Evolutionary Ecology Research, 2012, **14**: 147–167

Phenotypic divergence of exotic fish populations is shaped by spatial proximity and habitat differences across an invaded landscape

Peter A.H. Westley,* Corinne M. Conway and Ian A. Fleming

Ocean Sciences Centre, Memorial University of Newfoundland, St. John's, Newfoundland, Canada

ABSTRACT

Background: Brown trout (*Salmo trutta*) were introduced into, and subsequently colonized, a number of disparate watersheds on the island of Newfoundland, Canada (110,638 km^2), starting in 1883.

Questions: Do environmental features of recently invaded habitats shape population-level phenotypic variability? Are patterns of phenotypic variability suggestive of parallel adaptive divergence? And does the extent of phenotypic divergence increase as a function of distance between populations?

Hypotheses: Populations that display similar phenotypes will inhabit similar environments. Patterns in morphology, coloration, and growth in an invasive stream-dwelling fish should be consistent with adaptation, and populations closer to each other should be more similar than should populations that are farther apart.

Organism and study system: Sixteen brown trout populations of probable common descent, inhabiting a gradient of environments. These populations include the most ancestral (~130 years old) and most recently established (~20 years old).

Analytical methods: We used multivariate statistical techniques to quantify morphological (e.g. body shape via geometric morphometrics and linear measurements of traits), meristic (e.g. counts of pigmentation spots), and growth traits from 1677 individuals. To account for ontogenetic and allometric effects on morphology, we conducted separate analyses on three distinct size/age classes. We used the BIO-ENV routine and Mantel tests to measure the correlation between phenotypic and habitat features.

Results: Phenotypic similarity was significantly correlated with environmental similarity, especially in the larger size classes of fish. The extent to which these associations between phenotype and habitat result from parallel evolution, adaptive phenotypic plasticity, or historical founder effects is not known. Observed patterns of body shape and fin sizes were generally consistent with predictions of adaptive trait patterns, but other traits showed less consistent patterns with habitat features. Phenotypic differences increased as a function of straight-line distance (km) between watersheds and to a lesser extent fish dispersal distances, which suggests habitat has played a more significant role in shaping population phenotypes compared with founder effects.

Correspondence: P.A.H. Westley, School of Aquatic and Fishery Sciences, University of Washington, Box 355020, Seattle, WA 98195, USA. e-mail: resolute@uw.edu
Consult the copyright statement on the inside front cover for non-commercial copying policies.

© 2012 Peter A.H. Westley

Conclusion: Recently established brown trout populations exhibit phenotype-by-environment correlations consistent with adaptation to newly encountered environments, a characteristic that may aid their spread to additional systems.

Keywords: allometry, biological invasions, coloration patterns, geometric morphometrics, microevolution, phenotypic divergence with distance, phenotypic plasticity, salmonid fishes.

INTRODUCTION

Parallel evolution of similar traits in independently derived populations inhabiting similar environments provides strong evidence of natural selection as the causal agent (Nosil, 2012). Adaptive phenotypic change can occur within a small number of generations in populations experiencing abrupt environmental shifts and divergent regimes of natural selection (Hendry and Kinnison, 1999; Schluter, 2000; Ghalambor *et al.*, 2007). Empirical examples of this 'contemporary' phenotypic change occur among a range of taxa, including fruit flies (Huey *et al.*, 2000), aquatic invertebrates (Eroukhmanoff *et al.*, 2009), reptiles (Losos, 2009), fishes (Stearns, 1983; Haugen and Vollestad, 2001), birds (Johnston and Selander, 1964), and mammals (Williams and Moore, 1989). Biological invasions provide opportunities to examine population divergence in real time (Huey *et al.*, 2000; Hendry *et al.*, 2008; Westley, 2011). A first step towards understanding how exotic species may have responded to their new environments is to examine associations between habitat features and phenotypic traits likely linked to fitness (Haugen, 2000). Testing for signals of adaptation in introduced populations has additional applied value because rapid changes in invader phenotypes can feedback on the potential for further population expansion (Phillips *et al.*, 2006; Kinnison and Hairston, 2007).

Repeated global translocations of fishes, such as salmon, trout, and charr (family Salmonidae), afford excellent research opportunities to test patterns and processes of contemporary adaptive phenotypic change (e.g. Crawford and Muir, 2008). Salmon and trout are renowned for their remarkable diversity in behaviour, ecology, morphology, and life history both among species and among populations within species (Groot and Margolis, 1991; Elliott, 1994; Fleming, 1998; Klemetsen *et al.*, 2003; Quinn, 2005). This diversity is typically thought to reflect local adaptation to environmental conditions (Taylor, 1991; Garcia de Leaniz *et al.*, 2007) with much of it having evolved within 5000 to 15,000 years after the last glacial epoch (Kinnison and Hendry, 2004). Translocations allow refinement of the time-scales over which adaptive change can arise, as the precise ages of populations are often known from records (Hendry *et al.*, 2000; Quinn *et al.*, 2001; Ayllon *et al.*, 2006). Here we use brown trout (*Salmo trutta*) introduced to the island of Newfoundland, Canada, as such a model system.

In this paper, we quantify the phenotypic response of recently established populations to newly experienced abiotic habitat features. Specifically, we assess variation in body shape and fin sizes, coloration and pigmentation patterns, and growth rates of individuals within and among populations, and correlate this suite of phenotypic traits to habitat characteristics such as river size, water chemistry, and spatial proximity of watersheds to each other (relative to their distances to the putative source watershed of the initial introduction site). To the extent that phenotypes are shaped or selected by the environment, we predicted that rivers with similar habitat features would contain populations exhibiting similar phenotypic traits. Specifically, we predicted that: (1) habitats characterized by relatively high water discharge [stream width and depth used in lieu of discharge data

(Ward and Trimble, 2004)] would correlate with the expression of streamlined body shapes and larger fins, which in fishes relate to swimming efficiency (Langerhans, 2008); (2) coloration patterns would be inversely related to the extent of canopy cover and water clarity, where relatively drab and dark coloration patterns would be expressed in relatively dark environments; (3) water conductivity [a surrogate for nutrient input and productivity (Paul and Meyer, 2001)] would mediate trait expression indirectly through growth; and (4) populations in close spatial proximity would be phenotypically similar due to a combination of environmental similarity (watersheds close in space may be similar), greater shared ancestry, and greater potential for ongoing gene flow.

METHODS

Brown trout in Newfoundland

The brown trout is a member of the family Salmonidae native to Europe, North Africa, and western Asia. Its distribution has expanded rapidly via intentional introductions and the species is now established on every continent, except Antarctica (MacCrimmon and Marshall, 1968; Elliott, 1994). Brown trout display dramatic variation in morphology, colour, and life-history patterns, which has been recognized by astute naturalists since at least the seventeenth century (Walton, 1653). This variation has been revealed to reflect interactions between heritable divergence and environmentally induced plasticity (Ferguson and Mason, 1981; Ferguson, 1989; Ferguson and Taggart, 1991; Bernatchez *et al.*, 1992; Hutchings, 2011).

The founding trout in Newfoundland were primarily comprised of non-anadromous (freshwater resident) Loch Leven strain from the Howietoun Hatchery in Scotland. Fish were first introduced to the Windsor Lake watershed in 1883 and a year later to the Rennie's River watershed. Introduction to the Rennie's is notable because this watershed has a traversable connection to the ocean and represents the putative source watershed of the Newfoundland invasion. Trout from two other strains were also purportedly introduced, but apparently in much smaller numbers and to fewer locations (Hustins, 2007). Thus, most – if not all – established trout populations were founded by a common ancestral gene pool. The trout survived well upon introduction to lakes, established self-sustaining populations, and subsequently spread to additional watersheds (Westley and Fleming, 2011).

Field sampling of fish

We sampled a total of 1677 brown trout, during June to September 2008, from 16 watersheds in eastern Newfoundland (Fig. 1). These watersheds were selected to represent a variety of habitats along a gradient of increasing distance from the putative source watershed near St. John's (Fig. 1) and to cover a range of habitat types. Fish were collected with single-pass upstream electrofishing (Smith-Root LR-24 backpack shocker) and with beach seines and gill-nets (3 mm mesh size) in deeper pools of rivers where electrofishing was ineffective. We collected fish throughout river sections (mean section length 860 m, range 82–6800 m) to reduce the potential of sampling-related individuals. In addition, we attempted to collect across the size and age classes available in each site (~100 individuals were targeted), accounting for potential ontogenic variation in microhabitat use (Armstrong *et al.*, 2003).

