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Morphological variation of *Mytilus edulis* and *Mytilus trossulus* in eastern Newfoundland

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Abstract Allopatric populations of *Mytilus* species show distinct shell morphology which may be due to genetic and/or environmental effects. Sympatric populations of *Mytilus* species show similar shell morphology which may be due to hybridization eroding morphological differences and/or the influence of common environmental conditions. The present study examined shell morphology and shell shape from 16 sites in eastern Newfoundland where *M. edulis* L. and *M. trossulus* Gould coexist in common environments with limited hybridization. Shell morphology was based on measurements of eight characters, and shell shape was quantified by elliptic Fourier analysis of shell outlines. Significant differences were observed between species for both shell morphology and shell shape across 16 sites sampled. The relatively small differences in morphology and shape between the species were probably due to exposure to common environments rather than hybridization. Shell shape for *M. edulis* was more eccentric compared to *M. trossulus* which was more elongated. Shell shape analysis of a range of size classes at one site showed a change from an eccentric to an elongated shape going from the smaller to the larger size classes. Both species showed a similar trend, with the larger *M. edulis* more eccentric and the larger *M. trossulus* more elongated.

Introduction

Mussels within the *Mytilus edulis* complex have been divided into three species (*M. edulis*, *M. galloprovincialis*, *M. trossulus*) based on genetical and morphological differences (Koehn 1991; McDonald et al. 1991). These differences have been determined for samples collected world wide, but primarily from areas with only a single-species (McDonald et al. 1991). The genetical and morphological characteristics of each species in single-species populations have been useful for examining ecological and genetical interactions in areas where these species coexist. Species within this complex appear to hybridize whenever they come into contact (Skibinski et al. 1983; Koehn 1991). For example, *M. edulis* and *M. galloprovincialis* coexist on the coasts of England, France and Spain, where hybridization is common, ranging from 25 to 80% (e.g. Skibinski et al. 1978, 1983; Coustau et al. 1991; Gardner 1994, 1995; Sanjuan et al. 1994; Wilhelm and Hilbish 1998). Less well studied areas of hybridization include southern California, where the frequency of hybrids between *M. galloprovincialis* and *M. trossulus* has been estimated at about 7.5 to 29% (McDonald and Koehn 1988; Sarver and Foltz 1993; Rawson and Hilbish 1995; Rawson et al. 1996; Suchanek et al. 1997). *M. edulis* and *M. trossulus* coexist in the Baltic Sea, where hybridization and introgression are common enough that these two taxa can be considered as semispecies (Väinölä and Hvilsom 1991). *M. edulis* and *M. trossulus* also coexist on the Atlantic coast of Canada, where hybridization appears to be much less common. Bates and Innes (1995) sampled several sites along the east coast of Newfoundland and found a bimodal distribution of hybrid index values based on three partially diagnostic allozyme loci, consistent with limited if any hybridization. Mallet and Carver (1995) found $<2\%$ hybrids between the two species at two sites near Lunenburg, Nova Scotia. Saavedra et al. (1996) and Comesaña et al. (1999) used diagnostic genetic markers and estimated the frequency of hybrids (mostly backcross types) at sites in Nova Scotia and Newfoundland to be about 23% and 26%, respectively.

Mussel species that are morphologically distinct in allopatry may be less distinct in sympatry due to the effects of common environmental conditions and/or

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hybridization and introgression. McDonald et al. (1991) compared the shell morphology of the three species from single-species populations, avoiding areas of known hybridization. A canonical variates analysis, based on 18 shell measurements, produced three distinct clusters of individuals corresponding to the three species. Gardner (1996) found that *M. galloprovincialis* and *M. edulis* were morphologically distinct when allopatric populations were compared, but were less distinct when individuals were sampled from sympatric populations. The reduction in morphological distinction between the species was considered to be due to hybridization and introgression, but exposure to common environmental conditions could also play a role.

A principal components analysis of the allozyme data for all Northern Hemisphere populations of *Mytilus* spp. showed distinct clusters for each species, with some genetically intermediate individuals between *M. galla-provincialis* and *M. edulis*, and between *M. gallo-provincialis* and *M. trossulus* (Fig. 3b in McDonald et al. 1991). Almost no genetically intermediate individuals were found between *M. edulis* and *M. trossulus*. This appears to be due to the limited number of samples collected from areas where these two species coexist (Table 1 in McDonald et al. 1991). Observations from areas of Atlantic Canada where *M. edulis* and *M. trossulus* do coexist also showed few genetically intermediate individuals, suggesting limited hybridization (Koehn et al. 1984; Bates and Innes 1995). Although there is some variation in the level of hybridization between *M. edulis* and *M. trossulus* among sites in Atlantic Canada (Mallet and Carver 1995; Saavedra et al. 1996; Comesaña et al.1999), the frequency of individuals of mixed ancestory appears to be much less than that found for populations of *M. edulis* and *M. galloprovincialis* in Europe. Thus sites in Atlantic Canada offer an opportunity to study morphological variation between two mussel species that have been exposed to similar environments without the confounding influence of extensive hybridization.

