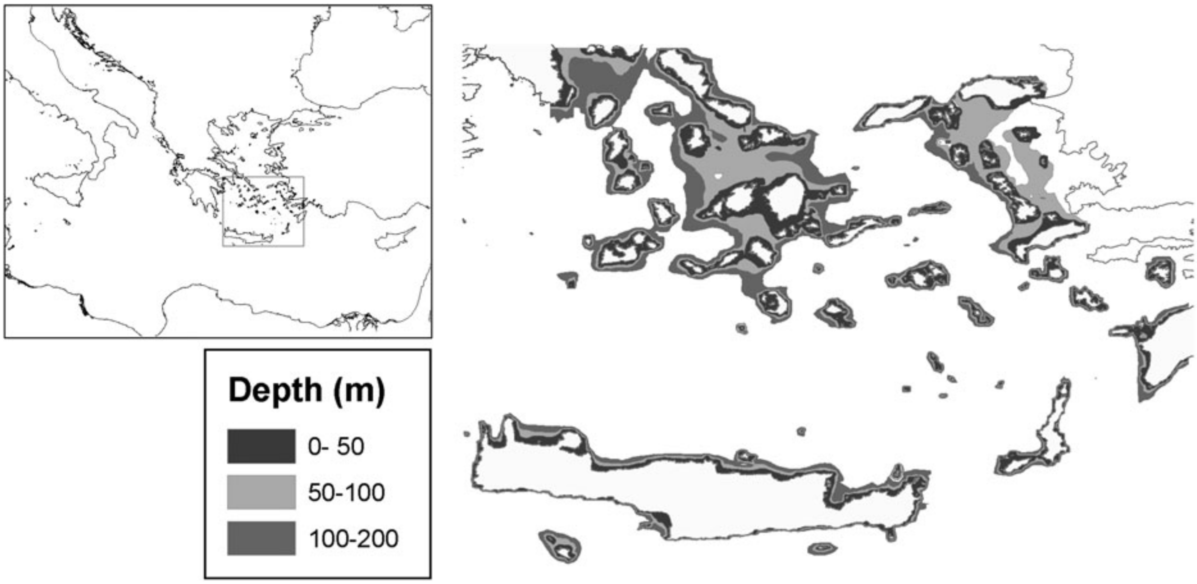


Fig. 1 Map of the examined area

of 60 stations distributed over the studied area. The data used for the purpose of this study comprise abundance indices by station, expressed in terms of kg per square km of swept area (kg/km^2).

For each species, the effect of depth on abundance was examined by means of Generalized Additive models (GAMs). GAMs, which are able to deal with non-linear relationships between a dependent variable and multiple predictors in the same model, are non-parametric generalizations of multiple linear regression that are less restrictive in assumptions about the underlying distribution of data (Hastie & Tibshirani, 1990). In GAMs, a pre-defined link function is related to predictor variables by scatterplot smoothers in lieu of least-squares fits. Based on the diagnostic residual plots of preliminary runs, we assumed a Gamma error structure model accompanied by a log-link function. The zero values have been excluded from the analysis, as their relatively high number did not allow proper modeling of the observed abundance variations. Apart from depth, the sampling year was also modeled to account for temporal fluctuations in stock abundance. Model fitting was accomplished by means of the “mgcv” package (Wood, 2004) under the R language environment (R Development Core Team, 2008) and statistical inference was based on the 95% confidence level.

At a next step, the relative (%) abundance by species and depth stratum (0–50, 50–100, 100–200, and >200 m) was estimated by grouping the GAM predicted estimates of abundance at depth. In addition, Geographic Information System (GIS) techniques were used to calculate the area within the zones defined by the 1.0 and 1.5 mile boundaries, respectively, for each depth stratum. Based on the above, the relative species abundance and thus the amount of resources available to the fishery under different management regimes was estimated by zone.

Results

GAM analysis revealed that the effect of depth was statistically significant for all species apart from *D. annularis*, while the effect of year was significant only in the case of *L. vulgaris* (Table 1). Plots of the standardized effect of depth on abundance indicated that the abundance of *M. barbatus* and *M. surmuletus* decreased linearly with increasing depth (smoothers were considered unnecessary by the “mgcv” algorithm and the GAM model dropped down to a linear model), while a rather monotonic pattern of decreasing abundance with depth was found for *L. vulgaris*,

Table 1 Analysis of deviance table for the applied GAMs by species

Species	Effect of year			Effect of depth			Deviance explained by the model
	df	*F*	*P*	df	*F*	*P*	
Boops boops	1	1.208	0.273	6.832	15.454	<0.001*	0.372
Diplodus annularis	1	2.578	0.115	1	2.857	0.098	0.079
Eledone moschata	1	0.058	0.810	8.378	12.161	<0.001*	0.349
Loligo vulgaris	3.223	2.138	0.047*	1.921	4.878	0.001*	0.192
Mullus barbatus	1.303	0.514	0.673	1	68.222	<0.001*	0.222
Mullus surmuletus	1.641	1.051	0.382	1.062	12.747	<0.001*	0.137
Octopus vulgaris	3.262	1.528	0.163	4.254	4.582	<0.001*	0.250
Pagellus erythrinus	1	3.374	0.068	8.725	7.235	<0.001*	0.274
Sepia officinalis	2.608	1.777	0.114	5.908	4.003	<0.001*	0.377
Spicara flexuosa	1.693	1.045	0.387	4.161	8.240	<0.001*	0.318
Spicara smaris	2.775	2.132	0.051	4.783	11.058	<0.001*	0.27

Asterisks indicate significant effects at the 0.05 confidence level

O. vulgaris, *S. smaris*, and *S. flexuosa*. The rest of the species had more complex polymodal depth distribution patterns (Fig. 2).

The estimates of relative species abundance by depth stratum revealed that the largest part (75–100%, depending on the species) of the populations of the examined species inhabit the continental shelf zone (0–200 m). Moreover, a significant part appears in more shallow depths, with percentages ranging from 14 to 30% and from 22 to 55% for the 0–50 and 50–100 m depth strata, respectively, (Table 2).

The GIS analysis of the topography of the area revealed that an increase of the trawling ban up to 1.5 NM from the coast would reduce by 22.41 and 11.12% the trawlable area of the 50–100 and 100–200 m depth strata, respectively, with reference to the current situation (Table 3). Based on this, and the estimated allocation of species by depth stratum (Table 2), the proportion of the resources not available to the fishery under different management regimes was calculated, assuming homogeneous species distribution in a given depth stratum (Table 4). Under the present management status (bottom-trawl fishing prohibition in depths less than 50 m and within 1 NM from the coast), more than 25% of the population is not accessible to the bottom trawlers for the majority of the species. With the additional 0.5 NM fishery ban, this amount increases by 20–80%, resulting in a total of 30–45% of inaccessible populations, depending on the species.

Discussion

It is well documented in the literature that depth plays an important role in determining the distribution and abundance patterns of marine populations (Macpherson, 2003; Reynolds, 2003; Katsanevakis & Maravelias, 2009), and depth is also a crucial driver for the composition of marine assemblages (Moranta et al., 1998; Tserpes et al., 1999; Demestre et al., 2000; Magnussen, 2002; Gaertner et al., 2005; Juan-Jordá et al., 2009). In line with those findings, our results clearly demonstrate the existence of statistically significant relationships between depth and abundance for all examined species apart from *D. annularis*, which is a species highly associated to the coastal zone and in the present case was not found in depths greater than 30 m. Taking into account that our sampling covered a depth range of 20–800 m, this explains why the depth-abundance relationship for this species was found not to be significant. For several commercially important species (e.g. *M. barbatus*, *P. erythrinus*, *O. vulgaris*, etc.), a large part of the population (over 50%) is found in depths up to 100 m, indicating that shallow waters of the continental zone are very important for fisheries targeting those species.

As depth determines, to a large extent, the distribution pattern of the species, it constitutes a variable of crucial importance for the spatial management of marine fisheries and should be taken into account when adopting relevant management schemes. In the case of areas such as the southern

Fig. 2 GAM derived standardized effect of depth on species abundance. The relative density of data points is shown by the "rug" on the *x*-axis

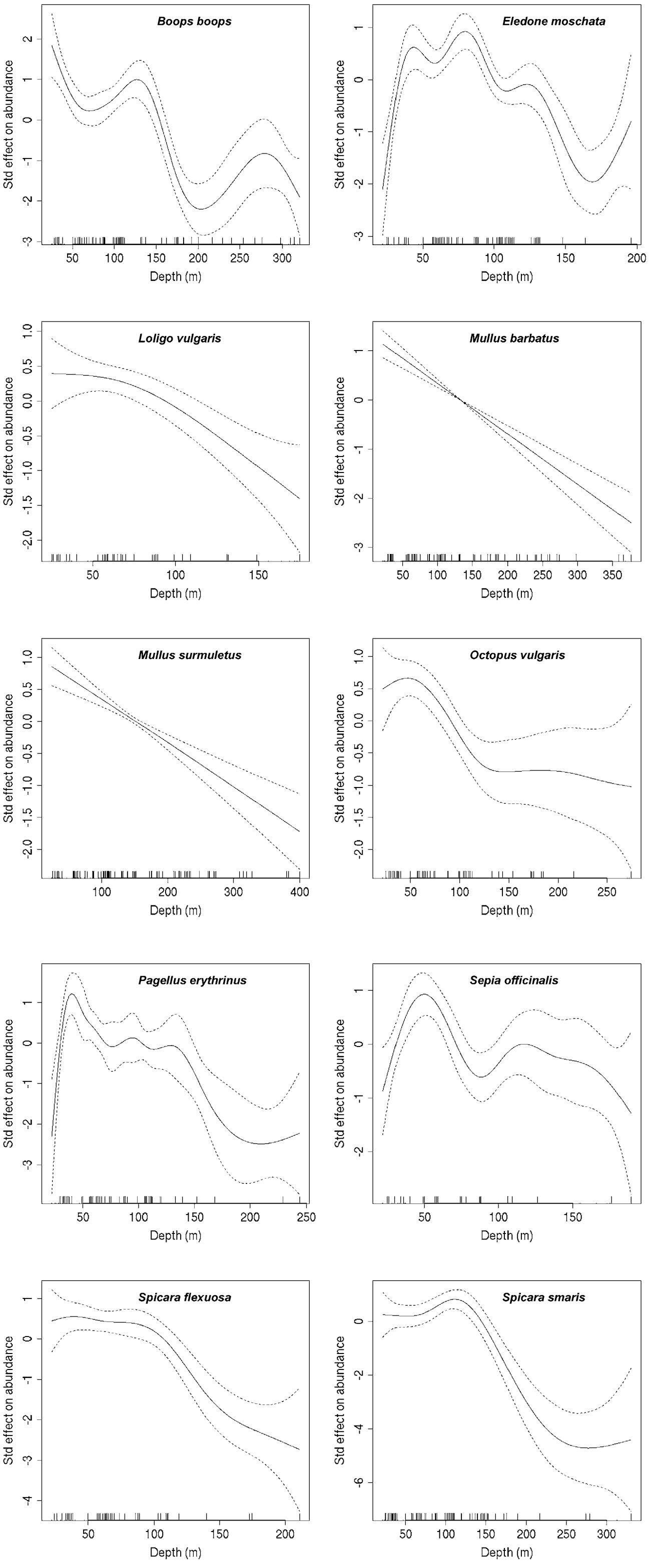


Table 2 Relative (%) GAM abundance estimates of the examined species by depth stratum

Species	Abundance (%)			
	0–50 m	50–100 m	100–200 m	>200 m
Boops boops	29.93 (8.46–51.40)	22.25 (13.30–31.20)	38.22 (19.55–56.89)	9.6 (1.92–17.38)
Eledone moschata	13.78 (8.11–19.45)	54.66 (35.14–74.17)	31.56 (17.83–50.71)	–
Loligo vulgaris	26.75 (7.43–46.08)	44.34 (15.89–72.79)	28.91 (7.66–50.15)	–
Mullus barbatus	23.25 (15.25–31.25)	31.92 (22.65–41.19)	30.57 (22.84–38.30)	14.26 (8.60–19.91)
Mullus surmuletus	16.92 (10.69–23.14)	26.38 (18.08–34.68)	32.29 (23.46–41.11)	24.42 (14.74–34.10)
Octopus vulgaris	23.04 (9.99–36.09)	35.62 (18.23–53.01)	25.86 (10.23–41.48)	15.48 (3.02–29.45)
Pagellus erythrinus	21.9 (8.84–34.96)	46.61 (21.10–72.13)	31.49 (7.81–56.17)	–
Sepia officinalis	23.56 (10.34–36.78)	36.91 (16.89–56.93)	39.53 (11.04–72.31)	–
Spicara flexuosa	26.98 (12.11–41.85)	48.55 (28.34–68.76)	23.4 (10.62–36.30)	1.06 (0.61–2.74)
Spicara smaris	14.82 (4.19–25.45)	36.34 (15.46–57.22)	47.75 (17.62–77.89)	1.09 (0.63–2.81)

Numbers in parentheses indicate the 95% confidence intervals of the corresponding estimates

Table 3 Total surface by depth stratum of the potentially accessible continental shelf fishing grounds under different management regimes

	Depth stratum (m)	Surface (km^2)	Remaining (%)
Total area	50–100	7826.83	
	100–200	7966.70	
Minus 1 NM from the shore (current situation)	50–100	7183.12	91.78
	100–200	7469.17	93.75
Minus 1.5 NM from the shore (proposal)	50–100	5573.71	71.21
	100–200	6638.61	83.33

Table 4 Estimated proportion (%) of species resources not available to the bottom-trawl fishery under different management regimes

Species	Current management	1.5 NM fishery ban
Boops boops	34.2 (10.8–57.5)	43.3 (15.9–70.6)
Eledone moschata	20.3 (12.1–28.7)	35.7 (21.8–50.6)
Loligo vulgaris	32.2 (9.2–55.2)	45.1 (13.5–76.7)
Mullus barbatus	27.8 (18.5–37.0)	38.2 (26.0–50.3)
Mullus surmuletus	21.1 (13.6–28.5)	30.5 (20.2–40.7)
Octopus vulgaris	27.6 (12.1–43.0)	38.3 (17.2–59.2)
Pagellus erythrinus	27.7 (11.0–44.4)	41.4 (16.6–66.4)
Sepia officinalis	29.1 (12.4–45.9)	41.5 (17.3–66.4)
Spicara flexuosa	32.4 (15.1–49.7)	45.7 (22.5–68.9)
Spicara smaris	20.8 (6.5–35.0)	34.0 (11.9–56.1)

Numbers in parentheses indicate the 95% confidence intervals of the corresponding estimates

Aegean Sea, with a narrow continental shelf, spatial fishery prohibitions that do not consider the depth parameter may seriously affect the viability of certain fisheries. In the present case, the enforcement of the 1.5 NM fishery ban would drastically decrease (over 30%) the availability to the bottom trawlers of fishery grounds along the continental shelf, a zone inhabited by several important target species. It will also significantly increase (20–80%) the proportion of the populations that are inaccessible to this gear. Provided that the fishing pressure caused by bottom trawlers in the coastal zone will not be replaced by other fisheries, the introduction of the new ban would be beneficial for the stocks, and consequently for the fishery, in the medium to long-term. In the short-term, however, significant catch reductions are expected for several species. Furthermore, the extension of the spatial trawling prohibition may favor the inclusion of unmapped coralligenous habitats that usually host important fish communities (Georgiadis et al., 2009; Ordines & Massutí, 2009; Ordines et al., 2009), into the protected zone.

However, if such a ban is not accompanied by a capacity reduction of the bottom-trawl fishery (e.g., number of vessel licenses), it would result in higher competition for fishery resources in deeper areas; thus increasing fishery pressure on stocks inhabiting the continental slope (e.g., hake, shrimps). Taking into account that currently about 34% of the commercial hauls in the studied area are accomplished within 1.5 NM off the coast (Skarvelis et al., 2009), it is expected that a considerable proportion of the fishing effort will be shifted to deeper living resources. The impact of such a change in the fishery exploitation pattern needs to be evaluated. In any case, management planning should ensure that any further bottom-trawling prohibitions will not result in the replacement of trawling with other fishing activities that maintain high fishing mortality rates.

Although there are no assessment studies for the exploited stocks in the area, the lack of significant temporal variation suggests that the populations are in relative stability. Significant abundance variations over time were found only for squid which is a species with a short life span, governed by an r-strategy life history and subject to wide population fluctuations (Natsukari & Komine, 1992; Guerra & Rocha, 1994); thus temporal abundance variations are not surprising. The general overall stability could perhaps be linked to an "overfishing steady state" situation that has been debated for the whole Mediterranean (Lleonart & Maynou, 2003). However, the fact that a significant part of the examined stocks is currently unavailable to the bottom-trawl fishery under the existing legislation (no fishing up to 50 m depth and within 1 NM from the coast) may also contribute to the aforementioned population stability.

Our estimates may to a certain extent be biased, as they are based on an annual survey; thus ignoring intra-annual variability in species distribution patterns. Our approach, however, is the first attempt to evaluate the impact that can be estimated to occur from the enforcement of the 1.5 NM fishery ban. Further studies that take into account the seasonal variability in species distribution patterns may provide more precise evaluations of the full impact of the proposed spatial management regime.

Acknowledgments This study has been supported by the Greek Ministry of Agricultural Development and Food.

References

Bertrand, J. A., L. Gil de Sola, C. Papaconstantinou, G. Relini & A. Souplet, 2000. An international bottom trawl survey in the Mediterranean: the MEDITS programme. In Bertrand, J. A. & G. Relini (eds), Demersal Resources in the Mediterranean. Actes de Colloques 26, Ifremer, Plouzané France: 76–93.

Bertrand, J. A., L. Gil de Sola, C. Papaconstantinou, G. Relini & A. Souplet, 2002. The general specifications of the Medits surveys. Scientia Marina 66: 9–17.

Caddy, J. F., 1993. Some future perspectives for assessment and management of Mediterranean fisheries. Scientia Marina 57(2–3): 121–130.

Caddy, J. F., F. Carocci & S. Coppola, 1998. Have peak fishery production levels been passed in continental shelf areas? Some perspectives arising from historical trends in production per shelf area. Journal of Northwest Atlantic Fishery Science 23: 191–219.

Demestre, M., P. Sánchez & P. Abelló, 2000. Demersal fish assemblages and habitat characteristics on the continental shelf and upper slope of the north-western Mediterranean. Journal of the Marine Biological Association of the UK 80: 981–988.

Gaertner, J. C., J. A. Bertrand, L. De Sola, J. P. Durbec, E. Ferrandis & A. Souplet, 2005. Large spatial scale variation of demersal fish assemblage structure on the continental shelf of the NW Mediterranean Sea. Marine Ecology Progress Series 297: 245–257.

Georgiadis, M., G. Papatheodorou, E. Tzanatos, M. Geraga, A. Ramfos, C. Koutsikopoulos & G. Ferentinos, 2009. Coralligène formations in the eastern Mediterranean Sea: morphology, distribution, mapping and relation to fisheries in the southern Aegean Sea (Greece) based on high-resolution acoustics. Journal of Experimental Marine Biology and Ecology 369: 44–58.

Guerra, A. & F. Rocha, 1994. The life history of *Loligo vulgaris* and *Loligo forbesi* (Cephalopoda: Loliginidae) in Galician waters (NW Spain). Fisheries Research 21(1–2): 43–69.

Halpern, B. S., S. Walbridge, K. A. Selkoe, C. V. Kappel, F. Micheli, C. D'Agrosa, J. F. Bruno, K. S. Casey, C. Ebert, H. E. Fox, R. Fujita, D. Heinemann, H. S. Lenihan, E. M. P. Madin, M. T. Perry, E. R. Selig, M. Spalding, R. Steneck & R. Watson, 2008. A global map of human impact on marine ecosystems. Science 319: 948–952.

Hastie, T. J. & R. J. Tibshirani, 1990. Generalized Additive Models. Chapman and Hall, London: 335 pp.

Juan-Jordá, M. J., J. A. Barth, M. E. Clarke & W. W. Wakefield, 2009. Groundfish species associations with distinct oceanographic habitats in the northern California Current. Fisheries Oceanography 18(1): 1–19.

Kaiser, M. J., M. J. Attrill, S. Jennings, D. N. Thomas, D. K. A. Barnes, A. S. Brierley, N. V. C. Polunin, D. G. Raffaelli & P. J. B. Williams, 2005. Marine Ecology: Processes, Systems and Impacts. Oxford University Press, Oxford: 557 pp.

Katsanevakis, S. & C. D. Maravelias, 2009. Bathymetric distribution of demersal fish in the Aegean and Ionian Seas based on generalized additive modelling. Fisheries Science 75(1): 13–23.

Lleonart, J. & F. Maynou, 2003. Fish stock assessments in the Mediterranean: state of the art. Scientia Marina 67(Suppl. 1): 37–49.

Macpherson, E., 2003. Species range size distributions for some marine taxa in the Atlantic Ocean. Effect of latitude and depth. Biological Journal of the Linnean Society 80(3): 437–455.

Magnussen, E., 2002. Demersal fish assemblages of Faroe Bank: species composition, distribution, biomass spectrum and diversity. Marine Ecology Progress Series 238: 211–225.

Moranta, J., C. Stefanescu, E. Massutí, B. Morales-Nin & D. Lloris, 1998. Fish community structure and depth-related trends on the continental slope of the Balearic Islands (Algerian basin, western Mediterranean). Marine Ecology Progress Series 171: 247–259.

Natsukari, Y. & N. Komine, 1992. Age and growth estimation of the European squid, *Loligo vulgaris*, based on statolith microstructure. Journal Marine Biological Association of the United Kingdom 72(2): 271–280.

Nielsen, J. R., P. J. Sparre, H. Hovgaard, H. Frost & G. Tserpes, 2006. Effort and capacity-based fisheries management. In Motos, L. & D. C. Wilson (eds), The Knowledge Base for Fisheries Management. Developments in Aquaculture and Fisheries Science, Vol. 36. Elsevier, Oxford: 163–216.

Ordines, F. & E. Massutí, 2009. Relationships between macro-epibenthic communities and fish on the shelf grounds of the western Mediterranean. Aquatic Conservation: Marine and Freshwaters Ecosystems 19: 370–383.

Ordines, F., A. Quetglas, E. Massutí & J. Moranta, 2009. Habitat preferences and life history of the red scorpion fish, *Scorpaena notata*, in the Mediterranean. Estuarine Coastal and Shelf Science 85: 537–546.

Papaconstantinou, C. & H. Farrugio, 2000. Fisheries in the Mediterranean. Mediterranean Marine Science 1: 5–18.

Pauly, D. & V. Christensen, 1995. Primary production required to sustain global fisheries. Nature 374: 255–257.

R Development Core Team, 2008. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, ISBN 3-900051-07-0, URL http://www.R-project.org.

Reynolds, J. A., 2003. Quantifying habitat associations in marine fisheries: a generalization of the Kolmogorov–Smirnov statistic using commercial logbook records linked to archived environmental data. Canadian Journal of Fisheries and Aquatic Sciences 60(4): 370–378.

Skarvelis, C., G. Lazarakis, P. Peristeraki & G. Tserpes, 2009. Particularities of the bottom trawl fishery in the S. Aegean: effects from the implementation of EC Regulation 1967/2006. In Proceedings of the 9th Panhellenic Symposium of Oceanography and Fisheries, Vol. II. Hellenic Centre for Marine Research: 959–964.

Tserpes, G. & P. Peristeraki, 2002. Trends in the abundance of demersal species in the southern Aegean Sea. Scientia Marina 66: 243–252.

Tserpes, G., P. Peristeraki, G. Potamias & N. Tsimenides, 1999. Species distribution in the southern Aegean sea based on bottom-trawl surveys. Aquatic Living Resources 12(3): 167–175.

Tsimenides, N., G. Tserpes, A. Machias & A. Kallianiotis, 1991. Distribution of fishes on the Cretan shelf. Journal of Fish Biology 39(5): 661–672.

Wood, S. N., 2004. Stable and efficient multiple smoothing parameter estimation for generalized additive models. Journal American Statistical Association 99: 673–686.

Hydrobiologia (2011) 670:275–287
DOI 10.1007/s10750-011-0677-5

ECOSYSTEMS AND SUSTAINABILITY

Contrasting multispecies patterns in larval fish production trace inter-annual variability in oceanographic conditions over the N.E. Aegean Sea continental shelf (Eastern Mediterranean)

Stylianos Somarakis · Alexis Ramfos ·
Andreas Palialexis · Vasilis D. Valavanis

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The response of larval fish communities of the northeastern Aegean Sea (NEA) to interannual environmental changes is analyzed using data from four ichthyoplankton surveys covering the NEA continental shelf during June 1993, 1994, 1995, and 1996. Waters were significantly cooler, less saline and richer in zooplankton in 1993 and 1996 ('cold' years) than in 1994 and 1995 ('warm' years). A comparison of monthly SST series (1993–1997) between the NEA, the Marmara Sea, and the Western Black Sea revealed high correlations and similar trend components among these areas implying that oceanographic conditions over the NEA (and observed inter-annual differences) were most likely dominated by the properties and relative amount of Black Sea water inflow in the NEA. The relative composition of the larval fish community was significantly related to the 'cold/warm' regime and larval diversity was higher during the warm years. Larvae of the small-sized pelagic species, such as anchovy (*Engraulis encrasicolus*), and most mesopelagic fishes were relatively more abundant during the cold, zooplankton-rich years. Larvae of the middle-sized pelagics (*Sardinella aurita*, *Scomber japonicus*, *Trachurus mediterraneaus*, *Auxis rochei*) and certain benthopelagic species exhibited an opposite trend, i.e., they were more abundant during the warm years or absent during the cold years. Most of these species are known to be typical summer spawners (e.g., *Serranus cabrilla*, *Lisa saliens*, *Trachinus draco*, and *Symphurus nigrescens)*. Co-variation in larval fish production might be indicative of similar responses among species to changing physical and/or trophic regimes.

Keywords Ichthyoplankton · North Aegean Sea · Black Sea water

Introduction

Variability in recruitment of fish stocks is primarily determined by inter-annual changes in oceanographic conditions. These may act directly on adults, affecting the spatiotemporal pattern of egg production, timing and duration of spawning (e.g., Ganias et al., 2007; Stratoudakis et al., 2007), geographical distribution of spawning habitats (e.g., Planque et al., 2007; Bernal et al., 2007), fecundity and spawning

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

S. Somarakis (✉) · A. Palialexis · V. D. Valavanis
Institute of Marine Biological Resources, Hellenic Centre for Marine Research, Thalassocosmos, P.O. Box 2214, Heraklio, Crete, Greece
e-mail: somarak@her.hcmr.gr

A. Ramfos
Department of Aquaculture and Fisheries Management, Technological and Educational Institute of Messolonghi, 30200 Messolonghi, Greece

frequency (e.g., Somarakis et al., 2004; Somarakis, 2005), and/or on developing eggs and larvae, determining their drift, development, growth, and survival (e.g., Heath, 1992, Allain et al., 2007).

As climate impacts on marine ecosystems and synoptic-scale recruitment processes are difficult to address experimentally, the comparative method is the best way to improve our knowledge on the reaction of ecosystems and populations embedded in them to physical forcing. The existence, scale, and interspecies relationships of repeated patterns may yield valuable insight into the nature of the processes driving recruitment variability (Koslow, 1984; Koslow et al., 1987; Cohen et al., 1991; Myers et al., 1997; Hsieh et al., 2005; Mueter et al., 2007). Furthermore, multispecies patterns may provide basic information required to devise effective multispecies and ecosystem fishery management strategies (Ludwig et al., 1993; Christensen, 1996).

The importance of multispecies ichthyoplankton investigations for our understanding of fish reproductive strategies has been often emphasized in recent years (Moser & Smith, 1993; Somarakis et al., 2000). According to Frank & Leggett (1983), multispecies larval fish associations are adaptive and result from similar responses among species to the pelagic environment. In that sense, covariation in the abundance of larvae of different fish species in the plankton might indicate similarities in patterns of egg production and subsequent recruitment for these species (Somarakis et al., 2000). Long-term inter-annual trends in larval abundance mostly reflect trends in adult biomass (Hsieh et al., 2005). Short-term fluctuations are generally considered to be related to episodes of high or low reproductive output or geographical shifts due to animal movement (Moser et al., 2000; Hsieh et al., 2005).

This paper extends a previous, 2-year, study (1995 and 1996) on epipelagic fish larvae in the northeastern Aegean Sea (Somarakis et al., 2000), to examine multispecies trends in mean abundance for the entire larval fish community during early summer from 1993 to 1996 (4 years). In the early 1990s, the northeastern Aegean Sea (NEA) experienced marked inter-annual fluctuations in oceanographic conditions that were recorded during the anchovy ichthyoplankton surveys in the region (Somarakis, 1999, 2005; Somarakis et al., 2000, 2002). This has afforded a unique opportunity to investigate the response of larval fish communities to dramatic environmental changes.

The NEA is the most important area of the eastern Mediterranean in terms of fisheries production (Stergiou et al., 1997). It has a relatively wide continental shelf and receives the direct influence of the cooler, less saline, Black Sea water (BSW) that significantly enhances the productivity of the area (Isari et al., 2006, 2007, and references therein). The structure and physical characteristics of the water column in the NEA are primarily determined by the seasonal and interannual variability in the amount of BSW entering into the Aegean Sea (Poulos et al., 1997; Zervakis & Georgopoulos, 2002). The advection of BSW induces high hydrological and biological complexity that is directly reflected in the structure, composition, and distribution of the assemblages of holoplankters (Isari et al., 2006, 2007). However, the formation of assemblages of early fish larvae is primarily determined by the bathymetric and spatial distribution of adults (Somarakis et al., 2002; Isari et al., 2008).

Horizontal distribution patterns of larval fishes over the broader area of the northern Aegean Sea (including NEA) have been described by Somarakis et al. (2002) for the June 1995 and June 1996 surveys. The focus here is on inter-annual changes in overall abundance of different species and in relative composition of the larval assemblage in relation to changes in the abiotic and biotic environment. Time series of mean SST over the study area are compared with those of Bosporus (Marmara Sea) and the Black Sea in order to provide the first evidence that oceanographic conditions in the Black Sea directly influence the NEA.