Fish were anaesthetized in clove oil (0.25 $mL \cdot L^{-1}$), measured (fork length to the nearest millimetre), weighed (to the nearest 0.1 g), and photographed with a 12.1 mega-pixel Canon

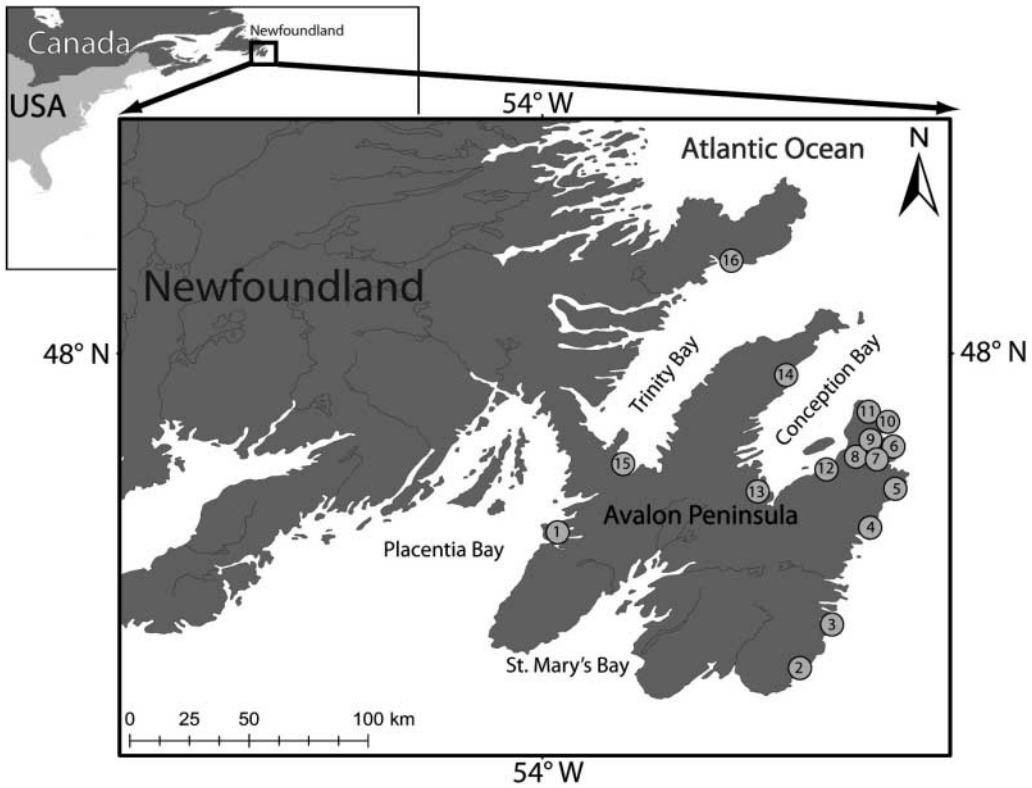

Fig. 1. Approximate locations of sampled brown trout populations on the island of Newfoundland, Canada: (1) Southeast Placentia River, (2) Chance Cove River, (3) Renews River, (4) Witless Bay/ Pierre's Brook, (5) Raymond's Brook, (6) Waterford River, (7) Rennie's River, (8) Middle Rocky Brook, (9) Virginia River, (10) Savage Creek, (11) Torbay River, (12) Topsail River, (13) Avondale River, (14) Salmon Cove River, (15) Chapel Arm River, (16) Port Rexton River. For coordinates of these systems, see Table 2 in Westley and Fleming (2011). The city of St. John's is located in the vicinity of numbers 7, 8, and 9.

digital camera (PowerShot A650 IS) using a low compression JPEG format. Each photograph included a unique label for identification, and a scale bar to allow standardization among photos taken at different heights (deemed necessary because fish ranged in size from 30 to 362 mm). Height was standardized *within* the three primary size classes described below, and an X-Rite mini colorchecker card (X-Rite Inc., Grand Rapids, MI) was included in each image. This colour card contains vignettes designed to express a known colour according to RGB (Red-Green-Blue) digital colour space allowing for standardization of colour balance among images [for examples of colour standardization, see Bergman and Beehner (2008) and Whiteley *et al.* (2009)]. Salmonids, like other fish species, can change melanin-based colour rapidly during periods of stress but adapt their coloration to environmental conditions over periods of days or weeks (Donnelly and Dill, 1984; Sumpter *et al.*, 1985; Sugimoto, 2002). To limit the effect of our handling on physiological colour change, we minimized the time from capture to photographing, and used standardized white-coloured storage containers and a consistent concentration of

anaesthetic. After photographing, a sample of scales was collected for subsequent ageing and the adipose fin was removed and stored in 95% ethanol for future genetic analyses. Removal of the adipose fin also provided an external mark to avoid resampling individuals between days. Fish were allowed to recover and released.

Quantifying phenotypic variation

Growth and condition

To examine how specific growth rates may mediate morphological shape, we mounted scales on microscope slides and digitally photographed them with a Lumenra Infinity 2 camera affixed to an M420 1.25× compound microscope under 10–20× magnification. Individuals were assigned to a year class where the number designation corresponded to the number of winter marks on the scale. We then calculated specific growth rates for each individual following Wootton (1990), where we assumed that all fish emerged at 25 mm on 15 May of their first spring. These values are based on laboratory and field observations with these populations (P.A.H. Westley and I.A. Fleming, unpublished data) and from literature (Elliott, 1994; Klemetsen *et al.*, 2003). In addition, we used a length-adjusted measure of weight as a proxy for fish condition (see Statistical analysis below).

Geometric body shape

Differences in body shape among populations were assessed with geometric-morphometrics (Adams *et al.*, 2004). Two-dimensional body shape was quantified by placing 14 homologous landmarks on digital images in the program tpsDig2 version 2.12 (Rohlf, 2005). The landmarks (Fig. 2) were modified from Michaud *et al*. (2008). Landmark data were aligned to a single consensus shape configuration using Procrustes superimposition using the program tpsRelw version 1.46 (Rohlf, 2006). After alignment, relative warp scores (analogous to principal component scores) were calculated for each individual. Interpretation of how each relative warp contributed to body shape was based on visualizations of thin-plate spline transformations generated in tpsRelw.

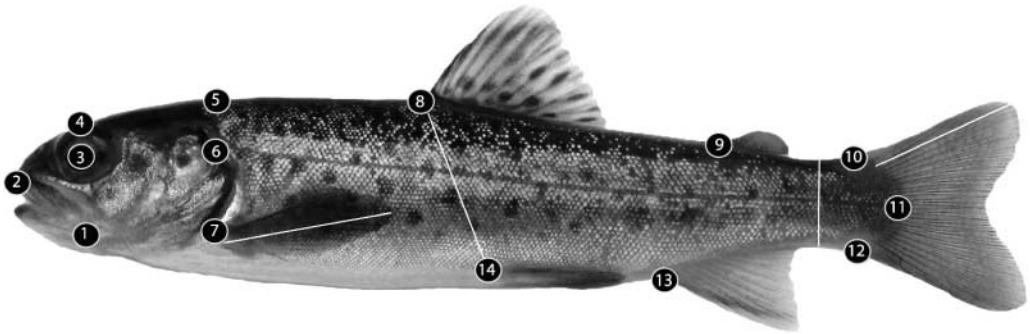

Fig. 2. Location of homologous landmarks and linear measures used to quantify shape differences in Newfoundland brown trout populations. The surface area of the head and eye were also quantified using the Freehand polygon tool in ImageJ. Count of pigment spots and quantification of red and overall coloration were quantified from lighting-adjusted photographs (see Methods).

Dimensional and meristic measures

From a subset of photographs ($n = 661$), we quantified 10 additional morphological, meristic, and colour traits. Direct linear measures of six traits were obtained from photographs using ImageJ version 1.42q (freely available at: http://www.rsbweb.nih.gov/ij/). Traits measured included: (1) surface area of the eye (mm^2), (2) surface area of the head (mm^2), (3) body depth (mm), (4) length of the pectoral fin (mm), (5) length of the caudal fin (mm), and (6) depth of the caudal peduncle (mm). Measurements were taken by the same person to reduce variability and a haphazardly selected sample of 100 fish was re-measured to assess error in data collection. Duplicate measures were highly correlated for all traits ($r^2 = \sim 0.99$).

We recorded the number of pigmented spots and quantified two metrics of coloration on each fish because colour patterns are often used to differentiate among brown trout populations (Aparicio *et al.*, 2005), are herititable (Blanc *et al.*, 1994), hypothesized to be linked to reproductive fitness (Wedekind *et al.*, 2008), and are apparently correlated with distinct behavioural syndromes (Kittilsen *et al.*, 2012). We counted spots irrespective of their colour on the left flanks of each fish, ignoring spots on fins because fin arrangement was not standardized sufficiently to always allow counts. To assess coloration, we first standardized all photographs to a common colour vignette to account for differences in lighting conditions during photographing (reviewed by Stevens *et al.*, 2007). Overall amount of red coloration (hereafter, 'red') was then measured as the percentage of pixels where the red value of the RGB colour space fell above a threshold of 50 points (~20%) above both the green and blue pixel values. Next, we extracted the mean value of pixels in the red, green, and blue spectrum and used these values to interpret the overall lightness or darkness of body coloration from individuals (hereafter, 'colour'). This is justified, as higher mean RGB values are associated with bright colours and lower mean values with darker colours. Colour values for each individual represent the first principal component scores extracted from a principal components analysis on RGB data (Whiteley *et al.*, 2009). Colour analysis was conducted using the Image Processing Toolbox™ of Matlab and automated with custom-written routines, which are available upon request.

