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Trophically mediated divergence of Arctic charr (*Salvelinus alpinus* L.) populations in contemporary time

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ABSTRACT

Hypothesis: Trophic specializations can evolve in contemporary time in response to changes in trophic opportunities afforded by different ecosystems.

Organism: Lacustrine populations of Arctic charr (*Salvelinus alpinus* L.), a model species in studies of trophic specialization and divergence.

Time and location: A natural experiment in divergence began when piscivorous Arctic charr were translocated from Floods Pond to Long Pond, Maine (USA) in the late 1970s.

Analytical methods: Stomach contents, carbon and nitrogen stable isotope ratios, head and body shape, gill raker morphology, and size-at-age were compared between the translocated and indigenous populations after approximately six generations (25 years) of divergence. The relative rate at which divergence arose in growth and morphological traits was estimated using haldanes.

Results: Despite the fact that ancestrally preferred prey items (fish) were available in both lakes, the translocated population of Arctic charr exhibited a clear shift in both diet and phenotype relative to its source. Differences in diet were primarily attributed to changes in the timing of an ontogenic shift, with Arctic charr from the translocated population becoming piscivorous at a later age. Divergence was also detected in several phenotypic characteristics, including body depth, fin lengths, eye width, maxilla length, gill raker design, and size-at-age.

Conclusions: Significant phenotypic differences between a translocated population of Arctic charr and its ancestral source suggest trophic specializations can diverge in contemporary time. The phenotypic differences noted in this case appear broadly consistent with long-term patterns of trophic specialization, and arose at a relatively rapid rate, even for a contemporary time scale. This suggests that the rudiments of post-glacial diversification, or perhaps even speciation, may arise in response to ecological opportunities very early in the divergence process.

Keywords: contemporary rapid evolution, geometric morphometrics, ontogenic shift, rate of divergence, resource polymorphism, trophic specialization, stable isotopes.

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INTRODUCTION

Population divergence in characters related to differential food resource use has been observed for a wide variety of taxa, including birds (Smith, 1987; Grant and Grant, 2002), amphibians (Collins *et al.*, 1993), insects (Carroll *et al.*, 1997), and freshwater fishes (Malmquist *et al.*, 1992; Robinson *et al.*, 1993; Schluter, 1995; Adams *et al.*, 1998; Lu and Bernatchez, 1999). Correlations of diet with behavioural, morphological, and life-history characteristics further suggests that selection driven by differential trophic ecology likely plays a major role in phenotypic divergence and, ultimately, ecological speciation (Schluter, 2000). Some of the most widely known cases of intraspecific trophic specialization are found among the freshwater fishes of northern temperate freshwater systems (Snorrasson and Skulason, 2004). Although trophic specializations had potentially thousands of years to develop in these regions, we hypothesize that incipient patterns of divergence in traits related to prey acquisition and handling can be observed over much shorter, even contemporary, time frames (less than a few centuries).

A growing body of literature suggests that phenotypic change can be measured in populations over humanly observable time scales (Hendry and Kinnison, 1999; Kinnison and Hendry, 2001). However, relatively few studies of contemporary divergence have focused on trophic specialization, and the focus of those that have has primarily been on terrestrial systems. For example, phenotypic divergence in diet and resource use arose in Galapagos finches (Grant and Grant, 2006) and phytophagous insects (Carroll *et al.*, 1997) over just a few generations in response to changes in the availability of different food items. In contrast, contemporary trophic divergence has rarely been examined for aquatic organisms, despite the aforementioned extensive and influential literature on post-glacial trophic specializations in northern temperate fish species. The dominant hypothesis concerning trophic specialization among post-glacial fishes proposes that divergence begins with behavioural changes in foraging and prey choice, which are themselves shaped by opportunities to use resources that are under-exploited in a given system (Skulason and Smith, 1995). Divergence in behaviour and related phenotypic plasticity then sets the stage for selection on morphological, physiological, and life-history traits, which may in turn lead to further behavioural specialization as the efficiency of feeding on alternative prey items is enhanced. A reinforcing model such as this would likely work through a combination of direct heritable trait change and genetic accommodation of behavioural and phenotypic plasticity (West-Eberhard, 1989; Skulason and Smith, 1995; Robinson and Parsons, 2002). Clearly, the most direct way to evaluate models of the tempo and mode of trophic specialization would be to directly observe the earliest stages of divergence. However, these stages are difficult if not impossible to recognize in the wild. Recent species introductions or invasions could therefore provide important opportunities to observe the processes that lead to trophic specialization, if indeed the translocated population and its source diverge.

Arctic charr (*Salvelinus alpinus* L.) have become a classic system for studies of trophic specialization (reviewed in Jonsson and Jonsson, 2001). Much of the research on trophic divergence among charr has explored resource polymorphisms in sympatric populations (e.g. Sandland *et al.*, 1992; Adams *et al.*, 1998; Alekseyev *et al.*, 2002, Guiguer *et al.*, 2002, Power *et al.*, 2005), but recent work demonstrates that allopatric populations can also show substantial trophic specialization (Morita and Suzuki, 1999; Adams *et al.*, 2007). In either case, trophically mediated selection has driven divergence of behavioural, morphological, and life-history traits among populations (Skulason and Smith, 1995). Patterns of divergence for these traits are typically correlated with size and hardness of the preferred prey type, as well as the limnological region where Arctic charr forage (Jonsson and Jonsson, 2001). But how and when did these diet specializations arise?