Materials and methods

Ichthyoplankton data

Larval fishes were collected during four surveys carried out during 7–11 June 1993, 19–23 June 1994, 15–22 June 1995, and 6–14 June 1996 in the northern Aegean Sea (Eastern Mediterranean). Samples from a grid of 36 plankton stations spaced every 10 nautical miles are analyzed here (Fig. 1). These stations were sampled in all years, except, for two (stations 39 and 58) which were not sampled in 1994 due to time

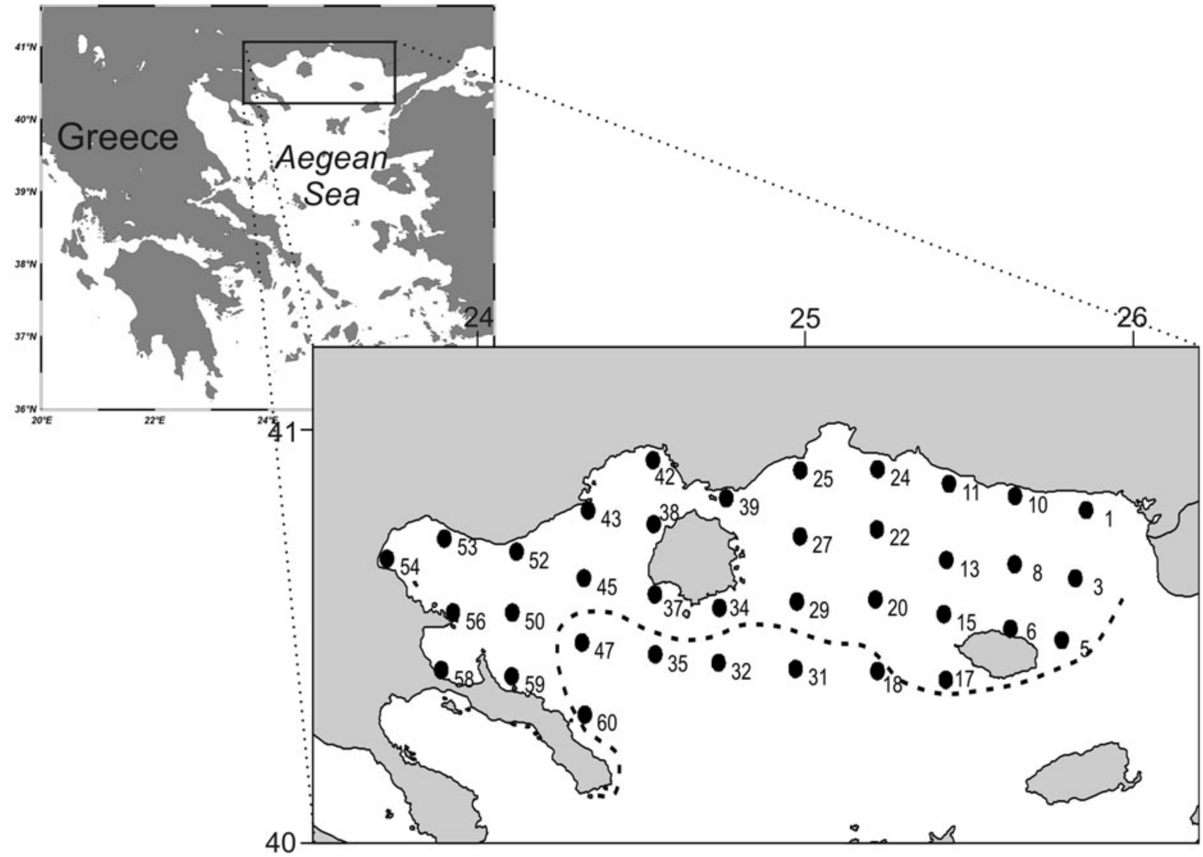


Fig. 1 Topography and sampling grid of the surveyed area. *Dotted line*: the 200 m isobath

constraints. Hydrographic sampling (temperature and salinity profiles) was also performed at each station using a Seabird 19 CTD profiler.

A 60-cm bongo-net sampler was used to sample ichthyoplankton. Mesh sizes on the sampler were 335 and 250 μm. Tows were double-oblique from within 5 m of the bottom to the surface or from 120 m depth to the surface at deep stations. Catches were standardized to numbers per square metre using information on maximum tow depth and volume of water filtered. More details are provided in Somarakis et al. (1998). Immediately after collection, samples were preserved in 5% borax-buffered formalin.

In the laboratory, larvae from the 0.250-mm mesh net were sorted and identified to the lowest possible taxonomic level. Zooplankton displacement volume (ZDV), which can be considered a rough index of zooplankton production (Smith & Richardson, 1977), was measured for each sampling site from the catch of the 0.250-mm mesh net. ZDV values were standardized to ml m^{-2}.

Analysis of variance was performed on [$\log_{10}(x + 1)$] transformed abundance values to test for differences in abundance of dominant larval taxa (i.e., those with frequency of occurrence > 10% in at least two surveys) between years. The Student–Newman–Keuls (SNK) test was used to define homogenous groups.

The ($\log_2$) Shannon–Wiener information index (H') and Pielou's evenness index (J') were calculated to assess the diversity of the larval fish in the samples. Only larvae identified to the species level were used for these indices.

A taxon-by-sampling site matrix, expressed as abundance values (ind. m^{-2}), was subsequently analyzed using non-metric multidimensional scaling (NMDS) (Field et al., 1982; Clarke & Warwick, 1994). Data were transformed using the [$\log_{10}(x + 1)$]

transformation and similarities were computed using the Bray-Curtis similarity index. 'Outlier' samples (e.g., samples biasing or dominating the ordination, often compressing the distribution of the remaining sites) were removed from the data set to obviate problems associated with such outliers (Gauch, 1982; Hosie & Cochran, 1994). These three outlier samples, collected in 1994, were identified by prior exploratory data analysis using group-average clustering (Hosie & Cochran, 1994; Somarakis et al., 2002).

Ordination scores produced by the NMDS were compared to various environmental parameters using multiple regression analysis in order to determine which of these parameters were significantly related to the larval assemblage compositions (Kruscal & Wish, 1978; Hosie & Cochran, 1994). In the regression analysis the NMDS scores were treated as the independent variables and each environmental parameter as the dependent variable. The rationale for selecting this method (vs. other methods, e.g., canonical analysis, non-parametric methods) is described in Somarakis et al. (2002). Regression lines and their directions were plotted in the NMDS graphs according to Kruscal & Wish (1978): The direction of maximum correlation of each regression line is at an angle φ_r with the rth MDS axis. The direction cosine, or regression weight c_r, of that angle is given by the formula:

$$c_r = b_r / \sqrt{b_1^2 + b_2^2}$$

where b_1 and b_2 are the coefficients from the multiple regression $a + b_1x_1 + b_2x_2$, and x_1 and x_2 are the scores in the first and second MDS axis, respectively. The parameters examined were latitude (Lat), longitude (Lg), distance from the coast (CD), haul depth (HD) and ZDV, as well as temperature (T) and salinity (S) of the upper water column (means for the 0–40 m layer).

Satellite data

The hydrological and biological properties of waters in the NEA are highly influenced by Black Sea Water (e.g., Isari et al., 2006). In order to better understand whether the inter-annual differences in the biotic and abiotic environment observed in this area (e.g. differences in temperature) are affected by BSW, i.e., reflect processes in the Black Sea, we examined monthly time series of sea surface temperature for the study area, the Marmara Sea and the Black Sea, covering the period 1993–1997. Satellite images of sea surface temperature (SST) distribution (in °C) were downloaded from the German Aerospace Agency's (DLR, Germany) online EOWEB data archive (eoweb.dlr.de:8080) derived from the Advanced Very High Resolution Radiometer (AVHRR) datasets on a spatial resolution of 1.3 km.

These monthly averaged datasets were processed as regular grids under a Geographic Information Systems (GIS) environment using ArcInfo GRID software (ESRI, 1994). The mean monthly values for the period March 1993–December 1997 were estimated for the western half of Black Sea (west of 34°E parallel), Marmara Sea and the northeastern Aegean Sea (north of the 40°N parallel).

A seasonal-trend decomposition procedure based on Loess (STL) was used for the decomposition of time-series into trend, seasonal, and remainder components, as described by Cleveland et al. (1990). The trend component represents a low frequency variation in the data together with non-stationary, long-term changes in level. The seasonal component represents the variation in the data at or near the seasonal frequency, while the remainder component is the remaining variation beyond that in the seasonal and trend component. The STL was implemented in Brodgar software (www.brodgar.com). The time-series frequency was set to 12 since monthly time-series were used. The 'periodic' value was selected for the Loess window trend and seasonal, which means that mean values per month are taken. For the trend and seasonal degree, a linear regression model was selected to be used by the Loess algorithm.

Results

The water column in the NEA presented typical early-summer conditions in all years. A seasonal thermocline was coupled with a strong halocline, separating the upper 40 m of the water column from the colder and more saline deeper layer (Fig. 2). Mean thermocline depth was at ~18 m and mean thermocline temperature range was 9°C during all years. In 1993 and 1996 (hereafter referred to as 'cold' years), temperature in the upper 100 m was about 2°C lower than in 1994 and 1995 (hereafter referred to as 'warm'

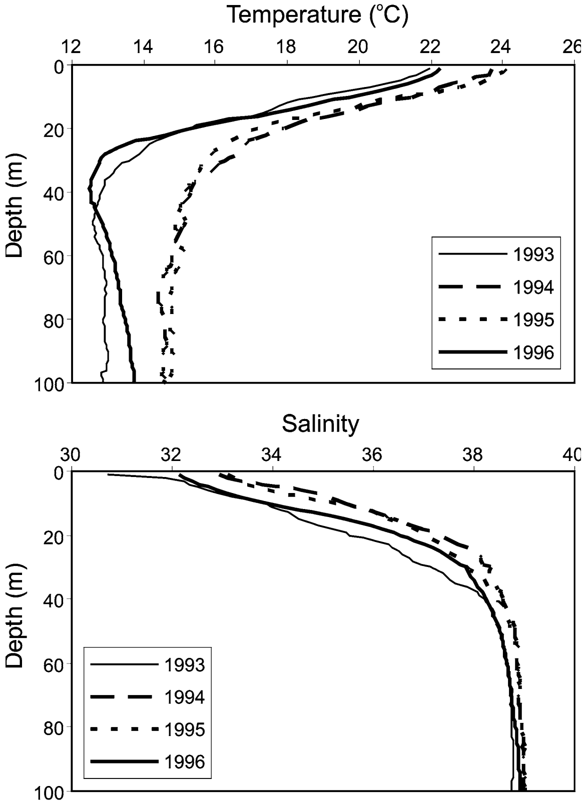


Fig. 2 Average profiles of temperature and salinity for the four surveys

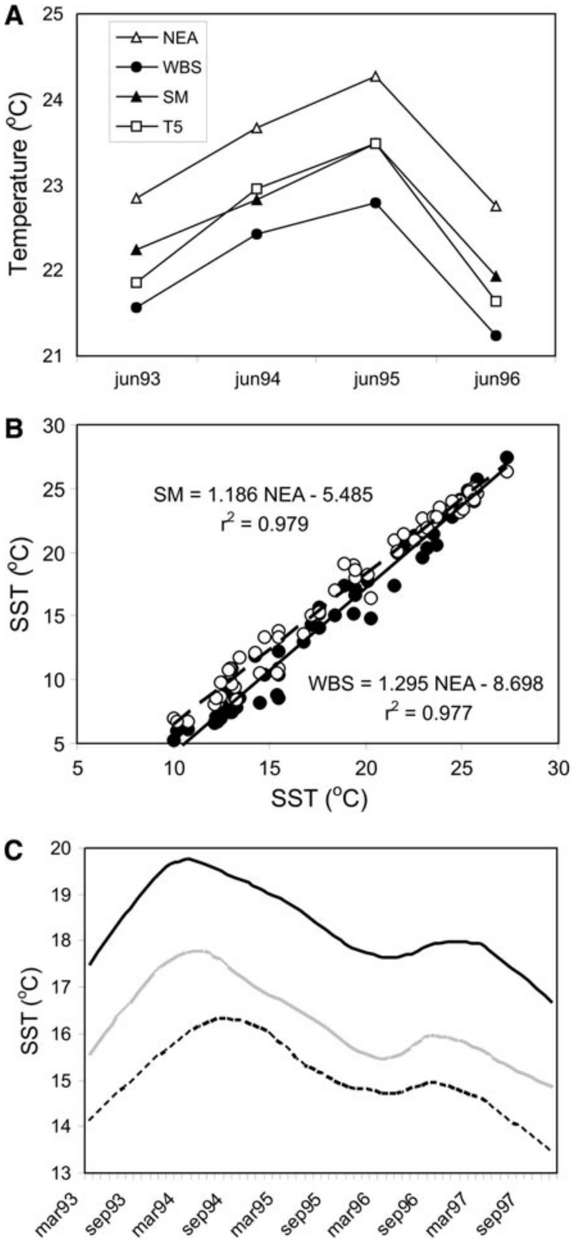


Fig. 3 **A** Plot of mean satellite SST for Western Black Sea (WBS), the Marmara Sea (SM) and the Northeastern Aegean Sea (NEA) in June 1993, 1994, 1995 and 1996. T5: mean temperature at 5 m measured during the ichthyoplankton surveys. **B** Regressions of mean monthly (March 1993–December 1997) SST in the WBS on SST in the NEA (*closed circles*) and SST in the SM on SST in the NEA (*open circles*). **C** The trend components of the SST data series in WBS (*dotted line*), SM (*grey line*), and NEA (*black line*)

years) (Fig. 2). The warm-cold pattern was also apparent in the mean SST values of the study area (NEA), Sea of Marmara (SM) and western Black Sea (WBS), which exhibited interannual patterns similar to in situ CTD measurements (Fig. 3A).

Mean salinity values increased sharply from the surface to 40 m depth and were lower in 1993 and 1996 (Fig. 2). Analysis of variance indicated that mean temperature and salinity of the upper water column (0–40 m) was significantly higher in 1994 and 1995 (Fig. 4, left panel; $F = 66.00$, $P < 0.0001$ and $F = 23.60$, $P < 0.0001$ for temperature and salinity, respectively). Mean ZDV was significantly higher during the cold years, especially in 1996 (Fig. 4, left panel; $F = 11.54$, $P < 0.0001$).

The analysis of mean monthly SST time series for NEA, SM, and WBS showed high linear correlations between NEA and both SM and WBS (Fig. 3B). The trend component of the three SST time series (Fig. 3C) exhibited similar general pattern with an increasing part from March 1993 to spring–summer 1994 and a decreasing part thereafter until spring–early summer of 1996.

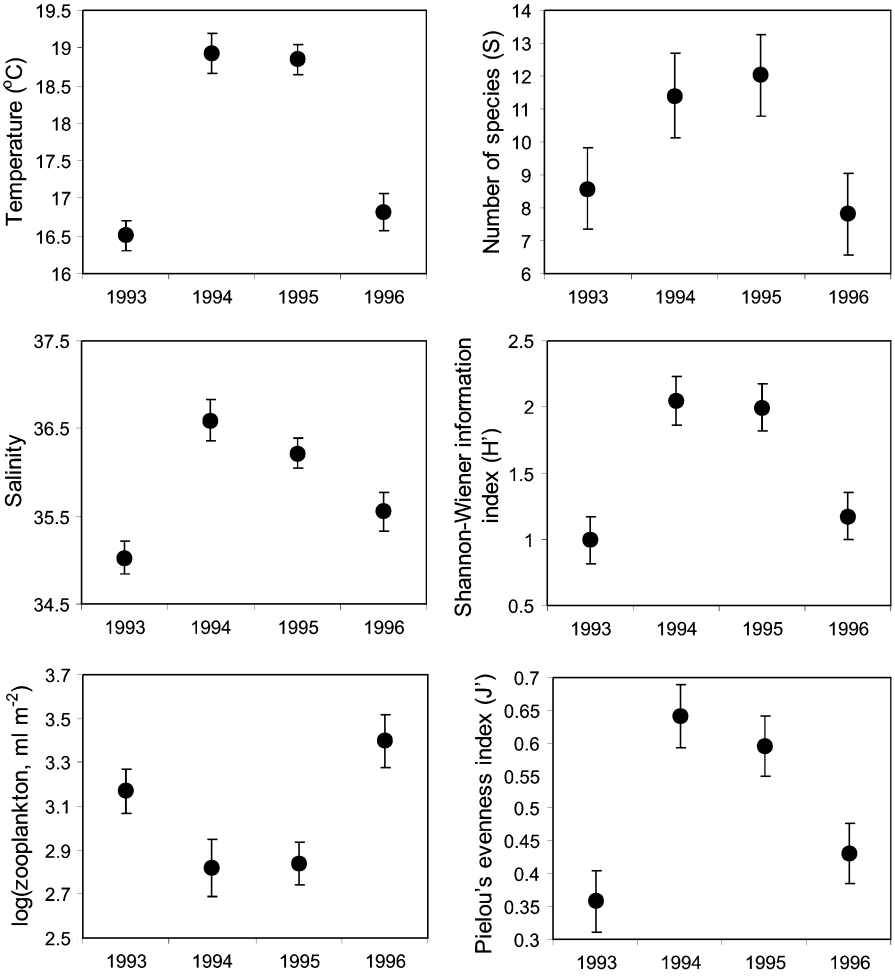


Fig. 4 Means and 95% confidence intervals for temperature (0–40 m), salinity (0–40 m) and zooplankton (ZDV) (*left panel*) and selected diversity indices for ichthyoplankton (*right panel*)

A total of 57 larval taxa were identified in the collections (40 in 1993, 44 in 1994, 48 in 1995, and 41 in 1996) from which 30 taxa were common in all years (Table 1). The most common species were the small- and middle-sized pelagics, namely *Engraulis encrasicolus*, *Sardinella aurita*, *Trachurus mediterraneaus* and *Scomber japonicus*, certain mesopelagic species, e.g., *Ceratoscopelus maderensis*, *Hygophum benoiti*, *Myctophum punctatum*, *Maurolicus muelleri*, and the benthopelagics *Serranus hepatus*, *Callionymus* spp., *Cepola macrophthalma*, *Arnoglossus* spp., *Serranus cabrilla*, *Mullus barbatus*, *Chromis chromis*, and *Coris julis*.

Species diversity and evenness were significantly higher during the warm, 1994 and 1995, years (Fig. 4 right; $F = 18.18$, $P < 0.0001$ and $F = 15.83$, $P < 0.0001$ for H' and J', respectively). These differences were mainly due to the high dominance of anchovy (*E. encrasicolus*) larvae (Table 1) and the lower mean number of species observed during the cold years (Fig. 4 right; $F = 5.42$, $P < 0.01$ for S). Certain species, such as *Callanthias ruber*, *Trachinus draco*, *Lepidopus caudatus*, *Parophidion vassali*, *Lisa saliens*, *Microchirus variegatus* and *Symphurus nigrescens* were collected only during the warm years.

When examining inter-annual trends in mean abundance, it was quite evident that certain species exhibited contrasting patterns between cold and warm years (Fig. 5; Table 1). Anchovy (*E. encrasicolus),* which was the most abundant species, exhibited higher mean abundance during 1993 and 1996. A similar pattern was observed for most mesopelagic species (e.g., *M. muelleri*, *H. benoiti*, *M. punctatum*, *Cyclothone braueri*, *Bentosema glaciale*, *Vinciguerria* spp., *Lestidiops* spp., *Arctozenus risso*, *Argyropelecus*

Table 1 Frequency of occurrence (N%) and mean abundance values (ind. m^{-2}) of larval fish taxa identified in the collections (June 1993–1996)

Taxa	1993		1994		1995		1996		*P*
	N%	ind. m^{-2}	*N%*	ind. m^{-2}	*N%*	ind. m^{-2}	*N%*	ind. m^{-2}	
Sardinella aurita	44	10.87^{b}	67	19.80^{a}	94	35.38^{a}	56	6.80^{b}	*
Engraulis encrasicolus	100	301.04^{a}	89	110.12^{b,c}	100	80.52^{c}	100	187.12^{a,b}	*
Cyclothone braueri	11	0.88	11	0.14	22	0.17	11	0.96	ns
Argyropelecus hemigymnus	17	0.30					3	0.04	
Maurolicus muelleri	31	1.34^{a}	14	0.34^{a,b}	11	0.15^{b}	22	1.06^{a,b}	*
Vinciguerria spp.	17	0.50	14	0.27	14	0.15	19	1.03	ns
Stomias boa boa			6	0.05	8	0.07	8	0.12	
Glossanodon leioglossus							3	0.12	
Bentosema glaciale	17	0.37	11	0.19	17	0.20	22	1.24	ns
Ceratoscopelus maderensis	53	4.10	56	16.77	58	17.54	61	18.01	ns
Diaphus holti			3	0.03	6	0.05			
Hygophum benoiti	58	13.26	31	4.67	44	5.98	39	12.50	ns
Lampanyctus crocodilus	11	0.18	19	0.75	31	0.39	19	0.63	ns
Lobianchia dofleini	14	0.58	14	0.20	28	0.61	14	0.47	ns
Myctophum punctatum	44	4.14	19	1.62	31	2.08	33	5.45	ns
Lestidiops spp.	11	0.24	8	0.13	8	0.10	22	0.55	ns
Arctozenus risso	3	0.04					3	0.12	
Macrouridae					3	0.04			
Merluccius merluccius	3	0.05			3	0.02	6	0.09	
Anthias anthias			6	0.05					
Callanthias ruber			14	0.14	11	0.07			ns
Epinephelus spp.	3	0.04	8	0.14	3	0.03			
Serranus cabrilla	19	0.34^{c}	67	2.76^{a}	58	1.46^{b}	17	0.45^{c}	*
Serranus hepatus	83	10.25^{a}	78	11.34^{a}	86	7.76^{a}	67	2.69^{b}	*
Cepola macrophthalma	75	4.54^{b}	81	8.17^{a}	81	3.53^{b}	58	2.19^{b}	*
Trachurus mediterraneus	64	5.31^{b}	75	10.74^{a}	83	6.61^{a,b}	69	2.75^{b}	*
Trachurus trachurus	14	0.20	25	0.26	25	0.26	14	0.17	ns
Brama brama					3	0.01			
Mullus barbatus	17	0.33^{c}	58	2.75^{a}	47	1.27^{b}	8	0.24^{c}	*
Boops boops	17	0.26			11	0.07	8	0.16	ns
Dentex dentex	6	0.08	3	0.02			3	0.03	
Pagellus erythrinus	6	0.03^{b}	44	1.09^{a}	11	0.19^{b}	19	0.26^{b}	*
sparids/centracanthids	72	5.24^{a,b}	78	6.77^{a}	92	3.09^{a,b}	61	2.40^{b}	*
Chromis chromis	22	1.64^{b}	69	4.42^{a}	72	2.51^{a}	11	1.52^{b}	*
Coris julis	50	1.22^{b,c}	64	6.65^{a}	64	2.12^{b}	22	0.48^{c}	*
Labridae	33	0.85	56	1.68	64	1.82	42	1.75	ns
Trachinus draco			17	0.26	8	0.05			
Uranoscopus scaber	3	0.03							
Lepidopus caudatus			8	0.11	3	0.02			
Auxis rochei			25	0.39^{b}	44	1.04^{a}	3	0.11^{b}	*
Scomber japonicus	53	2.13^{b,c}	61	7.62^{a}	53	6.81^{a,b}	33	1.56^{c}	*
Gobiidae	94	16.09^{a,b}	81	5.18^{c}	97	10.03^{b,c}	100	18.78^{a}	*

Table 1 continued

Taxa	1993		1994		1995		1996		*P*
	N%	ind. m^{-2}	*N*%	ind. m^{-2}	*N*%	ind. m^{-2}	*N*%	ind. m^{-2}	
Callionymus spp.	97	13.07^{a}	81	4.99^{b}	89	12.09^{a}	94	9.68^{a}	*
Blennius ocelaris					3	0.02			
Blenniidae	17	0.37	36	0.44	42	0.39	22	0.40	ns
Parophidion vassali			8	0.08	22	0.22			
Carapus acus	3	0.03					3	0.03	
Liza saliens			6	0.06	19	0.18			
Scorpaena spp.	3	0.05^{b}	28	0.33^{a}	14	0.11^{b}	3	0.02^{b}	*
Lepidotrigla cavillone	3	0.04	6	0.05			3	0.03	
Arnoglossus spp.	72	4.66	72	2.39	81	2.28	72	1.97	ns
Buglossidium luteum	17	0.45	8	0.17	17	0.33	14	0.99	ns
Microchirus variegatus			6	0.06	3	0.02			
Solea lascaris	3	0.04					17	0.18	
Symphurus nigrescens			3	0.02	17	0.11			
Remora remora					3	0.01			
Lophius budegassa					3	0.02			

Results for analysis of variance and SNK multiple range tests on $\log(x + 1)$ abundance of dominant species are also given

For ANOVA *P* values: * $P < 0.001$, *ns* non significant, a, b, c: SNK test homogeneous groups (a > b > c)

hemigymnus) in terms of either abundance, frequency of occurrence or both (Fig. 5; Table 1). With the exception of three species (*C. maderensis*, *Lampanyctus crocodilus*, and *Lobianchia dofleini*), all remaining mesopelagic larvae showed relatively increased abundance during the cold years, especially in 1996 when zooplankton concentration was significantly higher (Fig. 4). In contrast, larvae of middle-sized pelagics, i.e., *S. aurita*, *T. mediterraneus*, *Auxis rochei* and *S. japonicus* presented higher abundance during the warm years (1994 and 1995). A similar pattern was also observed for certain demersal species, such as *M. barbatus*, *S. cabrilla*, *C. chromis*, *C. julis* and *C. ruber* (Fig. 5; Table 1). Larvae of most remaining benthopelagic species did not present any particular between-years pattern.

Finally, the NMDS results based on larval composition data showed that samples were differentiated according to depth but also according to the 'cold-warm' regime (Fig. 6). It must be noted here that three samples (i.e., stations 24, 25, and 42) collected in 1994 were not used in the MDS because they were biasing the ordination. These "outlier" stations were characterized by both very low total abundance and low number of species.

Haul depth, latitude and distance from coast explained 67, 60, and 28% of the variation in the ordination, along the axia separating shallow sites (<40 m), sites of intermediate depth (>40 m and <110 m) and deeper sites (>110 m) (Table 2; Fig. 6A). In addition, temperature, salinity and zooplankton (ZDV) explained a significant amount of variation in the sense of cold (1993 and 1996) versus warm (1994–1995) years (Table 2; Fig. 6B).

Discussion

Significant inter-annual differences in temperature, salinity, and zooplankton concentration have been recorded in the NEA during the four ichthyoplankton surveys which took place in the 1990s (1993–1996). The high correlation of the SST time series among the NEA, SM, and WBS as well as the similarity of the SST trend components among the three areas indicate that the Black Sea water (BSW) might have a dominant role in determining the oceanographic regime of the NEA. Recently, Oguz et al. (2003) provided evidence that an intense warming event with ~2°C increase in the SST of the open (>200 m

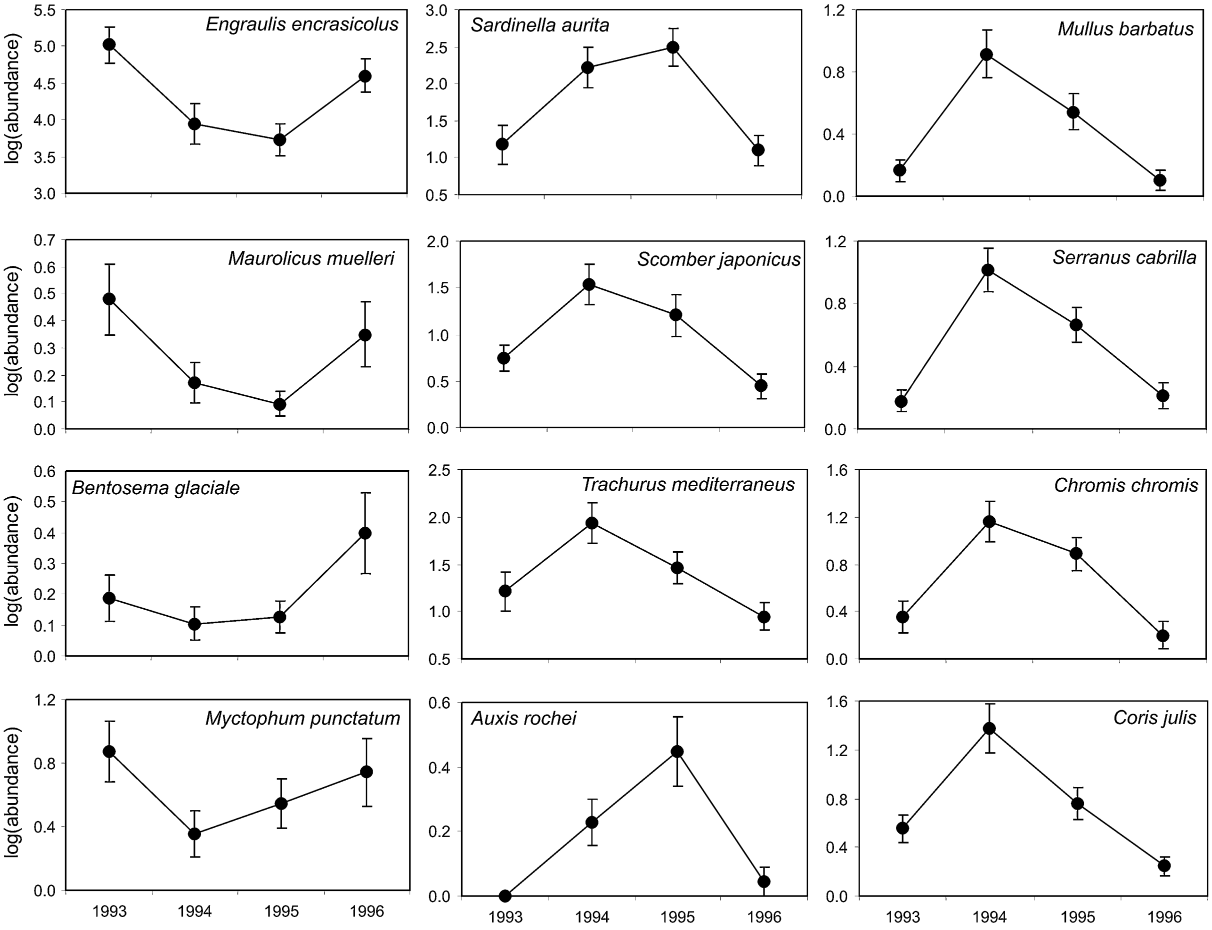


Fig. 5 Mean abundance (ind. m^{-2}) of selected larval species exemplifying among-year patterns. *Bars* indicate standard errors

depth) Black Sea took place during winters of the period 1995–1996, triggering changes in the ecological regime of the Black Sea. Furthermore, Stanev & Peneva (2002) have shown that negative anomalies in the outflow of BSW from the Bosporus Straits can result in increased salinity in the Aegean Sea. Hence, high salinity and lower productivity during the 1994 and 1995 surveys (warm years) might imply a decreased BSW influence (outflow).

Summing up, inter-annual differences in oceanographic conditions observed in this study are most likely related to changes in BSW properties and/or relative influence (e.g., amount of BSW entering into the NEA). We may hypothesize here that physical forcing affecting the Black Sea ecosystem might in turn affect the pelagic ecosystem of the Aegean Sea, a hypothesis that warrants further investigations.

Inter-annual differences in environmental conditions over the NEA were noticeably reflected in the structure and diversity of the larval fish assemblage. In the cold, less saline and richer in zooplankton regime (June 1993 and June 1996) larval diversity was significantly lower (Fig. 4). Factors determining the taxonomic composition and abundance were primarily depth-related (Fig. 6A), however inter-annual differences in temperature, salinity, and zooplankton (i.e., the cold-warm regime) were also significantly correlated with the NMDS scores (Fig. 6B).