Morphological analysis of *Mytilus* spp. shells (reviewed by Seed 1992) has relied primarily on univariate (Beaumont et al. 1989) and multivariate analyses (McDonald et al. 1991; Karakousis and Skibinski 1992; Mallet and Carver 1995; Gardner 1996) of several shell characteristics.Variation in shell shape has also been examined using ratios of shell length, height and width (Seed 1968; Beaumont et al. 1989). Direct analysis of bivalve shell shape, based on a digitized outline, has been developed using elliptic Fourier analysis (Ferson et al. 1985; Crampton 1995), which analyses complex outlines with little loss of shape information (Rohlf and Archie 1984; McLellan and Endler 1998). Elliptic Fourier analysis of shell outlines from two sites in eastern Newfoundland revealed small but statistically significant differences in shell shape between two electrophoretically distinct groups of *Mytilus* spp. (Ferson et al. 1985), which were subsequently identified as *M. edulis* and *M. trossulus* (McDonald and Koehn 1988; McDonald et al. 1991). Data from a limited number of sites in Newfoundland originally suggested the presence of pure species and mixed species populations (Koehn et al. 1984; Varvio et al. 1988), but more extensive sampling revealed that the species appear to coexist at most sites in eastern Newfoundland (Bates and Innes 1995).

In the present study we examined shells from a larger number of sites than examined by Ferson et al. (1985) to determine the degree of morphological differentiation between *Mytilus edulis* and *M. trossulus* coexisting in a variety of habitats in eastern Newfoundland. The two species may have a similar shell morphology due to exposure to a common environment rather than hybridization eroding morphological differences as has been previously documented for *M. edulis* and *M. galloprovincialis* (Gardner 1996).

Table 1 *Mytilus* spp. Mean values (standard errors in *parentheses*) of eight shell characters for *M. edulis* ($N = 117$) and *M. trossulus* ($N = 100$). See "Materials and methods" for character abbreviations. Standardized canonical coefficients were obtained from length-standardized data and residuals from the common within-groups regression line

Character	Mean values (mm)		Canonical coefficients	
	M. edulis	*M. trossulus*	Length-standardized	Residuals
aam	4.5 (0.14)	3.0 (0.09)	0.622	0.291
hp	5.2 (0.12)	3.8 (0.10)	0.605	0.399
pal	3.2 (0.11)	2.6 (0.08)	−0.387	−0.406
lig	24.7 (0.70)	19.3 (0.57)	−0.266	−0.335
padv	15.0 (0.33)	11.8 (0.31)	−0.177	−0.308
padp	15.3 (0.36)	12.6 (0.41)	−0.446	−0.510
ht	25.0 (0.53)	18.6 (0.52)	0.663	0.768
wid	9.7 (0.24)	7.5 (0.18)	0.482	0.347
len	47.8 (1.16)	36.8 (1.04)		

Materials and methods

Samples of *Mytilus edulis* L. and *M. trossulus* Gould, and their putative hybrids used in the present study are a random subset of 227 individuals from the samples described by Bates and Innes (1995). For each of the 16 sample sites [see Bates and Innes (1995) for site abbreviations and locations] the following sample sizes were analysed in the present study (sample size in parentheses): BB (11), BV0 (24), BV1 (16), BV2 (13), BV3 (14), CC1 (17), CC2 (15), EM (13), FH (9), IA (16), LH (6), THL (16), TT1 (13), TT2 (20), TT3 (16) and TW (8). Shells from individuals genotyped for four partially diagnostic enzyme loci (phosphoglucomutase, EC 5.4.2.2; esterase, EC 3.1.1.1; aminopeptidase-I, EC 3.4.11.-; aminopeptidase, EC 3.4.-.-) were labelled for subsequent morphometric analysis. A principal components analysis of the allozyme data resulted in a bimodal distribution of PC 1 scores (Fig. 3 in Bates and Innes 1995), and homozygous genotypes associated with each mode distinguished *M. edulis* (*Pgm* 100/100, *Est* 100/100) from *M. trossulus* (*Pgm* 111/111, *Est* 90/90). Assignment of each individual to *M. edulis* or *M. trossulus* was based on the PC 1 score using a similar approach to that described by Sarver and Foltz (1993); individuals with a PC 1 score < 0.0 were assigned to *M. edulis* and individuals with a PC 1 score > 0.0 were assigned to *M. trossulus*. This approach assigns any hybrid or backcross individuals to one

or the other species. However, the frequency of hybrid individuals appears to be low in this area (Bates and Innes 1995), and hybrids consist primarily of backcross individuals (Comesaña et al.1999).

The left valve was measured for the nine characteristics (including length) previously shown to be most useful for discriminating *Mytilus edulis* from *M. trossulus* (Mallet and Carver 1995). Following Fig. 2 in McDonald et al. (1991): (1) aam: length of anterior adductor muscle scar, (2) hp: length of hinge plate, (3) pal: distance between pallial line and ventral shell margin midway along shell, (4) padv: distance between ventral edge of posterior adductor muscle scar and ventral shell margin, (5) padp: distance between anterior edge of posterior adductor muscle scar and posterior shell margin, (6) lig: distance between umbo and posterior end of ligament, (7) len: shell length, (8) wid: shell width and (9) ht: shell height. All measurements were made with digital calipers to the nearest 0.1 mm except aam, hp, and pal, which were measured with an ocular micrometer on a stereo dissecting microscope. Some of the shells could not be reliably measured for all of the characteristics leaving a total of 217 shells for the morphometric analysis.

