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ECOSYSTEMS AND SUSTAINABILITY

Survey and management of mussel *Mytilus* species in Scotland

Patricia Joanna Dias · Stuart B. Piertney · Mike Snow · Ian M. Davies

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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

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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

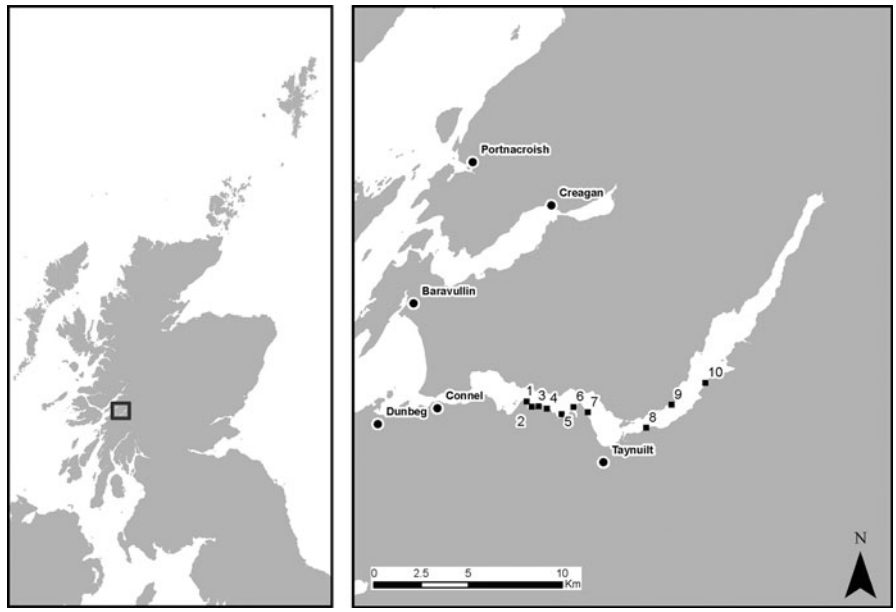

Fig. 1 Map (ArcGis©) showing sampling sites at Loch Etive (*right image*) in Scotland (*left*). Sites were numbered 1 to 10, site 1 being the closest to the Loch entrance and site 10 the most distant

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.

