

Pedro R. Peres-Neto · Pierre Magnan

## The influence of swimming demand on phenotypic plasticity and morphological integration: a comparison of two polymorphic charr species

Received: 28 October 2003 / Accepted: 22 March 2004 / Published online: 1 May 2004  
© Springer-Verlag 2004

**Abstract** In northern freshwater lakes, several fish species have populations composed of discrete morphs, usually involving a divergence between benthic and limnetic morphs. Although it has been suggested that swimming demand plays an important role in morphological differentiation, thus influencing habitat selection, it is unclear how it affects reaction norms, patterns in character correlation, and levels of morphological integration. We examined whether swimming demand could induce morphological plasticity in the directions expected under divergent habitat selection, and evaluated its influence on the morphological integration in Arctic charr (*Salvelinus alpinus*) and brook charr (*S. fontinalis*), two congeneric species exhibiting conspicuous and subtle resource polymorphism, respectively. We found that changes in morphology were induced by differential swimming demands in both species. The length of the pectoral fin was the character that responded most strongly according to the predicted morphological expectations under divergent habitat selection. High levels of morphological plasticity, relatively low levels of integration, and differences found in the morphological correlation structure among water velocity treatments suggest that constraints on morphological change are unlikely in either species, thus allowing great potential for phenotypic flexibility in both species. The magnitude of character integration, however, was larger for Arctic charr than for brook charr. This latter result is discussed in the light of the differences in the level of polymorphism between the two species in the wild. The results of the present study indicate that swimming

demand alone may not be sufficient to generate the polymorphism encountered in nature. Given that both diet and swimming demands can induce morphological changes, it would be important to conduct experiments targeting the interaction between the morphological modules related to trophic and swimming demands.

**Keywords** Charr · Morphological integration · Phenotypic plasticity · Polymorphism · Swimming ability

### Introduction

Environmentally induced changes in the developmental process of a given genotype are denominated phenotypic plasticity, and in certain cases they may constitute adaptive solutions for coping with environmental heterogeneity. Phenotypic plasticity has been regarded as an important ecological agent in expanding the habitat range of a species (Stearns 1989; Kawata 2002) and creating potential opportunities for new selective pressures to act on (Waddington 1953; Stearns 1989; Pigliucci 2001). Although phenotypic plasticity may be regarded as a property of individual traits, their expression along environmental gradients may be correlated with other characters, leading to phenotypic integration (Schlichting 1989). Phenotypic integration may be strong, where characters present similar levels of correlation independent of the environment (e.g., canalization), or plastic, where correlations between characters vary as a function of the environment. The study of phenotypic integration may bring to light important ecological and evolutionary forces determining phenotypic variation. For instance, high levels of phenotypic integration may impose restrictions on the range of environments that a species may inhabit (Schlichting 1989). In contrast, depending on the pattern of selection, characters having greater levels of independence may respond better to selection than characters that are highly associated (Lewontin 1978; but see Berg 1960). Therefore, if levels of plasticity depend on how characters are associated, then the potential for evolutionary changes

P. R. Peres-Neto · P. Magnan  
Groupe de recherche sur les écosystèmes aquatiques,  
Département de chimie-biologie, Université du Québec à Trois-  
Rivières,  
C.P. 500 Trois-Rivières, Québec, Canada, G9A 5H7

Present address:

P. R. Peres-Neto (✉)  
Département des Sciences Biologiques, Université de Montréal,  
C.P. 6128 succ. A Montréal, Québec, Canada, H3C 3J7  
e-mail: pedro.peres.neto@umontreal.ca  
Tel.: +1-514-3436111

may depend on how phenotypic integration varies across environments.

Differential selective pressures in response to habitat use affect fish body morphology. In northern freshwater lakes, several species of fishes have populations composed of discrete morphs, involving usually a divergence between benthic (littoral) and limnetic (pelagic) morphs (Skúlason and Smith 1995; Schluter 1996). This common pattern of resource-based segregation, coupled with the functional expectations related to habitat divergence and involving multiple independent taxa, suggests that these evolutionary responses are indeed selective (Robinson and Parsons 2002). Morphologically, deeper body shape improves burst swimming performance and is advantageous when swimming in complex habitats, such as littoral areas, whereas a streamlined shape enhances prolonged swimming ability and is energetically efficient in foraging for patchily distributed prey in open water or when migrating (Webb 1982; Taylor and McPhail 1985). Although it has been suggested that swimming demand plays an important role in morphological differentiation, influencing habitat selection, it is unclear how it affects reaction norms, patterns in character correlation, and levels of integration.

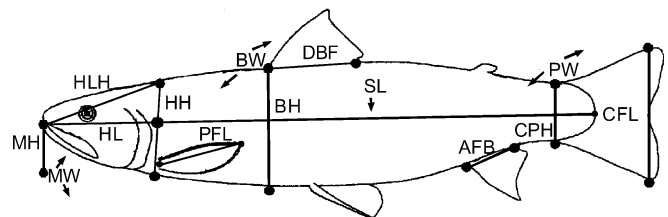
In the present study, we investigated experimentally the effects of swimming demand on the levels of morphological variation and integration of two charr species. We reared young-of-the-year brook charr (*Salvelinus fontinalis*) and Arctic charr (*S. alpinus*) under four different levels of water velocity. Brook charr and Arctic charr exhibit interindividual differences in spatial distribution and diet (Bourke et al. 1997, 1999; Jonsson and Jonsson 2001; Andersson 2003). Differences between the benthic and limnetic forms in Arctic charr are conspicuous (Skúlason et al. 1989; Jonsson and Jonsson 2001; Andersson 2003), whereas in brook charr differences are subtle (Bourke et al. 1997; Dynes et al. 1999). Given that these two congeneric species present different levels of phenotypic divergence between the limnetic and benthic morphs in the wild, their comparison may prove useful in understanding how phenotypic integration and phenotypic divergence are connected. The two main determinants of correlation between characters are genetic constraints and selection (Pigliucci 2001, p 172). Thus, the comparison of patterns of phenotypic integration between related species under different ecological pressures (i.e., swimming demands) may provide insights on how selection regimes and genetic constraints on development may contribute to polymorphism. In the present study, we address the following questions: (1) Is there plasticity to water velocity for the morphological traits investigated? And if so, how do they contrast across species? (2) Can differential swimming demands induce phenotypes in accordance with the expectations of benthic versus limnetic morphs found in these species? (3) How do the levels of integration compare between species, that is, does the more plastic species (i.e., Arctic charr) present lower or higher levels of phenotypic integration? (4) Is there

variation in character integration in relation to water velocity for the traits investigated?

