

Contribution of phenotypic plasticity and heredity to the trophic polymorphism of lacustrine brook charr (*Salvelinus fontinalis* M.)

Raphaël Proulx and Pierre Magnan*

Département de chimie-biologie, Université du Québec à Trois-Rivières,
C.P. 500, Trois-Rivières, Québec G9A 5H7, Canada

ABSTRACT

The objectives of this study were to determine: (1) if morphological characters of littoral and pelagic brook charr are inherited by their progeny when both forms are raised in the same conditions; (2) if sexual dimorphism would account for part of the variation in littoral and pelagic brook charr morphology; (3) the relative contribution of genetic and environmental factors to trophic polymorphism of brook charr; and (4) if a fish that had already performed one plastic response can reverse this response in a functional direction when shifted to an alternative habitat (e.g. littoral to pelagic). We conducted a reciprocal transplant experiment over 16 months after hatching in which fish of both ecotypes were fed in artificial pelagic (prey captured in the water column) and littoral (prey captured on the bottom) habitats. The results show that morphological differences between littoral and pelagic brook charr are heritable and related to both genetic and environmental factors. The percent variation of fish morphology explained by the effects of genetic and environmental factors was 17% and 15% respectively when the effect of sex was controlled, and 13% and 26% respectively when the effect of sex was not accounted for, indicating that sexual dimorphism accounts for an important part of the overall variation in brook charr morphology. The shift of some individuals from their initial to the opposite habitat from months 12–16 highlighted the role of the genetic and environmental factors as well as the magnitude of morphological plasticity: some characters remained unchanged during this 4-month shift, while some others exhibited a complete reversal, in line with the predictions of functional morphology. This result indicates that some characters are under pure environmental control and are not fixed after a fish has adopted a given strategy.

Keywords: adaptive morphology, brook trout, common garden experiment, functional morphology, heredity, phenotypic plasticity, reciprocal transplant experiment, resource polymorphism, sexual dimorphism.

INTRODUCTION

Trophic polymorphism appears to be common in fish inhabiting northern latitude lakes (Skúlason and Smith, 1995; Schluter, 1996; Robinson and Parsons, 2002). These lakes offer

* Author to whom all correspondence should be addressed. e-mail: pierre_magnan@uqtr.ca
Consult the copyright statement on the inside front cover for non-commercial copying policies.

two discrete functional habitats, the littoral and pelagic zones, and studies of trophic polymorphisms almost always include co-existing benthic and pelagic forms (Robinson and Wilson, 1994; Skúlason and Smith, 1995). These forms have been observed to partition the available resources by developing specific local adaptations to different habitats, such as variation in morphology and feeding habits (Skúlason and Smith, 1995; Jonsson and Jonsson, 2001; Adams and Huntingford, 2002; McKinnon and Rundle, 2002; Robinson and Parsons, 2002). Brook charr, *Salvelinus fontinalis* (Mitchill), exhibit such a trophic polymorphism in lakes of the Canadian Shield, where some individuals are specialists better adapted to feeding in the littoral habitat, whereas others are specialists better adapted to feeding in the pelagic habitat (Venne and Magnan, 1995; Bourke *et al.*, 1997, 1999; Dynes *et al.*, 1999; Proulx and Magnan, 2002). Individual differences in habitat preference were related to functional differences in body morphology (Bourke *et al.*, 1997; Dynes *et al.*, 1999; Proulx and Magnan, 2002): pelagic individuals are more fusiform and have shorter pectoral fins than littoral ones. In one of two study lakes in the same area, genetic data (microsatellites) also suggested that the pelagic and littoral forms are two populations with partial reproductive isolation and non-random mating (Dynes *et al.*, 1999). Finally, when restricted to feeding in pelagic zone enclosures, littoral brook charr spent more energy foraging (Marchand *et al.*, 2003) and exhibited lower physiological performance (Proulx and Magnan, 2002) than pelagic individuals, suggesting that trophic diversification is adaptive in this species.

Many studies have documented morphological plasticity in fishes (reviewed by Robinson and Parsons, 2002) and some, through reciprocal transplant experiments, have shown that morphological plasticity, together with a genetic component, explains a significant proportion of trophic polymorphism (Day and McPhail, 1996; Robinson and Wilson, 1996; Adams and Huntingford, 2002). However, few studies have accounted for the potential effect of sexual dimorphism, which could increase the relative contribution of the environmental component. Furthermore, no study has examined whether a fish that had already performed one plastic response can reverse this response in a functional direction when shifted into an alternative habitat (e.g. littoral to pelagic). Such a shift would provide independent support to the contribution of the genetic and environmental components to trophic polymorphism: if the reaction norm of a plastic character can be reversed, it implies that this character is largely under environmental control and is not fixed after the fish adopts a given strategy. In the same way, if the reaction norm of a character does not change following a shift to an alternative habitat, this suggests that this character is under genetic control. Such an experiment would also highlight the magnitude of plasticity as well as the time response of changes in flexible characters.

The objectives of the study were to determine: (1) if morphological characters of littoral and pelagic brook charr are heritable; (2) if sexual dimorphism accounts for part of the variation in littoral and pelagic brook charr morphology; (3) the relative contribution of genetic and environmental factors to trophic polymorphism in brook charr; (4) the degree of plasticity in morphological characters of littoral and pelagic individuals following a shift from their current to the alternative habitat. With a highly flexible and reversible reaction norm, we would expect that morphology will change in a functional way following a shift to the alternative habitat.

MATERIALS AND METHODS

Experimental fish and holding conditions until 4 months old

From 18 to 24 October 1998, littoral and pelagic brook charr were sampled on the spawning grounds of Lake Ledoux, Mastigouche Reserve, north of Trois-Rivières (Québec), Canada (46°40'N, 73°20'W). For each ecotype, allometric relationships between body length and fin length were obtained from Bourke *et al.* (1997). Their results showed that littoral individuals have longer size-adjusted dorsal and pectoral fins than pelagic ones. A littoral individual was selected if the length of both fins was above the size-adjusted regression lines predicted for the littoral form, while a pelagic individual was selected if the length of both fins was below the size-adjusted regression lines of the pelagic form. The artificial fertilization procedure was done according to the dry method described by Piper *et al.* (1982). We fertilized the sexual products of males and females to yield three full-sib broods of each pelagic and littoral form. Adults were killed with an overdose of tricaine methanesulphonate (MS 222) and kept frozen for further morphological analyses.

We incubated eggs at $6 \pm 0.5^\circ\text{C}$ in six ascending-current incubators (MariSource, Milton, WA). The incubators were connected to a glycol cooling system ($\pm 1^\circ\text{C}$). Water alkalinity and hardness were adjusted to $65 \text{ mg} \cdot \text{l}^{-1} \text{ CaCO}_3$ by adding sodium bicarbonate (Na_2HCO_3) and calcium chloride (CaCl_2). Light intensity (40 lux), water temperature ($12 \pm 1^\circ\text{C}$) and photoperiod (12 : 12) were held constant during the whole experiment. Ammonia (NH_3 ; $\mu\text{g} \cdot \text{l}^{-1}$), nitrites (NO_2 ; $\text{mg} \cdot \text{l}^{-1}$) and water hardness ($\text{mg} \cdot \text{l}^{-1}$ of CaCO_3) were estimated using standard procedures (American Public Health Association, 1989) and kept within the tolerance limits for salmonid aquaculture (MAPAQ, 1990). Hatching occurred from 18 to 25 February 1999.

The offspring of each brood were then counted and transferred into six 76-litre tanks (one for each brood). After the resorption of the yolk sac, food was distributed from overhead automatic feeders that functioned continuously over the 12-h daylight period. Such a feeding system is known to stimulate feeding of recently emerged salmonids on commercial food pellets. Until they were 4 months old, young brook charr were fed *ad libitum* with Biodiet starter, Corey #0.7 and Corey #1.5. The very slow sinking rate of these pellets simulated water column feeding of recently emerged brook charr (Power, 1980). Experimental units included bedrock and biological filters, and water quality and temperature ($12 \pm 0.5^\circ\text{C}$) were controlled as for the incubators. Thirty 4-month-old individuals from each brood were randomly sampled and killed with an overdose of MS 222 for morphological comparisons (heritability of morphological characters in littoral and pelagic brook charr; Objective 1).