In ichthyoplankton studies, individual species abundance among sampling stations generally exhibits high variability (in this study, coefficients of variation for dominant taxa ranged from 111 to 453%) and the number of stations feasible to sample is usually limited (here, $n = 36$). Hence, between-years

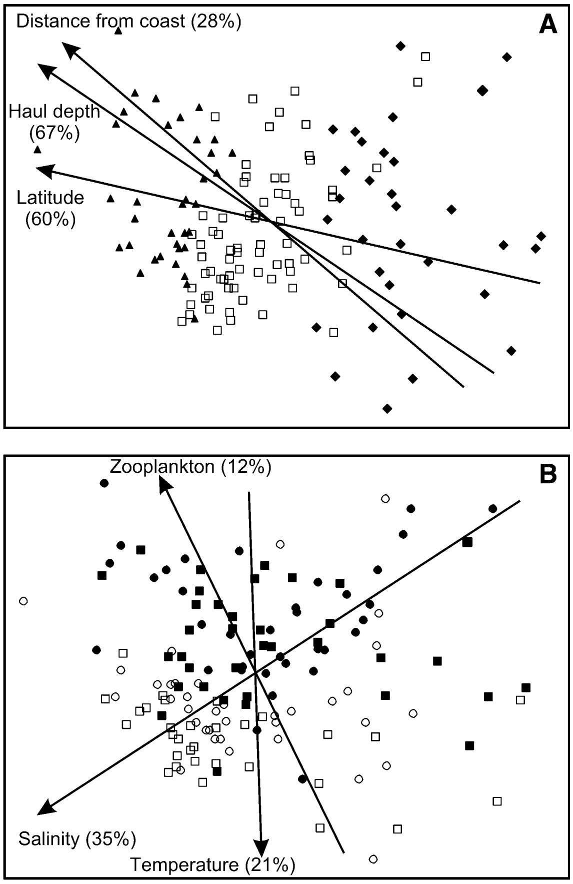


Fig. 6 Ordination plot of the comparison of sampling sites using non-metric multidimensional scaling and Bray-Curtis similarity index. **A** symbols according to station depth: *filled diamonds* < 40 m, *open squares* 40–110 m, *filled triangle* > 110 m. **B** Symbols according to sampling year (*open symbols* warm years, *closed symbols* cold years): *filled square* 1993, *open square* 1994, *open circle* 1995, *closed circle* 1996. Significant multiple regressions between ordination scores and environmental parameters are shown, as well as the fraction (%) of variance explained (see also Table 2)

Table 2 Multiple regression analysis between various environmental parameters and the NMDS scores for two-axis ordination of sampling sites

Variable	*X*	*Y*	Adjusted r^2	*F*	*P*
Haul depth	−33.44	15.32	67.15	141.0	<0.001
Latitude	0.15	−0.01	60.44	106.4	<0.001
Distance from coast	−2.62	1.25	27.9	27.7	<0.001
Temperature (0–40 m)	0.24	−1.4	21.37	19.4	<0.001
Salinity (0–40 m)	−0.78	−0.11	35.29	37.8	<0.001
Zooplankton (ZDV)	−4.84	5.17	12.49	10.9	<0.001
Longitude				2.26	>0.05

X and *Y* are the direction cosines (regression weights)

changes in mean abundance of most species are hard to be detected statistically (unless they are quite large) because the power of the Analysis of Variance and a posteriori multiple comparisons (i.e., avoiding Type II error) is low (Rotenberry & Wiens, 1985). This was especially true for mesopelagic species in this study (Table 1) because the number of stations sampled beyond the continental shelf was very low. However, despite the intrinsically low power for statistical inferences, when examining inter-annual trends in mean abundance, it was quite apparent that certain species exhibited contrasting patterns between the cold and warm years (Fig. 5; Table 1). Larvae of the small-sized species (anchovy and most mesopelagic species) were more abundant during the cold, less saline and zooplankton-rich years. A similar association of larvae of Engraulidae and Myctophidae with increased plankton production has also been reported off the U.S. northwest coast (Doyle et al., 1993). Other pelagic species, namely *S. aurita*, *T. mediterraneus*, *S. japonicus*, and *A. rochei* were more abundant during the warm regime (1994 and 1995). The same trend (higher relative abundance during the warm years) was observed for certain benthopelagic species (*M. barbatus, S. cabrilla, C. julis, and C. chromis*). Others, namely *C. ruber*, *T. draco*, *L. caudatus*, *P. vassali*, *L. saliens*, *Microchirus variegatus* and *S. nigrescens* were only collected during the warm years.

The period of spring-early summer is a transitional period in the reproduction of Mediterranean fish species (Sabatés & Maso, 1992; Sabatés & Olivar, 1996). It marks the end of the spawning season of those species that reproduce during the winter months (e.g., *Trachurus trachurus*, *Boops boops*) and the start of spawning for summer spawners (Tsikliras et al., 2010). This period coincides with the peak of spawning for anchovy (Palomera, 1992; Somarakis, 1999). There is only a single detailed study on seasonal patterns of larval fish occurrence in the Mediterranean (Sabatés, 1990). It shows that species such as *S. aurita*, *T. mediterraneus*, *S. japonicus κaι A. rochei, S. cabrilla*, *L. saliens, T. draco* and

S. nigrescens exhibit their peak abundance after the peak of anchovy and they comprise the group of the so called 'summer spawners'. The lower abundance (or absence) of these species in the NEA during the cold 1993 and 1996 years could be attributed to higher temperature thresholds for the onset of spawning in typically summer spawners.

Inter-annual differences in the abundance trends of the different larval taxa could not be explained in terms of changes in specific hydrographic structures (e.g., fronts, gyres) or currents differentially modifying the advection of larvae (Somarakis, 1999). Patterns of species distribution as well as horizontal patterns of hydrological properties (temperature, salinity, density) and inferred baroclinic currents, were very similar among years (not shown here, but see Somarakis et al. (2002) for the 'warm' June 1995 and the 'cold' June 1996). The semi-permanent anticyclones (like the Samothraki gyre) that are present in the area, always resided on the shelf and their effect on distributions could hardly be identified for pre-flexion larvae that dominate the bongo-net collections (e.g., Somarakis & Nikolioudakis, 2007).

In conclusion, the data presented here imply: (a) a temporal (inter-annual) covariation in larval production for the small-sized anchovy and mesopelagic species that is likely related to zooplankton production and (b) a similar covariation for typically summer spawners that can be explained in terms of temperature effects on the onset of the reproductive period. Larval abundances are primarily measures of the spawning biomass and reproductive effort of the adult stock, because most larvae sampled by low-speed plankton nets are in early stage of development (e.g. Somarakis et al., 1998). Since sudden changes in adult biomass would not be expected, short-term inter-annual changes in larval abundance are most likely related to adult reproductive output (Moser et al., 2000; Hsieh et al., 2005; Doyle et al., 2009). Regarding the pelagic species in this study, observed inter-annual differences have been explained in terms of the life history theory (Somarakis et al., 2000) and, specifically, the hypothesis of contrasting reproductive strategies among pelagic species (capital vs. income breeding, Stearns (1992)), coupled with differences in morphology and performance abilities of the larvae (Somarakis et al., 2000). At least for anchovy, for which concurrent adult fish sampling was carried out in June 1993 and June 1995, it has been shown that somatic condition, egg size, batch fecundity, and spawning frequency were significantly higher in 1993 (when adult prey fields -zooplankton- were higher) but spawning stock biomass was similar between 1993 and 1995 (Somarakis, 2005).

According to Frank & Leggett (1983), temporal and/or spatial covariation in the abundance of different species in the plankton implies similar inter-specific responses to the pelagic environment, i.e., co-adaptation of reproductive strategies (Moser & Smith, 1993). The synchrony in species larval abundance, similarities in links with physical and trophic variables and commonality of reproductive and early life history strategies imply common response to physical forcing and regime shifts and may explain the phenomenon of synchrony in recruitment and biomass trends among different populations (Doyle et al., 2009).

References

Allain, G., P. Petitgas, P. Lazure & P. Grellier, 2007. Biophysical modelling of larval, drift, growth, and survival for the prediction of anchovy recruitment in the Bay of Biscay (NE Atlantic). Fisheries Oceanography 16: 489–505.

Bernal, M., Y. Stratoudakis, S. Coombs, M. M. Angelico, A. L. de Lanzós, C. Porteiro, Y. Sagarminaga, M. Santos, A. Uriarte, E. Cunha, L. Valdés & D. Borchers, 2007. Sardine spawning off the European Atlantic coast: characterization of and spatio-temporal variability in spawning habitat. Progress in Oceanography 74: 210–227.

Christensen, V., 1996. Managing fisheries involving predator and prey species. Reviews in Fish Biology and Fisheries 6: 417–442.

Clarke, K. R. & R. M. Warwick, 1994. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation. Natural Environment Research Council, Plymouth Marine Laboratory, Plymouth.

Cleveland, R. B., W. S. Cleveland, J. E. McRae & I. Terpenning, 1990. STL: a seasonal-trend decomposition procedure based on Loess. Journal of Official Statistics 6: 3–73.

Cohen, E. B., D. G. Mountain & R. O'Boyle, 1991. Local-scale versus large scale factors affecting recruitment. Canadian Journal of Fisheries and Aquatic Science 48: 1003–1006.

Doyle, M. J., W. W. Morse & A. W. Kendall Jr, 1993. A comparison of larval fish assemblages in the temperate zone of the northeast Pacific and northwest Atlantic oceans. Bulletin of Marine Science 53: 588–644.

Doyle, M. J., S. J. Picquelle, K. L. Mier, M. C. Spillane & N. A. Bond, 2009. Larval fish abundance and physical forcing in the Gulf of Alaska, 1981–2003. Progress in Oceanography 80: 163–187.

ESRI, 1994. ARC Macro Language. Environmental Systems Research Institute Inc, Redlands: 3–37.

Field, J. G., K. R. Clarke & R. M. Warwick, 1982. A practical strategy for analysing multispecies distribution patterns. Marine Ecology Progress Series 8: 37–52.

Frank, K. T. & W. C. Leggett, 1983. Multispecies larval fish associations: accident or adaptation? Canadian Journal of Fisheries and Aquatic Sciences 40: 754–762.

Ganias, K., S. Somarakis, A. Machias & C. Koutsikopoulos, 2007. Factors affecting the spawning period of sardine in two highly oligotrophic Seas. Marine Biology 151: 1559–1569.

Gauch, H. G. Jr., 1982. Multivariate Analysis in Community Ecology. Cambridge University Press, Cambridge.

Heath, M. R., 1992. Field investigations of the early life stages of marine fish. Advances in Marine Biology 28: 1–174.

Hosie, G. W. & T. G. Cochran, 1994. Mesoscale distribution patterns of macrozooplankton communities in Prydz bay, Antarctica—January to February 1991. Marine Ecology Progress Series 106: 21–39.

Hsieh, C. -H., C. Reiss, W. Watson, M. J. Allen, J. R. Hunter, R. N. Lea, R. H. Rosenblatt, P. E. Smith & G. Sugihara, 2005. A comparison of long term trends and variability in populations of larvae of exploited and un-exploited fishes in the Southern California region: a community approach. Progress in Oceanography 67: 160–185.

Isari, S., A. Ramfos, S. Somarakis, C. Koutsikopoulos, A. Kallianiotis & N. Fragopoulu, 2006. Mesozooplankton distribution in relation to hydrology of the Northeastern Aegean Sea (eastern Mediterranean). Journal of Plankton Research 28: 241–255.

Isari, S., S. Psarra, P. Pitta, P. Mara, M. O. Tomprou, A. Ramfos, S. Somarakis, A. Tselepides, C. Koutsikopoulos & N. Fragopoulu, 2007. Differential patterns of mesozooplankters' distribution in relation to physical and biological variables of the northeastern Aegean Sea (eastern Mediterranean). Marine Biology 151: 1035–1050.

Isari, S., N. Fragopoulu & S. Somarakis, 2008. Interranual variability in horizontal patterns of larval fish assemblages in the northeastern Aegean Sea (eastern Mediterranean) during early summer. Estuarine, Coastal and Shelf Science 79: 607–619.

Koslow, A. J., 1984. Recruitment patterns in northwest Atlantic fish stocks. Canadian Journal of Fisheries and Aquatic Sciences 41: 1722–1729.

Koslow, A. J., K. R. Thompson & W. Silvert, 1987. Recruitment to Northwest Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) stocks: Influence of stock size and climate. Canadian Journal of Fisheries and Aquatic Sciences 44: 26–39.

Kruscal, J. B. & M. Wish, 1978. Multidimensional Scaling. Sage Publications, Beverly Hills.

Ludwig, D., R. Hilborn & C. Walters, 1993. Uncertainty, resource exploitation, and conservation: Lessons from history. Science 260: 17–36.

Moser, H. G. & P. E. Smith, 1993. Larval fish assemblages and oceanic boundaries. Bulletin of Marine Science 53: 283–289.

Moser, H. G., R. L. Charter, W. Watson, D. A. Ambrose, J. L. Butler, S. R. Charter & E. M. Sandknop, 2000. Abundance and distribution of rockfish (*Sebastes*) larvae in the Southern Calofornia Bight in relation to environmental conditions and fisheries exploitation. California Cooperative Oceanic Fisheries Investigations Reports 41: 132–147.

Mueter, F. J., J. L. Boldt, B. A. Megrey & R. M. Peterman, 2007. Recruitment and survival of Northeast Pacific Ocean fish stocks: temporal trends, covariation, and regime shifts. Canadian Journal of Fisheries and Aquatic Sciences 64: 911–927.

Myers, R. A., J. A. Hutchings & N. J. Barrowman, 1997. Why do fish stocks collapse? The example of cod in Atlantic Canada. Ecological Applications 7: 91–106.

Oguz, T., T. Cokacar, P. Malanotte-Rizzoli & H. W. Ducklow, 2003. Climatic warming and accompanying changes in the ecological regime of the Black Sea during 1990s. Global Biogeochemical Cycles 17(3): 1088. doi: 10.1029/2003GB002031.

Palomera, I., 1992. Spawning of anchovy *Engraulis encrasicolus*, in the North-Western Mediterranean relative to hydrographic features in the region. Marine Ecology Progress Series 79: 215–223.

Planque, B., E. Bellier & P. Lazure, 2007. Modelling potential spawning habitat of sardine (*Sardina pilchardus*) and anchovy (*Engraulis encrasicolus*) in the Bay of Biscay. Fisheries Oceanography 16: 16–30.

Poulos, S. E., P. G. Drakopoulos & M. B. Collins, 1997. Seasonal variability in sea surface oceanographic conditions in the Aegean Sea (Eastern Mediterranean): an overview. Journal of Marine Systems 13: 225–244.

Rotenberry, J. T. & J. A. Wiens, 1985. Statistical power analysis and community-wide patterns. American Naturalist 125: 164–168.

Sabatés, A., 1990. Distribution pattern of larval fish populations in the Northwestern Mediterranean. Marine Ecology Progress Series 59: 75–82.

Sabatés, A. & M. Maso, 1992. Unusual larval fish distribution pattern in a coastal zone of the western Mediterranean. Limnology and Oceanography 37: 1252–1260.

Sabatés, A. & M. P. Olivar, 1996. Variation of larval fish distributions associated with variability in the location of a shelf-slope front. Marine Ecology Progress Series 135: 11–20.

Smith, P. E. & S. L. Richardson, 1977. Standard techniques for pelagic fish eggs and larval surveys. FAO Fisheries Technical Paper 175: 1–107.

Somarakis, S., 1999. Ichthyoplankton of the Northeastern Aegean Sea with emphasis on anchovy, *Engraulis encrasicolus* (Linnaeus, 1758) (June 1993, 1994, 1995, 1996). PhD Thesis, University of Crete.

Somarakis, S., 2005. Marked inter-annual differences in reproductive parameters and daily egg production of anchovy in the northern Aegean Sea. Belgian Journal of Zoology 135: 247–252.

Somarakis, S. & N. Nikolioudakis, 2007. Oceanographic habitat, growth and mortality of larval anchovy (*Engraulis encrasicolous*) in the northern Aegean Sea (eastern Mediterranean). Marine Biology 152: 1143–1158.

Somarakis, S., B. Catalano & N. Tsimenides, 1998. Catchability and retention of larval European anchovy, *Engraulis encrasicolus*, with bongo nets. Fishery Bulletin 96: 917–925.

Somarakis, S., E. Maraveya & N. Tsimenides, 2000. Multispecies ichthyoplankton associations in epipelagic species:

is there any intrinsic adaptive function? Belgian Journal of Zoology 130(Suppl 1): 125–129.

Somarakis, S., P. Drakopoulos & V. Filippou, 2002. Distribution and abundance of larval fishes in the northern Aegean Sea—eastern Mediterranean—in relation to early summer oceanographic conditions. Journal of Plankton Research 24: 339–357.

Somarakis, S., I. Palomera, A. Garcia, L. Quintanilla, C. Koutsikopoulos, A. Uriarte & L. Motos, 2004. Daily egg production of anchovy in European waters. ICES Journal of Marine Science 61: 944–958.

Stanev, E. V. & E. L. Peneva, 2002. Regional sea level response to global climatic change: Black Sea examples. Global and Planetary Changes 32: 33–47.

Stearns, S. C., 1992. The Evolution of Life Histories. Oxford University Press, New York.

Stergiou, K. I., E. D. Christou, D. Georgopoulos, A. Zenetos & C. Souvermezoglou, 1997. The Hellenic Seas: physics, chemistry, biology and fisheries. Oceanography and Marine Biology Annual Review 35: 415–538.

Stratoudakis, Y., S. Coombs, A. L. De Lanzós, N. Halliday, G. Costas, B. Caneco, C. Franco, D. Conway, M. B. Santos, A. Silva & M. Bernal, 2007. Sardine (*Sardina pilchardus*) spawning seasonality in European waters of the northeast Atlantic. Marine Biology 152: 201–212.

Tsikliras, A. C., E. Antonopoulou & K. I. Stergiou, 2010. Spawning period of Mediterranean marine fishes. Reviews in Fish Biology and Fisheries 20(4): 499–538.

Zervakis, V. & D. Georgopoulos, 2002. Hydrology and circulation in the North Aegean (eastern Mediterranean) throughout 1997 and 1998. Mediterranean Marine Science 3: 5–19.

Hydrobiologia (2011) 670:289–306
DOI 10.1007/s10750-011-0665-9

ECOSYSTEMS AND SUSTAINABILITY

An overview of commercial fishers' attitudes towards marine protected areas

Cristina Pita · Graham J. Pierce ·
Ioannis Theodossiou · Karen Macpherson

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract Marine protected areas (MPAs) are attracting widespread attention worldwide as a tool for fishery management and marine ecosystem conservation. The establishment of MPAs has increased greatly in recent years mostly due to international commitments to the establishment of a global network of MPAs by 2012. MPAs have the potential to strongly affect the fishing industry, and their success depends, at least partly, on fishers' attitudes towards this management measure. However, research on MPAs tends to focus on the ecological and conservation aspects of this management approach and not on its human dimensions. Studies in attitudes, perceptions, beliefs and preferences related to MPA issues have been identified as priority social science topics in need of research. We present a 'rapid review', conducted systematically, of the literature published up to September 2009 and aimed at identifying the most investigated topics related to commercial fishers' attitudes towards MPAs, describing the main findings from these studies, and analysing the implications for management. Most published work focuses on fishers' attitudes towards issues of governance, conservation of biodiversity and the environment, and the impact of MPAs on fishing activity. Despite the recent increase in the literature on the human dimensions of MPAs, the present review reveals that little of this literature originates from empirical studies. Hence, given the forthcoming increase in the implementation of MPAs in the near future, research on fishers' attitudes towards these management measures is critically needed.

Keywords Fishers · Rapid review · Systematic · Marine protected areas · Attitudes · Perceptions · Beliefs

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

---

C. Pita · I. Theodossiou
Business School, Department of Economics, University of Aberdeen, Edward Wright Building, Dunbar Street, Aberdeen AB24 3QY, UK

C. Pita (✉)
Aberdeen Centre for Environmental Sustainability (ACES), School of Biological Sciences, University of Aberdeen, Tillydrone Avenue, Aberdeen AB24 2TZ, UK
e-mail: c.pita@abdn.ac.uk

G. J. Pierce
Institute of Biological and Environmental Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen AB24 2TZ, UK

G. J. Pierce
Instituto Español de Oceanografía,
Centro Oceanográfico de Vigo,
P.O. Box 1552, 36200 Vigo, Spain

K. Macpherson
NHS Quality Improvement Scotland, Delta House,
50 West Nile Street, Glasgow G1 2NP, UK

Introduction

A marine protected area (MPA) is a generic term used to describe a wide range of marine areas which afford different protection and conservation strategies. MPAs have been established for a multitude of purposes but mostly as tools for fishery management and the conservation of species or habitats (Jones, 1994; Sumaila & Charles, 2002; Agardy et al., 2003; Guarderas et al., 2008; Guidetti et al., 2008). As such, MPAs vary widely in type and level of protection applied, ranging from areas that allow multiple-use to areas that restrict all human access (Gubbay, 1995; Kelleher, 1999; IUCN-WCPA, 2008). Sites which fit the definition of MPAs have been given a variety of names, including amongst others, marine reserves, sanctuaries, parks, no-take zones or areas, fishery exclusion zones, fishery reserves, closed areas. Besides the broad range of terms, the definition of the same term may vary significantly from one region or country to another (Christie & White, 2007). For example, in Kenya ‘marine reserves’ allow for non-destructive forms of fishing, whereas in Tanzania ‘marine reserves’ are no-take areas (IUCN-WCPA, 2008).

Marine protected areas have existed for hundreds of years, however, most statutory MPAs are quite recent and their establishment has increased greatly in recent years. Silva et al. (1986) listed 430 MPAs created by 1985, 10 year later Kelleher et al. (1995) estimated that this number had increased to more than 1,300 and, more recently, Wood (2007) reported that approximately 5,000 MPAs had been designated worldwide. This figure is now (in 2010) likely an under-estimate given the rapid progress in the implementation of this management approach. The recent rapid growth in the global establishment of MPAs observed so far has resulted mostly from international commitments initiated at the Johannesburg 2002 World Summit on Sustainable Development (WSSD), which called for the establishment of a global representative network of MPAs to restore degraded aquatic ecosystems and fish stocks to healthy levels by 2012. Following this, several other international commitments, such as the Evian agreement signed by the G8 group of nations in 2003 (Evian Summit—Marine Environment and Tanker Safety: G8 Action Plan, Evian, June 3, 2003, France), and the United Nations Convention on Biological Diversity in 2004, further reinforced the call for a network of MPAs. These global undertakings were complemented by various international agreements at more regional levels. For instance, at the European level the agreement by OSPAR and HELCOM members in 2003 (‘OSPAR Convention’—Convention for the Protection of the Marine Environment of the North-East Atlantic; ‘Helsinki Convention’—Convention on the Protection of the Marine Environment of the Baltic Sea Area), and recent legislation (the Marine Strategy Framework Directive (MSFD), Directive 2008/56/EC, of 17 June 2008) reinforce the call for the use of MPAs and MPA networks within European waters.

Marine protected areas are increasingly recognised as having linked social and ecological dynamics (Agardy, 2000; Mascia, 2004; Pomeroy et al., 2007; Charles & Wilson, 2009; Pollnac et al., 2010). However, most published research tends to explore the ecological and conservation aspects of MPAs and not its social, economic, cultural, political and institutional implications. Only recently has the literature started to address the human dimensions of MPAs; covering topics on socio-economic and cultural aspects of MPAs, MPA governance, and a broad exploration of the human dimensions of MPAs (e.g. Bunce et al., 1999; Mascia, 1999, 2003; Pollnac et al., 2001; Cicin-Sain & Belfiore, 2005; Christie & White, 2007; Jentoft et al., 2007; Pomeroy et al., 2007; Charles & Wilson, 2009).

Studies in attitudes, perceptions, beliefs and preferences related to MPA issues have been recently identified as priority social science topics in need of research by governmental agencies and NGOs highly involved in the implementation of MPA (see Wahle et al., 2003; Pomeroy et al., 2004, 2005; NOAA, 2005).

As mentioned previously, although MPAs have been established for a multitude of reasons, they are principally established either for marine ecosystem conservation or for sustainable fisheries exploitation. As such, commercial fishers are amongst those most directly affected by MPAs (Badalamenti et al., 2000; Jones, 2008) and that can impact on MPAs the most. The performance of MPAs depends, at least partly, on fishers’ behaviour (Dimech et al., 2009; Suuronen et al., 2010) and a failure to understand their attitudes and perceptions towards issues related to MPAs may undermine their success (Himes, 2007; Jones, 2008; Charles & Wilson, 2009; Dimech et al., 2009).

Research on fishers' attitudes, beliefs, perceptions and preferences related to MPA issues examines the underlying motivations that may influence fishers' preferences, choices and actions (Wahle et al., 2003). As such, understanding the attitudes and perceptions of fishers towards MPAs could help predict their likely behaviour towards this management tool and contribute to its success.

Given the importance of the attitudes of commercial fishers to the success of MPAs we carried out a 'rapid review' in order to compile the literature which reports empirical evidence on commercial fishers' attitudes, perceptions, opinions and beliefs about MPAs. We also aimed to identify which are the most investigated issues, synthesise the main findings and analyse the implications for management and governance.

The rapid review was carried out systematically following Petticrew & Roberts (2006) guidelines. Systematic reviews are a form of structured literature review which aim to comprehensively identify, appraise and synthesize all relevant studies on a given topic in a systematic and critical fashion. Research results on a specific topic are sought using a 'search strategy' designed to locate as many sources of information as possible. Once the sources of data have been identified, they are assessed against pre-set criteria. All the stages of the process are clearly documented such that the work can be repeated and the same results obtained (Petticrew & Roberts, 2006). Systematic reviews are widely used as an aid to evidence-based decision-making. They are extensively used in the medical sciences, and more recently systematic reviews have been undertaken in a variety of fields, including biology, ecology and economics (Petticrew, 2001; Petticrew & Roberts, 2006). Several sets of guidelines for conducting systematic reviews in the social sciences and in conservation and environmental management have been described (Oxman, 1994; Petticrew & Roberts, 2006; Pullin & Stewart, 2006; CEBC, 2009).

Increasingly, due to time and financial constraints, rapid reviews rather than full systematic reviews are being undertaken (Watt et al., 2008a, b). The scope of rapid reviews are limited to enable the work to be undertaken as robustly as a systematic review but in a shorter period of time (Ganann et al., 2010). For instance, the search may be limited to certain years, range of databases searched, range of languages included, or by not extending the search beyond electronic searches (Petticrew & Roberts, 2006; Watt et al., 2008a, b; Ganann et al., 2010). All such limitations should be clearly specified in the reporting of the study.

The rapid review approach was chosen for the purposes of this study due to time and financial constraints and because it was considered that this would be suitable for providing this first overview on this topic.

Methodology

Search strategy and inclusion criteria

The current review consists of a literature search for relevant studies published in peer-reviewed journals, up to September 2009. The literature search was carried out by searching all databases in ISI Web of Knowledge, Scopus, Science Direct, Blackwell Synergy, IngentaConnect and JSTOR. A sensitive systematic search strategy which combined the terms 'marine protected areas', together with 'attitudes', 'perceptions', 'beliefs' or 'opinions', and 'fishers' and their synonyms, and used the truncation features of the databases was employed. The search strategy is available from the authors on request. Titles and abstracts were scanned by C.P. to identify studies potentially eligible for inclusion. A 20% sample of the retrieved records was also scanned by a second reviewer (K.M.). No major disagreements arose and any discrepancies in the studies selected for inclusion were resolved by discussion between the authors. The full text of the initially selected studies was then retrieved and a further selection process undertaken. References in all relevant papers were screened for additional papers. Criteria for inclusion in the rapid review were restricted to the following: (1) the study included a survey of commercial fishers; (2) the study reported data on attitudes, perceptions, opinions or beliefs about MPAs; (3) the study was published in a peer-reviewed journal indexed in the databases before September 2009.

The review question was intentionally left broad with the aim of identifying all articles investigating fishers' attitudes, perceptions and opinions on issues related to MPAs. Our rationale for specifying a broad research question was that no previous attempt has been made to review systematically any of the work published in this field. Also, and although systematic reviews often benefit from spatial restrictions as country or cultural context may severely impact outcomes (Egan et al., 2009), no country or language restrictions were included in the search in order to collect all available data (i.e. although the search terms were in English, due to all the databases searched being indexed and having titles and abstracts available in English, no studies were excluded on the basis of being published in another language).

Data extraction and analysis

Data extraction followed the guidelines developed by Petticrew & Roberts (2006). Data extraction of the included studies was undertaken by C.P. The data extraction process was quality assured by K.M. who checked a random 20% sample of the data extracted. Extracted data included: descriptions of study sites (geographical boundaries, history of implementation of MPA, and management, governance, socio-economic and cultural aspects); information regarding the MPA (type, year of implementation, objectives, size, purpose of designation); description of the fishing activity; methodological information (number of participants in the survey, response rates, sample design, selection criteria, number of study sites, population size); method of data analysis; main results and conclusions. Some of the methodology and analysis undertaken in the studies are reported in Table 1.

The included articles were analysed descriptively. Statistical analysis or meta-analysis was not attempted due to the variability among included studies in their research questions, research methods, data collection methodologies, type of data collected and analysis conducted.

Results

Included studies

The initial literature search identified a total of 115 potentially relevant articles. After the examination of the abstracts, 84 were excluded due to not meeting the inclusion criteria and full text of the remaining 31 were obtained and considered for inclusion; of those, 17 were subsequently excluded due to not meeting the inclusion criteria. The screening of the reference lists of the studies considered for inclusion identified a further 44 potential relevant articles, 42 of which were subsequently found not to meet the selection criteria. In total, 16 articles met the inclusion criteria and were selected to be used in the review. See Fig. 1 for the flowchart showing the stages of identification of studies in the review.

The articles included in the review are summarised in Table 2. The articles reported surveys conducted in both developed and developing countries, related to a wide array of types of MPA designations, implemented both with conservation and fishery management purposes. The studies gathered information either solely from commercial fishers or from commercial fishers and other stakeholders. Furthermore, the review revealed that articles about fishers' attitudes, perception, opinions and beliefs regarding issues related to MPAs focused basically on three different topics; fishers' attitudes, perceptions and opinions regarding: (i) governance issues related to MPAs, (ii) environment and biodiversity conservation issues related to MPAs, and (iii) the impact of MPAs on the fishing activity. Most surveys (81%) gathered information on more than one of these topics.