## Materials and methods

We used a laboratory-rearing experiment to determine the extent to which variation in morphology among individuals could be induced by differential swimming demands. The experimental protocol followed East and Magnan (1987). Yearling brook and Arctic charr were raised in a set of 24 tanks under four different water velocity treatments (three replicates per velocity treatment per species), each containing 150 individuals. Water velocity was kept counter-clockwise. Individuals per tank were measured and averaged weekly. The water velocity was then adjusted so that individuals in a tank would experience a constant water velocity (in terms of body lengths per second) throughout the experiment. The experiment consisted of raising fish for 6 months in four water velocity treatments 0.0 (still), 0.5 (slow), 1.0 (medium) and 1.5 (high) body length/s<sup>-1</sup> (bl/s<sup>-1</sup>). Note that 0.5 bl/s<sup>-1</sup> is the lowest speed at which salmonid fishes consistently orient upstream and hold station in the current (Drucker and Lauder 2003). Individuals were obtained from domestic breeding stocks where brook charr belonged to the NASH strain and Arctic charr came from the “Fraser” strain. Note that individuals were submitted to experimental conditions soon after the first time they fed. Average standard lengths and weights at the beginning of the experiment were 27.9 mm and 0.44 g for brook charr, and 36.5 mm and 0.20 g for Arctic charr. Fish were fed four times a day to satiation. Experimental tanks were set with bedrock, sand and biological filters, and were connected to a central glycol cooling system that kept water temperature at 10°C (±0.5°C). Water alkalinity and hardness were adjusted to 65 mg l<sup>-1</sup> CaCO<sub>3</sub> by addition of sodium bicarbonate (Na<sub>2</sub>HCO<sub>3</sub>) and calcium chloride (CaCl<sub>2</sub>). Light intensity (~40 lux), water temperature (10±1°C) and photoperiod (12:12) were held constant during the whole experiment. Ammonia (NH<sub>3</sub>; µg l<sup>-1</sup>), nitrites (NO<sub>2</sub>; mg l<sup>-1</sup> of CaCO<sub>3</sub>) and water hardness (mg l<sup>-1</sup> of CaCO<sub>3</sub>) were estimated using standard procedures (APHA 1989) and kept within tolerance limits for salmonids’ aquaculture (Ministère de l’agriculture, des pêcheries et de l’alimentation du Québec, 1990). Mortality occurred at the beginning of the experiment and was not significantly different among treatments so that any morphological difference would not arise by differential mortality (see Robinson and Parsons 2002 for a discussion). At the end of the experiment, 14 morphological traits were measured on ten randomly chosen individuals from each tank, adding up to 30 measured individuals for each species in each water velocity treatment (Fig. 1). Characters were measured to the nearest 0.1 mm with vernier calipers on the left side of the fish.

In order to adjust for size variation, the Aitchinson (1986) log-ratio transformation was applied:  $y_{ij} = \log x_{ij} - \frac{1}{p} \sum_i^p \log x_{ij}$ , where  $p$  denotes the number of characters being considered and  $x_{ij}$  represents



**Fig. 1** Fourteen morphometric measures used in this study. *HL* head length, *HH* head height, *BH* body height, *BW* body width, *CPH* caudal peduncle height, *PW* peduncle width, *CFL* caudal fin length, *DFB* dorsal fin base, *AFB* anal fin base, *HLH* head length at head height, *MH* mouth height, *PFL* pectoral fin length, *SL* standard length, *MW* mouth width. Measures having two arrows (*MW*, *BW* and *PW*) indicate the position where the width was taken. Error bars represent 95% confidence intervals

the value for the  $i$ th individual and  $j$ th character. This method has advantages over the more commonly applied method based on residuals from size–character regressions (Reist 1986; Fleming et al. 1994). First, it does not pose a problem in cases where regressions are heterogeneous among groups (e.g., species, treatments; see Reist 1986 for a discussion) given that each individual is scaled independently. Second, it applies a “general” measure of size based on the composite of all variables being considered. Finally, it does not eliminate the variable that is used as surrogate of size (e.g., standard length) from the data set.

To tackle the first two questions (i.e., induced phenotype and matched with expectations under the benthic/limnetic morphs), we started by using univariate factorial analysis of variance to test for phenotypic plasticity among water velocities (treatment effect) and for variation in plasticity between species (species by treatment interaction) for each morphological trait where tanks (random factor) were nested within species and treatments (fixed factors) as recommended by Ling and Cotter (2003). Note that in this case, the error term in the analysis was measured by the variation between tanks. Although we recognize it as an important issue (Peres-Neto 1999), 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; Peres-Neto et al. 2003). In addition, given that characters present some level of inherent dependence, these corrections may be not the most appropriate when studying integration. Standard reaction norm plots (e.g. Pigiucci et al. 1999) were produced to visualize the treatment effect on individual characters. To assess whether overall multivariate differences between species were greater than differences between treatment levels regarding phenotypic plasticity (i.e., treatment means), we compared shape differences using Discriminant Function Analysis (DFA). Jackknifing (also commonly called leave-one-out or  $n$ -fold cross validation) was applied to estimate correct classification (Olden et al. 2002). We applied principal component analysis (PCA) to evaluate the correlation structure among characters across treatments within species.

We addressed questions 3 and 4 (i.e., levels of integration between species and character integration across treatments) by using the variance of the eigenvalues from a correlation matrix as an overall measure of integration (Cheverud et al. 1989; Herrera et al. 2002). If correlations are high, the first few dimensions present large eigenvalues in relation to the latter ones and the variance of eigenvalues is relatively high. If correlations are close to zero, all axes have similar eigenvalues and variance is low. The maximum expected variance equals the number of characters. Having a

measure of integration, the first step is to assess whether a particular magnitude of integration is larger than would be expected if characters were just correlated by chance (Wagner 1984). In order to perform this assessment, we propose a test that has a similar basis to the parallel analysis (Peres-Neto et al. 2003) where a Monte Carlo approach generates critical values that are used to assess whether a level of integration is significantly different from the levels expected under chance alone (i.e., uncorrelated variables). The Monte Carlo protocol used here is as follows: (1) generate random normally distributed variables  $N(0,1)$  respecting the original dimensions (i.e., the same number of observations and variables) as in the data set being analyzed. In this case, we sampled data containing 30 (within-treatment) or 120 (all treatments) observations and 14 variables; (2) calculate the eigenvalue variance of the correlation matrix of the data matrix generated in step 1; (3) perform steps 1 and 2 a total of 1,000 times; and (4) based on all eigenvalue variance values accumulated in step 3, calculate percentile confidence intervals (e.g., 95% for an  $\alpha=0.05$ ), which are then used as critical values.