First transplant experiment: heredity versus phenotypic plasticity

The experimental set-up consisted of six 600-litre circular tanks, with three simulating a littoral habitat and three a pelagic habitat. Each unit was divided into two sections by a plastic grid (4 mm square mesh) fixed on a stainless steel frame, giving a total of six littoral and six pelagic habitats. Each littoral habitat consisted of eight flower pots (top internal diameter = 10 cm; bottom internal diameter = 7 cm; depth = 10 cm) fixed into a polyethyl plastic floor (Fig. 1). Trout pellets were distributed on the bottom of the pots with a tea infuser spoon to simulate benthic feeding (i.e. foraging on large prey items on the substrate).

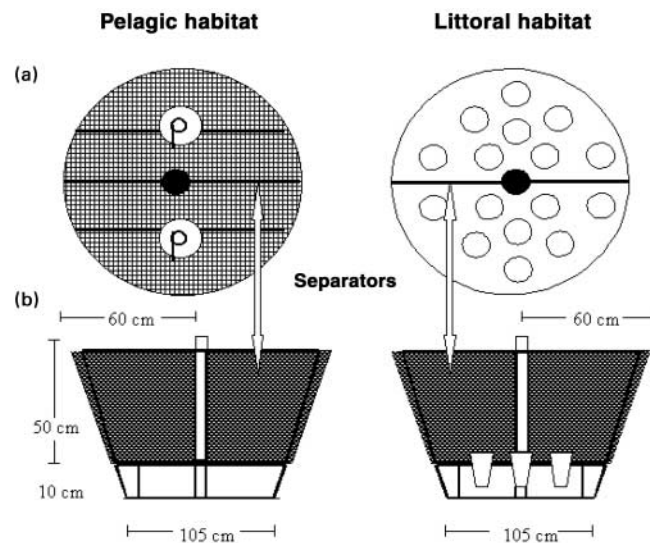


Fig. 1. Schematic representation of the pelagic and littoral habitat treatments: (a) top and (b) lateral views of one tank that mimicked each habitat. Each tank was divided into two experimental units to allow for pairing two ecotypes in the same habitat. Small food pellets simulating zooplankton sinking in the water column were distributed by automatic feeders (white circles above each pelagic unit) positioned at the top of the pelagic habitat. In the littoral habitat, trout pellets were distributed on the bottom of the flower pots (i.e. 8 circles in each littoral section), simulating bottom prey.

The floor of the pelagic habitats was made of a plastic grid (5 mm square mesh), which prevented fish from swimming underneath but allowed uneaten food particles to sink through (Fig. 1). Small food pellets were distributed by automatic feeders positioned at the top of each pelagic habitat that ran for 12 h per day (artificial daylight period). Fish kept in pelagic habitats fed on trout pellets sinking in the water column, thus simulating pelagic feeding (i.e. foraging on small prey items in the water column). The daily ration given to the fish was identical in all treatments and was estimated from a bioenergetic model (Cho and Bureau, 1998) based on fish weight and water temperature. The size of trout pellets varied from month 8 to better simulate larger littoral versus smaller pelagic prey (from 1.5 to 2.5 mm in the pelagic habitat and from 1.5 to 3.0 mm in the littoral habitat). Experimental units included bedrock and biological filters, and water quality and temperature ($12 \pm 0.5^\circ\text{C}$) were controlled as for the incubators.

The reciprocal transplant experiment consisted of two artificial habitats (littoral and pelagic) and two ecotypes (littoral and pelagic), giving the following treatments:

- littoral fish in the littoral habitat (LL);
- littoral fish in the pelagic habitat (LP);
- pelagic fish in the littoral habitat (PL); and
- pelagic fish in the pelagic habitat (PP).

The reciprocal transplant experiment started when young-of-the-year brook charr were 4 months old. At this time, fish were large enough to feed on bottom prey in our experimental set-up. This could also correspond to the shift of young-of-the-year littoral individuals

from open-water to benthic feeding (Venne and Magnan, 1995). At the beginning of the experiment, each of the six littoral and six pelagic habitats (half tank) was stocked with 120 fish of one given brood, providing three independent experiments for each of the above treatments. The true experimental unit in this design is the tank ($n = 6$). However, since each tank was divided into two parts, to allow for stocking two ecotypes in the same habitat, we assumed that half tanks were independent experimental units ($n = 12$). A pair of tanks (each tank simulating one littoral and one pelagic habitat) thus represented one independent reciprocal transplant experiment, for a total of three reciprocal transplant experiments in the study. To reduce the amount of dependence within habitats, a given littoral brood was always paired with a given pelagic brood in each of the three reciprocal transplant experiments. To control for potential differences among the reciprocal transplant experiments, we included a 'brood pair effect' in the statistical design described hereafter. Each fish was measured (total length, mm), weighed (g) and colour-marked with a biocompatible elastomer (Bailey *et al.*, 1998) to ensure *a posteriori* that no fish from one ecotype was accidentally transferred with fish from another ecotype (neighbouring section). Fish were held in the set-up for one year, between June 1999 and June 2000. Thirty fish were sampled one by one with a dip net in each of the 12 experimental units at 8, 12 and 16 months for morphological analyses (we assumed that this was a random sampling). This experimental design provided 12 fish samples at each time interval for three time intervals, giving an overall sample size of 36.

Second transplant experiment: response of fish to a shift to the alternative habitat

After 12 months, 30 fish from each of the 12 experimental units were shifted from their current to the alternative habitat (paired tanks). These fish were marked by clipping their adipose fins. Again, we assumed that half tanks were independent experimental units and tested for the effect of brood pairing in our statistical design. After 16 months, the morphology of these fish was compared with that of the non-shifted fish. This experimental design provided 12 fish samples of shifted and non-shifted individuals, for an overall sample size of 24.

Morphological measurements

We measured 10 morphological characters associated with swimming (body depth, body width, pectoral fin length, dorsal fin base, peduncle depth; Malmquist *et al.*, 1992; Robinson and Wilson, 1994; Dynes *et al.*, 1999) and with feeding (head length, head depth, mouth width, snout length, lower jaw length; Tabachek, 1988; Kotrschal, 1989) (Fig. 2). The total length (mm) and weight (g) of each fish were also measured. Morphological measurements were taken using a Mytutoyo digital calliper (± 0.01 mm) connected to a data transmitter and a computer database. We identified the sex of every fish when it was 12 and 16 months old; the sex of younger fish could not be determined.

Statistical analysis

Objective 1: heritability in morphological characters of littoral and pelagic brook charr

Logarithmic transformations were performed on all morphological variables to meet conditions of linearity and to stabilize the variances. We performed a stepwise discriminant

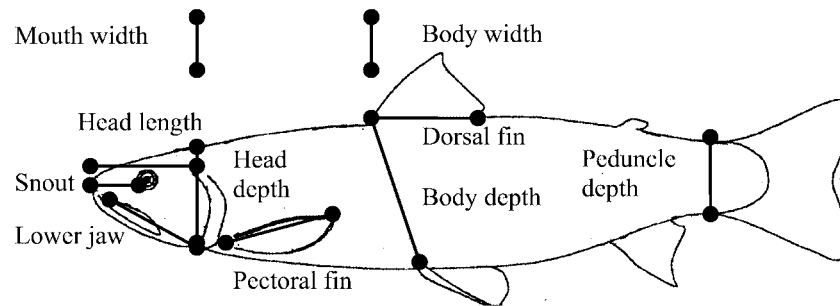


Fig. 2. The 10 morphological characters associated with swimming and feeding efficiency used in this study. The mouth and body widths were taken from a dorsal view.

function analysis (backward selection procedure, P to remove = 0.05) on the size-adjusted residuals of the 10 morphological characters to detect which ones best described the 4-month-old ecotypes (i.e. before the onset of the reciprocal transplant experiments). We used the jackknife method of classification to cross-validate group attribution. Unlike a normal classification matrix, a jackknife matrix excludes the data while the coefficient used to assign ecotype group is computed, which makes the method more accurate (Wilkinson, 1998). In this way, we could determine whether the morphological characters used to select adult ecotypes in the wild (pectoral and dorsal fins) were inherited by the progeny.