The shell characters were standardized for size by transforming each character (x) using $\log_{10}(x)/\log_{10}(\text{len})$ as described previously (McDonald et al. 1991; Mallet and Carver 1995; Gardner 1996). The degree of morphometric difference between *Mytilus edulis* and *M. trossulus* was determined using a canonical variates analysis (PROC CANDISC, SAS 1996) based on the eight length-standardized characters (McDonald et al. 1991; Sarver and Foltz 1993; Mallet and Carver 1995; Gardner 1996). The length standardization still resulted in a significant correlation ($r = 0.49$ to 0.80, all $p < 0.001$) between each transformed character and length. Furthermore, the canonical variate from the analysis of the length-standardized data was significantly correlated ($r = 0.54$, $p < 0.001$) with length. Thus, the canonical variates analysis may be partially confounded by differences in size between the two species rather than morphological differences independent of size. To reduce the influence of size, the morphological data were analysed as suggested by Reist (1985, 1986). An analysis of covariance (Sokal and Rohlf 1995) was performed for each $\log_{10}$ transformed character [with $\log_{10}$ (length) as the covariate] between the two species. The residual variation for each character and each individual was calculated using the common within-groups regression line (Reist 1986). Residual variation should be free from the direct influence of size. The canonical variates analysis was repeated using the residuals for each character, which were also used in a principal components analysis (PROC PRINCOMP) in order to account for morphological variation within and between the two species. The distributions of the first two principal components were found to be normal with homogeneous variances (PROC UNIVARIATE). Geographic variation in morphology was tested in a two-way (species × sites) ANOVA using the PC 1 and PC 2 scores for each individual as an index of the morphological variation.

In order to analyse variation in shell shape, the outlines of each of the 227 shells were digitized with a video image capture system (JAVA v. 1.20, Jandel Scientific), a procedure similar to that described by Ferson et al. (1985). The left valve of each mussel was placed in the same orientation, and the outline digitized in the same direction, starting at the umbo. The outline of each shell was described by about 300 to 600 point pairs (x and y coordinates), depending on the size of the shell, then analysed by elliptic Fourier analysis (Ferson et al. 1985; Crampton 1995) using the first ten harmonics. Normalization for starting position, orientation, and size resulted in each outline being represented by 37 Fourier coefficients. Principal components analysis of the variance-covariance matrix (Rohlf and Archie 1984; Crampton 1995) was used to summarize shape variation based on the Fourier coefficients for each shell. A canonical variates analysis summarized the degree of variation in shape differences between the two species. All multivariate analyses (PROC PRINCOMP, PROC CANDISC) were performed using SAS (1996). Average shell shape was reconstructed by averaging the Fourier coefficients for a group of shells and inverting the Fourier transform (Crampton 1995).

One site (Traytown) was selected for more detailed sampling because both species were common there (sample TT3 of Bates and Innes 1995). A random subset consisting of 141 mussels sampled from the Traytown site and described by Bates and Innes (1995) was divided into eight 5 mm shell-length classes (sample sizes in parentheses): 16–20 (14), 21–25 (19), 26–30 (16), 31–35 (19), 36–40 (20), 41–45 (18), 46–50 (21), and >50 mm (14). Each of the 124 shells that could be measured for all nine morphological characters were used to summarize morphometric differentiation between the two species based on a canonical variates analysis. The length-standardized data were compared to size adjustment using the residuals from an ANCOVA as described above. The outline of each of the 141 shells was also digitized, and the Fourier coefficients analysed in a principal components analysis and a canonical variates analysis. An average shell shape was reconstructed as described above for each size class for individuals identified by the genetic markers as *Mytilus edulis* and *M. trossulus*.

Results

Morphometric variation – length standardized

All mean character values were greater in *Mytilus edulis* (Table 1). The mean shell length of *M. edulis* was significantly ($F_{(1,215)} = 48.5$, $p < 0.001$) larger than that of *M. trossulus*. Canonical variates analysis, based on the length-standardized characters, produced a canonical correlation of 0.66 ± SE 0.03, which was significantly greater than 0.0 (Wilks' lambda = 0.556, $p < 0.001$), with approximately 45% of the variation in the canonical variable accounted for by the two species. Standardized canonical coefficients indicated that aam, hp, ht, wid and padp contributed most to discriminating between the two species (Table 1), as in previous studies on morphometric differences between *M. edulis* and *M. trossulus* based on length-standardized characters (McDonald et al. 1991; Mallet and Carver 1995). The canonical variate showed a significant correlation ($r = 0.54$, $p < 0.001$) with shell length. The two species were distributed with a broad overlap on the canonical variate axis (Fig. 1). A similar histogram was obtained using 85 individuals with genotypes likely to be pure *M. edulis* (*Pgm* 100/100, *Est* 100/100) and 33 individuals likely to be pure *M. trossulus* (*Pgm* 111/111, *Est* 90/90), suggesting that any overlap in the two distributions for the whole sample was not due to pooling of hybrid individuals. A cross validation analysis showed that, based on the eight morphometric characters, 24 of 117 (21%) *M. edulis* were misclassified as *M. trossulus* and 22 of 100 (22%) *M. trossulus* were misclassified as *M. edulis*. Thus, the a posteriori error rate was slightly less than half the error rate expected by chance alone.