We performed comparisons between the levels of integration within species across treatments and between species. Here, we applied a protocol adapted from the parametric bootstrap (Efron and Tibshirani 1993) in order to estimate confidence intervals around levels of integration. If two confidence intervals overlap then differences in phenotypic integration are considered non-significant. The protocol for generating confidence intervals around eigenvalue variances was as follows: (1) generate a sample matrix following the correlation matrix of the original data (see computational details below); (2) calculate the eigenvalue variance for the correlation matrix of the sample generated in step 1; (3) perform steps 1 and 2 a total of 1,000 times; and (4), based on all eigenvalue variance values computed in step 3, calculate percentile confidence intervals. In order to generate one single matrix in step 1 (see Peres-Neto et al. 2003 for more details), the following protocol was applied: (1) generate random normally distributed variables  $N(0,1)$  respecting the original dimensions of the data (30 individuals by 14 characters); (2) decompose the correlation matrix using Cholesky decomposition; and (3) post-multiply the upper triangular matrix resulting from the matrix factorization of step 2 by the matrix of step 1. Note that we chose the parametric bootstrap over the more commonly used non-parametric bootstrap (see Herrera et al. 2002 for an example) given that we found that under certain circumstances confidence intervals based on the latter may appear larger than the sample estimates (Peres-Neto and Magnan, unpublished results).

As for phenotypic means, levels of character integration may also vary. In order to assess the level of integration of each character, we calculated the eigenvalue variance within each treatment level for

**Table 1** Analyses of variance to test for the effects of treatment, species and their interaction. Error is measured by tanks nested within treatment  $\times$  species. The table reports means squares and

degrees of freedom ( $df$ ) for each effect. Values in bold indicate values that were significant ( $\alpha=0.05$ )

Factor ( $df$ )	Treatment (3)	Species (1)	Treatment $\times$ species (3)	Error tanks (treatment $\times$ species) (19)
Head length	<b>0.0026</b>	<b>0.0474</b>	<b>0.0017</b>	0.0004
Head height	0.0000	0.0006	0.0006	0.0007
Body height	<b>0.0039</b>	0.0003	0.0012	0.0006
Body width	<b>0.0075</b>	0.0007	0.0058	0.0014
Caudal peduncle height	<b>0.0035</b>	<b>0.1671</b>	0.0008	0.0006
Peduncle width	<b>0.0090</b>	<b>0.1705</b>	0.0053	0.0018
Caudal fin length	0.0059	<b>0.0305</b>	<b>0.0099</b>	0.0012
Dorsal fin base	0.0002	<b>0.0676</b>	<b>0.0017</b>	0.0011
Anal fin base	0.0059	<b>0.0744</b>	<b>0.0084</b>	0.0019
Head length at head height	<b>0.0014</b>	<b>0.0455</b>	<b>0.0011</b>	0.0003
Mouth height	<b>0.0172</b>	<b>0.3773</b>	0.0114	0.0020
Pectoral fin length	<b>0.0633</b>	<b>0.0708</b>	0.0221	0.0061
Standard length	<b>0.0008</b>	<b>0.0458</b>	<b>0.0007</b>	0.0002
Mouth width	<b>0.0088</b>	0.0007	0.0031	0.0014

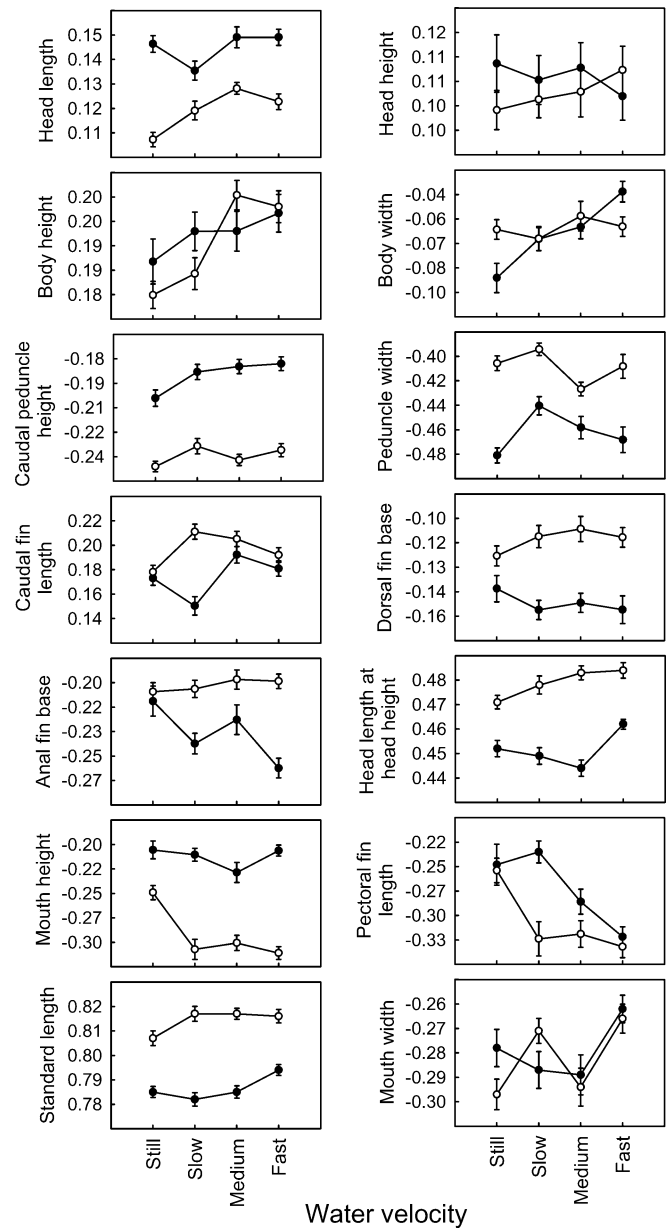
each species considering all characters but the one of interest. Thus, when comparing values between any given two characters calculated in this way, the one presenting the smallest eigenvalue variance would be more correlated (i.e., integrated) with the others than the one having the largest eigenvalue variance. To assess the overall magnitude of integration of individual characters, the same procedure as above was used, but considering all water velocity levels in one analysis. These values were also assessed for randomness (i.e., character independence) applying the procedure explained previously. Although a particular character may present high levels of integration, the patterns of correlation among characters may vary across treatments. In order to perform this contrast, we first calculated the eigenvectors of the first two principal components based on a correlation matrix for each treatment level and species separately. Eigenvectors were scaled so that loadings were equivalent to the Pearson product-moment correlation between the PC scores and the individual variables.

