Genetic and environmental components of phenotypic variation in body shape among populations of Atlantic cod (Gadus morhua L.)

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A common-garden experiment was conducted on larvae to test for genetic differences in body shape among populations of Atlantic cod (Gadus morhua). Offspring from four north-west Atlantic regions were reared from hatching to postmetamorphosis at two temperatures (7 ± 1 °C and 11 ± 1 °C) and two food levels (1500 and 4500 prey L⁻¹). Body shape differed between populations and treatments. Population differences were greatest between south-west Scotian Shelf cod and those further north; the former were characterized by a deeper body, larger head, and longer caudal peduncle than cod from the other populations. Significant differences were also observed between two putative populations on the south-west Scotian Shelf, suggesting genetic divergence between spawning aggregations at small spatial scales (< 100 km). Temperature and food supply also influenced body shape, with the effect of the former being more pronounced. Individuals reared at the higher temperature or food level had a deeper body and a larger head than those reared at the lower temperature or food supply. Phenotypic responses to changes in the rearing environment also differed among populations, indicating genetic differences in phenotypic plasticity. Differences between populations in morphology and in phenotypic plasticity suggest genetic divergence at both large (> 1000 km) and small (< 100 km) spatial scales. The genetic differences at large spatial scales counteracted the expected effects of temperature differences in the wild, suggesting countergradient variation in morphology among these populations. © 2006 Her Majesty the Queen in Right of Canada. Journal compilation © 2006 The Linnean Society of London, Biological Journal of the Linnean Society, 2006, 88, 351–365.


INTRODUCTION

Most documented cases of genetic divergence between fish populations originate from studies on freshwater and anadromous fishes (Hendry & Stearns, 2004). For marine fishes, there are generally fewer geographical barriers to gene flow and most species have high dispersal capability, either as planktonic eggs and larvae (which can be widely dispersed by ocean currents) or as migratory adults. As a consequence, marine fish are often assumed to be more genetically homogeneous than freshwater and anadromous fishes, an assumption that earlier genetic studies appeared to support (Gyllensten, 1985; Ward, Woodwark & Skibinski, 1994). However, an absence of geographical barriers does not exclude the possibility of local adaptation.

Genetic divergence in response to natural selection can be rapid (Reznick et al., 1997; Conover & Munch, 2002; Koskinen, Haugen & Primmer, 2002) and can occur despite a level of gene flow that would prevent or eliminate neutral genetic differentiation (Allendorf, 1983, 1995; Carvalho, 1993). Consequently, genetic divergence can develop more rapidly in adaptive than in selectively neutral characters and may persist in adaptive characters despite gene flow (Hard, 1995; Conover, 1998). Given the prominence of genetic
examinations of gene flow based on molecular markers presumed to be neutral to selection, it is possible that genetic differentiation for adaptive traits may have been under-estimated (Karhu et al., 1996; Reed & Frankham, 2001; Koskinen et al., 2002). The few studies that have examined adaptive characters in marine fishes have indeed found evidence of genetic differentiation between populations in terms of growth rate (Conover & Present, 1990; Schultz, Reynolds & Conover, 1996; Puvanendran & Brown, 1998), food conversion efficiency (Purchase & Brown, 2000), and vertebral number (Billerbeck, Orti & Conover, 1997).

Fishes that exploit different resources in terms of food, space, and habitat tend to evolve different morphologies, reflecting adaptation to these ecological differences (Alexander, 1974). Evidence of local adaptation in morphology due to differences in selection pressure has been documented in a number of fish species. For example, trophic specialization between benthic and pelagic habitats has been observed among populations of Arctic charr, Salvelinus alpinus (Schorrason et al., 1994) and between sympatric groups of stickleback, Gasterosteus spp. (McPhail, 1984). Similarly, differences in body shape attributable to contrasting selection pressures related to swimming performance have been documented for northern redbelly dace, Phoxinus eos (Toline & Baker, 1993) and coho salmon, Onchorhynchus kisutch (Taylor & McPhail, 1985a, b; Swain & Holthby, 1989).

