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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

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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),

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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

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[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).

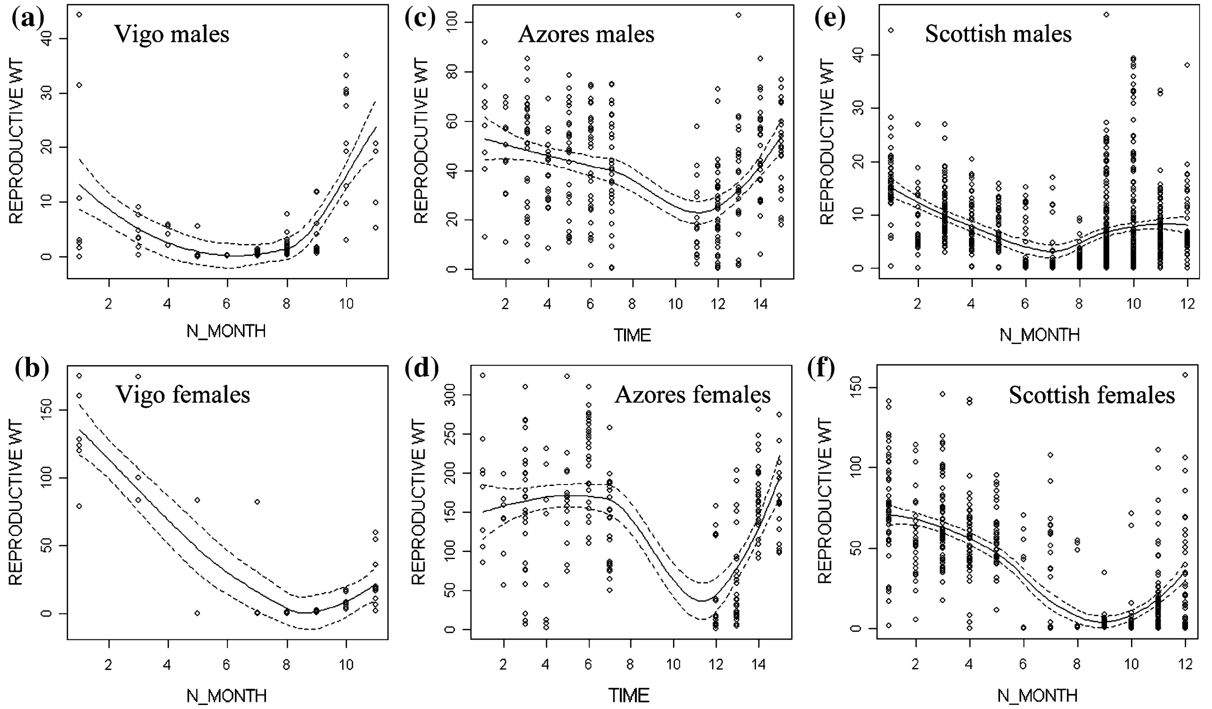
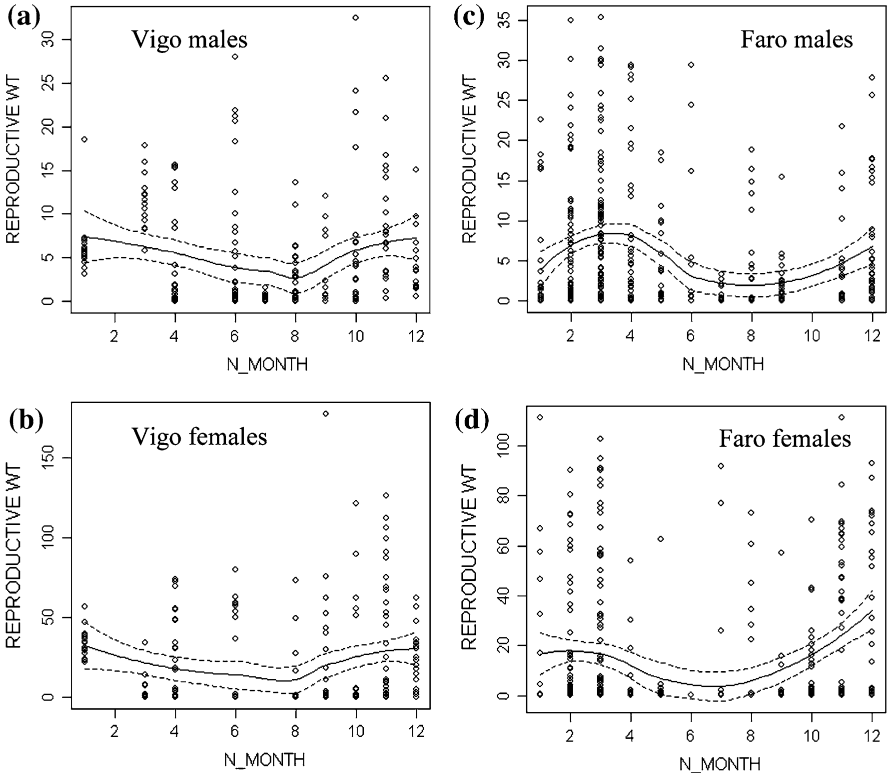


