Small but diverse: patterns of sexual trait differentiation among fragmented trout populations of varying size

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A Thesis

in

The Department

of

Biology

Presented in Partial Fulfillment of the Requirements

for the Degree of Master of Science (Biology) at

Concordia University

Montreal, Quebec, Canada

January 2017

CONCORDIA UNIVERSITY
School of Graduate Studies

This is to certify that the thesis prepared

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Entitled: Small but diverse: patterns of sexual trait differentiation among fragmented trout populations of varying size

and submitted in partial fulfillment of the requirement for the degree of

Master of Science (Biology)

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ABSTRACT

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A reduction in population size due to habitat fragmentation can alter the relative roles of different evolutionary mechanisms in phenotypic trait differentiation. While deterministic (selection) and stochastic (genetic drift) mechanisms are expected to affect trait evolution, genetic drift may be more important than selection in small populations. We examined relationships between mature adult traits and ecological (abiotic and biotic) variables among 14 populations of brook trout. These naturally fragmented populations have a common ancestor but experienced considerable variability in habitat characteristics and differ by up to two orders of magnitude in population size ($49 < N_c < 10{,}032$; $3 < N_b < 567$). Populations differed markedly in body size, shape, and colouration, with a tendency for more variation among small populations in both phenotypic trait mean and CV when compared to large populations. These differences were more frequently and directly linked to habitat variation or operational sex ratio than to population size, suggesting that selection may overcome genetic drift at small population size. Phenotype-environment associations were also stronger in females than males, suggesting that natural selection due to abiotic conditions may act more strongly on females than males. Our results suggest that natural and sexual-selective pressures on phenotypic traits related to mating systems change during the process of habitat fragmentation, and that these changes are largely contingent upon existing habitat conditions within isolated fragments. Our study provides an improved understanding of the ecological and evolutionary consequences of habitat fragmentation, and lends insight into the ability of some small populations to respond to selection and environmental change.

Acknowledgements

Contributions of authors

C. Zastavniouk conducted fieldwork, statistical analyses, and redaction of the manuscript. D.J. Fraser provided field and logistical support, theoretical guidance, and writing assistance. L. K. Weir provided theoretical guidance and writing assistance.

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INTRODUCTION

Human disturbances are resulting in the widespread depletion, fragmentation and isolation of natural populations (World Wildlife Fund 2016). As a result, populations can enter an extinction vortex through increased inbreeding and genetic drift, a resulting loss of genetic diversity, and reduced adaptive responses to environmental change (Gilpin & Soulé 1989; Hanski & Gilpin 1991; Blomqvist et al. 2010). Yet, before such a genetic extinction vortex commences, emerging evidence suggests that evolution in small populations is highly affected by selective pressures within habitat fragments; these can either improve population persistence or exacerbate extinction risk in the face of future environmental change (Willi et al. 2007; Fraser et al. 2014; Wood et al. 2014, 2016). Hence, further investigation is required to understand how phenotypic evolution changes among populations as they are fragmented, isolated, and reduced in population size. Such knowledge could allow for differentiating between populations that have or do not have a chance of persisting with resource input.

Both natural selection (arising from variance in fitness as a result of abiotic factors) and sexual selection (arising from variance in mating success) can act on phenotypic traits as a result of specific ecological conditions (Arnold & Wade 1984; Wellborn & Langerhans 2015). Strong associations between phenotype and abiotic factors across populations, henceforth phenotype-environment associations, provide support that traits are under natural selection (Langerhans et al. 2007). Conversely, population differences in secondary sexual characteristics independent of habitat may point to sexual selection (Panhuis et al. 2001). How both natural and sexual selection on phenotypes change when populations have become fragmented, isolated, and small is understudied, but is thought to provide key information on the fate of a population under ongoing

environmental change (Haugen et al. 2008; Palkovacs et al. 2012; Franssen et al. 2013; Heinen-Kay et al. 2014; Murphy et al. 2016).

