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ORIGINAL ARTICLE

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Genotypes of *Mytilus* from waters of different salinity around Bergen, Norway

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Abstract Samples of *Mytilus* were collected at eight sites located in and around Bergen, Norway, and analysed by starch gel electrophoresis for the two highly polymorphic loci *PGM** and *PGI**. The genotype distribution and allele frequencies varied significantly among samples from the different locations. The variations were most significant between localities with full strength seawater and brackish water, and this difference was so large that it indicated the presence of two populations, possibly representing two species. The brackish water mussels may represent the species *Mytilus trossulus*, while the species *Mytilus edulis* may be distributed on the outer shores where salinity is normally around 30‰. Differential survival, as a result of specific adaptation to different salinities, may be the mechanism that maintains the populations (or species) and prevents gene flow between them.

Keywords *Mytilus* · Allozymes · Western Norway · Salinities

Introduction

Three species of blue mussels of the genus *Mytilus* are found in European coastal waters: *Mytilus edulis* L., 1758; *M. galloprovincialis* Lamarck, 1819; and *M. trossulus* Gould, 1850. In Europe, *M. edulis* is the coastal species generally associated with the seas of the north-eastern Atlantic. In the north of Europe, *M. trossulus* is found in waters with a lower salinity, such as the Baltic Sea. *M. galloprovincialis* generally has a more southern distribution than the other two species. All three species may hybridise in narrow zones where their distributions overlap (Cousteau et al. 1991; Gosling 1992; Saavedra et al. 1996; Comensaña et al. 1999). Several studies of allozyme frequencies at hybrid zones of these three species have been completed in the north-western Atlantic (Koehn et al. 1975, 1984; Mallet and Carver 1989; McDonald et al. 1991; Pedersen et al. 2000; Hilbish et al. 2002) and in the north-eastern Atlantic (Väinölä and Hvilsom 1991; Hummel et al. 2001).

Previous studies have revealed very high intra- and interpopulation diversity in blue mussels from the areas around Bergen (Ridgway 2001). Especially pronounced are the differences in genotype distribution which were observed between localities with brackish and full strength seawater. This indicates that the natural differences in salinity may maintain high genetic diversity, or that two groups (possibly species) may be present, one adapted to high salinity and one to low salinity with possible hybridisation in locations where both are present. If two species are present, it is reasonable to postulate that the brackish form is *M. trossulus*, while the species distributed in localities with full strength saltwater is *M. edulis*, as also indicated by Vaïnölä (personal communication).

The present paper describes genetic diversity of two enzymes in eight localities with different salinities. The results are discussed with respect to the proposed existence of two species and to possible differential selection due to differential adaptation to different salinities.

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Methods

Description of locations

Blue mussels were sampled at eight locations that were chosen to represent a broad distribution of habitats with mussels in and around Bergen. The locations are listed in Table 1, together with salinity ranges and a general description of the surface on which the mussels grew. The environmental conditions at these sites, or nearby locations, are described by Botnen et al. (2000). Hylkje and Garnes are both located in Sørfjorden, north-east of Bergen. The salinity in the fjord is low at the surface and is influenced by the amount of rainfall and air temperature. At Hylkje, the mussels were

Table 1 Sampling location of blue mussels, with salinity ranges and substrate characters

Location	Salinity range (‰)	Substrate
1 Solheim, Bergen	10–30	Granite sea wall
2 Store Lungegaardsvann, Bergen	10–20	Silt mud bank
3 Hylkje, Sørfjorden	6–22	Granite sea wall
4 Garnes, Sørfjorden	6–22	Granite sea wall
5 Espeland, Raunefjorden	30–33	Pebble sand shore
6 Syltøy, Sotra	30–33	Granite sea wall
7 Angeltveit, Sotra	30–33	Cement slipway
8 Herdla	27–33	Granite sea wall