Governance issues related to MPAs

Most articles reported on fishers' perception, attitudes, opinions or beliefs concerning governance issues related to MPAs. Information on this topic was collected in 14 out of the 16 studies, and the studies assessed fishers' perceptions on a wide array of issues related to governance. Ten articles reported on fishers' attitudes, perceptions or opinions regarding MPAs as management tools (Blyth et al., 2002; Mangi & Austen, 2008) and on the acceptance and effectiveness of these management measures (Suman et al., 1999; Himes, 2003; Gelcich et al., 2005; McClanahan et al., 2005, 2008; Jones, 2008; Oikonomou & Dikou, 2008; Suuronen et al., 2010). Three articles also reported on fishers' perceptions of costs and benefits of MPAs (Gelcich et al., 2005, 2009; McClanahan et al., 2008). Five articles reported information on fishers' attitudes regarding empowerment and participation in the management and

Table 1 Summary of methodology and analysis undertaken in the studies included in the systematic review

Study	Year of survey	Data collection method	Sampling method	Sample size		Main questions format	Comparison groups			Statistical analysis	
				Commercial fishers	Other		None	Different groups fishers	Fishers and other groups	Univariate/ bivariate	Multivariate
Blyth et al. (2002)	NA	Face-to-face semi-structured interviews	NA	17	3	Open-ended	–	+	–	+	+
Dimech et al. (2009)	2006	Questionnaires by telephone interview	Stratified	194	47	Likert-scale	–	+	–	+	+
Gelcich et al. (2005)	2003	Questionnaires and PRA	Random	60		Likert-scale	–	+	–	+	+
Gelcich et al. (2008a)	2004	Group sessions and face-to-face structured questionnaires	Random	217		Likert-scale	+	+	–	+	+
Gelcich et al. (2009)	2006	Face-to-face questionnaires, semi-structured interviews and focus groups	Judgement (representatives from fishing unions), random (fishers)	143		Likert-scale	+	+	–	+	+
Himes (2003)	2001	Face-to-face questionnaires and unstructured interviews	Snowball	94		Open-ended, categorical	–	+	–	–	–
Jiménez-Badillo (2008)	2005	Observational study, structured questionnaires, focus groups	Random	396		Multiple-choice	–	+	–	–	–
Jones (2008)	2005	Semi-structured interview	NA	37	20	Open-ended, categorical	+	+	+	–	–
Mangi & Austen (2008)	2007	Face-to-face questionnaires	NA	48	172	Ranking and rating approaches, Likert-scale	–	–	+	+	+
McClanahan et al. (2005)	2003	Self-completion questionnaires	Interview large proportion fishers	224	44	Rating scale	–	+	+	+	+
McClanahan et al. (2008)	2007	Face-to-face questionnaires	Systematic	184[a]	14	Likert-scale, other rating scale	–	+	+	+	+
Oikonomou & Dikou (2008)	2005	Self-completion questionnaires	Random	30	151	Likert-scale	–	–	+	+	–
Stump & Kriwoken (2006)	2002	Face-to-face and telephone surveys	Random	51		Likert-scale, categorical	–	+	–	–	–

Table 1 continued

Study	Year of survey	Data collection method	Sampling method	Sample size		Main questions format	Comparison groups			Statistical analysis	
				Commercial fishers	Other		None	Different groups fishers	Fishers and other groups	Univariate/ bivariate	Multivariate
Suman et al. (1999)	1996	Face-to-face questionnaires and mail surveys	Random	337	332	Likert-scale	–	–	+	+	–
Suuronen et al. (2010)	2007	Focus groups and survey	Judgment	22		Open-ended	+	–	–	–	–
Tonder & Jurvelius (2004)	1999	Face-to-face semi-structured interviews	NA	4	34	Open-ended	–	–	+	–	–

NA information not available, *PRA* participatory rural appraisal

[a] Sample size. Survey was carried out to head of households and all were involved in fisheries (information provided by authors)

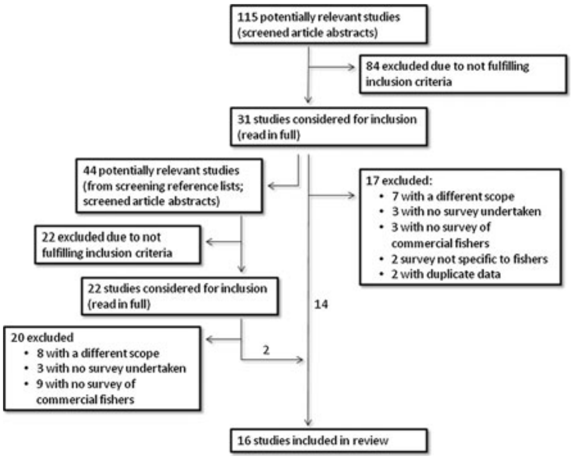


Fig. 1 Flowchart showing the stages of identification of studies in the systematic review

decision-making processes (Suman et al., 1999; Himes, 2003; Stump & Kriwoken, 2006; Oikonomou & Dikou, 2008; Gelcich et al., 2009). Three studies reported on fishers' perceptions regarding conflicts between different resource-users (Blyth et al., 2002; Dimech et al., 2009; Suuronen et al., 2010). And finally, eight articles reported on fishers' opinions concerning issues of compliance with, and enforcement of, rules and regulations (Blyth et al., 2002; Himes, 2003; Stump & Kriwoken, 2006; Jiménez-Badillo, 2008; Jones, 2008; Dimech et al., 2009; Gelcich et al., 2009; Suuronen et al., 2010).

Marine protected areas implemented for fishery management purposes (fishery MPAs) were generally accepted by fishers as effective and beneficial management tools (Suman et al., 1999; Himes, 2003; Gelcich et al., 2005; McClanahan et al., 2005; Jones, 2008; Dimech et al., 2009; Suuronen et al., 2010), while MPAs implemented for conservation purposes (conservation MPAs) were perceived less favourably (Suman et al., 1999; Himes, 2003; Tonder & Jurvelius, 2004; McClanahan et al., 2005, 2008; Jones, 2008; Oikonomou & Dikou, 2008; Gelcich et al., 2009).

Fishers' perceptions about the benefits and effectiveness of fishery MPAs over conservation MPAs do not seem to be affected by geographical location or different cultural backgrounds. Himes (2003) noticed that the majority of Italian fishers from within the fishery MPA, the Gulf of Castellammare Fishery Reserve (GCFR), perceived that overall this measure was successful and beneficial, while fishers from the

Table 2 Summary of studies included in the systematic review

Study	Country	MPA designation	MPA name	Year implemented	Purpose of MPA	Fishing within the MPA	Stakeholders in the survey		Topics investigated in the survey		
							Commercial fishers	Other	Governance	Conservation	Impact on fishing
Blyth et al. (2002)	UK	Voluntary fishery management system	Inshore Potting Agreement (IPA)	1978 (legally recognised in 2002)	Fishery management	Fishing allowed, with varying levels of temporal and spatial restrictions	+	−	+	+	+
Dimech et al. (2009)	Malta	Fisheries management Zone (FMZ)	Maltese Fisheries Management Zone	1971	Fishery management	Fishing allowed, with several restrictions (vessel sizes, fishing effort, spatial restrictions)	+	+	+	+	+
Gelcich et al. (2005)	Chile	Marine Management Area	Management and Exploitation Areas for Benthic Resources (MEABR)	Prior to implementation	Fishery management	Fishing allowed, with spatial restrictions in some areas	+	−	+	+	−
Gelcich et al. (2008a)	Chile	Marine Management Area	Management and Exploitation Areas for Benthic Resources (MEABR)	NA	Fishery management	Fishing allowed, with spatial restrictions in some areas	+	−	−	+	−
Gelcich et al. (2009)	Chile	Marine Management Area	Management and Exploitation Areas for Benthic Resources (MEABR)	1997	Fishery management	Fishing allowed, with spatial restrictions in some areas	+	−	+	+	−

Table 2 continued

Study	Country	MPA designation	MPA name	Year implemented	Purpose of MPA	Fishing within the MPA	Stakeholders in the survey		Topics investigated in the survey		
							Commercial fishers	Other	Governance	Conservation	Impact on fishing
Himes (2003)	Italy	Fishery reserve and Marine reserve	Egadi Islands Marine Reserve (EIMR), Gulf of Castellammare Fishery Reserve (GCFR)	1990 (EIMR), 1991 (GCFR)	Conservation and fishery management	Fishing allowed, EIMR divided into 4 zones with varying levels of spatial restrictions and GCFR closed to all bottom-towed gears	+	–	+	–	+
Jiménez-Badillo (2008)	Mexico	Marine park	Veracruz reef system marine park	1992	Conservation	Fishing allowed, with seasonal and gear closures	+	–	+	+	–
Jones (2008)	UK	No-take MPAs (NTMPAs)	NTMPAs in the South-west of England	Prior to implementation	Conservation	No fishing allowed	+	+	+	–	+
Mangi & Austen (2008)	International	Several designations of MPAs	Several MPAs in the Mediterranean Sea	Oldest in 1974, youngest to be implemented	Conservation and fishery management	Ranged from no-take zones to areas with limited fishing (seasonal or gear closures)	+	+	+	+	+
McClanahan et al. (2005)	Kenya	Several designations of MPAs (park and reserve)	3 MPAs (Diani, Malindi-Watamu, Mombasa)	Oldest 1968, youngest 1994	NA	Ranged from closed area to gear-restricted areas	+	+	+	–	+

Table 2 continued

Study	Country	MPA designation	MPA name	Year implemented	Purpose of MPA	Fishing within the MPA	Stakeholders in the survey		Topics investigated in the survey		
							Commercial fishers	Other	Governance	Conservation	Impact on fishing
McClanahan et al. (2008)	Tanzania	Marine park	Mafia Island Marine Park	1995	Conservation	Ranged from no-take zones to areas with general use (closed to illegal and destructive gear)	+	+	+	−	+
Oikonomou & Dikou (2008)	Greece	National Marine Park	National Marine Park of Alonissos (NMPANS)	1992	Conservation	Fishing allowed, with several prohibitions	+	+	+	+	+
Stump & Kriwoken (2006)	Australia	MPAs in general	System of MPAs in Tasmania	Prior to expansion in number	Conservation	NA	+	−	+	−	+
Suman et al. (1999)	USA	Marine sanctuary	Florida Keys National Marine Sanctuary (FKNMS)	1990	Conservation	Several degrees of protection (closed areas to areas where fishing is allowed)	+	+	+	−	−
Suuronen et al. (2010)	Sweden	Network of box closures	Box closures in the Baltic	First 1997 (subsequent increase in area)	Fishery management	No fishing allowed	+	−	+	−	+
Tonder & Jurvelius (2004)	Finland	Nature 2000 site	Lake Pihlajavesi Natura 2000 site	NA	Conservation	Fishing allowed	+	+	−	+	−

NA information not available

conservation MPA, the Egadi Islands Marine Reserve (EIMR), did not perceive benefits from this management measure. McClanahan et al. (2005) noted this same trend amongst Kenyan fishers, whom perceived more benefits from the gear-restricted areas (reserves) than from fisheries exclusion zones (parks). Blyth et al. (2002) noticed that UK fishers from the South-west of England, perceived the Inshore Potting Agreement (IPA), a fishery MPA, as a good system which protects traditional practices and the local fishing industry. More recently, Jones (2008) surveyed fishers also from the South-west of England about their general attitudes towards no-take MPA (prior to their implementation) and noticed that these fishers perceived these conservation MPAs should not be the way forward. Gelcich et al. (2005, 2009) noticed this same trend amongst Chilean fishers. The former noticing that most fishers perceived the Management and Exploitation Areas for Benthic resources (MEABR), a fishery MPAs, to be beneficial and effective and the latter that fishers did not see any benefits from no-take MPAs.

Only one study investigated fishers' attitudes towards a fishery MPA where no fishing was allowed (i.e. a no-take fishery MPAs). The survey was carried out to Swedish fishers targeting cod in the Baltic and collected information about their attitudes towards the network of box closures in the Baltic (closed in order to reduce total effort and fishing mortality of cod) (Suuronen et al., 2010). The survey found that fishers were overly pessimistic with respect to the no-take MPAs, considering their objectives to be inconsistent; they didn't think that reducing effort through the implementation of the network of box closures actually reduced the fishing mortality of cod. They found the no-take MPAs inefficient in the conservation of cod stocks; frequently emphasising that no-take MPAs cannot effectively protect migrating fish such as cod. They were also of the opinion that the enlargement of one of the boxes was unfair, and that the locations of the boxes was inappropriate and ineffective from a conservation point of view. Three other surveys investigated fishers' attitudes towards no-take MPAs, but they collected information about fishers' attitudes prior to the implementation of the MPAs. Stump & Kriwoken (2006) investigated Tasmanian fishers' attitudes towards the establishment of additional no-take MPAs. They found that fishers were of the opinion that an increase on the number of no-take MPAs was not necessary and that they were concerned with the possible deleterious impact an increase would have on the sustainability of Tasmania's commercial fisheries. Jones (2008) and Gelcich et al. (2009) investigated, respectively, UK and Chilean fishers' general attitudes towards no-take MPAs. Jones (2008) found that overall fishers were of the opinion that no-take MPAs are not good management measures, and that they should not be the way forward, considering that improving the wider-scale fisheries management approaches to be best. Gelcich et al. (2009) found that the vast majority of fishers showed a lack of understanding of the benefits associated with no-take MPAs. Furthermore, they perceived that the implementation of no-take MPAs would be highly controversial and that it would result in conflicts with authorities. UK and Tasmanian fishers also recognised the difficulty of achieving compliance with and enforcement of these particular measures (Stump & Kriwoken, 2006; Jones, 2008).

Swedish fishers perceived no-take fishery MPAs to increase gear conflicts outside the MPA (Suuronen et al., 2010). While, fishery MPAs in which fishing is allowed were perceived to avoid conflicts between different gear-users (Blyth et al., 2002; Dimech et al., 2009). In the case of the UK this is actually the primary function of the IPA (Blyth et al., 2002). However, Maltese fishers also perceived the Fisheries Management Zone (FMZ) (a 25-NM zone around Malta's waters created in the interests of fishery conservation which has limitations on fishing effort, vessels size and spatial restrictions on certain types of fishing activities) to reduce conflicts among users and that it was a good management measure for the local economy (Dimech et al., 2009).

Fishers' perceptions on issues of compliance with and enforcement of MPA rules and regulation were somewhat more variable. Chilean fishers perceived compliance with policy requirements of the MEABR to be good; however, they saw the lack of effective enforcement as a problem (Gelcich et al., 2009). On the other hand, UK and Mexican fishers recognised a lack of compliance with rules (Blyth et al., 2002; Jiménez-Badillo, 2008). All UK mobile-gear fishers admitted to fishing inside the IPA static-gear only zones (at the time of the survey the IPA was a voluntary agreement, nowadays its statutory) (Blyth et al., 2002). Mexican fishers recognised a lack of compliance with regulations in general and specially with the closed season (Jiménez-Badillo, 2008). Furthermore, Maltese and Italian fishers perceived MPAs to have no impact on illegal fishing (Himes, 2003; Dimech et al., 2009). The Italian fishers also perceived the enforcement of regulations to be lacking. In fact, local fishers from the areas around the marine reserve (EIMR) perceived the lack of enforcement to be one of the main causes of management failure in the reserve (Himes, 2003). Swedish fishers also perceived the enforcement of regulations to be inadequate. They were of the opinion that the inadequate landings inspection system in the Baltic cod fishery had ruined fishers rule compliance and undermined the credibility of all management measures (Suuronen et al., 2010).

Participation in the management and decision-making processes was generally regarded as deficient (Suman et al., 1999; Himes, 2003; Stump & Kriwoken, 2006; Oikonomou & Dikou, 2008) and fishers who already were involved in the management process sought further empowerment (Gelcich et al., 2009). Suman et al. (1999) noticed that US fishers from the Florida Keys National Marine Sanctuary (FKNMS) displayed a high degree of alienation from the public process. Stump & Kriwoken (2006) noticed that less than half of the Tasmanian fishers interviewed participated in the public consultation process that accompanied the development of the MPA strategy. They were dissatisfied with the consultation process and the way the information had been delivered. Oikonomou & Dikou (2008) found that Greek fishers from the National Marine Park of Alonissos (NMPANS) perceived a total lack of communication with management bodies. Himes (2003) also noticed that most Italian fishers perceived communication to be lacking.

The length of time of MPAs existence affects fishers' perceptions of MPAs in different ways. McClanahan et al. (2005) noticed that Kenyan fishers from areas adjacent to older MPAs perceived more benefits from these management measures. While Oikonomou & Dikou (2008), on the other hand, noticed that Greek fishers' acceptance and support for the MPA decreased over time. These fishers emphasised that although they had been initially supportive of the establishment of the marine park, they were currently upset with the decrease in fish stocks and fishing grounds, and the lack of compensations for damages to gear caused by the Mediterranean monk seal. Mangi & Austen (2008) also noticed that fishers from several other Mediterranean countries believed that the potential of MPAs to deliver fisheries objectives declines the longer the MPA has been designated.

Several articles compared attitudes of fishers and other stakeholder groups towards several issues related to MPAs and noticed that in some cases attitudes varied and that in other cases fishers and other stakeholder groups expressed the same attitudes. Suman et al. (1999) noticed that the support for the FKNMS varied between different stakeholders groups, with fishers not supporting the MPAs while other stakeholders (divers and environmental group members) supported it. Mangi & Austen (2008) carried out surveys in several countries in the Mediterranean in order to assess the perceptions of stakeholders of the importance of MPAs as areas for conservation, fisheries management, research and education and tourism development. They found that fishers and other stakeholders (governmental officials, MPA managers, researchers, conservationists, recreational users and local residents) tended to have divergent views about what should be the most important objective of implementing MPAs. Fishers perceived fisheries management as the most important reasons to establish MPAs and conservation the less important, while government officials and MPA managers expressed exactly the opposite view. All other stakeholder groups scored conservation higher than fisheries management as a reason to establish MPAs (Mangi & Austen, 2008). On the other hand, several other surveys found that fishers and other stakeholder groups expressed similar attitudes towards MPAs. Jones (2008) found that, overall, neither fishers nor other stakeholders related to fishing (mainly enforcement officers) in the South-West Coast of England perceived no-take MPAs as good management measures and both groups expressed strong disagreement with their implementation, considering that it would be best to improve the wider-scale fisheries management approaches instead. Oikonomou & Dikou (2008) reported that there was a general conviction amongst the local community (fishers and other stakeholders) in the marine park in Greece that the management of the MPA was ineffective. McClanahan et al. (2005, 2008) found that Kenyan and Tanzanian fishers and other stakeholders (park services, marine attendants, fisheries officers and fisheries department personnel) perceived MPAs to benefit the government more than fishers and local communities.

Conservation issues related to MPAs

Nine articles reported information regarding fishers' perceptions, attitudes, opinions or beliefs concerning issues related to the conservation of biodiversity or the environment. Studies gathered information mostly on fishers' opinions about the role and importance of MPAs as sites for the conservation of marine biodiversity (Mangi & Austen, 2008; Oikonomou & Dikou, 2008; Dimech et al., 2009; Gelcich et al., 2009), protection of marine species (Blyth et al., 2002; Gelcich et al., 2005) and protection of marine habitats (Blyth et al., 2002). Some studies also reported on fishers' general attitudes towards conservation and the value of natural resources (Tonder & Jurvelius, 2004; Gelcich et al., 2005; Jiménez-Badillo, 2008), perceptions regarding environmental issues (Gelcich et al., 2008a), and attitudes towards conservation measures (Tonder & Jurvelius, 2004).

Fishers displayed a range of attitudes regarding the conservation value of MPAs. Some studies found that fishers perceived MPAs to protect biodiversity and the environment (Blyth et al., 2002; Gelcich et al., 2005, 2008a, 2009; Jiménez-Badillo, 2008) and some that they did not (Oikonomou & Dikou, 2008; Dimech et al., 2009).

Differences in fishers' perception regarding MPAs as conservation measures were observed between different groups of fishers within the same study. Blyth et al. (2002) noticed that different gear-users in the south coast of England had different perceptions regarding MPAs ability to protect biodiversity. While most static-gear fishers were of the opinion the IPA protected benthic habitats, mobile-gear fishers perceived it did not, being of the opinion that static gears also caused damage. Regardless of this, fishers from both sectors agreed that the IPA functions as a reserve for species targeted by the towed sector. Gelcich et al. (2005, 2008a, 2009), surveying Chilean fishers from different fishing syndicates and livelihood strategies in different times, also found that they perceived MEABRs to be important for the maintenance of benthic resources and fish biodiversity, acting as reserves for resources. However, differences were observed between fishers from different fishing syndicates regarding their perceptions about conservation and the value of natural resources, with some having strong positive attitudes towards conservation and the intrinsic value of natural resources and others perceiving natural resources primarily as a source of income (Gelcich et al., 2005, 2008a). Jiménez-Badillo (2008) also noticed that Mexican fishers had a positive attitude towards conservation and that they were conscious of the need to conserve resources. The vast majority of Mexican fishers in the survey recognised that local marine resources had deteriorated and nearly half of them regarded overfishing as the cause of resource change. However, they perceived fishing resources primarily as a source of income. On the other hand, Dimech et al. (2009) noticed that most Maltese fishers had a negative or neutral perception that the FMZ helps to protect biodiversity.

Tonder & Jurvelius (2004) and Oikonomou & Dikou (2008) investigated fishers and other stakeholders' perceptions towards MPAs implemented for the protection of seals in different locations, the first in a Natural Marine Park in Greece and the latter in a Natura 2000 site in Finland. Both studies found that local individuals were of the opinion that the MPA was unnecessary. Furthermore, the Finnish fishers were unsympathetic towards the MPA even though the restrictions in the area did not apply to the gear most often used (Tonder & Jurvelius, 2004).

In general, stakeholders expressed more favourable attitudes towards MPAs as conservation tools the longer the MPA had been established. Mangi & Austen (2008), comparing fishers and other stakeholders from recent and longer established MPAs in the Mediterranean, found that their perceptions about the conservation value of MPAs progressively increased with the length of time that an MPA had been in place. Gelcich et al. (2008a) comparing Chilean fishers from different fishing syndicates, which were involved with the MEABR policy for various periods of time, noticed that there was a significant relationship between the amount of time engaged with the policy and positive environmental perceptions.

Impact of MPAs on the fishing activity

Information related to fishers' perceptions, attitudes, opinions or beliefs regarding the impact of MPAs on the fishing activity was reported in 10 articles. Most of the studies gathered information precisely on fishers' perceptions regarding the impact of MPAs on their fishing activity (Himes, 2003; Stump & Kriwoken, 2006; Dimech et al., 2009; Suuronen et al., 2010). A few studies also collected information of fishers' perceptions of the benefits of MPAs for fish abundance and enhancement of stocks inside and outside the MPA area (Blyth et al., 2002; Stump & Kriwoken, 2006; Jones, 2008; Oikonomou & Dikou, 2008; Dimech et al., 2009; Suuronen et al., 2010). Five studies collected information on fishers' preferences for different types of closures (e.g. seasonal closures, spatial restrictions, gear restrictions) (McClanahan et al., 2005, 2008; Jones, 2008; Mangi & Austen, 2008; Suuronen et al., 2010).

For the most part, fishers perceived conservation MPAs and fishery MPAs with severe restrictions to have an overall negative impact on their fishing activity (Himes, 2003; Jones, 2008; Oikonomou & Dikou, 2008; Dimech et al., 2009; Suuronen et al., 2010). They tended to prefer gear-restriction zones or seasonal closures over more restrictive measures (McClanahan et al., 2005, 2008; Jones, 2008; Mangi & Austen, 2008; Suuronen et al., 2010).

Fishers had rather negative perceptions regarding the ability of MPAs to enhance fish abundance inside the protected areas or benefit stocks outside MPA boundaries (Stump & Kriwoken, 2006; Jones, 2008; Oikonomou & Dikou, 2008; Dimech et al., 2009; Suuronen et al., 2010). Swedish fishers found the no-take MPAs inefficient in the conservation of cod stock. They were of the opinion that in practice the cod boxes only displaced effort and increased the by-catch of juvenile cod out of the no-take area, mostly due to the intensified competition between different fleet segments outside the cod boxes (Suuronen et al., 2010). Maltese fishers mostly thought the FMZ did not benefit stocks outside the area and they had neutral perception about its ability to enhance fish abundance inside the area (Dimech et al., 2009). Greek fishers perceived fish stocks to be decreasing within the NMPANS (Oikonomou & Dikou, 2008). Both Tasmanian and UK fishers were concerned with the possible decline in catches in areas outside the no-take MPAs, due to perceiving that spillover will not compensate for loss of access and that the increase in effort in areas outside the no-take MPA will cancel any benefits from such areas (Stump & Kriwoken, 2006; Jones, 2008). Furthermore, UK fishers regarded fish stocks around south-west England to be too diverse, mixed and migratory for no-take MPAs to result in any benefits. They were of the opinion that the benefits for spawning grounds could be achieved through partial/seasonal closures (Jones, 2008). On the other hand, other surveys did find that fishers were of the opinion that MPAs acted as reserves to fishing resources (Blyth et al., 2002; Gelcich et al., 2005; Jiménez-Badillo, 2008).

Shortcomings of studies

Several problems which affect the reliability and validity of the studies were identified. The major shortcoming identified in several studies, which according to Petticrew & Roberts (2006) is quite common in the social sciences, was the vague reporting of methodological information. Some studies carrying quantitative research or that had a quantitative component as part of the research failed to report which survey sampling method was employed (Blyth et al., 2002; Mangi & Austen, 2008). Some studies failed to identify what proportion of the population was sampled (Gelcich et al., 2005, 2008a, 2009; Jiménez-Badillo, 2008; Mangi & Austen, 2008; McClanahan et al., 2005, 2008; Oikonomou & Dikou, 2008). Most failed to give information about the response rate (Himes, 2003; Gelcich et al., 2005, 2008a, 2009; Jiménez-Badillo, 2008; McClanahan et al., 2008; Oikonomou & Dikou, 2008). Some studies failed to carry any type of statistical data analysis to the quantitative data collected (Himes, 2003; Stump & Kriwoken, 2006; Jiménez-Badillo, 2008). In general, qualitative studies had clearly defined aims, and gave adequate descriptions of the sampling and data collection, but only two studies (Himes, 2003; Blyth et al., 2002) provided a justification for the sample size. There was also limited evidence of sample validation, with the exception of Jones (2008) and Suuronen et al. (2010) who report having carried informant feedback, no other article reported any kind of validation.

Discussion and conclusion

Understanding commercial fishers' attitudes and perceptions can be critical for the success of MPAs

(Himes, 2007; Jones, 2008; Charles & Wilson, 2009; Dimech et al., 2009). However, the views of the fishing industry are often neglected (Jones, 2008) and studies that actually collect information concerning commercial fishers' attitudes, perceptions and opinions regarding issues related to MPA make up only for a small proportion of the literature. Indeed, the present review revealed that although interest in the human dimensions of MPAs is growing, little literature originates from empirical studies aiming at examining commercial fishers' attitudes and perceptions about MPAs and issues of relevance to MPAs.

The unique social and ecological context of each location plays a critical role in MPA design, implementation and impacts (Pomeroy et al., 2007). The diversity of topics under investigation identified in this review is an indication of the heterogeneity of MPAs and of the variety of issues of concern surrounding MPAs in the different locations. Divergent attitudes and perceptions were observed amongst fishers from different locations and cultural backgrounds and within different groups of fishers from the same location. However, common trends in fishers' attitudes were also observed irrespectively of geographical locations and cultural contexts.

The review points to the fact that fishers' attitudes and perceptions tend to reflect their personal interests and concerns. Fishers who generally benefit the most from the implementation of the MPA or that are less affected by them tend to be more accepting and supportive of this management measures.

Fishers' attitudes in respect to their acceptance of MPAs were quite homogeneous. The review showed that fishers are more accepting of MPAs implemented for the purpose of fishery management rather than for conservation. This trend was observed amongst fishers from geographical locations and cultural contexts as different as the UK, Italy, Chile and Kenya.

The review also showed that fishers' perceptions about very restrictive MPAs are quite homogeneous. Fishers expressed negative attitudes towards no-take MPAs and tended to favour gear-restriction zones or temporal closures over more restrictive measures. As pointed out by Helvey (2004), the fishing industry is highly regulated and thus it may be unreasonable to expect fishers to accept very restrictive measures when other fishery management measures are already in place and the effectiveness of no-take MPAs is still poorly understood (Polunin, 2002; Willis et al., 2003; Kaiser, 2005). The strong emphasis given to no-take MPAs as a means to address broad conservation and sustainable fisheries exploitation has been questioned by several authors (Kaiser, 2005; Jones, 2006, 2007). While, there is general consensus amongst the scientific community on the use of no-take MPAs for restoring ecosystems (Murray et al., 1999; Pauly et al., 2002; Roberts et al., 2005) it is also suggested that the improvement of conventional fisheries management approaches (such as quotas, effort reduction, seasonal closures and technical measures) will work better in promoting sustainable fish stock yields (Shipp, 2003; Hilborn et al., 2004, 2006; Kaiser, 2004, 2005). In addition, despite empirical evidence showing that no-take MPAs have the ability to significantly increase species richness, biomass and density of fish (Halpern & Warner, 2002; Halpern, 2003; Stewart et al., 2009), the considerable uncertainty and shortage of information about the effect of no-take MPAs in temperate waters is a problem (Kaiser, 2005; Jones, 2007; Stewart et al., 2009). As such, several authors argue for a combined use of both no-take MPAs and conventional fisheries management approaches since both have limitations and the use of both together complement each other (Shipp, 2003; Hilborn et al., 2004, 2006; Kaiser, 2004, 2005; Roberts et al., 2005).

Fishers displayed a range of attitudes regarding the conservation value of MPAs, with some perceiving MPAs as good tool to protect biodiversity and the environment and some not. Indeed, empirical evidence from biological surveys suggests that MPAs have a positive effect in species richness, biomass and density in some of the places where the questionnaire surveys took place and not in others. The findings from the present review show that in some cases fishers' perceptions about the conservation values of MPAs are in accordance with biological findings and in some other cases not. For instance, fishers in Chile and the UK perceived the MPAs in which they were involved to act as a reserve for resources. However, while Gelcich et al. (2005, 2008a, 2009) noted that Chilean fishers perceived the MPA to be important for the maintenance of benthic resources and fish biodiversity, Blyth et al. (2002) observed that different gear-users in the UK had divergent opinions about the MPA's ability to protect benthic habitats. In fact, benthic surveys, in both locations, showed an increase in benthic resource abundance in the protected areas (Kaiser et al., 2000; Gelcich et al., 2008b). In Malta, Dimech et al. (2009) noted that fishers had a negative or neutral perception that the FMZ helps to protect biodiversity. However, trawl surveys revealed significant differences between the inside and outside of the restricted fishing zone, with the inside area having twice as much biomass and larger individuals of some species of demersal communities than the outside (Dimech, 2008). On the other hand, Himes (2003) noted that the majority of Italian fishers from within the fishery reserve (area which is closed to all bottom-towed gears) perceived that overall this measure was successful and beneficial for their fishing activity. And indeed, studies demonstrated that catch per unit effort were much higher inside partial exclusion zones (which banned trawls) and that artisanal fishers within the area achieved higher catch rates than those outside (Pipitone et al., 2000; Whitmarsh et al., 2002). Again, fishers and the scientific community are in agreement regarding the inefficiency of the Baltic cod boxes for the conservation of cod stock. Suuronen et al. (2010) reported that Swedish fishers who targeted Baltic cod found these boxes to be inefficient in the conservation of cod stocks and indeed scientific advice indicates that the cod boxes have neither effectively reduced total effort of the cod fleet nor conserved the Baltic cod stock (ICES, 2004; Suuronen et al., 2010).

The review revealed that fishers' perceptions about their participation in the MPA decision-making process were also quite homogeneous, generally fishers perceived participation to be either lacking or insufficient. This lack of active involvement has consequences for the success of MPAs since if fishers feel alienated from the decision-making process it will be difficult to obtain their support for and compliance with the rules and regulations of the MPAs (Agardy et al., 2003; Mascia, 2004; Pomeroy et al., 2007; Viteri & Chávez, 2007).

The review showed a relationship between fishers' attitudes towards MPAs and the length of time MPAs have been established. Several studies found that fishers' attitudes towards MPAs tend to be more favourable the longer the MPA has been established. This positive relationship was found both regarding the benefits fishers perceive to arise from these management measures and the value they ascribe to MPAs as sites for conservation. However, it was also observed that Mediterranean fishers tended to perceive the potential of MPAs to deliver fisheries objectives to decline the longer the MPA had been designated. Mangi & Austen (2008) emphasise the need for management bodies to see fishers perceptions about this issue as a reason for concern, since a number of studies in the Mediterranean have demonstrated that the present MPAs satisfy both fisheries and ecosystem management objectives (e.g. Badalamenti et al., 2000; Whitmarsh et al., 2002, 2003; Goni et al., 2006). Fishers' participation in MPAs decision-making process could contribute to change their point of view on this matter. Gelcich et al. (2008a) noticed that there was a significant relationship between the amount of time engaged with the co-management of the MPA and positive environmental perceptions. They concluded that such a relationship establishes the potential for co-management to change fishers' perceptions and suggests that artisanal fishers may demonstrate more sustainable behaviours in the future.

