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Inherited differences in foraging behaviour in the offspring of two forms of lacustrine brook charr

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ABSTRACT

Hypothesis: Behavioural differences observed in the field between littoral and pelagic brook charr, *Salvelinus fontinalis* (Mitchill), persist in their progeny when progeny are reared in a common-garden laboratory environment.

Methods: Young-of-the-year of the pelagic and littoral forms were raised in the laboratory. Experiments began when the fry were approximately 4 months after the first feeding. Swimming and foraging behaviour, as well as the morphological characteristics of individuals, were measured.

Results: Morphological characters used to identify brook charr ecotypes in the field (pectoral fin length and dorsal fin base length) were inherited in the progeny. Littoral offspring exhibited a deeper body and longer pectoral fins and dorsal fin base than pelagic offspring. Furthermore, the foraging experiments indicated that naive littoral offspring were significantly more efficient than pelagic offspring when feeding on bloodworms (higher capture rate and lower rejection rate). There were no significant differences between the pelagic and littoral individuals when feeding on *Daphnia* sp. Although subtle, these results suggest that there is a genetic component to trophic polymorphism in brook charr and that behavioural diversification is not constrained by morphological traits: the few differences in feeding-related morphology between littoral and pelagic individuals cannot explain the observed differences in the capture and rejection rates of bloodworms.

Keywords: activity pattern, brook charr, capture rate, foraging behaviour, morphology, swimming behaviour, trophic polymorphism.

INTRODUCTION

Trophic polymorphism occurs when individuals of the same species express different phenotypes related to differential niche use. Usually, divergent forms (or ecotypes) can be distinguished on the basis of several traits including behaviour, colouration, morphology, and life history (Smith and Skúlason, 1996). In northern latitudes, lakes offer two main discrete functional habitats, the littoral and the pelagic areas (Schluter, 1996), and studies of trophic

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polymorphism generally involve littoral and pelagic forms associated with these habitats (Robinson and Wilson, 1994; Smith and Skúlason, 1996; Robinson and Parsons, 2002).

Among divergent traits, behaviour may constitute an early stage in the development and evolution of trophic polymorphism (Wimberger, 1994; Smith and Skúlason, 1996; McLaughlin, 2001; Swanson *et al.*, 2003). Ecological contexts that promote the initial diversification of behaviours are still not well understood, in part because the literature on trophic polymorphism reports cases where behavioural differences are related to well-defined ecotypes (e.g. Malmquist, 1992; Skúlason *et al.*, 1993; Adams and Huntingford, 2002; Klemetsen *et al.*, 2002; Rogers *et al.*, 2002). There are essentially two kinds of trophic polymorphism in fishes: one involves conspicuous differences between forms, while the other is much more subtle, where morphological differences can only be detected with accurate measurements and multivariate statistical analysis (Skúlason and Smith, 1995; Robinson and Wilson, 1996; Bourke *et al.*, 1999; Peres-Neto and Magnan, 2004). It is thought that behavioural diversification precedes morphological diversification (Wimberger, 1994; Skúlason and Smith, 1995; Bourke *et al.*, 1999; Adams *et al.*, 2003). However, few studies have reported examples of behavioural differences in foraging tactics, where individuals exhibit no or only subtle morphological segregation (McLaughlin, 2001; Svanbäck and Eklöv, 2006). Recently emerged *S. fontinalis* foraging in still-water pools along the sides of streams exhibited conspicuous differences in foraging tactics without exhibiting morphological segregation (see McLaughlin *et al.*, 1994, 1999; McLaughlin, 2001). Studies in this kind of system could allow a better understanding of conditions and mechanisms promoting behavioural specialization before its reinforcement by morphological differences. Heredity (Skúlason *et al.*, 1993; Adams and Huntingford, 2002), behavioural flexibility related to spatial and temporal variations in food availability (Dill, 1983; Day and McPhail, 1996), or some kind of learning (Ehlinger, 1989, 1990) have been proposed to explain behavioural divergences.

Brook charr inhabiting oligotrophic lakes of the Canadian Shield exhibit a subtle trophic polymorphism, where some individuals are specialists better adapted to feeding in the littoral habitat and 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). In a study using telemetry, Bourke *et al.* (1997) found that adult brook charr exhibited individual differences in habitat use in two lakes of this system: 50% of radio-tracked individuals were found mainly in the littoral zone, 18% in the pelagic zone, and 32% travelled between the two zones. Furthermore, individual differences in habitat preference were related to functional differences in body morphology: pelagic individuals are more fusiform and have shorter pectoral fins than littoral individuals (Bourke *et al.*, 1999; Dynes *et al.*, 1999; Proulx and Magnan, 2002, 2004; Marchand *et al.*, 2003). In this example, differences in morphology are related to swimming modes (cruising versus manoeuvring) but not to feeding traits.