found on a rock surface. At Garnes, the mussels were picked from a sea wall. Store Lungegårdsvann is a semi-closed small fjord located in the city of Bergen, and surface salinity is depressed by water flowing from the surrounding mountains. The mussels here were sampled at a sheltered shore with silt, while at Solheim, located at the outlet about 200 m further south, the mussels were sampled from a sea wall. Angeltveit and Syltøy are located on the west coast of the island of Sotra and are not directly influenced by the euryhaline environments of the fjords. The Syltøy location is entirely sheltered from waves by a sea wall, and the Angeltveit location was also relatively sheltered by its position in a bay, but other environmental factors, such as water flow and substrate, were different compared to Syltøy. At Espeland, the mussels were picked from a pebble beach which is slightly influenced by some minor streams, and the location is also relatively sheltered. The surface salinity was derived from environmental reports (Botnen et al. 2000). The given salinities are accurate for the general area and reveal the lower and upper levels of the annual variation, but the actual salinity at the exact location may vary a little from the values in Table 1. In general, precipitation on the shore will create salinities at the actual locations which are lower than those recorded for the centre of the fjord. So, at all locations, the lowest values are probably slightly lower than recorded here.

Electrophoresis

A piece of the adductor muscle was homogenised in a drop of water in a micro-well tray and centrifuged at 3,000 rpm for 10 min. Starch-gel electrophoresis of the supernatant was carried out on 12% starch gels cooled to around 4°C. After an initial screening of several enzymes and buffers, phosphoglucose isomerase (PGI, EC 5.3.1.9) and phosphoglucose mutase (PGM, EC 2.7.5.1) were chosen for routine analysis. A buffer made of amine-citrate morpholine (0.04 M) and citric acid monohydrate (pH adjusted to 6.8), with an inclusion of 0.01 M EDTA, as described by Murphy et al. (1990), was used. It was applied undiluted at the electrode, and a 1:19 dilution of the stock was applied for the gel.

Statistics

The program "Tools for Population Genetic Analyses" (Miller 1997) was used to calculate allele frequencies, Hardy-Weinberg distribution and heterozygosity. χ^2 homogeneity tests were used to compare genotype distributions. The Mann-Whitney test was used for non-parametric testing of the two-tailed null hypothesis that there is no difference in genotype distribution among locations, especially between the samples from salt and brackish water.

Results

Polymorphism and genotype distribution

Both *PGI** and *PGM** loci displayed a high number of alleles. For *PGM** some alleles had to be pooled because the difference in migration distance was so small that they could not always be identified properly. Allele frequencies for both loci are presented in Table 2. The allele frequencies are illustrated in Figs. 1 and 2. Herdla and Espeland displayed intermediate distributions between those shown for the outer marine and the inner fjord locations. Several of the distributions were not in Hardy-

Table 2 Allele frequencies for the enzyme loci *PGM** and *PGI** for eight samples of blue mussels, together with test of accordance between observed and expected Hardy-Weinberg distributions of genotypes, and observed and expected heterozygosities for each locus

Locus	Allele	Sample no.							
		1	2	3	4	5	6	7	8
*PGM**	100	0.46	0.53	0.70	0.71	0.07	0.02	0.03	0.08
	90	0.38	0.34	0.28	0.23	0.28	0.21	0.26	0.30
	80	0.09	0.10	0.02	0.04	0.58	0.76	0.71	0.39
	70	0.05	0.03	–	0.02	0.07	0.01	0.01	0.21
	60	0.02	–	–	–	–	0.01	–	0.02
n		122	188	160	210	132	144	144	106
H-W exact probability		1.00	0.00	0.00	0.01	0.26	1.00	0.04	0.03
Expected heterozygosity		0.49	0.51	0.43	0.45	0.58	0.38	0.43	0.71
Direct count heterozygosity		0.50	0.45	0.30	0.32	0.29	0.33	0.38	0.68
*PGI**	130	0.21	0.19	0.09	0.01	0.67	0.69	0.91	0.40
	125	–	–	–	–	–	–	–	0.04
	115	0.02	0.05	0.04	0.02	0.22	0.04	0.06	0.12
	100	0.72	0.66	0.78	0.82	0.10	0.22	0.03	0.39
	85	0.05	0.09	0.09	0.14	0.01	0.06	0.00	0.04
n		130	110	158	210	130	144	144	180
H-W exact probability		0.00	1.00	0.00	1.00	0.00	0.00	0.09	0.05
Expected heterozygosity		0.48	0.51	0.38	0.30	0.49	0.48	0.17	0.67
Direct count heterozygosity		0.35	0.45	0.25	0.26	0.29	0.31	0.14	0.61