Objective 2: the effect of sexual dimorphism on the variation of littoral and pelagic brook charr morphology (first transplant experiment)

We performed a one-way multivariate analysis of covariance (MANCOVA; with body length as the covariate) to test for the presence of sexual dimorphism among our set of morphological characters for 12- and 16-month-old fish (data of the first transplant experiment: heredity vs phenotypic plasticity).

Objective 3: contribution of genetic and environmental factors (and their interaction) to trophic polymorphism in brook charr (first transplant experiment)

We accounted for the variance associated with sex and body length by computing the residuals of the allometric relationship between each morphological variable and body length for each sex separately in 12- and 16-month-old fish. As the sex of the 8-month-old fish could not be determined, we randomly attributed a sex ratio of 1 : 1 to the residuals of the relationship between each morphological variable and body length for these fish. The standardized residuals were calculated by applying a group regression line (Fleming *et al.*, 1994). These residuals were independent of fish length and sex-related differences in morphology. We then applied a principal component analysis (PCA on a correlation matrix) to the morphological variables at 8, 12 and 16 months to determine the degree of association among all individuals (canonical scores). For any principal component (PC) retained (eigenvalues above 1), we computed the mean canonical score for each experimental unit ($n = 12$) at each time interval (8, 12 and 16 months; $n = 36$). We then performed an analysis of variance (ANOVA) on the mean canonical scores to test for the effects of ecotype, habitat, time, brood pair and ecotype-habitat interaction. By performing analyses of variance on the mean PC scores, we reduced the overall degrees of freedom,

making the analysis more conservative and more appropriate for estimating the amount of variance due to ecotype and habitat effects (Robinson and Wilson, 1996). We were not interested in testing other higher interaction terms, since our objective was to assess the relative contribution of ecotype and habitat effects. Considering all possible interaction terms would have greatly reduced the degrees of freedom and the power of our analysis (e.g. 10 independent terms out of 36 statistical units).

We estimated the percent variation of fish morphology explained by the pure contribution of the genetic and environmental factors by summing the percent variation of fish morphology explained by each independent effect on PC1, PC2 and PC3 (MANCOVA based on canonical scores; see also Robinson and Wilson, 1996). This analysis was performed with and without controlling for the effect of sex to determine the potential effect of sexual dimorphism on the estimation of the contribution of the genetic and environmental factors to trophic polymorphism in brook charr.

Objective 4: plasticity in morphological characters of littoral and pelagic individuals following a shift from their initial to the alternative habitat (second transplant experiment)

We performed a principal components analysis on morphological variables at 16 months (data of the second transplant experiment) to determine the degree of association among all individuals (canonical scores). Factorial analyses of variance using the mean canonical scores from the principal components analyses were then used to determine how the morphology of fish that had been shifted from their original habitat changed relative to the morphology of the non-shifted fish. To do so, we tested the effects of ecotype, habitat, brood pair, shift and interactions with shift (ecotype–shift, habitat–shift, brood pair–shift) in 16-month-old fish. This allowed us to measure the morphological plasticity of a specific set of characters. Following the shift, changes in a fish's morphology would result in a significant effect of the habitat–shift interaction.

The above statistical design is more robust and conservative than other multivariate approaches for two main reasons. Principal components analysis, in contrast to other factor analyses, is a unique mathematical solution (e.g. empirical solution) that analyses all the variance comprised in the observed variables, not only the shared variance between variables (Tabachnick and Fidell, 1996). Although we recognize this as an important issue, we did not attempt to correct probability values for multiple tests given that well-established standard corrections such as the Holm's sequential Bonferroni correction can be extremely conservative (Moran, 2003). In addition, given that characters present some level of inherent dependence, these corrections may not be the most appropriate when studying plasticity. Furthermore, our statistical design is conservative and decreases the possibility of a Type I error. All statistical analyses were done using SYSTAT 8.0 (Wilkinson, 1998).

RESULTS

Objective 1: heritability in morphological characters of littoral and pelagic brook charr

The discriminant function analysis indicated that 4-month-old fish differed significantly in their morphology between ecotypes (Table 1). The stepwise procedure retained five morphological characters: young littoral fish possessed longer pectoral and dorsal fins, a larger body, a more robust head (greater head depth) and a larger peduncle than young pelagic fish. The jackknife method allowed us to correctly reclassify 71% of the littoral and

Table 1. Results of the discriminant function analysis on the morphology of laboratory-reared 4-month-old brook charr (beginning of the experiment)

Character	Littoral ecotype (mm)	Pelagic ecotype (mm)	Canonical score
Head depth	9.31 $\pm$ 0.04	9.08 $\pm$ 0.04	0.31
Body width	7.45 $\pm$ 0.04	7.16 $\pm$ 0.04	0.38
Pectoral fin	8.50 $\pm$ 0.08	7.71 $\pm$ 0.08	0.69
Dorsal fin	7.65 $\pm$ 0.05	7.40 $\pm$ 0.05	0.26
Peduncle depth	6.22 $\pm$ 0.03	6.04 $\pm$ 0.03	0.32

Note: Mean size-adjusted ($\pm$ standard error) data of the five selected morphological characters are presented here (backward selection procedure; P to remove = 0.05). The jackknife method allowed us to correctly reclassify 71% of the littoral and 70% of the pelagic individuals to their appropriate ecotype. Samples for the analysis comprised 90 littoral and 90 pelagic individuals. Wilks' lambda = 0.67, F statistic = 16.66, $P < 0.0001$.

70% of the pelagic individuals to their appropriate ecotype. The relationships between the size-adjusted pectoral and dorsal fins for both the parental stock and their progeny showed two striking patterns (Fig. 3): first, the pectoral and dorsal fin characters of young brook charr are similar (in direction and amplitude) to those of their parents (especially the females) and, second, the lengths of the two fins are still correlated after adjusting for body size ($r = 0.49$, $P < 0.001$), suggesting a morphological integration between these two characters. The overall mean difference in the pectoral and dorsal fin lengths between ecotypes was 0.79 and 0.25 mm, respectively (Table 1).

Objective 2: the effect of sexual dimorphism on the variation of littoral and pelagic brook charr morphology (first transplant experiment)

The MANCOVA showed significant differences in the overall slope of morphological characters–body length relationships between male and female brook charr (Table 2). An inspection of the mean differences in the slope and the canonical loading between sexes indicates that males had longer characters associated with trophic-related traits than females: males had more robust heads (greater head length and depth), longer snouts, longer lower jaws and wider mouths than females of the same length (Table 2). Males were also significantly larger (mean weight: $t = 5.17$, $P < 0.0001$) and longer (mean length: $t = 4.25$, $P < 0.0001$) than females at the end of the experiments (i.e. 16 months old). At 12 and 16 months old, fish measuring less than 150 mm (145 females and 81 males) showed no significant difference in morphology between sexes (MANCOVA: sex–length interaction, $F = 1.19$, $P > 0.05$; sex, $F = 1.16$, $P > 0.05$). Although the sex of the 8-month-old fish could not be determined, it was of little concern because only two fish in this age group were above the 150-mm threshold (mean length at 8 months $\pm$ standard deviation: 109 $\pm$ 15.6 mm).