Habitat sampling

We quantified seven abiotic habitat variables from each watershed that we thought might influence population trait variation. Habitat surveys were conducted within a two-week span during periods of similar water conditions. Within each watershed, we haphazardly chose sites corresponding to the downstream, middle, and upstream end of the sample section. At each of these three sites within each watershed, we measured: (i) stream wetted width (m); (ii) depth (cm, an average of five equidistant points along a perpendicular transect); (iii) the ratio of wetted width to depth; (iv) stream gradient (%); (v) the extent of riparian canopy cover using the categories: 1 = no cover or only grasses, 2 = alders and willows along banks, branches encroached the stream, 3 = large alders and conifers present, branches and woody debris in channel, 4 = alders, conifers, and deciduous species present, large amount of wood in the channel, and little light reaching stream; (vi) water transparency (cm) using a standard 1.3 m transparency tube (Dahlgren *et al.*, 2004); and (vii) water conductivity ($\mu S \cdot cm^{-1}$) with an Accumet® AP 85 handheld meter.

We quantified 'dispersal distance' and 'straight-line distance' from the mouth of each sample watershed to the mouth of the Rennie's River watershed. In doing so, we obtained

pairwise distances ($n = 120$) between the mouths of each watershed. We calculated dispersal distances using the least-cost distance tool in ArcGIS with an approach that provided a realistic distance that a fish would have to swim between locations, thereby capturing the dynamics of potential gene flow and colonization (for details, see Westley and Fleming, 2011). We used Google Earth version 6.2.2.6613 to measure straight-line distances. The dispersal distances between sample locations are interpreted as an indication of potential gene flow and time since colonization assuming a stepping-stone type dispersal process (Kimura and Weiss, 1964). In contrast, we interpreted a significant contribution of straight-line distance to primarily be the influence of habitat similarity across the landscape.

Statistical analysis

Our primary analytical approach was to: (1) construct a matrix of mean size-adjusted phenotypic traits and a matrix of habitat features for each population; (2) calculate the pairwise similarity of each population based on their respective mean phenotypic traits and habitat features; (3) visualize similarity in multidimensional space; (4) correlate and test for significance of phenotypic similarity and habitat similarity values using the BIO-ENV routine and Mantel tests; (5) regress the best environmental correlates identified in the previous step against specific phenotypic traits to explore the mechanistic and potential adaptive significance of the relationships; and (6) regress phenotypic and habitat similarity against two measures of spatial proximity. The matrix of phenotypic values in the three size classes and habitat data are shown in Supplementary Tables 1–3 and 4, respectively (see evolutionary-ecology.com/data/2706_appendix.pdf). Shape (warp scores) and morphological and meristic traits (linear measures, spot counts, and coloration) were size-adjusted prior to statistical analyses. This was necessary because traits such as body shape can change markedly during ontogeny (Loy *et al.*, 1998), complicating interpretation among individuals of different sizes and ages. Indeed, exploratory data analysis revealed distinct patterns of allometry among fish sizes and supported a division of our samples among the three primary size and age classes observed (small: <60 mm, intermediate: 60–150 mm, and large: >150 mm; Fig. 3a). Because fish sizes varied among sites, not all populations are included in each size class (Population $n_{<60\text{mm}} = 13$, $n_{60\text{–}150\text{mm}} = 16$, $n_{>150\text{mm}} = 7$). We then conducted separate analyses using these subsets of data (hereafter, 'size class'). All phenotypic traits varied significantly with size, thus each trait was adjusted to the mean length of each size class (45, 100, and 200 mm, respectively) using common within-class allometric coefficients (Reist, 1986; McCoy *et al.*, 2006). Allometric coefficients for each trait represent the slope coefficient of analysis of covariance (ANCOVA) on log $(x + 1)$ transformed trait and body length values. We verified common within-class slope by testing for significant body size-by-population interaction terms of ANCOVA for each trait. We found homogeneity of slopes within each size class, but slopes differed among classes supporting the presence of size-dependent allometric effects. There was a strong linear relationship between body length and centroid size (length = 0.7575 * centroid + 7.0728; $r^2 = 0.998$), and we used the former for size standardization to aid in biological interpretation.

We visualized phenotype and habitat similarity (based on Euclidean distances) among populations with non-metric multidimensional scaling (NMDS) plots fitted using the ecodist package in R version 2.10.1 (R Development Core Team, 2009). Phenotypic traits and habitat features were scaled to within-columns means and standard deviations to avoid large values

having unequal weighting in the similarity calculations. Coordinates on the first and second dimension represent the positions in multivariate space that best maintained the order in the original similarity matrix (i.e. minimum stress) after 100 random starting configurations.

We employed the BIO-ENV routine (Clarke and Ainsworth, 1993) to assess the correlative relationship between phenotypic similarity of populations with similarity values for specific habitat features (for each size class). In short, this routine calculates a similarity matrix of scaled and centred phenotypic values, selects all possible subsets of habitat features, calculates Euclidean distances for this subset, and finds the correlation between the two matrices. Mantel tests (Legendre and Legendre, 1998) were then used to test the significance of the correlations generated from the BIO-ENV routine. Mantel statistics (R_m) and probabilities based on 999 permutations were calculated in the vegan package of R.

Finally, we used linear regression based on population mean traits to examine the mechanistic and potentially adaptive relationships between phenotype and the habitat features identified with the BIO-ENV routine. To test for patterns consistent with phenotypic divergence with distance, we calculated pairwise similarities (Euclidean distance) based on phenotypic traits in all 16 populations and used these similarities (response variable) in a multiple regression with pairwise dispersal distances and straight-line distance between watersheds as predictors. To investigate whether watersheds in close spatial proximity were indeed similar in habitat features, we conducted a separate analysis using similarity based on habitat features. Specifically, we regressed habitat similarity, again based on Euclidean distance, against the straight-line distance between the mouths of watersheds.

RESULTS

We observed marked variation in the suite of phenotypic traits measured both among populations and size classes (Supplementary Tables 1–3; see evolutionary-ecology.com/data/2706_appendix.pdf). For example, growth rates varied by 43%, 73%, and 37% for the small, intermediate, and largest size classes of fish, respectively.

The first relative warp explained 43% of the variation in geometric body shape and described a decreasing relative size of the head and deepening of the body and caudal areas (see Fig. 3 for thin-plate spline visualizations). The second and third warps explained 14% and 8% of the variation in shape, respectively, and suggested dorsal-ventral bending of the fish during photographing. This 'arch effect' has been described elsewhere and attributed to error in placement of specimens during photo capture (Michaud *et al.*, 2008; Valentin *et al.*, 2008). Furthermore, the 21 remaining warps individually explained little variation and were not retained for subsequent analyses. Thus, we limited our analyses of shape to scores of the first relative warp.

Overall, the first relative warp varied significantly and non-linearly with body size (Fig. 3a). However, three distinct *linear* allometric trajectories were detected within size classes (Fig. 3b). In general, shape changed most rapidly in fish less than 60 mm (linear coefficient of shape vs. body size = 0.07), intermediate in fish 60–150 mm (coefficient = 0.04), and slowest in fish larger than 150 mm (coefficient = –0.01) (Fig. 3c). Moreover, the sign of the coefficient changed from positive (a trend towards smaller heads and a deepening body) in the first two size classes to negative (larger heads and streamlined bodies) in the largest size class (see Fig. 3 for thin-plate spline visualizations).