The objective of the present study was to determine if behavioural differences between forms observed in the field persist in their progeny when progeny are reared under similar laboratory conditions. Based on habitat use by the two forms in the field (Bourke *et al.*, 1997), it was hypothesized that offspring of the pelagic form would spent more time swimming in the water column while those of the littoral form would spend more time near the bottom. Furthermore, experiments on feeding performance were conducted to assess differences between the two forms when they feed on typical zooplankton (*Daphnia* sp.) and benthic (bloodworms) prey. Based on diet segregation observed in the field (see Bourke *et al.*, 1999), it was hypothesized that naive offspring of littoral individuals will have higher capture rates than the pelagic ones when feeding on benthic prey and vice versa if the feeding behaviour of ecotypes is heritable. Even though the heritability of morphological traits was recently demonstrated in this case of subtle polymorphism (Proulx and Magnan, 2004), we investigated the

morphology of our experimental fish to confirm that behavioural comparisons were based on the proper phenotypes.

MATERIALS AND METHODS

Experimental fish and rearing conditions

During the fall of 2003, littoral and pelagic brook charr were sampled on the main spawning ground of Lake Ledoux in the Mastigouche Reserve in Québec (46°48′10″N, 73°16′45″W) using gill nets. To identify ecotypes in the field, we used allometric relationships between body and fin lengths that had been developed for each form in a previous study (Bourke *et al.*, 1997). In the field, pelagic individuals were selected if the lengths of both the pectoral and dorsal fins were below those expected from the size-adjusted regression lines for the pelagic form, while littoral fish were selected if the lengths of both fins were above the size-adjusted regression line for the littoral form. Eggs were fertilized artificially according to the dry method described by Piper *et al.* (1982) to produce three full-sib families for each of the pelagic and littoral forms.

Eggs were incubated at 6 ± 0.1°C in ascending-current incubators (MariSource, Milton, WA) connected to a glycol cooling system. After hatching, 300 individuals per family were transferred into separate 76-litre rearing tanks. Upon yolk sac resorption, exogenous feeding was initiated with commercial food pellets distributed from automatic overhead feeders that functioned continuously over the 12 h daylight period. The offspring were fed with *Biodiet starter* Corey #0.5, #0.7, and #1.0 food pellets as they grew. The daily quantity of food was estimated from bioenergetic models for salmonid rearing (Ralston Purina, Canada). Rearing tanks were equipped with biological filters and were connected to a glycol cooling system (± 0.1°C). Water alkalinity and hardness were adjusted to 65 $mg \cdot l^{-1}$ of $CaCO_3$ by adding sodium bicarbonate (Na_2HCO_3) and calcium chloride ($CaCl_2$). Light intensity (~40 lux), water temperature (12°C), and photoperiod (12 h light/12 h dark) were held constant during the rearing period. One month before the beginning of the experiment, fish were acclimated to a water temperature of 15°C. Ammonia (NH_3; $\mu g \cdot l^{-1}$), nitrites (NO_2; $mg \cdot l^{-1}$), and water hardness ($mg \cdot l^{-1}$ of $CaCO_3$) were estimated using standard procedures (American Public Health Association, 1989) and kept within the tolerance limits for salmonid aquaculture (MAPAQ, 1990). All manipulations involving fish met Canadian Council on Animal Care regulations and were supervised by the Animal Care Committee of the Université du Québec à Trois-Rivières.

Experimental conditions

Experiments began when the fry were approximately 4 months after their first feeding. The experimental set-up consisted of twelve 50-litre aquaria (50 cm long × 25 cm wide × 40 cm high) set on shelves. To ensure that the experimental conditions were as homogeneous as possible, all aquaria were equipped with an overhead fluorescent light (Aqua-Glo Fluorescent, 14 W). This system provides a light spectrum similar to natural conditions. The photoperiod remained 12 h light/12 h dark and the lights were switched on one hour before the beginning of the experiments. The water was kept at room temperature (range 14–16°C throughout the experiment). The back of each aquarium was covered with a white plastic plate to facilitate observations of fish and their prey during the trials. Black curtains were

placed on the sides to prevent any interaction between adjacent aquaria. All experiments were video-recorded using a camera (Panasonic WV-BP130 series; ⅓″ B/W CCD) set in front of the aquaria to allow later viewing. For feeding trials, direct observations were also made behind a black curtain to avoid disturbing the fish. The fish did not show any signs of stress during the experiments.

Morphological analyses

Morphological characters (Fig. 1) were measured on the left side of the fish at the end of each trial with a Mytutoyo digital calliper (± 0.01 mm) connected to a computer. The swimming and trophic characters selected are among the most representative to discriminate ecotypes in several polymorphic species (e.g. Malmquist, 1992; Adams *et al.*, 1998; Jonsson and Skúlason, 2000; Robinson *et al.*, 2000; Proulx and Magnan, 2002). Total length (± 0.1 mm) and mass (± 0.01 g) were also measured. Sex could not be determined due to the small size of individuals.

Activity patterns

The time in seconds spent performing each of the following behaviours was recorded to compare the activity patterns of both ecotypes:

- *Fast swimming*: when the fish was using caudal propulsion for swimming.
- *Slow swimming*: when the fish was using mostly its pectoral fins for displacement.