Several allopatric populations of Arctic charr occur in the state of Maine, USA. Among these is a recently translocated population of lacustrine Arctic charr, which was created as part of efforts to preserve an endemic piscivorous population of this species (Kircheis, 1980). Although this particular translocation site was selected for its superficial similarity to the source habitat (e.g. temperature, dissolved oxygen, forage species), differences in the ecological community structure could afford a semi-natural opportunity to witness contemporary population divergence as it unfolds (e.g. Carroll *et al.*, 1997; Morita and Suzuki, 1999; Stockwell and Weeks, 1999; Streelman *et al.*, 2004). The specific goals of this study were to use the translocated Arctic charr population and its source to determine: (1) the scope for diet shifts to occur in Arctic charr populations in contemporary time; (2) whether such a shift was associated with divergence in related morphological and life-history traits; and (3) the degree to which such contemporary patterns of divergence are consistent with the patterns of resource specialization described in post-glacially diverged Arctic charr populations.

METHODS

Translocation and lake characteristics

Arctic charr in Floods Pond have long been recognized as phenotypically distinct among charr populations in Maine (Kendall, 1914; Kircheis, 1980), and are commonly referred to as 'Sunapee Trout'. Recent diet, morphology, and life-history analyses confirm this population represents a relatively specialized pelagic piscivore, and is indeed phenotypically distinct among Arctic charr populations in Maine (W.K. Michaud and M.T. Kinnison, unpublished). Microsatellite analyses, however, suggest that the Floods Pond population derives from the same glacial lineage as all other Arctic charr in Maine (Bernatchez *et al.*, 2002).

As part of an effort to preserve the Floods Pond lineage, sexually mature Arctic charr were translocated from Floods Pond to Long Pond during the fall of 1977 (40 males and 49 females) and again in 1979 (48 males and 65 females). These individuals were captured on a known spawning shoal in Floods Pond, then transported by aeroplane to an area of Long Pond that appeared to provide suitable spawning habitat. Both lakes are physically similar, and contain the preferred forage species of Floods Pond charr, the rainbow smelt (*Osmerus mordax*) (Table 1). Brook charr (*Salvelinus fontinalis*), a potential competitor and predator that is ubiquitously sympatric with Arctic charr in Maine, is also found in both locations. However, the fish communities found in each lake do differ (Table 1).

Table 1. Sample site characteristics and sampling dates

Lake	Area (hectares)	Mean depth (m)	Oxygen ($mg \cdot l^{-1}$)	Average Secchi (m)	Fish species[a]	Sampling Dates
Long Pond	107	12	10 @ 27 m	8.3	4	July 2003
Floods Pond	257	12	7 @ 39 m	7.3	10	August 2003
						July 2004
						August 2004

[a] Long Pond: *Salvelinus alpinus*, *Salvelinus fontinalis*, *Osmerus mordax*, *Couesius plumbeus*. Floods Pond: *Salvelinus alpinus*, *Salvelinus fontinalis*, *Osmerus mordax*, *Castostomus commersonii*, *Lepomis gibbosus*, *Fundulus diaphanous*, *Luxilus cornutus*, *Semotilus corporalis*, *Notemigonus crysoleucas*, *Pungitius pungitius*, *Gasterosteus aculeatus*.

Fish collection and processing

Arctic charr were sampled from Floods Pond and Long Pond during the summers of 2003 and 2004 (Table 1). Fish were caught using monofilament gillnets (1–5 cm bar mesh), each set at a depth of approximately 30 m for 12–24 h. All fish were placed immediately on ice, and digital photographs were taken of the left side of every fish within 12 h of capture. Images were standardized using a camera set at a fixed focal length, and a ruler was included in each photograph for size reference. Head width (measured as inter-orbital distance) was also recorded soon after capture. Individuals were then frozen to preserve them for later dissection. Gill arches, sagittal otoliths, and stomach contents were removed from each fish, and sex and maturity status were assessed during dissection by examining gonadal development.

All statistical analyses noted below were performed using SPSS, Version 14.0 (SPSS Inc., 2005). Statistical significance was set at $\alpha = 0.05$, unless otherwise noted.

Diet

Stomach contents from all individuals were examined to determine the presence or absence of items from each of three prey categories: zooplankton, insect larvae, and fish. Differences in diet based on stomach contents were then tested using a chi-squared test for each prey category.