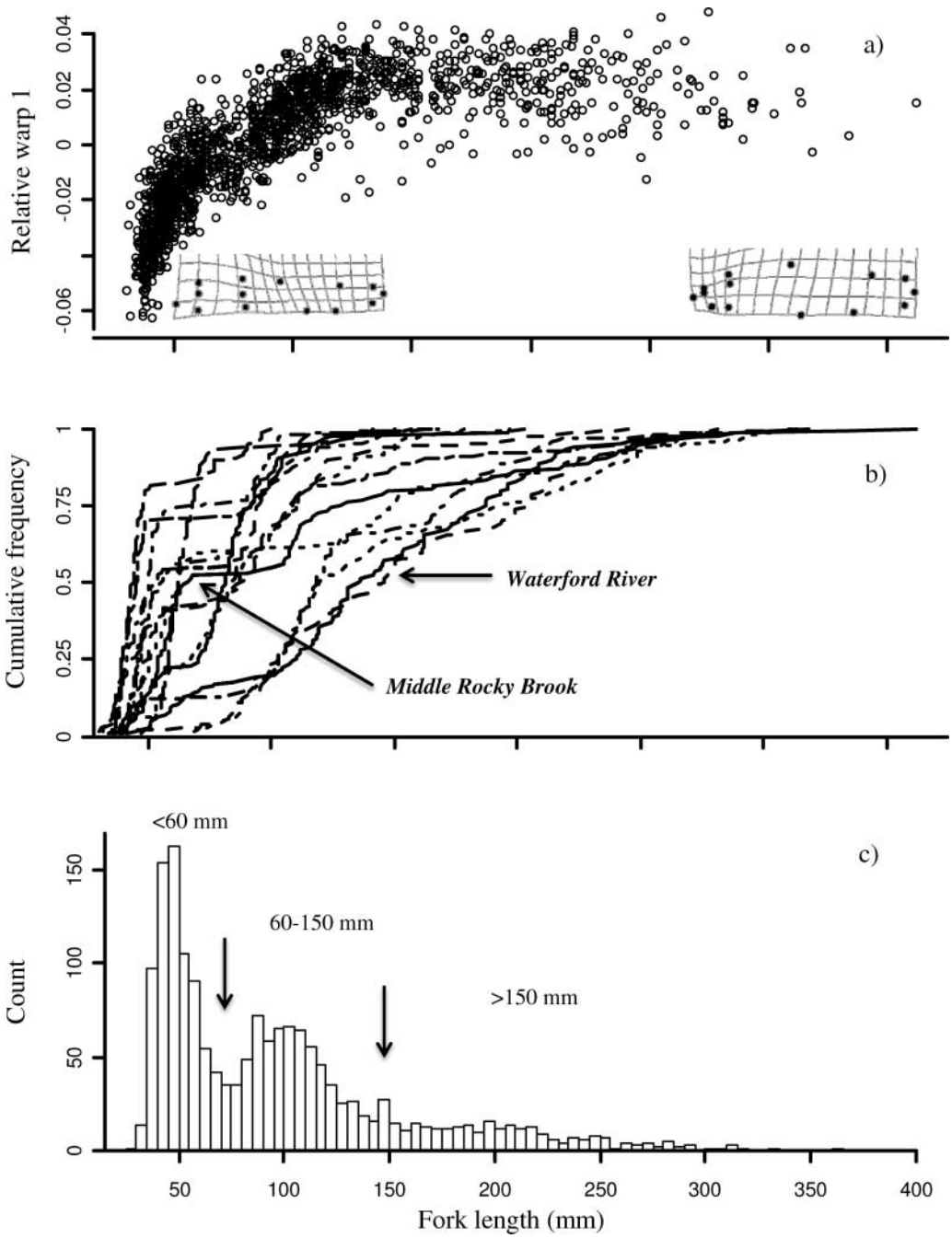

Fig. 3. (a) Relative warp scores and thin-plate spline visualizations depicting the extreme values of the first relative warp. (b) Cumulative length frequency of total sampled fish in 16 trout populations based on fork length, showing, for example, the Middle Rocky Brook where 50% of the sampled fish were less than ~55 mm and the Waterford River where 50% of the fish were less than ~150 mm. (c) Histogram of fork lengths pooled across populations; arrows denote breaks corresponding to size classes in statistical analyses.

Phenotypic similarity

Visualization of population similarity based on phenotypic traits with non-metric multidimensional scaling (NMDS) yielded several salient insights (Fig. 4). First, stress values (measures of fit between placement in multidimensional space and similarity in the original phenotypic matrix) for the small, intermediate, and large size class was 0.19, 0.22, and 0.19, respectively. These values suggest that visualization in two-dimensional space is sufficient for interpretation of population similarity (Clarke and Warwick, 2001). Second, the NMDS revealed considerable variation among populations, indicative of marked divergence based on individuals in the small (Fig. 4a), intermediate (Fig. 4b), and largest size classes (Fig. 4c). Third, patterns of similarity among populations were inconsistent among size classes, and the phenotypic correlations explaining population similarity also varied among classes.

Habitat similarity

In line with the analysis above, NMDS successfully placed watersheds in two dimensions (stress = 0.21) based on similarity of habitat features (Fig. 5). The visualization of watershed similarity based on habitat features revealed that locations close on the landscape (as measured by straight-line distance and indicated by number order) tended to be more similar than watersheds further apart. For example, the Southeast Placentia, Chance Cove, and Renews watersheds were more similar to each other based on their distance to the south of the putative source watershed and their large width–depth ratios compared with the Waterford, Rennie's, Virginia, and Topsail watersheds, which were similar to each other based on close proximity to the source watershed and high water conductivity. NMDS also indicated habitat correlations that are intuitive based on tenets of river ecology and geomorphology. Specifically: (1) increasing stream gradient was positively correlated with increasing canopy cover and decreasing wetted-width; (2) the width–depth ratio was positively correlated with both width and depth but more so with width, as we observed greater variation in stream width vs. depth (Supplementary Table 4; see evolutionary-ecology.com/data/2706_appendix.pdf); and (3) conductivity was positively correlated with distance towards the putative source watersheds (indicating that rivers near St. John's have higher conductivity), but negatively correlated with water clarity (indicating that as conductivity increases the amount of turbidity in the rivers also increases).

Phenotype-by-habitat correlations

In support of our overarching prediction, we detected significant correlations between phenotypic similarity (visualized in Fig. 4) and habitat similarity (Fig. 5). We observed that the strength of the similarity correlations was strongest in the intermediate and largest size classes of fish (Table 1) and that the habitat similarities correlated most strongly with phenotypic similarities varied among size classes. Based on individuals in the smallest size class, the BIO-ENV routine identified the strongest ($R_m = 0.25$) and marginally significant ($P = 0.07$) correlation to be between phenotypic similarity and the combined habitat similarity effects of canopy cover, width–depth ratio, and stream gradient. In contrast, population phenotypic similarities for the intermediate size class of fish were strongly and significantly correlated with similarity values for all of the habitat variables extracted from the BIO-ENV routine ($R_m = 0.33$, $P = 0.007$). However, the strongest correlation was

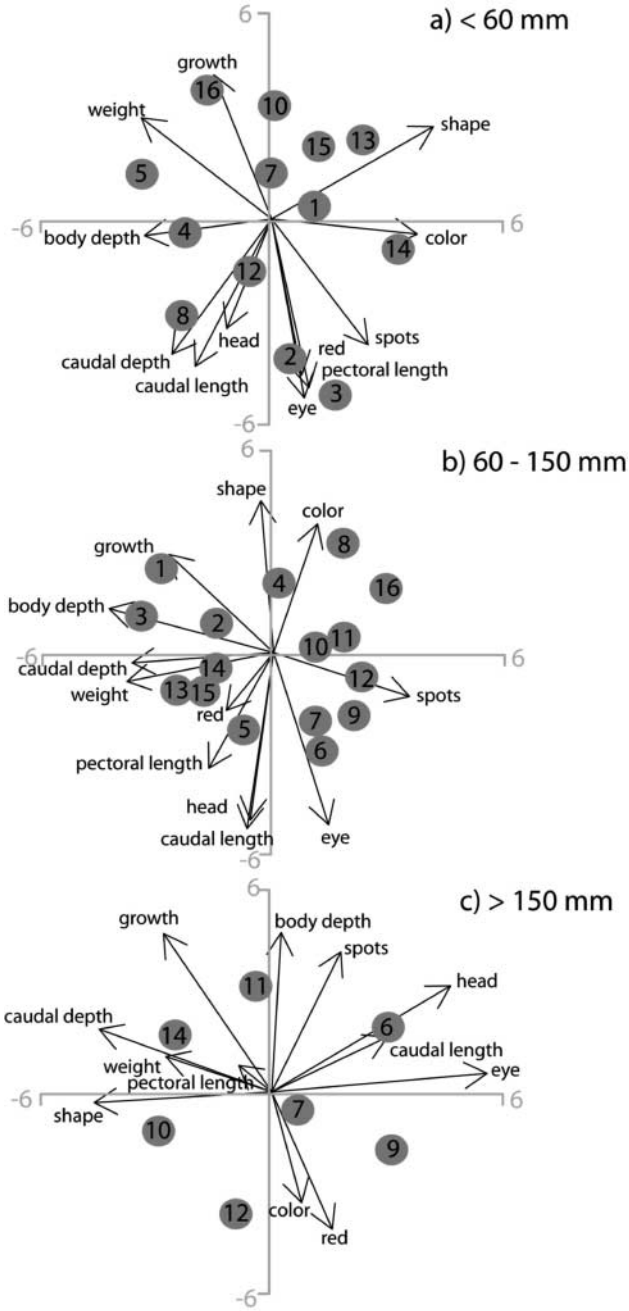

Fig. 4. Non-metric multidimensional scaling plots to visualize phenotypic similarity and correlations with 12 phenotypic traits of brown trout populations in Newfoundland. Similarity based on Euclidean distance in populations from fish in three size classes: (a) fish <60 mm, (b) fish 60–150 mm, and (c) fish >150 mm. Stress values associated with these ordinations are (a) 0.19, (b) 0.22, and (c) 0.19. See Supplementary Tables 1–3 (evolutionary-ecology.com/data/2706_appendix.pdf) for more details. Numbers correspond to watersheds (see Fig. 1).

