

The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology

E. R. KEELEY,* E. A. PARKINSON† & E. B. TAYLOR‡

*Department of Biological Sciences, Idaho State University, Pocatello, ID, USA

†British Columbia Ministry of Environment, University of British Columbia, Vancouver, BC, Canada

‡Department of Zoology, Biodiversity Research Centre, Native Fishes Research Group, University of British Columbia, Vancouver, BC, Canada

Keywords:

ecotypic differentiation;
environmental plasticity;
genetic basis;
morphology;
rainbow trout.

Abstract

Although morphological plasticity has been observed in a variety of taxa, few experimental studies have compared the relative proportion of morphological variability that is accounted for by environmentally induced plasticity, and how much is because of genetically based differences among populations. We compared the morphology of six rainbow trout (*Oncorhynchus mykiss*) populations from different ecotypic categories that were raised under flowing vs. standing-water conditions. Our data indicate that both environmental conditions and ecotypic differences account for a significant proportion of variation in morphology. Among ecotype effects, however, accounted for a much larger proportion of morphological variability than environmental conditions. Rainbow trout from stream populations had deeper caudal peduncles, and longer fins than lake populations, and rainbow trout from a piscivorous population had larger mouth and head lengths than all other ecotypes. Environmentally induced differences in morphology were primarily related to differences in mouth and head lengths, as well as fin length. Relative to morphometric differences from natural rainbow trout populations, most characteristics deviated in the same direction in our experimental populations. Our data indicate that morphological differences across rainbow trout populations have a genetic basis and may represent locally adaptive characteristics and highlight the role of ecology in promoting phenotypic divergence.

Introduction

Observations of phenotypic diversity between populations and species play a crucial role in understanding the processes that lead to diversification of species within ecosystems (Schluter, 2000). The chain of evidence linking phenotypic diversity to adaptive radiation requires the demonstration of both the genetic basis and the fitness consequences of phenotypic variation. Differences in morphology, behaviour or life-history characteristics between populations result from a combination of adaptive plastic responses to environmental heterogeneity at one extreme (cf. Robinson & Parsons, 2002) and relatively fixed, or genetically based differences, at the other (cf. Hard, 1995).

Temperate freshwater fishes have proven to be excellent taxa to study the process of adaptive radiation (Schluter, 1996; Taylor, 1999). Most extant diversity within these species is thought to be of recent origin (< 15 000 years) because they occupy areas that were covered with ice during the last glaciation (McPhail & Lindsey, 1986; Pielou, 1991). In addition, freshwater fish are confined by watershed boundaries, which often results in hundreds of populations that are both isolated from each other and experience contrasting environmental conditions. Salmonid fishes represent some of the best examples of diversification among freshwater fishes (Taylor, 1991, 1999) and rainbow trout (*Oncorhynchus mykiss*) have some of the most variable life-history and morphological differences documented among salmonids (Scott & Crossman, 1973; Irvine, 1978; Northcote & Hartman, 1988). Past studies have documented a genetic basis for behavioural (Skúlason *et al.* 1993) or morphological (Riddell & Leggett, 1981; Hard *et al.*, 1999;

Correspondence: Ernest R. Keeley, Department of Biological Sciences, Box 8007, Idaho State University, Pocatello, ID 83209, USA.
Tel.: 208 282 3145; fax: 208 282 4570; e-mail: keeleer@isu.edu

Sheehan *et al.*, 2005) differences in some species of salmonid fishes, which are often thought to reflect locally adaptive characteristics among populations (Taylor, 1991). In contrast, other studies have found that morphological differences in salmonid fishes may be environmentally induced (Pakkasmaa & Piironen, 2001; Imre *et al.*, 2002; Adams *et al.*, 2003). A comparison of 34 rainbow trout populations concluded that pervasive morphological variation observed in nature is associated with environmental and life-history differences rather than ancestry (major phylogenetic group), a result consistent with adaptation to local environments as the major process leading to such diversity (Keeley *et al.*, 2005). To date, however, there are no data available to assess the second important criterion for adaptive variation: a genetic basis to the variation in rainbow trout morphology (Taylor, 1991).

In this study, we selected representative populations from each of the six major ecotypes described by Keeley *et al.* (2005) to raise in a common garden experiment designed to test for the genetic basis of the variation observed among the 34 populations that we had previously examined. The six ecotypes represent contrasts in water velocity, diet and competitive environment. Salmonids from flowing water or stream environments typically have larger morphological characteristics than those from habitats with slower moving water (Swain & Holtby, 1989; Pakkasmaa & Piironen, 2001; Imre *et al.*, 2002), and this pattern of variation in wild-collected rainbow trout was consistent with these observations (Keeley *et al.*, 2005). Similarly, piscivorous fishes often exhibit a morphology that reflects feeding on relatively large prey items, which includes large mouths for a given body size in comparison to nonfish-eating species (Skúlason *et al.* 1989; Scharf *et al.*, 2000). As predicted, Keeley *et al.* (2005) found that piscivorous rainbow trout had significantly longer mouths and heads than similar-sized individuals from nonpiscivorous lake populations. Interspecific competition is also thought to drive morphological diversification among some fish species (Magnan, 1988; Robinson & Wilson, 1994), but the differences observed by Keeley *et al.* (2005) were relatively small in comparison to the differences associated with diet and water velocity. These observations suggest that ecological conditions influence the phenotype of rainbow trout, but do not distinguish between the hypotheses of plastic responses to environmental conditions vs. locally adaptive differences that are genetically based.

The six populations in the current study include three from streams and three from lakes that are a subsample of ecotypes selected by Keeley *et al.* (2005). The first stream ecotype is a 'dwarf' or headwater population because they are commonly isolated above a waterfall barrier on small stream (< 5 m wide) and they mature at a small body size (10–20 cm in length) while retaining juvenile parr markings (Northcote & Hartman, 1988). The second occupies a large river (> 50 m wide), and

matures at a relatively large body size (35–60 cm), does not retain juvenile markings at maturity and is isolated from the ocean by a natural barrier. The third stream ecotype is an anadromous rainbow trout population, commonly referred to as steelhead trout. Although steelhead trout migrate between stream and ocean environments, they typically spend a significant component (2–7 years) of their life cycle in streams and therefore may be under the same ecological influences of other stream populations (Quinn, 2005). We compare each of these three stream ecotypes with three lake ecotypes to provide an overall comparison between stream and lake populations. To investigate how interspecific competition might drive diversification among lake populations, we selected two of our lake populations based on the presence of benthivorous (Catostomidae) and zooplanktivorous (Cyprinidae) fish competitors (mixed ecotype) or by the absence of these other species, when rainbow trout were the only fish species present (solitary ecotype). Finally, to investigate whether a piscivorous morphology (characterized by a large mouth and head) persists under laboratory conditions, we also selected a rainbow trout population that was known to routinely feed on another fish species in nature (piscivorous ecotype). Our objective was to use these six populations to draw general conclusions concerning the genetic basis of the patterns of phenotypic variation observed in the 34 populations sampled by Keeley *et al.* (2005). The genetic basis to the phenotypic variation described by Keeley *et al.* (2005) is important to assess and help understand the relative roles of deterministic (e.g. natural selection) or random effects (e.g. drift) in explaining variation in morphology. Demonstration of a genetic basis to morphological variation coupled with the environment–phenotype associations demonstrated previously (Keeley *et al.*, 2005) would support our overall hypothesis that phenotypic variation in native rainbow trout populations is, to a large extent, driven by evolved responses to divergent natural selection. This result would contribute to a general understanding of the role of ecology (habitat and feeding biology) in generating biodiversity – a fundamental goal of evolutionary ecology and essential for conservation programmes.