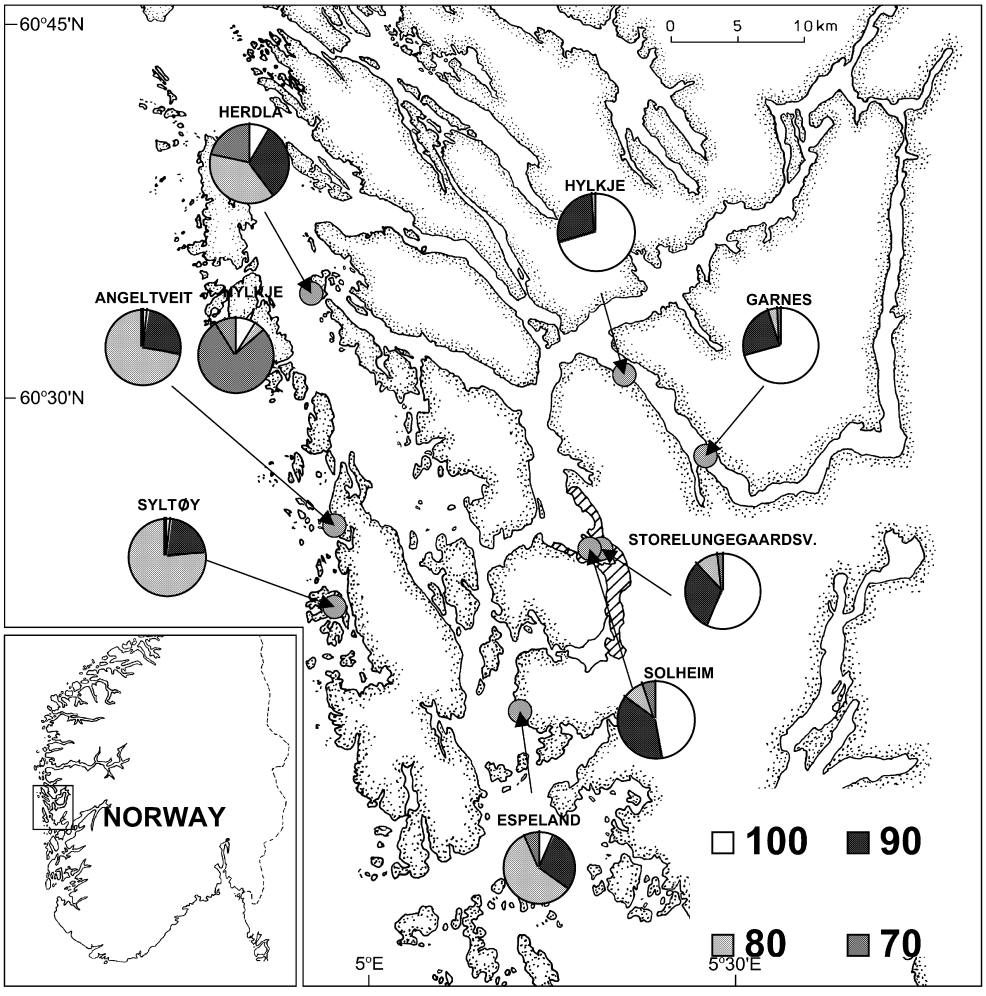

Fig. 1 *Mytilus PGM** allele frequencies (the four most common alleles) on the coast around Bergen, Norway

Weinberg equilibrium, as indicated by the differences between observed and expected heterozygosities (Table 2). In most cases fewer heterozygotes were observed compared to expected numbers.

Significance between samples

Using pair-wise χ^2-homogeneity tests, all but two pairs of samples were shown to be significantly different (Table 3). The samples from Solheim and Store Lungegaardsvann were collected close to each other, and although they grew on very different substrates they were not expected to represent different populations. Neither did the samples from Hylkje and Garnes differ significantly. These two locations share a similar environment within the same fjord, and thus could be expected to support the same population. Also, Angeltveit and Syltøy were rather close, although significantly different. Samples from these locations also differed in shell morphology (Ridgway, unpublished observation).