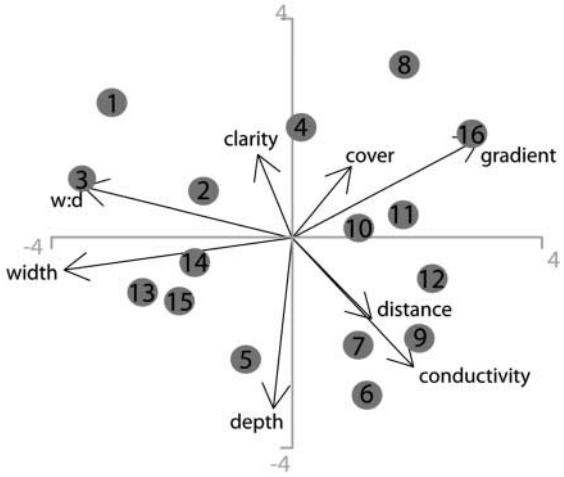

Fig. 5. Non-metric multidimensional scaling plot (stress = 0.21) to visualize habitat similarity of watersheds with established populations of brown trout in Newfoundland. Vectors denote correlations with specific features where the length of the arrow is proportional to the strength of the correlation. Similarity represents Euclidean distance on eight habitat features (for more details, see Supplementary Table 4; evolutionary-ecology.com/data/2706_appendix.pdf). Numbers correspond to watersheds (see Fig. 1). w:d = width–depth ratio.

between phenotypic similarity and habitat similarity based on geographic proximity (i.e. distance), canopy cover, and width–depth ratio ($R_m = 0.54$, $P = 0.001$), which were two of the three variables also identified in the strongest model for the smallest size class. Finally, we observed that population phenotypic similarity for the largest fish size class correlated most strongly with habitat similarity based on canopy cover and conductivity ($R_m = 0.53$, $P = 0.006$).

Mechanistic and adaptive underpinnings of phenotype-by-habitat correlations

Body streamlining (shallower body depth, narrower caudal depth, and overall body shape) and fin sizes tended to increase with stream width–depth ratios but were not significant ($P > 0.05$) in the small size class. Similar patterns were detected in the intermediate size class, but now the effect of width–depth ratio on geometric body shape was significant ($F_{1,14} = 6.07$, $r^2 = 0.30$, $P = 0.02$) and it explained more variation in linear measures of body depth, caudal depth, and fin sizes (~11–17%), though these relationships remained non-significant. Width–depth ratio was not important in explaining phenotypic similarity in the largest size class and thus is not explored here.

For all size classes, the relationships between canopy cover and fish coloration were very weak ($r^2 = 0$–2%) and non-significant ($P > 0.05$). Similarly, water clarity and fish coloration patterns were weak (0–7% variation explained) and not significant. However, although growth rate in the smallest class was independent of canopy cover ($F_{1,11} = 0.01$, $r^2 = 0.0$, $P = 0.92$), increasing canopy cover was associated with decreased growth in the intermediate ($F_{1,14} = 1.8$, $r^2 = 0.11$, $P = 0.2$) and largest size class ($F_{1,5} = 7.2$, $r^2 = 0.59$, $P = 0.04$).

Table 1. Role of habitat similarity in explaining phenotypic similarity of Newfoundland brown trout populations

Size group	*k*	Habitat feature	R_m	*P*-value
<60 mm	1	w:d	0.16	0.150
	2	cover, w:d	0.23	0.080
	3	cover, w:d, gradient	0.25	0.070
	4	cover, w:d, gradient, clarity	0.24	0.060
	5	cover, width, w:d, gradient, clarity	0.21	0.070
	6	cover, width, w:d, depth, gradient, clarity	0.2	0.080
	7	cover, width, w:d, depth, gradient, conduct, clarity	0.11	0.200
	8	distance, cover, width, w:d, depth, gradient, conduct, clarity	0.03	0.390
60–150 mm	1	distance	0.39	0.015
	2	distance, w:d	0.46	0.002
	3	distance, cover, w:d	0.54	0.001
	4	distance, cover, width, w:d	0.50	0.002
	5	distance, cover, width, w:d, clarity	0.45	0.003
	6	distance, cover, width, w:d, depth, clarity	0.42	0.004
	7	distance, cover, width, w:d, depth, gradient, clarity	0.38	0.003
	8	distance, cover, width, w:d, depth, gradient, conduct, clarity	0.33	0.007
>150 mm	1	conduct	0.43	0.032
	2	cover, conduct	0.53	0.006
	3	cover, depth, conduct	0.49	0.017
	4	distance, cover, depth, conduct	0.43	0.021
	5	distance, cover, depth, gradient, conduct	0.42	0.027
	6	distance, cover, w:d, gradient, conduct, clarity	0.31	0.200
	7	distance, cover, w:d, depth, gradient, conduct, clarity	0.20	0.230
	8	distance, cover, width, w:d, depth, gradient, conduct, clarity	0.14	0.290

Note: Correlates represent the best combinations of habitat variables (highest R_m) for a given number of habitat features (*k*) permitted to enter the BIO-ENV model selection procedure. *P*-values are for Mantel tests. w:d = width–depth ratio; conduct = conductivity.

As we predicted, growth rate variation (independent of size) influenced the expression of other phenotypic traits. Growth rate was inversely related to eye ($F_{1,14} = 4.85$, $r^2 = 0.25$, $P = 0.04$) and head size ($F_{1,14} = 4.26$, $r^2 = 0.23$, $P = 0.06$) of intermediate size fish. That is, fast growing individuals (after adjusting for differences in body size) had smaller heads and eyes than their slow growing counterparts. Among the largest size class, however, there were no clear patterns, as head and eye size vectors were orthogonal to that of growth (Fig. 4c). Counter to predictions, we found that water conductivity was inversely associated with growth rates in the largest size class ($F_{1,5} = 9.8$, $r^2 = 0.66$, $P = 0.02$).

Phenotypic and habitat divergence with distance

The BIO-ENV results identified geographic proximity as an important explanatory habitat feature of phenotypic similarity in the intermediate size class (Table 1) and thus we focused on this size group in the following analysis. Using multiple regression, we found that 24% of

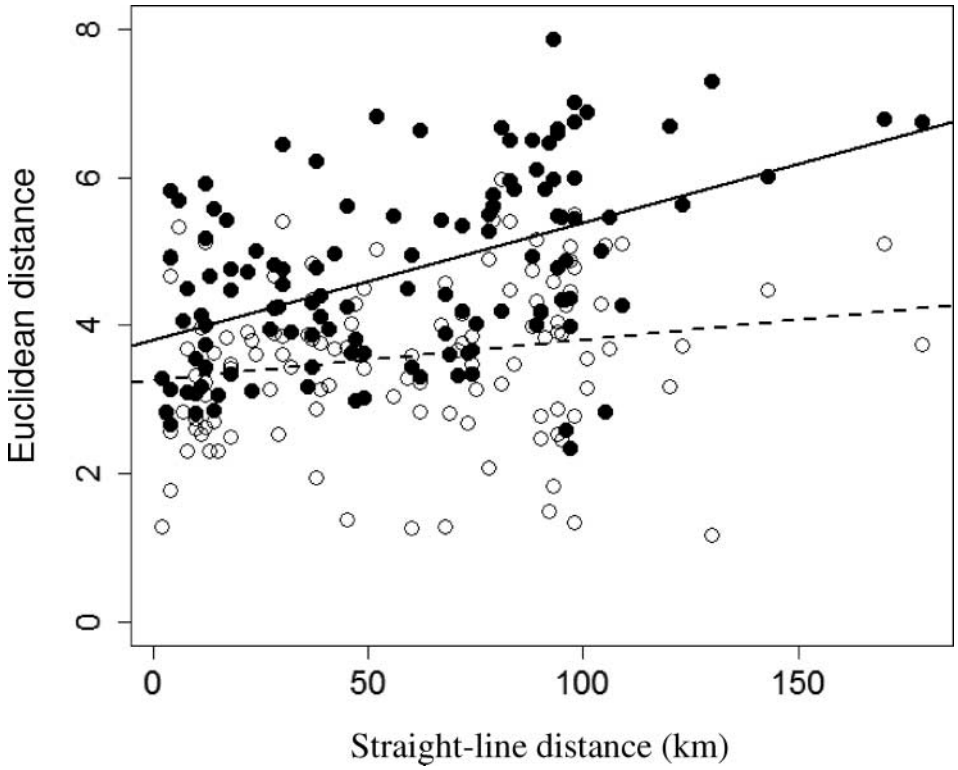

Fig. 6. Pairwise relationships between Euclidean distance based on phenotype (solid circles and unbroken line) and habitat (open circles and dashed line) vs. straight-line distances among 16 watersheds with established populations of brown trout in Newfoundland. Phenotypic distance represents dissimilarity (Euclidean distance) based on 12 phenotypic characters (Supplementary Tables 1–3; evolutionary-ecology.com/data/2706_appendix.pdf) and habitat distance represents dissimilarity (Euclidean distance) based on 7 habitat features (Supplementary Table 4).

the variation in 120 pairwise phenotypic differences between populations was explained by pairwise spatial proximity between populations, as measured by the combination of straight-line distance and dispersal distance ($F_{2,117} = 18.8$, $r^2 = 0.24$, $P < 0.01$; Fig. 6). Of these two distance metrics, however, straight-line distance was statistically significant ($P < 0.001$), but dispersal distance was not ($P = 0.22$). Similarly, we found that watersheds with more similar habitat features (based on the limited set we measured) tended to be, based on straight-line distance, closer in proximity ($F_{1,118} = 4.7$, $r^2 = 0.04$, $P = 0.03$; Fig. 6).