Fig. 1 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and month (or 'time') in *L. forbesii*. **a** Vigo males, **b** Vigo females, **c** Azores males, **d** Azores females, **e** Scottish males and **f** Scottish females

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



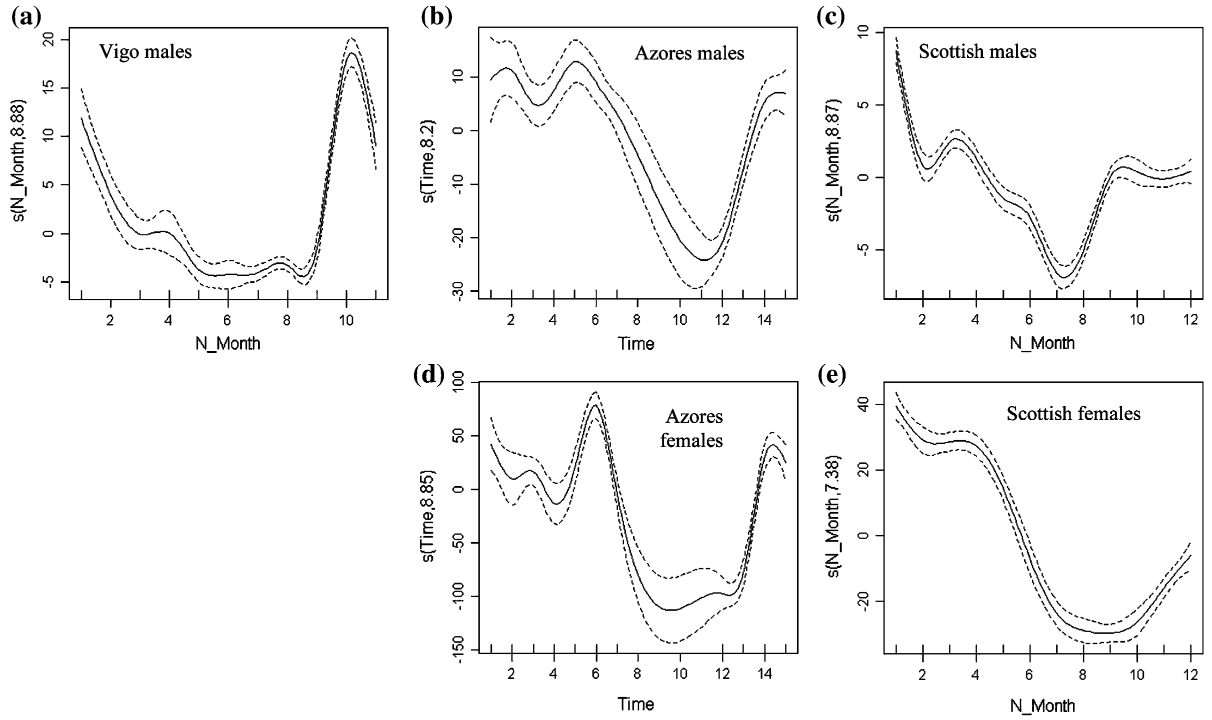


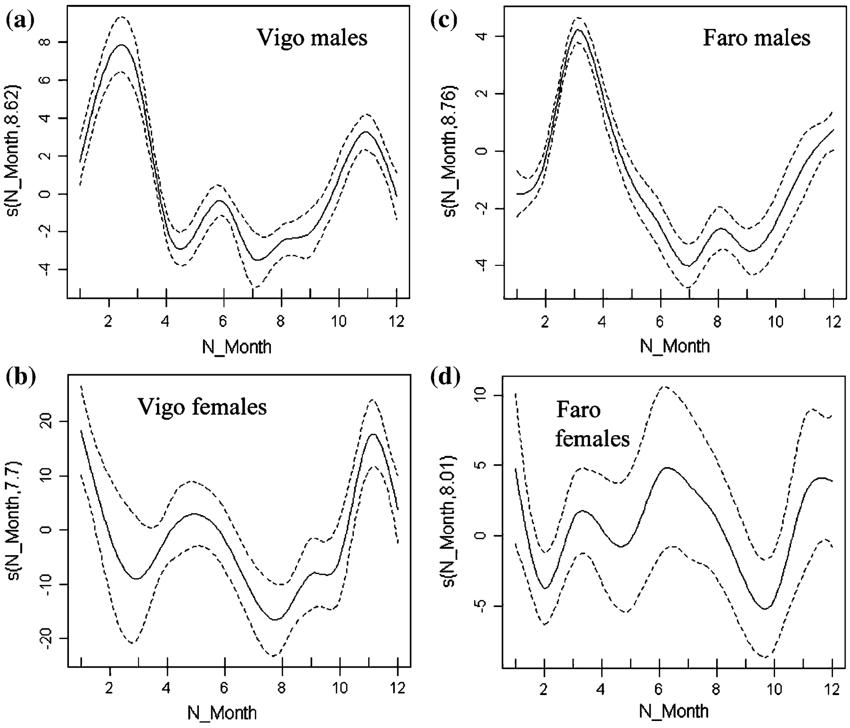
Fig. 3 GAM smoothing curves fitted to partial effects of the explanatory variable month (or 'time') on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of month in **a** Vigo males, **a** Azores males, **c** Azores females, **d** Scottish males and **e** Scottish 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