## Results

Most characters were plastic as they responded to the water velocity treatment (Table 1). Most traits also showed marked differences between species and interactions were significant for many characters (Table 1). The DFA confirmed that the two species were quite different in terms of shape, regardless of the treatment level (Table 2).

Reaction norm plots to swimming demand show that for both species, the largest difference occurred for pectoral fin length, which decreased dramatically with the increase of water velocity (Fig. 2; Table 1). Body height and standard length increased with water velocity, but body width only increased in brook charr. Brook charr individuals in the still treatment had smaller standard length than in the other treatments, whereas for Arctic charr only individuals under the fast treatment were larger than the others. Head length increased with water velocity mainly for Arctic charr, whereas for brook charr the slow and fast treatments presented comparable means. Head length at head height increased with water velocity for Arctic charr, whereas for brook charr it was higher only for the fast treatment level. Body height increased in both species. Some characters (e.g., caudal fin length) presented complex patterns of variation (polynomial) with values that were most comparable in the still and fast treatments.

A PCA considering all treatments for each species provided an overall description of induced changes in phenotypes, at least for characters that correlated across treatments (Fig. 3). The first two principal components

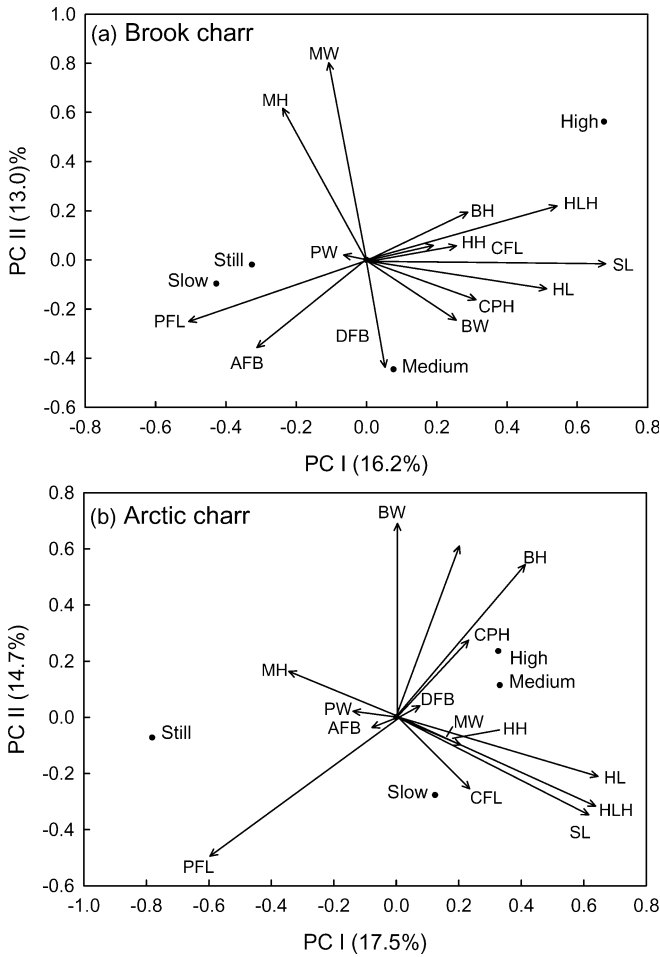


**Fig. 2** Reaction norms of brook charr (filled circles) and Arctic charr (open circles) to swimming demand. Values represent characters after Aitchinson log-ratio transformation (see methods)

summarized 29.2 and 32.2% of the total variation in the

**Table 2** Number of individual brook charr (*Salvelinus fontinalis*) and Arctic charr (*S. alpinus*) (out of 30 in each case) correctly classified according to each treatment level and species by a discriminant function analysis. Estimates were produced by jackknife

		Brook charr				Arctic charr			
		Still	Slow	Medium	Fast	Still	Slow	Medium	Fast
Brook charr	Still	19	4	3	3	1	0	0	0
	Slow	6	19	3	2	0	0	0	0
	Medium	4	2	17	7	0	0	0	0
	Fast	1	4	4	21	0	0	0	0
Arctic char	Still	0	0	0	0	24	1	3	2
	Slow	0	0	1	1	4	15	2	7
	Medium	0	0	0	0	2	1	18	9
	Fast	0	0	0	0	0	10	4	16



**Fig. 3a, b** Principal component analysis including all treatments for **a** brook charr and **b** Arctic charr. Arrows represent correlations between individual scores and morphometric variables. Each point represents the mean of individual scores within treatments

data for brook charr and Arctic charr, respectively. Treatment values represent the mean of individual scores within each treatment. Comparing the solutions between species, the most relevant pattern was that individuals in the still and slow treatment showed relatively larger pectoral fins and smaller standard and head lengths, suggesting that individuals raised in faster waters tended to be more streamlined than the ones raised in slower waters. In addition, body width and body height were larger for

individuals under higher swimming demands, especially in brook charr.

Characters examined were significantly integrated with-in all treatments for the two species, with the exception of the fast water treatment for brook charr (Table 3). Differences between species within each treatment level were significant with the exception of the medium velocity level, as indicated by the confidence intervals for the index of integration that did not overlap. In all cases, the magnitude of character integration was larger for Arctic charr than for brook charr. The magnitude of integration was negatively associated with water velocity, especially for Arctic charr. For brook charr, this tendency is not as strong as the medium water velocity presented the highest magnitude of integration (Table 3). The magnitude of integration for each character differed extensively among treatments levels, suggesting that swimming demand had a strong effect on the plasticity of character integration (Table 4). When computing the magnitude of integration for individual characters considering all treatments together, the length of pectoral fin, standard length, head length and head length at head height appeared to be more integrated than the other characters within treatment levels (Table 4).

Examining the distribution of eigenvectors along the first two principal components suggests that character correlations are both species and treatment specific (Table 5). We observed two patterns. First, the standard length and the length of the pectoral fin were usually associated with the first two components, but they usually loaded in different components, indicating an independence of these two characters within treatments. Second, the length of the pectoral fin is usually negatively correlated with either body height or head height, or both at times. Considering these two patterns aside, each treatment within species seemed to impose a somewhat distinctive pattern of integration among characters.