Morphometric variation – residuals

In the ANCOVA, three (aam, hp, ht) of the four characters with homogeneous slopes had significantly different intercepts between the two species (Table 2). All three mean character values were significantly greater in *Mytilus edulis*, after adjusting for differences in shell length. Residuals in Table 1 were calculated from the common within-groups regression line of each species

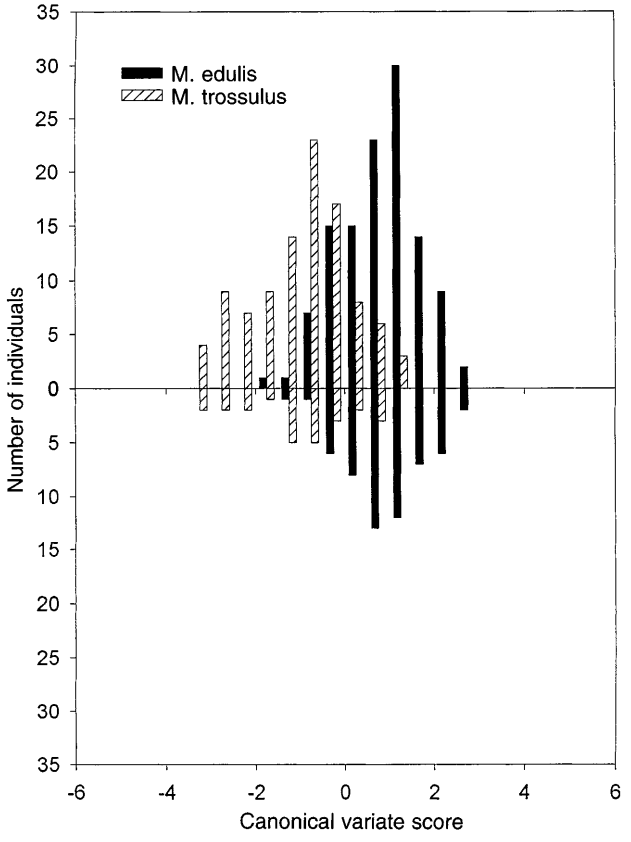

Fig. 1 *Mytilus* spp. Histogram of canonical variate scores for *M. edulis* and *M. trossulus* based on eight length-standardized shell characters. *Upper histogram* for all 217 individuals, *lower histogram* for 85 individuals most likely to be pure *M. edulis* (genotype *Pgm* 100/100, *Est* 100/100) and for 33 individuals most likely to be pure *M. trossulus* (genotype *Pgm* 111/111, *Est* 90/90) (see Bates and Innes 1995)

for each character (Table 2). Although four of the eight regression coefficients (slopes) were significantly different between the species (Table 2), the common within-groups regression coefficient provided a standard for comparison between the species with the direct influence of size removed from each character (Reist and Crossman 1987). The residuals for the eight characters showed much smaller correlations with length ($r = -0.18$ to 0.20, with $p < 0.05$ for three of the eight characters) than did the length-standardized values. A canonical variates analysis of the residuals produced results similar to the analysis using length-standardized characters, despite the reduced correlation between the canonical variate and length ($r = 0.26$, $p < 0.001$). The canonical correlation (0.60 ± SE 0.04) was significantly greater than 0.0 (Wilks' lambda = 0.60, $p < 0.001$), with approximately 40% of the variation in the canonical variable accounted for by the two species. The standardized canonical coefficients showed that ht, padp, pal and hp contributed most to discriminating between the two species (Table 1). In the cross validation analysis, 25 of 217 (21%) *M. edulis* were misclassified as *M. trossulus* and 24 of 100 (24%) of *M. trossulus* were misclassified as *M. edulis*, comparable to the results using the length-standardized data.

The first two principal components from the principal components analysis, based on the residuals, explained about 24% and 21% of the variation in shell morphology, respectively. A plot of data from all individuals showed that the two species differed morphologically as defined primarily by the PC 2 axis (Fig. 2). Both PC 1 and PC 2 showed significant but weak correlation with length ($r = 0.19$, $p < 0.01$ and $r = 0.13$, $p < 0.05$, respectively). PC 2 exhibited significant variation between the two species ($F_{(1,15)} = 10.9$, $p < 0.005$) as well as among the 16 sites ($F_{(15,185)} = 2.9$, $p < 0.001$). A significant species-by-site interaction term ($F_{(15,185)} = 1.9$, $p = 0.03$) suggested that the pattern of morphological variation among sites was different for each species. Morphological variation, as defined by PC 1, also exhibited significant variation between the species ($F_{(1,15)} = 7.3$, $p < 0.05$) and among sites ($F_{(15,185)} = 6.3$, $p < 0.001$), but the species-by-site interaction was not significant.