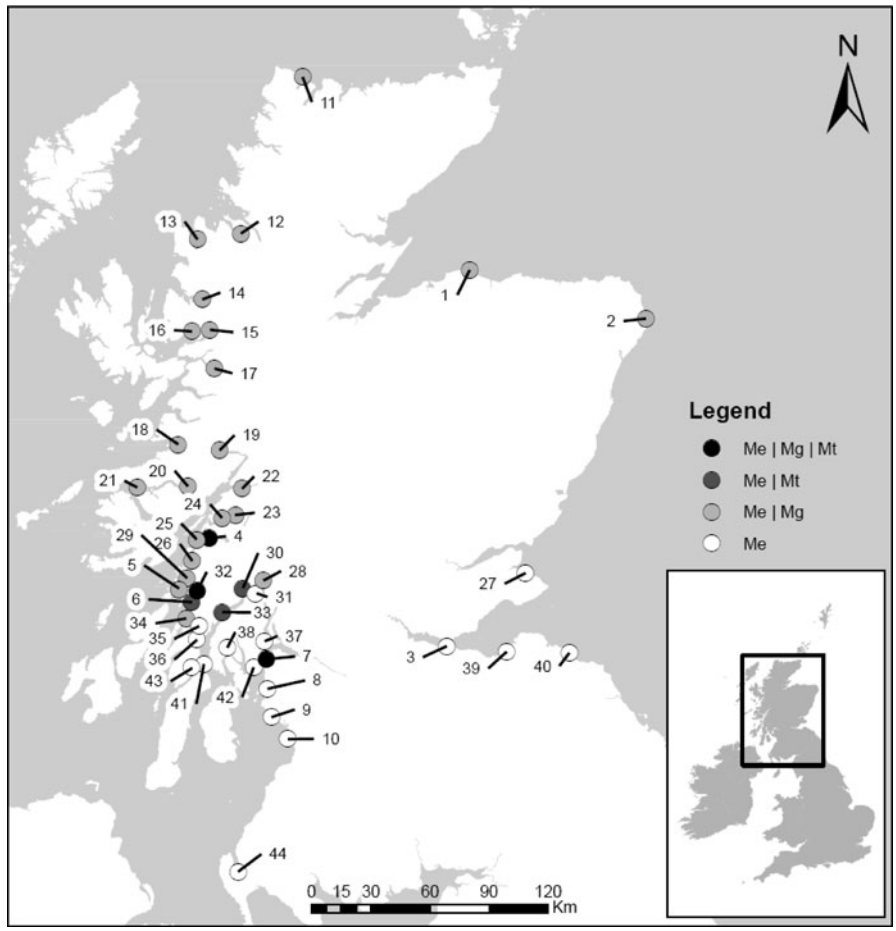

Fig. 2 Map showing sampling sites (1–44) from the intertidal zone and marinas in Scotland, and also the sites detected positive for Me (*M. edulis*), Mg (*M. galloprovincialis*) and Mt (*M. trossulus*)

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

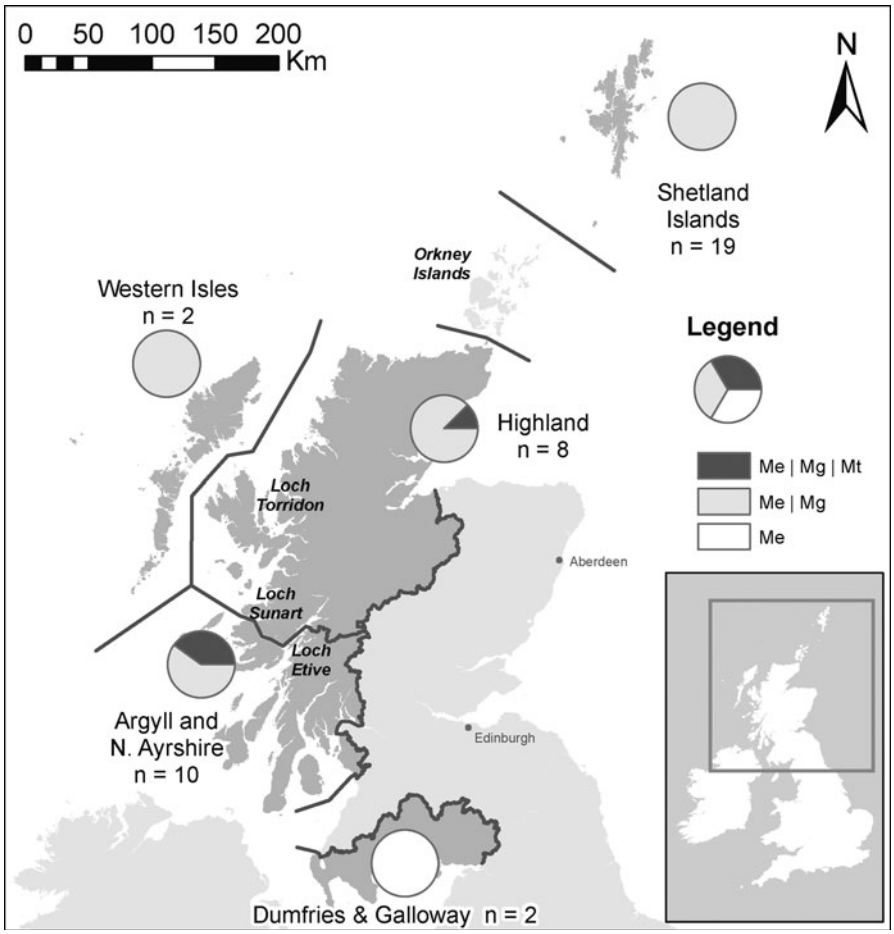

Fig. 3 Map showing the distribution of samples taken at mussel aquaculture sites in Scotland. Number of aquaculture sites sampled, and detections obtained of the *M. edulis* (Me), *M. galloprovincialis* (Mg) and *M. trossulus* (Mt) species-specific alleles at the Me 15/16 locus, are given per local authority 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