Methods and materials

Collection and rearing of experimental animals

We used a sample of adult fish from six populations in British Columbia (BC), Canada, as the source of gametes to create cohorts of juvenile fish that were used in this experiment. We collected fish from three stream populations to represent stream ecotypes, including fish from Murray Creek (121°22.2'W, 50°25.5'N) representing a headwater population, fish from the Chilliwack River (122°42.8'W, 49°04.8'N) to represent an anadromous population, and we used fish from the Blackwater River

(123°30.9'W, 53°05.5'N) to represent a resident large river population. We collected fish from three lakes to represent a sample of lake ecotypes, including fish from Pimainus Lake (121°04.5'W, 50°24.3'N) to represent a solitary population, fish from Tzenziacut Lake (122°50.2'W, 52°35.8'N) as a mixed species lake population, and fish from Kootenay Lake (116°51.5'W, 49°36.3'N) as a piscivorous population. All six represent naturally occurring native populations that have not been artificially established by humans. Because some populations spawn in unknown or inaccessible tributaries, we collected broodstock fish from Pimainus Lake, Murray Creek and Blackwater River the previous fall (September 1999) and held the fish in the Fraser Valley Trout Hatchery (49°0.9'N, 122°16.4'W), near Abbotsford, BC until they were ready to spawn in the spring of 2000. Broodstock fish from Kootenay Lake, Chilliwack River and Tzenziacut Lake were collected as fish moved to their spawning areas in April or May of 2000.

Once female rainbow trout had ovulated, we collected eggs from each female rainbow trout and fertilized them with the sperm from one male rainbow trout from its respective population to create full-sib families (i.e. one male rainbow trout crossed with one female rainbow trout), except in the Kootenay Lake population where, unavoidably, five female rainbow trout were individually crossed with the sperm from seven male rainbow trout mixed together. We established 3, 8, 7, 5 and 4 full-sib families from the Chilliwack, Tzenziacut, Blackwater, Pimainus and Murray populations respectively. Embryos were then placed in incubation trays at the Fraser Valley Trout Hatchery and held on spring water until they had completely absorbed their yolk sac. Because of large differences in the natural spawning time of the six populations, we were forced to incubate embryos from early spawning populations in cooler water and later spawning populations in warmer water to ensure that the cohorts of juvenile fish were similar in size during the experiment. Although we know of no study that has shown an influence of incubation temperature on the morphological characters we considered (but see Watkins & Vraspir, 2006 for an example with amphibians), we treated fish incubated at the cooler temperature (Chilliwack River, anadromous ecotype, and Kootenay Lake, piscivorous ecotype; mean temperature = 7.3 ± 1 °C) separately in initial analyses than those from the warmer incubation temperature (Pimainus Lake, Tzenziacut Lake, Murray Creek, Blackwater River, mean temperature = 10 ± 1 °C). When juvenile fish were ready to begin feeding exogenously, we placed each cohort of rainbow trout in a fibreglass trough supplied with the same spring water and fed them a maintenance ration. A week before being used in the experiment, fish were transferred to the Cultus Lake Laboratory (49°3.3'N, 122°1.4'W) and held in fibreglass troughs supplied by water from the nearby Cultus Lake, BC.

Experimental set-up

We used two types of experimental environments to rear all six of the source populations of rainbow trout. To simulate a stream or lotic environment, we used 18 stream channels, each measuring 5 m long by 0.92 m wide and 0.40 m deep. Channels were constructed from plywood sheets supported by lumber frames, lined with polyurethane tarpaulins, and then sealed with silicone to make them watertight. To reduce the large volume of water required to simulate flow from a natural stream, the channels were arranged in a blocked, staircase design (also see Keeley, 2000). Channels were grouped into six columns of three, with water introduced to the upper channels supported by cinder blocks 117 cm high. The second group of channels, 78 cm off the ground, was connected to the first with plastic troughs, 68 cm long and 32 cm across. Hence, water could flow from the uppermost channels to the next highest channels, and then to a third set of channels that was placed level with the ground. The downstream end of each connecting trough was screened with 3-mm mesh to prevent the escape of any fish placed into the channel. To simulate a stream bottom, we placed a single layer of gravel, 5–10 cm in diameter, into each channel. To prevent mortality caused by aerial predators, we placed a canopy of burlap, supported by a wooden frame 132 cm high, above the top edge of each channel. The burlap also equalized the shade over the channels, while still permitting enough light to pass through to the water to allow the fish to feed and interact with each other.

To simulate a standing water or lentic environment, we also reared the six types of rainbow trout in three concrete ponds. Each pond measured 5 m in diameter and was 1.5 m deep. A hole in the bottom centre of each pond allowed water to drain from the ponds at the level of a stand pipe that regulated water depth at 1.25 m. To separate each of the six types of rainbow trout, we divided each pond into six equally sized sections with dividers constructed by lumber frames and opaque plastic sheeting that was reinforced with screening. We secured the dividers to the bottom of the pond by fastening the wooden frames to a steel bracket in the centre of the pond that was embedded into the concrete. We then sealed the dividers to the concrete with silicone. A plywood wall 1.5 m high surrounded each pond and the top opening of the walls was covered with a layer of burlap to prevent any predators from entering and to equalize shading.

Water was delivered to the channels and ponds from two large reservoirs supplied by water drawn from both above and below the thermocline of the nearby Cultus Lake. By mixing the two sources, we maintained the experimental water temperature at 13.3°C (± 1.1 SD). For the channels, we mixed incoming water in a 1700-L tank positioned above the top of the array of channels and allowed water to flow into the uppermost channels

through pipe, 5 cm in diameter. Each channel received 435 L of water per minute, producing a flow that averaged 7 cm s⁻¹ and 10 cm deep. In the case of the ponds, flexible plastic pipes, 3 cm in diameter, delivered water from each reservoir and were split with a series of valves to allow water to be delivered to each section of the ponds, using water from both above and below the thermocline.

Stocking protocol

We stocked stream channels at densities to promote competition for food as they would in a natural stream. Because of the range in body size at maturity and egg size, offspring size at stocking varied significantly among the six populations, ranging from 2.7 cm for the Pimainus Lake population to 3.7 cm for the Kootenay Lake population. Hence, to correct for differences in biomass across populations, we stocked stream channels to account for size differences using Grant & Kramer's (1990) allometric model of territory size vs. body size for salmonid fishes. Stocking densities were 31 fish m⁻² for Pimainus Lake fish, 33 fish m⁻² for Chilliwack and Blackwater rivers, Murray Creek and Tzenziacut Lake fish and 20 fish m⁻² for Kootenay Lake fish.