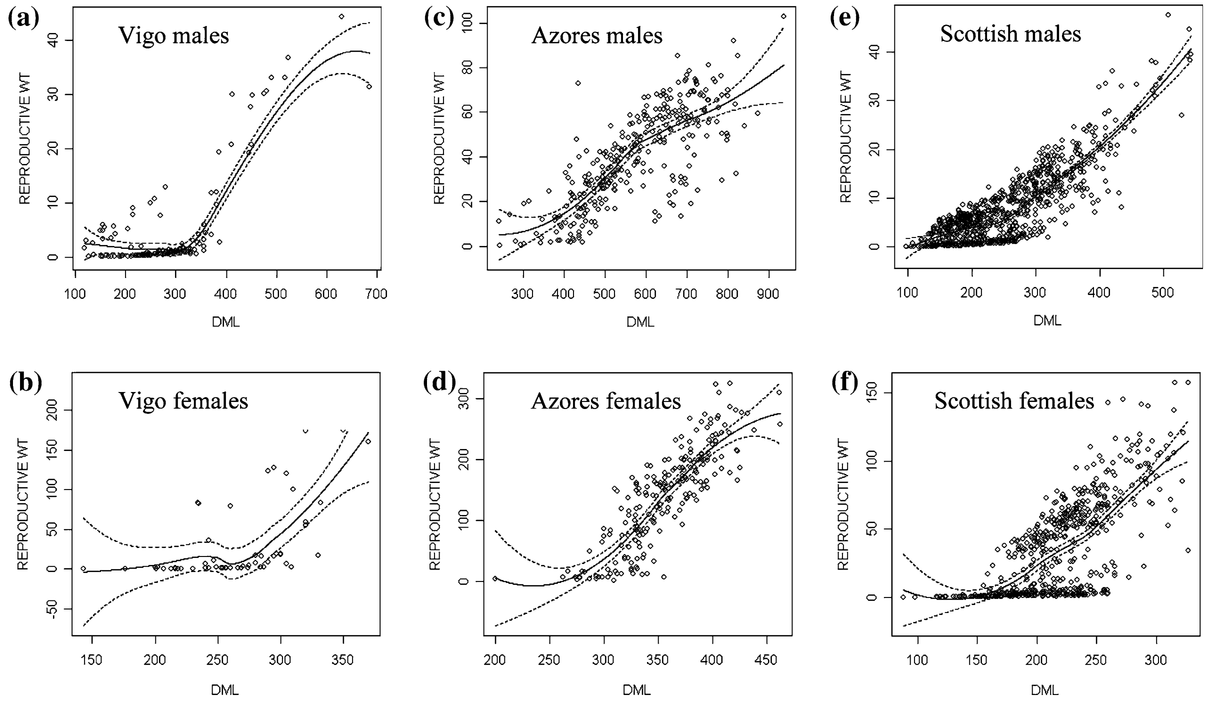


Fig. 5 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and DML in *L. forbesii*. **a** Vigo males, **b** Vigo females, **c** Azores males, **d** Azores females, **e** Scottish males and **f** Scottish females