This study has provided the first comprehensive and systematically derived overview of this topic. The inclusion criteria for the present systematic review will have resulted in some studies having been missed. For example the limitation to studies published in peer-reviewed journals means that interesting unpublished studies may have been excluded. However, it should be noted that there can be no guarantee of the quality of studies that have not been through a peer review process. Also the focus on commercial fishers will have inevitably resulted in other important studies examining non-commercial fishers and other stakeholders, being excluded. However, as is accepted practice in systematic reviews, the inclusion criteria are clearly documented in the report and the conclusions drawn in the paper relate only to the topics (i.e. attitudes, perceptions, beliefs and opinions about MPAs) and target population (i.e. commercial fishers) covered in the included studies.

In conclusion, this review of the literature on fishers' attitudes, perceptions, opinions and beliefs about MPAs revealed above all that the number of studies which communicate primary research in these topics is still considerably small. MPAs have different social, cultural, economical and environmental settings, and the unique context of each MPA plays a critical role in the way they will impact and be impacted by the fishing industry. Although some homogeneity in commercial fishers' attitudes towards some issues related to MPAs can be observed, they also showed varied attitudes, both among and within groups, towards other issues.

Forecasting the impact of the fishing industry on MPAs, and vice versa, is critical for the management process and knowledge about fishers' attitudes and perceptions will lead to more successful planning and management of MPAs. Given the forthcoming increase in the implementation of MPAs, and more specifically no-take MPAs, in the near future research on commercial fishers' attitudes towards MPAs is all the more pressing.

Acknowledgments C. Pita was funded by an FCT grant (Foundation for Science and Technology—Portugal) (contract number SFRH/BD/17783/2004). G. J. Pierce is in receipt of a Marie Curie Excellence grant (MEXC-CT-2006-042337). The funding sources played no part in the design, analysis, interpretation or writing-up of the study or in the decision to publish.

References

Agardy, T., 2000. Information needs for marine protected areas: scientific and societal. Science 66(3): 875–888.

Agardy, T., P. Bridgewater, M. Crosby, J. Day, P. Dayton, R. Kenchington, D. Laffoley, P. McConney, P. Murray, J. Parks & L. Peau, 2003. Dangerous targets? Unresolved issues and ideological clashes around marine protected areas. Aquatic Conservation-Marine and Freshwater Ecosystems 13: 353–367.

Badalamenti, F., A. Ramos, E. Voultsiadou, L. Lizaso, G. D'Anna, C. Pipitone, J. Mas, J. Fernandez, D. Whitmarsh & S. Riggio, 2000. Cultural and socio-economic impacts of Mediterranean marine protected areas. Environmental Conservation 27: 110–125.

Blyth, R., M. Kaiser, G. Edwards-Jones & P. Hart, 2002. Voluntary management in an inshore fishery has conservation benefits. Environmental Conservation 29: 493–508.

Bunce, L., K. Gustavson, J. Williams & M. Miller, 1999. The human side of reef management: a case study analysis of the socioeconomic framework of Montego Bay Marine Park. Coral Reefs 18: 369–380.

CEBC, 2009. Guidelines for Systematic Review in Conservation and Environmental Management. Centre for Evidence-Based Conservation. Report Version 3.1. Bangor University, UK.

Charles, A. & L. Wilson, 2009. Human dimensions of marine protected areas. ICES Journal of Marine Science 66: 6–15.

Christie, P. & A. White, 2007. Best practices in governance and enforcement of marine protected areas: an overview. In FAO (ed.), FAO Expert Workshop on Marine Protected Areas and Fisheries Management: Review of Issues and Considerations. FAO Fisheries Report No. 825. FAO, Rome: 183–220.

Cicin-Sain, B. & S. Belfiore, 2005. Linking marine protected areas to integrated coastal and ocean management: a review of theory and practice. Ocean & Coastal Management 48: 847–868.

Dimech M., 2008. Differences in demersal community structure and biomass size spectra within and outside the Maltese Fishery Management Zone (FMZ). Scientia marina 72.

Dimech, M., M. Darmanin, I. Smith, M. Kaiser & P. Schembri, 2009. Fishers' perception of a 35-year old exclusive Fisheries Management Zone. Biological Conservation 142: 2691–2702.

Egan, M., C. Bambra, M. Petticrew & M. Whitehead, 2009. Reviewing evidence on complex social interventions: appraising implementation in systematic reviews of the health effects of organisational-level workplace interventions. Journal of epidemiology and community health 63: 4–11.

Ganann, R., D. Ciliska & H. Thomas, 2010. Expediting systematic reviews: methods and implications of rapid reviews. Implementation Science 5: 1–10.

Gelcich, S., G. Edwards-Jones & M. Kaiser, 2005. Importance of attitudinal differences among artisanal fishers toward co-management and conservation of marine resources. Conservation Biology 19: 865–875.

Gelcich, S., M. Kaiser, J. Castilla & G. Edwards-Jones, 2008a. Engagement in co-management of marine benthic resources influences environmental perceptions of artisanal fishers. Environmental Conservation 35: 36–45.

Gelcich, S., N. Godoy, L. Prado & J. Castilla, 2008b. Add-on conservation benefits of marine territorial user rights fishery policies in central Chile. Ecological Applications 18: 273–281.

Gelcich, S., N. Godoy & J. Castilla, 2009. Artisanal fishers' perceptions regarding coastal co-management policies in Chile and their potentials to scale-up marine biodiversity conservation. Ocean and Coastal Management 52: 424–432.

Goni, R., A. Quetglas & O. Renones, 2006. Spillover of spiny lobsters *Palinurus elephas* from a marine reserve to an adjoining fishery. Marine Ecology Progress Series 308: 207–219.

Guarderas, A., S. Hacker & J. Lubchenco, 2008. Current status of marine protected areas in Latin America and the Caribbean. Conservation Biology 22: 1630–1640.

Gubbay, S., 1995. Marine protected areas: past, present and future. In Gubbay, S. (ed.), Marine Protected Areas: Principles and Techniques for Management. Chapman & Hall, London: 1–14.

Guidetti, P., M. Milazzo, S. Bussotti, A. Molinari, M. Murenu, A. Pais, N. Spanò, R. Balzano, T. Agardy, F. Boero, G. Carrada, R. Cattaneo-Vietti, A. Cau, R. Chemello, S. Greco, A. Manganaro, G. Notarbartolo di Sciara, G. F. Russo & L. Tunesi, 2008. Italian marine reserve effectiveness: does enforcement matter? Biological Conservation 141: 699–709.

Halpern, B., 2003. The impact of marine reserves: do reserves work and does reserve size matter? Ecological Applications 13: 117–137.

Halpern, B. & R. Warner, 2002. Marine reserves have rapid and lasting effects. Ecology Letters 5: 361–366.

Helvey, M., 2004. Seeking consensus on designing marine protected areas: keeping the fishing community engaged. Coastal Management 32: 173–190.

Hilborn, R., A. E. Punt & J. Orensanz, 2004. Beyond band-aids in fisheries management: fixing world fisheries. Bulletin of Marine Science 74: 493–507.

Hilborn, R., F. Micheli & G. De Leo, 2006. Integrating marine protected areas with catch regulation. Canadian Journal of Fisheries and Aquatic Sciences 63: 642–649.

Himes, A., 2003. Small-scale Sicilian fisheries: opinions of artisanal fishers and sociocultural effects in two MPA case studies. Coastal Management 31: 389–408.

Himes, A., 2007. Performance indicators in MPA management: using questionnaires to analyze stakeholder preferences. Ocean & Coastal Management 50: 329–351.

ICES, 2004. Report of the study group on closed spawning areas of eastern Baltic cod. Report ICES CM 2004/ACFM:17. WGBFAS, International Council for the Exploration of the Sea.

IUCN-WCPA, 2008. Establishing Marine Protected Area Networks: Making it Happen. World Conservation Union – World Commission on Protected Areas (IUCN-WCPA), National Oceanic and Atmospheric Administration (NOAA) and the Nature Conservancy, Washington, DC.

Jentoft, S., T. van Son & M. Bjorkan, 2007. Marine protected areas: a governance system analysis. Human Ecology 35: 611–622.

Jiménez-Badillo, L., 2008. Management challenges of small-scale fishing communities in a protected reef system of Veracruz, Gulf of Mexico. Fisheries Management and Ecology 15: 19–26.

Jones, P., 1994. A review and analysis of the objectives of marine nature reserves. Ocean Coastal Management 24: 149–178.

Jones, P., 2006. Collective action problems posed by no-take zones. Marine Policy 30: 143–156.

Jones, P., 2007. Arguments for conventional fisheries management and against no-take marine protected areas: only half of the story? Reviews in Fish Biology and Fisheries 17: 31–43.

Jones, P., 2008. Fishing industry and related perspectives on the issues raised by no-take marine protected area proposals. Marine Policy 32: 749–758.

Kaiser, M., 2004. Marine protected areas: the importance of being earnest. Aquatic Conservation: Marine and Freshwater Ecosystems 14: 635–638.

Kaiser, M., 2005. Are marine protected areas a red herring or fisheries panacea? Canadian Journal of Fisheries and Aquatic Sciences 62: 1194–1199.

Kaiser, M., F. Spence & P. Hart, 2000. Fishing-gear restrictions and conservation of benthic habitat complexity. Conservation Biology 14: 1512–1525.

Kelleher, G., 1999. Guidelines for Marine Protected Areas. World Commission on Protected Areas of the World Conservation Union (WCPA-IUCN), Gland, Switzerland and Cambridge, UK.

Kelleher, G., C. Bleakley & S. Wells, 1995. A Global Representative System of Marine Protected Areas. Great Barrier Reef Marine Park Authority, The World Bank and IUCN, Washington, DC.

Mangi, S. & M. Austen, 2008. Perceptions of stakeholders towards objectives and zoning of marine-protected areas in southern Europe. Journal for Nature Conservation 16: 271–280.

Mascia, M., 1999. Governance of marine protected areas in the Wider Caribbean: preliminary results of an international mail survey. Coastal Management 27: 391–402.

Mascia, M., 2003. The human dimension of coral reef marine protected areas: recent social science research and its policy implications. Conservation Biology 17: 630–632.

Mascia, M., 2004. Social dimensions of marine reserves. In Sobel, J. & C. Dahlgren (eds), Marine Reserves: A Guide to Science, Design, and Use. Island Press, Washington, DC: 164–186.

McClanahan, T., J. Davies & J. Maina, 2005. Factors influencing resource users and managers' perceptions towards marine protected area management in Kenya. Environmental Conservation 32: 42–49.

McClanahan, T., J. Cinner, A. Kamukuru, C. Abunge & J. Ndagala, 2008. Management preferences, perceived benefits and conflicts among resource users and managers in the Mafia Island Marine Park, Tanzania. Environmental Conservation 35: 340–350.

Murray, S., R. Ambrose, J. Bohnsack, L. Botsford, M. Carr, G. Davis, P. Dayton, D. Gotshall, D. Gunderson, M. Hixon, J. Lubchenco, M. Mangel, A. MacCall, D. McArdle, J. Ogden, J. Roughgarden, R. Starr, M. Tegner & M. Yoklavich, 1999. No-take reserve networks: sustaining fishery populations and marine ecosystems. Fisheries 24: 11–25.

NOAA, 2005. Social Science for Marine Protected Areas Website. NOAA Coastal Services Center and National Marine Protected Area Center, USA.

Oikonomou, Z. & A. Dikou, 2008. Integrating conservation and development at the national marine park of Alonissos, Northern Sporades, Greece: perception and practice. Environmental management 42: 847–866.

Oxman, A., 1994. Preparing and Maintaining Systematic Reviews: The Cochrane Collaboration Handbook. Cochrane Collaboration, Oxford, UK.

Pauly, D., V. Christensen, S. Guénette, T. Pitcher, U. Sumaila, C. Walters, R. Watson & D. Zeller, 2002. Towards sustainability in world fisheries. Nature 418: 689–695.

Petticrew, M., 2001. Systematic reviews from astronomy to zoology: myths and misconceptions. British Medical Journal 322: 98–101.

Petticrew, M. & H. Roberts, 2006. Systematic Reviews in the Social Sciences: A Practical Guide. Blackwell Publishing, Oxford, UK.

Pipitone C., F. Badalamenti, G. D'Anna, D. Whitmarsh, C. James & H. Pickering, 2000. Trawling ban in the Gulf of Castellammare: Effects on the small-scale fishery economics and on the abundance of fish. Report 97/063, Final Report to the European Commission.

Pollnac, R., B. Crawford & M. Gorospe, 2001. Discovering factors that influence the success of community-based marine protected areas in the Visayas, Philippines. Ocean & Coastal Management 44: 683–710.

Pollnac R., P. Christie, J. Cinner, T. Dalton, T. Daw, G. Forrester, N. Graham & T. McClanahan, T., 2010. Marine reserves as linked social-ecological systems. Proceedings of the National Academy of Sciences (Special Feature): 1–4.

Polunin, N., 2002. Marine protected areas, fish and fisheries. In Hart, P. & J. Reynolds (eds), Handbook of Fish Biology and Fisheries. Blackwell Publishing, Malden, USA: 293–318.

Pomeroy, R., J. Parks & L. Watson, 2004. How is Your MPA Doing? A Guidebook of Natural and Social Indicators for Evaluating Marine Protected Areas Management Effectiveness. IUCN, WWF, and US National Oceanic and Atmospheric Administration (NOAA), Gland, Switzerland and Cambridge, UK.

Pomeroy, R., L. Watson, J. Parks & G. Cid, 2005. How is your MPA doing? A methodology for evaluating the management effectiveness of marine protected areas. Ocean & Coastal Management 48: 485–502.

Pomeroy R., M. Mascia & R. Pollnac, 2007. Marine protected areas, the social dimension. In FAO (ed.), FAO expert workshop on marine protected areas and fisheries management: Review of issues and considerations. FAO, Rome: 149–275.

Pullin, A. & G. Stewart, 2006. Guidelines for systematic review in conservation and environmental management. Conservation Biology 20: 1647–1656.

Roberts, C., J. Hawkins & F. Gell, 2005. The role of marine reserves in achieving sustainable fisheries. Philosophical Transactions: Biological Sciences 360: 123–132.

Shipp, R., 2003. A perspective on marine reserves as a fishery management tool. Fisheries 28: 10–21.

Silva, M., E. Gately & I. Desilvestre, 1986. A Bibliographic Listing of Coastal and Marine Protected Areas: A Global Survey. Woods Hole Oceanographic Institution, Report WHOI-86, Massachusetts, USA.

Stewart, G., M. Kaiser, I. Côté, B. Halpern, S. Lester, H. Bayliss & A. Pullin, 2009. Temperate marine reserves: global ecological effects and guidelines for future networks. Conservation Letters 2(6): 243–244.

Stump, N. & L. Kriwoken, 2006. Tasmanian marine protected areas: attitudes and perceptions of wild capture fishers. Ocean and Coastal Management 49: 298–307.

Sumaila, U. & A. Charles, 2002. Economic models of marine protected areas: an introduction. Natural Resource Modelling 15: 261–272.

Suman, D., M. Shivlani & J. Milon, 1999. Perceptions and attitudes regarding marine reserves: a comparison of stakeholder groups in the Florida Keys National Marine Sanctuary. Ocean and Coastal Management 42: 1019–1040.

Suuronen, P., P. Jounela & V. Tschernij, 2010. Fishermen responses on marine protected areas in the Baltic cod fishery. Marine Policy 34: 237–243.

Tonder, M. & J. Jurvelius, 2004. Attitudes towards fishery and conservation of the Saimaa ringed seal in Lake Pihlajavesi, Finland. Environmental Conservation 31: 122–129.

Viteri, C. & C. Chávez, 2007. Legitimacy, local participation, and compliance in the Galápagos Marine Reserve. Ocean and Coastal Management 50: 253–274.

Wahle, C., S. Lyons, K. Barva, L. Bunce, P. Fricke, C. Pomeroy, H. Recksiek & J. Uravitch, 2003. Social Science Research Strategy for Marine Protected Areas. NOAA National Marine Protected Areas Center, MPA Science Institute, Santa Cruz, CA, USA.

Watt, A., A. Cameron, L. Sturm, T. Lathlean, W. Babidge, S. Blamey, K. Facey, D. Hailey, I. Norderhaug & G. Maddern, 2008a. Rapid versus full systematic reviews: validity in clinical practice. ANZ Journal of Surgery 78(11): 1037–1040.

Watt, A., A. Cameron, L. Sturm, T. Lathlean & W. Babidge, 2008b. Rapid reviews versus full systematic reviews: an introduction of current methods and practice in health technology assessment. International Journal of Technology Assessment in Health Care 24(2): 133–139.

Whitmarsh, D., C. James, H. Pickering, C. Pipitone, F. Badalamenti & G. D'Anna, 2002. Economic effects of fisheries exclusion zones: a Sicilian case study. Marine Resource Economics 17: 239–250.

Whitmarsh, D., C. Pipitone, F. Badalamenti & G. D'Anna, 2003. The economic sustainability of artisanal fisheries: the case of the trawl ban in the Gulf of Castellammare, NW Sicily. Marine Policy 27: 489–497.

Willis, T., R. Millar, R. Babcock & N. Tolimieri, 2003. Burdens of evidence and the benefits of marine reserves: putting Descartes before des horse? Environmental Conservation 30: 97–103.

Wood, L., 2007. MPA Global: A database of the world's marine protected areas. Sea Around Us Project. UNEP-WCMC & WWF. www.mpaglobal.org.

Hydrobiologia (2011) 670:307–315
DOI 10.1007/s10750-011-0668-6

ECOSYSTEMS AND SUSTAINABILITY

Functional diversity and species turnover of benthic invertebrates along a local environmental gradient induced by an aquaculture unit: the contribution of species dispersal ability and rarity

Charalampos Dimitriadis · Drosos Koutsoubas

Published online: 25 March 2011
© Springer Science+Business Media B.V. 2011

Abstract The relation of macrobenthic species turnover (beta diversity) and species plylogenetic variation with functional diversity patterns, across an environmental gradient induced by an aquaculture unit, in a coastal area of the island of Lesvos (NE Aegean) has been investigated in this study. The contribution of rare species response and species dispersal ability in the variation of functional diversity patterns along the environmental gradient, on a spatio-temporal scale, has been also examined. Our results revealed that benthic functional diversity was decreasing monotonically with increasing species turnover rate and hence with increasing spatial variability along the environmental gradient. Increased environmental stress which was detected in the immediate vicinity of the fish cages resulted to low species functional redundancy, since different species didn't perform the same functional role at the most disturbed part of the established gradient. Functional diversity patterns were found to be correlated with species population size, whereas a strong linear relationship was also detected with phylogenetic diversity patterns, thus supporting the claim that wider local taxonomic trees can support a wider range of species functions even in small spatial scales. Rare species loss seemed to be one of the dominant factors ruling functional diversity variation. Species with the minimum possible dispersal ability, which were mostly rare, tend to diminish both in species number and population size faster than species with wider dispersal ability towards the most disturbed areas. The aforementioned results indicate that rare species variation and endemic species loss are critical factors in determining functional diversity loss across a human-induced environmental gradient in soft bottom benthic communities.

Keywords Functional diversity · Beta diversity · Aquaculture · Benthos

Introduction

Biodiversity, as a multi-facet concept, can be distinguished in various sub-concepts such as beta or turnover diversity and functional diversity (Ricotta, 2007). Beta diversity represents the rate of species identities shift and measures species turnover rate along a given spatial scale (Whittaker, 1960, 1972) thus capturing differences in species composition either between two or more local assemblages or between local and regional assemblages (Koleff

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

C. Dimitriadis · D. Koutsoubas (✉)
Department of Marine Sciences, Faculty of Environment, University of the Aegean, Lesvos Island 81100, Greece
e-mail: drosos@aegean.gr

et al., 2003). Despite the fact that it provides a fundamental tool in spatial diversity patterns decoding (Wilson & Shmida, 1984), it has received limited attention especially in the marine biome (Gray, 2000; Becking et al., 2006). However, a large variety of beta diversity indices have been proposed and used in the ecological literature, mainly concerning species presence/absence data rather than species abundance (Chao et al., 2005), while the original Whittaker's measure has been the most frequently employed (Koleff et al., 2003).

There is growing evidence that ecosystem functioning is primarily governed by species-specific functional traits rather than species richness per se (Loreau et al., 2001; Bolam et al., 2002; Giller et al., 2004; Hooper et al., 2005). The importance of species biological traits related to ecosystems functions have been highlighted in many biodiversity-function experiments, especially on small spatio-temporal scales, in a wide range of ecosystems (Cardinale et al., 2004). The value and range of species traits that influence ecosystem functioning in a given system have been used by Tilman (2001) in order to describe functional diversity. Species traits have been also used in several other definitions of functional diversity (e.g. Díaz & Cabido, 2001; Tesfaye et al., 2003; Petchey & Gaston, 2006). Therefore, functional diversity has progressively been used as a tool in exploring ecosystem mechanisms, such as resource use complementarity and facilitation (Díaz & Cabido, 2001; Petchey & Gaston, 2006), as well as in predicting functional consequences of human-induced pressure on the biota (Loreau et al., 2002). Since threats resulting to marine ecosystems degradation are rapidly increasing nowadays there is a great need to assess the impacts of man-made stress on marine ecosystems functioning (Gaston, 2000; Olsen et al., 2002; Pandolfi et al., 2003; Bremner 2008) and many indices that take into account functional differences between species have been proposed and reviewed in several studies (e.g. Petchey et al., 2004; Ricotta, 2005; Bremner et al., 2006; Petchey & Gaston, 2006; Podani & Schmera, 2006; Walker et al., 2008). However, investigation of functional diversity has been mostly focused in terrestrial ecosystems, while coastal areas have attracted less attention (e.g. Covich et al., 2004; Gessner et al., 2004). Furthermore, functional diversity patterns of benthic invertebrates and macroalgae in aquatic habitats have been examined in rather few studies (e.g. Emmerson & Raffaelli, 2000; Emmerson et al., 2001; Biles et al., 2003; Raffaelli et al., 2003; Mermillod-Blondin et al., 2005; Griffin et al., 2009), while response of benthic invertebrates' functional diversity in coastal areas along a human-induced disturbance gradient is even less examined (Papageorgiou et al., 2009).

The present study aims to address marine benthic functional diversity levels, on a small spatial scale, along an environmental gradient caused by the presence of an aquaculture unit in a coastal area of the NE Mediterranean. In addition, we present the relation of species turnover (beta diversity) and species plylogenetic variation with functional diversity patterns across this gradient. Finally, we examined the hypothesis whether a decline in rare species number and species geographic range size contributes in the variation of functional diversity patterns along the environmental gradient.

Materials and methods

Study area

The study area is located at NE Aegean Sea (Eastern Mediterranean Sea) and particularly at the entrance of the semi-enclosed Bay of Gera in Lesvos Island (Fig. 1). The presence of an aquaculture unit (with an average annual production of 150 tones of sea-bream and sea-bass) in the study area has proven to cause a series of modifications in the environmental conditions across a gradient (stations N, M, F—Fig. 1) which subsequently have an effect on the structure and dynamics of benthic fauna (Dimitriadis & Koutsoubas, 2008).

Data analysis

The Molluscan taxocoenosis data set (Dimitriadis & Koutsoubas, 2008) has been used in the analysis of the present study. This analysis involved the calculation of beta and functional diversity along the environmental transect (Fig. 1) and between the different sampling seasons. Beta diversity measurement involved the use of two quantitative and two qualitative indices based on species presence–absence and species abundance data correspondingly.

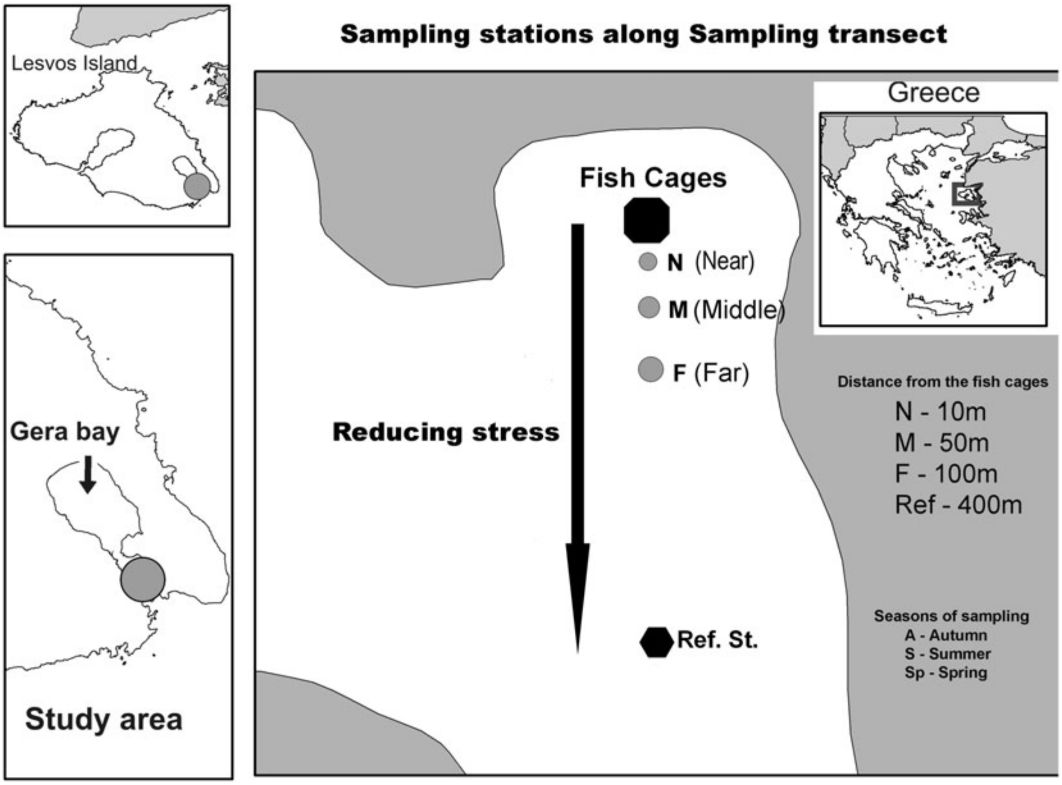


Fig. 1 Study area and sampling design for macrofauna samples (after Dimitriadis & Koutsoubas, 2008, slightly modified)

Considering the qualitative beta diversity measures Whittaker's (1960) index β_w of species continuity and index β_{gl} (Lennon et al., 2001), which is specially designed for species richness gradients (Koleff et al., 2003), have been used. Quantitative beta diversity CJE and CSE indices involved the use of the modified Jaccard and Sørensen indices correspondingly for species abundance data (Chao et al., 2005). The later have been also corrected for the effect of unseen shared species (i.e. species that are likely to be present in a theoretical larger sample of the assemblage, but that are missing from actual sample data) (Chao et al., 2005). According to Chao et al. (2005), higher values of CJE and CSE index correspond to lower beta diversity levels, whereas lower values of CJE and CSE correspond to higher beta diversity levels. Beta diversity indices were calculated between adjacent pairs of sampling sites along the sampling transect as it is proposed and discussed by Wilson & Shmida (1984). Quantitative beta diversity indices have been calculated by means of the ESTIMATES v8 software package (Colwell, 2005).

Considering species functional traits nine different functional characteristics of benthic molluscs related to resource use requirements (i.e. detritus feeders, suspension feeders, deposit feeders, herbivores, parasitic), life mode (positioning in the sediment—i.e., sub-surface infauna, epifauna) and movement in the sediment (i.e. mobile, sessile) (Norling et al., 2007; Papageorgiou et al., 2009) have been incorporated in the analysis. Functional diversity was calculated based on functional attribute diversity index (FAD) which reflects the sum of the pair-wise functional dissimilarities of species (Walker et al., 1999). The properties and performance of FAD index are discussed by Ricotta (2005) and Schmera et al. (2009). According to Walker et al. (2008), FAD is probably more appropriate index in revealing the resilience of a community in the face of an environmental change and this has been the reason to select this index instead of other functional diversity indices (see Petchey & Gaston, 2006). All pair-wise distances between species in functional trait space were calculated with the use of Euclidean distance in SPSS v12 (SPSS, 2002).

Correlation of functional diversity with species richness, total taxonomic distinctness sΔ^+, variation of taxonomic distinctness Λ^+ (Warwick & Clarke, 2001), species abundance and rare species number was carried out with the use of Spearman's correlation index (ρ) provided by SPSS v12 software. A regression model was also used in order to describe the relationship between the aforementioned variables. Finally, significant differences among measurements were detected with the non-parametric Mann–Whitney test (Zar, 1984).

Results

Species turnover

Calculation of beta diversity indices β_w and β_{gl} based on species presence/absence data revealed a pattern of gradual decrease in species turnover towards the environmental gradient with decreasing disturbance levels. Maximum species turnover rate was detected close to the areas of the immediate vicinity of the fish-cages during summer and autumn, while minimum rate was noticed at the less impacted sampling sites further away from the fish-cages during spring. However, significant alterations in beta diversity levels were noticed only between the pairs of stations N-M and F-Ref (Mann–Whitney test results, $P <$ 0.05), indicating that the area located at the middle of the environmental gradient consists a transitional point in species turnover (Fig. 2). An alteration in species turnover rate was also detected from the quantitative indices CJE and CSE along the environmental gradient. Hence, maxima of species turnover (minimum values of CJE and CSE index) were recorded to the pair of stations close to the cages while minima (maximum values of CJE and CSE index) were obtained further away. Significant modifications in the values of CJE and CSE indices were obtained for the pairs of stations N-M and F-Ref between all sampling seasons denoting an important shift in species turnover rate therein (Mann–Whitney test results, $P < 0.05$; Fig. 3). Increased turnover rates between the most impacted sites were found to be driven by both quantitative and qualitative changes in species composition since those areas were only sharing a few species, whereas common species populations were roughly decreasing from intermediate to highly impacted sites. In addition, rare species number seemed to strongly affect beta diversity levels rather than the fluctuations of common species abundance. On the contrary, seasonal modifications of beta diversity were not statistically significant for the pooled abundance data in the study area (Mann–Whitney test results, $P > 0.05$) implying that seasonal variation was not a major factor governing species turnover rate.

Functional diversity

Regarding functional diversity, maxima were recorded to the area of low environmental stress (station SpF) during spring sampling period, while minima were obtained to the area of the immediate vicinity of the fish cages during summer (station SN).