Objective 3: contribution of genetic and environmental factors (and their interaction) to trophic polymorphism in brook charr (first transplant experiment)

Principal components PC1, PC2 and PC3 explained 32, 12 and 12%, respectively, of the total variation in morphology of 8-, 12- and 16-month-old brook charr. The results of analyses of variance on the canonical score of the PCA revealed that brood pair effects and ecotype–habitat interactions were never significant (Table 3). PC1 separated individuals with large characters from those with small characters and did not explain any significant

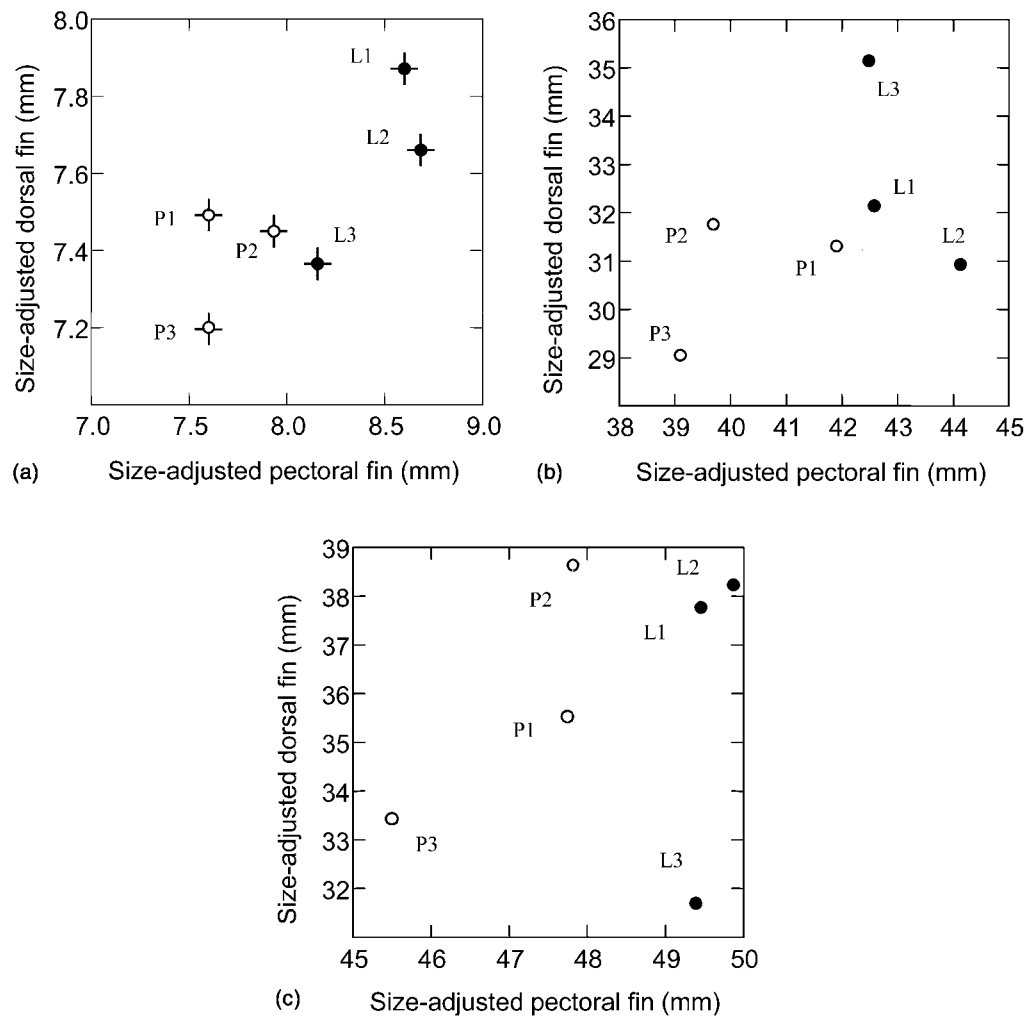


Fig. 3. Size-adjusted pectoral and dorsal fins for (a) the mean progeny, (b) each adult female brook charr and (c) each adult male brook charr from Lac Ledoux. Error bars ($\pm$ standard error) in (a) were obtained from the distributions of thirty 4-month-old brook charr from each brood. Filled circles represent the littoral ecotype and open circles the pelagic ecotype. Numbers in (b) and (c) identify the Lac Ledoux parents used to create the experimental broods used in laboratory experiments.

variation of the effects studied (ecotype, habitat, time and brood pair; Table 3). In contrast, we found a significant effect of habitat and ecotype on PC2 (Table 3). The loading of canonical coefficients on PC2 indicated that the length of pectoral and dorsal fins, body width and lower jaw length were the four best descriptors for separating treatments (Table 4). PC3 was not significantly related to any effect (Table 3). To better illustrate which characters were related to habitat, ecotype or both effects, we reported the reaction norms of the four traits explaining most of the variation across treatments on PC2 (Fig. 4; $P < 0.0001$). Littoral fish in the littoral habitat (LL) had longer pectoral fins than fish in

Table 2. Results of MANCOVA for sexual dimorphism on the set of morphological characters studied in 12- and 16-month-old brook charr (with body length as the covariate)

Character	Females	Males	Canonical score
Swimming-related traits			
Body depth	1.18 ± 0.02	1.30 ± 0.03	0.37
Body width	1.22 ± 0.02	1.32 ± 0.03	0.29
Pectoral fin	1.19 ± 0.03	1.24 ± 0.04	0.11
Dorsal fin	1.02 ± 0.02	1.04 ± 0.03	0.06
Peduncle depth	1.03 ± 0.01	1.03 ± 0.02	0.01
Trophic-related traits			
Head length	0.87 ± 0.01	0.97 ± 0.02	0.53
Head depth	0.93 ± 0.01	1.07 ± 0.02	0.61
Mouth width	0.99 ± 0.03	1.24 ± 0.04	0.45
Snout length	1.00 ± 0.03	1.23 ± 0.03	0.53
Lower jaw length	0.97 ± 0.01	1.22 ± 0.02	0.85

Note: Least-squares slopes (± standard error) for the 10 morphological characters are presented. Samples comprised 510 males and 640 females. Wilks' lambda = 0.92, *F* statistic = 9.78, *P* < 0.0001.

Table 3. Results of ANOVA of the first transplant experiment for each principal component testing the effect of ecotype, habitat, time, brood pair and ecotype–habitat interaction on the morphology of 8-, 12- and 16-month-old brook charr

Principal component	Effect	<i>F</i> statistic	<i>P</i>
PC1	Ecotype	0.16	0.69
	Habitat	0.09	0.77
	Time	0.56	0.46
	Brood pair	0.01	0.96
	Ecotype–habitat	1.09	0.30
PC2	Ecotype	30.19	<0.0001
	Habitat	50.76	<0.0001
	Time	0.18	0.73
	Brood pair	0.01	0.99
	Ecotype–habitat	0.06	0.80
PC3	Ecotype	2.43	0.12
	Habitat	1.39	0.24
	Time	0.81	0.37
	Brood pair	0.01	0.99
	Ecotype–habitat	0.17	0.68

Note: Sample size was 9 for each analysis in each treatment (LL, LP, PL, PP; see Fig. 4 legend for treatment descriptions). Significant effects are in **bold**.