**Discussion**

Phenotypic plasticity and integration are important subjects for understanding whether and how environmental pressures contribute in shaping phenotypes. The ecological processes that induce phenotypic plasticity are still not well known, though they would bring light into how the interaction between selective pressures and plasticity may

**Table 3** Within-treatment integration among morphometric characters for brook charr and Arctic charr, as estimated by the variance of eigenvalues of their correlation matrices. Confidence interval

under the expectation of no correlation between characters is 0.352–0.624. Confidence intervals were estimated by parametric bootstrap (see Materials and methods)

Treatment	Brook charr		Arctic charr	
	Integration index	95% confidence interval	Integration index	95% confidence interval
Still	0.873	0.783–0.913	1.500	1.330–1.552
Slow	0.769	0.692–0.813	1.000	0.889–1.044
Medium	0.980	0.888–1.018	0.901	0.799–0.951
Fast	0.545	0.495–0.588	0.738	0.671–0.773

**Table 4** Level of integration of each character, as measured by the variance of eigenvalues of correlation matrices based on all characters but the one of interest (see Materials and methods for details). Bootstrap confidence interval is presented only for overall integration, which was based on correlation matrices considering all treatments. Within-treatment confidence interval under the expectation of no correlation between characters is 0.352–0.624 (matrices based on 30 individuals), whereas for the overall estimates is 0.087–0.154 (matrices based on 120 individuals). Values in bold indicate the largest levels of integration within a column (see Methods for interpretation)

	Still	Slow	Medium	Fast	Overall	95% confidence interval
<b>Brook charr</b>						
Head length	0.822	0.751	<b>0.835</b>	0.525	<b>0.389</b>	0.374–0.394
Head height	0.788	0.711	0.930	0.512	0.400	0.385–0.406
Body height	0.848	0.704	0.951	<b>0.484</b>	0.388	0.373–0.395
Body width	0.849	<b>0.640</b>	0.917	0.520	0.409	0.393–0.416
Caudal peduncle height	0.836	0.783	0.978	0.495	0.412	0.396–0.418
Peduncle width	0.887	0.739	0.952	<b>0.473</b>	0.405	0.390–0.411
Caudal fin length	0.820	0.658	0.919	0.501	0.406	0.390–0.412
Dorsal fin base	0.848	0.768	0.971	0.550	0.418	0.401–0.424
Anal fin base	<b>0.714</b>	0.769	0.890	0.512	0.389	0.373–0.395
Head length at head height	0.783	0.740	0.962	0.506	<b>0.368</b>	0.353–0.372
Mouth height	0.781	0.686	0.879	0.524	0.378	0.362–0.384
Pectoral fin length	<b>0.767</b>	<b>0.651</b>	0.896	0.494	<b>0.348</b>	0.334–0.355
Standard length	<b>0.760</b>	<b>0.680</b>	<b>0.884</b>	0.499	<b>0.353</b>	0.340–0.359
Mouth width	0.845	0.715	<b>0.777</b>	<b>0.488</b>	0.374	0.359–0.381
<b>Arctic charr</b>						
Head length	1.392	1.019	0.885	0.685	<b>0.380</b>	0.364–0.386
Head height	1.409	<b>0.858</b>	0.833	0.723	0.416	0.399–0.421
Body height	1.349	0.905	0.933	<b>0.637</b>	0.397	0.382–0.403
Body width	1.500	0.910	<b>0.753</b>	<b>0.651</b>	0.400	0.385–0.406
Caudal peduncle height	1.509	0.955	0.833	0.734	0.438	0.421–0.445
Peduncle width	1.438	<b>0.831</b>	0.801	0.690	0.449	0.432–0.456
Caudal fin length	1.408	1.028	0.876	0.680	0.424	0.408–0.431
Dorsal fin base	1.529	0.947	0.805	0.762	0.455	0.437–0.461
Anal fin base	<b>1.283</b>	0.975	0.921	0.670	0.449	0.430–0.455
Head length at head height	1.361	0.908	<b>0.789</b>	0.659	<b>0.349</b>	0.334–0.355
Mouth height	1.404	0.972	0.862	0.722	0.420	0.404–0.427
Pectoral fin length	<b>1.128</b>	<b>0.815</b>	<b>0.776</b>	0.719	<b>0.332</b>	0.318–0.339
Standard length	1.464	0.914	0.799	<b>0.610</b>	<b>0.359</b>	0.344–0.366
Mouth width	<b>1.312</b>	0.963	0.844	0.653	0.441	0.423–0.449

drive morphological evolution (Day et al. 1994). The study of the variation of single characters and their reaction norms is important in determining functional responses and the direction of changes under potential selective pressures. Correspondingly, the study of phenotypic integration may help to recognize, among other issues, how characters are coordinated with each other and to what extent this organization allows or constrains morphological plasticity, and ultimately how characters may respond to selection (Waite and Levin 1993). The aim of this study was to address whether swimming demand could induce morphological plasticity in the directions expected under divergent habitat selection and to evaluate its influence on the plasticity of integration. Because the two species present different levels of plasticity in the wild, we were interested in how these differences may reflect in their levels of plasticity and integration under a potential selective factor (i.e., swimming demand). To our knowledge, this is the first study to evaluate morphological integration and the plasticity of morphological integration in fishes.

#### Phenotypic plasticity

We found that differential swimming demands influenced the reaction norms and phenotypic integration of both brook charr and Arctic charr. Although there were some traits for which the direction of changes was qualitatively similar across treatments (e.g., decrease or increase), their functional responses were generally quite different (e.g., linear versus polynomial) among traits and between species. Differences in reaction norms between species may either reflect adaptations to habitat levels (i.e., microhabitats) that are finer than we perceive (Robinson and Parsons 2002) or simply that evolutionary constraints (e.g., differential historic patterns of evolution) influence differently their levels of plasticity (Witte et al. 1990).

Has differential swimming demand induced some of the morphological differences that are expected to have evolved in the benthic and limnetic habitats? Robinson and Parsons (2002) provided a qualitative description of the expected morphological differences between the two morphs. Benthic morphs are characteristically more robust (i.e., deeper bodies and larger heads), having longer paired fins (pectoral or pelvic) and deeper, but compressed,

**Table 5** Within-treatment eigenvector structure for the first two principal components (PC) based on the correlation matrix of morphological characters of brook charr and Arctic charr. Largest values are presented in bold