In stream fishes, abiotic factors such as water temperature, depth, velocity, and pH regularly shape phenotypes. Temperature controls fish metabolism and growth; growth and temperature are positively related (McCormick et al. 1972) as are stream depth and body depth (Quinn et al. 2001). Shallower streams are associated with more streamlined, easily maneuverable body shapes in fish, whereas longer pelvic and pectoral fins are expected in deeper streams (Pease et al. 2012). Higher stream velocity levels are also associated with fusiform fish body types and longer fins to maintain feeding positions (Riddell & Leggett 1981). Finally, dark water environments host fish with deeper colour (Kelley et al. 2012); commonly such waters are low in pH and high in dissolved organic compounds (DOC) in peatland environments (Ishikawa & Gumiri 2006).

Among stream fish populations, salmonids in particular (salmon, trout, charr) are often physically isolated from one another in different habitats and vary greatly in phenotypic traits and sexual dimorphism (Riddell & Leggett 1981; Hutchings 1996; Westley et al. 2013). Thus, stream salmonids provide a unique opportunity to understand the natural and sexual selective consequences of habitat fragmentation on phenotypic evolution. Salmonid males compete aggressively for access to females and exhibit exaggerated secondary sexual traits such as a dorsal hump, a deep body shape and bright colouration in ventral areas, which are indicators of social status, fighting capabilities, and/or intersexual mechanisms for mate attraction (Fleming & Foote 1994; Quinn & Foote 1994; Blanchfield & Ridgway 1999; Nitychoruk *et al.* 2013). In addition, the operational sex ratio (OSR; the ratio of males to females that are ready to mate; Emlen 1976) is a predictor of the intensity of competition for mates (Emlen & Oring 1977; Weir

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et al. 2011) and thus can influence the evolution of secondary sexual characteristics. Collectively, both biotic and abiotic factors are important to consider as putative drivers of selection, as salmonid trait differences often directly relate to individual fitness in local environmental conditions (Fraser et al. 2011).

We investigated relationships between adult morphological traits and abiotic and biotic ecological variables among 14 naturally fragmented stream populations of a wild salmonid (brook trout, *Salvelinus fontinalis*). First, we examined whether or not populations differed in body size, shape, and colouration, and whether there were differences between the sexes in these traits. We then explored possible relationships between phenotype and abiotic habitat factors to determine if population trait differences are putatively driven by natural selection, as well as the influence of OSR on trait differences between the sexes.

We complemented these analyses with two general hypotheses regarding how trait characteristics might change as both habitat fragment size and population size are reduced during habitat fragmentation (see Figure 1). A first, 'directional' hypothesis predicts that consistent shifts in habitat characteristics occur during ongoing fragmentation and isolation, and hence so do species traits characteristics (Willi & Hoffmann 2012; Fraser et al. 2014; Wood et al. 2014, 2016). A second, 'variable' hypothesis suggests that habitat characteristics and selection pressures become more variable as fragment and population size decrease, leading to more trait variation among and within small populations (Willi et al. 2007; Fraser et al. 2014; Wood et al. 2014, 2016).

Our study was specifically conducted on isolated brook trout populations in Cape Race, Newfoundland, Canada. Phylogeographic work suggests that population isolation occurred from a common ancestor during the late Wisconsonian deglaciation (10 000-12 000 ybp; Danzmann et

al. 1998). Like human-induced fragmentation, the natural fragmentation experienced by Cape Race populations appears to have arisen rapidly (Fraser et al. 2014). Previous research has also found that standing neutral genetic diversity within these populations is directly proportional to population size (Wood *et al.* 2014). These populations vary 200-fold in census population size (N_c) and 100-fold in their effective number of breeders (N_b - analogous to the effective population size (N_e) but for an individual cohort) (Table S3; Bernos & Fraser 2016). Population sizes reflect those that are typically very small to very large in vertebrates, including several below minimum viable population sizes for conservation (Reed et al. 2003); thus our study's results may have general implications for fragmented and isolated vertebrate populations.

MATERIALS AND METHODS

STUDY SITE

Cape Race is a barren coastal region situated in south-eastern Newfoundland, Canada (Figure S1). Throughout it are brook trout populations harboured within a parallel series of relatively short, low-order streams (0.27-8.10km), which create an ideal environment for investigating phenotypic and breeding traits in fragmented vertebrate populations of varying size ($49 < N_c < 10,032$; $3 < N_b < 567$; Bernos & Fraser 2016). Due to their small size, Cape Race streams can be sampled comprehensively to obtain reliable population size estimates. The trout populations are pristine (i.e., there has been little to no exploitation due to the small size of the fish), completely isolated (most streams end in a waterfall entering the ocean), and genetically distinct (Wood et al. 2014).