Levels of food abundance represented an average level of the dry mass of invertebrates drifting per area of stream profile (Keeley & Grant, 1997) and representing 72% of the maximum ration predicted by Marschall & Crowder's (1995) model for a 3-cm salmonid. A channel thus received a daily ration of 1.44 g day⁻¹ of crumble-type trout feed. Using invertebrate drift as the model, we chose a size grade of food that was similar in size to the average size (~0.75 mm) of invertebrates encountered by stream salmonids (grade 1 Nutra Plus®; Moore Clark, Surrey, BC, Canada). We introduced the daily ration of food over a 12-h period (beginning at 07:00 hours) to simulate a natural encounter rate (Keeley & Grant, 1997), using automated belt feeders 20 cm wide and 50 cm long (Zeigler Bros Inc., Gardners, PA, USA), attached to the top of each channel.

We assigned populations across the array of channels using the channel position as a blocking factor (Quinn & Keough, 2002) to remove any potential upstream-downstream effects of treatment position. Each population appeared once in the upper, middle and lower channel positions in the first three columns and once again in the next three columns. Food particles naturally occurring in the lake water were filtered out at the inflow with a 250- μ m nylon mesh.

When stocking each section of a divided pond, we again corrected for differences in biomass across experimental units to account for differences in body size between populations, using Grant & Kramer's (1990) allometric model. Stocking densities were 31 fish m⁻² for Pimainus Lake fish, 33 fish m⁻² for Chilliwack and Blackwater rivers, Murray Creek and Tzenziacut Lake

fish and 20 fish m⁻² for Kootenay Lake fish. To avoid any position effect of having two populations consistently beside each other across the three ponds, we alternated the position of populations across the ponds to minimize this potential problem. We again used a ration of 1.44 g day⁻¹ of dry crumble food per experimental unit. Rather than using a belt feeder to simulate the encounter rate of drifting aquatic invertebrates in streams, we sprinkled the ration of food across the surface of the pond section once a day, between 07:00 and 09:00 hours.

Every 12 h, we checked each channel and pond to ensure that water continued to flow equally in all experimental units. At the end of the 30-day experiment, we collected surviving individuals by removing all of the substrate in the stream channels and capturing fish by dip net. In the ponds, we allowed the water level to drop to a level of approximately 20 cm before capturing survivors by dip net. We haphazardly sampled 50 individuals from each channel or pond and measured them for fork length (\pm 0.5 mm) and wet mass (\pm 0.01 g) to estimate the size of surviving fish. We killed fish before collecting size measurements and finally placed individuals in preservative (10% formalin) for later morphometric analyses.

Morphological measurements

We measured external morphological characteristics by taking a digital photograph of a haphazardly selected subsample of 25 fish from each channel or pond. Each fish was placed on its right side, pinned to a flat board to allow its fins to be positioned in an erect manner, and then photographed with a digital camera. We repeated this procedure to photograph the ventral surface of the fish by placing the dorsal side down and pinning the specimen to the board. We then used an image analysis software package (IMAGE PRO PLUS, ver. 1.3; Media Cybernetics, Silver Spring, MD, USA) to measure the size of external morphological features. A scale bar of known length was included in every photograph to translate distances in pixels into distances in millimetres. We selected a suite of 12 external morphological features that reflected characteristics of the fish that we hypothesized would be selected for under different ecotypic conditions (Fig. 1). For comparing differences in feeding morphology, we used measurements of mouth, eye and head dimensions (Skúlason *et al.*, 1989), whereas swimming morphology was assessed by comparing differences in paired fin length, caudal peduncle depth and body depth (Boily & Magnan, 2002).

Statistical analyses

In order to correct for body size-related differences across individuals and populations, we regressed each morphological characteristic against fork length to obtain the slope of the relationship. We then used the separate

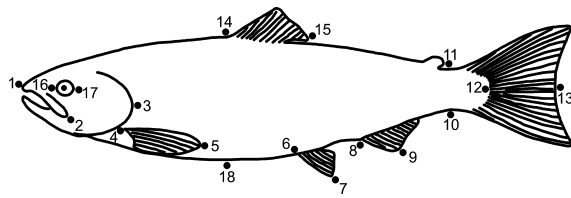


Fig. 1 Position of the landmarks used to measure the size of 12 morphological features on rainbow trout. Features are defined as follows: 1–2 = premaxilla length; 1–3 = head length; 4–5 = pectoral fin length; 6–7 = pelvic fin length; 8–9 = anal fin length; 10–11 = caudal peduncle depth; 12–13 = caudal fin length; 14–15 = dorsal fin length; 16–17 = eye diameter; 14–18 = body depth. Other morphological measures not shown include head width (the distance between the left and right side of the head measured at the centre of the operculum) and mouth width (the internal breadth of the mouth measured proximate to point 2 on either side of the buccal cavity).

within-group slope to adjust each trait to a common body size across all fish in the experiment based on the method of Thorpe (1976). Because we were interested in comparing the relative size of characteristics according to ecological and environmental categories, rather than overall differences in body size between populations, we used the size adjusted characters in all subsequent analyses. All measurements were \log_{10} -transformed to ensure that we met the assumption of homogeneity of variance, and we visually examined the residuals from regressions by plotting them against body length to ensure that we met this assumption.

To provide an objective assessment of whether individuals from replicates of the same ecotype or environmental condition tended to have a suite of more similarly sized morphological features than those from different ecotypes or conditions, we used a principal component analysis (PCA) to summarize differences. We only considered principal components with eigenvalues that accounted for a significant proportion of the total variation, as determined by the broken-stick method (see Jackson, 1993). We considered each stream channel or pond as our unit of observation; hence, we calculated a mean value for each size-adjusted morphological trait for a given channel or pond, and then used these values in the PCA. We compared PC scores across ecotypes and environmental conditions using a two-factor ANOVA to determine whether principal axes explained a significant proportion of the variation in morphology. Because of differences in incubation temperatures, we initially treated populations from the 'cool' group separately from the 'warm' group, to evaluate differences among ecotypes and environmental conditions. We did not use temperature as a covariate because with 36 replicates distributed over 12 treatment combinations, there were not sufficient degrees of freedom to include an additional variable in the model. All tests of significance were based on type III sum-of-squares (SAS Institute, 1999).

If the relative size of characteristics in this experimental study follows the same pattern of deviation observed in natural populations, we expected to see the same direction of change when the two are compared. To determine whether the relative size of morphological characteristics in this study deviated from the mean size in the same direction as those observed in natural populations, we compared the per cent deviation from the mean in this study with the per cent deviation from the same six ecotypes in Keeley *et al.* (2005). Because some measurements in Keeley *et al.* (2005) were made with slightly different methods, we limited our comparison of individual characteristics to those eight which were the same in both studies.

Results

Anadromous and piscivorous ecotypes

Because their incubation temperature differed from the other four ecotypes, we first compared the anadromous ecotype with the piscivorous ecotype separately from all other ecotypes. Our comparison indicated that there were significant differences in rainbow trout morphology that were accounted for by differences among ecotypes as well as between flowing vs. standing-water environments. Differences between the anadromous and piscivorous ecotypes accounted for 69% of the variation in PC1 scores (Fig. 2a, ANOVA, $F_{1,11} = 18.39$, $P < 0.01$). These differences were primarily because of the strong loading of caudal peduncle depth, head length, premaxilla length, eye diameter and anal fin length (Table 1). There were no significant differences between ecotypes based on scores from PC2 (Fig. 2a, ANOVA, $F_{1,11} = 1.26$, $P = 0.29$) or PC3 (Fig. 2b, ANOVA, $F_{1,11} = 0.53$, $P = 0.49$).