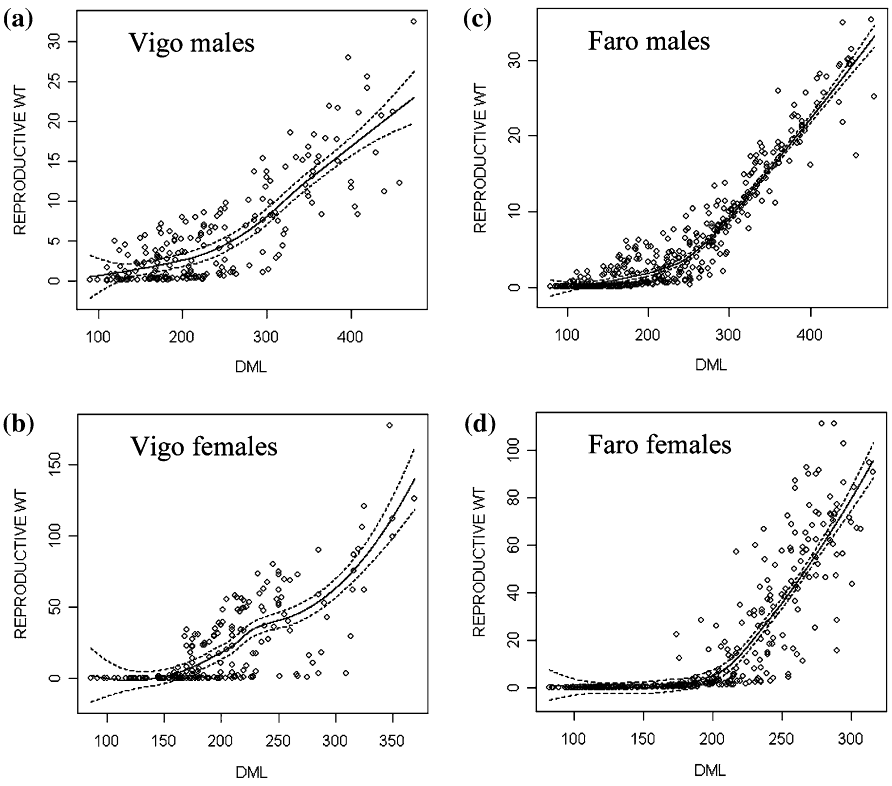


Fig. 6 Scatterplots with fitted smoothing curves for the relationship between reproductive weight and DML in *L. vulgaris*. **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females

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.