	Still		Slow		Medium		Fast	
	PC		PC		PC		PC	
	I	II	I	II	I	II	I	II
<b>Brook charr</b>								
Percentage of explanation	21.88	18.86	21.33	15.85	21.88	17.43	18.01	14.21
Head length	0.52	-0.04	0.15	0.43	<b>0.82</b>	0.12	0.43	0.04
Head height	0.46	<b>-0.62</b>	-0.33	<b>0.63</b>	-0.01	<b>0.68</b>	-0.03	<b>0.64</b>
Body height	0.45	-0.24	-0.20	<b>0.76</b>	-0.29	0.48	-0.29	<b>0.69</b>
Body width	-0.13	<b>0.59</b>	<b>-0.76</b>	0.18	0.50	0.21	0.48	0.08
Caudal peduncle height	0.40	0.44	-0.15	0.18	-0.12	0.24	<b>0.55</b>	0.21
Peduncle width	0.07	0.35	-0.47	-0.17	-0.17	<b>0.64</b>	-0.49	-0.37
Caudal fin length	0.41	0.54	<b>0.74</b>	-0.03	-0.09	-0.27	0.35	0.33
Dorsal fin base	0.04	<b>0.63</b>	-0.13	-0.33	0.38	-0.02	0.16	0.17
Anal fin base	<b>-0.74</b>	0.44	0.16	0.21	<b>0.58</b>	-0.34	-0.46	0.36
Head length at head height	<b>0.73</b>	0.04	0.41	0.06	-0.06	0.28	<b>0.60</b>	-0.10
Mouth height	<b>-0.58</b>	-0.08	<b>0.59</b>	<b>-0.48</b>	<b>-0.61</b>	0.04	-0.34	-0.01
Pectoral fin length	0.01	<b>-0.62</b>	-0.51	<b>-0.71</b>	0.11	<b>-0.83</b>	0.18	<b>-0.69</b>
Standard length	<b>0.74</b>	0.23	<b>0.59</b>	0.22	0.54	<b>0.50</b>	<b>0.61</b>	-0.19
Mouth width	-0.27	-0.47	0.54	0.16	<b>-0.92</b>	-0.13	<b>-0.50</b>	<b>-0.38</b>
<b>Arctic charr</b>								
Percentage of explanation	33.01	15.85	24.07	19.61	24.66	16.19	20.05	15.83
Head length	<b>0.64</b>	-0.14	-0.28	0.25	0.24	0.57	0.24	<b>0.60</b>
Head height	0.47	<b>0.70</b>	0.65	0.47	<b>-0.60</b>	-0.06	-0.42	-0.14
Body height	0.63	<b>0.48</b>	<b>0.71</b>	0.11	-0.19	0.03	<b>-0.77</b>	0.16
Body width	-0.15	<b>0.76</b>	<b>0.68</b>	0.04	<b>-0.75</b>	0.21	<b>-0.65</b>	0.30
Caudal peduncle height	0.43	-0.01	0.31	-0.47	<b>-0.57</b>	-0.11	-0.10	-0.27
Peduncle width	-0.51	0.44	<b>0.81</b>	-0.12	<b>0.66</b>	0.32	-0.14	<b>-0.57</b>
Caudal fin length	0.52	<b>-0.55</b>	-0.07	-0.29	0.41	-0.34	0.35	<b>-0.62</b>
Dorsal fin base	-0.31	-0.36	-0.42	0.46	-0.52	<b>0.60</b>	-0.03	0.00
Anal fin base	<b>-0.74</b>	-0.09	0.44	0.34	-0.09	-0.13	0.56	-0.10
Head length at head height	0.64	-0.14	-0.08	<b>0.75</b>	0.56	<b>0.60</b>	<b>0.57</b>	0.49
Mouth height	0.52	0.15	0.18	<b>-0.56</b>	-0.45	0.23	0.12	0.33
Pectoral fin length	<b>-0.90</b>	-0.27	<b>-0.83</b>	0.01	0.48	<b>-0.70</b>	0.22	-0.11
Standard length	0.50	-0.28	-0.04	<b>0.73</b>	0.51	<b>0.62</b>	<b>0.74</b>	0.34
Mouth width	<b>0.70</b>	-0.27	-0.21	<b>-0.63</b>	0.48	-0.11	-0.40	<b>0.67</b>

caudal peduncles. Pelagic morphs have slender elongated bodies, smaller paired fins and shallower, but widened, caudal peduncles. This contrast is expected mainly due to differences in search tactics that the benthic and limnetic habitats impose correspondingly. Whereas a streamlined shape enhances prolonged swimming ability and is energetically efficient in foraging for patchily distributed prey in open water, deeper body shape improves burst swimming performance and is advantageous when swimming in complex habitats, such as littoral areas. Longer pectoral fins enhance maneuverability in complex habitats (Webb 1984), but shortening the pectoral fins decreases drag (Drucker and Lauder 2003).

Contrasting the reaction norms and the PCAs considering all treatments (Fig. 3), the pectoral fin, standard length, head length and body height were consistently the most important characters differentiating individuals across treatments. As expected, pectoral fins were larger in individuals raised in slower waters. Individuals raised in

faster waters seemed to present relatively longer bodies and head, suggesting a more streamlined body when compared with individuals under the slow water treatments. Dynes et al. (1999) compared wild benthic and limnetic individuals of brook charr and found that limnetic fish had shorter pectoral fins, higher caudal peduncle, and longer body length posterior to the dorsal fin in comparison to benthic fish. In both species, benthic individuals present larger pectoral fins in comparison to lentic individuals in the wild (Bourke et al. 1997; Dynes et al. 1999; Proulx and Magnan 2002; Jonsson and Jonsson 2001). In the present study, we also observed that higher caudal peduncles were induced in brook charr at lower water velocities. Contrary to predictions, body height increased with water velocity. Pakkasmaa and Piironen (2001) also found that the Atlantic salmon (*Salmo salar*), but not brown trout (*Salmo trutta*), were higher-bodied when reared in fast water velocity, revealing a taxon-specific response. Interestingly, Andersson (2003) found

that Arctic charr individuals under a zooplankton diet (i.e., pelagic environment) achieved relatively deeper bodies than individuals fed with chironomids (i.e., benthic environment). In sum, changes in morphology were largely induced by differential water velocity in both species and the length of the pectoral fin was the character that responded most strongly according to the predicted morphological expectations under divergent habitat selection.

The only study that parallels ours in terms of phenotypic plasticity (Imre et al. 2002) found that only caudal fin height and caudal peduncle depth showed a significant change with water velocity in brook charr. Here we found that water velocity induced changes in most of the traits measured, including those two traits. These differences may be related to two possible aspects. First, we have applied four water velocity treatments, whereas Imre et al. (2002) applied only two. In many pairwise differences between two treatments, a number of traits would have not demonstrated significant effects. Second, and most importantly, the water velocity was modified to maintain a constant rate in relation to the standard length of the fish, whereas they kept water velocity constant throughout the entire experiment. In their experiment, since they were interested in contrasting their results with stream populations, keeping water constant may reflect better the ecological conditions that fish encounter in streams while growing. Conversely, in lakes, fish may adjust their swimming speed as a function of size in order to maximize their chances of encountering patches of food, especially in the pelagic zone. Andersson (2003; see his Fig. 5) found that as fish grow, their swimming speeds increase, thus supporting our assumption. This could explain why polymorphism has been observed for brook charr in lakes (Bourke et al. 1997; Dynes et al. 1999), but not in streams (McLaughlin 2001).