Analyses of carbon and nitrogen stable isotopes were conducted to further explore the trophic status of each population. Muscle stable isotope signatures were examined in particular because they are reflective of an individual's diet over a period of months, which complements the diet 'snapshot' obtained from examination of stomach contents. Dorsal muscle tissue, taken just anterior of the dorsal fin, was removed from a representative set of 20 charr from each lake and processed according to the methods described in Guiguer *et al.* (2002). Individuals included in this analysis were selected from the larger samples to span the range of age classes (as determined from otoliths) obtained from each lake. All stable isotope values are reported in conventional δ notation, where $\delta^{13}C$ or $\delta^{15}N = [(R_{sample} - R_{standard})/R_{standard}] \times 1000$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The relationships between fork length and both mean $\delta^{13}C$ or $\delta^{15}N$ were also examined using regression and analysis of variance (ANOVA) to evaluate ontogenic changes in diet for each population.

Gill raker morphology

The most anterior, left gill arch was used for comparisons of gill raker morphology between populations. Individual arches were pressed flat between two sheets of plexiglass and photographed (along with a ruler for size reference) using a digital camera mounted at a fixed focal length. From these images we obtained measurements of mean gill raker length, width at the base, and spacing (measured from the edge of one raker to the next at the base), for the first three rakers below the apex on the ventral side of the arch, as well as a count of total number of gill rakers per arch. All the above measurements were obtained using the program ImageJ, Version 1.32 (Rasband, 2004). Since we were primarily interested in the ability of fish to handle prey of different sizes, we decided to examine gill raker morphology in relation to a surrogate for head size, gill arch size (measured as the distance between the apex and the last gill raker on the ventral side of the arch). Differences in the allometric

relationship of each gill raker measurement and gill arch size (using $\log_{10}$-corrected values) was initially tested using an analysis of covariance (ANCOVA) model containing a population × size interaction term. Population differences in these traits were then tested using ANCOVA, but with the interaction term removed for traits where it was non-significant. A size-adjusted data set was also created for the purpose of estimating population divergence rates (see below). In this case, the trait variables were size-corrected using a common within-groups slope adjustment as suggested by Reist (1986), with all individuals adjusted to a hypothetical gill arch size of 8.00 mm. Difference in the mean gill raker number between populations was assessed using ANOVA.

Body morphology

The morphological analyses presented here use only females to avoid confounding trophic-related divergence with disparate sex ratios between populations and secondary sexual trait development in males. Two-dimensional body shape variation was quantified using geometric morphometric approaches. Relative warp scores were calculated in tpsRelw, Version 1.42 (Rohlf, 2005a) using 18 homologous landmarks placed on standardized digital images of each fish (Fig. 1) using the program tpsDig, Version 2.0 (Rohlf, 2004). Analysis of covariance was first used to test for population differences in the allometric relationship between each relative warp and size (using centroid size as the covariate). Differences in relative warp scores between populations were then tested using ANCOVA, but with the interaction term removed for traits where it was insignificant. Relative warp scores were also used in a discriminant functions analysis to test for and summarize the morphological divergence between groups. The resulting discriminant functions were regressed back onto the partial warps using tpsRegr, Version 1.31 (Rohlf, 2005b), to produce thin-plate spline transformation images depicting the shapes associated with the extremes of each discriminant function. Since the above analysis only accounts for isometric effects of size scaling, ANCOVA was subsequently used to test for any allometric relationship between the discriminant function and body size, using centroid size as the covariate for body size.

In addition to these shape differences, we also considered several linear measurements that could not be incorporated into the relative warps analysis either because of the inconsistent location of their associated landmarks (e.g. fin tips), or because they did not fit into the two dimensions described by the relative warps (e.g. head width). These linear

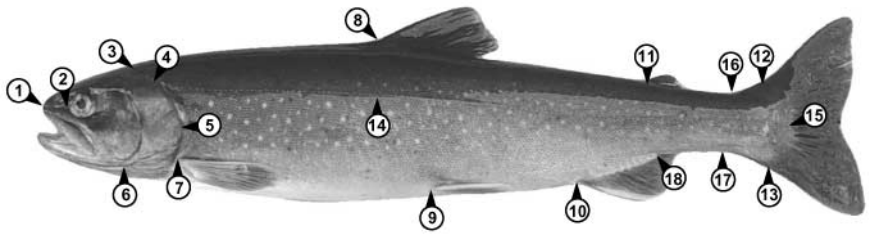

Fig. 1. Anatomical landmarks used for morphological analyses: (1) tip of snout; (2) left of eye socket; (3) posterior edge of skull; (4) top of operculum; (5) most posterior point of operculum; (6) bottom of operculum directly below lowermost point of pre-operculum; (7–13) fin insertions; (14) point on lateral line directly below insertion of dorsal fin; (15) posterior centre point of caudal peduncle; (16, 17) narrowest part of caudal peduncle; and (18) insertion of last anal fin ray.

measurements included pectoral fin length, dorsal fin length, pelvic fin length, anal fin length, caudal fin length, eye width, maxilla length, and head width. Allometric effects were tested for in population comparisons by using analyses of covariance on $\log_{10}$-transformed trait values and $\log_{10}$ standard length (measured as the distance from the posterior edge of the eye to the tip of the caudal peduncle) as a covariate. Population differences in these traits were then tested using ANCOVA, but with the interaction term removed for traits where it was insignificant. We also created a size-adjusted data set of these traits for estimating population divergence rates using allometric coefficients from the common within-groups slopes and a standard length of 187.29 mm.