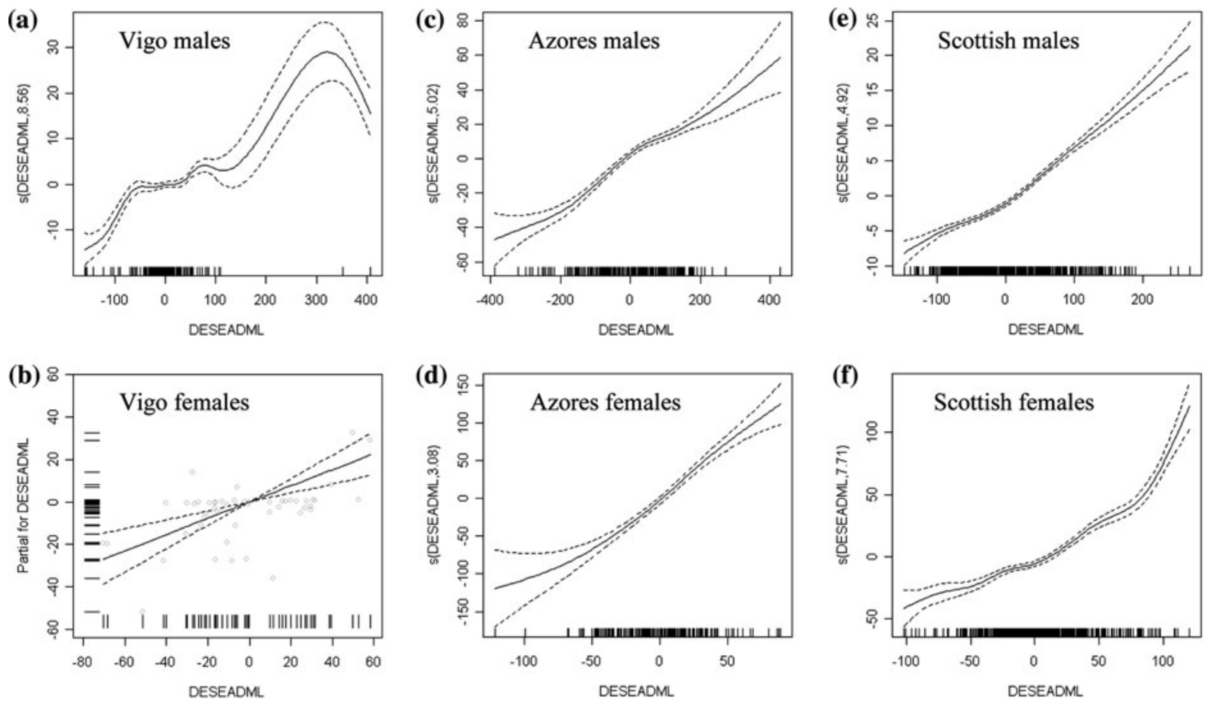


Fig. 7 GAM smoothing curves fitted to partial effects of the explanatory variable de-seasonalised DML on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of de-seasonalised DML in **a** Vigo males, **b** Vigo females (linear regression model), **c** Azores males, **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