DATA COLLECTION

From September through October 2014, we photographed 1059 fish for phenotypic analyses described below from 14 Cape Race populations with multi-year population size and habitat data (Bernos & Fraser 2016). Individuals were randomly sampled throughout streams using a backpack electrofisher; however, spawning aggregates were targeted in those streams where they were found. Only fish that were reproductive that season were chosen for photographs; immature individuals and post-spawn females were not included. This selection was done by assessing individuals visually and physically for determining spawning readiness (i.e., gently squeezing abdomens). Each population was sampled during its spawning period, allowing for standardized inter-population comparisons.

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Morphological traits

Body size and shape

We compared body size and shape between populations using length and mass measurements and photography. Five fish were anesthetized at a time using tricaine mesylate (MS-222) at 0.2g/L. A wooden platform was used to ensure a level tripod and camera, which was set up at the same angles, distance, and zoom for each picture. A size reference, ruler and individual label were included and placed in a similar position in each photograph. The sex, spawning readiness, length, and mass were collected. Fish were then placed in a recovery container for 10 minutes before release back into the stream. Condition factor was calculated using the formula $K = \frac{10^5 \times Mass}{Length^3}$ (Weatherly and Gill 1987).

To calculate body shape, geometric morphometric analysis was conducted. In each photograph, seventeen landmarks were placed along the fish outline and assigned an x,y coordinate to produce a consensus shape using the program tpsDig2 (v.154, Rohlf 2014; see Figure S2). These landmarks were then used to produce relative warps (RWs), a multivariate description of shape variation, through tpsRelw (v.154) (Bookstein 1991; Zelditch et al. 2004; Rohlf 2014). The first four of thirty total RWs were used for statistical analysis of body shape, as these explained most (64.6%) of the total variation and were related to secondary sexual characteristics. ImageJ (Schneider et al. 2012) was used to measure pelvic and pectoral fin length, measured as the maximum distance from the proximal to distal margin of the fin (Pease et al. 2012).

Body colour

Redness in body colour (total area and saturation) was compared between populations. Inclusion of a X-Rite® ColorChecker Passport (a colour palette used as a standardization tool) in

each photo allowed for the removal of any changing lighting conditions, using nip2 (VIPS software; Martinez & Cupitt 2005). Following the methods of Wedekind et al. 2008 (using ImageJ), the total area of redness and its saturation level was calculated on each individual (excluding dorsal, adipose, and anal fins). We also used ImageJ to count red spots with blue halos on fish abdomens (limited to below the lateral line to reduce confounding from glare).

Abiotic habitat characteristics

Summer habitat variables were taken from stream measurements during mid-June to mid-July in 2012-2015, from 19 to 64 transects per stream. We considered the following variables: water temperature, pH, velocity, and depth. A WTW Multiline P4 universal meter was used to measure temperature and pH. Velocity (m/s) was measured by releasing a ball attached to a one metre string from an upstream position and recording the time required for the ball to travel one metre with the current. Mean velocity and depth per transect (measured using a metre stick to a precision of 0.1mm) were calculated as the average of three to six measurements spaced equally across the width of the stream channel. Overall habitat means within streams were calculated by bootstrapping values to account for differences in sampling effort between years (ensuring that all years were weighted equally).

Biotic factors

Operational Sex Ratio

We calculated operational sex ratio (OSR) as the ratio of potentially receptive males to potentially receptive females in a population (fish that were classified as 'almost ready' and 'ready') (*sensu* Emlen 1976). Potentially receptive individuals were determined from stream

surveys assigning spawning readiness for each fish caught (i.e. not close/almost ready/ready/spawned for females, and ready/not ready for males, with 'not ready' meaning several days to weeks away from spawning and 'almost ready' meaning spawning would happen within 1-4 days). Average OSR was used as datum for each population.

Population size

Mean population estimates for N_c and N_b were taken from Bernos & Fraser (2016). Harmonic means were used to ensure averages that were not biased by outliers. Additional calculations were done to obtain an N_b estimate for two populations (FW and PD), using a model describing the relationship between N_c and N_b in Cape Race trout populations (see Bernos & Fraser 2016, Table 2, Full N model).