The only significant difference between flowing and standing-water habitats involved PC3 (Fig. 2b, ANOVA, $F_{1,11} = 10.37$, $P < 0.05$), based primarily on body depth, pelvic fin length and dorsal fin length (Table 1). Differences between the water flow treatments were not significant for PC1 (Fig. 2a, ANOVA, $F_{1,11} = 0.23$, $P = 0.65$) or PC2 (Fig. 2a, ANOVA, $F_{1,11} = 1.18$, $P = 0.31$) scores, even though both ecotypes had larger PC scores in flowing-water treatment. There were no significant interactions between ecotype effects and water flow effects for principal component scores from all three principal axes (ANOVA, all F -values for interactions ≤ 0.07 , all P -values ≥ 0.79).

River and lake ecotypes

The four ecotypes that were incubated at higher temperatures also had differences in morphology that were accounted for by among ecotype differences and by differences between water flow treatments. Significant differences among ecotypes in PC1 (Fig. 3, ANOVA,

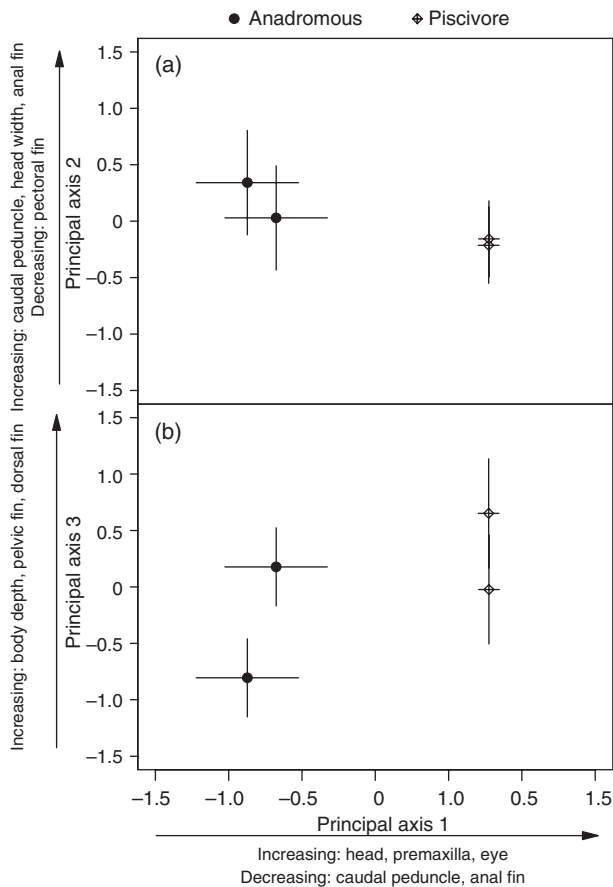


Fig. 2 Principal component scores (± 1 SE) from two rainbow trout ecotypes based on (a) the first and second principal axes or (b) the first and third principal axes of external morphological features. Symbols define populations as follows: diamond = piscivore; circles = anadromous. Uppermost and lowermost symbols for each pair represent stream channel and pond reared fish respectively.

$F_{3,23} = 6.95$, $P < 0.01$) were related to differences in caudal peduncle, head width, eye diameter and pelvic, anal and dorsal fin sizes (Table 2). We also detected significant differences between ecotypes in PC2 scores (Fig. 3, ANOVA, $F_{3,23} = 7.38$, $P < 0.01$). In order to determine which groups differed from each other, we compared ecotypic categories along principal axes and found that some of the populations representing the four ecotypes were separated from each other based on two principal axes. Stream ecotypes were not significantly different from each other based on scores from the first principal axis (Fig. 3, ANOVA, $F_{1,11} = 4.06$, $P = 0.061$) and lake ecotypes did not differ between each other along the first (Fig. 3, ANOVA, $F_{1,11} = 0.26$, $P = 0.66$) or second axis (Fig. 3, ANOVA, $F_{1,11} = 5.00$, $P = 0.066$); however, stream ecotypes had higher scores along the first axis than lake ecotypes (Fig. 3, ANOVA, $F_{1,23} = 16.48$, $P < 0.001$). We also detected significant

Table 1 Loading coefficients from a principal component analysis for 12 external morphological characteristics for anadromous and piscivorous rainbow trout (*Oncorhynchus mykiss*).

Morphological variable	Principal component 1	Principal component 2	Principal component 3
Body depth	0.25	0.45	0.66
Caudal peduncle depth	-0.61	0.60	0.18
Head length	0.96	-0.20	0.041
Head width	-0.31	0.83	-0.13
Mouth width	0.13	-0.36	0.17
Premaxilla length	0.94	-0.15	0.16
Eye diameter	0.87	0.29	0.18
Pectoral fin length	-0.13	-0.86	-0.098
Pelvic fin length	-0.056	-0.0038	0.96
Caudal fin length	0.34	-0.23	-0.060
Anal fin length	-0.60	0.50	0.24
Dorsal fin length	0.12	0.10	0.89
Eigenvalue	4.17	2.73	2.19
Proportion of total	0.35	0.23	0.18

Eigenvalues from each principal component are listed below the column of coefficients.

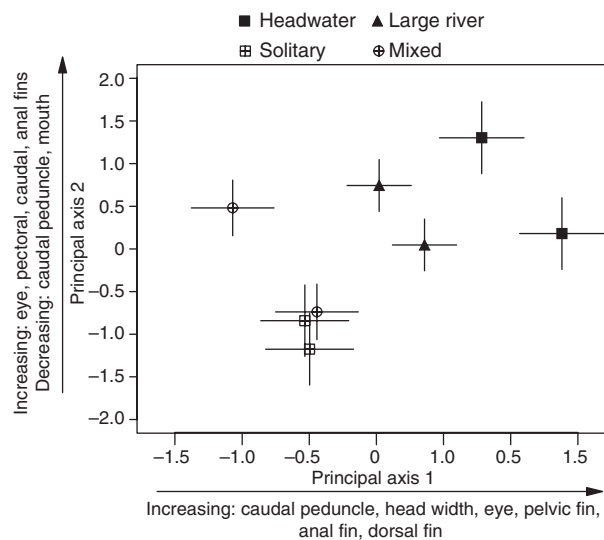


Fig. 3 Principal component scores (± 1 SE) from four rainbow trout ecotypes based on the first and second principal axes. Symbols define populations as follows: crossed square = solitary lake; crossed circle = mixed lake; square = headwater stream; triangle = large river. Uppermost and lowermost symbols for each pair represent stream channel and pond reared fish respectively.

differences in morphology that were associated with water flow treatments based on scores from PC2 (ANOVA, $F_{1,23} = 9.05$, $P < 0.01$) but not based on PC1 (ANOVA, $F_{1,23} = 1.62$, $P = 0.22$). Fish from the flowing-water treatment had higher scores than those from the standing-water treatment based on scores from PC2 (Fig. 3). There were no significant interactions between ecotype

Table 2 Loading coefficients from a principal component analysis for 12 external morphological characteristics for headwater stream, large river, solitary lake and mixed lake rainbow trout (*Oncorhynchus mykiss*).