Growth

Whole sagittal otoliths were immersed in a 50% glycerin solution and examined to determine individual ages. Annuli were counted on both left and right otoliths (when possible), and age was determined based on agreement between these counts. The following model was used to compare mean-size-at-age between populations, using fork length as a measure of size:

$$\text{size} = b_0 + b_1(\text{age}) + b_2(\text{population}) + b_3(\text{age} \times \text{population}) + \varepsilon$$

Individual population growth curves were estimated using the Von Bertalanffy growth function. This was fitted to the length-at-age data using the least squares method, although biological information was also considered in selecting reasonable parameter estimates since the model was affected by a lack of data for age 0–3 Arctic charr in both populations. The growth curves were compared between populations using the analysis of residual sums of squares method described by Chen *et al.* (1992).

Rates of divergence

Rates of phenotypic divergence in body morphology (individual traits and discriminant function values), gill raker morphology, and size at age 9 (mean age) were estimated in haldanes as described in Hendry and Kinnison (1999), using size-adjusted data where appropriate (see above). Since the actual generation time for each population is unknown, a conservative mean generation time estimate of $g = 6.25$ was employed, based on the assumption that most trait change occurred in the Long Pond population since the time of introduction (see Kinnison and Hendry, 2001). This generation length assumes that most individuals in each population mature at age 4, which is the age of first maturation previously reported for Arctic charr in Floods Pond (Kircheis, 1976), and accounts for 25 years of isolation (1979–2004). The significance of each haldane estimate was tested using a modified two-sample *t*-test (A. Hendry, personal communication).

RESULTS

Diet

Stomach contents were obtained for 91 Arctic charr from Long Pond and 38 from Floods Pond. Individuals with empty stomachs (Long = 12, Floods = 13) were omitted from the diet analysis. Most individuals sampled from each population consumed insect larvae;

however, a significantly greater percentage of Long Pond charr utilized invertebrate prey resources, while more Arctic charr from Floods Pond included fish in their diet (Table 2). This suggests that the population of Arctic charr in Floods Pond relies more heavily on piscivory than those in Long Pond.

Stable isotope signatures were consistent with the differences in diet inferred from stomach contents, and provided further resolution of trophic distinctions. Based on $\delta^{15}N$ values, all Arctic charr sampled from Floods Pond appear to feed at approximately the same trophic level (mean ± 95% CI: $\delta^{15}N = 10.03 \pm 0.15$), while two trophic level clusters are apparent among the $\delta^{15}N$ values for Arctic charr from Long Pond (overall mean $\delta^{15}N = 9.13 \pm 0.29$) (Fig. 2a). Plotting $\delta^{15}N$ against length, which is correlated with age for both populations (Flood: $r = 0.79$; Long: $r = 0.71$), reveals that the clustering among the Long Pond charr may be explained by an ontogenic shift occurring at a fork length of approximately 210 mm (Fig. 2b). When each population is split into a small (< 210 mm) and a large (> 210 mm) group, the size groups in Long Pond have significantly different $\delta^{15}N$ values (mean ± 95% CI: small = 8.45 ± 0.15, large = 9.90 ± 0.18, $P < 0.001$), while the equivalent groups in Floods Pond do not (small = 9.93 ± 0.38, large = 10.24 ± 0.17, $P = 0.380$).

Table 2. Percentage of individuals from each population with organisms from a given prey category present in stomach contents

	Plankton	Insect	Fish
Floods Pond	5%	79%	50%
Long Pond	1%	90%	14%
P-value	**	0.039	<0.001

Note: *P*-values were obtained from Pearson's chi-squared tests of frequency differences between the groups.
** Insufficient data for significance test.

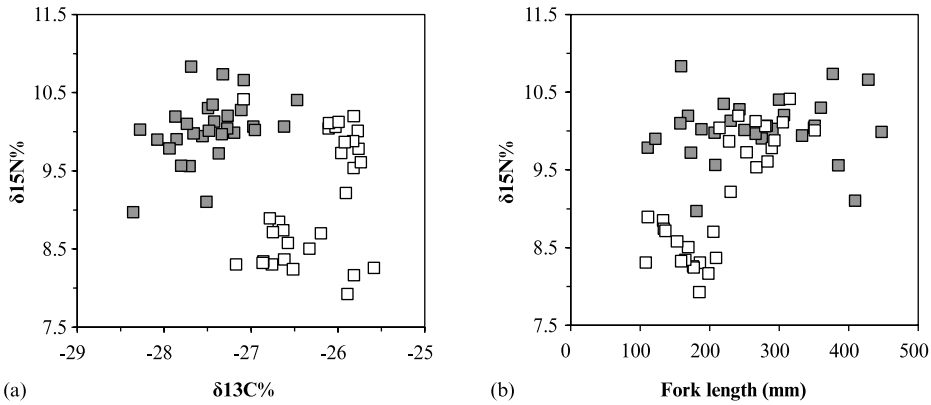

Fig. 2. (a) Stable isotope plots for Arctic charr from Floods Pond (■) and Long Pond (□). (b) $\delta^{15}N$, as an indication of relative trophic position, vs. individual fork length.