Table 3 Heterogeneity tests of significant differences among samples for the loci *PGM** and *PGI** combined given as chi-square values. Chi-square values >26.1 correspond to $P<0.001$(*df* 8)

Sample no.	2	3	4	5	6	7	8
1	11.8	42.6	47.6	208.2	212.8	176.0	109.0
2	–	29.1	47.2	256.5	268.7	340.6	146.7
3		–	19.2	345.1	369.2	404.9	228.2
4			–	433.6	425.2	515.5	293.8
5				–	44.3	31.3	32.9
6					–	27.7	78.6
7						–	135.5

Salinity and allele distributions

The most significant χ^2 values were found between locations with different salinities. The most common alleles at the sites with low salinity (Hylkje, Solheim, Store Lungegaardsvann and Garnes) were *PGI*100* and *PGM*100*. At the more saline sites (Herdla, Angeltveit, Syltøy and Espeland) the most common alleles were *PGI*130* and *PGM*80*. To test this apparent main difference in allele distribution, the Mann-Whitney test was applied. The mussels were divided into two main groups,

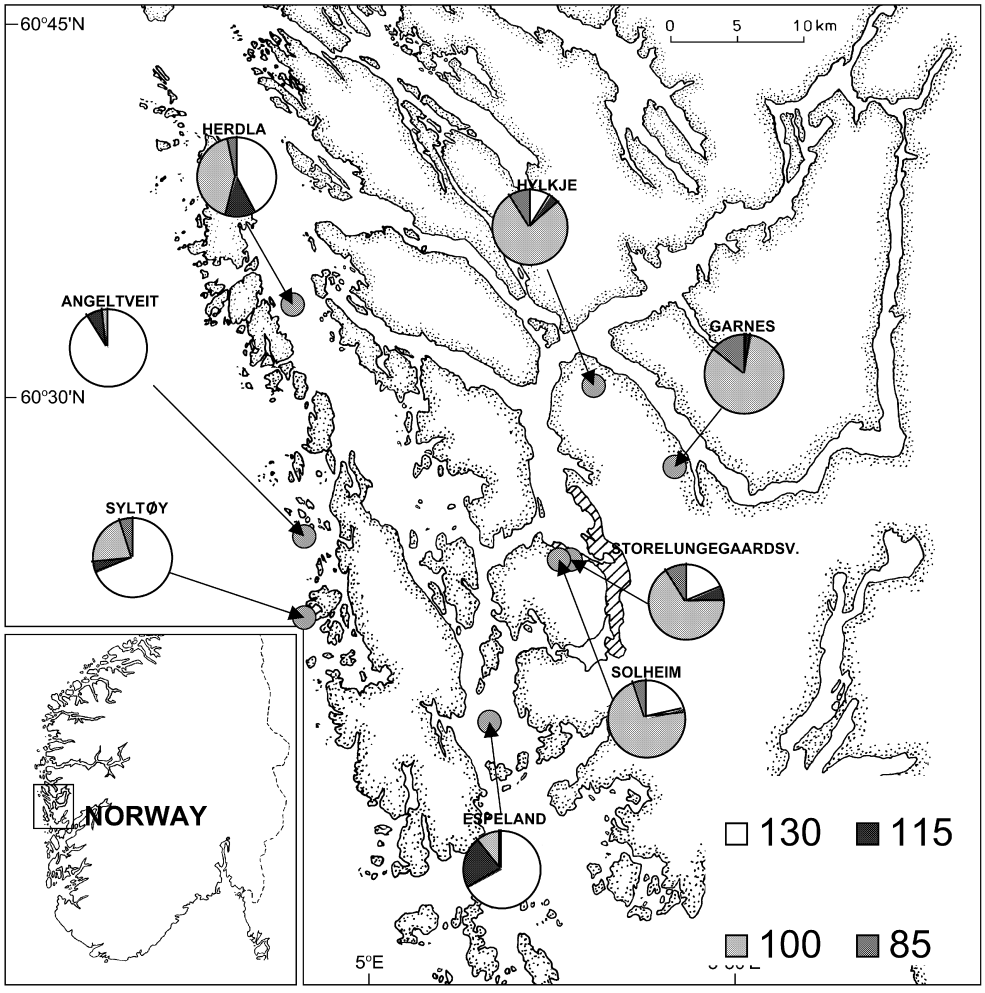

Fig. 2 *Mytilus PGI** allele frequencies (the four most common alleles) on the coast around Bergen, Norway