DISCUSSION

We found evidence that 130 years after first introduction – or approximately 32 generations – the phenotypic similarity of brown trout populations in Newfoundland is correlated with habitat similarity across a recently colonized landscape. Consistent with our hypotheses, some phenotypic patterns matched adaptive predictions for habitat features, such as the predicted relationship between fish morphology and inferred stream flow. In addition, we

observed that phenotypic dissimilarity and habitat dissimilarity increased with straight-line distance among watersheds, which we interpret as complementary evidence that similar habitats shape or select for similar phenotypes, a defining feature of parallel adaptive divergence. Other traits, such as coloration patterns, did not reveal easily interpretable patterns and overall we observed marked 'noise' in many of the relationships between phenotypic values and habitat features. Such noise suggests substantial opportunity for random founder effects and idiosyncratic habitat features to shape local trait variation following colonization. Hence, our findings suggest a mixture of determinism and contingency shaping the phenotypes of new populations. The extent to which parallel phenotypic patterns resulted from site-specific selection and genetic adaptation following colonization, recurrent phenotypic plasticity to similar environmental conditions during development, or colonization by pre-selected founders is not yet known.

Phenotypic similarity among watersheds varied across size classes and the phenotypic traits underlying the observed phenotypic similarities (visualized as correlation arrows in Fig. 4) also varied among classes. We interpret these patterns to indicate that the factors underlying expression of these traits vary with size and are presumably related to ontogenetic shifts in habitat and resource use and availability (Bisson *et al.*, 1988; Nicieza, 1995; Michaud *et al.*, 2008). Similarly, although we observed some commonalities in the specific habitat features that correlated most strongly with phenotypes among size classes, we also observed differences as well as an increase in the strength of the correlations in the larger class. The relatively weak associations between phenotypic similarity and habitat similarity in the smallest size class may reflect an insufficient amount of developmental time for plasticity and selection to act on younger fish, or reflect responses to habitat features at the microhabitat spatial scale (i.e. below the habitat spatial scale sampled here). Previous work has demonstrated the influence of microhabitat environments on shaping phenotypes in recently emerged salmonids of similar size and age (Pakkasmaa and Piironen, 2000; Pavey *et al.*, 2010; Drinan *et al.*, 2012). Differences among size classes may also partly reflect inclusion of different watersheds and different numbers of watersheds in each class comparison (not all watersheds had small or large individuals). Thus, we focus the discussion based on the results of the intermediate size class because all populations were represented and correlations with habitat features were strongest.

Phenotypic similarity among watersheds, based on individuals of the intermediate size class, was most strongly correlated with three environmental similarity predictors: dispersal distance between watersheds, the extent of canopy cover, and stream width–depth ratios. We interpret the importance of dispersal distance to reflect the combination of serial founder effects and the correlative effects of spatial segregation of habitat types (i.e. habitats in closer proximity tend to be more similar than those further apart). Despite all watersheds in Newfoundland with established trout populations having descended from a common European ancestral source, the stepping-stone manner of dispersal identified in this biological invasion (Westley and Fleming, 2011) makes it likely that watersheds closer in spatial proximity would also share closer common ancestry and phenotypic attributes. Ongoing isolation-by-distance patterns of gene flow would also contribute to such a pattern. Interestingly, recent work has shown that body shape in brown trout varies significantly as a function of longitudinal distance *within* a watershed (Stelkens *et al.*, 2012). The potential role of founder effects and gene flow notwithstanding, we detected greater explanatory power of straight-line distance between watersheds, than of dispersal distance, on phenotypic similarity. Straight-line distance was also a stronger predictor of habitat similarity. These

lines of evidence suggest a greater role for habitat-specific influences on population phenotypes – genetic or plastic – than those of founder effects and gene flow. However, these processes are not mutually exclusive. Phenotypic convergence among closely related populations occupying similar habitats may result from some combination of 'favoured' founder effects by colonization of individuals particularly suited to specific environments (*sensu* Quinn *et al.*, 2001; Kinnison and Hairston 2007), recurrent phenotypic plasticity (Losos *et al.*, 2000), or adaptive parallel evolution (Schluter, 2000).

Canopy cover was an important correlate of phenotypic similarity among populations. We attribute this, at least in part, to the effect of canopy cover on resource availability, stream temperatures, and growth patterns. In addition to providing physical habitat structure, riparian and canopy cover determines the amount of light reaching streams and can influence photosynthesis and primary productivity. In Trinidad, canopy cover explains 93% of the variation observed in guppy growth rates by influencing the standing crop of algae and food availability (Grether *et al.*, 2001). Furthermore, the amount of light reaching streams will affect water temperatures and thus metabolic processes, with the optimal temperature for growth in brown trout being ~15°C (reviewed in Jonsson and Jonsson, 2011). Similarly, but to a lesser extent, we found that canopy cover was inversely related to growth rates and explained 11% and 66% of the variation in growth among the intermediate and larger size class, respectively. Growth, in turn, has the capacity to influence the expression of other morphological traits. Specifically, we detected negative allometries between growth rate and eye size and between growth rate and head size (in intermediate size fish only) as reported elsewhere (McDowall and Pankhurst, 2005; Devlin *et al.*, 2012).

The width–depth ratio similarity of streams was also an important correlate of phenotypic similarity, especially in the intermediate size fish. The width–depth ratio is a frequently used surrogate for stream size and water discharge (Ward and Trimble, 2004) and high ratios suggest limited areas of deeper, slower moving water for individuals to seek refuge from high flows. The causal link of width–depth ratio to phenotypic similarity emerges through its apparent influence on geometric body shape and fin sizes. We observed that individuals inhabiting streams with high width–depth ratios exhibited relatively streamlined morphology and tended to have large fins, whereas robust morphology and relatively smaller fins were observed in individuals and populations inhabiting habitats of lower ratios. These observations are consistent with other studies (Pakkasmaa and Piironen, 2000; Imre *et al.*, 2002; Keeley *et al.*, 2007; Haas *et al.*, 2010; Franssen, 2011; Drinan *et al.*, 2012), which together suggest a biomechanical advantage of streamlined morphology for life in high flow habitats (Langerhans, 2008). Moreover, the repeated convergence of streamlined phenotypes in similar habitats strongly implicates a selective advantage (Nosil, 2012). The associations between geometric body shape and fin sizes observed in this study are likely maintained by a combination of phenotypic plasticity and local adaptation. At least some populations of salmonids display adaptive plasticity with regards to shape, where plasticity acts in the direction believed to be favoured by selection (Pakkasmaa and Piironen, 2000; Haas *et al.*, 2010; Franssen, 2011).

Not all traits showed marked or predicted relationships between population similarity and habitat similarity or proximity. Although some of this 'noise' may capture the role of contingency (e.g. founder effects) in shaping trait diversity, it is also possible such instances reflect the challenges of interpreting trait and habitat variation. The weak and highly variable relationships between colour and habitat features suggest that the habitat or genetic factors underlying the expression of coloration may be more complex than captured in this study. Indeed, coloration patterns have recently been found as a correlate of behavioural

syndromes and parasite resistance in the closely related Atlantic salmon (Kittilsen *et al.*, 2012). Counter to predictions for system productivity, we found that conductivity was negatively correlated with growth rates among fish in the largest size class. However, our prediction may have missed the importance of conductivity as an indirect indicator of human-induced disturbance. Indeed, conductivity typically increases in urban streams from water running off modified landscapes (Paul and Meyer, 2001). Growth of salmonids (albeit of smaller size classes) in urban rivers of Newfoundland can be high (Gibson and Haedrich, 1988), yet we found fast growth in rivers outside the urban areas and in habitats with low conductivity.

CONCLUSION

The colonization success of exotic brown trout populations around the globe is often attributed to their wide environmental tolerances and the ability to respond plastically to environmental change (Elliott, 1994; Jonsson and Jonsson, 2011). Indeed, the success of invasive species as a whole is frequently linked to adaptive phenotypic plasticity (Davidson *et al.*, 2011). Plasticity can allow persistence in novel environments (Yeh and Price, 2004; Ghalambor *et al.*, 2007) and is an important route towards genetically determined local adaptation (West-Eberhard, 2003; Lande, 2009; Chevin *et al.*, 2010; Chevin and Lande, 2011). The similarity between phenotype and environmental features, combined with the observation that traits are often correlated with growth, strongly implicates the underlying influence of phenotypic plasticity in shaping brown trout populations in Newfoundland. This does not, however, preclude the possibility that the phenotypic variation observed also represents a degree of heritable local adaptation. Moreover, correlations between phenotype and environment may arise from the successful colonization by a subset of pre-adapted individuals to certain conditions ['favoured founders' *sensu* Quinn *et al.* (2001)]. Minor changes in trait values can have disproportionately large influences on fitness and population vital rates (Kinnison *et al.*, 2008), irrespective of whether the changes result from environmentally induced plasticity, genetic adaptation, or a combination of the two. Changes in vital rates and fitness can feedback on the potential for species to spread and colonize new environments, thus understanding the realized fitness consequences of the observed phenotypic variation in Newfoundland brown trout populations is an important next step towards testing for local adaptation and assessing their potential to further invade.