Shell shape analysis

The first two principal components (PC 1, PC 2) explained about 58% and 15%, respectively, of the total variation in shell shape as analysed by the 37 Fourier coefficients. PC 1 showed no significant correlation with length ($r = -0.04$), while PC 2 was negatively correlated ($r = -0.26$, $p < 0.001$) with length. Data from individuals plotted on the PC 1 and PC 2 axes showed a broad overlap between species (Fig. 3), although the cluster of points for *Mytilus edulis* centered towards the negative side of 0.0 for both PC 1 and PC 2, while the

Table 2 *Mytilus* spp. ANCOVA for eight shell characters of *M. edulis* and *M. trossulus* using shell length as the covariate

Character	Regression coefficient, *b*			Slopes	Intercepts
	M. edulis	*M. trossulus*	common		
aam	1.0248	0.8925	0.9550	ns	$p < 0.001$
hp	0.8069	0.7230	0.7610	ns	$p < 0.001$
pal	1.1099	0.8047	0.9428	$p < 0.001$	ns
lig	1.0785	0.9521	1.0093	$p < 0.001$	$p < 0.05$
padv	0.9212	0.8829	0.9002	ns	ns
padp	1.0916	0.1458	1.0257	$p < 0.001$	$p < 0.001$
ht	0.8998	0.9525	0.9287	ns	$p < 0.001$
wid	0.9445	0.7583	0.8428	$p < 0.001$	ns

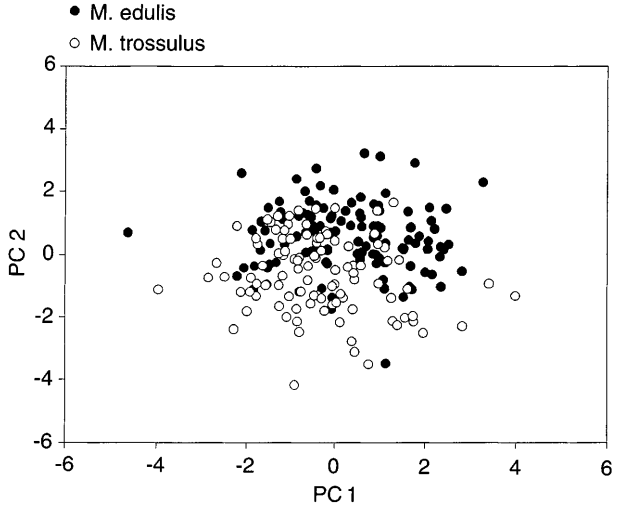

Fig. 2 *Mytilus* spp. Plot of principal components (PC 1, PC 2) for *M. edulis* and *M. trossulus* based on residuals from the ANCOVA for eight shell characters

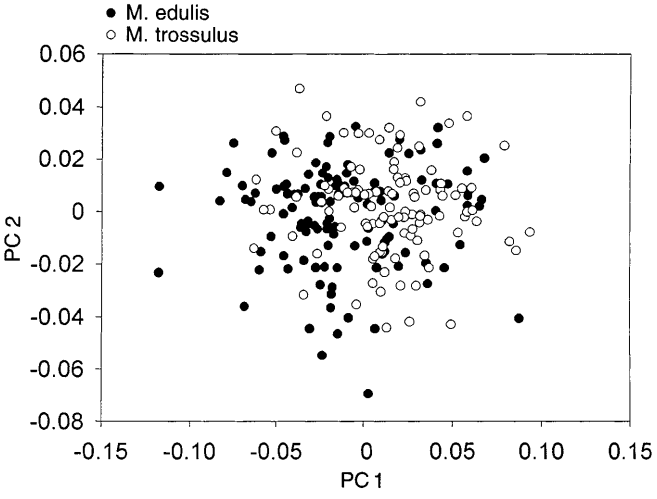

Fig. 3 *Mytilus* spp. Plot of principal components (PC 1, PC 2) for *M. edulis* and *M. trossulus* based on 37 Fourier coefficients from shell outlines used to summarize shell shape

cluster of *M. trossulus* individuals centered on the positive side of 0.0 for both principal component axes. The PC 1 axis was associated with variation in the degree of eccentricity of the dorsal margin and the degree of elongation. The PC 2 axis was associated with the degree of convexity of the ventral margin. Both species exhibited a similar range of shape variation and a similar average shell shape. However, *M. trossulus* was slightly more elongated, and *M. edulis*, slightly more eccentric. Shell shape, as summarized by PC 1 and PC 2, exhibited significant variation among sites (PC 1: $F_{(15,195)} = 3.06$, $p < 0.001$; PC 2: $F_{(15,195)} = 1.97$, $p < 0.05$), but only PC 1 showed significant variation ($F_{(1,15)} = 9.11$, $p < 0.05$) between the two species. Neither PC 1 nor PC 2 showed a significant species-by-site interaction.

The canonical variates analysis of the Fourier coefficients produced a canonical correlation (0.61 ± SE 0.04) significantly different from 0.0 (Wilks' lambda = 0.625, $p < 0.001$), with approximately 38% of the variation in shell shape being explained by the two species. The canonical variate showed a significant ($r = -0.34$, $p < 0.001$) correlation with length. Shell shape, as described by the canonical variates analysis, showed significant discrimination between the two species, but with a broad overlap in canonical variate scores (Fig. 4). The a posteriori cross-validation analysis showed that 38 of 122 (31%) *M. edulis* shells were misclassified as *M. trossulus* and 39 of 105 (37%) *M. trossulus* shells were misclassified as *M. edulis*.