Table 4 Allele frequencies for *PGI*100* and *PGM*100* together with rank of samples from saline and brackish water, respectively

*PGI*100*				*PGM*100*			
Saline water		Brackish water		Saline water		Brackish water	
Allele frequencies	Rank	Allele frequencies	Rank	Allele frequencies	Rank	Allele frequencies	Rank
0.39	8	0.82	4	0.08	8	0.71	4
0.22	6	0.78	3	0.07	5	0.70	3
0.10	5	0.66	2	0.03	6	0.53	2
0.03	7	0.61	1	0.02	7	0.46	1
n=4	26	*n*=4	10	*n*=4	26	*n*=4	10

from saline and brackish water. Their common alleles, *PGM* 100* and *PGI*100* (Table 4), were ranked and probability values derived for testing the two-tailed hypothesis that salt and brackish water mussels display equal allele frequencies. Because the *U* values were found to be greater than the values expected at a significance level of 0.05, the hypothesis was rejected, indicating that the main difference in allele frequencies is connected to salinity at the locations where the mussels grow.

Discussion

In general, mussels display a high degree of allozyme variation, including *PGM** and *PGI**, and the results obtained by different authors are summarised by Gosling (1992). Except possibly for *MPI**, no loci show diagnostic variations between any of the European species, but they have been show to possess very different allele frequencies. Within species, allele frequencies seem to be very similar over wide areas, as should be expected for species with planktonic stages. However, in several cases (summarised by Gosling 1992), as in the present study,

considerable variation in allele frequencies over short distances has been observed. In most cases, this occurs in zones where two *Mytilus* species overlap, for instance in several locations on the west coast of the British Isles. At such locations there is clear evidence of hybridising and segregation of the two species with respect to positions on the shore beds. Evidently, there is no reproductive barrier between the *Mytilus* species in the zones of overlap.

Heterozygote deficiency is a common phenomenon in marine bivalves (Gosling 1992, and references therein). Different explanations have been put forward to explain the phenomenon, e.g. selection governed by underdominance in early stages and overdominance in later stages, Wahlund effects and null alleles. None of these explanations can fully explain the observations.

Beaumont (1994) showed that *PGM** and *PGI** are not linked in *M. edulis*. Non-linkage of these two loci was also observed in mussels by Gardner et al. (1996), and thus the two loci *PGI** and *PGM** make independent contributions to the identification of different groups of blue mussels.

In the present study, significant differences between populations were observed over relatively short distances. The allele *PGI*100* was found at high frequencies in all samples from brackish water (Store Lungegaardsvann, Solheim, Hylkje and Garnes). In the samples from Espeland, Syltøy and Angeltveit the allele *PGI*130* was very frequent, while the sample from Herdla displayed intermediate frequencies. Although exposed to some freshwater from local small streams, the latter localities were, on the whole, more exposed to the open sea. The difference in *PGI** allele frequencies between the low salinity locations on the one hand, and the high salinity locations on the other hand, was found to be greater than that between *M. edulis* and *M. trossulus* in other surveys (Bulnheim and Gosling 1988; Penney and Hart 1999). *PGM** alleles differentiate the locations in a similar way. The *PGM*100* allele is common at the four brackish locations, and slow *PGM*80* alleles are common at the four saltwater locations.

In her overview, Gosling (1992) gives data for *PGM** and *PGI** for both *M. edulis* and *M. trossulus* from Denmark and the Baltic (Tvärrminne), respectively. In the former species, the allele *PGI*107* is the more common, while in the latter species the allele *PGI*98* is the most common. Likewise for *PGM**, an allele with high anodic mobility (*PGM*111*) is the most common for *M. trossolus*, while two alleles with lower anodic mobility (*PGM*93* and **100*) are the common ones for *M. edulis*.