Morphological variable	Principal component 1	Principal component 2
Body depth	-0.052	-0.38
Caudal peduncle depth	0.61	-0.47
Head length	0.042	-0.059
Head width	0.90	0.068
Mouth width	0.39	-0.73
Premaxilla length	0.37	0.14
Eye diameter	0.76	0.44
Pectoral fin length	0.093	0.84
Pelvic fin length	0.93	0.13
Caudal fin length	0.36	0.78
Anal fin length	0.63	0.60
Dorsal fin length	0.87	-0.076
Eigenvalue	4.69	3.10
Proportion of total	0.39	0.26

Eigenvalues from each principal component are listed below the column of coefficients.

effects and water flow effects for principal component scores from both principal axes (ANOVA, all *F*-values for interactions ≤ 1.81 , all *P*-values ≥ 0.19).

Multivariate analysis of all ecotypes

When we summarized the suite of 12 morphological features by PCA for all six ecotypes, we extracted three principal component axes with eigenvalues > 1, which accounted for 74% of the variation of morphology (Table 3). The first principal component axis was primarily composed of factors such as caudal peduncle

Table 3 Loading coefficients from a principal component analysis of 12 external characteristics for anadromous, piscivorous, headwater stream, large river, solitary lake and mixed lake rainbow trout (*Oncorhynchus mykiss*) morphology.

Morphological variable	Principal component 1	Principal component 2	Principal component 3
Body depth	0.20	0.64	-0.27
Caudal peduncle depth	0.76	-0.011	-0.44
Head length	-0.15	0.93	0.016
Head width	0.89	-0.10	-0.036
Mouth width	0.25	0.38	-0.70
Premaxilla length	0.036	0.90	0.17
Eye diameter	0.51	0.40	0.45
Pectoral fin length	-0.051	0.31	0.71
Pelvic fin length	0.82	0.19	0.10
Caudal fin length	0.22	-0.031	0.84
Anal fin length	0.66	-0.15	0.50
Dorsal fin length	0.68	0.39	-0.0082
Eigenvalue	4.65	2.27	1.89
Proportion of total	0.39	0.19	0.16

Eigenvalues from each principal component are listed below the column of coefficients.

depth, head width and eye diameter as well as pelvic fin, dorsal fin and anal fin lengths (Table 3, Fig. 4a). Body depth, head length, premaxilla length and eye diameter were the variables that loaded most strongly on the second principal component axis (Table 3). Caudal peduncle depth, mouth width, pectoral fin, caudal fin and anal fin lengths loaded most strongly on the third principal component axis (Table 3, Fig. 4b).

Ecotypic differences influenced the separation of groups, with principal component scores on all three axes differing significantly among ecotypes. Ecotypic differences accounted for 50% of the variation in PC1 scores (ANOVA, $F_{5,35} = 5.16$, $P < 0.01$), 56% of the variation in PC2 scores ($F_{5,35} = 16.84$, $P < 0.0001$) and 35% of the variation in PC3 scores ($F_{5,35} = 15.52$, $P < 0.0001$). When we compared principal component

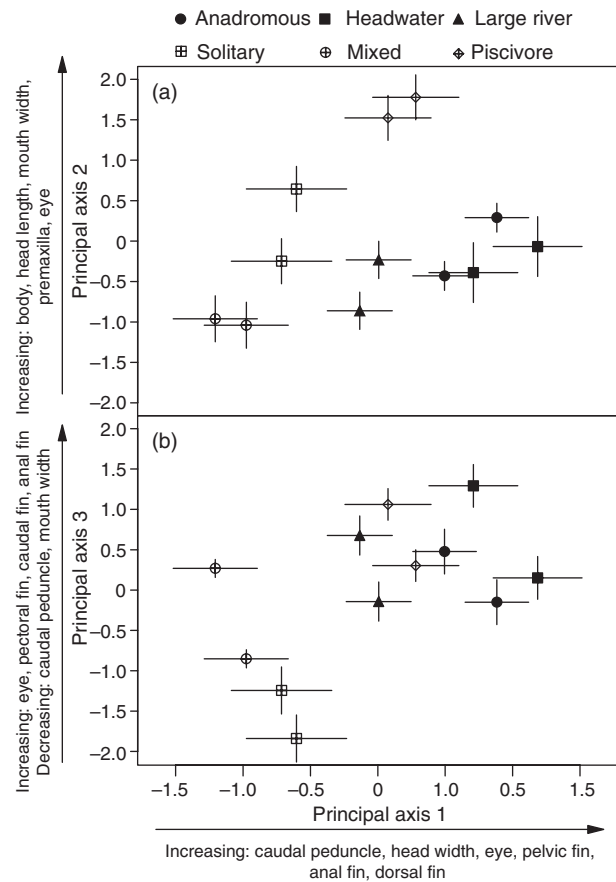


Fig. 4 Principal component scores (± 1 SE) for six experimental populations of rainbow trout based on (a) the first and second principal axes or (b) the first and third principal axes of external morphological features. Symbols define populations as follows: crossed square = solitary lake; crossed circles = mixed lake; diamond = piscivore; square = headwater stream; circle = anadromous; triangle = large river. Uppermost and lowermost symbols for each pair represent stream channel and pond reared fish respectively.

scores by ecotypic categories, we found that a combination of external morphological features grouped some, but not all, categories apart from each other (Fig. 4a,b). Although stream ecotypes were not significantly separated from each other based on scores from the first principal component axis (Fig. 4a, ANOVA, $F_{2,17} = 2.52$, $P = 0.12$), stream ecotypes had higher scores than lake ecotypes (Fig. 4a, ANOVA, $F_{1,35} = 5.94$, $P < 0.05$). In contrast, there were significant differences in morphology among lake ecotypes along the first principal component axis (Fig. 4a, ANOVA, $F_{2,17} = 5.84$, $P < 0.05$). The piscivorous ecotype had higher scores than the mixed species ecotype (ANOVA, $F_{1,11} = 15.61$, $P < 0.01$) but not the solitary lake ecotype (Fig. 4a, ANOVA, $F_{4,14} = 0.55$, $P = 0.70$). Similarly, the piscivorous lake ecotype did not differ from stream ecotypes along the first principal component axis (Fig. 4a, ANOVA, $F_{1,23} = 0.04$, $P = 0.85$), but was distinguished from the stream ecotypes based on scores from the second principal component axis, associated with piscivorous fish having relatively large premaxilla and head lengths (Fig. 4a, ANOVA, $F_{1,23} = 12.01$, $P < 0.01$).

In addition to ecotypic differences, the variation in morphology was also related to differences in water flow treatments. Based on scores from PC1, fish raised under flowing-water conditions, on average, had higher scores than those raised under standing water conditions (Fig. 4a), but the differences were not significant ($F_{1,35} = 1.35$, $P = 0.26$) and accounted for only 2.6% of the variation. The association between higher scores and the flowing-water treatment (Fig. 4a), however, was significantly different based on scores from PC2 ($F_{1,35} = 7.92$, $P < 0.01$), accounting for 6.4% of the variation. Similarly, differences between flowing- and standing-water environments accounted for 25% of the variation in morphology based on scores from PC3 (Fig. 4b, $F_{5,35} = 34.43$, $P < 0.0001$). Analysis of variance of PC scores from all three axes revealed no significant interaction between ecotype or water flow treatments (all F -values for interactions ≤ 1.46 , all P -values ≥ 0.23).