Phenotypic plasticity arises when the same genotype produces different phenotypes in different environments (Hutchings, 2004). The set of phenotypic responses across different levels of an environmental factor is termed the reaction norm (Via et al., 1995) and is specific to each genotype. Body shape in fishes has been demonstrated to be influenced by environmental factors such as temperature (Martin, 1949; Beacham, 1990), food ration (Currens et al., 1989), and type of food or feeding mode (Day, Pritchard & Schluter, 1994; Robinson & Wilson, 1996). Plasticity in fish body shape has been hypothesized to be adaptive (Robinson & Parsons, 2002). Adaptive phenotypic plasticity in fish morphology has been demonstrated in crucian carp, Carassius carassius, in response to the presence or absence of a predator (Brönmark & Miner, 1992), and in pumpkinseed sunfish, Lepomis gibbosus (Robinson & Wilson, 1996) and stickleback, Gasterosteus spp. (Day et al., 1994), in response to differences between benthic and pelagic habitats.

Morphological differences observed in the wild that appear to be adaptive are often assumed to reflect genetic divergence between groups when in fact they may be simply cases of adaptive phenotypic plasticity. Conversely, a lack of phenotypic differences in the wild may not always reflect genetic similarity between groups. When genotypic influences oppose environmental influences, a pattern termed countergradient variation (Conover & Schultz, 1995), phenotypic similarity in the wild may reflect genetic divergence among groups (Conover, 1998). Therefore, when studying genetic divergence using phenotypic characters such as body shape, it is necessary to separate the genetic and environmental components of variation. Common-garden experiments have proven to be a useful tool for determining the genetic basis of phenotypic differences between putative groups (Conover & Schultz, 1995; Conover, 1998; Swain & Foote, 1999). They consist of rearing the different groups under identical conditions, thus eliminating the environmental differences between groups. The observed differences can then be attributed to genetic divergence.

Atlantic cod, Gadus morhua, is a widespread, commercially important marine fish. Adults from many populations undergo seasonal migrations between summer/spawning grounds and over-wintering grounds (ICES, 1994; Sinclair & Currie, 1994; Lawson & Rose, 2000a; Comeau, Campana & Chouinard, 2002). During those migrations, fish from one stock sometimes mix with those from other adjacent stocks (Lawson & Rose, 2000a). The egg and larval stages are pelagic and are subject to dispersal by water currents. Most Atlantic cod populations in the north-west Atlantic have been depleted and remain at a low level of abundance (Hutchings & Reynolds, 2004). A better understanding of the spatial scale of population structure and local adaptation would contribute to the effective management and conservation of this species.

In the present study, a common-garden experiment was used to test for genetic differences in juvenile body shape between four spatially separated populations of Atlantic cod. The effects of temperature and food density on body shape were also examined to test for genetic differences in phenotypic plasticity between the populations. This study tests for genetic divergence between populations in a marine fish with high potential for dispersal and may serve as an example for other widespread marine fish species that have evolved over a range of environments and have been subjected to similar levels of gene flow between groups.

MATERIAL AND METHODS

STUDY POPULATIONS

Atlantic cod larvae were compared from four areas (Fig. 1): (1) south-western Scotian Shelf (Northwest Atlantic Fisheries Organization [NAFO] division 4X); (2) southern Gulf of St Lawrence (NAFO division 4T); (3) Placentia Bay, Newfoundland (NAFO subdivision 3Ps); and (4) Bonavista Bay on the north-eastern coast of Newfoundland (NAFO division 3L). The
spawning period for the 4T, 3Ps, and 3L populations occurs in spring and early summer, with peak spawning in May and June (Lett, 1980; Myers, Mertz & Bishop, 1993; Hutchings & Myers, 1994; Lawson & Rose, 2000b). Spawning for the 4X population occurs during the winter from February to March (ICES, 1994). Because of this temporal difference in spawning, 4X larvae are expected to experience colder temperatures than those from the other three populations (Marcil, 2004). Adult cod from the 4T population undertake extensive seasonal migrations between spawning and feeding grounds inside the Gulf of St Lawrence and overwintering grounds outside the Gulf (Sinclair & Currie, 1994; Comeau et al., 2002). Adults in the other populations exhibit seasonal movements at a smaller scale (ICES, 1994; Lawson & Rose, 2000a).

**COMMON-GARDEN EXPERIMENT**

Mature, prespawning cod (54–77 individuals per population) were collected from each of the four populations (Fig. 1) and allowed to spawn in the laboratory. Cod were collected from the 4X area on 7 January 2002 and 16 January 2003, from the 3Ps area on 12 April 2002, from the 4T area on 15 May 2003, and from the 3L area on 12 June 2003. A number of spawning components are believed to occur in the 4X area (ICES, 1994). The date of peak spawning differed by more than 1 month between the two collections from 4X, suggesting that they may have been from different spawning components.