TRAIT ANALYSIS

Inter-population trait variation

We used linear models to compare trait differences between populations (in R Studio 0.99.484, Team 2015). Body size, shape, and colour data were firstly tested for normality within each population using a Shapiro-Wilks test and by examining residual distributions. Mass and condition factor were log transformed as they were non-normal in several populations. Red area (area of red colour/total body area) was analyzed using a beta regression to account for data over-dispersion and heteroscedasticity (Cribari-Neto & Zeileis 2009). Independent predictor variables in our linear models were population, sex, and a population × sex interaction, tested through backwards step-wise model selection. Centroid size, a geomorphometric measure of overall body size, was included in our models as a covariate, but was removed from body size

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models to avoid redundancy due to a high correlation with mass and length. For traits with a significant population × sex interaction, least-square means (R package lsmeans; Lenth 2016) were used to identify significant differences between population, sex, as well as within-population sex differences. Statistical significance levels were adjusted to control for type I error via a FDR correction, and also divided in half ($p<0.025$) for length and mass to account for their non-independence.

Phenotype-environment associations

We tested whether mean stream habitat variables were putative drivers of inter-population variation in body size, shape and colour using linear mixed models (LMMs). Habitat variables were centered around zero. Collinearity between variables was tested through variance inflation factors (vif); those variables with vif values higher than 5 were discarded (two interactions: stream depth × velocity and depth × temperature). Interactions between habitat variables that were not collinear and biologically relevant were included in LMMs, specifically stream pH × temperature, and stream temperature × velocity. Population size (to test the directional hypothesis, N_c and N_b in separate analyses), sex (to account for putative differences between sexes), and a random effect of population (to control for population level variation) were also included in the models. Backwards step-wise model selection was conducted for each trait individually. As red area/total body area is proportional data, a logit transformation was performed prior to modeling to create continuous values along a real line [-inf, inf] instead of proportions [0,1]. For those models that showed a significant difference in sex, within-sex models were used to test for population differences within each sex separately.

<u>Directional and variable hypotheses</u>

The directional hypothesis was first tested for each trait using linear models described above. We also visually assessed plots of phenotypic traits against N_b and N_c to find corresponding patterns relating to consistent (directional hypothesis) or more variable (variable hypothesis) trait changes with population size, using both trait means and coefficients of variation (CV; a normalized measure of dispersion where $CV = \sigma/\mu$ [standard deviation/mean]). To further test whether trait variability (both trait means and CVs) specifically increased at smaller population sizes, we used White's test for heteroscedasticity (White 1980).

RESULTS

<u>Inter-population and inter-sex trait variation</u>

Details of percent variation explained at each RW, body shape characteristics associated with each RW, and average consensus shapes are found in Figure 2. All twelve morphological traits assessed were significantly different among populations ($p < 2.20E-16$; Table 1), ranging from a 1.32-fold mean difference in red saturation (106.73 units to 141.49 units) to a 2.96-fold mean difference in mass (13.35 g to 39.51 g; Table S1). Across populations, 10 of 12 traits also varied significantly between sexes ($p < 0.001$) (exceptions were the number of red spots and pelvic fin length; Table 1), ranging from a 1% mean difference in condition factor (female 1.179 to male 1.184) to a 15% mean difference in red saturation (female 118.89 units to male 138.30 units; Table S2). In most cases, males had greater trait values than females (exceptions: mass, length, and RW4). F-values were much higher for population than sex for 9 of 12 traits, indicating that among-population differences were much larger than sex differences (exceptions: RW1, RW2, and red colour saturation; Table 1).

F-values for the population $\times$ sex interaction were much lower than those for population and sex separately, but this interaction was significant for 7 of 12 traits (Table 1). Of these traits, three or more populations had sex differences inconsistent with the general trend, driving the interaction (see Figures 3 and Figure S3 for examples); for body depth (RW1), only UO were not much deeper than UO females to primarily drive the interaction (Figure 3).