Morphological traits that separated ecotypes within an incubation temperature continued to separate groups when all ecotypes were combined in a single analysis. Stream vs. lake ecotypes were initially separated along PC1 by caudal peduncle depth, head width and eye diameter, and by pelvic, anal and dorsal fin lengths (Table 2, Fig. 3a). The same characters again separated ecotypes when the piscivorous and anadromous ecotypes were added (Table 2, Fig. 4a). Similarly, head and premaxilla lengths separated the piscivorous ecotype from the anadromous ecotype (Table 1, Fig. 2a). When combined with the four other ecotypes, the piscivorous ecotype was primarily separated by these same characters (Table 3, Fig. 4a); however, the characters separated ecotypes most strongly along PC2 rather than PC1 (Tables 3 and 4).

Table 4 Mean size-adjusted characteristics (mm) and the range as a proportion of the mean for the six rainbow trout ecotypes.

Morphological variable	Mean (mm)	Range	Per cent deviation from the mean
Anal fin length	6.64	0.37	6
Caudal fin length	5.00	0.58	12
Dorsal fin length	9.28	0.52	6
Pectoral fin length	6.26	0.64	10
Pelvic fin length	5.50	0.38	7
Body depth	9.76	0.41	4
Caudal peduncle depth	4.50	0.18	4
Eye diameter	3.21	0.37	12
Head length	10.95	0.66	6
Head width	5.78	0.34	6
Mouth width	3.53	0.26	7
Premaxilla length	4.95	0.50	10

The range is the maximum minus the minimum value over the six ecotypes (i.e. $n = 6$ ecotypes rather than 36 replicates), per cent deviation represents the absolute mean per cent deviation from the mean across the six ecotypes. Measurements have been adjusted to a common body length of 45.83 mm.

Deviation in individual traits

For individual characteristics, the average per cent deviation from the mean size across all categories was 5.2%; however, for some traits, the difference was more than double (range = 4–12%; Table 4). The variation in 11 of 12 characteristics was significantly related to differences among ecotypes (all characters except mouth width; ANOVA, all F -values ≤ 3.19 , P -values ≤ 0.021). After controlling for differences according to ecotypic variation, 6 of 12 characteristics were related to differences according to environmental conditions (anal, caudal and dorsal fin lengths, caudal peduncle depth, mouth width and premaxilla length; ANOVA, all F -values ≤ 5.74 , P -values ≤ 0.023). When we compared the direction of change from the mean for eight morphological characteristics from this study with the same measures from natural populations (Keeley *et al.*, 2005), we found that seven of eight characteristics deviated in the same, positive direction (binomial test, $P < 0.05$; Fig. 5). Only body depth varied negatively from the mean in this study when compared with the same trait from Keeley *et al.* (2005).

Discussion

Parallel divergence in wild and laboratory-reared trout

Our results suggest that the pattern of variation in morphology among rainbow trout populations is strongly influenced by inherited differences. On average, 52.7% of the variation in rainbow trout morphology was explained by differences between ecotypes (range = 20.9–77.1),

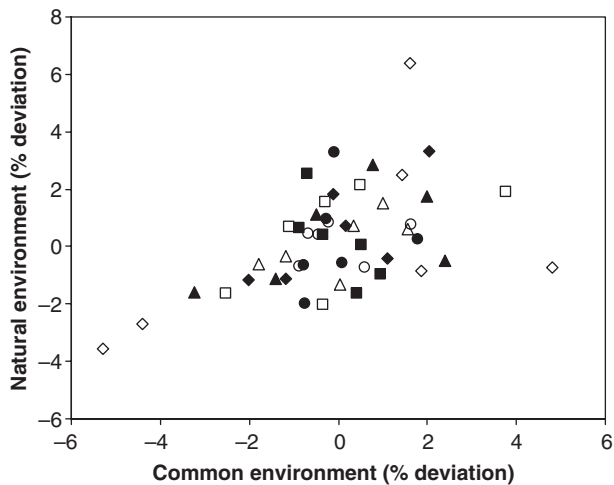


Fig. 5 Per cent deviation from the mean for eight size-adjusted morphological characteristics of rainbow trout raised under common rearing conditions (this study) vs. the same characteristics for rainbow trout from natural populations (Keeley *et al.*, 2005). Values from the common rearing conditions represent the average deviation between flowing- and standing-water environments. Symbols are defined as: circles = caudal peduncle depth ($r_s = 0.49$); squares = body depth ($r_s = -0.77$); triangles = pectoral fin length ($r_s = 0.60$); diamonds = pelvic fin length ($r_s = 0.77$); open circles = head length ($r_s = 0.26$); open squares = premaxilla length ($r_s = 0.77$); open triangles = head width ($r_s = 0.66$); open diamonds = eye diameter ($r_s = 0.54$).

whereas only 7.3% of the variation was explained by environmental effects (range = 0.01–28.3). These results imply that much of the diversity among wild-collected populations documented by Keeley *et al.* (2005) stems from genetic differentiation rather than plastic phenotypic responses to environmental differences.

Many of the detailed patterns of variation in the current data set parallel those observed by Keeley *et al.* (2005), who argued that the morphological variation matched the expected phenotypic differences for trout occupying and adapted to different ecological conditions. For example, piscivorous rainbow trout from three different lakes in BC had larger head and mouth features than nonpiscivorous lake populations. Piscivorous trout achieve a large body size by feeding on nonanadromous sockeye salmon (*Oncorhynchus nerka*) commonly called kokanee (Irvine, 1978). In this study, the Kootenay Lake piscivorous ecotype had the largest head and premaxilla lengths relative to any other ecotype compared. Piscivorous fishes are able to achieve a large body size by feeding on relatively large prey items that confer a growth advantage even when large prey are rare (Kerr, 1971). Maximum prey size, however, is constrained by mouth size in fishes (Scharf *et al.*, 2000) and relatively large body sizes may only be achieved if individuals can ingest increasingly larger prey items (Kerr, 1971). Hence, piscivorous populations may be under selective pressure

for large mouth size to maximize the benefit of feeding on large prey items. Piscivorous phenotypes have also been noted in a number of additional species of salmonids such as Arctic char (*Salvelinus alpinus*; Snorrason *et al.*, 1994) or brown trout (*Salmo trutta*; Campbell, 1979). Our earlier work on rainbow trout also found that piscivorous rainbow trout had significantly larger heads and mouths, even after controlling for differences in body size across populations (Keeley *et al.*, 2005).

In addition to differences related to feeding morphology, we found morphological differences between populations that may also be related to differences in swimming ecology. Populations that spend an extended period of their lifespan living in stream habitats may have morphological features that provide an advantage to living in a flowing-water environment. For example, past studies have noted that salmonids living in higher water velocities tend to have deeper caudal peduncles and longer paired fins than populations experiencing slower- or standing-water habitats (Swain & Holtby, 1989; Pakkasmaa & Piironen, 2001; Imre *et al.*, 2002). Although such differences are sometimes attributed to environmentally plastic responses (Robinson & Parsons, 2002), other studies have found a fixed genetic basis for such differences between populations (Riddell & Leggett, 1981). In our study, the anadromous ecotype had the deepest caudal peduncle of all the trout examined and all three stream ecotypes tended to have long paired fins, which separated the stream ecotypes from the lake ecotypes along the first principal axis. These patterns are similar to those reported for wild-collected lake and stream ecotypes by Keeley *et al.* (2005).