Examination of $\delta^{13}C$ values also indicated an ontogenic diet shift in the Long Pond population. As seen in Fig. 2a, there is a concurrent shift in the $\delta^{13}C$ signatures of individuals from Long Pond, which also occurs at a fork length of approximately 210 mm. If we again split each population into groups of small and large size, we find that the $\delta^{13}C$ values for these groups again differ in Long Pond (mean ± 95% CI: small = −26.50 ± 0.23, large = −25.99 ± 0.20, $P = 0.001$), but also in Flood Pond (small = −27.79 ± 0.29, large = −27.34 ± 0.19, $P = 0.008$). In this case, both populations likely include more items from the littoral food web as they grow. However, among Floods Pond Arctic charr there is a gradual shift along the carbon gradient with increasing trophic position, whereas the Long Pond Arctic charr experience an abrupt shift to an entirely different food source and a greater littoral affiliation overall.

Gill raker morphology

Gill rakers were examined for 69 Arctic charr (Long = 44, Floods = 25). Of the four gill raker variables examined, only spacing and length demonstrated significantly different allometric relationships with gill arch size (Table 3). The intercept of the relationship between gill raker spacing and gill arch size was significantly greater for Floods Pond charr (intercept: Floods = −0.75, Long = −1.17, $P = 0.013$), but spacing increased at a greater rate with gill arch size for the Long Pond fish (slope: Floods = 0.64, Long = 1.11, $P = 0.011$). The

Table 3. Mean trait values (all in millimetres) and *P*-values associated with the population effect and population × size interaction effect of the univariate analyses of covariance for morphological trait variables

	Mean ± 95% CI		*P*-value	
Trait	Long Pond	Floods Pond	Population	Population × size
Gill arch size	7.25 ± 0.58	9.44 ± 1.26	0.006	—
Gill raker width	0.46 ± 0.04	0.58 ± 0.10	0.545	0.387
Gill raker spacing	0.63 ± 0.07	0.75 ± 0.10	0.013	0.011
Gill raker length	2.32 ± 0.23	2.55 ± 0.32	0.052	0.015
Standard length	177.90 ± 10.70	206.73 ± 27.22	0.107	—
Head width	17.70 ± 1.09	18.60 ± 2.34	<0.001	0.124
Eye width	7.94 ± 0.25	9.93 ± 0.58	<0.001	0.926
Maxilla length	15.63 ± 0.96	19.88 ± 2.67	<0.001	0.986
Pectoral fin length	29.30 ± 1.72	33.07 ± 3.75	0.001	0.001
Pelvic fin length	20.72 ± 1.05	22.39 ± 2.74	0.030	0.723
Dorsal fin length	27.56 ± 1.52	30.40 ± 3.66	0.130	0.866
Anal fin length	20.68 ± 1.52	24.75 ± 3.85	0.723	0.199
Caudal fin length	31.78 ± 1.73	38.43 ± 3.99	0.004	0.011
Centroid size	1137.30 ± 67.38	1114.32 ± 144.725	0.739	—
Relative warp 2	0.0016 ± 0.002	−0.0033 ± 0.005	<0.001	<0.001
Relative warp 5	−0.0027 ± 0.001	0.0056 ± 0.003	<0.001	0.679

Note: Gill arch size was included as the size covariate for each gill raker variable, centroid size for the relative warps, and standard length was included in analyses for fin size and head traits. Univariate ANOVA results for each of the size covariates are also included.

same pattern occurred for gill raker length, with Floods Pond fish initially having longer gill rakers (intercept: Floods = −0.42, Long = −0.66), but gill raker length increased at a greater rate with gill arch size for the Long Pond charr (Floods = 0.84, Long = 1.18, $P = 0.015$). The number of gill rakers (Floods = 16.04, Long = 15.50, $P = 0.298$) and gill raker width did not differ significantly between populations (Table 3).

Body morphology

Analysis of relative warp scores for 86 female Arctic charr (Floods = 28, Long = 58) revealed significant differences in two-dimensional body shape between the transplant population and its ancestral source. Before any analyses, the first relative warp was eliminated as it described variance in shape due mainly to fish not being placed perfectly straight in each photograph ('bending' along the dorsal–ventral axis). Analysis of covariance on the remaining relative warps revealed significant population differences for only relative warps 2 and 5 (Table 3, Fig. 3). Centroid size did not differ significantly between the populations, although allometric (slope) differences were detected for relative warp 2 (Table 3). Smaller fish from Floods Pond are associated with more negative values of relative warp 2 (intercept: Floods = −0.036, Long = −0.005, $P < 0.001$), while larger fish from Floods Pond have more positive values than those from Long Pond (slope: Floods = 0.00003, Long = 0.00001, $P < 0.001$).