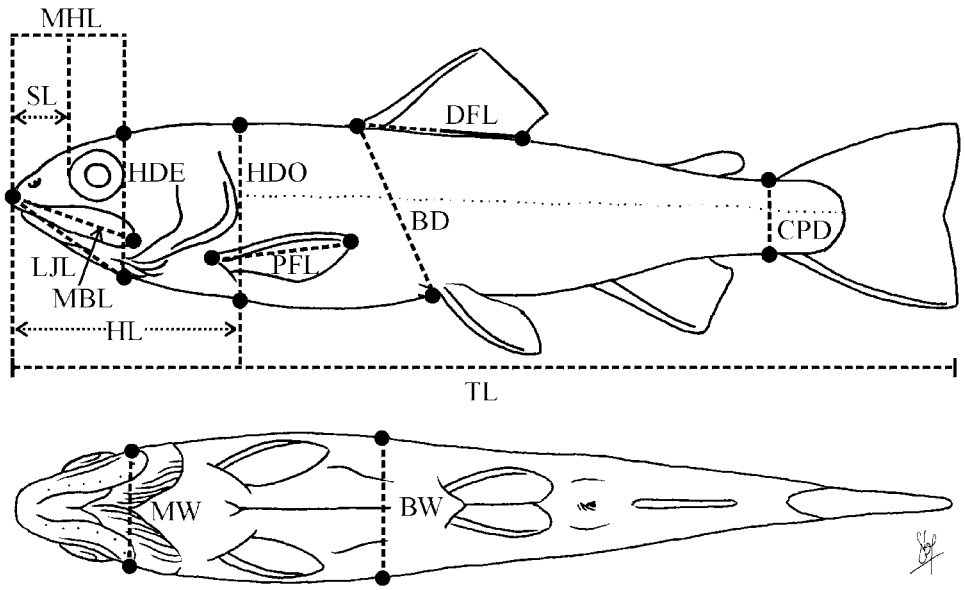

Fig. 1. Locations of the morphological characters related to feeding efficiency and swimming measured on the left side of the young-of-the-year brook charr. MHL: mid-head length; SL: snout length; LJL: lower jaw length; MBL: maxillary bone length; HL: head length; HDE: head depth at the eye; HDO: head depth at the operculum; PFL: pectoral fin length; DFL: dorsal fin base length; BD: body depth; CPD: caudal peduncle depth; BW: body width; MW: mouth width; TL: total length.

- *Hovering in open water*: when the fish was stationary, using its pectoral fins to maintain its position.
- *Resting*: when the fish was resting on its pectoral and pelvic fins on the bottom.

One fish was placed in an aquarium for 15 min. A single fish was used because interactions were observed among fish when there were two or more in the same aquarium during pilot experiments. Ten trials per family were performed for a total of 30 fish per ecotype. Twelve trials were performed each morning between 08.00 and 11.00 h for five consecutive days. Each aquarium was divided into five depth intervals of 8 cm from the bottom to the surface to determine the proportion of time spent hovering in each section of the water column. Fish were placed in the aquarium 24 h before the beginning of the experiment and were not fed during this acclimation period.

Foraging behaviour

Four behavioural traits were estimated when fish were feeding on bloodworms (benthic prey) and *Daphnia* sp. (open-water prey) to compare the foraging behaviour of both ecotypes.

1. *Capture rate*: the number of prey consumed per minute per fish during an experiment. The duration of an experiment was defined as the time elapsed between the prey delivery and when all prey were consumed or when fish stopped feeding for one minute. Fish stopped feeding before the consumption of all prey in only three trials with benthic prey.
2. *Reaction time*: the time in seconds between prey delivery and the first attack by a fish.
3. *Number of rejected prey per fish*: a prey ejected from the mouth after being grabbed.
4. *Number of fish participating in a given feeding trial*: the fish were in groups of five per trial during these experiments.

The number of fish participating in a given feeding trial and the number of rejected prey were used to correct the capture rate and the number of rejected prey per fish, respectively.

Ten trials (i.e. five fish per trial) per family (i.e. three families per ecotype) were performed for a total of 150 fish per ecotype and for each prey type (benthic and open-water prey). Six trials were performed every morning between 08.00 and 10.00 h. The fish used in the foraging experiments were naive (they had never been in contact with bloodworms or *Daphnia*). At this time, fish were approximately 4–6 months past the date of first feeding and were large enough to feed on the bottom prey. Venne and Magnan (1995) found benthic organisms such as chironomids and *Daphnia* in the diet of young-of-the-year of similar size in the field. The fish were placed in the aquarium 48 h before the beginning of the experiments and were not fed during this period. Pilot experiments confirmed that the response of the fish to benthic and zooplanktonic prey were better when they had been starved for 48 h rather than 24 h before a trial. Thirty commercially available frozen bloodworms [mean length = 11.18 mm, standard deviation = 1.18, $n = 30$) were placed on the bottom of the aquarium with a tea infuser spoon or 30 *Daphnia* sp. (raised in our laboratory; mean length = 1.81 mm, standard deviation = 0.34, $n = 30$) were added below the water surface with a syringe (60 ml, 3.5 mm diameter opening). The experiments with these two prey were performed separately. Two trials with benthic prey were excluded because both direct observation and the video recording did not allow for an accurate

estimation of the capture rate (many fish were feeding at the same time in the same area of the aquarium).