ACKNOWLEDGEMENTS

We thank Haley Cohen, Claire Lewis, Chris Corcoran, and Cameron Tobin for their assistance during the 2008 field season. Ryan Stanley assisted us greatly in the colour analysis and Mike Kinnison provided useful comments on an earlier version of the manuscript. Funding was provided by The Conservation Corps of Newfoundland and Labrador, the Institute of Biodiversity and Ecosystem Sustainability, Department of Fisheries and Oceans Canada, and the Natural Sciences and Engineering Research Council of Canada.

REFERENCES

Adams, D.C., Rohlf, F.J. and Slice, D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Ital. J. Zool.*, **71**: 5–16.

Aparicio, E., Berthou-Garcia, E., Araguas, R.M., Martinenz, P. and Marin-Garcia, L. 2005. Body

pigmentation pattern to assess introgression by hatchery stocks in native *Salmo trutta* from Mediterranean streams. *J. Fish. Biol.*, **67**: 931–949.

Armstrong, J.D., Kemp, P.S., Kennedy, G.J.A., Ladle, M. and Milner, N.J. 2003. Habitat requirements of Atlantic salmon and brown trout in rivers and streams. *Fish. Res.*, **62**: 143–170.

Ayllon, F., Davaine, P., Beall, E. and Garcia-Vazquez, E. 2006. Dispersal and rapid evolution in brown trout colonizing virgin Subantarctic ecosystems. *J. Evol. Biol.*, **19**: 1352–1358.

Bergman, T.J. and Beehner, J.C. 2008. A simple method for measuring colour in wild animals: validation and use on chest patch colour in geladas (*Theropithecus gelada*). *Biol. J. Linn. Soc.*, **94**: 231–240.

Bernatchez, L., Guyomard, R. and Bonhomme, F. 1992. DNA sequence variation of the mitochondrial control region among geographically and morphologically remote European brown trout *Salmo trutta* populations. *Mol. Ecol.*, **1**: 161–173.

Bisson, P.A., Sullivan, K. and Nielsen, J.L. 1988. Channel hydraulics, habitat use, and body form of juvenile coho salmon, steelhead, and cutthroat trout in streams. *Trans. Am. Fish Soc.*, **117**: 262–273.

Blanc, J.M., Chevassus, B. and Krieg, F. 1994. Inheritance of the number of red spots on the skin of the brown trout. *Aquat. Living Resources*, **7**: 133–136.

Chevin, L.M. and Lande, R. 2011. Adaptation to marginal habitats by evolution of increased phenotypic plasticity. *J. Evol. Biol.*, **24**: 1462–1476.

Chevin, L.M., Lande, R. and Mace, G.M. 2010. Adaptation, plasticity, and extinction in a changing environment: towards a predictive theory. *PLoS Biol.*, **8**: e1000357.

Clarke, K.R. and Ainsworth, M. 1993. A method of linking multivariate community structure to environmental variables. *Mar. Ecol. Progr. Ser.*, **92**: 205–219.

Clarke, K.R. and Warwick, R.M. 2001. *Change in Marine Communities: An Appraoch to the Statistical Analysis and Interpretation*, 2nd edn. Plymouth: Primer-E.

Crawford, S.S. and Muir, A.M. 2008. Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870–2007. *Rev. Fish Biol. Fish.*, **18**: 313–344.

Dahlgren, R.A., Van Nieuwenhuyse, E. and Litton, G. 2004. Transparency tube provides reliable water-quality measurements. *Calif. Agric.*, **58**: 149–153.

Davidson, A.M., Jennions, M. and Nicotra, A.B. 2011. Do invasive species show higher phenotypic plasticity than native species and, if so, is it adaptive? A meta-analysis. *Ecol. Lett.*, **14**: 419–431.

Devlin, R.H., Vandersteen, W.E., Uh, M. and Stevens, E.D. 2012. Genetically modified growth affects allometry of eye and brain in salmonids. *Can J. Zool.*, **90**: 193–202.

Donnelly, W.A. and Dill, L.M. 1984. Evidence for crypsis in coho salmon, *Oncorhynchus kisutch* (Walbaum), parr: substrate colour preference and achromatic reflectance. *J. Fish Biol.*, **25**: 183–195.

Drinan, T.J., McGinnity, P., Coughlan, J.P., Cross, T.F. and Harrison, S.S.C. 2012. Morphological variability of Atlantic salmon *Salmo salar* and brown trout *Salmo trutta* in different river environments. *Ecol. Freshw. Fish*, **21**: 420–432.

Elliott, J.M. 1994. *Quantitative Ecology and the Brown Trout*. New York: Oxford University Press.

Eroukhmanoff, F., Hargeby, A. and Svensson, E.I. 2009. Rapid adaptive divergence between ecotypes of an aquatic isopod inferred from F_{ST}–Q_{ST} analysis. *Mol. Ecol.*, **18**: 4912–4923.

Ferguson, A. 1989. Genetic differences among brown trout, *Salmo trutta*, stocks and their importance for the conservation and management of the species. *Freshw. Biol.*, **21**: 35–46.

Ferguson, A. and Mason, F.M. 1981. Allozyme evidence for reproductively isolated sympatric populations of brown trout *Salmo trutta* L. in Loch Melvin, Ireland. *J. Fish Biol.*, **18**: 629–642.

Ferguson, A. and Taggart, J.B. 1991. Genetic differentiation among the sympatric brown trout (*Salmo trutta*) populations of Lough Melvin, Ireland. *Biol. J. Linn. Soc.*, **43**: 221–237.

Fleming, I.A. 1998. Patterns and variability in the breeding systems of Atlantic salmon, with comparisons to other salmonids. *Can. J. Fish. Aquat. Sci.*, **55**(suppl. 1): 59–76.

Franssen, N.R. 2011. Anthropogenic habitat alteration induces rapid morphological divergence in a native stream fish. *Evol. Appl.*, **4**: 791–804.

Garcia de Leaniz, C., Fleming, I.A., Einum, S., Verspoor, E., Jordan, W.C., Consuegra, S. *et al.* 2007. A critical review of adaptive genetic variation in Atlantic salmon: implications for conservation. *Biol. Rev.*, **82**: 173–211.

Ghalambor, C.K., McKay, J.K., Carroll, S.P. and Reznick, D.N. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptations to new environments. *Funct. Ecol.*, **21**: 394–407.

Gibson, R.J. and Haedrich, R.L. 1988. The exceptional growth of juvenile Atlantic salmon (*Salmo salar*) in the city waters of St. John's, Newfoundland, Canada. *Polish Arch. Hydrobiol.*, **35**: 385–407.

Grether, G.F., Millie, D.F., Bryant, M.J., Reznick, D.N. and Mayea, W. 2001. Rain forest canopy cover, resource availability, and life history evolution in guppies. *Ecology*, **82**: 1546–1559.

Groot, C. and Margolis, L. 1991. *Pacific Salmon Life Histories*. Vancouver: UBC Press.

Haas, T.C., Blum, M.J. and Heins, D.C. 2010. Morphological responses of a stream fish to water impoundment. *Biol. Lett.*, **6**: 803–806.

Haugen, T.O. 2000. Growth and survival effects on maturation pattern in populations of grayling with recent common ancestors. *Oikos*, **90**: 107–118.

Haugen, T.O. and Vollestad, L.A. 2001. A century of life-history evolution in grayling. *Genetica*, **112**: 475–491.

Hendry, A.P. and Kinnison, M.T. 1999. The pace of modern life: measuring rates of contemporary microevolution. *Evolution*, **53**: 1637–1653.

Hendry, A.P., Wenburg, J.K., Bentzen, P., Volk, E.C. and Quinn, T.P. 2000. Rapid evolution of reproductive isolation in the wild: evidence from introduced salmon. *Science*, **290**: 516–518.

Hendry, A.P., Farrugia, T.J. and Kinnison, M.T. 2008. Human influences on rates of phenotypic change in wild animal populations. *Mol. Ecol.*, **17**: 20–29.

Huey, R.B., Gilchrist, G.W., Carlson, M.L., Berrigan, D. and Serra, L. 2000. Rapid evolution of a geographic cline in an introduced species of fly. *Science*, **287**: 308–309.

Hustins, D. 2007. *Brown Trout and Rainbow Trout: A Journey into Newfoundland Waters*. St. John's: Tight Line Publishers.

Hutchings, J.A. 2011. Old wine in new bottles: reaction norms in salmonid fishes. *Heredity*, **106**: 421–437.

Imre, I., McLaughlin, R.L. and Noakes, D.L.G. 2002. Phenotypic plasticity in brook charr: changes in caudal fin induced by water flow. *J. Fish Biol.*, **61**: 1171–1181.

Johnston, R.F. and Selander, R.K. 1964. House sparrows – rapid evolution of races in North America. *Science*, **144**(3618): 548–550.

Jonsson, B. and Jonsson, N. 2011. *Ecology of Atlantic Salmon and Brown Trout: Habitat as a Template for Life Histories*. New York: Springer.