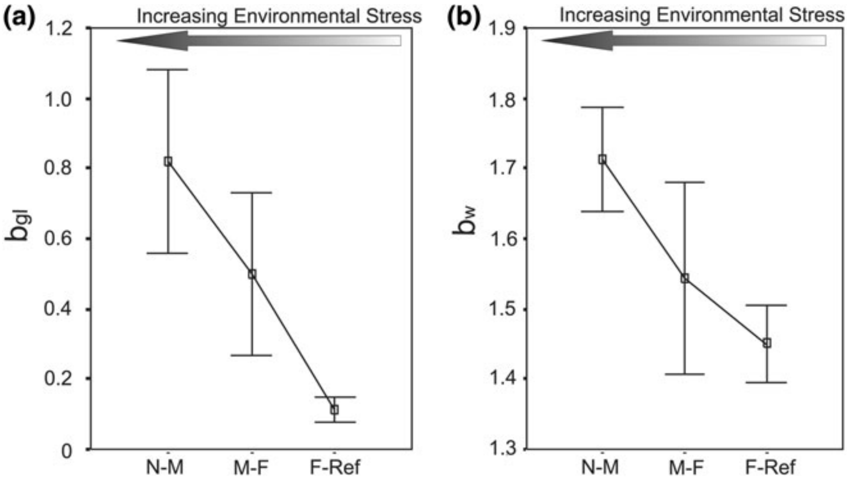


Fig. 2 Beta diversity variation according to **a** β_{gl} index and **b** β_w index along the environmental gradient for the pooled species presence–absence data of Molluscan fauna (*error bars* represent standard error of mean)

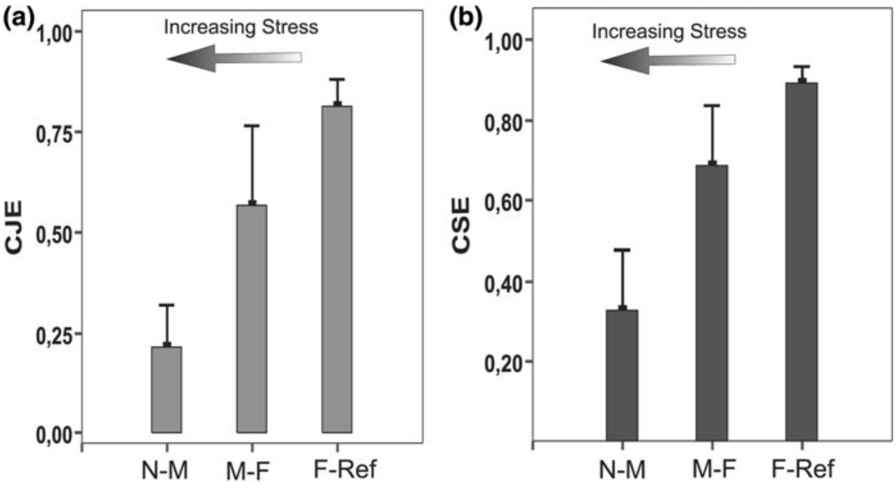


Fig. 3 Beta diversity variation according to **a** CJE index and **b** CSE index along the environmental gradient for the pooled species abundance data of Molluscan fauna (*error bars* represent standard error of mean)

Functional diversity presented a linear decrease ($R^2 = 0.61$, $P < 0.05$) with increasing environmental stress across the sampling transect revealing a pattern of seriation in functional diversity variation (Fig. 4), since functional diversity was gradually decreasing towards the most disturbed end of the gradient. Significant modifications of FAD index values were obvious between the group of areas of high and moderate disturbance effects with the rest of the study area where higher functional diversity values were present during all sampling seasons (Mann–Whitney test results, $P < 0.05$). Though, in seasonal basis, FAD values didn't show any significant alterations in functional diversity levels between the three sampling seasons (Mann–Whitney test results, $P > 0.05$) a fact that should be mainly attributed to the effect of the increased functional diversity levels during spring throughout the whole length of the sampling transect. Correlation of functional diversity with species richness of benthic molluscs revealed a strong linear relationship between them (Spearman's $\rho = 0.876$, $P < 0.05$) suggesting that increasing species diversity leads to species assemblages of higher functional complexity along the environmental gradient towards the undisturbed areas. In addition, a positive linear relationship was also detected when functional diversity index FAD and taxonomic distinctness index $s\Delta^+$ are considered (Spearman's $\rho = 0.867$, $P < 0.05$).

The latter denotes that wider phylogenetic trees can support a greater number of species functions. On the other hand, variation of taxonomic distinctness L+ seemed to be more or less constant with increasing values of functional attribute diversity index. Correlation of functional diversity with species abundance across the environmental gradient showed a hump-shaped relationship between these two variables (Fig. 4). A hump-shaped relationship was also detected in the correlation of species richness and species abundance. Functional diversity was highly correlated with rare species number variation along the environmental gradient. Thus, loss of rare species seemed to be linearly correlated with functional diversity loss across the environmental gradient ($R^2 = 0.92$, $P < 0.05$; Fig. 5).

Discussion

According to the P–R (Pearson–Rosenberg) model (Pearson & Rosenberg, 1978), marine benthic community succeeds in several progressive stages towards a disturbance gradient in space and time from diverse community fauna to a transitional community structure where opportunistic species are proliferating in terms of abundance and species number and eventually to an azoic area at the most disturbed end of the gradient. Changes in benthic species composition, species diversity patterns (Pearson & Rosenberg, 1978; Gray, 1981), life history traits (Diaz & Rosenberg, 1995; Cheung et al., 2008), feeding guilds (Pagliosa, 2005; Dimitriadis & Koutsoubas, 2008) and species range size representation (Dimitriadis & Koutsoubas, 2008) along environmental gradients are well documented in contrast with benthic functional diversity patterns

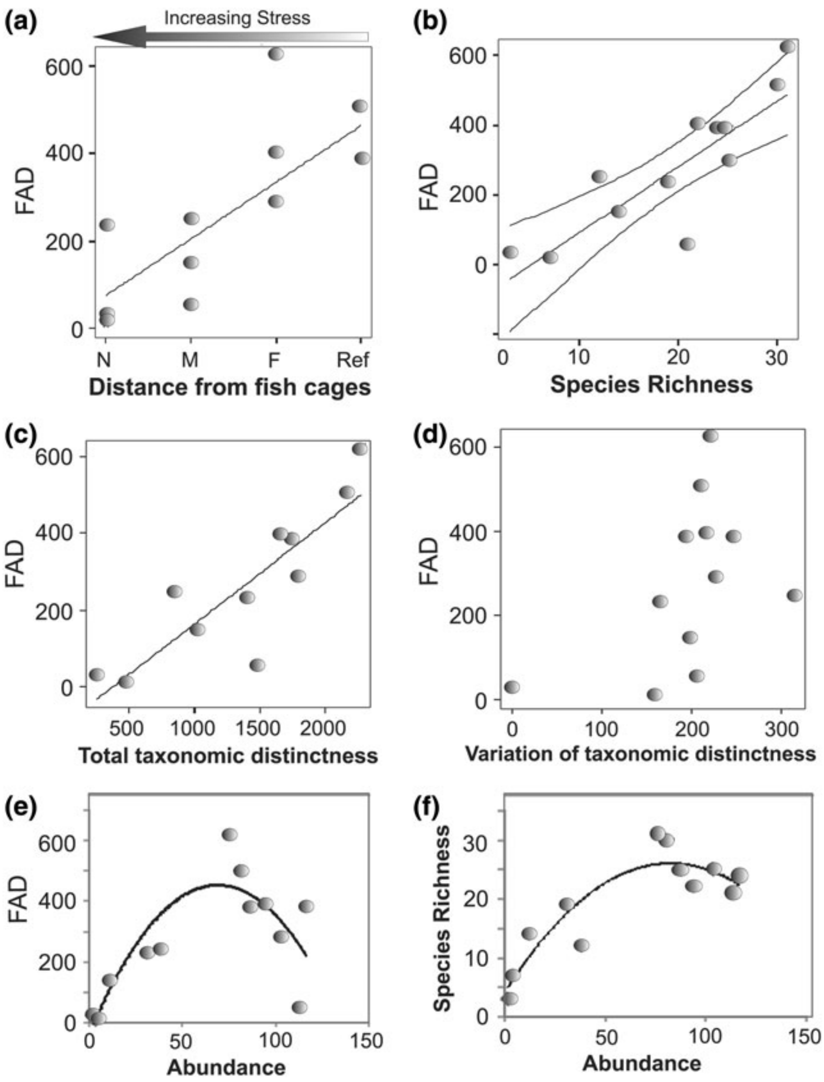


Fig. 4 Scatter plots of **a** FAD index and distance from the fish cages (linear regression, $R^2 = 0.61$, $P < 0.05$), **b** FAD index and species richness (linear regression, $R^2 = 0.72$, $P < 0.05$), **c** FAD index and total taxonomic distinctness (linear regression, $R^2 = 0.75$, $P < 0.05$), **d** FAD index and variation of taxonomic distinctness (linear regression, $R^2 = 0.02$, $P > 0.05$), **e** FAD index and species abundance (polynomial regression of second order, $R^2 = 0.70$, $P < 0.05$) and **f** species richness and species abundance (polynomial regression of second order, $R^2 = 0.83$, $P < 0.05$)

which are not widely understood yet along gradients (e.g. Norling et al., 2007; Papageorgiou et al., 2009). The results of the present study revealed that benthic functional diversity was decreasing monotonically with increasing species turnover rate and hence with increasing spatial variability of macrofauna along the established environmental gradient. Furthermore, beta diversity patterns were strongly governed by the loss rate of rare species across the environmental gradient, a fact which has also been reported in several studies for other major taxonomic groups (e.g. Gaston, 1994). Functional diversity was strongly correlated to rare species number denoting that the loss of rare species leads to the gradual loss of functional complexity of benthic communities across the environmental gradient. It was also evident that increased environmental stress resulted to low species functional redundancy (Lawton & Brown, 1993; Loreau, 2004) since different species didn't perform the same functional role across the gradient. Low functional redundancy across the environmental gradient can be possibly attributed to the fact that the smothering of the habitats towards the disturbance source acts as selective mechanism resulting in the reduction of species with similar functional traits. Hence, the gradual loss of certain species' functional traits with increasing environmental stress led to a strong and successive decline in benthic functional diversity suggesting that species diversity (i.e. species richness) changes can trigger shifts in benthic community functional stability. The latter has also been reported in several studies which have also shown a strong correlation between species diversity and functional diversity in marine sediments (Papageorgiou et al., 2009). However, low functional redundancy levels which were observed in the study area do not necessarily imply that functional redundancy were not present among some of the species since, for example, the bivalve *Nucula nitidosa* was replaced by the functionally similar bivalve *Nuculana pella* at the area of the immediate vicinity of the fish cages. Similar results have also been recorded when species functional redundancy was investigated within functional groups (Loreau et al., 2001).

Benthic functional diversity patterns across the environmental gradient were found to be strongly correlated with species population size. The hump-shaped relation between functional diversity and species abundance denotes that functional diversity increases when low and intermediate levels of species abundance are involved. However, when species abundance is reaching its maximum values, functional diversity is decreasing. This pattern was also the case for species richness and species abundance correlation which was also described from a hump-shaped relationship. Hence, species richness was decreasing when abundance was reaching intermediate and maximum values in species communities. These results suggest that the most populated communities do not necessarily encompass increased functional complexity since there was an upper limit in species abundance from which functional diversity was starting to decline.

A strong linear relationship was detected between functional and phylogenetic diversity patterns thus supporting the claim that wider local taxonomic trees can support a wider range of species functions even in small spatial scales. However, the proliferation of disturbance regimes results to narrower taxonomic trees with a smaller range of species functions. This narrow range of species functions is maintained by certain species which were also present in the wider taxonomic trees. Therefore, the results of this study confirm the hypothesis that some species are more important than other in ecosystem functioning (Tilman et al., 1997). Rare species loss seemed to be one of the dominant factors ruling functional diversity variation at different disturbance levels in space since it is well known that these species are characterized by high niche differentiation and are usually intolerant in disturbance regimes (Smith & Knapp, 2003). Therefore, it seems that rare species are important in maintaining the stability of ecosystem functioning in changing environments (Walker et al., 1999; Loreau et al., 2001). Rare species included a broad range of functional traits which were comparable to the ones noticed for the total species pool, a fact which was also observed by Ellingsen et al. (2007) for soft bottom macrofauna. According to Naeem & Li (1997) and Ellingsen et al. (2007), the latter pattern demonstrates that species with restricted geographic dispersal ability can have an impact on stability and resilience of soft bottom communities. Earlier publications concerning species geographic range size in the study area revealed that endemic species (i.e. species with the minimum possible dispersal ability) which were mostly rare tend to diminish their species number and population size faster than species with wider dispersal ability with increasing environmental stress (Dimitriadis & Koutsoubas, 2008). Hence, benthic rare species do not invest in functional resilience but they can still have an important influence in community and ecosystem functioning (Ellingsen et al., 2007). Although it is widely acknowledged that species dispersal ability consist a primary factor governing biodiversity distribution patterns in nature (Tilman, 1999), relatively less attention has been paid in the influence of species dispersal ability on the variation of functional diversity (Giller et al., 2004; France & Duffy, 2006) and especially in cases of environmental gradients.

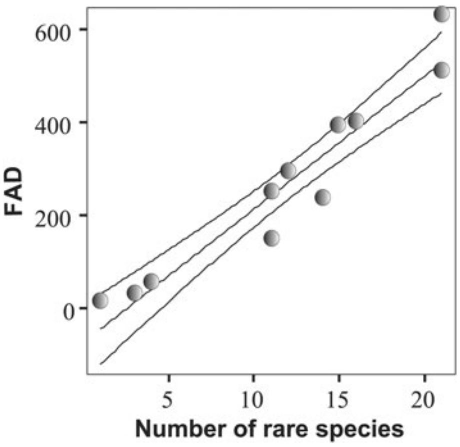


Fig. 5 Functional attribute diversity index plotted against rare species number in the study area (linear regression, $R^2 = 0.92$, $P < 0.05$)

Conclusions

Understanding the relationship between benthic diversity loss and functional diversity modifications can increase our knowledge in predicting the functional consequences of human induced pressure on the marine environment. From the above, it was evident that human activities such as fish farming can modify benthic biodiversity which in return can alter functional diversity of sediment communities and affect ecosystem functioning in coastal areas. However, functional diversity loss was restricted to the immediate vicinity of the fish cages, a fact that clearly demonstrates that impacts of aquaculture on benthic communities functioning had a strictly local character. The results from the present study have also revealed that species dispersal ability increment (i.e. loss of species with narrow geographic range size—endemic species) and rare species number decrement influenced the magnitude of functional diversity loss with increasing levels of stress on soft bottom benthic communities.

References

Becking, L. E., D. F. R. Cleary, N. J. De Voogd, W. Renema, M. De Beer, R. W. M. Van Soest & B. W. Hoeksema, 2006. Beta diversity of tropical marine benthic assemblages in the Spermonde Archipelago, Indonesia. Marine Ecology 27: 76–88.

Biles, C. L., M. Solan, I. Isaksson, D. M. Paterson, C. Emes & D. G. Raffaelli, 2003. Flow modifies the effect of biodiversity on ecosystem functioning: an in situ study of estuarine sediments. Journal of Experimental Marine Biology and Ecology 286: 165–177.

Bolam, S. G., T. F. Fernandes & M. Huxham, 2002. Diversity, biomass, and ecosystem processes in the marine benthos. Ecological Monographs 72: 599–615.

Bremner, J., 2008. Species traits and ecological functioning in marine conservation and management. Journal of Experimental Marine Biology and Ecology 366: 37–47.

Bremner, J., S. I. Rogers & C. L. J. Frid, 2006. Methods for describing ecological functioning of marine benthic assemblages using biological traits analysis (BTA). Ecological Indicators 6: 609–622.

Cardinale, B. J., A. R. Ives & P. Inchausti, 2004. Effects of species diversity on the primary productivity of ecosystems: extending our spatial and temporal scales of inference. Oikos 104: 437–450.

Chao, A., R. L. Chazdon, R. K. Colwell & T. J. Shen, 2005. A new statistical approach for assessing similarity of species composition with incidence and abundance data. Ecology Letters 8: 148–159.

Cheung, S. G., N. W. Y. Lam, R. S. S. Wu & P. K. S. Shin, 2008. Spatio-temporal changes of marine macrobenthic community in sub-tropical waters upon recovery from eutrophication. II. Life-history traits and feeding guilds of polychaete community. Marine Pollution Bulletin 56: 297–307.

Colwell, R. K., 2005. EstimateS: Statistical Estimation of Species Richness and Shared Species from Samples. Version 8. User's Guide and Application, http://purl.oclc.org/estimates.

Covich, A. P., M. C. Austen, F. Barlocher, B. J. Cardinale, C. L. Biles, P. Inchausti, O. Dangles, M. Solan, M. O. Gessner, B. Statzner & B. Moss, 2004. The role of biodiversity in the functioning of freshwater and marine benthic ecosystems. Bioscience 54: 767–775.

Díaz, S. & M. Cabido, 2001. Vive la différence: plant functional diversity matters to ecosystem processes. Trends in Ecology and Evolution 16: 646–655.

Diaz, R. J. & R. Rosenberg, 1995. Marine benthic hypoxia: a review of its ecological effects and the behavioural responses of benthic macrofauna. Oceanography and Marine Biology Annual Review 33: 245–303.

Dimitriadis, C. & D. Koutsoubas, 2008. Community properties of benthic molluscs as indicators of environmental stress induced by organic enrichment. Journal of Natural History 42: 559–574.

Ellingsen, K. E., J. E. Hewitt & S. F. Thrush, 2007. Rare species, habitat diversity and functional redundancy in marine benthos. Journal of Sea Research 58: 291–301.

Emmerson, M. C. & D. G. Raffaelli, 2000. Detecting the effects of diversity on measures of ecosystem function: experimental design, null models and empirical observations. Oikos 91: 195–203.

Emmerson, M. C., M. Solan, C. Emes, D. M. Paterson & D. G. Raffaelli, 2001. Consistent patterns and the idiosyncratic effects of biodiversity in marine ecosystems. Nature 411: 73–77.

France, K. E. & J. E. Duffy, 2006. Diversity and dispersal interactively affect predictability of ecosystem function. Nature 441: 1139–1143.

Gaston, K. J., 1994. Rarity. Chapman and Hall, London.

Gaston, K. J., 2000. Global patterns in biodiversity. Nature 405: 220–228.

Gessner, M., P. Inchausti, L. Persson, D. G. Raffaelli & P. S. Giller, 2004. Biodiversity effects on ecosystem functioning: insights from aquatic systems. Oikos 104: 419–422.

Giller, S. P., H. Hillebrand, U. G. Berninger, O. M. Gessner, S. Hawkins, P. Inchausti, C. Inglis, H. Leslie, B. Malmqvist, T. M. Monaghan, J. P. Morin & G. O'Mullan, 2004. Biodiversity effects on ecosystem functioning: emerging issues and their experimental test in aquatic environments. Oikos 104: 423–436.

Gray, J. S., 1981. The Ecology of Marine Sediments. Cambridge University Press, Cambridge.

Gray, J. S., 2000. The measurement of marine species diversity, with an application to the benthic fauna of the Norwegian continental shelf. Journal of Experimental Marine Biology and Ecology 250: 23–49.

Griffin, J. N., V. Méndez, A. F. Johnson, S. R. Jenkins & A. Foggo, 2009. Functional diversity predicts overyielding effect of species combination on primary productivity. Oikos 118: 37–44.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer & D. A. Wardle, 2005. Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. Ecological Monographs 75: 3–35.

Koleff, P., K. J. Gaston & J. J. Lennon, 2003. Measuring beta diversity for presence–absence data. Journal of Animal Ecology 72: 367–382.

Lawton, J. H. & V. K. Brown, 1993. Redundancy in ecosystems. In Schulze, E. D. & H. A. Mooney (eds), Biodiversity and Ecosystem Function. Springer, Berlin: 255–270.

Lennon, J. J., P. Koleff, J. J. D. Greenwood & K. J. Gaston, 2001. The geographical structure of British bird distributions: diversity, spatial turnover and scale. Journal of Animal Ecology 70: 966–979.

Loreau, M., 2004. Does functional redundancy exist? Oikos 104: 3.

Loreau, M., S. Naeem, P. Inchausti, J. Nengtsson, J. P. Grime, A. Hector, D. U. Hooper, M. A. Huston, D. Raffaelli, B. Schmid, D. Tilman & D. A. Wardle, 2001. Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294: 804–808.

Loreau, M., S. Naeem & P. Inchausti, 2002. Biodiversity and Ecosystem Functioning: Synthesis and Perspectives. Oxford University Press, Oxford.

Mermillod-Blondin, F., F. François-Carcaillet & R. Rosenberg, 2005. Biodiversity of benthic invertebrates and organic matter processing in shallow marine sediments: an experimental study. Journal of Experimental Marine Biology and Ecology 315: 187–209.

Naeem, S. & S. Li, 1997. Biodiversity enhances ecosystem reliability. Nature 390: 507–509.

Norling, K., R. Rosenberg, S. Hulth, A. Grémare & E. Bonsdorff, 2007. Importance of functional biodiversity and species-specific traits of benthic fauna for ecosystem functions in marine sediment. Marine Ecology Progress Series 332: 11–23.

Olsen, D. M., E. Dinerstein, G. V. N. Powell & E. D. Wikramanayake, 2002. Conservation biology for the biodiversity crisis. Conservation Biology 16: 1–3.

Pagliosa, P. R., 2005. Another diet of worms: the applicability of polychaete feeding guilds as a useful conceptual framework and biological variable. Marine Ecology 26: 246–254.

Pandolfi, J. M. R. H., E. Bradbury, T. P. Sala, K. A. Hughes, R. G. Bjorndal, D. Cooke, L. McArdle, M. J. H. McClenachan, G. Newman, R. R. Paredes, J. B. C. Warner & Jackson, 2003. Global trajectories of long-term decline of coral reef ecosystems. Science 301: 955–958.

Papageorgiou, N., K. Sigala & I. Karakassis, 2009. Changes of macrofaunal functional composition at sedimentary habitats in the vicinity of fish farms. Estuarine, Coastal and Shelf Science 83: 561–568.

Pearson, T. H. & R. Rosenberg, 1978. Macrobenthic succession in relation to organic enrichment and pollution of the marine environment. Oceanography and Marine Biology: An Annual Review 16: 229–311.

Petchey, O. L. & K. J. Gaston, 2006. Functional diversity: back to basics and looking forward. Ecology Letters 9: 741–758.

Petchey, O. L., A. Hector & K. J. Gaston, 2004. How do different measures of functional diversity perform? Ecology 85: 847–857.

Podani, J. & D. Schmera, 2006. On dendrogram-based measures of functional diversity. Oikos 115: 179–185.

Raffaelli, D., M. Emmerson, M. Solan, C. Biles & D. Paterson, 2003. Biodiversity and ecosystem processes in shallow coastal waters: an experimental approach. Journal of Sea Research 49: 133–141.

Ricotta, C., 2005. A note on functional diversity measures. Basic and Applied Ecology 6: 479–486.

Ricotta, C., 2007. A semantic taxonomy for diversity measures. Acta Biotheoretica 55: 23–33.

Schmera, D., T. Erős & J. Podani, 2009. A measure for assessing functional diversity in ecological communities. Aquatic Ecology 43: 157–167.

Smith, M. D. & A. K. Knapp, 2003. Dominant species maintain ecosystem function with non-random species loss. Ecology Letters 6: 509–517.

SPSS Inc, 2002. SPSS Base 11.5. User's guide: 613 pp.

Tesfaye, M., N. S. Dufault, M. R. Dornbusch, D. L. Allan, C. P. Vance & D. A. Samac, 2003. Influence of enhanced malate dehydrogenase expression by alfalfa on diversity of rhizobacteria and soil nutrient availability. Soil Biology and Biochemistry 35: 1103–1113.

Tilman, D., 1999. The ecological consequences of changes in biodiversity: a search for general principles. The Robert H. MacArthur Award Lecture. Ecology 80: 1455–1474.

Tilman, D., 2001. Functional diversity. In Levin, S. A. (ed.), Encyclopedia of Biodiversity. Academic Press, San Diego, CA.

Tilman, D., J. Knopps, D. Wedin, P. Reich, M. Ritchie & E. Siemann, 1997. The influence of functional diversity and composition on ecosystem processes. Science 277: 1300–1302.

Walker, B., A. Kinzig & J. Langridge, 1999. Plant attribute diversity, resilience, and ecosystem function: the nature and significance of dominant and minor species. Ecosystems 2: 95–113.

Walker, S. C., M. S. Poos & D. A. Jackson, 2008. Functional rarefaction: estimating functional diversity from field data. Oikos 117: 286–296.

Warwick, R. M. & K. R. Clarke, 2001. Practical measures of marine biodiversity based on relatedness of species. Oceanography and Marine Biology 39: 207–231.

Whittaker, R. H., 1960. Vegetation of the Siskiyou Mountains, Oregon and California. Ecological Monographs 30: 279–338.

Whittaker, R. H., 1972. Evolution and measurement of species diversity. Taxon 21: 213–251.

Wilson, M. V. & A. Shmida, 1984. Measuring beta diversity with presence–absence data. Journal of Ecology 72: 1055–1064.

Zar, J. H., 1984. Biostatistical Analysis. Prentice-Hall Inc, Englewood Cliffs, NJ.

Hydrobiologia (2011) 670:317–333
DOI 10.1007/s10750-011-0721-5

ECOSYSTEMS AND SUSTAINABILITY **Review Paper**

Fishery discards and bycatch: solutions for an ecosystem approach to fisheries management?

**Jose M. Bellido · M. Begoña Santos ·
M. Grazia Pennino · Xulio Valeiras ·
Graham J. Pierce**

© Springer Science+Business Media B.V. 2011

Abstract It has been widely acknowledged that fishery discard practices constitute a purposeless waste of valuable living resources, which plays an important role in the depletion of marine populations. Furthermore, discarding may have a number of adverse ecological impacts in marine ecosystems, provoking changes in the overall structure of trophic webs and habitats, which in turn could pose risks for the sustainability of current fisheries. The present review aims to describe the current state-of-the-art in discards research, with particular emphasis on the needs and challenges associated with the implementation of the Ecosystem Approach to Fisheries Management (EAFM) in European waters. We briefly review the international and European policy contexts of discarding, how discard data are collected and incorporated into stock assessments, selectivity in fishing and the main consequences of discarding for ecosystem dynamics. We then review implementation issues related to reducing discards under the EAFM and the associated scientific challenges, and conclude with some comments on lessons learned and future directions.

Keywords Discards · Bycatch · Ecosystem approach to fisheries management · EAFM

Introduction

Fisheries management has evolved over the years, from being uniquely concerned with single stocks and quotas to the realization that individual fisheries should be managed taking into account their effects on, and interactions with, the ecosystems to which the target species belong, and taking account the human dimensions of fisheries and their relationships with other marine and coastal zone activities, for example, by working in partnership with stakeholders. This has led to the coining of the term 'Ecosystem Approach to Fisheries Management' (EAFM). The EAFM (also named Ecosystem Approach to Fisheries, EAF and Ecosystem-Based Fisheries Management, EBFM) is defined as an integrated approach to management that considers the entire ecosystem, including humans. The goal is to maintain an ecosystem in a healthy,

---

Guest editors: Graham J. Pierce, Vasilis D. Valavanis, M. Begoña Santos & Julio M. Portela / Marine Ecosystems and Sustainability

J. M. Bellido (✉) · M. G. Pennino
Instituto Español de Oceanografía, Centro Oceanográfico de Murcia, Murcia, Spain
e-mail: josem.bellido@mu.ieo.es

J. M. Bellido · M. B. Santos · G. J. Pierce
School of Biological Sciences, University of Aberdeen, Aberdeen, UK

M. B. Santos · X. Valeiras · G. J. Pierce
Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, Spain

productive and resilient condition so that it can continue to provide the services that humans want and need (FAO Fisheries Glossary, http://www.fao.org/fi/glossary/default.asp).

Discarding is currently one of the most important topics in fisheries management, both from economic and environmental points of view (Alverson & Hughes, 1996; Alverson, 1997; Kelleher, 2005; Catchpole & Gray, 2010). The FAO Fisheries Glossary describes discards as ‘that proportion of the total organic material of animal origin in the catch, which is thrown away or dumped at sea, for whatever reason. It does not include plant material and post-harvest waste such as offal. The discards may be dead or alive’. Discarding is an integral part of most fishing operations, since practically all fishing gears catch, at some time, species or specimens that are subsequently thrown back into the sea. Although the two concepts are obviously linked, it is nevertheless not necessarily the same as bycatch, which is the part of a catch that is ‘taken incidentally in addition to the target species towards which fishing effort is directed. Some or all of it may be returned to the sea as discards, usually dead or dying’. Another related concept is ‘slippage’, a common practice in pelagic seine net fishing, whereby unwanted catches are released from the net and not taken on board. This is also destructive because the fish are often killed during the capture process (e.g. FAO, 2010; Huse & Vold, 2010).

Discarding involves a conscious decision made by fishers to reject some part of the catch. Discarding of target species can occur for reasons related to fishing regulations, e.g. if fish are below the minimum landing size or the fisher holds insufficient quota for the species or economic reasons: differences in market prices of different species and size-classes and limited availability of storage space can lead to so-called ‘high grading’, whereby less valuable species and size-classes are discarded to leave space for more valuable catch (e.g. Punt et al., 2006). Other reasons for discarding include damage or degradation of the catch and catching of non-commercial species. When the quota for a species is exceeded, the decision is often taken, especially in mixed fisheries, to continue fishing for other species even if this implies discarding individuals of the species for which the quota has been exceeded. In most EU fisheries, this is both legally permitted and economically justified (since the alternative would usually be to stop fishing), albeit clearly wasteful. It is generally illegal to sell undersized fish or catches of protected species such as corals, some sharks or rays, and marine mammals.

Bycatch and discarding have numerous, generally undesirable, consequences. Clearly these are to some extent no different from the consequences of fishing per se, since all fishing causes mortality of marine animals and potentially also affects marine ecosystem structure and function. The main distinction to be drawn therefore is that discards (and any landed bycatch of no economic value) offer no obvious economic benefit to fishers and therefore represent additional ‘unnecessary’ mortality.

Kelleher (2005) estimated worldwide discards at an average of 7.3 million tonnes per year, or around 8% of the total catch, although the discard rate was much higher in certain fisheries. Thus, shrimp fisheries, particularly in tropical waters, had the highest total amount and highest proportion of discards with a weighted average discard rate of 62% (see Table 1, based on Kelleher, 2005). Demersal finfish trawling had a relatively low discard rate but because of its ubiquity contributed a substantial total amount of discards worldwide. The third most important contribution to total discards was from tuna longlines. Most other line fisheries have low or negligible discards although they may have significant bycatches of seabirds and turtles, an issue which gained prominence in the 1990s (e.g. Brothers, 1991; Cherel et al., 1996; Barnes et al., 1997; Hall et al., 2000). Fisheries with very low or negligible discards included small-scale and artisanal fisheries in general. However, although small-scale and artisanal fisheries usually have low levels of discards per vessel, in certain areas with very large artisanal fleets (e.g. the Mediterranean, some parts of Africa), the total amount of discards can still be very substantial (Stergiou et al., 2003; Nunoo et al., 2009).

Global fishery discards have significantly declined in recent years (Kelleher, 2005; Zeller & Pauly, 2005; Davies et al., 2009). However, there are important exceptions, including (poorly regulated) deepwater fisheries in international waters and some of the most highly regulated fisheries, where severe quota restrictions have resulted in high grading (Kelleher, 2005). There is no unique and simple explanation for the overall decline, but it appears to have been due to, among other factors, improved selectivity of fishing technology and greater utilization of the bycatch for aquaculture and human consumption. Obviously, the

Table 1 Annual landings and discards in the main types of fisheries worldwide (in thousand tonnes), the percentage of discards to catch and the range of discard rates (based on Kelleher, 2005)

Fishery	Landings	Discards	Weighted average discard rate (%)	Range of discard rates (%)
Shrimp trawl	1126.3	1865.1	62.3	0–96
Demersal finfish trawl	16051.0	1704.1	9.6	0.5–83
Tuna and HMS longline (high migratory species)	1403.6	560.5	28.5	0–40
Midwater (pelagic) trawl	4133.2	147.1	3.4	0–56
Tuna purse seine	2679.4	144.2	5.1	0.4–10
Multigear and multispecies	6023.1	85.4	1.4	na
Mobile trap/pot	240.6	72.5	23.2	0–61
Dredge	165.7	65.4	28.3	9–60
Small pelagic purse seine	3882.9	48.9	1.2	0–27
Demersal longline	581.6	47.3	7.5	0.5–57
Gillnet (surface/bottom/trammel)	3350.3	29.0	0.5	0–66
Handline	155.2	3.1	2.0	0–7
Tuna pole and line	818.5	3.1	0.4	0–1
Hand collection	1134.4	1.7	0.1	0–1
Squid jig	960.4	1.6	0.1	0–1

latter is unlikely to have contributed much to reducing fishing mortality or reducing damage to ecosystems. Indeed, the growth of aquaculture potentially represents one of the greatest threats to marine ecosystems through the increased demand for fishmeal derived from so-called ‘reduction fisheries’—although Asche & Tveterås (2004) argue that the threat can be avoided by efficient management of such fisheries.