Table 4. Canonical loading of coefficients for morphological characters from the principal components analysis of the first transplant experiment

Morphological character	PC1	PC2	PC3
Pectoral fin length*	0.285	0.602	0.385
Body width*	0.397	0.375	−0.663
Dorsal fin length*	0.428	0.363	0.313
Mouth width	0.536	0.206	−0.491
Peduncle depth	0.569	0.237	−0.072
Body depth	0.617	0.208	0.337
Upper jaw length	0.644	−0.347	0.047
Head length	0.652	−0.330	0.034
Lower jaw length*	0.708	−0.464	0.039
Head depth	0.718	−0.088	0.051

Note: Coefficients represent the correlation of each character with component scores. Coefficients are listed in increasing order based upon the first principal component (PC1). Asterisks identify the four morphological characters with the highest coefficients on PC2 ($P < 0.05$).

intermediate situations (LP or PL), whereas pelagic fish in the pelagic habitat (PP) had smaller pectoral fins than fish in intermediate situations (Fig. 4a). Although the same pattern was expected among treatments in the length of dorsal fins, fish in the LP condition showed mean dorsal fins below the overall group average residuals (dashed line in Fig. 4b). The reaction norms for habitat-related characters such as body width and lower jaw length responded to habitat but not to the effect of the ecotype (Fig. 4c,d). Absolute size-adjusted mean ($\pm$ standard error) differences for the pectoral and dorsal fins were 1.34 mm (littoral, 19.13 ± 0.10 ; pelagic, 17.79 ± 0.10) and 0.20 mm (littoral, 15.80 ± 0.05 ; pelagic, 15.60 ± 0.05) respectively between ecotypes, while their mean differences between habitats reached 1.48 mm (littoral, 19.20 ± 0.10 ; pelagic, 17.72 ± 0.10) and 0.25 mm (littoral, 16.83 ± 0.05 ; pelagic, 16.58 ± 0.05) respectively. The percent variation of fish morphology explained by the pure effects of genetic and environmental components was 17% and 15% respectively when the effect of sex was controlled, and 13% and 26% respectively when the effect of sex was not accounted for.

Objective 4: plasticity in morphological characters of littoral and pelagic individuals following a shift from their initial to the alternative habitat (second transplant experiment)

Principal components PC1, PC2 and PC3 explained 30, 14 and 11%, respectively, of the total variation in morphology of shifted and non-shifted brook charr. The results of the analyses of variance on the canonical scores showed a significant effect of habitat and the habitat–shift interaction on PC2 among 16-month-old brook charr shifted from their original habitat compared with fish that were not shifted (Table 5). The significant effects of the habitat and habitat–shift interaction indicate that the effect of habitat was significant but not in the same direction among shifted and non-shifted individuals. The inspection of canonical coefficients indicated that body width and lower jaw were the two characters responding to the shift of individuals to the alternative habitat (Table 6). In addition, the pectoral and dorsal fin lengths were not significantly related to PC2 but to an effect of

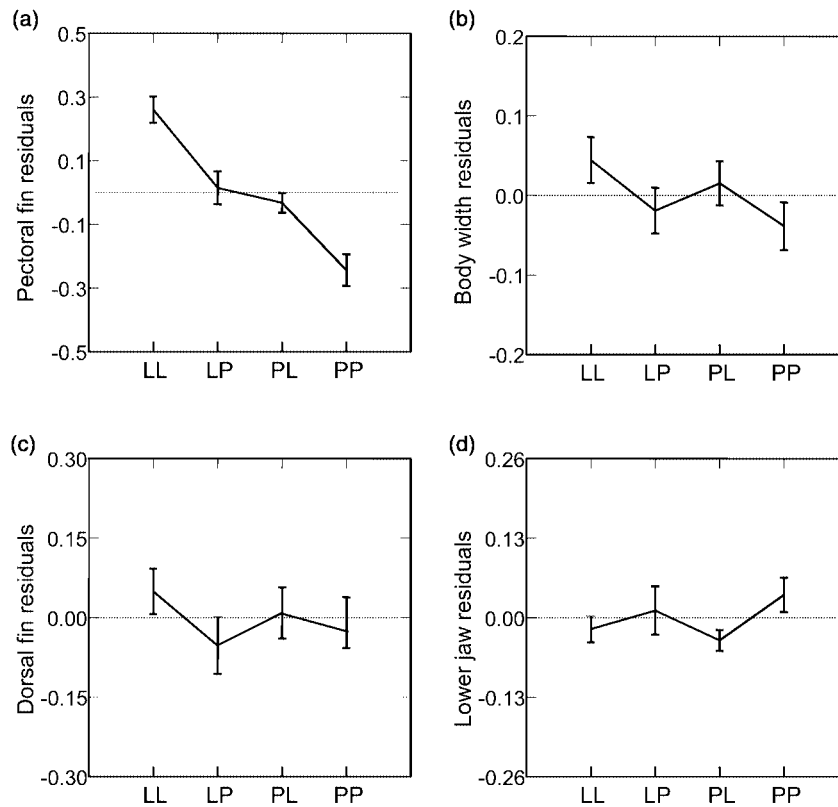


Fig. 4. Reaction norms of four characters in the first transplant experiment. LL, littoral fish in the littoral habitat; LP, littoral fish in the pelagic habitat; PL, pelagic fish in the littoral habitat; PP, pelagic fish in the pelagic habitat. Pectoral (a) and dorsal (b) fins, body width (c) and lower jaw (d) were the best morphological descriptors for which there was a significant effect of ecotype and habitat on PC2. Morphological descriptors are expressed as the residuals ($\pm$ standard error) of the allometric relationship between the morphological variable and body length. The error bars represent the brood effect within treatments.

ecotype on PC3 (Tables 5 and 6). This suggested that the pectoral and dorsal fins did not respond to the shift of individuals to the alternative habitat.

The body width of littoral fish tended to adjust to the new habitat following their 4-month shift (becoming smaller when shifted to the pelagic habitat and larger when shifted to the littoral habitat; Fig. 5a,c). This trend was not as clear for the pelagic fish. However, one striking result of this experiment is the large increase of the standard error of the body width and lower jaw residuals following the shift (Fig. 5a,c). This pattern was similar in other morphological characters loading high on PC2 (i.e. lower jaw and peduncle depth). This indicates that the different broods did not respond to the shift with the same magnitude (i.e. brood effect). In contrast, there was a significant effect of ecotype on PC3 (Table 5). The pectoral fin residuals of each ecotype in its own habitat (LL and PP) remained in the same direction (positive *vs* negative residuals, respectively) while those of each ecotype in the contrasting habitat (PL and LP) remained near zero (Fig. 5b,d). With the exception

Table 5. Results of ANOVA of the second transplant experiment for each principal component testing the effects of ecotype, habitat, brood pair and shift (and all interactions with shift) on 16-month-old shifted and non-shifted fish

Principal component	Effect	<i>F</i> statistic	<i>P</i>
PC1	Ecotype	1.78	0.20
	Habitat	0.63	0.44
	Brood pair	0.06	0.81
	Shift	0.01	0.93
	Ecotype–shift	0.08	0.77
	Habitat–shift	0.31	0.56
	Brood pair–shift	0.01	0.97
PC2	Ecotype	0.24	0.62
	Habitat	10.49	0.005
	Brood pair	0.01	0.93
	Shift	0.01	0.92
	Ecotype–shift	0.23	0.64
	Habitat–shift	7.20	0.016
	Brood pair–shift	0.01	0.93
PC3	Ecotype	5.78	0.029
	Habitat	0.32	0.58
	Brood pair	0.02	0.90
	Shift	0.01	0.93
	Ecotype–shift	1.32	0.26
	Habitat–shift	2.34	0.15
	Brood pair–shift	0.02	0.90

Note: Sample size was 6 for each analysis in each treatment (LL, LP, PL, PP; see Fig. 4 legend for treatment descriptions). Significant effects are in **bold**.

of the LP to LL shift (Fig. 5b,d), the standard error of the pectoral fin residuals remained of the same magnitude after the shift. This pattern was also similar in the other morphological characters loading high on PC3 (i.e. dorsal fin).

DISCUSSION

The results of the present study show that morphological differences between littoral and pelagic brook charr are heritable and regulated by both genetic and environmental factors after controlling for the effect of sexual dimorphism. Genetic factors explained 17% of the variation in morphological characters, while environmental factors explained 15%. The shift of some individuals from their initial to the alternative treatments from months 12 to 16 also highlighted the role of the genetic and environmental components as well as the magnitude of morphological plasticity: some characters remained unchanged during this 4-month shift while others were more flexible. Finally, the direction of morphological changes was consistent with the predictions of functional morphology, suggesting that morphological differences between the two brook charr ecotypes are adaptive.