Keeley, E.R., Parkinson, E.A. and Taylor, E.B. 2007. The origins of ecotypic variation of rainbow trout: a test of environmental vs. genetically based differences in morphology. *J. Evol. Biol.*, **20**: 725–736.

Kimura, C. and Weiss, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. *Genetics*, **49**: 561–576.

Kinnison, M.T. and Hairston, N.G. 2007. Eco-evolutionary conservation biology: contemporary evolution and the dynamics of persistence. *Funct. Ecol.*, **21**: 444–454.

Kinnison, M.T. and Hendry, A.P. 2004. From macro- to micro-evolution: tempo and mode in Salmonid evolution. In *Evolution Illuminated* (A.P. Hendry and S.C. Stearns, eds.), pp. 209–231. Oxford: Oxford University Press.

Kinnison, M.T., Unwin, M.J. and Quinn, T.P. 2008. Eco-evolutionary vs. habitat contributions to invasion in salmon: experimental evaluation in the wild. *Mol. Ecol.*, **17**: 405–414.

Kittilsen, S., Johansen, I.B., Braastad, B.O. and Øverli, Ø. 2012. Pigments, parasites and personality: towards a unifying role for steroid hormones? *PLoS ONE*, **7**: e34281.

Klemetsen, A., Amundsen, P.A., Dempson, J.B., Jonsson, B., Jonsson, N., O'Connell, M.F. *et al.* 2003. Atlantic salmon *Salmo salar* L., brown trout *Salmo trutta* L. and Arctic charr *Salvelinus alpinus* (L.): a review of aspects of their life histories. *Ecol. Freshw. Fish*, **12**: 1–59.

Lande, R. 2009. Adaptation to an extraordinary environment by evolution of phenotypic plasticity and genetic assimilation. *J. Evol. Biol.*, **22**: 1435–1446.

Langerhans, R.B. 2008. Predictability of phenotypic differentiation across flow regimes in fishes. *Integr. Comp. Biol.*, **48**: 750–768.

Legendre, L. and Legendre, P. 1998. *Numerical Ecology*, 2nd edn. Amsterdam: Elsevier.

Losos, J.B. 2009. *Lizards in an Evolutionary Tree: Ecology and Adaptive Radiation of Anoles*. Berkeley, CA: University of California Press.

Losos, J.B., Creer, D.A., Glossip, D., Goellner, R., Hampton, A., Roberts, G. *et al.* 2000. Evolutionary implications of phenotypic plasticity in the hind limb of the lizard *Anolis sagrei*. *Evolution*, **54**: 301–305.

Loy, A., Mariani, L., Bertelletti, M. and Tunesi, L. 1998. Visualizing allometry: geometric morphometrics in the study of shape changes in the early stages of the two-banded sea bream, *Diplodus vulgaris* (Perciformes, Sparidae). *J. Morphol.*, **237**: 137–146.

MacCrimmon, H.R. and Marshall, T.L. 1968. World distribution of brown trout, *Salmo trutta*. *J. Fish. Res. Bd. Can.*, **25**: 2527–2548.

McCoy, M.W., Bolker, B.M., Osenberg, C.W., Miner, B.G. and Vonesh, J.R. 2006. Size correction: comparing morphological traits among populations and environments. *Oecologia*, **148**: 547–554.

McDowall, R.M. and Pankhurst, N.W. 2005. Loss of negative eye-size allometry in a population of *Aplochiton zebra* (Teleostei: Galaxiidae) from the Falkland Islands. *NZ J. Zool.*, **32**: 17–22.

Michaud, W.K., Power, M. and Kinnison, M.T. 2008. Trophically mediated divergence of Arctic charr (*Salvelinus alpinus* L.) populations in contemporary time. *Evol. Ecol. Res.*, **10**: 1051–1066.

Nicieza, A.G. 1995. Morphological variation between geographically disjunct populations of Atlantic salmon: the effects of ontogeny and habitat shift. *Funct. Ecol.*, **9**: 448–456.

Nosil, P. 2012. *Ecological Speciation*. New York: Oxford University Press.

Pakkasmaa, S. and Piironen, J. 2000. Water velocity shapes juvenile salmonids. *Evol. Ecol.*, **14**: 721–730.

Paul, M.J. and Meyer, J.L. 2001. Streams in the urban landscape. *Annu. Rev. Ecol. Syst.*, **32**: 333–365.

Pavey, S.A., Nielsen, J.L., Mackas, R.H., Hamon, T.R. and Breden, F. 2010. Contrasting ecology shapes juvenile lake-type and riverine sockeye salmon. *Trans. Am. Fish. Soc.*, **139**: 1584–1594.

Phillips, B.L., Brown, G.P., Webb, J.K. and Shine, R. 2006. Invasion and the evolution of speed in toads. *Nature*, **439**(7078): 803.

Quinn, T.P. 2005. *The Behavior and Ecology of Pacific Salmon and Trout*. Seattle, WA: University of Washington Press.

Quinn, T.P., Kinnison, M.T. and Unwin, M.J. 2001. Evolution of chinook salmon (*Oncorhynchus tshawytscha*) populations in New Zealand: pattern, rate, and process. *Genetica*, **112**: 493–513.

R Development Core Team. 2009. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing.

Reist, J.D. 1986. An empirical evaluation of coefficients used in residual and allometric adjustment of size covariation. *Can. J. Zool.*, **64**: 1363–1368.

Rohlf, F.J. 2005. *tpsDig2.12*. New York: State University of New York, Stony Brook.

Rohlf, F.J. 2006. *tpsRelW*, Verson 1.46. New York: State University of New York, Stony Brook.

Schluter, D. 2000. *The Ecology of Adaptive Radiation*. New York: Oxford University Press.

Stearns, S.C. 1983. The evolution of life-history traits in mosquito fish since their introduction to Hawaii in 1905: rates of evolution, heritabilities, and developmental plasticity. *Am. Zool.*, **23**: 65–75.

Stelkens, R.B., Jaffuel, G., Escher, M. and Wedekind, C. 2012. Genetic and phenotypic population divergence on a microgeographic scale in brown trout. *Mol. Ecol.*, **21**: 2896–2915

Stevens, M., Parraga, C.A., Cuthill, I.C., Partridge, J.C. and Troscianko, T.O.M.S. 2007. Using digital photography to study animal coloration. *Biol. J. Linn. Soc.*, **90**: 211–237.

Sugimoto, M. 2002. Morphological color changes in fish: regulation of pigment cell density and morphology. *Microscopy Res. Technique*, **58**: 496–503.

Sumpter, J.P., Pickering, A.D. and Pottinger, T.G. 1985. Stress-induced elevation of plasma [alpha]-MSH and endorphin in brown trout, Salmo trutta L. *Gen. Comp. Endocrinol.*, **59**: 257–265.

Taylor, E.B. 1991. A review of local adaptation in Salmonidae, with particular reference to Pacific and Atlantic Salmon. *Aquaculture*, **98**: 185–207.

Valentin, A.E., Penin, X., Chanut, J.P., Sévigny, J.M. and Rohlf, F.J. 2008. Arching effect on fish body shape in geometric morphometric studies. *J. Fish Biol.*, **73**: 623–638.

Walton, I. 1653. *The Compleat Angler, or the Contemplative Man's Recreation: Being the Discourse of Rivers, Fishponds, Fish and Fishing not Unworthy of the Perusal of Most Anglers*. London: T. Maxey for R. Marriot.

Ward, A.D. and Trimble, S. 2004. *Environmental Hydrology*, 2nd edn. Boca Raton, FL: CRC Press.

Wedekind, C., Jacob, A., Evanno, G., Nussle, S. and Muller, R. 2008. Viability of brown trout embryos positively linked to melanin-based but negatively to carotenoid-based colours of their fathers. *Proc. R. Soc. Lond. B*, **275**: 1737–1744.

West-Eberhard, M.J. 2003. *Developmental Plasticity and Evolution*. Oxford: Oxford University Press.

Westley, P.A.H. 2011. What invasive species reveal about the rate and form of contemporary phenotypic change in nature. *Am. Nat.*, **177**: 496–509.

Westley, P.A.H. and Fleming, I.A. 2011. Landscape factors that shape a slow and persistent aquatic invasion: brown trout in Newfoundland 1883–2010. *Divers. Distrib.*, **17**: 566–579.

Whiteley, A.R., Gende, S.M., Gharrett, A.J. and Tallmon, D.A. 2009. Background matching and color-change plasticity in colonizing freshwater sculpin populations following rapid deglaciation. *Evolution*, **63**: 1519–1529.

Williams, C.K. and Moore, R.J. 1989. Phenotypic adaptation and natural selection in the wild rabbit, *Orcytolagus cuniculus*, in Australia. *J. Anim. Ecol.*, **58**: 495–507.

Wootton, R.J. 1990. *Ecology of Teleost Fishes*. London: Chapman & Hall.

Yeh, P.J. and Price, T.D. 2004. Adaptive phenotypic plasticity and the successful colonization of a novel environment. *Am. Nat.*, **164**: 531–542.