Traytown size-class variation

The mean shell length of *Mytilus edulis* (44.5 ± SE 1.51 mm) was significantly ($F_{(1,122)} = 29.15$, $p < 0.001$) larger than *M. trossulus* (34.6 ± 1.05 mm) for the sample from Traytown. In the ANCOVA of the Traytown size-class sample, there were no significant differences between the species for the slopes of the regressions between $\log_{10}$ of each character and $\log_{10}$ length. The means of four characters (aam, hp, pal, ht) were significantly greater in *M. edulis*, but padp was significantly greater in *M. trossulus* when the character means had been adjusted for differences in length between the two species. The canonical variate scores, based on the length-standardized data, showed a bimodal distribution for the total sample (Fig. 5a), with each mode associated with one of the species (Fig. 5b). In contrast, the total sample based on the residuals (Fig. 6a) had a single mode composed of two broadly overlapping distributions for each species (Fig. 6b).

The principal component scores, based on the Fourier coefficients for individuals from the Traytown sample, showed a significant correlation between length

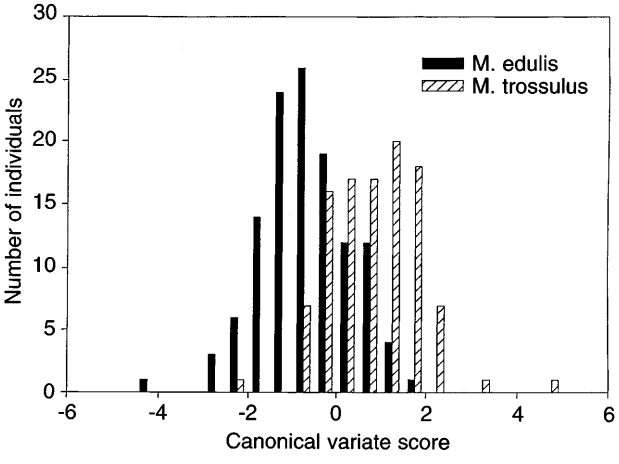

Fig. 4 *Mytilus* spp. Histogram of canonical variate scores for *M. edulis* and *M. trossulus* based on 37 Fourier coefficients from shell outlines used to summarize shell shape

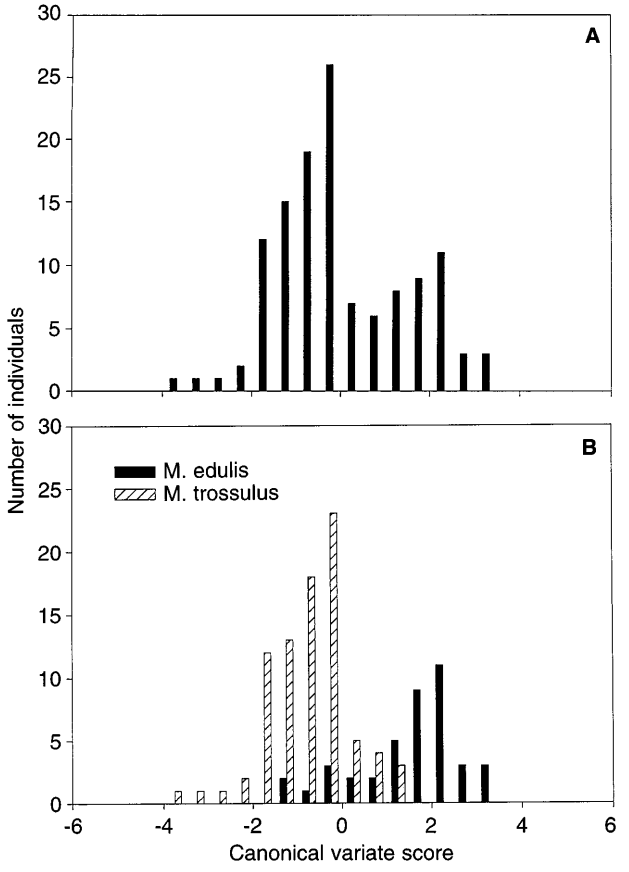

Fig. 5 *Mytilus* spp. Histograms of the canonical variate scores for Traytown size-class sample based on eight length-standardized shell characters. **A** Total sample, **B** total sample separated into *M. edulis* and *M. trossulus*

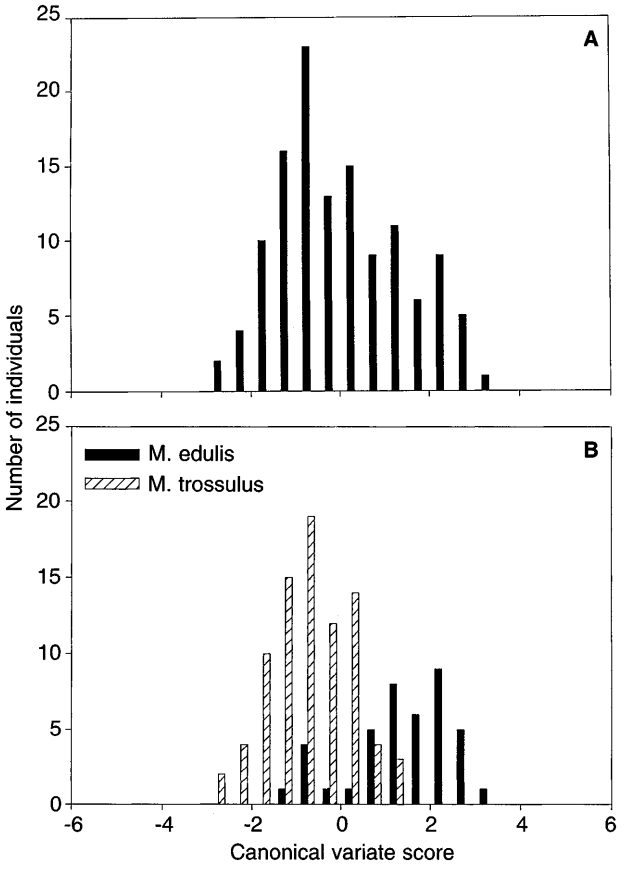

Fig. 6 *Mytilus* spp. Histograms of the canonical variate scores for the Traytown size-class sample based on the residuals from the ANCOVA for the eight shell characters. **A** Total sample, **B** total sample separated into *M. edulis* and *M. trossulus*