Discriminant functions analysis summarized the two-dimensional shape differences between populations (Wilks' $\lambda = 0.349$, $P < 0.001$) along a gradient defined mainly by head and body depth (Fig. 3). Floods Pond charr cluster towards the deeper-bodied, larger-headed end of this spectrum, while those from Long Pond appear to have a thinner, more streamlined body shape. The reclassification rates of individuals from both groups also indicate strong divergence in shape between populations (percent correct classification: Floods = 89%, Long = 98%). Analysis of covariance indicated centroid size did not explain a significant portion of the variation in our discriminant function ($P = 0.406$), although population, not surprisingly, did ($P < 0.001$). This result indicates that the geometric shape differences observed between populations are independent of body size.

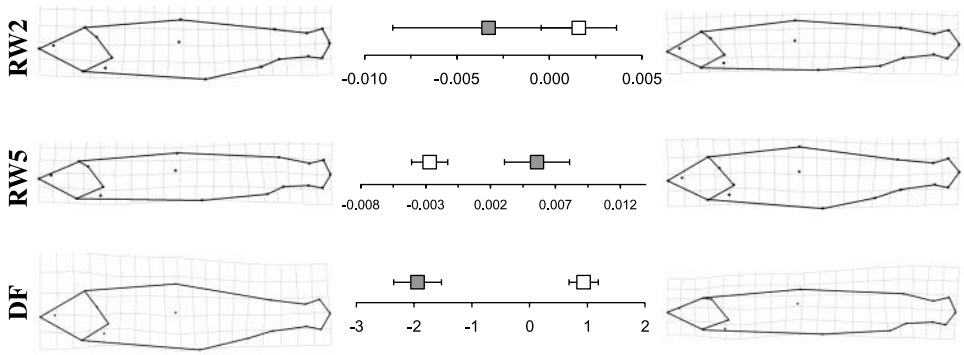

Fig. 3. Mean relative warp (RW) and discriminant function (DF) scores for Floods Pond (■) and Long Pond (□) (± 95% CI). Interpretations of high negative and positive scores are indicated at the end of each axis. Images show thin-plate spline transformations depicting body shapes at the extremes of each function.

Significant differences between populations were also found for some of the linear traits not included in the relative warps analysis (Table 3). Comparison of standard length between populations revealed no significant difference between populations; however, covariance analysis indicated significant allometric (slope) divergence for pectoral and caudal fin lengths. Smaller Floods Pond fish have longer pectoral fins for their size (intercept: Floods = −0.36, Long = −0.66, $P = 0.001$), but pectoral fin length increases at a greater rate with size in the Long Pond population (slope: Floods = 0.81, Long = 0.94, $P = 0.001$). Caudal fin length was also greater among the smaller Floods Pond charr (intercept: Floods = −0.17, Long = −0.49, $P = 0.004$), while Long Pond fish exhibited a greater increase in caudal fin length with size (slope: Floods = 0.76, Long = 0.89, $P = 0.011$), with the two regression lines converging at the upper end of the observed size range. Analysis of covariance also indicated that head width, eye width, maxilla length, and pelvic fin length differed significantly among populations (Table 3).

Size-at-age

The two populations exhibited significant differences in mean size-at-age ($P < 0.001$), as well as a marginal difference in the Von Bertalanffy growth functions estimated for each group ($P = 0.052$) (Fig. 4). The Floods Pond population was estimated to have a larger asymptotic length value ($L\infty = 810$ mm) and smaller growth coefficient ($k = 0.03$) than the Long Pond population ($L\infty = 550$ mm, $k = 0.04$). However, it should be noted that k values for both populations might have been underestimated due to the lack of young individuals in the data set (those less than 4 years of age).

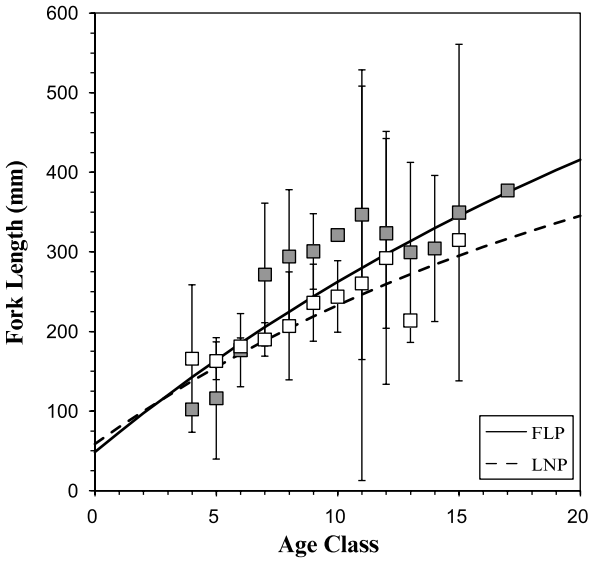

Fig. 4. Plot of mean size-at-age (± 95% CI) for individuals collected from Floods Pond (FLP, ▩) and Long Pond (LNP, □), overlaid with the Von Bertalanffy growth function estimated for each population.