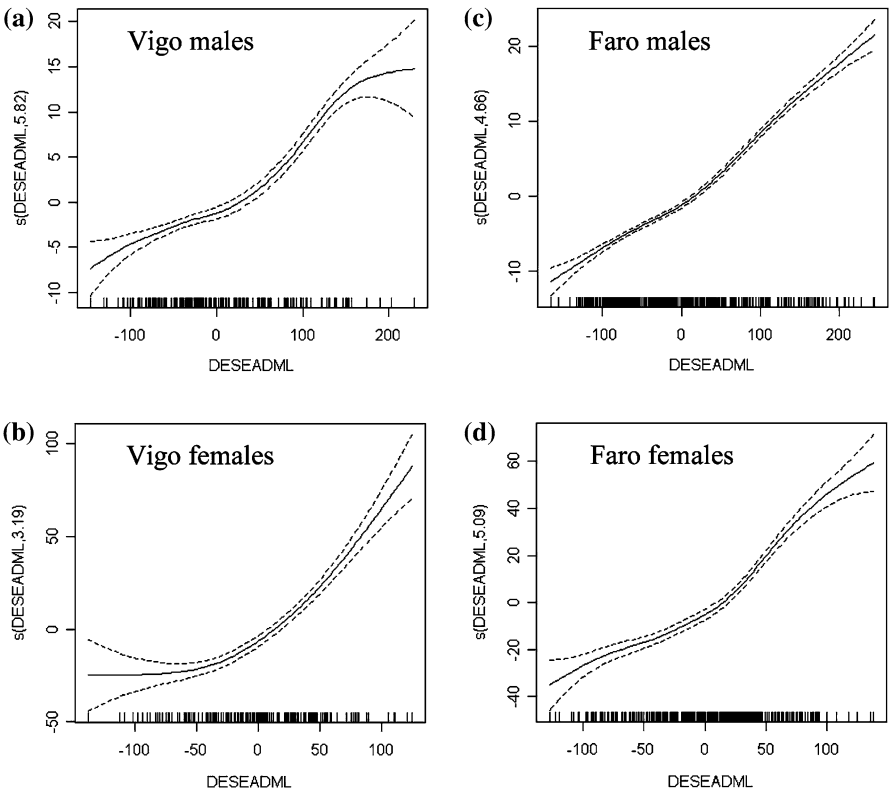


Fig. 8 GAM smoothing curves fitted to partial effects of the explanatory variable de-seasonalised DML on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of de-seasonalised DML in **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females. *Dashed 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

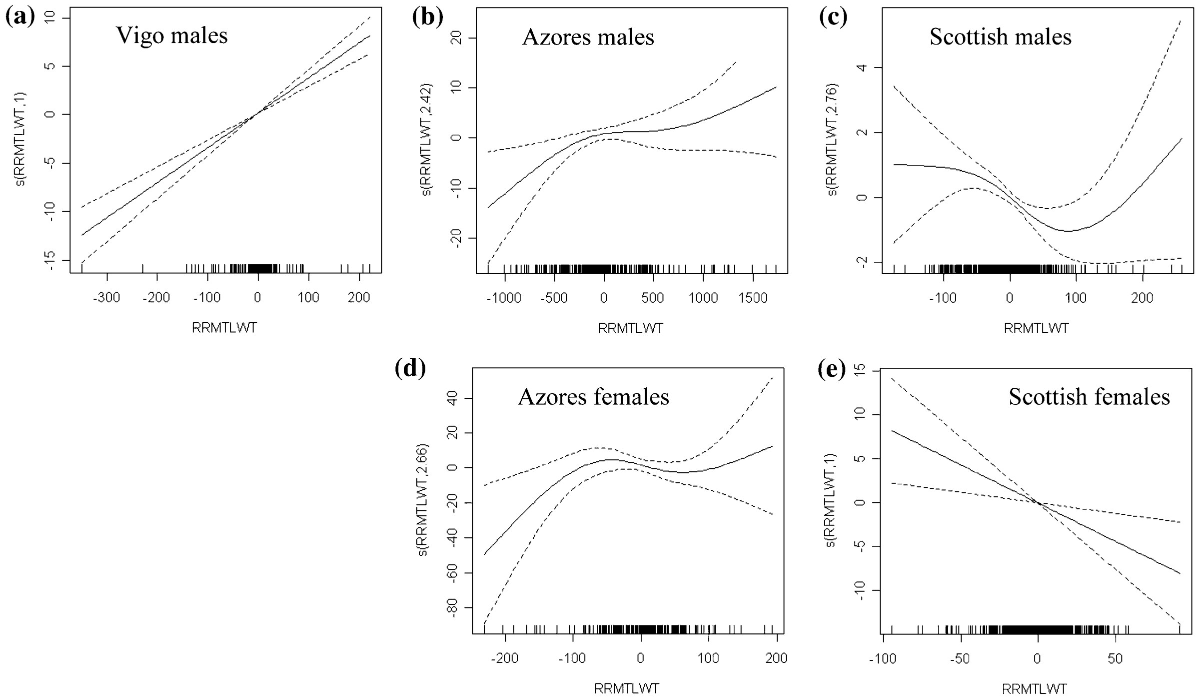


Fig. 9 GAM smoothing curves fitted to partial effects of the explanatory variable mantle weight (residuals) on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of residuals of mantle weight in **a** Vigo males, **b** Azores males, **c** Azores females, **d** Scottish 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