Broodstock from the 4X and 4T populations were held in the Aquatron facility at Dalhousie University whereas broodstock from the 3Ps and 3L populations were held at the Ocean Sciences Centre in St John’s, Newfoundland. Four batches of fertilized eggs were collected from each of the populations. Each batch consisted of eggs from two consecutive days of spawning. All the eggs were incubated at the Ocean Sciences Centre in 250-litre flow-through tanks held between 5 °C and 8 °C until hatching. When almost all the eggs in a batch had hatched, samples of larvae were transferred to 30-litre aquaria for rearing.

Larvae were stocked in 30-litre aquaria at a density of 40 larvae per litre. Samples from each batch were stocked in four aquaria, one for each of the following treatments: (1) high temperature–high food (HTHF); (2) high temperature–low food (HTLF); (3) low temperature–high food (LTHF); and (4) low temperature–low food (LTLF). The high and low temperature treatments were 11 ± 1 °C and 7 ± 1 °C, respectively. Temperature was recorded twice daily. The high food treatment was 4500 prey per litre; the low food treatment was 1000 prey per litre in 2002 and 1500 prey per litre in 2003. Larvae were fed three times daily: morning, afternoon, and evening. Larvae were fed Isochrasis-enriched rotifers from days 2–10 and Algomat-enriched rotifers from days 11–31. The larvae were then weaned to a diet of Artemia. Larvae were fed a mixture comprised of 50% rotifers and 50% Artemia from days 32–39 and then Artemia only from day 40 until the end of the experiment. Larvae were reared under continuous light at an intensity of 2000 lux. Other physical variables were kept constant among populations throughout the experiment.

**MORPHOMETRIC DATA COLLECTION**

Larvae were reared until they had reached an average standard length of 25 mm. A photograph of each individual was taken using a 1.2 megapixel digital camera and the programme VCS (Visual Communication Suite) version 2.1.1. Homologous landmarks, corresponding to 12 anatomical structures, were digitized along the profile of the larvae (Fig. 2) using the TpsDig programme (Rohlf, 2003a). Larvae were not fed for at least 12 h before sampling to ensure that variation in stomach fullness would not influence the shape analysis.

A generalized least-squares Procrustes superimposition (Rohlf & Slice, 1990) was performed on all the digitized sets of coordinates using the TpsRelw programme (Rohlf, 2003b). This procedure aligned each
set of coordinates by translation, scaling, and rotation, so that corresponding homologous landmarks lay as close as possible to each other. A consensus configuration was then computed on the set of scaled and aligned specimens. This configuration served as a reference to compute the partial warps and uniform components that serve as shape variables in geometric morphometric analyses. Uniform components represent the shape change associated with uniform stretching or compression of all landmarks along their x and y axes (Rohlf & Bookstein, 2003). Partial warps are parameters describing the non-uniform deformations in shape between the consensus configuration and the specimens (Rohlf, 1990). The 12 pairs of x and y coordinates were transformed into 18 partial warps and two uniform components for each specimen.

A key step when studying shape is to account for the size differences among specimens. There are two types of size effects: (1) isometric effects—a proportional increase in all morphometric measurements as size increases and (2) allometric effects—a change in shape as size increases. Size and shape can be strongly related because of allometric growth, differential rates of growth for different body parts (Gould, 1966). In the present study, the aim was to test for shape differences between populations and experimental treatments that could not be attributed to differences in size between individuals from the different populations or treatments. Thus, there was the need to adjust for both isometric and allometric effects of size.

Centroid size, the square root of the sum of the squared distances between the landmarks and their centroid, is used in the Procrustes superimposition to scale each specimen to a common unit size by dividing each coordinate of a specimen by its centroid size (Bookstein, 1986), thus removing isometric effects of size. To account for allometric effects of size, each morphometric variable was adjusted to a common size based on its regression on centroid size. Within-group slopes were estimated from an analysis of covariance of the model:

\[ D_{ijklm} = \mu + P_i + B_{ij} + F_k + T_l + \beta X_{ijklm} + e_{ijklm} \]