### Morphological integration

The degree of integration affecting characters may have important consequences in the evolution of phenotypes. To a greater extent (e.g., canalization—genetic but no phenotypic variability), integration may impose constraints on the plasticity of characters, limiting phenotypic variability, and it may, by consequence, restrain adaptation to novel conditions (Badyaev and Foresman 2000). To a lesser extent, if characters are too independent, it may indicate a lack of coordination among functionally important structures, which in turn may affect fitness (Schlichting 1989).

The magnitude of character integration varied between 3.9 and 10.7% of the maximum possible (i.e., the number of traits involved = 14). These values are consistent with field estimates for brook charr (unpublished results). The relatively low levels of integration and differences found in the morphological correlation structure among treatments suggest that constraints on morphological change are unlikely in both species, thus allowing great potential

for phenotypic flexibility. This decoupling of morphological traits suggests that most characters do not make consistent joint contributions to individual fitness (Merila and Bjorklund 1999) under differential swimming demands. However, pectoral fin, standard length and head length appeared to be more integrated despite the fact that their level of correlation with other characters varied across treatments. This may suggest the existence of some complex pattern of joint variation in which, if selection favors any particular set of characters, pectoral fin, for instance, would be also selected.

There seems to be little limit to plasticity in the studied species, which begs the question of why Arctic charr is more polymorphic in the wild than brook charr. Age of speciation can probably be ruled out, as brook charr is more basal than Arctic charr (Westrich et al. 2002). We see at least two possible, and in no way mutually exclusive, explanations of why polymorphism is more conspicuous in Arctic charr than brook charr. The first possibility is genetics. In brook charr, although existent, it seems that the degree of genetic differentiation between benthic and limnetic morphs is very small (Dynes et al. 1999), whereas in Arctic charr there is evidence that the genetic differentiation can be quite large (see Jonsson and Jonsson 2001 for a review). Thus, the absence of genetic isolation between morphs may promote difficulties in the fixation of characters in brook charr when compared to Arctic charr.

The second possibility comes from evidence found in the present study. Although both species were relatively plastic under differential swimming demands, we found that the levels of phenotypic integration were slightly higher in Arctic charr than brook charr. Plasticity may increase evolutionary opportunities and even genetic divergence in contrast to the hypothesis that plasticity would obscure genetic variation, hence constraining divergence between populations (see Robinson and Parsons 2002, for a discussion). However, some intermediate level of correlation may appear advantageous. If selection favors the increase or decrease of the phenotypic value of a particular character and there is positive genetic correlation of this character with another character that is also favored by the same selection regime, the population will respond more quickly (Pigliucci 2001). Thus selection might favor stronger correlations between characters that are functionally related (Berg 1960; Cheverud 1995; Armbruster et al. 1999) or as Dobzhansky (1970, p 210) posed: “the correlated responses to selection for desirable traits.” Most characters studied here are expected to correlate with swimming performance. It follows that Arctic charr populations may diverge more quickly as they may present a better balance between flexibility and coordination of characters than brook charr populations. However, making evolutionary inferences of these types assumes that phenotypic correlations are a sufficient depiction of genetic correlations (Waitt and Levin 1993), which are shared with previous studies that have examined phenotypic plasticity and integration under an evolutionary viewpoint (Merilä and Björklund 1999; Herrera et al. 2002 and references therein).

The lake environment presents habitat pressures that require differential demands regarding resource acquisition and swimming abilities. Given the high plastic potential and independence of characters, the interaction of swimming demand with other selective pressures may be relevant. Much of the direct evidence suggesting that habitat choice can induce phenotypic variation in lake fishes comes from experiments designed to evaluate the influence of benthic versus limnetic feeding environments on phenotypic variation (Day et al. 1994; Adams et al. 2003; Andersson 2003; see Robinson and Parsons 2002 for a review). However, some of the expected morphological responses imposed by the littoral versus pelagic environments would seem more in accordance with swimming demands than the mechanics involved in handling prey. In contrast to morphological characters related to swimming, at least for Arctic charr, there is some evidence that characters related directly to trophic morphology (head anatomy) are more integrated compared to the ones studied here (Adams et al. 2003; the first principal component based on nine morphological variables retained 48% of the variation whereas here only between 16.2% for brook charr and 17.5% for Arctic charr was retained). Therefore, if characters act somewhat independently under swimming demands, they may be easily “allocated” separately under other ecological demands such as feeding, perhaps inducing a greater match between morphology and ecology under a mix of ecological pressures. However, the results of the present study indicate that swimming demand alone may not be sufficient to generate the polymorphism encountered in nature. Andersson (2003) also concluded that diet itself was not enough to induce the differences found in Arctic charr in nature. Therefore, given that both diet and swimming demands can induce morphological changes, it would be central to conduct experiments targeting the interaction between group of characters related to trophic (head anatomy) and swimming demands (body anatomy).

**Acknowledgements** We would like to thank Jens Andersson, Steven Kohler, and an anonymous reviewer for providing valuable comments on our manuscript. We thank Sophia Balcomb and Craig Osenberg for editorial comments. We thank Pierre East for his invaluable assistance in designing and running the laboratory experiments, and Steve Garçeau for morphological measurements. Raphaël Proulx gave helpful comments at different stages of the present study. The laboratory work was supported by a grant from le Fonds pour la formation des chercheurs et d’aide à la recherche (FCAR) and la Société de recherche et de développement en aquaculture continentale (SORDAC) (Action concertée FCAR-SORDAC program) to P. Magnan. The analytical work was supported by the Canada Research Chair program to P. Magnan. P.R. Peres-Neto was supported by a Postdoctoral fellowship from the Canada Research Chair in Freshwater Ecology.