Statistical analysis

Before statistical analysis, each morphological character was first log-transformed to meet the conditions of linearity and to stabilize the variances. Allometric adjustments were used to standardize trait sizes to the overall mean total length (70.73 mm) using the equation $M_{std} = M_{obs}(70.73/L_{obs})^b$, where M is the trait size, L is the total length, b is the slope obtained from the analysis of covariance (ANCOVA) without the interaction term, and the subscripts 'std' and 'obs' refer to standardized and observed measurements respectively. These allometric standardizations use a common within-group slope that allows for different intercepts (ecotypes) (for details, see Reist, 1986; Hendry *et al.*, 2002). Morphological differences between ecotypes with subtle polymorphisms can be detected with multivariate statistical analysis (Skúlason and Smith, 1995; Robinson and Wilson, 1996; Bourke *et al.*, 1999; Peres-Neto and Magnan, 2004). Discriminant function analysis was used to assess which, if any, of the morphological characters were the most efficient to predict group membership of the littoral and pelagic individuals. Tolerance values were low for some descriptors when all shape variables were included in the model, indicating collinearity among some morphological characters. Thus, a stepwise procedure (backward selection) was used to eliminate the redundant characters. The cut-off significance values for variable selection, p-to-enter and p-to-remove, were both set to a probability $P = 0.15$ to ensure that important discriminating characters were not excluded from the analysis (Tabachnick and Fidell, 2001). The jackknife method of classification was used to cross-validate group attribution.

Linear mixed models [procedure MIXED (SAS Institute Inc., 2000)] were used to compare activity patterns and foraging behaviours of the two ecotypes (Littell *et al.*, 1996). The models were built using the ecotype and the family (ecotype) as fixed and random effects. The linear mixed models were run on log-transformed (reaction time and capture rate) or on rank-transformed data (Quinn and Keough, 2002) when the data did not meet the conditions of normality and homogeneity of variances (percent of time spent in fast swimming, slow swimming, hovering at a given depth and resting, and the number of rejected prey per fish). Similar analyses were performed on pooled variables: fast and slow swimming, the time spent hovering at the five depth intervals, and percent total time stationary (hovering + resting). Due to logistic problems with the production of *Daphnia*, the trials with this prey were performed after those with benthic prey were finished. As the trials with these two prey were not randomized, the analyses with zooplanktonic and benthic prey were treated separately. We used SAS version 8.0 to run our analyses.

RESULTS

Morphological variations

The discriminant function analysis indicated that the two ecotypes were significantly different in terms of body shape (Table 1). The frequency distributions of the discriminant function scores show the overlap between the pelagic and littoral groups (Fig. 2). Among the six traits retained by the stepwise procedure, inspection of the standardized canonical coefficients revealed that littoral offspring exhibited a deeper body, longer pectoral fins, and

Table 1. Results of the discriminant function analysis to compare shape characteristics of littoral and pelagic brook charr reared in the laboratory (backward selection procedure) (mean ± standard error)

Discriminant morphological character	Mean size-adjusted length (mm)						
	Littoral ecotype	Pelagic ecotype	% Difference	Canonical score	Wilks' lambda	*F*-value	*P*-value
Swimming-related traits							
Pectoral fin length	8.63 ± 0.03	8.11 ± 0.03	6.0	0.73	0.71	40.50	<0.0001
Dorsal fin base length	6.81 ± 0.03	6.57 ± 0.03	3.5	0.36			
Body height	11.94 ± 0.03	11.76 ± 0.03	1.5	0.16			
Feeding-related traits							
Lower jaw length	9.39 ± 0.03	9.06 ± 0.04	3.5	0.39			
Snout length	2.84 ± 0.02	2.82 ± 0.02	0.7	−0.14			
Mid-head length	6.67 ± 0.02	6.54 ± 0.02	2.0	0.13			

Note: Sample size for the analysis comprised 300 littoral and 300 pelagic individuals from the foraging experiments with benthic and zooplanktonic prey pooled together. The mean lengths for each morphological character were size-adjusted to facilitate comparisons between the two ecotypes.