Table 4. Rates of divergence for mean phenotypic traits between Floods Pond and Long Pond over 6.25 generations (listed in descending order)

Trait	Haldanes
Eye width	0.351*
Relative warp 5	0.232*
Caudal fin length	0.205*
Maxilla length	0.187*
Length at mean age	0.172*
Head width	0.170*
Gill raker width	0.106*
Gill raker spacing	0.086*
Pelvic fin length	0.085*
Relative warp 2	0.079*
Dorsal fin length	0.055
Gill raker length	0.048
Gill raker number	0.041
Anal fin length	0.020
Pectoral fin length	<0.001

* Traits whose rates of divergence differed significantly from zero ($\alpha = 0.05$).

Rates of divergence

Significant rates of divergence were calculated for 10 traits, with eye width exhibiting the greatest rate of divergence (Table 4). Nearly all of the significant rate estimates are larger than the expected mean for this time scale (0.091 haldanes) based on a review by Kinnison and Hendry (2001). As such, it is reasonable to suggest that these rates are as rapid, if not more rapid, than many other documented rates of contemporary trait change in the wild.

DISCUSSION

Significant phenotypic divergence has occurred between the translocated Arctic charr population in Long Pond and its ancestral source population in Floods Pond. These differences developed over only 25 years (approximately six generations), and constitute phenotypic rates of change ($h_{p(0.796)} = 0.079$–0.351) that are equivalent to some of the fastest rates of divergence observed over comparable time scales in nature (see Kinnison and Hendry, 2001, for a general review). Importantly, the specific morphological and life-history differences observed here arose concurrently with a shift in resource use by the translocated population, as clearly evidenced by stomach contents and stable isotope signatures. As we shall discuss, the combined evidence from behavioural (diet), life-history (size-at-age), and morphological traits suggest an integrated contemporary analog to the patterns of post-glacial divergence commonly noted in Arctic charr in other parts of their range. Although the relative degree to which the observed population differences are heritable remains to be determined, the analogous nature of these divergence patterns would suggest that much of the phenotypic

template that characterizes post-glacial specializations in this species is established very early in the divergence process.

Individuals from both populations consume fish as part of their diets; however, Arctic charr in Floods Pond clearly utilize this resource much more heavily than those in Long Pond. Although this was evident from both stomach contents and stable isotope analyses, the isotope signatures provide the additional insight that trophic divergence in this instance is perhaps best characterized by a shift in the timing of an ontogenic switch to piscivory (Fig. 2). The $\delta^{15}N$ values suggest all Arctic charr sampled from Floods Pond feed at a trophic level indicative of piscivory for this population. Arctic charr from Long Pond, however, do not undergo a clear shift towards piscivory until approximately 210 mm in length. Based on stomach contents, it is likely that this reflects a shift from a primarily chironomid or invertebrate diet to one that includes small fish. Interestingly, this change in diet is consistent with that observed for piscivorous Arctic charr from Thingvallavatn, where a shift to piscivory occurs between 200 and 250 mm (Malmquist *et al.*, 1992), and small-form Arctic charr from Lake Hazen, where a noted $\delta^{13}C$ enrichment associated with a shift in resource use also occurs in the 220–250 mm range (Guiguer *et al.*, 2002).

It is worth noting that the shift in diet of Arctic charr in Long Pond occurred despite the availability of similar prey items in both lakes. Piscivorous charr translocated from Floods Pond to Long Pond could have continued to feed primarily on their reportedly preferred prey, pelagic rainbow smelt, but this does not appear to be the case. This observation has important implications for conservation efforts and species invasions. Namely, this demonstrates simple availability of an ancestrally dominant food item in a new location may not be sufficient to maintain a particular feeding specialization in the new environment. Rather, it would appear that retention of a diet specialization involves a more complicated interaction between the relative costs, benefits, and opportunities for utilizing alternative prey resources in the new environment. Superficial similarities in lake geomorphology and fish community structure are, therefore, unlikely to provide an accurate picture of the relative trophic opportunities and potential for divergence when a species is moved into a new system. If this is typically the case, then translocations may generally prove to be a risky approach for conserving specific trophic specializations and the unique phenotypes of some threatened populations (Stockwell *et al.*, 2003).