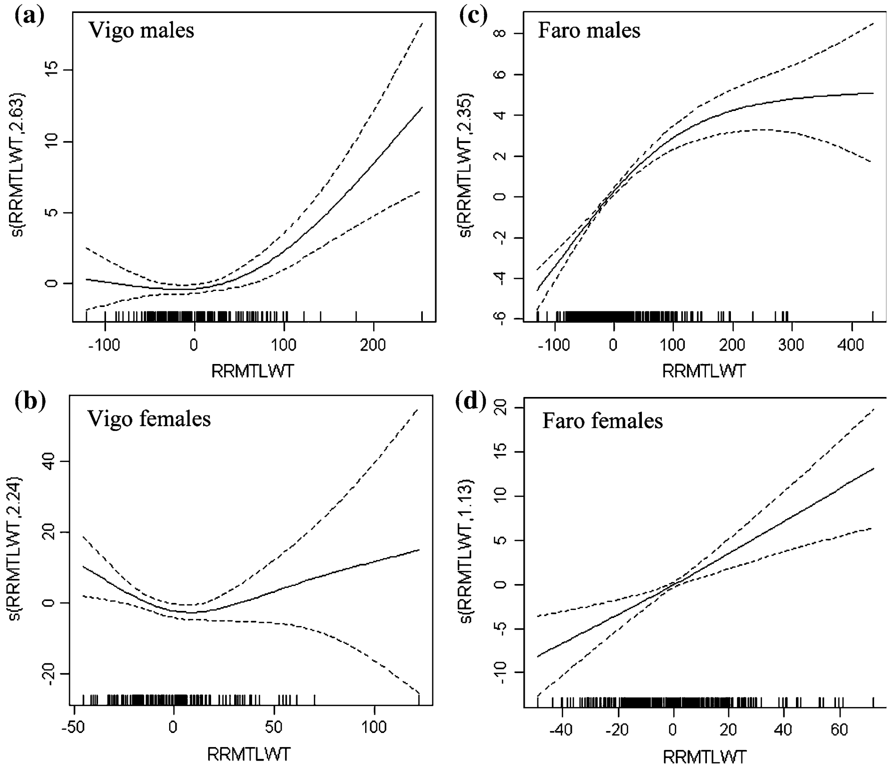


Fig. 10 GAM smoothing curves fitted to partial effects of the explanatory variable mantle weight (residuals) on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of residuals of mantle weight in **a** Vigo males, **b** Vigo females, **c** Faro males and **d** Faro females. *Dashed lines* represent 95% confidence intervals around the main effects