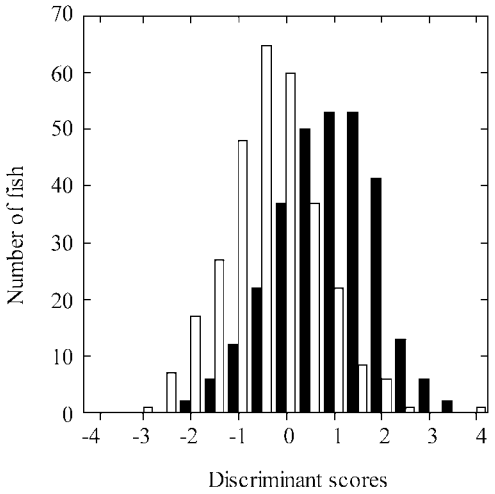

Fig. 2. Frequency distribution of the discriminant scores for littoral (■) and pelagic (□) individuals. Sample size for the analysis comprised 300 littoral and 300 pelagic individuals from the foraging experiments with benthic and zooplanktonic prey (pooled).

a longer dorsal fin base than the pelagic offspring (Table 1). The two latter characters were used to identify the ecotypes in the field, indicating that these morphological characters were inherited by the progeny. Furthermore, pectoral and dorsal fin lengths of the progeny follow the same pattern (in direction and amplitude) as the parental stock (Fig. 3). The mean size-adjusted (± standard error) pectoral fin and the dorsal fin base were 8.63 mm

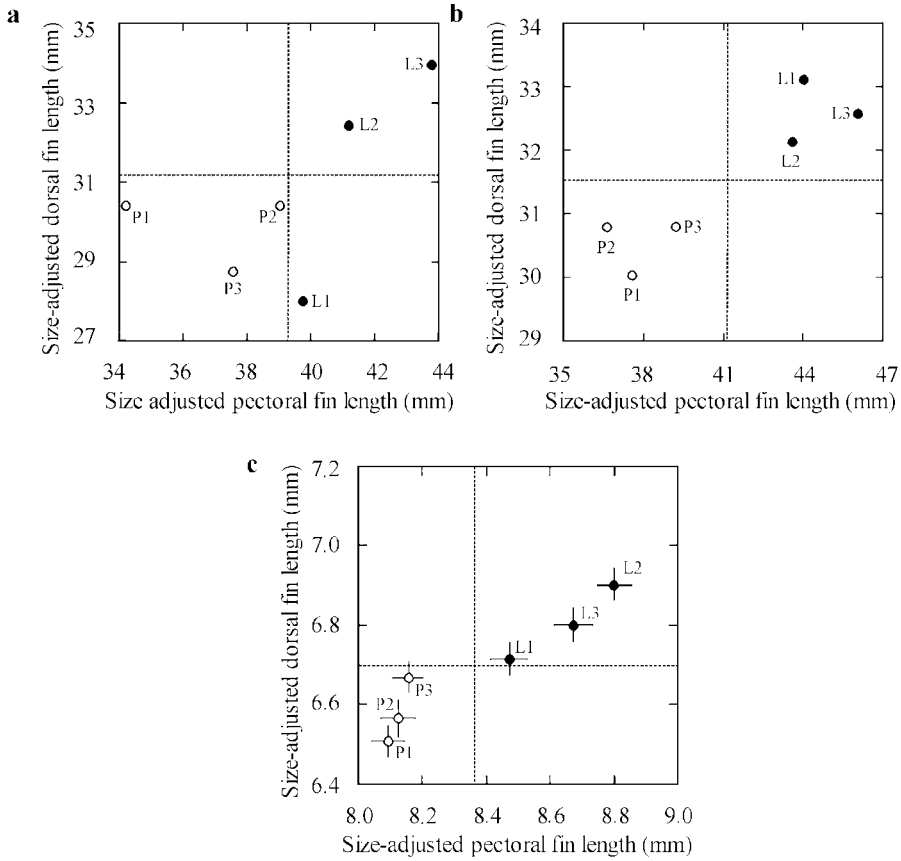

Fig. 3. Size-adjusted pectoral and dorsal fin lengths for each adult female brook charr (a), each adult male brook charr (b), and the means (± standard errors) for their progeny (c). ●, littoral families; ○, pelagic families. The morphological characters were adjusted to a total length of 300 mm and 71 mm for the adults and the progeny respectively.

(± 0.03) and 6.81 mm (± 0.03) respectively for the littoral form, and 8.11 mm (± 0.03) and 6.57 mm (± 0.03) respectively for the pelagic form. These values represent an overall difference of 6% for the pectoral fin and 4% for the dorsal fin base between the two ecotypes. The *a posteriori* classification accuracy of the discriminant function analysis (the jackknife classification procedure) correctly reclassified 220 of 300 (73%) littoral individuals and 226 of 300 (75%) pelagic individuals to their appropriate group, for an overall classification of 74%.