The differences among ecotypes are small, but are in the same range of differences among characteristics that vary between species. The range as a proportion of the mean among ecotypes averaged 7.9% for fin lengths, 4.0% for body depth and 8.2% for head shape characteristics. In comparison, jaw and head lengths are used in distinguishing cutthroat trout (*Oncorhynchus clarkii*) from cutthroat-rainbow trout hybrids, even though the average difference is < 3% (Weigel *et al.*, 2002). Divergence in closely related species can also be much larger than those we observed. For example, the difference in jaw length, one of the three key distinguishing characters between bull trout (*Salvelinus confluentus*) and the closely related Dolly Varden char (*Salvelinus malma*), is 19% of the mean of the two species (Haas & McPhail, 1991), whereas the range of jaw lengths that we observed was only 10% of the mean. Truss analysis measurements of headwater *O. mykiss* spp. in Mexico differed by 10–30% between mainland and Baja populations but by < 5% between two mainland *Oncorhynchus* species (Ruiz-Campos *et al.*, 2003).

Small differences in morphology have been shown to have significant implications for performance measures that are important for growth and survival. Inherited morphological differences, such as median fin area, which was 18% larger (~8% longer) in coho salmon

(*Oncorhynchus kisutch*) from coastal vs. interior areas of BC (Taylor & McPhail, 1985a), were associated with significant differences in acceleration and swimming stamina (Taylor & McPhail, 1985b). In brook trout (*Salvelinus fontinalis*), age-0 fish from littoral vs. pelagic areas of a single lake differed in pectoral fin length by 3.7% and in mandible length by 1.6% as predicted by the literature on foraging behaviour in the two habitats (Proulx & Magnan, 2002). When confined to the pelagic habitat, these differences were associated with differences in performance as indicated by proximate tissue composition.

Ecotypic and environmental influences on variation

Adaptive phenotypic plasticity is also becoming increasingly viewed as an important source of variation in morphological characteristics observed within- and between-fish populations (Robinson & Parsons, 2002). Although morphological plasticity can be viewed as nonadaptive variation that is ecologically unimportant, an alternative view is that such variation can be adaptive if some characteristics respond to environmental variation in a direction that increases the fitness of individuals. By comparing populations from different ecological categories and then raising those populations in two habitats that have been found to be correlated with morphological variation, we were able to assess the relative influence of environmentally induced vs. fixed differences between populations. In addition to between ecotype differences, we also found that a significant proportion of the remaining morphological variation was related to differences in rearing conditions. Of the 12 external morphological characteristics we measured, six exhibited a significant component of variation explained by flowing vs. standing-water rearing conditions that separated treatment groups along the second and third principal axes. All median fins and caudal peduncle depth were significantly different between rearing environments. Caudal and anal fins were longer in the standing-water treatment, whereas dorsal fin length and caudal peduncle depth were greater in the flowing-water treatment. Past studies of salmonids in fast vs. slow-flowing water conditions have found that caudal fin height increases with increasing current velocity (Pakkasmaa & Piironen, 2001; Imre *et al.*, 2002). Given their common anterior position, longer caudal and anal fins may provide some manoeuvrability benefit when there is no directional flow in the water. In contrast, we found a deeper caudal peduncle, which is often associated with salmonids that live in fast-flowing water, probably as a result of higher levels of physical exercise (Imre *et al.*, 2002).

Implications for fitness and conservation of trout in nature

An important step in evaluating the implications of phenotypic variation in nature is to determine if any

differences in morphology translate into performance differences for individuals under different ecological conditions. Although experimental data exist for some species of fishes (e.g. Schluter, 1995; Reznick *et al.*, 1997), no such data exist for salmonid fishes. The poor success of transplanted salmonid populations suggests that native populations have a local adaptive advantage (Taylor, 1991), but few studies have attempted to determine whether patterns of morphological variation found across ecotypic categories provide a fitness advantage. Our study provides an initial step in evaluating the adaptive significance of morphology from a sample of rainbow trout populations. In addition to uncovering unknown morphological variation, future studies will need to demonstrate that patterns of morphological variation do provide feeding or locomotory benefits to individuals under natural conditions and whether these patterns persist at many different spatial scales.

The presence of adaptive differences among populations within a species emphasizes the importance of conserving intraspecific diversity because such variation is clearly central to the persistence of populations across a diversity of habitats. Although north-western North America has relatively few species of freshwater fishes when compared with other parts of North America, the 'species' level of classification does not recognize all levels of diversity that are important in maintaining populations of a species in a range of environments. Our study indicates that ecology is an important factor promoting intraspecific biodiversity and provides an example of how ecological categories can be used to recognize and categorize such biodiversity. In combination with phylogenetic and population genetic aspects of intraspecific biodiversity, adaptive variation in phenotypic traits such as that which we and Keeley *et al.* (2005) have described provides a comprehensive framework within which to help prioritize populations for conservation.

Acknowledgments

We thank Ken Scheer, Dale Larson and staff of the Fraser Valley Trout Hatchery for help in collecting and raising fish as well as the staff of the Kootenay Trout Hatchery who helped in collecting Gerrard rainbow trout from Kootenay Lake. We thank the Department of Fisheries and Oceans (Canada) for loan of equipment and for use of the Cultus Lake Laboratory facilities. Bryan Smith, Ken Morton and staff of the Cultus Lake Laboratory provided a great deal of support for our work. S. Seiler and two anonymous reviewers provided helpful comments on an earlier version of this work. Thanks are also due to J. Scroggie, M. Phillotts and I. Imre for help in setting up and running the experiment. Financial support was provided by grants from Forest Renewal British Columbia, by the Natural Sciences and Engineering Research Council of Canada, by the National Science

Foundation, by The University of British Columbia, and by a Faculty Research Grant from Idaho State University.