and both PC 1 ($r = 0.710$, $p < 0.001$) and PC 2 ($r = -0.28$, $p < 0.01$), indicating that the shape variation was strongly associated with length. There was a similar pattern of change in shape with length for both species. The smaller size classes had a more eccentric shape compared with a more elongated shape for the larger size classes. In the larger size classes, *Mytilus trossulus* was slightly more elongated, and *M. edulis* was slightly more eccentric. The canonical variates analysis, based on shell shape, produced two distributions (Fig. 7), with a greater separation between the two species than for the sample from the 16 sites shown in Fig. 4.

Discussion

Mytilus edulis and *M. trossulus* differed in shell morphology among 16 sites along the east coast of Newfoundland. The shells of the two species differed as measured by both a multivariate analysis of several shell characteristics and a multivariate analysis of shell outline shape. The morphological differences between the species were small and not as reliable as genetic markers (Bates and Innes 1995) for discriminating between the species, agreeing with the conclusions of Ferson et al. (1985). Nevertheless, the morphological differences were consistent between the two species, despite the morphological variation among individuals of each species collected from different sites. Both species also exhibited a similar pattern of morphological variation among sites with only a slight suggestion of a species-by-site interaction.

McDonald et al. (1991) also determined that *Mytilus edulis* and *M. trossulus* differ in shell morphology, but found a greater degree of morphological separation between *M. edulis* and *M. trossulus* than in the present study. The increased discrimination may be explained, in part, by the use of 18 shell characters by McDonald et al. (1991) compared with the eight used in the present study. In addition, McDonald et al. (1991) sampled individuals from single-species populations, which would be expected to accentuate differences in shell morphology due to the influence of local environmental conditions (Seed 1968). Nevertheless, differences in local environmental conditions were not sufficient to obscure species-specific differences for each species sampled from a broad survey of several Northern Hemisphere sites (McDonald et al. 1991). Most of the sites sampled in the

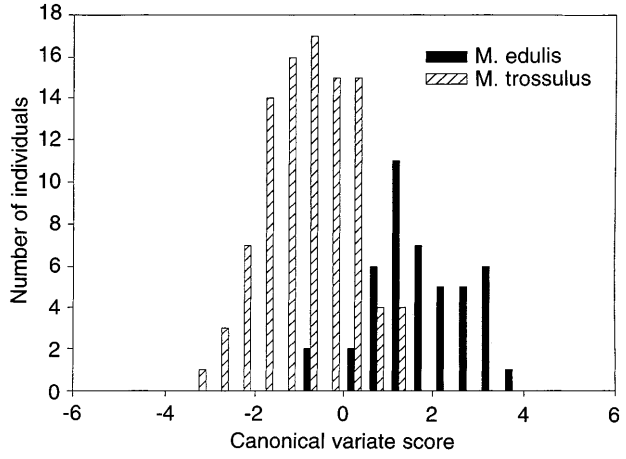

Fig. 7 *Mytilus* spp. Histogram of canonical variate scores for Traytown size-class sample based on 37 Fourier coefficients from shell outlines used to summarize shell shape for *M. edulis* and *M. trossulus*

present study contained a mixture of both species (Bates and Innes 1995), and exposure to common environmental conditions may have resulted in an increase in morphological similarity. Although hybridization occurs between *M. edulis* and *M. trossulus* (Comesaña et al. 1999), morphological differences between individuals most likely to be pure *M. edulis* and *M. trossulus* (homozygous for species-specific alleles at the *Pgm* and *Est* loci) were no greater than for the whole sample.

Another factor that may accentuate differences among species is the comparison of samples differing in average length. In the present study, the log-transformed length standardization only partially removed the influence of size, and the canonical variate was significantly correlated with length. Use of residuals reduced the influence of size, but the results from the multivariate analysis were not much different from those obtained from the length-standardized data. At the Traytown site, where shells from different size classes were examined, the canonical variates analysis on the length-standardized data produced a bimodal distribution, with each mode associated with one of the species. This is because the distribution of shell lengths at this site was bimodal. The same analysis using the residuals produced a unimodal distribution, suggesting that the size difference between the two species contributed to the bimodal distribution of the length-standardized canonical variate. Thus the results from a multivariate analysis of individual morphological characters may be strongly influenced by size differences despite attempts to remove the effect of size by dividing each character by shell length. Studies reporting morphological differences among species of *Mytilus* may have exaggerated the differences if the taxa being compared differ in shell length (McDonald et al. 1991; Karakousis and Skibinski 1992; Sarver and Foltz 1993; Mallet and Carver 1995). McDonald et al. (1991) based their analysis on shells with a length of 23 to 80 mm and Mallet and Carver (1995) analysed shells with a length of 15 to 60 mm, but neither study reported if the average shell length differed among the species.