Activity pattern

Linear mixed models on activity pattern did not show any significant differences in the swimming behaviours of littoral and pelagic ecotypes ($P > 0.05$; active swimming: $F = 0.14$, $P = 0.724$; slow swimming: $F = 0.42$, $P = 0.553$; total active pattern: $F = 0.21$, $P = 0.674$). The two ecotypes did not show significant differences in inactive behaviours ($P > 0.05$; hovering 0–8 cm depth: $F = 0.10$, $P = 0.765$; 8–16 cm depth: $F = 0.32$, $P = 0.602$; 16–24 cm

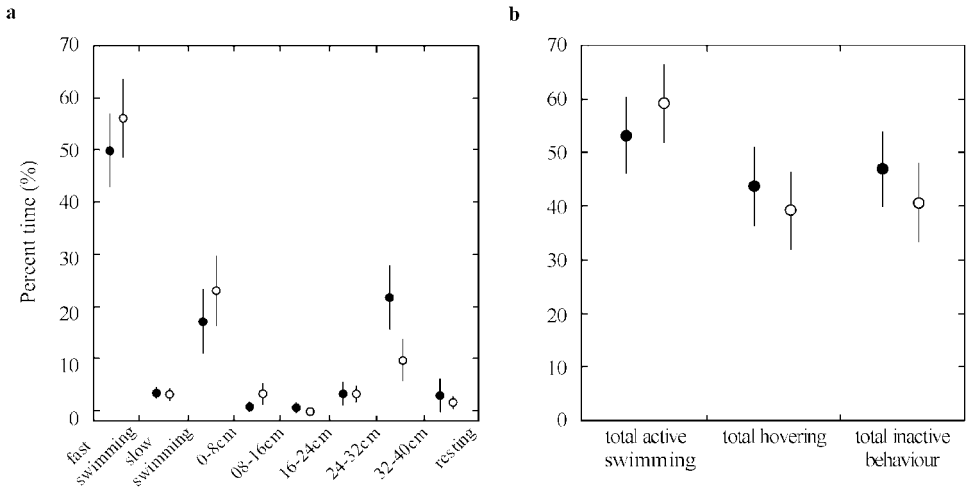

Fig. 4. (a) Percent time (± standard error) spent by the littoral (●) and pelagic (○) individuals for each activity pattern. The five depth intervals (cm) correspond to the time spent hovering at the given depth. (b) Percent time (± standard error) spent by the littoral and pelagic individuals on total active swimming, hovering, and total inactive behaviour.

depth: $F = 0.10$, $P = 0.763$; 24–32 cm depth: $F = 0.05$, $P = 0.831$; 32–40 cm depth: $F = 2.13$, $P = 0.219$; total hovering: $F = 0.07$, $P = 0.805$; resting: $F = 0.30$, $P = 0.615$; total inactive pattern: $F = 0.21$, $P = 0.674$) (Fig. 4a,b).

Foraging behaviour

There were no significant differences in the reaction time of the two ecotypes when feeding on *Daphnia* sp. ($F = 0.11$, $P = 0.746$) or on bloodworms ($F = 1.84$, $P = 0.248$). The capture rate of bloodworms was significantly higher in littoral than in pelagic individuals ($F = 13.36$, $P = 0.001$; mean ± standard error: 3.35 ± 0.48 vs. 1.51 ± 0.20 captures $\cdot$ min^{-1} $\cdot$ fish^{-1} respectively, $n = 58$; Fig. 5b). In contrast, the capture rate of *Daphnia* sp. was not significantly different between the two ecotypes ($F = 0.38$, $P = 0.541$; mean ± standard error: 1.95 ± 0.25 vs. 1.55 ± 0.15 captures $\cdot$ min^{-1} $\cdot$ fish^{-1} respectively, $n = 60$; Fig. 5a). Also, the number of rejected bloodworms was significantly higher in pelagic (mean ± standard error: 1.88 ± 0.30) than in littoral (0.43 ± 0.10) individuals ($F = 16.19$, $P = 0.016$). Rejection of *Daphnia* sp. was not observed by individuals of either ecotype during the experiments.

DISCUSSION

This study has confirmed that the characters used to identify brook charr ecotypes in the field (pectoral fin length and dorsal fin base length) were inherited in the progeny when offspring were reared in a common-garden laboratory environment. Furthermore, the foraging experiments indicated that naive littoral offspring were significantly more efficient than pelagic ones when feeding on benthic prey (higher capture rate and lower rejection rate), whereas there was no significant difference between the pelagic and littoral individuals when feeding on *Daphnia* sp. These results suggest that there is a genetic component to