It is not possible to directly compare the alleles found in the present study with those described in the literature. However, it is striking that low mobility *PGM** alleles are found with high frequencies in locations with full strength saltwater, while in locations with brackish water the high mobility alleles are more frequent. In *PGI** it is the opposite; the low mobility alleles are found to be more frequent in brackish water, while the high mobility alleles are found in localities with high salinity. This is what should be expected if the species *M. trossulus* is present in brackish water, i.e. inner fjord localities and other localities which are highly influenced by freshwater, while *M. edulis* is found at localities with full strength saltwater. Genotypic differences have also been described by Bulnheim and Gosling (1988), Varvio et al. (1988) and Väinölä and Hvilsom (1991). These authors all revealed significant differences at the *PGI** and *PGM** loci between North Sea and Baltic mussels in a similar direction as in the present study.

The fact that in the present study the greatest difference between samples is connected to salinity also immediately points to the differences between the species *M. edulis* and *M. trossulus* which in other areas are mainly found in full strength seawater and brackish water, respectively. This supports the hypothesis that the extensive variation observed in the present study could be explained by the existence of two species in coastal waters of western Norway. It is known that *M. trossulus* and *M. edulis* meet in a narrow zone of a relatively short distance of 150 km in the Danish Belt Sea (Väinölä and Hvilsom 1991), but the Norwegian coast with very variable salinity may well be such a mixing zone for the brackish water *M. trossulus* and the more salt-dependent *M. edulis*.

Based on calculations (Lessios 1992), it has been claimed that these enzyme variations are maintained by selection. Salinity has previously been regarded as the main cause of differential selection at these loci (Gardner and Kathiravetpillai 1997; Gardner and Palmer 1998). The temporal environmental variability hypothesis that heterozygotes are superior in temporally unstable environments cannot be refuted by the results, but the results present no support to this theory either.

Different levels of salinity are reasoned to be the cause for the existence and maintenance of the two main genotype distributions observed in the present study. Bayne (1965) investigated the fertilisation success of mussels in different salinities and found that their range of salinity tolerance varied depending on which salinity they were derived from. The lower level of tolerance for *M. edulis* from saltwater (between 30 and 32‰) was 20‰. Below 20‰ there was no growth of the larvae, and it has been suggested that salinity affects the level of expression of genes promoting larval growth (Innes and Haley 1977). These early observations offer an explanation for the limited distribution of saltwater genotypes in brackish water areas along the coast. Thus, salinity may represent the isolation mechanism that maintains the genetically different groups or species. It is highly probable that larvae from the different groups will be distributed both to brackish water and full strength seawater, but adaptation to local environmental conditions, possibly governed by different salinities, may prevent larvae from one location from growing up in locations with different salinities. To survive in salinities lower than ~20‰, a differentially adapted genotype has evolved. It may correspond to the species recognised as *M. trossulus*, which can be found throughout the Baltic (Bulnheim and Gosling 1988; Väinölä 1990), and possibly also along the Norwegian coast.

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References

Bayne BL (1965) Growth and delay of metamorphosis of the larvae of *Mytilus edulis* (L.). Ophelia 2:1–47

Beaumont A (1994) Linkage studies in *Mytilus edulis*, the mussel. Heredity 72:557–562

Botnen HB, Hjohlman S, Johannessen PJ (2000) 'Byfjordsundersøkelsen' overvåking av fjordene rundt Bergen—Miljøundersøkelse i 1999 (in Norwegian). IFM Rapport no. 18, Universitetet i Bergen

Bulnheim HP, Gosling E (1988) Population genetic structure of mussels from the Baltic Sea. Helgol Meeresunters 42:113–129

Comensaña AS, Toro JE, Innes DJ, Thompson RJ (1999) A molecular approach to the ecology of a mussel (*Mytilus edulis—M. trossulus*) hybrid zone on the east coast of Newfoundland, Canada. Mar Biol 133:213–221

Cousteau C, Renaud F, Delay B (1991) Genetic characterization of the hybridization between *Mytilus edulis* and *M. galloprovincialis* on the Atlantic coast of France. Mar Biol 111:87–93