where \( P_i \) is the effect of population \( i \) (4X-02, 4X-03, 4T, 3Ps, or 3L), \( B_{ij} \) is the effect of batch \( j \) nested within population \( i \), \( F_k \) is the effect of food treatment \( k \), \( T_l \) is the effect of temperature treatment \( l \), \( D_{ijklm} \) and \( X_{ijklm} \) are the partial warps or uniform components and log_{10} centroid size of fish \( m \) in treatment \( kl \) of batch \( j \) nested within population \( i \), \( \beta \) is the covariate slope, and \( e_{ijklm} \) is a random normal deviation. Batch nested within population was treated as a random effect and all other terms were treated as fixed effects. Terms for interactions between the main effects (population, food treatment, and temperature treatment) were also included in the model if significant (\( P < 0.05 \)). Heterogeneity of slopes between populations and between temperature and food treatments (and their combinations) was tested based on type I sums of squares. Adjustment was based on the common within-group slope if there was no significant (\( P < 0.05 \)) heterogeneity of slopes between groups, and on separate within-group slopes otherwise. Analyses used the SAS procedures GLM and REG (SAS, 1999–2001).

The partial warps and uniform components of the Procrustes analysis were adjusted to a common size using:

\[ a_{ij} = d_{ij} + b_{ij}(\overline{CS} - CS_i) \]

where \( a_{ij} \) is the adjusted partial warp or uniform component for specimen \( i \) in group \( j \), \( d_{ij} \) is the original partial warp or uniform component, \( b_{ij} \) is the estimated regression slope for group \( j \) (or the common within-group slope), \( \overline{CS} \) is the grand mean log_{10} centroid size, and \( CS_i \) is the specimen’s log_{10} centroid size.

**Statistical Analysis of Shape Variation**

Morphometric variation was summarized by extracting principal components (PCs) from the covariance matrix of the shape variables (i.e. the adjusted partial warps and uniform components). The component scores of the first three principal components were used as dependent variables in a nested analysis of variance (ANOVA) to test for significant shape differences among populations and among treatment groups. Type III sums of squares were used because of unequal sample size among groups. Batch nested within population was treated as a random effect whereas all other terms were treated as fixed effects. Because sample sizes were unequal, Satterthwaite’s approximation (Sokal & Rohlf, 1981) was used to construct the denominator mean square (and to determine its degrees of freedom) for tests of population effects.

Very few 4X larvae survived the low temperature treatment; none survived beyond metamorphosis in this treatment in both years. Rearing larvae under high light intensity may have been a possible cause of the low survival of the 4X larvae (Puvanendran & Brown, 1998). As a consequence, two sets of analyses were performed. The first analysis comprised all four
populations (the two 4X year-groups and the groups from 4T, 3Ps, and 3L) compared at the two food levels of the high temperature treatment. The second analysis included the 4T, 3Ps, and 3L larvae compared at all levels of both the food and temperature treatments.

The assumption of normality of the component scores was tested within each treatment group for the first three principal components using the Shapiro–Wilk statistic. Statistical significance was assessed following sequential Bonferroni adjustments (Rice, 1989). In most cases (64 out of 66 cases), the distributions did not depart significantly from normality. Homogeneity of variances was tested using an F-max test for the first three principal components of each analysis section. Variance was homogeneous in all cases.

Because slopes were heterogeneous between groups in many cases, differences between populations or experimental treatments will vary depending on the size to which morphometric variables are adjusted. Analyses were repeated adjusting the shape variables to a larger or smaller centroid size (the largest and smallest centroid sizes common to all groups in the analysis). Results, both in terms of the differences in PC score between groups and their statistical significance, were very similar regardless of the centroid size to which shape variables were adjusted. Thus, over the range of sizes produced in our experiments, the differences in shape between groups are not sensitive to the size to which specimens are adjusted (Marcil, 2004).

In addition to the analysis reported here using geometric morphometrics, parallel analyses were conducted using truss morphometrics (Strauss & Bookstein, 1982). Both approaches revealed very similar shape differences between populations or experimental treatments (Marcil, 2004), and only the results using geometric morphometrics are reported here.

RESULTS

VARIATION IN ALLOMETRY AMONG POPULATIONS AND ENVIRONMENTAL TREATMENTS

Significant allometric differences were found between populations and treatment groups. In many instances, allometric size adjustment of the partial warps used separate within-group slopes between treatment groups, populations, or both. The highest percentage of significant allometric differences was found between populations when examining shape variation among all populations reared at high temperature (Table 1: Analysis 1). In the analysis of the three populations (4T, 3Ps, and 3L) reared at two temperature and two food levels, significant allometric differences most frequently occurred in the temperature (C × T), food (C × F), or population × temperature (C × P × T) model terms (Table 1, analysis 2).