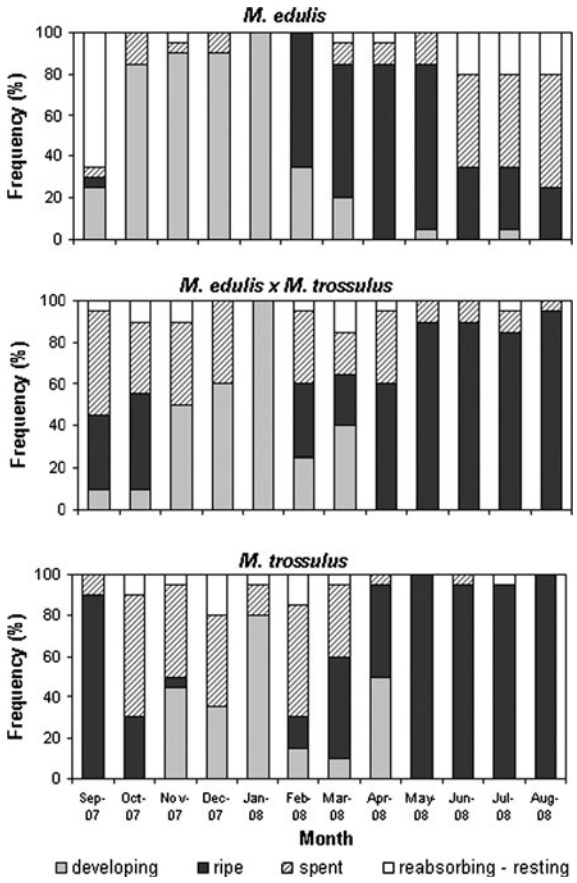

Fig. 4 *Mytilus edulis*, *M. trossulus* and *M. edulis* × *M. trossulus* frequency distribution of gonad maturation stages observed between September 2007 and August 2008

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

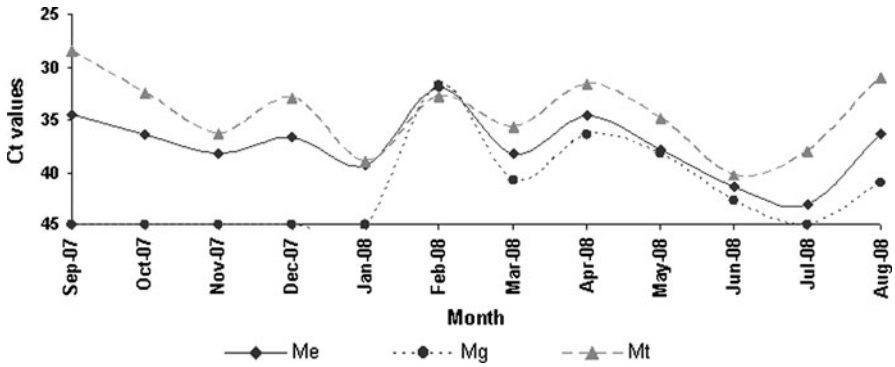

Fig. 5 Real-time PCR cycle threshold (Ct) detection values obtained for *M. edulis* (Me), *M. trossulus* (Mt) and *M. galloprovincialis* (Mg) alleles present in the plankton samples analysed by real-time PCR. Because higher Ct values correspond to initially lower template DNA quantity, Ct values in the 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.

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