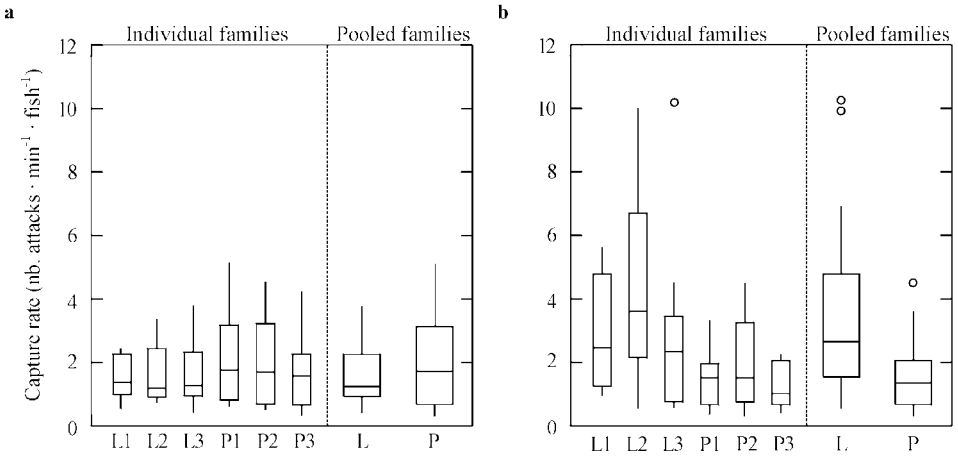

Fig. 5. Box plots illustrating feeding performance of individual littoral and pelagic families and of the littoral and pelagic families pooled together on (a) zooplanktonic prey and (b) benthic prey. Actual capture rate data are presented here, but statistical analyses were performed on log-transformed data.

trophic polymorphism in brook charr. As we did not observe inherited differences in feeding-related morphology between the two forms (Table 1), our results suggest that behavioural diversification is not constrained by morphological diversification in brook charr (at least not by the traits we measured). This supports the hypothesis that behavioural diversification precedes morphological diversification in the evolution of trophic polymorphism of fishes (Wimberger, 1994; Smith and Skúlason, 1996).

Morphological variations

The results of the present study show that morphological differences between littoral and pelagic brook charr are heritable from both males and females (Fig. 3). Littoral offspring exhibited a deeper body (i.e. were stouter) as well as longer pectoral fins and dorsal fin base than the pelagic offspring after controlling for the effect of body size. Also, the discriminant function analysis on morphological characters allowed for an effective reclassification (74%) of individuals into their appropriate group. These morphological differences between littoral and pelagic brook charr from Lake Ledoux are stable: they have been observed in six independent studies investigating wild fish (Bourke *et al.*, 1997; Dynes *et al.*, 1999; Proulx and Magnan, 2002, 2004; Marchand *et al.*, 2003; the present study). Furthermore, morphological differences appear adaptive because each ecotype seems better adapted to forage in its own habitat (i.e. benthic individuals in the littoral zone and pelagic individuals in the open-water habitat) based on the usual relationships established between swimming ability and morphology at the interspecific level (Gatz, 1979; Webb, 1984; Svanbäck and Eklöv, 2002). Given that pelagic brook charr feed mainly on zooplankton (Bourke *et al.*, 1999), a sustained swimming ability that provides high search rates might be advantageous in exploiting such a patchily distributed resource (e.g. Webb, 1984; Ehlinger, 1990). In this context, a fusiform body shape and short pectoral and dorsal fins are expected to reduce the energetic cost of swimming by minimizing drag (Gatz, 1979; Webb, 1984; Drucker and Lauder, 2003) and thus should improve searching and feeding performances on dispersed and mobile prey, like zooplankton in open water. In contrast,

littoral brook charr feed mostly on zoobenthos (Bourke *et al.*, 1999), thus the ability for slow and precise manoeuvring should help when searching and capturing benthic organisms in a complex habitat like the littoral zone. A deep body and well-developed median fins (i.e. dorsal, anal, and caudal fins) are associated with the slow and precise manoeuvring required to feed on benthic organisms in a complex habitat (such as the littoral zone) (Gatz, 1979; Webb, 1984; Elhinger, 1990; Lauder and Drucker, 2004). A similar relationship between the length of the pectoral fin and foraging behaviour has been found in other freshwater fish, such as pumpkinseed (Ehlinger, 1990) and Arctic charr (Malmquist, 1992), suggesting a strong relationship between this character and foraging behaviour in fishes. Such relationships between morphology and swimming ability provide only indirect evidence that diversification is adaptive in fish. Although they did not perform a complete reciprocal experiment, the results of Proulx and Magnan (2002) suggest that pelagic individuals spend less energy (body lipids and proteins) than littoral fish when restricted to swimming in pelagic enclosures.

Apart from the differences in body depth and the pectoral fin and dorsal fin base lengths, the other morphological descriptors had little significance for group discrimination. Taken together, the pattern of the canonical scores indicated the presence of two forms with a high degree of overlap (Fig. 2). This high overlap could indicate that the diversification between littoral and pelagic brook charr is constrained by random or near-random mating within the population [i.e. gene flow constrains adaptation (see Hendry and Taylor, 2004)]. This would also fit with the fact that these lakes show little differences between morphs. Dynes *et al.* (1999) studied the microsatellite DNA of littoral and pelagic brook charr in two lakes of the same system (Bondi and Ledoux). While they found no evidence of microsatellite differentiation between forms in Lake Ledoux, the genetic markers revealed that the degree of divergence was low in Lake Bondi.