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<thead>
<tr>
<th>Model term</th>
<th>Analysis 1</th>
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<tr>
<td>C × P</td>
<td>65%</td>
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<td>C × T</td>
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<td>C × T × F</td>
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The percent of morphometric variables (partial warps or uniform components) with heterogeneous slopes in the regression on log centroid size (C) is shown for each model effect (e.g. C × P gives the percent of cases in which slopes were heterogeneous between populations and C × P × F the percent of cases in which slopes differed between each combination of population and food level).

VARIATION IN BODY SHAPE AMONG FOUR POPULATIONS (4X, 4T, 3PS, AND 3L) REARED AT HIGH TEMPERATURE

The first three principal components accounted for 50% of the variation in shape (Table 2). They summarized shape differences related to both population and food level, although the effect of population was the most significant.

The first PC, accounting for 25.3% of the variation in body shape, summarized variation in body depth, caudal peduncle length, and head size (Fig. 3A). Fish having a high PC1 score were characterized by a deep body, large head, short posterior body, and long caudal peduncle (Fig. 3A). This component showed a strong
Figure 3. Mean phenotypic values of the principal components of the size-adjusted partial warps of Atlantic cod from four populations reared at high temperature and two food levels. Symbols show the mean principal component value with its standard error for each population. Thin-plate spline deformations corresponding to the extreme positive and negative phenotypic values are depicted on the right hand side of each graph. Deformations are exaggerated three-fold.
and highly significant population effect and a weak but significant food effect (Table 2). Population differences were greatest between the two 4X groups and the three other populations (Figs 3A, 4). The two 4X groups tended to have a deeper body, larger head, and longer caudal peduncle than the other populations (Figs 3A, 5A, B). Fish fed high food densities also tended to have a slightly deeper body, larger head and longer caudal peduncle than those fed a low food density, although this effect was slight compared with the population effect.

The second PC, accounting for 13.4% of the variation in body shape, summarized variation in bending along the lateral profile of the body (Fig. 3B); this bending was an artifact of how a fish was positioned when its picture was taken. The geometric morphometrics analysis, which accounts for the relative position of the landmarks, was able to incorporate this small variation in body position even though the bending effect was reduced by careful positioning of the larvae and the selection of only the best photographs. In addition to this artifact of fish positioning, variation in body depth contributed somewhat to this PC (Fig. 3B). Fish having a high PC1 score were characterized by a shallow body, elongated posterior body and caudal peduncle regions, and a slight upward bending of the body (Fig. 3B).

The third PC, accounting for 11.5% of the variation in shape, summarized shape variation in head, posterior body and caudal peduncle length, and in abdomen size (Fig. 3C). Fish having a high PC3 score were characterized by a short head, extended abdomen, and a short caudal region (Fig. 3C). There was a significant population effect along PC3 with a marked difference between the two 4X groups (Fig. 4). At both food levels, the 4X-02 group had higher mean PC3 scores than the 4X-03 group (Fig. 3C). There was also a strong population × food interaction for PC3 (Table 2). PC3 score increased with an increase in food density for the 4T group, decreased with an increase in food density for 3Ps and 4X-02 cod, and showed little change between food densities for 3L and 4X-03 cod (Fig. 3C).

**Figure 4.** Principal component scores from the analysis of size-adjusted partial warps of Atlantic cod from four populations reared at high temperature and two food levels. Symbols show batch means within each treatment. Error bars indicate the standard error.

**Variation in body shape among three populations (4T, 3Ps, and 3L) reared at high and low temperature**

The first three principal components accounted for 52% of the variation in the adjusted partial warp scores (Table 3). They summarized shape differences due to all terms in the model but primarily differences related to temperature level.