Gardner JPA, Kathiravetpillai G (1997) Biochemical genetic variation at a leucine aminopeptidase (LAP) locus in blue (*Mytilus galloprovincialis*) and greenshell (*Perna canaliculus*) mussel populations along a salinity gradient. Mar Biol 128:619–625

Gardner JPA, Palmer NL (1998) Size-dependent, spatial and temporal genetic variation at a leucine aminopeptidase (LAP) locus among blue mussel (*Mytilus galloprovincialis*) populations along a salinity gradient. Mar Biol 132:275–281

Gardner JPA, Eyles RF, Pande A (1996) Biochemical—genetic variation in a wild and a cultured population of the greenshell mussel, *Perna canaliculus*. NZ J Mar Freshw Res 30:435–441

Gosling E (1992) Genetics of *Mytilus*. In: Gosling E (ed) The mussel *Mytilus*: ecology, physiology, genetic and culture. Elsevier, Amsterdam, pp 309–382

Hilbish TJ, Carson EW, Plante JR, Weaver LA, Gily MR (2002) Distribution of *Mytilus edulis*, *M. galloprovincialis*, and their hybrids in open-coast populations of mussels in southwestern England. Mar Biol 140:137–142

Hummel H, Colucci F, Bogaards RH, Strelkov P (2001) Genetic traits in the bivalve *Mytilus* from Europe, with an emphasis on Arctic populations. Polar Biol 24:44–52

Innes DJ, Haley LE (1977) Genetic aspects of larval growth under reduced salinity in *Mytilus edulis.* Biol Bull 153:312–321

Koehn RK, Milkman R, Mitton JB (1975) Population genetics of marine pelecypods. IV. Selection, migration and genetic differentiation in the blue mussel *Mytilus edulis.* Evolution 30:2–32

Koehn RK, Hall JG, Innes DJ, Zera AJ (1984) Genetic differentiation of *Mytilus edulis* in eastern North America. Mar Biol 79:117 –126

Lessios HA (1992) Testing electrophoretic data for agreement with Hardy-Weinberg expectations. Mar Biol 112:517–523

Mallet AL, Carver CEA (1989) Growth, mortality, and secondary production in natural populations of the blue mussel, *Mytilus edulis.* Can J Fish Aquat Sci 46:1154–1159

McDonald JH, Seed R, Koehn RK (1991) Allozymes and morphometric characters of three species of *Mytilus* in the northern and southern hemispheres. Mar Biol 111:323–333

Miller MP (1997) Tools for population genetic analyses (TFPGA) 1.3. Arizona State University

Murphy RW, Sites JW, Buth DG, Haufler CH (1990) Proteins I: isozyme electrophoresis. In: Hillis DM, Moritz C, Mable BK (eds) Molecular systematics. Sinauer, Sunderland, Mass., pp 51–120

Pedersen EM, Hunt HL, Scheibling RE (2000) Temporal genetic heterogeneity within a developing mussel (*Mytilus trossulus* and *M. edulis*) assemblage. J Mar Biol Assoc UK 80:843–854

Penney RW, Hart MJ (1999) Distribution, genetic structure and morphometry of *Mytilus edulis* and *M. trossulus* within a mixed species zone. J Shellfish Res 18:367–374

Ridgway G (2001) Interpopulation variation in blue mussels, *Mytilus edulis* L., over short distances. Sarsia 86:157–161

Saavedra C, Stewart DT, Stanwood RR, Zouros E (1996) Species-specific segregation of gender-associated mitochondrial DNA types in an area where two mussel species (*Mytilus edulis* and *M. trossulus*) hybridize. Genetics 143:1359–1367

Väinölä R (1990) Allozyme differentiation between Baltic and North Sea *Mytilus* populations: a reassessment of evidence from transplantations. Mar Ecol Prog Ser 67:305–308

Väinölä R, Hvilsom MM (1991) Genetic divergence and a hybrid zone between Baltic and North Sea *Mytilus* populations. Biol J Linn Soc Lond 43:127–148

Varvio S-L, Koehn RK, Väinölä R (1988) Evolutionary genetics of the *Mytilus edulis* complex in the North Atlantic region. Mar Biol 98:51–60