Behavioural variations

The hypothesis of differences in activity patterns between the two forms raised in the laboratory was not confirmed. The dimensions of the experimental set-up (small 50-litre aquaria; 50 cm long × 25 cm wide × 40 cm high) compared to the natural habitat may partially explain why differences in activity patterns, including depth selection, were not observed. In contrast, two studies involving sympatric forms of Arctic charr (Skúlason *et al.*, 1993; Klemetsen *et al.*, 2002) found differences in activity patterns in aquaria similar to ours (i.e. 30-litre and 65-litre). As for the foraging experiments with zooplankton, it can be hypothesized that fish from our system exhibited more subtle differences than those observed in other polymorphic species like Arctic charr. Nevertheless, Venne and Magnan (1995) observed that young-of-the-year brook charr were spatially segregated into two groups in relation to water depth in a lake of the same system. Contrary to the homogeneous experimental conditions maintained during the laboratory experiments, habitat and resource diversity in the field are likely to play an important role in fostering resource polymorphism in fish (Smith and Skúlason, 1996). Thus, predictable patterns of phenotypic variation and stable behavioural polymorphisms for some behavioural traits (e.g. activity patterns) may be more pronounced in the field than in laboratory experiments because captive individuals are severely restricted with respect to their ability to select their habitat (Stamps, 2003).

It was predicted that naive littoral and pelagic individuals would react more rapidly (reaction time) and be more efficient (capture rate) and more familiar (rejection rate) with

benthic and pelagic prey, respectively, based on the hypothesis that the feeding behaviour of brook charr ecotypes is heritable. The significantly higher capture rate and lower rejection rate of bloodworms by littoral individuals support this hypothesis. In contrast, the absence of a significant difference in the capture rate of zooplankton between ecotypes could be an artefact related to the way the fish were fed in the laboratory. From their first feeding (i.e. after yolk sac resorption) until the beginning of the experiments, the fish of both forms were fed with very small commercial food pellets distributed from overhead automatic feeders. Fish from both forms were thus 'acclimated' to feeding on trout pellets slowly sinking in the water column, a foraging mode closely resembling that used on zooplankton. If this is the case, this highlights that fishes of the genus *Salvelinus* exhibit a high potential for plasticity in response to environmental changes (Noakes, 1989; McLaughlin *et al.*, 1994; Hutchings, 1996). The environment and the resources experienced in the field may expose individuals to a strong selection pressure that improves their resource exploitation efficiency (Ruzzante *et al.*, 1998). In this context, evolutionary mechanisms are most likely to act initially on behaviour because changes in behaviour are expected to be more flexible than changes in morphology (see discussions by Wimberger, 1994; Smith and Skúlason, 1996; Bourke *et al.*, 1999; Adams *et al.*, 2003).

From a mechanistic point of view, the significantly higher capture rate and lower rejection rate of bloodworms by littoral than pelagic individuals could be related to an inherited propensity to feed on benthic prey. Diet choices and high rejection rates are usually based on constraints imposed by morphology of both predators and prey (Skúlason *et al.*, 1993; Gill and Hart, 1994). For example, Adams and Huntingford (2002) found that inherited differences in gape placed constraints on the foraging ability of two sympatric morphs of Arctic charr and were associated with their inherited differences in dietary preference. The prey size spectrum accessible to a fish is closely related to its mouth width (Wankowski and Thorpe, 1979; Wainwright, 1988), while prey rejection is associated with difficulty in manipulating them (Wankowski, 1979). However, in the context of the subtle polymorphism in brook charr, the differences in feeding-related morphology between littoral and pelagic individuals cannot explain the observed differences in capture and rejection rates of bloodworms. The mouth width of the two ecotypes did not differ significantly (mean mouth width ± standard deviation: littoral vs. pelagic individuals, 3.83 ± 0.23 vs. 3.79 ± 0.22 mm; $n = 300$, $t = 1.78$; $P = 0.125$). Furthermore, the lack of differentiation in feeding-related morphology in brook charr ecotypes from Lake Ledoux (Dynes *et al.*, 1999; Proulx and Magnan 2002, 2004; the present study) suggests that traits associated with swimming demands are more important than those used for food acquisition and manipulation in this system. This suggests that behavioural diversification is not constrained by morphological diversification in brook charr.

In conclusion, this study supports the hypothesis of inherited differences in morphology and foraging behaviour in brook charr. Even though the differences between the two forms are subtle, behavioural and morphological divergences observed in the field persist in the progeny reared in the laboratory, suggesting a genetic component to trophic polymorphism. Nevertheless, these differences are less contrasting than those observed in other polymorphic species (e.g. Malmquist, 1992; Skúlason *et al.*, 1993; Pigeon *et al.*, 1997; Adams and Huntingford, 2002; Klemetsen *et al.*, 2002; Rogers *et al.*, 2002). Our study and that of Svanbäck and Eklöv (2006) are among the first to show the occurrence of inherited behaviour in ecotypes from a subtle trophic polymorphism. A more striking diversification between ecotypes of these species could be tempered by random or near-random mating within populations [i.e. gene flow constrains adaptation (Hendry and Taylor, 2004)]. These results, together with those of McLaughlin (2001),

also support the hypothesis that behavioural diversification precedes morphological diversification in the evolution of trophic polymorphism in fishes.

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