## References

- Adams CE, Woltering C, Alexander G (2003) Epigenetic regulation of trophic morphology through feeding behaviour in Arctic charr, *Salvelinus alpinus*. *Biol J Linn Soc* 78:43–49
- Aitchinson J (1986) The statistical analysis of compositional data. Chapman and Hall, New York
- Andersson J (2003) Effects of diet-induced resource polymorphism on performance in Arctic char (*Salvelinus alpinus*). *Evol Ecol Res* 5:213–228
- APHA (American Public Health Association), American Water Work Association, and Water Pollution Control Federation (1989) Standard methods for the examination of water and wastewater, 17th edn. APHA Press, Washington
- Armbruster WS, Di Stilio VS, Tuxill JD, Flores TC, Runk JLV (1999) Covariance and decoupling of floral and vegetative traits in nine neotropical plants: A re-evaluation of Berg’s correlation-pleiades concept. *Am J Bot* 86:39–55
- Badyaev AV, Foresman KR (2000) Extreme environmental change and evolution: stress-induced morphological variation is strongly concordant with patterns of evolutionary divergence in shrew mandibles. *Proc R Soc Lond B* 267:371–377
- Berg RL (1960) The ecological significance of correlation pleiades. *Evolution* 14:171–180
- Bourke P, Magnan P, Rodriguez MA (1997) Individual variations in habitat use and morphology in brook charr. *J Fish Biol* 51:783–794
- Bourke P, Magnan P, Rodriguez, MA (1999) Phenotypic responses of lacustrine brook charr in relation to the intensity of interspecific competition. *Evol Ecol* 13:19–31
- Cheverud JM (1995) Morphological integration in the saddle-back tamarin (*Saguinus fuscicollis*) cranium. *Am Nat* 145:63–89
- Cheverud JM, Wagner GP, Dow MM (1989) Methods for the comparative-analysis of variation patterns. *Syst Zool* 38:201–213
- Day T, Pritchard J, Schluter D (1994) A comparison of two sticklebacks. *Evolution* 48:1723–1734
- Dobzhansky T (1970) Genetics of the evolutionary process, 1st edn. Columbia University Press, New York
- Drucker EG, Lauder G (2003) Function of pectoral fins in rainbow trout: behavioral repertoire and hydrodynamic forces. *J Exp Biol* 206:813–826
- Dynes J, Magnan P, Bernatchez L, Rodriguez MA (1999) Genetic and morphological variation between two forms of lacustrine brook char. *J Fish Biol* 54:955–972
- East P, Magnan P (1987) The effect of locomotor activity on the growth of brook char, *Salvelinus fontinalis* Mitchell. *Can J Zool* 65:843–846
- Efron B, Tibshirani RJ (1993) An introduction to the bootstrap. Chapman & Hall, New York
- Fleming IA, Jonsson B, Gross MR (1994) Phenotypic divergence of sea-ranched, farmed, and wild salmon. *Can J Fish Aquat Sci* 51: 2808–2824
- Herrera CM, Cerda X, Garcia MB, Guitian J, Medrano M, Rey PJ, Sanchez-Lafuente AM (2002) Floral integration, phenotypic covariance structure and pollinator variation in bumblebee-pollinated *Helleborus foetidus*. *J Evol Biol* 15:108–121
- Imre I, McLaughlin RL, Noakes DLG (2002) Phenotypic plasticity in brook char: changes in caudal fin induced by water flow. *J Fish Biol* 61:1171–1181
- Jonsson B, Jonsson N (2001) Polymorphism and speciation in Arctic char. *J Fish Biol* 58:605–638
- Kawata M (2002) Invasion of vacant and subsequent sympatric speciation. *Proc R Soc Lond B* 269:55–63
- Lewontin RC (1978) Adaptation. *Sci Am* 239:213–231
- Ling EN, Cotter D (2003) Statistical power in comparative aquaculture studies. *Aquaculture* 224:159–168
- McLaughlin RL (2001) Behavioural diversification in brook char: adaptive responses to local conditions. *J Anim Ecol* 70:325–337
- Mëriila J, Bjorklund M (1999) Population divergence and morphometric integration in the greenfinch (*Carduelis chloris*)—evolution against the trajectory of least resistance? *J Evol Biol* 12:103–112

- 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. Gouvernement du Québec, Québec
- Moran MD (2003) Arguments for rejecting the sequential Bonferroni in ecological studies. *Oikos* 100:403–405
- Olden JD, Jackson DA, Peres-Neto PR (2002) Predictive models for fish species distributions: a note on proper validation and chance predictions. *Trans Am Fish Soc* 131:329–336
- Pakkasmaa S, Piironen J (2001) Water velocity shapes salmonids. *Evol Ecol* 14:721–730
- Peres-Neto PR (1999) How many statistical tests are too many? The problem of conducting multiple inferences revisited. *Mar Ecol Prog Ser* 176:303–306
- Peres-Neto PR, Jackson DA, Somers KM (2003) Giving meaningful interpretation to ordination axes: assessing loading significance in principal component analysis. *Ecology* 84:2347–2363
- Pigliucci M (2001) Phenotypic plasticity: beyond nature and nurture. The Johns Hopkins University Press, Baltimore
- Pigliucci M, Cammell K, Schmitt J (1999) Evolution of phenotypic plasticity: a comparative approach in the phylogenetic neighbourhood of *Arabidopsis thaliana*. *J Evol Biol* 12:779–791
- Proulx R, Magnan P (2002) Physiological performance of two forms of lacustrine brook char, *Salvelinus fontinalis*, in the open-water habitat. *Environ Biol Fish* 64:127–136
- Reist JD (1986) An empirical-evaluation of coefficients used in residual and allometric adjustment of size covariation. *Can J Zool* 64:1363–1368
- Robinson BW, Parsons KJ (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
- Schlichting, CD (1989) Phenotypic integration and environmental change. *BioScience* 39:460–464
- Schluter D (1996) Ecological speciation in postglacial fishes. *Phylos Trans R Soc Lond B* 351:807–814
- Skúlason S, Smith TB (1995) Resource polymorphism in vertebrates. *Trends Environ Ecol* 10:366–370
- Skúlason S, Noakes DLG, Snorrason SS (1989) Ontogeny of trophic morphology of four sympatric morphs of Arctic char *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biol J Linn Soc* 38:281–301
- Stearns SC (1989) The evolutionary significance of phenotypic plasticity. *BioScience* 39:436–445
- Taylor EB, McPhail JD (1985) Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Can J Fish Aquat Sci* 42:2020–2028
- Waddington CH (1953) Genetic assimilation of an acquired character. *Evolution* 7:118–126
- Wagner GP (1984) On the eigenvalue distribution of genetic and phenotypic dispersion matrices—evidence for a nonrandom organization of quantitative character variation. *J Math Biol* 21:77–95
- Waite DE, Levin DA (1993) Phenotypic integration and plastic correlations in *phlox-drummondii* (polemoniaceae). *Am J Bot* 80:1224–1233
- Webb PW (1982) Locomotor patterns in the evolution of the actinopterygian fishes. *Am Zool* 22:329–342
- Webb PW (1984) Body form, locomotion and foraging in aquatic invertebrates. *Am Zool* 24:107–120
- Westrich KM, Konkola NR, Matsuokab MP, Phillips RB (2002) Interspecific relationships among charrs based on phylogenetic analysis of nuclear growth hormone intron sequences. *Environ Biol Fish* 64:217–222
- Witte F, Barel CDN, Hoogerhoud RJC (1990) Phenotypic plasticity of anatomical structures and its ecomorphological significance. *Neth J Zool* 40:278–298