References

- Adams, C.E., 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.
- Boily, P. & Magnan, P. 2002. Relationship between individual variation in morphological characters and swimming costs in brook charr (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). *J. Exp. Biol.* **205**: 1031–1036.
- Campbell, R.N. 1979. Ferox trout, *Salmo trutta* (L.), and charr, *Salvelinus alpinus* (L.), in Scottish lochs. *J. Fish Biol.* **14**: 1–29.
- Grant, J.W.A. & Kramer, D.L. 1990. Territory size as a predictor of the upper limit to population density of juvenile salmonids in streams. *Can. J. Fish. Aquat. Sci.* **47**: 1724–1737.
- Haas, G.R. & McPhail, J.D. 1991. Systematics and distributions of dolly varden (*Salvelinus malma*) and bull trout (*Salvelinus confluentus*) in North America. *Can. J. Fish. Aquat. Sci.* **48**: 2191–2211.
- Hard, J.J. 1995. A quantitative genetic perspective on the conservation of intraspecific diversity. *Am. Fish. Soc. Symp.* **17**: 304–326.
- Hard, J.J., Winans, G.A. & Richardson, J.C. 1999. Phenotypic and genetic architecture of juvenile morphometry in chinook salmon. *J. Hered.* **90**: 597–606.
- Imre, I., McLaughlin, R.L. & Noakes, D.L.G. 2002. Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. *J. Fish Biol.* **61**: 1171–1181.
- Irvine, J.R. 1978. The Gerrard rainbow trout of Kootenay Lake, British Columbia – a discussion of their life history with management, research and enhancement recommendations. Prov. B.C. Fish. Manag. Rep. No. 72.
- Jackson, D.A. 1993. Stopping rules in principal components analysis: a comparison of heuristical and statistical approaches. *Ecology* **74**: 2204–2214.
- Keeley, E.R. 2000. An experimental analysis of territory size in juvenile steelhead trout. *Anim. Behav.* **59**: 477–490.
- Keeley, E.R. & Grant, J.W.A. 1997. Allometry of diet selectivity in juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **54**: 1894–1902.
- Keeley, E.R., Parkinson, E.A. & Taylor, E.B. 2005. Ecotypic differentiation of native rainbow trout (*Oncorhynchus mykiss*) populations from British Columbia. *Can. J. Fish. Aquat. Sci.* **62**: 1523–1539.
- Kerr, S.R. 1971. Prediction of growth efficiency in nature. *J. Fish. Res. Board Can.* **28**: 809–814.
- Magnan, P. 1988. Interactions between brook trout and non-salmonid species: ecological shift, morphological shift, and their impact on zooplankton communities. *Can. J. Fish. Aquat. Sci.* **45**: 999–1009.
- Marschall, E.A. & Crowder, L.B. 1995. Density-dependent survival as a function of size in juvenile salmonids in streams. *Can. J. Fish. Aquat. Sci.* **52**: 136–140.
- McPhail, J.D. & Lindsey, C.C. 1986. Zoogeography of freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In: *The Zoogeography of North American Freshwater Fishes* (C. H. Horcutt & E. O. Wiley, eds), pp. 615–637. John Wiley and Sons, New York.
- Northcote, T.G. & Hartman, G.F. 1988. The biology and significance of stream trout populations (*Salmo* spp.) living above and below waterfalls. *Pol. Arch. Hydrobiol.* **35**: 409–442.
- Pakkasmaa, S. & Piironen, J. 2001. Water velocity shapes juvenile salmonids. *Evol. Ecol.* **14**: 721–730.
- Pielou, E.C. 1991. *After the Ice Age, the Return of Life to Glaciated North America*. The University of Chicago Press, Chicago, IL.
- Proulx, R. & Magnan, P. 2002. Physiological performance of two forms of lacustrine brook charr, *Salvelinus fontinalis*, in the open-water habitat. *Environ. Biol. Fishes* **64**: 127–136.
- Quinn, T.P. 2005. *The Behavior and Ecology of Pacific Salmon and Trout*. University of Washington Press, Seattle, WA.
- Quinn, G.P. & Keough, M.J. 2002. *Experimental Design and Data Analysis for Biologists*. Cambridge University Press, Cambridge, UK.
- Reznick, D.N., Shaw, F.H., Rodd, F.H. & Shaw, R.G. 1997. Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). *Science* **275**: 1934–1937.
- Riddell, B.E. & Leggett, W.C. 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (*Salmo salar*). *Can. J. Fish. Aquat. Sci.* **38**: 308–320.
- Robinson, B.W. & 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. & Wilson, D.S. 1994. Character release and displacement in fishes: a neglected literature. *Am. Nat.* **144**: 596–627.
- Ruiz-Campos, G., Camarena-Rosales, F., Varela-Romero, A., Sánchez-González, S. & Rosa-Vélez, J. 2003. Morphometric variation of wild trout populations from northwestern Mexico (Pisces: Salmonidae). *Rev. Fish Biol. Fish.* **13**: 91–110.
- SAS Institute. 1999. *SAS Online Doc, Version 8*. SAS Institute Inc., Cary, NC.
- Scharf, F.S., Juanes, F. & Rountree, R.A. 2000. Predator size – prey size relationships of marine fish predators: interspecific variation and effects of ontogeny and body size on trophic-niche breadth. *Mar. Ecol. Prog. Ser.* **208**: 229–248.
- Schluter, D. 1995. Adaptive radiation in sticklebacks: trade-offs in feeding performance and growth. *Ecology* **76**: 82–90.
- Schluter, D. 1996. Ecological speciation in postglacial fishes. *Philos. Trans. R. Soc. Lond. B* **351**: 807–814.
- Schluter, D. 2000. *The Ecology of Adaptive Radiation*. Oxford University Press, Oxford, UK.
- Scott, W.B. & Crossman, E.J. 1973. *Freshwater fishes of Canada*. *Bull. Fish. Res. Board Can.* **184**. Canadian Government Publishing Centre, Ottawa, ON.
- Sheehan, T.F., Kocik, J.F., Cadrin, S.X., Legault, C.M., Atkinson, E. & Bengtson, D. 2005. Marine growth and morphometrics for three populations of Atlantic salmon from eastern Maine, USA. *Trans. Am. Fish. Soc.* **134**: 775–788.
- Skúlason, S., Noakes, D.L.G. & Snorrason, S.S. 1989. Ontogeny of trophic morphology in four sympatric morphs of arctic charr *Salvelinus alpinus* in Thingvallavatn, Iceland. *Biol. J. Linn. Soc.* **38**: 281–301.
- Skúlason, S., Snorrason, S.S., Ota, D. & Noakes, D.L.G. 1993. Genetically based differences in foraging behaviour among sympatric morphs of arctic charr (Pisces: Salmonidae). *Anim. Behav.* **45**: 1179–1192.
- Snorrason, S., Skúlason, S., Jonsson, B., Malmquist, H.J., Jónasson, P.M., Sandlund, O.T. & Lindem, T. 1994. Trophic

- specialization in Arctic charr *Salvelinus alpinus* (Pisces; Salmonidae): morphological divergence and ontogenetic niche shifts. *Biol. J. Linn. Soc.* **52**: 1–18.
- Swain, D.P. & Holtby, L.B. 1989. Differences in morphology and behavior between juvenile coho salmon (*Oncorhynchus kisutch*) rearing in a lake and in its tributary stream. *Can. J. Fish. Aquat. Sci.* **46**: 1406–1414.
- Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific salmon and Atlantic salmon. *Aquaculture* **98**: 185–207.
- Taylor, E.B. 1999. Species pairs of north temperate freshwater fishes: evolution, taxonomy, and conservation. *Rev. Fish Biol. Fish.* **9**: 299–324.
- Taylor, E.B. & McPhail, J.D., 1985a. Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Can. J. Fish. Aquat. Sci.* **42**: 2020–2028.
- Taylor, E.B. & McPhail, J.D., 1985b. Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch*. *Can. J. Fish. Aquat. Sci.* **42**: 2029–2033.
- Thorpe, R.S. 1976. Biometrical analysis of geographical variation and racial affinities. *Biol. Rev.* **51**: 407–452.
- Watkins, T.B. & Vraspir, J. 2006. Both incubation temperature and posthatching temperature affect swimming performance and morphology of wood frog tadpoles (*Rana sylvatica*). *Physiol. Biochem. Zool.* **79**: 140–149.
- Weigel, D.E., Peterson, J.T. & Spruell, P. 2002. A model using phenotypic characteristics to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. *Trans. Am. Fish. Soc.* **131**: 389–403.

Received 9 August 2006; accepted 12 August 2006