Table 6. Canonical loading of coefficients for morphological characters from the principal components analysis of the second transplant experiment

Morphological character	PC1	PC2	PC3
Peduncle depth	0.370	-0.442	-0.026
Pectoral fin length*	0.378	0.202	-0.577
Dorsal fin length*	0.403	-0.029	-0.574
Body depth	0.443	-0.422	-0.414
Body width*	0.458	-0.555	0.327
Mouth width	0.543	-0.290	0.246
Head length	0.589	0.248	0.229
Upper jaw length	0.663	0.419	0.044
Lower jaw length*	0.722	0.464	0.223
Head depth	0.773	0.109	0.043

Note: Coefficients represent the correlation of each character with component scores. Coefficients are listed in increasing order based upon the first principal component (PC1). Asterisks identify the four morphological characters associated with habitat and ecotype effects in the first transplant experiment.

Different lines of evidence suggest that the pectoral fin, and to a lesser extent the dorsal fin, is genetically inherited in the brook charr system:

1. In previous studies on different lakes of the same system, dorsal and (or) pectoral fin lengths were invariably good descriptors of the ecotype in the field (Bourke *et al.*, 1997; Dynes *et al.*, 1999; Marchand, 2001; Proulx and Magnan, 2002).
2. In the present study, spawning adults were selected on the basis of inter-individual differences in the size-adjusted length of the pectoral and dorsal fins, and laboratory-reared offspring showed significant differences in these two characters.
3. The pectoral and dorsal fin lengths maximized the separation between offspring of the 4-month-old ecotypes (e.g. before the beginning of the experiments) among a set of 10 morphological characters.
4. Morphological differences in the dorsal and pectoral fins appeared early between ecotypes and were maintained regardless of the treatments throughout the duration of the experiment (i.e. over 1 year).

Furthermore, the relationship between the length of the pectoral fin and foraging behaviour (littoral *vs* pelagic) has been observed in many freshwater fishes (*Coregonidae*: Todd *et al.*, 1981; *Lepomis macrochirus*: Ehlinger and Wilson, 1988; Ehlinger, 1990; *Gasterosteus aculeatus*: Lavin and McPhail, 1986; *Cichlasoma citrinellum*: Meyer, 1987; *Coregonus artedii*: Hénault and Fortin, 1989; *Coregonus chupeaformis*: Fortin and Gendron, 1990; Chouinard *et al.*, 1996; *Lepomis gibbosus*: Robinson *et al.*, 1993; *Percichthys trucha*: Ruzzante *et al.*, 1998). This suggests a strong functional relationship between pectoral fin size and feeding mode in nature and a high potential for the selection of this character in lakes that have the two discrete habitats.

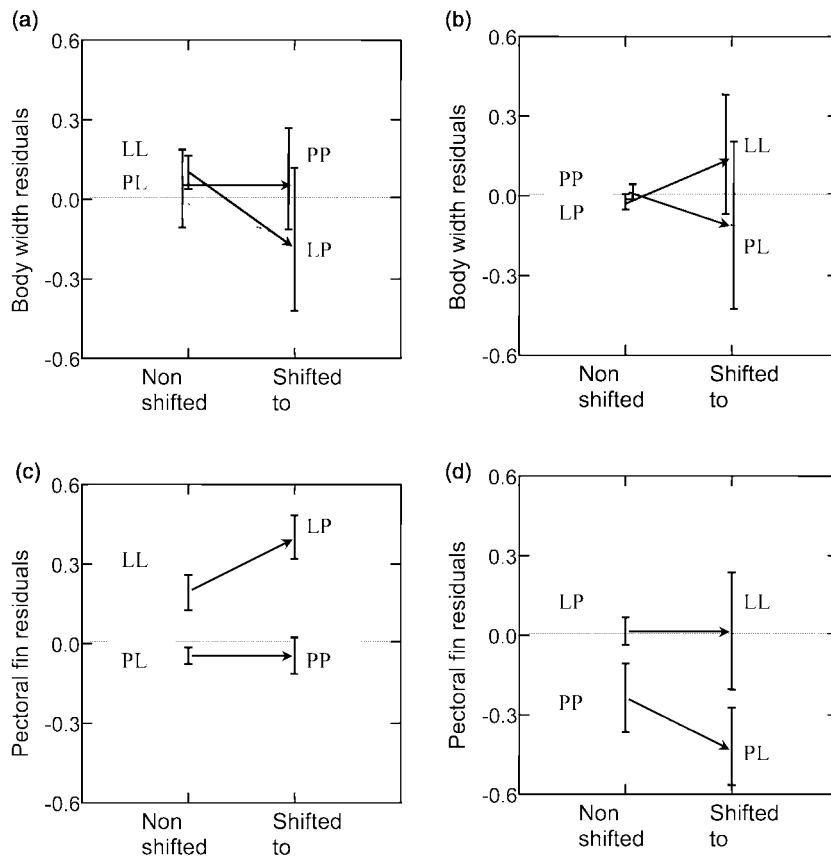


Fig. 5. Comparison of body width and pectoral fin residuals in the second transplant experiment. Panels (a) and (b) report means ($\pm$ standard error) for fish from LL and PL conditions shifted to LP and PP treatments, while (c) and (d) report means ($\pm$ standard error) for fish from LP and PP conditions shifted to LL and PL treatments (see Fig. 4 for treatment description). The error bars represent the brood effect within treatments.

Sexual dimorphism accounted for part of the variation in littoral and pelagic brook charr morphology. Based on 10 morphological characters, males longer than 150 mm differed from females in their morphology. These males had more robust heads, longer snouts, longer lower jaws and wider mouths than females of the same length. The ecological role of the phenotypic differences between sexes has received little attention in the context of trophic polymorphism (Ehlinger, 1990; Magurran and Garcia, 2000; Kristjánsson *et al.*, 2002).

The pure contribution of the environmental components identified in our experiment (15%) is much lower than that observed in pumpkinseed sunfish (53%; Robinson and Wilson, 1996), while the genetic contribution to ecotype differences is low and about the same in the two systems (15% *vs* 17%). However, Robinson and Wilson (1996) did not account for the potential effect of sexual dimorphism in their experiment, which could have increased the relative contribution of the environmental component. When not accounting

for the effect of sex, we found that the percentages of explained variation of morphological characters related to the pure genetic and pure environmental components were 13% and 26%, respectively, indicating that sexual dimorphism accounts for a large part of the overall variation in brook charr morphology. We were thus conservative in eliminating the effect of sexual dimorphism because we restricted our analyses to those characters important in explaining the trophic polymorphism in both sexes. Sexual dimorphisms, even subtle ones, might play important roles in non-mating behaviours of fish, such as feeding modes. A direct test of this hypothesis will be required to address this question. On the other hand, our experiment was not designed to differentiate the maternal effects, which are known to influence charr morphology (Heath *et al.*, 1999), from genetic effects. Rather, we were interested in determining if traits inherited from parents of the same form were maintained across treatments during the first year of life. In this context, our experimental design could have overestimated the genetic effect (Heath *et al.*, 1999). The contribution of maternal effect to the morphology of brook charr progeny and its potential consequence on the evolution of trophic polymorphism is an important issue that should be addressed in future studies.