At the time of writing, the European Commission is discussing the banning of discards as part of the reform of the CFP. In the present review, we examine the policy context of discarding in European fisheries and the current state-of-the-art in discards research. We discuss the main consequences of discarding for ecosystem dynamics, fishing exploitation and implications for management, with particular emphasis on the needs and challenges associated with the implementation of the Ecosystem Approach to Fisheries Management (EAFM) in European waters. We and then examine possible solutions to the issue in the context of the EAFM.

International regulations on discarding and bycatch

Before turning to focus on the situation in Europe, we here briefly outline the international context. As noted by Alverson et al. (1994) in their global assessment of fisheries bycatch and discards, awareness of discarding in fisheries can be seen in the bible, in parable of the net (Matthew 13: 47–48): ‘Again, the Kingdom of Heaven can be illustrated by a fisherman—he casts a net into the water and gathers in fish of every kind, valuable and worthless. When the net is full, he drags it up onto the beach and sits down and sorts out the edible ones into crates and throws the others away…’. Alverson et al. also point out that incidental catches and discards have received most attention in the USA, relating to primarily to mortality of marine mammals in the Eastern Tropical Pacific purse seine fishery for tuna, high seas driftnetting fisheries (in which seabird and salmon bycatches were also a major issue) and the high level of discarding in shrimp fisheries in the Gulf of Mexico. Two significant pieces of national legislation resulted in the 1970s, the Marine Mammal Protection Act (1972) and the Endangered Species Act (1973). The USA also had a leading role in the adoption in 1989 of United Nations General Assembly Resolution 44/225, which recommended that all members of the United Nations agreed to a Moratorium on all large-scale pelagic driftnet fishing on the high seas by 30 June 1992. The United Nations Convention on the Law of the Sea (UNCLOS) was concluded in 1982, finally coming into force in 1994. This covers, for example, the requirement for fishing within the Exclusive Economic Zones of another country to respect conservation measures and other laws and regulations of the country.

In recent decades, the Fishery and Agriculture Organization of the United Nations (FAO) has provided a range of legislative instruments and guidelines for fisheries, including the 1995 Code of Conduct for Responsible Fisheries, the 1999 FAO International Plan of Action for Reducing Incidental Catch of Seabirds in Longline Fisheries (IPOA-Seabirds, FAO, 1999), the 1999 FAO International Plan of Action for the Conservation and Management of Sharks (IPOA-Sharks, FAO 1999), and the 2009 FAO Guidelines to Reduce Sea Turtle Mortality in Fishing Operations (FAO, 2009). Arising from a Technical Consultation held in Rome in December 2010, FAO issued International Guidelines on By-catch Management and Reduction of Discards (FAO, 2010). These guidelines are intended to assist States and Regional Fisheries Management Organisations or Arrangements (RFMO/As) in the management of bycatch and reduction of discards in conformity with the FAO Code of Conduct for Responsible Fisheries. Among other initiatives, these Guidelines establish that States and RFMO/As should develop a framework for long-term cooperative work on bycatch management and discard reduction in association with stakeholders, management authorities at all levels, and other agencies and organizations, including providing accurate and timely information on bycatch-related issues, regulations and activities. They also establish the participation of scientists with appropriate expertise to conduct and evaluate bycatch and discard assessments, and propose mitigation strategies.

Discarding and fishery policy in Europe: towards an ecosystem approach

To the extent that obligatory discarding is part of a coherent management framework, it could be regarded as unfortunate but unavoidable collateral damage which nevertheless confers wider benefits for sustainability. In the European Union, however, such a viewpoint is increasingly untenable, not least because the European Common Fishery Policy has, at least in several important respects, failed to deliver sustainable fisheries. Important issues include fleet overcapacity, overexploitation of vulnerable species, wasteful practices such as discarding, environmental degradation and effects on non-target species: see Daw & Gray (2005) and Khalilian et al. (2010) for detailed critiques. Such failings are explicitly recognized in the Green Paper concerning the current process of CFP reform (EU COM, 2009). Some other countries, e.g. Norway, ban discarding and arguably also achieve more sustainable fisheries.

Any implementation of EAFM must consider discarding for several reasons: (a) it directly affects the balance, diversity and functioning of the ecosystem, (b) it potentially leads to reduced income from fisheries and (c) because it is widely perceived as being wasteful and ineffective, it undermines respect of fishers for the governance system and thereby leads to reduced compliance with, participation in and effectiveness of the regulatory system.

According to Hilborn (2011), there are ‘core’ and ‘extended’ aspects of EAFM. The ‘core’ consists of three primary features: (a) keeping fleet capacity and fishing mortality rates low enough to prevent ecosystem-wide overfishing, (b) reducing or eliminating bycatch and discards and (c) avoiding habitat-destroying fishing methods. The ‘extended’ EAFM takes into account trophic interactions and area-based management. Certainly such management objectives are not exclusive to EAFM and most fisheries management agencies around the world attempt to meet at least some of these objectives as part of existing single-species management regimes. In fact, the recent FAO International Guidelines on Bycatch Management and Reduction of Discards (FAO, 2010), in support of management measures to mitigate bycatch and discard problems, advised that ‘States and RFMO/As should, where appropriate, map seabed habitats, distributions and ranges of species taken as bycatch, in particular rare, endangered, threatened or protected species, to ascertain where species taken as bycatch might overlap with fishing effort’.

It is evident that the good intentions of the CFP have not borne fruit. Thus, the current UK government stance (as of April 2011) is that ‘The current Common Fisheries Policy is broken. It has not delivered its key objective of an economically viable fishing industry which minimizes impacts on marine ecosystems’. (http://www.defra.gov.uk/environment/marine/cfp/). A significant part of that problem appears to be that the scientific advice, which aims to address the CFP objectives, has been routinely ignored due to a decision-making process that clearly has rather different objectives, short-term political expediency being prominent among them. Cardinale & Svedang (2008) argued that, despite the limitations of using a deterministic single stock modelling framework for assessment, managers and politicians have had the necessary scientific instruments for managing stocks and avoid stock collapses (and by implication for achieving increased economic and social sustainability), but they failed to deliver since they tried to minimize the short-term negative impact of policy on those who are most affected (i.e. the fishing industry). The authors argued that is the practice of ignoring the scientific advice, more than the advice itself, which is to be blamed for the wasteful depletion of formerly abundant marine resources. Khalilian et al. (2010) offer similar arguments when discussing the failure of the CFP from biological, economical, legal and political perspectives. Excessive quotas set by the Council, regularly overriding scientific advice and payment of direct and indirect subsidies by both the EU and Member States, have resulted in too much fishing effort and excessive exploitation rates, leading in turn to low stock sizes, low catches and severely disturbed ecosystems. The lack of transparency of its regulations as well as insufficient control and enforcement of its provisions have contributed to the failure of the CFP. Khalilian et al. (2010) characterize the CFP as an opaque decision-making procedure with little approval by the public, which leads to a culture of non-compliance that undermines the CFP and the final goal of implementing sustainable fisheries management.

Several authors have argued that appropriate application of single-species management could actually achieve some of the goals of EAFM. Froese et al. (2008) show that setting fishing mortalities for several North Sea and Baltic species so as to achieve maximum sustainable yield (MSY) for individual stocks would be an improvement on the current regime, while taking only larger individuals (such that all fish are able to achieve maximum growth rate) would increase yield while at the same time rebuilding stocks and minimizing impact on the ecosystem. Although not specifying how catching smaller fish could be avoided, the authors point to fisheries elsewhere in the world where such objectives have been achieved (Hilborn, 2011). Hilborn (2011) raises the question ‘Would EAFM be unnecessary if we had implemented single-species management correctly?’ His answer is that successful single-species management could be a major step forward in many areas but, by itself, it is not sufficient because pure single-species management does not consider impacts on non-target species, trophic interactions among species and habitat-destroying fishing practices. However, he also notes that successful single-species management demands understanding of the ecosystem impacts of factors other than fishing, i.e. the need to deal with broader ecosystem concerns is already evident.

Nevertheless, even this latter analysis is based on the implicit assumption that the current assessment, management and governance system, whereby the different components are seen as independent, sequential, processes, is an appropriate framework. Environmental sustainability cannot be achieved in isolation from considerations of socioeconomic sustainability; the implementation of management measures must take into account the responses of the fishers. Thus, stock assessments must extend to offering predictions of stock trajectories under not only a range of possible management measures but a range of realistic outcomes in terms of compliance and enforcement of regulations. Furthermore, fisher buy-into the management and governance regime can itself be managed, through measures such as participatory management and co-management.

Collecting information on discards

Discards account for significant mortality in fisheries. However, few stock assessments take into account information on discards (Mesnil, 1996; Hammond & Trenkel, 2005; Punt et al., 2006; Aarts & Poos, 2009; Fernández et al., 2009). This is mainly due to limitations of the available data: long time series of onboard observation are not available for all the fleets involved in the exploitation of most stocks. In addition, a large amount of monitoring and research effort is needed to obtain this kind of information (Alverson et al., 1994; Kelleher, 2005).

One of the main problems with onboard observer data is the high spatial and temporal variation shown in discard patterns. Aside from the obvious difficulty of obtaining precise estimates for a highly variable phenomenon, if the sampling design does not account for it, this high variation could hide some bias in the estimation, which will be transferred and multiplied when raising estimates to the level of the whole fleet or stratum (Allen et al., 2001, 2002; Borges et al., 2004; Apostolaki et al., 2006). Rochet & Trenkel (2005) concluded that the factors underlying variation in discard rates are complex, noting that the amount of discards is rarely proportional to catch or effort, and commenting that although environmental conditions and fishing methods affect discards stratification, stratification of sampling to take this into account may not improve the precision of estimates.

The above-mentioned conclusions notwithstanding, one solution is to identify and measure auxiliary variables (e.g. environmental, biological, regulatory, market factors) which affect the nature and extent of discarding and use statistical modelling to control for these effects. For example, Stratoudakis et al. (1998) analysed sources of variation in proportions of three gadid species discarded at length by fishers using demersal gears in the North Sea. They found clear differences between inshore and offshore fishing areas (with more high grading observed in the latter) but also showed that discarding practices for haddock and cod were consistent over time and across gears—although discarding of (the less valuable) whiting was more variable and depended on catch composition. Borges et al. (2005) investigated both the best sampling unit and auxiliary variables for estimating discards in Irish fisheries. Their results showed that use of fishing trip rather than haul as sampling unit reduced the overall variability of estimates. Use of different auxiliary variables resulted in different estimates and although the authors observed that number of fishing trips is probably reported more reliably than hours fishing or weight of landings reliable, there was no reason to favour one estimate over another.

While spatial stratification of discard sampling is routinely undertaken (as described, for example, in Stratoudakis et al. 1998), it is worth considering that spatial patterns of discarding can occur at several scales and may differ between species. Such patterns can be quantified using spatial statistical methods, as shown by Sims et al. (2008) and Lewison et al. (2009) in relation to fishery bycatch. In the context of bycatches of megafauna, these authors point out the importance of considering bycatch relative to target catch as well as the relevance of identifying spatial patterns in bycatch to management and mitigation of bycatches. These are conclusions which are equally relevant to discarding.

Another aspect requiring more attention is the change of discarding behaviour over time, e.g. seasonally or over the course of a fishing trip, the latter being particularly important in distant water fleets that make long trips. Several factors, e.g. availability of storage space, temporal variation in abundance of target species or even changes in market price during the fishing trip can lead to changing decisions about which part of the catch to retain. Bellido & Pérez (2007) evaluated alternative sampling strategies for discarding by Spanish trawlers using computer resampling (bootstrapping) and identifying the strategy that minimized the coefficient of variation. They suggested sampling at least one vessel and one trip per vessel, monthly, sampling between 30 to 50 hauls within a trip, and sampling 8–15 hauls at the beginning, middle and end of the trip. Gray et al. (2005) reported seasonal differences in discard rates in an Australian estuarine commercial gillnet fishery. These differences were attributed to a seasonal difference in fishing regulations such that nets could be left in the water only 3 h during summer but could be set overnight in the winter. Although the discarding rate was generally low, the authors concluded that reducing maximum soak time (as well as increasing mesh size) would reduce the discard rate.

Most of the studies cited thus far have involved data collection by on-board observers. Observer programmes are generally thought to be essential for accurate quantification of discards in most fisheries. However, some authors have questioned whether observer at-sea trips can be used to make inferences about catch composition and discards. Thus Benoît & Allard (2009) highlight two issues, 'deployment' bias resulting from non-random distribution of observers among sampling units and observer effects due to changes in fishing practice or location when observers are on board.

A major limitation is the expense of using on-board observers to record discard data. Allard & Chouinard (1997) proposed using a combination of on-board and shore-based sampling, with the latter making use empirically determined changes in the length-frequency distribution of catches when discarding had taken place. The advent of on-board camera technology offers the prospect of a more comprehensive (if perhaps less detailed) picture of discarding practices. FAO (2010) recommend that management of bycatch and reduction of discards should be supported by technological development both in the harvest and the post-harvest and valorization sector.

Incorporating discard data into assessments

The omission of discard data from the stock assessment process may result in underestimation of fishing mortality and can lead to biased assessments, hampering achievement of sustainable resource use (e.g. Punt et al., 2006; Aarts & Poos, 2009). Some progress has been made recently on inclusion of discard data and survival estimates into stock assessment. For example, in the case of the Norwegian lobster (*Nephrops norvegicus*), one of the most valuable crustaceans landed in Europe, with most of the catches taken by bottom trawls, estimates of 25% discard survival rate have been used in the assessment of the stocks by the International Council for the Exploration of the Sea (ICES, 2010).

Several authors have used statistical modelling to estimate discards, based on the assumption that the main driver for discarding is minimum landing size regulations (e.g. Casey, 1996; Cotter et al., 2004; Punt et al., 2006). One limitation in such models has been the assumption that gear selectivity is constant. Aarts & Poos (2009) developed a statistical catch-at-age model with flexible selectivity functions to reconstruct historical discards of plaice in the North Sea and estimate stock abundance. Fernández et al. (2009) developed a Bayesian age-structured stock assessment model for the southern stock of European hake (*Merluccius merluccius*) and showed that incorporating information on discards into the model had an important effect on predicted stock trajectories.

Punt et al. (2006) point out that inclusion of discard data can also permit detection of strong year-classes before they are apparent in landings data—while stressing that discarding remains a poor use of the resource and that conducting pre-recruit surveys is a more appropriate way to predict future recruitment. The few fish stock assessments that include discards assume that all discarded fish die, which is not necessarily the case. Mesnil (1996) incorporated various levels of discard survival into stock assessments based on Virtual Population Analysis (VPA) and showed that this could significantly affect estimates of fishing mortality and stock size. The author also suggests that, from a management point of view, measures to improve the survival of released fish (if feasible and effective) might be as effective as increasing mesh size and potentially more acceptable to fishers. Although the inclusion of discard data into stock assessment models is a major improvement, most of the above-mentioned examples are based mainly on a single-species approach.

Selective fishing

More selective fishing should reduce discards by avoiding unwanted catches and maximizing the marketable portion of the catch. Zhou et al. (2010) refer to six types of selective fishing: by species, stock, size, sex, season and/or space. Increased selectivity is generally favoured by fishers, as they are by nature selective and do not want to catch fish that cannot be sold or that will create sorting difficulties. Recent work in this field covers topics such as mesh size regulation (Suuronen et al., 2007), technical measures (Catchpole et al., 2008; Enever et al., 2009a), mesh size and selectivity modifications (Revill & Holst, 2004; Guijarro & Massuti, 2006; Revill et al., 2007; Massuti et al., 2009), cost-benefit analysis (Macher et al., 2008), new designs to improve escapement of unwanted fish (Graham, 2003; Revill et al., 2006; Catchpole et al., 2007; Moore et al., 2009; Yamashita et al., 2009) and devices to reduce the impact of trawls on benthic communities (Revill & Jennings, 2005). There have also been important advances in reduction of bycatches of marine mammals and seabirds in gears such as purse seines, gill nets and long-lines. National Research Council (1992) describe how a combination of modified fishing gear, modified procedures and education of skippers dramatically reduced dolphin bycatches in the Eastern Tropical Pacific tuna fishery. Several studies have shown that acoustic alarms (pingers) can reduce porpoise bycatch in gill nets (e.g. Gearin et al., 2000), although their efficacy is by no means universally accepted and there is a need to monitor the success of deploying pingers. Goetz et al. (2011)

describe trials of modifications to long-lines to reduce seabird bycatches (see also references therein).

Although bycatch reduction has been achieved in some fisheries by modifying the gear, some well-publicised cases have not been successful. The fishery for Baltic cod (*Gadus morhua*) has been subject to a great number of technical regulations, with the aim of reducing juvenile mortality. However, a large increase in selectivity introduced in a single step may not be commercially acceptable and in this case the measures resulted in substantial short-term economic losses. Suuronen et al. (2007) note that fishers' willingness to comply with new regulations depends largely on their ability to deal with such short-term reductions in catch. When losses are too large, gears will be manipulated and rules will be circumvented. Apparently, a gradual increase in mesh size (or gradual introduction of any restrictive measure) would often be more acceptable to the fishers (Suuronen et al., 2007). In addition, fishers usually prefer mesh size regulations to fishing effort regulation, probably because the former still allows them the opportunity to apply the deep knowledge they have on fishing gears and the way they operate.

Although more selective fishing is always suggested as a key factor in reducing discards, Zhou et al. (2010) argue that less selective fishing gears may help to maintain diversity and functioning in certain marine ecosystems (although they do also point to the importance of the protection of vulnerable species and the need for regulation of fishing effort). This potential inconsistency between promoting more selective fishing and the 'ecosystem approach' requires attention from both theorists and practitioners in order to formulate the best scientific advice (Kelleher, 2005). Hall & Mainprize (2005) recommend diversifying our harvest and learning to utilize a wider variety of products, although they stress that this is not intended as a justification of extending fishing activity to other species, rather it should involve reduced fishing pressure on current target species.

On the impact of fishing and discards in the ecosystem

Knowledge of the impacts of bycatch and discarding at the community and ecosystem levels becomes increasingly necessary in the context of the multi-species and ecosystem-based approaches to fisheries management (Borges et al., 2001).

Disturbance by trawling is well known to affect the species composition and structure of marine benthic communities. Several authors have suggested that trawling disturbance is 'farming the sea'; ploughing the seabed to boost production. To others, trawling is assumed to damage key functional processes (Jennings & Kaiser, 1998). Also, the physical disturbance of the sediment by trawl nets could expose endobenthic organisms which can then be predated by carnivores (Jenkins et al., 2004). However, the effects on ecosystem structure and function (biodiversity, community structure, trophic links) of returning biomass directly to the ecosystem though discarding are not so well known (Dayton et al., 1995; Jennings & Kaiser, 1998; Lindeboom & de Groot, 1998; Hall, 1999; Collie et al., 2000; Kaiser & de Groot, 2000; Borges et al., 2001; Erzini et al., 2002). The effects of discarding on the stability of trophic webs may have negative consequences for commercial stocks due to the disruption of species interactions and cascading effects throughout the trophic chains (Monteiro et al., 2001). Tsagarakis et al. (2008) showed that the composition and/or trophic level of discards in relation to the marketed catch seemed to be indicative of the exploitation state of the demersal community.

Various seabird species use discards and offal as trophic resources, and some species are believed to have increased in numbers as a result of availability of food via discards (Furness, 2003; Valeiras, 2003; Votier et al., 2004). However, Grémillet et al. (2008) argue that, at least for gannets, fishery waste is basically 'junk food' and has a negative impact on growth rates of chicks.

Another fraction of the discards sinks in the water column and its fate is poorly known but some midwater scavengers such as sharks (Sánchez et al., 2005) may benefit from them. Finally, the remaining discarded biomass ends up on the seabed and is consumed by the benthic fauna (Jennings & Kaiser, 1998; Jenkins et al., 2004). The biomass made available by fisheries discards returning to the seabed may produce good conditions for a short-term increase of scavenger benthic species, including fish, crabs, shrimps and other invertebrates.

Long-term studies of the benthos communities in the southern and central North Sea suggest that biomass and production have increased (Kroncke et al., 1998). This could be a response to trawling disturbance, climate change and/or eutrophication (Rijnsdorp & van Leeuwen, 1996; Kroncke et al., 1998). The decrease in abundance of vulnerable species such as elasmobranchs, echinoderms, corals and sponges due to seafloor disturbance caused by trawling could be followed by increases of other benthic species.

Many elasmobranch species are thought to be threatened by bycatch and discarding, and it is also a serious issue for various species of turtles and seabirds (caught on long-lines), and marine mammals (caught in purse seines, gillnets and trawls). Elasmobranch fish have been reported to be more resistant to capture than teleosts, with several species of sharks and rays having a high probability of survival after being discarded from trawlers. Rodríguez-Cabello et al. (2005) quoted a mean survival rate of 78% for spotted catshark *Scyliorhinus canicula* in the Cantabrian Sea, while Enever et al. (2009b) found a short-term rate of survival of 55% for skates discarded in the skate fishery in the Bristol Channel.

Further important related issues that still need further research include the impact of abandoned gears (ghost fishing) and slippage of catches in pelagic fisheries. This is highlighted in the FAO International Guidelines on Bycatch Management and Reduction of Discards (FAO, 2010) which dedicates a section to pre-catch losses and ghost fishing, establishing that States and RFMO/As should consider measures to address the impact of pre-catch losses and ghost fishing on living aquatic resources. Recommendations include development methods for estimating pre-catch losses by various gear types, modification of gears and fishing methods, identification of gear ownership, reduction of gear losses, development of gear retrieval procedures and programs, and reducing, and where possible eliminating, fishing power of lost gear, e.g. through the use of degradable materials. FAO (2010) also remind us that abandoned and discarded gears should be considered as marine pollution and that

> States and RFMO/As should take account of current work at the International Maritime Organization on the revision of Annex V of the International Convention for the Prevention of Pollution from Ships, 1973 as modified by the Protocol of 1978 (MARPOL 73/78) and the Guidelines for the Implementation of Annex V in relation to reducing the impact of lost fishing gear.

Brown & Macfadyen (2007) reports that ghost fishing in depths shallower than 200 m is not a significant problem and declines rapidly once nets have been lost. This is due to lost, discarded, and abandoned nets have a limited fishing life, because many static-net fisheries take place in shallow water, where storm and tide action can quickly roll up the nets, and bio-fouling reduces their catching efficiency (Erzini et al., 1997; Pawson, 2003; Revill & Dunlin, 2003). Large et al. (2009) carried out retrieval exercises to recover lost and abandoned nets from deep-water gillnet fisheries in the Northeast Atlantic. They towed a retrieval gear that basically consisted of three grapnels connected by chains to a steel bar and towed at a speed of 1–2 knots, a technique called ‘creeping’. In terms of mitigation, they suggested that information should be collected from fishers and fisher organizations, and creeping should then be carried out at locations where fishers have reported incidences of lost or abandoned nets.

Huse & Vold (2010) showed that (short-term) mortality of mackerel in purse seines could be reduced by avoidance of ‘excessive crowding’ of the fish. Studies by Stratoudakis & Marcalo (2002) on sardine (*Sardine pilchardus*) taken by purse seiners in Portugal and for another sardine species (*Sardinops sagax*) taken with the same gear in western Australia (Mitchell et al., 2002) indicate that slippage mortality could be much higher in the long-term as, although fish are still alive when released, many are believed to have suffered physical damage (loss of scales, skin abrasions) by contact with other fish and the walls of the net.

Implementation of policy

Pikitch et al. (2004) state that the overall objective of EAFM is to sustain healthy marine ecosystem and the fisheries they support. EAFM is generally considered more conservative and more protective of marine ecosystems than is single-species management. Hilborn (2011) comments that he suspects the general public and legislators believe that if we can manage every species to its MSY level, there would be no significant ecosystem impacts. However, we should be aware that a healthier ecosystem does not automatically imply more productive fisheries. Additionally, EAFM objectives are quite often vague enough that different interpretations could lead to drastically different outcomes. The current legislative frameworks for EAFM often lack clarity, and management agencies will have insufficient guidance on appropriate policy unless international agreements and national legislation are made more specific.

Given that fisheries and conservation tend to be the responsibilities of different and independent government departments, it is perhaps unsurprising that some of the most important contributions to EAFM have arisen from non-fisheries legislation. Hall & Mainprize (2005) review several examples, including the US Marine Mammal Protection Act, which sets monitoring requirements and imposes tough and rigorously enforced limits on fishery bycatch of marine mammals. Other examples include the US Endangered Species Act which limits the incidental capture of the short-tailed albatross in Alaska and the Environmental Protection and Biodiversity Conservation Act in Australia, which requires fisheries to undertake ‘threat abatement plans’ if they impact on certain marine species, and to become accredited as ecologically sustainable. Aside from illustrating the power of non-fisheries legislation to effect changes in fishing practices, an important precautionary note is that these are all non-European examples. In the European context, it is apparent that fishery and conservation may be contradictory (e.g. the CFP and the Habitats Directive), and indeed, because national governments cede power to regulate fisheries beyond their immediate coastal waters to the European Union, they may be legally powerless to fulfil their species protection obligations under the Habitats Directive (Khalilian et al., 2010).

There is a clear need to take account of the interdependence of stocks and the effects on species associated with or dependent upon harvested species, with a view to maintaining or restoring populations of such associated or dependent species above levels at which their reproduction may become seriously threatened. The 1980 Convention on the Conservation of Antarctic Marine Living Resources provides that ‘ecological relationships between harvested, dependent and related species must be maintained’. This principle often refers specifically to endangered, threatened or protected species. A key-related objective is to minimize bycatch and discards. As it is impossible to optimize the exploitation for all species at the same time, compromise solutions will need to be found, reflecting decisions on which species may be more negatively affected. Optimal harvest strategies for multi-species fisheries have for some time been a focus of ICES work. A variety of mathematical approaches has been developed, among which the Fcube (Fleet and Fishery Forecast) model is particularly promising (J. Castro-Pampillon, pers. Comm.)

The Ecosystem Approach to Fisheries Management (EAFM) will provide some impetus to this process, in that it aims for an integral ecosystem-based management of fisheries. One of the main challenges of the EAFM is to understand the trade-offs resulting when a particular approach is chosen, and to develop the institutional and legislative frameworks that recognize and account for these trade-offs (Hall & Mainprize, 2005). While a measure may, at first glance, appear entirely reasonable and may well make fishery managers and conservationists feel better, the complexities of ecological systems and the biology and population dynamics of the species within them, the difficulty of measuring the outcomes, the inability or unwillingness on the part of the fishers to comply with the measure, and the inability of the regulatory agency to enforce compliance, can often conspire against good intentions and render a measure ineffective, unexpectedly costly or simply impossible to evaluate. As with most complex decisions, there are trade-offs that must be carefully weighed.

As is increasingly obvious across the spectrum of different fishery management measures, it is essential to engage fishers and stakeholders in the management system to find appropriate and agreed solutions. Furthermore, as the potential interactions between fisheries and other uses of the seas are increasingly recognized (and captured within concepts such as integrated coastal zone management, marine spatial planning and integrated marine management), there may be a need to involve experts and stakeholders from other management areas.

In very broad terms, there are two different approaches for managing discards in the world: regulating what it is allowed to be caught and regulating what can be retained on board and landed, with the latter being more easily enforceable since it requires inspection only at the landing port. In addition, the full utilization of the catch may be promoted, for example, by developing markets for 'non-commercial' species (e.g. Portela et al., 2004).

Measures to reduce may include modifications of gear and or fishing practices. While it is impossible to legislate against bycatch occurring, it can be discouraged by imposing penalties. Thus, in relation to marine mammal bycatch, measures available under the US Marine Mammal Protection Act include fishery-specific limits on bycatches, time and area closures, gear modifications and deployment of pingers (the latter being a measure originally proposed by the fishers, Bache, 2001). Bisack & Sutinen (2006) explored the idea of introducing Individual Transferable Quotas for porpoise bycatch and argue that it is a potentially more efficient measure than area closures.

One option for regulating discards is to pursue a no-discard policy, as implemented in, for example, Norway, Iceland and New Zealand, whereby all catches, desirable and non-desirable must be landed. However, unless combined with measures to reduce catches of unwanted fish and/or to provide for their utilization, the benefit in terms of environmental conservation and sustainable marine and coastal zones management may be limited or negative. Rather than ensuring zero waste, the policy potentially transfers the problem of marine waste onto the land, where its safe disposal becomes a problem for local authorities. If such waste is stored adjacent to the coast, there is the risk of pollution in the coastal and littoral area. A partial solution (at least providing benefits onshore) may be the development of processing facilities and markets to make use of fish waste, e.g. to produce feed and fertilizer. Catchpole et al. (2005) note that discard bans can create markets for incidental catches. While there may be cases for the development of markets for particular species or size classes, where there is pressure on resources and threats to sustainable fishing activities, the main objective must be reducing the capture of potential discards rather than their utilization. The above discussion highlights the importance of careful analysis before a measure is adopted.

The European Commission is at present reconsidering its discard policy, which represents a major shift in European fisheries management (Green Paper, EU COM, 2009). This is taking place in the context of a bigger and fascinating challenge, to develop holistic approaches to manage the use of the sea and its resources as a whole, as envisaged under EU Marine Strategy (Apitz et al., 2006; Jensen, 2006). EAFM thus represents the 'fishery' component within holistic marine management.

A no-discard policy changes the focus of management from landings to catches, in other words from production to total fishing mortality. This is exemplified in the contrasting Norwegian (it is prohibited to *catch*…) and EU legislation (it is prohibited to *have on board*…). This means that many of the no-discards management measures are designed to ensure that unwanted fish is not caught. Thus, the choice is not between returning unwanted fish to the sea and obligatory landings for fishmeal or animal feed, but between catching and not catching unwanted fish.

While the EU sees reducing excessive fishing effort as the main way to reduce the level of unwanted catch, other measures, already enforced in no-discard countries, should also be considered (Green Paper, EU COM, 2009): (a) temporary area closure for spawning stocks, vulnerable habitats or protecting juveniles; (b) real-time movement of vessels to another fishing area once their unwanted catches exceed a certain level; (c) adapting fishing gear so that threatened species or sizes can escape from nets and (d) reviewing existing management measures which may lead to discarding. The discard ban could be implemented progressively, for example, starting with a discard ban for pelagic species (mackerel, herring, blue withing, etc.) in the first year of the new CFP, and continuing with demersal target (cod, hake, nephrops, sole, etc.) and associated species (haddock, whiting, hake, plaice, etc.) as well as a discard ban in Mediterranean fisheries in the second year of the new CFP (EU High Level Meeting on banning discards, Brussels 1st March 2011).