The first and second PCs, accounting for 22.3% and 16.6% of the variation in body shape, respectively, summarized variation in body depth, and posterior body and caudal peduncle length (Fig. 6A, B). They also included shape variation corresponding to a bending of the body. Fish having a high PC1 score were characterized by a shallow body, elongated posterior body and caudal peduncle regions, and a slight upward bending of the body (Fig. 6A). Fish having a high PC2 score were also characterized by a shallow body and elongated posterior body and caudal peduncle regions, but with the bending of the body directed downward (Fig. 6B).
Figure 5. Representative specimens of juvenile Atlantic cod showing the mean shape differences between (A) 4X-03 and (B) 3Ps populations and between 4T cod reared at (C) high temperature and (D) low temperature. Differences in pigmentation between specimens are not indicative of population or treatment effects on pigmentation. SL, standard length.
There was a strong and highly significant effect of temperature on the aspects of body shape summarized by PC1 and PC2 (Table 3), with the difference between temperatures particularly strong along PC1. Fish reared at high temperature tended to have a deeper body, a larger head, and shorter posterior body and caudal peduncle regions (lower PC1 and PC2 scores) than those reared at low temperature (Figs 5C, D, 6A, B). The only exception was in the PC2 scores of 3Ps fish reared at low food density. Significant population effects also occurred along PC1 and PC2, although the differences between populations were complicated by interactions with temperature and food treatments (Table 3). Along PC1, 3Ps fish tended to have higher scores (shallow body, elongated caudal region) than did 4T and 3L fish, except in the HTLF treatment (Fig. 6A). Along PC2, population differences varied between both temperature and food treatments. Unlike PC1, there was also a significant effect of food density on PC2 (Table 3). Fish reared at high food density tended to have deeper bodies and shorter posterior body regions, except in the case of 3Ps at low temperature (Fig. 6B). Significant interactions between population and temperature and food on PC2 (Table 3) reflected the tendency for PC3 score to increase with food density for 4T and 3L fish reared at high temperature.

The third PC, accounting for 13.4% of the variation in body shape, summarized variation in body depth and mid-body, posterior body, and caudal peduncle lengths. Fish having a high PC3 score were characterized by a deep body, a short mid-body region, and long posterior body and caudal peduncle regions (Fig. 6C). Unlike the patterns observed in the first two PCs, an increased body depth along PC3 was also associated with a compression of the mid-body region and an elongation of the caudal peduncle region (Fig. 6C). There was also a slight but significant food effect reflecting the tendency for PC3 score to increase with food density for 4T and 3L fish reared at high temperature.

**DISCUSSION**

**GENETIC DIVERGENCE AMONG POPULATIONS OF ATLANTIC COD**

The present study provides evidence of genetic differences in morphology and allometry between populations of Atlantic cod. Significant morphometric differences were found between the 4T, 3Ps, and 3L populations when reared in a common environment. However, the greatest morphological differences were found between these populations and the two 4X groups. Fish from both 4X groups tended to have a deeper body shape than did those from the other populations. Just as morphological differences were greatest for the 4X population, ecological differences are expected to be greatest between this group and the other three populations. Cod from the south-west Scotian Shelf spawn during the winter, whereas the other three populations spawn in the spring and early summer. It can therefore be expected that environmental conditions experienced by the 4X cod during the larval and early juvenile stages are much different from the environmental conditions experienced by the other cod populations examined in the present study.

Significant morphometric differences were also found between the two 4X groups when reared in a common environment. This suggests the presence of very fine-scale genetic differentiation within the same
Figure 6. Mean phenotypic values of the principal components (PC) of the size-adjusted partial warps of Atlantic cod from three populations reared at two temperature and two food levels. Symbols show the mean PC value with its standard error for each population. Symbol shading indicates temperature treatment. Thin-plate spline deformations corresponding to the extreme positive and negative phenotypic values are depicted on the right hand side of each graph. Deformations are exaggerated three-fold.
stock. These fine-scale genetic differences are a possible consequence of the different spawning components suspected to exist in this division (ICES, 1994).

The genetic differences observed among the four populations of Atlantic cod in this study suggest the presence of local adaptation in morphology among these populations, as well as between different spawning components of the same population. For a commercially important species, such as the Atlantic cod, evidence of local adaptation among and within populations has important implications for management and conservation. A population adapted to its local environment will have reduced fitness in contrasting environments. In this case, a depleted or extirpated population might not be readily replaced by adjacent stocks, which would be poorly adapted to its environment. The existence of genetic subcomponents within populations, as suggested by the differences between the 4X groups in the present study, also complicates resource management. Management of stock complexes as a single unit may lead to the extinction of subunits, reducing overall productivity of the stock complex (Frank & Brickman, 2000).