Although significant, the relatively low genetic contribution found in our experiment might explain the genetic results obtained in a previous study on two lakes of the same system (Dynes *et al.*, 1999). Microsatellite DNA analyses at five loci showed a significant genetic differentiation between littoral and pelagic brook charr from Lake Bondi but not from Lake Ledoux (origin of the fish used in the present study). However, the genetic analyses failed to tell us whether the trophic polymorphism observed in Lake Ledoux is the result of natural selection through sympatric speciation or of contemporary evolution following anthropogenic impacts such as stocking (Stockwell *et al.*, 2003). For instance, Hendry *et al.* (2000) found evidence for the evolution of reproductive isolation (based on microsatellite DNA analyses) after fewer than 13 generations in sockeye salmon (*Onchorhynchus nerka*) following stocking of individuals from Baker Lake to Lake Washington, two lakes in Washington state (USA). Indeed, brook charr stocking occurred on four occasions between 1971 and 1989 in Lake Ledoux, but this practice has since been abandoned. Although Dynes *et al.* (1999) did not find any differences between littoral and pelagic individuals using microsatellite DNA analysis, it is possible that morphological differences between the stocked individuals and native fish from Lake Ledoux have contributed to the observed ecotype differences. If this is the case, the two forms were able to maintain these differences until now (approximately 6–15 generations) and might be experiencing the early stages of speciation. Given that trophic polymorphism was observed in other unstocked lakes of the same system, such as Lake Bondi, the most parsimonious hypothesis remains that these lakes, which were colonized about 15,000 years ago, represent early stages of evolution in brook charr.

The results of our first transplant experiment showed that body width and lower jaw length responded more markedly to the effect of habitat than did pectoral and dorsal fin lengths. To demonstrate phenotypic plasticity, a given morphological character should change its shape to rapidly adapt to the new habitat (Scheiner, 1993). In general, fishes of the genus *Salvelinus* show a high potential for plasticity in response to environmental changes (Noakes, 1989; McLaughlin *et al.*, 1994; Hutchings, 1996). Our experimental littoral and pelagic habitats forced brook charr to adopt different foraging behaviours, which, in turn, produced different phenotypes (i.e. reaction norms). Fish in the littoral

habitat had a more robust body, shorter jaw length, and longer pectoral and dorsal fins than those in the pelagic habitat. A fusiform shape is expected to reduce the energetic cost of swimming (minimizing drag) by allowing for efficient cruising (Gatz, 1979; Webb, 1984) and thus should improve searching and feeding performances on dispersed and mobile prey, like zooplankton in open water. In contrast, a stout body shape improves burst swimming, which is useful when catching larger and faster prey of the littoral habitat (Webb, 1984; Norton, 1991; Malmquist *et al.*, 1992; Walker, 1997). Also, Wainwright and Richard (1995) showed that the length of the lower jaw controls in part gape size and mouth closing speed. Because of their larger mouth gape, individuals with longer lower jaws are better adapted to feeding on mobile prey items in the water column (Kotrschal, 1989; Norton, 1991). Finally, the long pectoral fins of benthic specialists are predicted to be better adapted for precise manoeuvring in complex littoral habitat, while the short pectoral fins of pelagic specialists would help to reduce drag while swimming in the open-water zone (Gatz, 1979; Webb, 1984; Winemiller, 1991). Our experimental results are further supported by field observations: brook charr captured in the littoral zone were more stout, had greater head, body and peduncle depths, and longer dorsal and/or pectoral fins than charr captured in the pelagic zone (Bourke *et al.*, 1997; Dynes *et al.*, 1999; Marchand, 2001; Proulx and Magnan, 2002). Experimental and field observations suggest that swimming modes rather than feeding *per se* are important cues in maintaining trophic polymorphism.

The second transplant experiment was designed to provide independent support for the contribution of genetic and environmental components. The results of this experiment showed that the flexible characters identified in the first transplant experiment (pectoral and dorsal fins) did not change significantly following a shift of individuals from their initial to the alternative habitat, suggesting that these characters are under genetic control. In contrast, the other highly flexible characters identified in the first transplant experiment (body width and lower jaw length) exhibited a complete and a rapid reversal (within 4 months) when the individuals were shifted to the alternative habitat, indicating that these characters are under pure environmental control (and are not fixed after a fish has adopted a given strategy). Furthermore, changes in these plastic characters were in line with the predictions of functional morphology: pelagic individuals shifted to the littoral habitat became more stout, while littoral individuals shifted to the pelagic habitat became more fusiform, compared with their initial morphology 4 months earlier. Robinson and Parsons (2002) found a family-level convergence on the predicted reaction norms in response to littoral and pelagic habitats for the general body form (e.g. fusiform *vs* stout). This second transplant experiment also highlighted the magnitude of plasticity as well as the time response of changes in flexible characters. Such a plasticity in morphology could be adaptive if resources within a habitat (pelagic or littoral) diminish substantially, as the fish may decide to move to the other habitat type.

In our study, each brood represented the full-sib progeny of one male and one female. The increase in among-family variance (i.e. brood effect) of body width residuals in both ecotypes following the 4-month shift indicates that body width changed in a functional way in some broods but not in others (Fig. 5). The result of increased variance following the habitat shift can be interpreted as evidence of genetic variation in plastic responses, since all groups used in our experiments had experienced the same habitats. These results suggest that morphological plasticity has an evolutionary potential in brook charr and could respond to natural selection (Day *et al.*, 1994).

ACKNOWLEDGEMENTS

We thank Julie Adams, Claudia Cossette and Pierre East for their field and laboratory assistance. Dr Pedro Peres-Neto, Dr Beren Robinson and an anonymous reviewer provided helpful comments on earlier drafts of the manuscript. This research was supported by a grant from the Natural Sciences and Engineering Research Council (NSERC) of Canada to P. Magnan. R. Proulx was supported by postgraduate fellowships from the Fondation Universitaire du centre du Québec à Trois-Rivières.

REFERENCES

- Adams, C.E. and Huntingford, F.A. 2002. Inherited differences in head allometry in polymorphic Arctic charr from Loch Rannoch, Scotland. *J. Fish Biol.*, **60**: 515–520.
- American Public Health Association. 1989. *Standard Methods for the Examination of Water and Wastewater*, 17th edn. Washington, DC: APHA Press.
- Bailey, R.E., Irvine, J.R., Dalziel, F.C. and Nelson, T.C. 1998. Evaluations of visible implant fluorescent tags for marking coho salmon smolts. *N. Am. J. Fish. Manage.*, **18**: 191–196.
- Bourke, P., Magnan, P. and Rodríguez, M.A. 1997. Individual variations in habitat use and morphology in brook charr. *J. Fish Biol.*, **51**: 783–794.
- Bourke, P., Magnan, P. and Rodríguez, M.A. 1999. Phenotypic responses of lacustrine brook charr in relation to the intensity of inter-specific competition. *Evol. Ecol.*, **13**: 19–31.
- Cho, C.Y. and Bureau, D.P. 1998. Development of bio-energetic models and the Fish-PrFEQ software to estimate production, feeding ration and waste output in aquaculture. *Aquat. Living Resour.*, **11**: 199–210.
- Chouinard, A., Pigeon, D. and Bernatchez, L. 1996. Lack of specialization in trophic morphology between genetically differentiated dwarf and normal forms of lake whitefish (*Coregonus clupeaformis* Mitchell) in Lac de l'Est, Québec. *Can. J. Zool.*, **74**: 1989–1998.
- Day, T. and McPhail, J.D. 1996. The effect of behavioural and morphological plasticity on foraging efficiency in the threespine stickleback (*Gasterosteus* sp.). *Oecologia*, **108**: 380–388.
- Day, T., Pritchard, J. and Schluter, D. 1994. Ecology and genetics of phenotypic plasticity: a comparison of two sticklebacks. *Evolution*, **48**: 1723–1734.
- Dynes, J., Magnan, P. and Rodríguez, M.A. 1999. Genetic and morphological variation between two forms of lacustrine brook charr, *Salvelinus fontinalis*. *J. Fish Biol.*, **54**: 955–972.
- Ehlinger, T.J. 1990. Habitat choice and phenotype-limited feeding efficiency in bluegill: individual differences and trophic polymorphism. *Ecology*, **71**: 886–896.
- Ehlinger, T.J. and Wilson, D.S. 1988. Complex foraging polymorphism in bluegill sunfish. *Proc. Natl. Acad. Sci. USA*, **85**: 1878–1882.
- Fleming, I.A., Jonsson, B. and Gross, M.R. 1994. Phenotypic divergence of sea-ranched farmed and wild salmon. *Can. J. Fish. Aquat. Sci.*, **51**: 2808–2821.
- Fortin, R. and Gendron, M. 1990. Reproduction, croissance et morphologie comparée des Grands corégones (*Coregonus clupeaformis*) nains et normaux du réservoir Outardes-2 (Québec). *Can. J. Fish. Aquat. Sci.*, **47**: 2808–2821.
- Gatz, A.J. 1979. Community organisation in fishes as indicated by morphological features. *Ecology*, **60**: 711–718.
- Heath, D.D., Fox, C.W. and Heath, J.W. 1999. Maternal effects on offspring size: variation through early development of chinook salmon. *Evolution*, **53**: 1605–1611.
- Hénault, M. and Fortin, R. 1989. Comparison of meristic and morphometric characters among spring- and fall-spawning ecotypes of cisco (*Coregonus artedii*) in southern Québec, Canada. *Can. J. Fish. Aquat. Sci.*, **46**: 166–173.
- Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C. and Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Nature*, **290**: 516–518.