Scientific challenges to implement an EAFM

How can scientists provide answers and tools to meet such a huge challenge? Hilborn (2011) suggests that EAFM needs to be set in the context of risk analysis. The FAO guidelines for bycatch and discards reduction (FAO, 2010) also identify the need for 'a risk assessment to identify the specific nature and extent of bycatch and discard problems in the fishery as a basis for prioritization and planning'. However, before we can conduct risk analyses, the specific objectives of EAFM must be clear.

It is evident that complete knowledge of fisheries, and the ecosystems in which they take place, is impossible. For example, in some multispecies, multigear fisheries, reporting the full species composition of catches may not be practical. Consequently, alternative methods, such as reporting on indicator species or other suitable proxies, may be necessary. Levin et al. (2009) propose an Integrated Ecosystem Assessment (IEA) as a framework for organizing science in order to inform decisions in marine EAFM at multiple scales and across sectors. IEA comprises five key stages: scoping, indicator development, risk analysis, management strategy evaluation and ecosystem assessment. It develops ecosystem indictors through synthesis and quantitative analysis of information on relevant natural and socioeconomic factors, in relation to specified ecosystem management objectives, and integrates them into management measures.

Implementation of spatial management, with zoning for different kinds of fishing activity and use of seasonal or temporary closures, can be a useful tool for reducing discard rates and controlling effort exerted. Spatial management measures must be underpinned by a good knowledge of the biology, spatial distribution and abundance of both resource species and other species impacted by fisheries, including protected species. The effects of fleet displacement must also be understood, otherwise spatial management results can be disappointing.

There is a huge literature on the pros and cons of marine protected areas (MPAs). In the context of fisheries, successes have been decidedly mixed. Catchpole et al. (2005) note that temporary closure, through establishment of the 'Plaice Box', failed to protect the main nursery grounds for plaice in south-eastern North Sea, even after closure was made permanent, whereas a Norwegian system of temporary closures used in the Barents Sea is regarded as having an important contribution to the recovery of cod and haddock stocks. Robb et al. (2011) comment that 'no-take' MPA, in which all fishing is prohibited, can result in greater productivity of fish stocks. However, they highlight the need for effective management to ensure that only permitted activities occur within MPAs. The authors found that all but one of 161 MPAs on the Pacific coast of Canada are open to some kind of commercial fishing and attribute the mismatch between intent and practice to a lack of coordination between management of protected areas and management of fisheries.

Recent fisheries research has focused on the development of indicators that might underpin the implementation of an EAFM. Such indicators would provide information on the state of the ecosystem, the extent and intensity of effort or mortality and the progress of management in relation to objectives (Jennings, 2005). Papers on ecosystem or ecological indicators in the context of fisheries have flourished over the last 10 years (see, for example, Piet & Jennings, 2005; Piet et al., 2008; Cotter et al., 2009; Rochet & Trenkel, 2009; Van Hoey et al., 2010; Greenstreet et al., 2011). Trenkel et al. (2007) proposed such an approach for the assessment of two anglerfish (*Lophius piscatorious* and *L. budegassa*) stocks in the Bay of Biscay and the Celtic Sea. The authors used a set of indicators derived from scientific survey data and compared the results between traditional model-based and the indicator-based methods. Although their results were somewhat inconclusive, it is clear that the progressive implementation of an EAFM will need to be based on the behaviour of ecological indicators (Piet et al., 2008). Regarding discard and bycatch issues, some relevant pressure indicators have been suggested to address how fishing impacts on the ecosystem. The discarding rates of commercially exploited species and discard rates in relation to landings value have been suggested as pressure indicators to use as measures of the relative environmental impact of different fisheries (Piet et al., 2007). Indicators should guide the management of fishing activities that have led to, or are most likely to lead to, unsustainable impacts on ecosystem components or attributes (Jennings, 2005; Rice & Rivard, 2007).

Currently, the implementation of the Marine Strategy Framework Directive (MSFD Directive 2008/56/EC) is providing a new impetus to the process of indicator development. It calls for completion of an initial assessment of the current environmental status of EU waters and the environmental impact of human activities by 2012 and envisages EU Member States achieving (or maintaining) good environmental status (GES) across all European waters by 2020. In relation to fisheries, populations of commercially exploited fish and shellfish should be within safe biological limits and elements of marine food webs should occur at normal abundance and diversity. Reduction of bycatches and discarding should contribute to both objectives.

Heymans et al. (2011) modelled the deep-sea ecosystem of the Rockall area (200 miles off the west of Scotland) using Ecopath with Ecosim. They identified the lack of discard data from deepwater fisheries in the area as an important limitation and potentially a substantial source of error in the model. This emphasises the importance of having a deep knowledge and good quantification of discards throughout EU waters. This is needed to assess ecosystem status, as required for the implementation of EAFM and the MSFD. A common database of discarded species for different fishing gears and areas would provide a good starting point. Data are needed to make rational decisions, evaluate fisheries performance in relation to management objectives and fulfil regional, national and international obligations. The extent to which management objectives are achieved is assessed using indicators, which are generated from data. Appropriate indicators can be developed which measure the state of the resource, the performance of fishing controls, economic efficiency and social value (e.g. to coastal communities).

Conclusions and future directions

The history of fisheries management, like that of many human endeavours, is a tale of an increasingly detailed and sophisticated understanding of what we are doing wrong, while, on the whole, solutions are developed at a much slower pace. In the case of the EAFM, we increasingly recognize that the damage caused by fishing spreads far beyond the target fish population, and we are developing a range of metrics and indicators to quantify these negative effects and to help identify optimal states (good environmental status). However, it is arguable that (at least so far), we have been much less successful at devising management measures and governance systems that can deliver on these objectives.

There is also a common agreement that reduction of discarding will greatly benefit the health of marine ecosystems. The 'discards problem' is a key point in the EAFM. It is far from being an easy issue to solve, as it involves the 'hard core' of fishing operations, from economic, legal and biological points of view. Assuming that discards are unavoidable, the question of an acceptable level of discards has a moral dimension in addition to the more obvious biological and economic criteria (Kelleher, 2005). Additionally, the legal requirement (as under the current CFP) to carry out such an obviously wasteful practice undermines the legitimacy of the regulatory/management system. However, in spite of all these difficulties, there is a common and positive perception from all sides (citizens, NGOs, the fishing sector, policymakers, scientists, etc.) that discards are negative for all us. We all should work to find a better solution.

Of course, that desirable solution will most probably not come about implementing a few simple management measures, and it would require substantial changes in many fisheries, possibly with substantial economic consequences. Here we suggest the principles and goals that should be met to achieve a reduction of discards and finally a better and healthier marine environment as well sustainable fishing exploitation under the framework of the EAFM:

1. A better balance between fishing intensity exerted and the carrying capacity of the ecosystem: This requires, firstly, a deeper and more detailed knowledge on ecosystem dynamics, including spatial distribution, abundance patterns and fish behaviour, secondly supplementary discards-directed management measures within the EAFM framework, such as requirements to change fishing ground and real-time closures. The basic implementation principle is to regulate what is caught in the first place rather than to regulate landings.
2. Better selectivity without altering biodiversity and ecosystem functioning: Progressive introduction of discard reduction devices and encouragement to improve the selectivity of fishing gears but with a focus on maintaining the functionality of the ecosystem and the protection of vulnerable species or sizes.
3. Establishment of clear, simple and rapid indicators as fishery management tools: Ecosystems are complex and ecological indicators can help describe them in simpler terms that can be

understood and used by non-scientists to make management decisions. The use of indicators has not yet been fully developed in the context of discards and bycatch, but indices related to the species- and size-composition and amount of bycatch and discards could be useful indicators to support an EAFM.

4. Public engagement: Finally, as we commented above, (almost) everybody agrees that discarding is a bad thing. However, greater public awareness of the issues could prove to be the most crucial driver for change. Fox (1992, cited by Alverson et al., 1994) noted that aside from its economic, conservation and legal facets, discarding is a public ethics issue, the latter being the most overlooked as a driving force but undoubtedly important for the establishment of the Marine Mammal Protection Act in the USA. Cod may not be as charismatic as dolphins, but public opinion could also be crucial for success in tackling the discard and bycatch problem in Europe.

Acknowledgments We acknowledge our technician Hortensia Araujo for her great assistance in managing the literature database and Luis Taboada for some useful information given to prepare this manuscript. We would also like to thank the input of anonymous referees for their helpful comments on the manuscript.

References

Aarts, G. & J. J. Poos, 2009. Comprehensive discard reconstruction and abundance estimation using flexible selectivity functions. ICES Journal of Marine Science 4: 763–771.

Allard, J. & G. A. Chouinard, 1997. A strategy to detect fish discarding by combining onboard and onshore sampling. Canadian Journal of Fisheries and Aquatic Sciences 54: 2955–2963.

Allen, M., D. Kilpatrick, M. Armstrong, R. Briggs, N. Pérez & G. Course, 2001. Evaluation of sampling methods to quantify discarded fish using data collected during discards project EC 95/094 by Northern Ireland, England and Spain. Fisheries Research 49: 241–254.

Allen, M., D. Kilpatrick, M. Armstrong, R. Briggs, G. Course & N. Pérez, 2002. Multistage cluster sampling design and optimal sample sizes for estimation of fish discards from commercial trawlers. Fisheries Research 55: 11–24.

Alverson, D. L., 1997. Global assessment of fisheries bycatch and discards: a summary overview, Global Trends. Fisheries Management 20: 115–125.

Alverson, D. L. & S. E. Hughes, 1996. Bycatch: from emotion to effective natural resource management. Reviews in Fish Biology and Fisheries 6: 443–462.

Alverson, D. L., M. H. Freeberg, S. A. Murawski & J. G. Pope, 1994. A global assessment of fisheries bycatch and discards. FAO Fisheries Technical Paper 339, Rome: 233 pp.

Apitz, S. E., M. Elliott, M. Fountain & T. S. Galloway, 2006. European environmental management: moving to an ecosystem approach. Integrated Environmental and Management 2: 80–85.

Apostolaki, P., E. A. Babcock & M. K. McAllister, 2006. Contrasting deterministic and probabilistic ranking of catch quotas and spatially and size-regulated fisheries management. Canadian Journal of Fisheries and Aquatic Sciences 63: 1777–1792.

Asche, F. & S. Tveterås, 2004. On the relationship between aquaculture and reduction fisheries. Journal of Agricultural Economics 55: 245–265.

Bache, S. J., 2001. A primer on take reduction planning under the Marine Mammal Protection Act. Ocean and Coastal Management 44: 221–239.

Barnes, K. N., P. G. Ryan & C. Boix-Hinzen, 1997. The impact of the hake *Merluccius* spp. longline fishery off South Africa on Procellariiform seabirds. Biological Conservation 82: 227–234.

Bellido, J. M. & N. Pérez, 2007. An optimal allocation sampling design for estimating discards in the Spanish fleet operating in ICES areas VII. Boletín del Instituto Español de Oceanografía 23: 73–83.

Benoît, H. P. & J. Allard, 2009. Can the data from at-sea observer surveys be used to make general inferences about catch composition and discards? Canadian Journal of Fisheries and Aquatic Sciences 66: 2025–2039.

Bisack, K. D. & J. G. Sutinen, 2006. Harbor Porpoise Bycatch: ITQs or Time/Area Closures in the New England Gillnet Fishery. Land Economics 82: 85–102.

Borges, T. C., K. Erzini, L. Bentes, M. E. Costa, J. M. S. Goncalves, P. G. Lino & C. Pais, 2001. By-catch and discarding practices in five Algarve (southern Portugal) metiers. Journal of Applied Ichthyology 17: 104–114.

Borges, L., A. F. Zuur, E. Rogan & R. Officer, 2004. Optimum sampling levels in discard sampling programs. Canadian Journal of Fisheries and Aquatic Sciences 61: 1918–1928.

Borges, L., A. F. Zuur, E. Rogan & R. Officer, 2005. Choosing the best sampling unit and auxiliary variable for discards estimations. Fisheries Research 75: 29–39.

Brothers, N., 1991. Albatross mortality and associated bait loss in the Japanese longline fishery in the Southern Ocean. Biological Conservation 55: 255–268.

Brown, J. & G. Macfadyen, 2007. Ghost fishing in European waters: impacts and management responses. Marine Policy 31: 488–504.

Cardinale, M. & H. Svedang, 2008. Mismanagement of fisheries: policy or science? Fisheries Research 93: 244–247.

Casey, J., 1996. Estimating discards using selectivity data: the effects of including discard data in assessments of the demersal fisheries in the Irish Sea. Journal of Northwest Atlantic Fishery Science 19: 91–102.

Catchpole, T. L. & T. S. Gray, 2010. Reducing discards of fish at sea: a review of European pilot projects. Journal of Environmental Management 91: 717–723.

Catchpole, T. L., C. L. J. Frid & T. S. Gray, 2005. Discards in North Sea fisheries: causes, consequences and solutions. Marine Policy 29: 421–430.

Catchpole, T. L., A. N. Tidd, L. T. Kell, A. S. Revill & G. Dunlin, 2007. The potential for new *Nephrops* trawl designs to positively effect North Sea stocks of cod, haddock and whiting. Fisheries Research 86: 262–267.

Catchpole, T. L., A. S. Revill, J. Innes & S. Pascoe, 2008. Evaluating the efficacy of technical measures: a case study of selection device legislation in the UK *Crangon crangon* (brown shrimp) fishery. ICES Journal of Marine Science 65: 267–275.

Cherel, Y., H. Weimerskirch & G. Duhamel, 1996. Interactions between longline vessels and seabirds in Kerguelen waters and a method to reduce seabird mortality. Biological Conservation 75: 63–70.

Collie, J. S., S. J. Hall, M. J. Kaiser & I. R. Poiner, 2000. A quantitative analysis of impacts on shelf-sea benthos. Journal of Animal Ecology 69: 785–798.

Cotter, A. J. R., L. Burt, C. G. M. Paxton, C. Fernandez, S. T. Buckland & J. X. Pax, 2004. Are stock assessment methods too complicated? Fish and Fisheries 5: 235–254.

Cotter, J., B. Mesnil, P. Witthames & M. Parker-Humphreys, 2009. Notes on nine biological indicators estimable from trawl surveys with an illustrative assessment for North Sea cod. Aquatic Living Resources 22: 135–153.

Davies, R. W. D., S. J. Cripps, A. Nickson & G. Porter, 2009. Defining and estimating global marine fisheries bycatch. Marine Policy 33: 661–672.

Daw, T. & T. Gray, 2005. Fisheries science and sustainability in international policy: a study of failure in the European Union's Common Fisheries Policy. Marine Policy 29: 189–197.

Dayton, P. K., S. F. Thrush, M. T. Agardy & R. J. Hofman, 1995. Environmental effects of marine fishing. Aquatic Conservation 5: 205–232.

Enever, R., A. Revill & A. Grant, 2009a. Discarding in the North Sea and on the historical efficacy of gear-based technical measures in reducing discards. Fisheries Research 95: 40–46.

Enever, R., T. L. Catchpole, J. R. Ellis & A. Grant, 2009b. The survival of skates (Rajidae) caught by demersal trawlers fishing in UK waters. Fisheries Research 97: 72–76.

Erzini, K., C. C. Monteiro, J. Ribeiro, M. N. Santos, M. Gaspar, P. Monteiro & T. C. Borges, 1997. An experimental study of gillnet and trammel net 'ghost fishing' off the Algarve (southern Portugal). Marine Ecology Progress Series 158: 257–265.

Erzini, K., M. E. Costa, L. Bentes & T. C. Borges, 2002. A comparative study of the species composition of discards from five fisheries from the Algarve (southern Portugal). Fisheries Management and Ecology 9: 31–40.

EU COM, 2009. Green Paper – reform of the common fisheries policy. Commission of the European Communities, Brussels, launched April 2009 and open to submit comments by 31 December 2009. COM: 163 pp.

FAO, 1999. International plan of action for reducing incidental catch of seabirds in longline fisheries. International plan of action for the conservation and management of sharks. International plan of action for the management of fishing capacity. FAO, Rome: 26 pp.

FAO, 2009. Guidelines to reduce sea turtle mortality in fishing operations. FAO, Rome: 128 pp.

FAO, 2010. Report of the technical consultation to develop international guidelines on bycatch management and reduction of discards, Rome, 6–10 December 2010. FAO Fisheries and Aquaculture Report No. 957. FAO, Rome: 32 pp.

Fernández, C., S. Cerviño, N. Pérez & E. Jardim, 2009. Stock assessment incorporating discards estimates in some years and implications for prediction of future stock trajectories. ICES CM 2009/M:10.

Fox, W. W., 1992. Bycatch: a NMFS perspective. In Schoning, R. W., R. W. Jacobson, D. L. Alverson, T. G. Gentle & J. Auyong (eds.), Proceedings of the National Industry Bycatch Workshop, February 4–6, 1992, Newport, Oregon. Natural Resources Consultants, Inc., Seattle, WA: 180–190.

Froese, R., A. Stern-Pirlot, H. Winker & D. Gascuel, 2008. Size matters: how single species management can contribute to ecosystem-based fisheries management. Fisheries Research 92: 231–241.

Furness, R. W., 2003. Impacts of fisheries on seabird communities. Scientia Marina 67: 33–45.

Gearin, P. J., M. E. Gosho, J. L. Laake, L. Cooke, R. L. DeLong & K. M. Hughes, 2000. Experimental testing of acoustic alarms (pingers) to reduce bycatch of harbour porpoise, *Phocoena phocoena*, in the state of Washington. Journal of Cetacean Research and Management 2: 1–9.

Goetz, S., M. Laporta, J. M. Portela, M. B. Santos & G. J. Pierce, 2011. Experimental fishing with an "umbrella-and-stones" system to reduce interactions of sperm whales (*Physeter macrocephalus*) and seabirds with bottom-set longlines for Patagonian toothfish (*Dissostichus eleginoides*) in the Southwest Atlantic. ICES Journal of Marine Science 68: 228–238.

Graham, N., 2003. By-catch reduction in the brown shrimp, *Crangon crangon*, fisheries using a rigid separation Nordmore grid (grate). Fisheries Research 59: 393–407.

Gray, C. A., D. D. Johnson, M. K. Broadhurst & D. J. Young, 2005. Seasonal, spatial and gear-related influences on relationships between retained and discarded catches in a multi-species gillnet fishery. Fisheries Research 75: 56–72.

Greenstreet, S. P. R., S. I. Rogers, J. C. Rice, G. J. Piet, E. J. Guirey, H. M. Fraser & R. J. Fryer, 2011. Development of the EcoQO for the North Sea fish community. ICES Journal of Marine Science 68: 1–11.

Grémillet, D., L. Pichegru, G. Kuntz, A. G. Woakes, S. Wilkinson, R. J. M. Crawford & P. G. Ryan, 2008. A junk-food hypothesis for gannets feeding on fishery waste. Proceedings of the Royal Society B-Biological Sciences 275: 1149–1156.

Guijarro, B. & E. Massuti, 2006. Selectivity of diamond- and square-mesh codends in the deepwater crustacean trawl fishery off the Balearic Islands (western Mediterranean). ICES Journal of Marine Science 63: 52–67.

Hall, S. J., 1999. The effects of fishing on marine ecosystems and communities. Blackwell Science, Oxford, England.

Hall, S. J. & B. M. Mainprize, 2005. Managing by-catch and discards: how much progress are we making and how can we do better? Fish and Fisheries 6: 134–155.

Hall, M. A., D. L. Alverson & K. I. Metuzals, 2000. By-catch: problems and solutions. Marine Pollution Bulletin 41: 204–219.

Hammond, T. R. & V. M. Trenkel, 2005. Censored catch data in fisheries stock assessment. ICES Journal of Marine Science 62: 1118–1130.

Heymans, J. J., K. L. Howell, M. Ayers, M. T. Burrows, J. D. M. Gordon, E. G. Jones & F. Neat, 2011. Do we have enough information to apply the ecosystem approach to management of deep-sea fisheries? An example from the West of Scotland. ICES Journal of Marine Science 68: 265–280.

Hilborn, R., 2011. Future directions in ecosystem based fisheries management: a personal perspective. Fisheries Research 108: 235–239.

Huse, I. & A. Vold, 2010. Mortality of mackerel (*Scomber scombrus* L.) after pursing and slipping from a purse seine. Fisheries Research 106: 54–59.

ICES, 2010. Report of the working group on the assessment of demersal stocks in the North Sea and Skagerrak 2010. Available at www.ices.dk.

Jenkins, S. R., C. Mullen & A. R. Brand, 2004. Predator and scavenger aggregation to discarded by-catch from dredge fisheries: importance of damage level. Journal of Sea Research 51: 69–76.

Jennings, S., 2005. Indicators to support an ecosystem approach to fisheries. Fish and Fisheries 6: 212–232.

Jennings, S. & M. J. Kaiser, 1998. The effects of fishing on marine ecosystems. Advances in Marine Biology 34: 201–352.

Jensen, J. N., 2006. Guidance on the application of the ecosystem approach to management of human activities in the European marine environment. ICES Cooperative Research Report No. 273.

Kaiser, M. J. & S. J. de Groot, 2000. Effects of Fishing on Non-Target Species and Habitats. Biological, Conservation and Socio-Economic Issues. Blackwell Science, Oxford, England.

Kelleher, K., 2005. Discards in the world's marine fisheries. An update. FAO Fisheries Technical Paper No. 470. FAO, Rome: 131 pp.

Khalilian, S., R. Froese, A. Proelss & T. Requate, 2010. Designed for failure: a critique of the Common Fisheries Policy of the European Union. Marine Policy 34: 1178–1182.

Kroncke, I., J. W. Dippner, H. Heyen & B. Zeiss, 1998. Long-term changes in macrofaunal communities off Norderney (East Frisia, Germany) in relation to climate variability. Marine Ecology Progress Series 167: 25–36.

Large, P. A., N. G. Graham, N. R. Hareide, R. Misund, D. J. Rihan, M. C. Mulligan, P. J. Randall, D. J. Peach, P. H. McMullen & X. Harlay, 2009. Lost and abandoned nets in deep-water gillnet fisheries in the Northeast Atlantic: retrieval exercises and outcomes. ICES Journal of Marine Science 66: 323–333.

Levin, P. S., M. J. Fogarty, S. A. Murawski & D. Fluharty, 2009. Integrated ecosystem assessments: developing the scientific basis for ecosystem-based management of the ocean. PLoS Biology 7: 23–28.

Lewison, R. L., C. U. Soykan & J. Franklin, 2009. Mapping the bycatch seascape: multispecies and multi-scale spatial patterns of fisheries bycatch. Ecological Applications 19: 920–930.

Lindeboom, H. J., & S. J. de Groot, 1998. The effects of different types of fisheries on the North Sea and Irish Sea benthic eco-systems. EU-project AIR2-CT94 1664 (IMPACT-II), Final Report ISSN 0923-3210. Netherlands Institute for Sea Research, NIOZ-Rapport 198-1. RIVO-DLO Report C003/98: 404 pp.

Macher, C., O. Guyader, C. Talidec & M. Bertignac, 2008. A cost-benefit analysis of improving trawl selectivity in the case of discards: the *Nephrops norvegicus* fishery in the Bay of Biscay. Fisheries Research 92: 76–89.

Massuti, E., F. Ordines & B. Guijarro, 2009. Efficiency of flexible sorting grids to improve size selectivity of the bottom trawl in the Balearic Islands (western Mediterranean), with comparison to a change in mesh codend geometry. Journal of Applied Ichthyology 25(2): 153–161.

Mesnil, B., 1996. When discards survive: accounting for survival of discards in fisheries assessments. Aquatic Living Resources 9: 209–215.

Mitchell, R. W., S. J. Blight, D. J. Gaughan & I. W. Wright, 2002. Does the mortality of released *Sardinops sagax* increase if rolled over the headline of a purse seine net? Fisheries Research 57: 279–285.

Monteiro, P., A. Araújo, K. Erzini & M. Castro, 2001. Discards of the Algarve (southern Portugal) crustacean trawl fishery. Hydrobiologia 449: 267–277.

Moore, J. E., B. R. Wallace, R. L. Lewison, R. Zydelis, T. M. Cox & L. B. Crowder, 2009. A review of marine mammal, sea turtle and seabird bycatch in USA fisheries and the role of policy in shaping management. Marine Policy 33: 435–451.

National Research Council, 1992. Dolphins and the Tuna Industry. National Academy Press, Washington, DC.

Nunoo, F. K. E., J. O. Boateng, A. M. Ahulu, K. A. Agyekum & U. R. Sumaila, 2009. When trash fish is treasure: the case of Ghana in West Africa. Fisheries Research 96: 167–172.

Pawson, M. G., 2003. The catching capacity of lost static fishing gears: introduction. Fisheries Research 64: 101–105.

Piet, G. J. & S. Jennings, 2005. Response of potential fish community indicators to fishing. ICES Journal of Marine Science 62: 214–225.

Piet, G. J., F. J. Quirijns, L. Robinson & S. P. R. Greenstreet, 2007. Potential pressure indicators for fishing, and their data requirements. ICES Journal of Marine Science 64: 110–121.

Piet, G. J., H. M. Jansen & M. J. Rochet, 2008. Evaluating potential indicators for an ecosystem approach to fishery management in European waters. ICES Journal of Marine Science 65: 1449–1455.

Pikitch, E. K., C. Santora, E. A. Babcock, A. Bakun, R. Bonfil, D. O. Conover, P. Dayton, P. Doukakis, D. Fluharty, B. Heneman, E. D. Houde, J. Link, P. A. Livingston, M. Mangel, M. K. McAllister, J. Pope & K. J. Sainsbury, 2004. Ecosystem-based fishery management. Science 305: 346–347.

Portela, J., J. Wang, P. Brickle, M. Sacau, G. J. Pierce, X. Cardoso, E. Ulloa, M. Otero & V. Tato, 2004. Study of the variations of a potentially exploitable species (*Patagonotothen* spp.) in the southwest Atlantic, using GIS techniques. ICES CM 2004/K:58.

Punt, A. E., D. C. Smith, G. N. Tuck & R. D. Methot, 2006. Including discard data in fisheries stock assessments: two case studies from south-eastern Australia. Fisheries Research 79: 239–250.

Revill, A. & G. Dunlin, 2003. The fishing capacity of gillnets lost on wrecks and on open ground in UK coastal waters. Fisheries Research 64: 107–113.

Revill, A. & R. Holst, 2004. The selective properties of some sieve nets. Fisheries Research 66: 171–183.

Revill, A. S. & S. Jennings, 2005. The capacity of benthos release panels to reduce the impacts of beam trawls on benthic communities. Fisheries Research 75: 73–85.

Revill, A., G. Dunlin & R. Holst, 2006. Selective properties of the cutaway trawl and several other commercial trawls used in the Farne Deeps North Sea *Nephrops* fishery. Fisheries Research 81: 268–275.

Revill, A., T. L. Catchpole & G. Dunlin, 2007. Recent work to improve the efficacy of square-mesh panels used in a North Sea *Nephrops norvegicus* directed fishery. Fisheries Research 85: 321–327.

Rice, J. C. & D. Rivard, 2007. The dual role of indicators in optimal fisheries management strategies. ICES Journal of Marine Science 64: 775–778.

Rijnsdorp, A. D. & P. I. van Leeuwen, 1996. Changes in abundance of demersal fish species in the North Sea between 1906–1909 and 1990–1995. ICES Journal of Marine Sciences 53: 1054–1062.

Robb, C. K., K. M. Bodtker, K. Wright & J. Lash, 2011. Commercial fisheries closures in marine protected areas on Canada's Pacific coast: the exception, not the rule. Marine Policy 35: 309–316.

Rochet, M. J. & V. M. Trenkel, 2005. Factors for the variability of discards: assumptions and field evidence. Canadian Journal of Fisheries and Aquatic Sciences 62: 224–235.

Rochet, M. J. & V. M. Trenkel, 2009. Why and how could indicators be used in an ecosystem approach to fisheries management? Future of Fisheries Science in North America 31: 209–226.

Rodríguez-Cabello, C., A. Hernández, I. Olaso & F. Sánchez, 2005. Survival of small-spotted catshark (*Scyliorhinus canicula*) discarded by trawlers in the Cantabrian Sea. Journal of the Marine Biological Association of the United Kingdom 85: 1145–1150.

Sánchez, F., C. Rodríguez-Cabello & I. Olaso, 2005. The role of elasmobranchs in the Cantabrian Sea shelf ecosystem and impact of the fisheries on them. Journal of Northwest Atlantic Fishery Science 35: 267–480.

Sims, M., T. Cox & R. Lewison, 2008. Modeling spatial patterns in fisheries bycatch: improving bycatch maps to aid fisheries management. Ecological Applications 18: 649–661.

Stergiou, K. I., A. Machias, S. Somarakis & A. Kapantagakis, 2003. Can we define target species in Mediterranean trawl fisheries? Fisheries Research 59: 431–435.

Stratoudakis, Y. & A. Marcalo, 2002. Sardine slipping during purse-seining off northern Portugal. ICES Journal of Marine Science 59: 1256–1262.

Stratoudakis, Y., R. J. Fryer & R. M. Cook, 1998. Discarding practices for commercial gadoids in the North Sea. Canadian Journal of Fisheries and Aquatic Sciences 55: 1632–1644.

Suuronen, P., V. Tschernij, P. Jounela, D. Valentinsson & P. O. Larsson, 2007. Factors affecting rule compliance with mesh size regulations in the Baltic cod trawl fishery. ICES Journal of Marine Science 64: 1603–1606.

Trenkel, V. M., M. J. Rochet & B. Mesnil, 2007. From model-based prescriptive advice to indicator-based interactive advice. ICES Journal of Marine Science 64: 768–774.

Tsagarakis, K., A. Machias, M. Giannoulaki, S. Somarakis & I. Karakassis, 2008. Seasonal and temporal trends in metrics of fish community for otter-trawl discards in a Mediterranean ecosystem. ICES Journal of Marine Science 65: 539–550.

Valeiras, J., 2003. Attendance of scavenging seabirds at trawler discards off Galicia, Spain. Scientia Marina 67: 77–82.

Van Hoey, G., A. Borja, S. Birchenough, L. Buhl-Mortensen, S. Degraer, D. Fleischer, F. Kerckhof, P. Magni, I. Muxika, H. Reiss, A. Schröder & M. L. Zettler, 2010. The use of benthic indicators in Europe: from the Water Framework Directive to the Marine Strategy Framework Directive. Marine Pollution Bulletin 60: 2187–2196.

Votier, S. C., R. W. Furness, S. Bearhop, J. E. Crane, R. W. G. Caldow, P. Catry, K. Ensor, K. C. Hamer, A. V. Hudson, E. Kalmbach, N. I. Klomp, S. Pfeiffer, R. A. Phillips, I. Prieto & D. R. Thompson, 2004. Changes in fisheries discard rates and seabird communities. Nature 427: 727–730.

Yamashita, H., D. Shiode & T. Tokai, 2009. Longline hook selectivity for red tilefish *Branchiostegus japonicus* in the East China Sea. Fisheries Science 75: 863–874.

Zeller, D. & D. Pauly, 2005. Good news, bad news: global fisheries discards are declining, but so are total catches. Fish and Fisheries 6: 156–159.

Zhou, S., A. D. M. Smith, A. E. Punt, A. J. Richardson, M. Gibbs, E. A. Fulton, S. Pascoe, C. Bulman, P. Bayliss & K. Sainsbury, 2010. Ecosystem-based fisheries management requires a change to the selective fishing philosophy. Proceedings of the National Academic of Sciences of the United States of America 107(21): 9485–9489.