The analysis of the Traytown size-class sample showed a similar change in shell shape with shell length in both species. Smaller mussels were more eccentric compared to larger mussels, which were more elongated, comparable to observations made by Seed (1968). In the canonical variates analysis positive canonical variate scores were associated with *Mytilus edulis* because larger individuals had a more elongated shape compared to the smaller eccentric mussels, which were predominantly *M. trossulus*. This particular association between shell length and shell shape combined with the bimodal shell lengths observed in the Traytown sample produced a greater separation between the two species compared to a similar analysis involving individuals from the 16 sites.

In addition to environmental differences between sites, single-species populations would not be influenced by the effects of hybridization and introgression, which would be expected to erode any morphological distinctiveness between the species. Thus the greatest overlap in morphology for species within the *Mytilus edulis* complex would be expected in areas where species pairs coexist with a high degree of hybridization. This appears to be the situation for *M. edulis* and *M. galloprovincialis* in southwest England (Gardner 1996) for which a canonical variates analysis, based on ten shell characteristics, produced a bimodal distribution of canonical variate scores for allopatric populations of each species and a unimodal distribution for two populations consisting of a mixture of both species and hybrids. Genetic markers distinguished *M. edulis* from *M. galloprovincialis* and their hybrids, and revealed that the two species were less morphologically distinct in sympatry than allopatric populations of each species. The hybrids were generally morphologically intermediate but showed a broad overlap with each species. The high degree of morphological distinctiveness for the allopatric populations was not due to differences in size between the two species (J.P.A. Gardner, personal communication).

Three shell characteristics (aam, hp, ht) had the greatest contribution in discriminating *Mytilus trossulus* from *M. edulis* in both the Newfoundland and Nova Scotia studies (Mallet and Carver 1995). However, Mallet and Carver (1995) found a greater difference in shell morphology between *M. edulis* and *M. trossulus* than in the present study, possibly because they sampled mussels from a single commercial mussel growing site at Lunenburg, Nova Scotia rather than on natural substrate, where a more heterogeneous environment may reduce morphological differences between the species. Difference in average length of each species was not reported and may have also contributed to the degree of difference observed despite the use of length-standardized characters. A slightly greater growth rate was found for *M. edulis* compared to *M. trossulus*, which may contribute to the morphological differences observed at the Lunenburg site.

Although most studies on mussels have attempted to evaluate morphometric characters for discriminating taxa, morphometric analysis is also useful in highlighting potentially adaptive differences in morphology. Comparative studies have shown that *Mytilus* species exhibit several morphological adaptations to an epifaunal existence (Stanley 1970, 1972, 1983), such as a reduced anterior, flattened ventral surface combined with a well-developed byssal system to attach the mussel to the substrate. In addition, an expanded posterior margin of the shell may also be an adaptation to maintain adequate current flow under crowded conditions (Stanley 1972), although Seed (1968) found that crowding resulted in a more elongated shell and other studies (Seed 1968; Gardner et al. 1993; Stirling and Okumus 1994) have also found an association between slower growth rate and more elongated shells. Norberg and Tedengren (1995) showed that *M. edulis* with elongated shells were more prone to starfish predation than were mussels with a more eccentric shell shape. Shell shape appears to be involved in differential adaptation by *M. edulis* and *M. galloprovincialis* to wave exposure (Gardner and Skibinski 1991; Willis and Skibinski 1992). *M. galloprovincialis* has a taller shell height with a wider, flattened ventral margin. This shell shape, combined with a greater strength of attachment, may explain the dominance of *M. galloprovincialis* on shores with high wave exposure (Gosling and Wilkins 1981; Skibinski et al. 1983).

Mytilus edulis and *M. trossulus* from Newfoundland were found to differ in shell morphology, but the adaptive significance of this difference is presently unknown. The more eccentric shell shape of *M. edulis* may suggest a better adaptation to wave exposure based on the comparison between *M. edulis* and *M. galloprovincialis* (Willis and Skibinski 1992). However, two exposed sites sampled in a previous study (Bates and Innes 1995) were dominated by small *M. trossulus*. Adaptation of mussels to wave exposure in Newfoundland is also complicated by the occurrence of extensive ice scouring in the winter and spring. Gardner (1996) has commented on the presence of pairs of hybridizing *Mytilus* species in transitional zones between marine provinces. A mosaic of environments in these transitional areas, combined with migration and differential adaptation, may explain the coexistence of pairs of *Mytilus* species (Wilhelm and Hilbish 1998). This explanation constitutes a testable hypothesis for the maintenance of *M. edulis* and *M. trossulus* populations along the extensive coastline of Newfoundland. The observed variation in shell morphology may be one component of adaptive differences between *M. edulis* and *M. trossulus*. Further studies involving transplant experiments of both species between contrasting wave-exposure environments are required to establish the adaptive significance of the observed interspecific differences in morphology. Elliptic Fourier analysis of shell shape, in combination with other methods for measuring shape (McLellan and Endler 1998), will be a valuable approach for the quantification of morphological variation in species of *Mytilus*.

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