- Hutchings, J.A. 1996. Adaptive phenotypic plasticity in brook trout, *Salvelinus fontinalis*, life histories. *Ecoscience*, **3**: 25–32.
- Jonsson, B. and Jonsson, N. 2001. Polymorphism and speciation in Arctic charr. *J. Fish Biol.*, **58**: 605–638.
- Kotrschal, K. 1989. Trophic ecomorphology in eastern Pacific blennioid fishes: character transformation of oral jaws and associated change of their biological roles. *Environ. Biol. Fish.*, **24**: 199–218.
- Kristjánsson, B.K., Skúlason, S. and Noakes, D.L.G. 2002. Rapid divergence in a recently isolated population of threespine stickleback (*Gasterosteus aculeatus* L.). *Evol. Ecol. Res.*, **4**: 659–672.
- Lavin, P.A. and McPhail, J.D. 1986. Adaptive divergence of trophic phenotype among freshwater populations of the threespine stickleback (*Gasterosteus aculeatus*). *Can. J. Fish. Aquat. Sci.*, **43**: 2455–2463.
- Magurran, A.E. and Garcia, C.M. 2000. Sex differences in behaviour as an indirect consequence of mating system. *J. Fish Biol.*, **57**: 839–857.
- Malmquist, H.J., Snorrason, S.S., Skúlason, S. *et al.* 1992. Diet differentiation in polymorphic Arctic charr in Thingvallavatn, Iceland. *J. Anim. Ecol.*, **61**: 21–35.
- MAPAQ (Ministère de l'agriculture, des pêcheries et de l'alimentation Québec). 1990. *Atelier de travail sur la génétique des salmonidés d'élevage au Québec*, cahier de conférences. Québec: Gouvernement du Québec.
- Marchand, F. 2001. Facteurs biotiques et abiotiques influençant l'activité et les coûts énergétiques associés à l'alimentation chez les juvéniles (1+) de l'omble de fontaine (*Salvelinus fontinalis*). PhD thesis, Université du Québec à Montréal.
- Marchand, F., Magnan, P. and Boisclair, D. 2003. Differential time budget for two forms of juvenile (1+) brook charr *Salvelinus fontinalis* (Mitchill) in the open-water zone. *J. Fish. Biol.*, **63**: 687–698.
- McKinnon, J.S. and Rundle, H.D. 2002. Speciation in nature: the threespine stickleback model systems. *TREE*, **17**: 480–488.
- McLaughlin, R.L., Grant, J.W.A. and Kramer, D.L. 1994. Foraging movements in relation to morphology, water-column use, and diet for recently emerged brook trout (*Salvelinus fontinalis*) in still-water pools. *Can. J. Fish. Aquat. Sci.*, **51**: 268–278.
- Meyer, A. 1987. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution*, **41**: 1357–1369.
- Moran, M.D. 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos*, **100**: 403–405.
- Noakes, D.L.G. 1989. Early life history and behaviour of charrs. *Physiol. Ecol. Japan, Spec.*, **1**: 173–186.
- Norton, S.F. 1991. Capture success and diet of cottid fishes: the role of predator morphology and attack kinematics. *Ecology*, **72**: 1807–1819.
- Piper, R.G., McElwain, I.B., Orme, L.E. *et al.* 1982. *Fish Hatchery Management*. Washington, DC: US Department of the Interior, Fish and Wildlife Service.
- Power, G. 1980. The brook charr, *Salvelinus fontinalis*. In *Charrs: Salmonid Fishes of the Genus Salvelinus* (E.K. Balon, ed.), pp. 141–203. The Hague: Dr. W. Junk.
- Proulx, R. and Magnan, P. 2002. Physiological performance of two forms of lacustrine brook charr, *Salvelinus fontinalis*, in the open water habitat. *Environ. Biol. Fish.*, **64**: 127–136.
- Robinson, B.W. and Parsons, K.J. 2002. Changing times, spaces, and faces: tests and implications of adaptive morphological plasticity in the fishes of northern postglacial lakes. *Can. J. Fish. Aquat. Sci.*, **59**: 1819–1833.
- Robinson, B.W. and Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. *Am. Nat.*, **144**: 596–627.
- Robinson, B.W. and Wilson, D.S. 1996. Genetic variation and phenotypic plasticity in a trophically polymorphic population of pumpkinseed sunfish (*Lepomis gibbosus*). *Evol. Ecol.*, **10**: 631–652.

- Robinson, B.W., Wilson, A.S., Margosian, A.S. and Lotito, P.T. 1993. Ecological and morphological differentiation by pumpkinseed sunfish in lakes without bluegill sunfish. *Evol. Ecol.*, **7**: 451–464.
- Ruzzante, D.E., Walde, S.J., Cussac, V.E., Macchi, P.J. and Alonso, M.F. 1998. Trophic polymorphism, habitat and diet segregation in *Percichthys trucha* (Pisces: Percichthyidae) in the Andes. *Biol. J. Linn. Soc.*, **65**: 191–214.
- Scheiner, S.M. 1993. Genetics and evolution of phenotypic plasticity. *Annu. Rev. Ecol. Syst.*, **24**: 35–68.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Phil. Trans. R. Soc. Lond. B*, **351**: 807–814.
- Skúlason, S. and Smith, T.B. 1995. Resource polymorphism in vertebrates. *TREE*, **10**: 366–370.
- Stockwell, C.A., Hendry, A.P. and Kinnison, M.T. 2003. Contemporary evolution meets conservation biology. *TREE*, **18**: 94–101.
- Tabachek, J.L. 1988. The effect of feed particle size on growth and feeding efficiency of Arctic charr (*Salvelinus alpinus*). *Aquaculture*, **71**: 319–330.
- Tabachnick, B.G. and Fidell, L.S. 1996. *Using Multivariate Statistics*. Oxford: Harper Collins College Publishers.
- Todd, T.N., Smith, G.H. and Cable, L. 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (*Coregonidae*) of the Great Lakes. *Can. J. Fish. Aquat. Sci.*, **38**: 59–67.
- Venne, H. and Magnan, P. 1995. The impact of intra- and inter-specific interactions on young-of-the-year brook charr, in temperate lakes. *J. Fish Biol.*, **46**: 669–686.
- Wainwright, P.C. and Richard, B.A. 1995. Predicting patterns of prey use from morphology of fishes. *Environ. Biol. Fish.*, **44**: 97–113.
- Walker, J.A. 1997. Ecological morphology of lacustrine threespine stickleback *Gasterosteus aculeatus* L. (Gasterosteidae) body shape. *Biol. J. Linn. Soc.*, **61**: 3–50.
- Webb, P.W. 1984. Body form, locomotion and foraging in aquatic vertebrates. *Am. Zool.*, **24**: 107–120.
- Wilkinson, L. 1998. *SYSTAT 8.0 Statistics*. Evanston, IL: SYSTAT Inc.
- Winemiller, K.O. 1991. Eco-morphological diversification in lowland freshwater fish assemblages from five biotic regions. *Ecol. Monogr.*, **61**: 343–365.