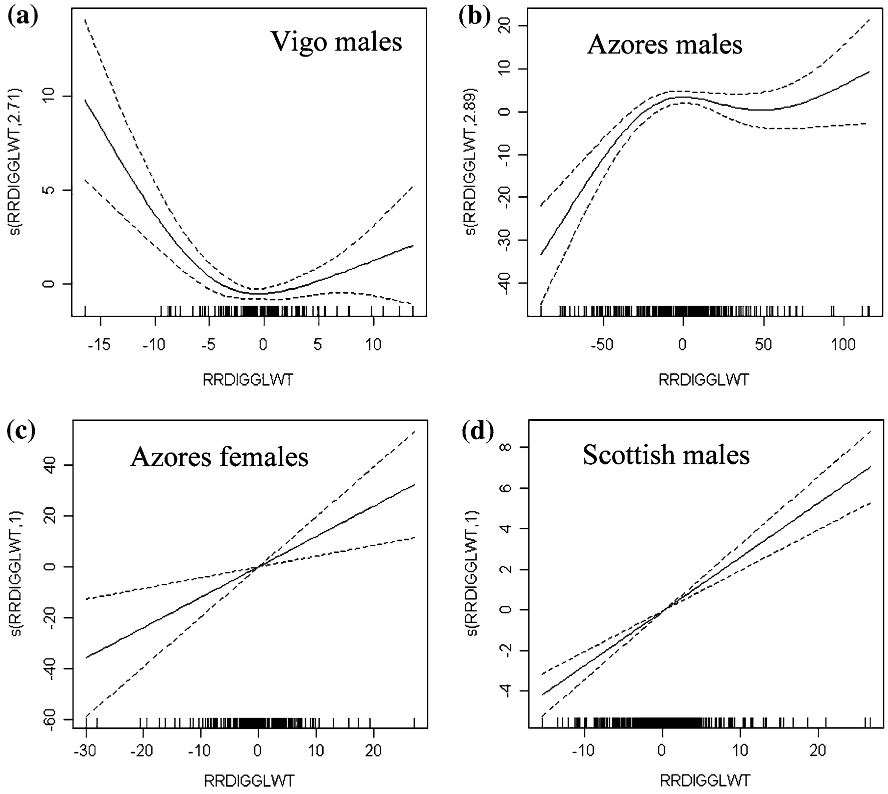


Fig. 11 GAM smoothing curves fitted to partial effects of the explanatory variable digestive gland weight (residuals) on reproductive weight in *L. forbesii*. Reproductive weight is represented as a function of residuals of digestive gland weight in **a** Vigo males, **b** Azores males, **c** Azores females and **d** Scottish males. *Dashed lines* represent 95% confidence intervals around the main effects

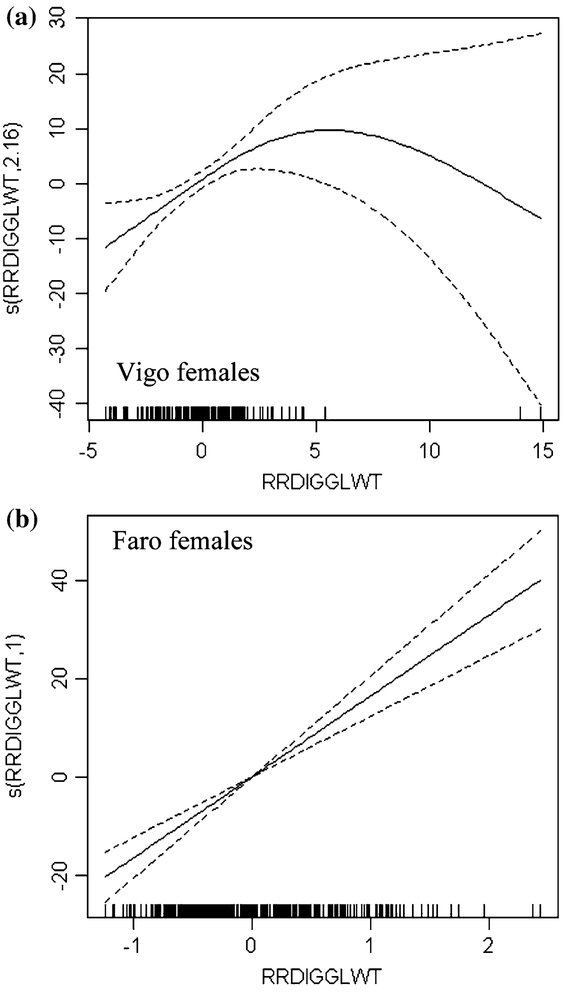


Fig. 12 GAM smoothing curves fitted to partial effects of the explanatory variable digestive gland weight (residuals) on reproductive weight in *L. vulgaris*. Reproductive weight is represented as a function of residuals of digestive gland weight in **a** Vigo females and **b** Faro females. *Dashed 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.

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