Differences in body shape and allometry between populations of Atlantic cod reared in a common environment are interpreted here as evidence for genetic divergence among the populations. However, these differences could also reflect maternal effects or the effects of variation in the extent of selective mortality. As expected in marine fish larvae, mortality was quite high for all populations and treatments. If selective mortality among phenotypes was the major cause of the shape differences among groups, the frequency distribution of PC scores for high-mortality groups would be expected to be a subset of the distributions for the low-mortality groups. This was clearly not the case in the present study: when the phenotypic distribution of a high- and a low-mortality group were plotted against each other, the first group had phenotypes more extreme than any observed among the latter group (Fig. 7). Furthermore, the extent of phenotypic differences between groups was unrelated to the difference in mortality between them (Marcil, 2004). Maternal effects could also contribute to the morphological differences between populations. However, maternal effects have a greater influence early in development and are less likely to be substantial in postmetamorphosis juveniles (Heath, Fox & Heath, 1999). The differences documented in the present study between populations reared in a common environment most likely reflect genetic differences rather than differences in selective mortality or maternal effects.

Evidence of genetic divergence in adaptive quantitative and neutral molecular traits has been documented between populations of marine fishes in a number of cases. Conover & Present (1990) reported differences in growth capacity of Atlantic silversides, *Menidia menidia*, from Nova Scotia, New York, and South Carolina when reared in a common environment. Purchase & Brown (2000) also observed differences in the growth rate of larvae and the food conversion efficiency of juveniles between Atlantic cod from the Grand Banks and Gulf of Maine when reared under identical conditions. Genetic studies using microsatellite DNA have reported differences at selectively neutral loci among Atlantic cod stocks at the large scale examined in the present study (Nova Scotia to Newfoundland) (Bentzen et al., 1996; Ruzzante, Taggart & Cooke, 1999) but not at the finer scale examined in the present study on the south-west Scotian Shelf. Taken together, the inferences of genetic differentiation in the present study coupled with previous evidence of spatially variable restrictions in gene flow are consistent with the hypothesis that Atlantic cod can differ genetically at comparatively large and small geographical scales.

**ENVIRONMENTAL EFFECTS ON FISH MORPHOLOGY**

Body shape in fishes is known to be affected by a number of environmental factors, including rearing temperature (Martin, 1949; Beacham, 1990; present study), water velocity (Imre, McLaughlin & Noakes, 2002), quantity of food (Currens et al., 1989; present study), and type of food or feeding mode (Meyer, 1987, 1990; Wainwright, Woodwark & Skibinski, 1991; Wimberger, 1991, 1992; Day et al., 1994; Robinson & Wilson, 1995; Day & McPhail, 1996). In the present study, rearing temperature had a strong effect on the body shape of juvenile Atlantic cod. Fish reared at high
temperature were characterized by a deeper body, a larger head, and shorter caudal region than fish reared at low temperature. This temperature effect on body shape was similar for all three populations tested, although the effect was slightly stronger for Placentia Bay (3Ps) cod. Similar effects of rearing temperature on body shape have been reported in studies on juvenile chum salmon, *Onchorhynchus keta* (Beacham, 1990), and on juvenile carp, *Cyprinus carpio* (Loy, Cataudella & Corti, 1996). In all three cases, juveniles reared at warmer temperatures tended to have deeper bodies, suggesting that this may be a general tendency in fishes.

Food ration also had a significant impact on cod body shape, although its effect was weak compared with the effects of population and temperature. Fish reared at high food density tended to be deeper bodied with larger heads and caudal peduncles. To our knowledge, no other study using a common-garden experimental protocol has investigated the effect of food ration on fish morphology, except for the extreme comparison of starved vs. fed fish (Currens et al., 1989).

Most studies demonstrating environmental effects on fish body shape have used freshwater or anadromous fishes. Studies on marine fishes are rare, presumably due to the difficulty of rearing these fish. However, the few studies available (Loy et al., 1996; Sarà, Favalaro & Mazzloa, 1999; present study) indicate that rearing environment may also have an important effect on morphology in these fishes.