Interestingly, the primary diet difference between Floods Pond and Long Pond charr is the delayed ontogenic shift to piscivory among the translocated, Long Pond fish. This could indicate that the relative benefits of, or opportunities for, piscivory are reduced in Long Pond relative to Floods Pond. For example, charr introduced to Long Pond may have encountered a reduced opportunity for utilizing forage fish, particularly at smaller sizes, or an especially abundant and high-quality invertebrate resource (or both conditions). While we do not have direct estimates of forage fish abundance, Floods Pond does contain more potential forage fish species than Long Pond (Table 1), which could provide a more stable and widely accessible resource for piscivorous Arctic charr over a wider prey size range. Conversely, there is no reason to presume piscivory is a universally superior diet strategy among Arctic charr, as opportunities to meet resource needs via invertebrate sources may be equally suitable. Floods Pond Arctic charr also exhibit slower growth (k) and smaller size-at-age than Long Pond fish during their early years (Fig. 4), when smaller invertebrates would comprise the bulk of their diets. This suggests invertebrate resources may be more limiting in Floods Pond, favouring an earlier shift to piscivory. However, explanations based on the abundance of forage fish or invertebrates are not mutually exclusive, and both factors

may contribute to diet divergence. Indeed, many of the potential forage fish species in Floods Pond feed on insect larvae, and are therefore likely to influence the availability of invertebrate prey for Arctic charr through competition for this resource. Irrespective of a proximate basis, the early emergence of ontogenic diet differences in our study would support the hypothesis that trophic specializations in post-glacial fishes may be at least in part associated with behavioural plasticity (Skulason *et al.*, 1999).

Divergence in ontogenic diet shifts, which may be expected to lay the groundwork for trophic specialization, was in this instance also coupled with divergence in a number of potentially related morphological traits. Floods Pond Arctic charr represent a highly piscivorous form of Arctic charr, while those from Long Pond have some characteristics that are more consistent with populations that forage for smaller, invertebrate prey items. Shape analysis reveals Long Pond fish have thinner, more streamlined bodies overall. This fusiform body shape is consistent with a pelagic form that must continuously cruise through the water column to pick-off small invertebrate prey. In contrast, the stouter body and larger caudal fins (among smaller individuals) observed for piscivorous Arctic charr from Floods Pond may reflect adaptations for the rapid burst speed and manoeuvrability needed to capture fast-moving fish prey (Webb, 1975). Individuals from the translocated population also tended to have smaller heads with shorter maxilla (indicative of a smaller gape size), and more closely spaced gill rakers (especially in smaller individuals), which all aid in capturing smaller food particles with greater efficiency. In combination, these differences appear roughly analogous to the distinctions between large and small pelagic forms of Arctic charr described for populations in other parts of the species' range (Adams *et al.*, 1998; Jonsson and Jonsson, 2001; Alekseyev *et al.*, 2002).

The trends in divergence observed for diet, body shape, gill raker design, and growth between Floods Pond and Long Pond charr suggest a broad-spectrum shift in trophic specialization. However, some indications of mosaic evolution are also evident (see Stebbins, 1983, for review), in that not all traits considered here diverged at the same rate (Table 4). This mosaic pattern may reflect an early premium on features linked to prey capture (e.g. visual detection and gape size), as opposed to features associated with the efficiency of processing prey items (such as gill raker morphology). Therefore, this could be reflective of an intermediate diet transition phase towards specializing on a smaller prey resource or a trade-off between juvenile and adult diets. The potential for incomplete specialization in the translocated population might best be demonstrated by placing the magnitude of this contemporary divergence into context with post-glacial divergence. For example, while morphologically divergent, the difference in geometric body shape between Floods Pond and Long Pond fish is still less than half the shape difference observed between Floods Pond charr and any other population thus far characterized in Maine (W.K. Michaud and M.T. Kinnison, unpublished data). Longer time frames may therefore be required to fully attain the scale and pattern of divergence seen in post-glacially isolated populations. Conversely, Long Pond Arctic charr may remain more similar to Floods Pond Arctic charr when compared with other populations, if the environmental characteristics of Long Pond are indeed more similar to those of Floods Pond than to those of other ecosystems in the region.

The variation in phenotypes observed between Arctic charr in Floods Pond and Long Pond indicate trophic divergence can arise very quickly in populations subject to shifts in community structure and associated resource opportunities. Although it was beyond the scope of this project to evaluate the genetic mechanisms behind these differences, previous surveys of contemporary trait change in the wild suggest that both evolution and

phenotypic plasticity are likely involved to some degree (e.g. Kinnison and Hendry, 2001), with plasticity playing a large role in cases such as this one, where humans act as agents of disturbance (Hendry *et al.*, 2008). Recent diet and habitat manipulation studies in other populations of Arctic charr clearly demonstrate a degree of plasticity is associated with changes in similar morphological traits (Adams *et al.*, 2003; Andersson, 2003; Peres-Neto and Magnan, 2004; Andersson *et al.*, 2005). However, the potential for phenotypic plasticity does not diminish the relevance of our findings for understanding the early stages of trophic specialization. Genetic assimilation or other forms of genetic accommodation of plasticity have long been invoked as part of theories of adaptation and speciation (see Crispo, 2008, for a review). This is particularly true of hypotheses for the origins of trophic polymorphisms in Arctic charr, wherein behavioural plasticity linked to diet is suggested to pave the way for genetic specialization (West-Eberhard, 1989; Skulason *et al.*, 1999). Disentangling the early interactions between plasticity and local adaptation should be a future goal in our understanding of the origins of post-glacial fish radiations.

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