**Evidence of Genetic Differentiation in Phenotypic Plasticity**

Phenotypic responses to a change in environmental conditions were different among populations of Atlantic cod. Phenotypic responses to temperature were similar between populations in terms of direction but differed in terms of magnitude. There was a general tendency for fish reared at high temperature to be deeper bodied with a large head and short caudal region, but this response was slightly more pronounced for the 3Ps fish than for the 4T and 3L populations. Greater differences in plasticity were evident between populations in their responses to a change in food ration. Fish from the 4T population showed greater plasticity to a change in food density than did the other three populations. These instances of population × temperature and population × food interaction represent cases of genotype × environment interaction. They suggest that there are genetic differences in plasticity among these populations of Atlantic cod.

Phenotypic plasticity is often assumed to be adaptive. If different phenotypes are optimal in different environments, a single genotype can produce the different optimal phenotypes through phenotypic plasticity (Hutchings, 2004). Phenotypic responses to food type and feeding mode often appear to be adaptive. For example, limnetic morphs feeding in open water tend to have a more streamlined body shape, whereas benthic morphs feeding in the littoral zone tend to have a deeper body shape (McPhail, 1992; Toline & Baker, 1993, 1997). In some fishes, limnetic forms fed benthic food develop a deeper body shape whereas benthic forms fed limnetic food develop a more streamlined body shape (Wimberger, 1991; Day et al., 1994; Robinson & Wilson, 1996). In the examples mentioned above, phenotypic plasticity was shown to be adaptive with the morphological change induced by a particular environment improving performance in that environment. However, the adaptive significance of the effects of temperature and food ration reported in the present study is unknown.

**Cogradient vs. Countergradient Variation in Morphology of Atlantic Cod**

Phenotypic variation across environmental gradients can be the result of both genotypic and environmental influences on the phenotype. Genotypic variation may be random across environmental gradients, or it may covary with environmental variation, either reinforcing environmental effects and increasing phenotypic variation across the gradient (cogradient variation) or opposing environmental effects and decreasing phenotypic variation across the gradient (countergradient variation; Conover & Schultz, 1995). Cogradient variation is expected to occur when different phenotypes are favoured in the different environments. On the other hand, countergradient variation is expected when the same phenotype is favoured in the different environments.

The strongest genetic difference observed in the present study was between 4X cod and cod from the three other populations. The 4X cod were characterized by a deeper body than cod from the other populations. In the wild, a strong environmental gradient is also expected between 4X cod and the other populations. Cod in the 4X population spawn in winter, whereas those in the 4T, 3Ps, and 3L populations spawn in spring and early summer. Consequently, at the larval and early juvenile stages when they feed on zooplankton in the upper water layers, 4X cod are expected to experience colder temperatures than do cod from the other populations (Fig. 8). In the present study, fish reared at colder temperatures tended to have a shallower body shape. Thus, the genetic differences between the 4X fish and the other populations appear to counteract the environmental differences expected in the wild. This suggests the presence of countergradient variation in body shape across the
temperature gradient between these populations in the wild. Countergradient variation in body shape has been demonstrated between early and late-spawning stocks of chum salmon, *O. keta* (Tallman, 1986; Tallman & Healey, 1991).

Most previous studies of genetic diversity in morphology between groups of fish comprise cases where different body shapes are favoured between groups, reflecting adaptation to ecological differences between the groups (e.g. limnetic vs. benthic groups, McPhail, 1984; coastal vs. inland populations, Taylor & McPhail, 1985a, b; stream- vs. lake-dwelling groups, Swain & Holtby, 1989). If genetic and environmental effects on morphology are countergradient between 4X cod and the other populations in the present study, this would be a case where the difference in morphology observed between groups in a common laboratory environment does not reflect a difference in the optimal body shape between groups in the wild. Instead, the genetic differences revealed in common-garden experiments would be an adaptation to counteract non-adaptive environmental effects on morphology to produce similar body shapes in the different populations in the wild. This would be consistent with our expectation that similar body shapes should be favoured in these populations given their similarity in lifestyle. Further work needs to be carried out to test the hypothesis that differences in body shape between these populations in the wild are minimized by countergradient variation. One consequence would be that adaptive genetic differences between populations would be overlooked in the wild because of the phenotypic similarity produced by countergradient variation.

**Figure 8.** Mean temperature in the upper water layer (0–30 m depth) following spawning in the areas occupied by each cod population in this study. Spawning is assumed to occur in February for the 4X population and May for the other populations. For details, see Marcil (2004).

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**REFERENCES**


Puvanendran V, Brown JA. 1998. Effect of light intensity on the foraging and growth of Atlantic cod larvae: interpopula-