<u>Phenotype-environment associations</u>

Biologically interpretable phenotype-environment associations were detected in all 12 traits; out of a possible 224 phenotypic trait vs. habitat or ecological variable comparisons, 54-72

were significant (p < 0.05) (Tables 2, S4). Visual plots of significant phenotype-environment associations can be found in Figure 4; additional associations can be found in Figure S4. Trout were significantly larger in warmer and faster streams, though these relationships appear to be weak. Deeper streams favoured deeper bodies (RW1) in females, and a larger dorsal hump (RW2) was strongly associated with warmer water. Body redness was greater in acidic streams for both sexes, and female redness increased in deeper, faster, and warmer streams. Male redness, however, was positively associated with colder streams. Fast streams also favoured longer pectoral and pelvic fin lengths in females only, and only pelvic fin length was positively associated with depth in females. Body size had a weak positive relationship with average OSR in females while body depth for males decreased with increasing OSR. Pelvic fin length was strongly positively associated with higher OSR in females, and number of spots also increased with OSR.

Overall, females had more significant differences (20 vs 9) and generally stronger relationships between phenotypic traits and habitat variables than males, suggesting stronger phenotype-environment associations. Of the significant trait-habitat combinations in both sexes, *F*-values were higher in females in 7 of 9 cases (Tables 2, S4).

Directional and variable hypotheses

Only two traits (red area and RW4) showed a pattern consistent with the directional hypothesis (a negative association was observed between each trait and N_c or N_b; Table 2, S4). Conversely, 6 of 12 traits showed the pattern expected through the variable hypothesis for both trait means and trait CV when plotted against N_b (trait means: red area/total body area, red saturation, RW1, RW2, condition factor, spot number; trait CV: red saturation, RW1, RW2,

length, pectoral fin size, pelvic fin size; Figures 3 and 5 respectively). However, White's tests did not show statistically significant heteroscedasticity for any trait (means or CVs) (Appendix C).

DISCUSSION

We found a large number of phenotype-environment associations in fragmented brook trout populations, consistent with the hypothesis that selection may be influencing inter-population differences in morphological traits. We also found little support that population size (and by extension the amount of genetic variation) affects sexual trait characteristics in these isolated populations. Previous studies (Fraser et al. 2014; Wood et al. 2014, 2015) found that across Cape Race trout populations (including the 14 in this study), the process of habitat fragmentation increased variability in spatial habitat, adaptive genetic differentiation, and in early life traits going from large to small fragment and population size, consistent with the variable hypothesis. Our study's results suggest that although there appears to be some semblance of more variation in the phenotypic traits of small populations, existing habitat characteristics can better explain trends in sexual phenotypic characteristics among Cape Race trout populations than the variable or directional hypotheses. This may be due to a difference in genetic factors between early life traits and adult phenotypic traits in brook trout, or a difference in habitat selective pressures that are influential in early life traits but perhaps not in later life stages. Additionally, our White's tests might have had reduced statistical power with a sample size of 14 populations. To some extent, this contrast may also be due to phenotypic plasticity within each population in response to habitat variables. Nevertheless, the associations between phenotypes and environments, combined with the large number of genetically-based trait differences observed among Cape Race populations using common garden experimentation, suggest that traits are under selection (Wood & Fraser 2015; Wood et al. 2015; D. Fraser, unpublished results).

Our results have three key implications. First, they suggest that natural selection on adult traits in fragmented populations may operate even under conditions of pronounced genetic drift (mean N_b ranging from 5 to 355, mean N_e 9 to 589 with four populations $N_e < 50$ and five populations $50 < N_e > 100$; Bernos & Fraser 2016), consistent with recent meta-analysis findings across taxa (Wood et al. 2016). Second, the strength and number of phenotype-environment associations were higher in females than in males, suggesting that female phenotypic traits may be under a stronger influence from natural selection. Lastly, across a fragmented landscape of many populations, trait differentiation that appears to be influenced by natural selection – a deterministic process – also appears to be highly influenced by starting conditions of initial fragmentation events that are largely random with respect to habitat patch characteristics and population size (see also Figure 1).

As suspected based on previous work (Hutchings 1993, 1996; Belmar-Lucero et al. 2012), Cape Race trout populations were highly differentiated in all 12 traits despite occupying a very small geographic scale. Phenotype-environment associations observed were consistent with theoretical expectations and previous works on stream fishes (McCormick et al. 1972; Riddell & Leggett 1981; Quinn et al. 2001; Ishikawa & Gumiri 2006; Kelley et al. 2012; Pease et al. 2012), again suggesting that traits are under selection in all Cape Race populations despite their large spread in population size. However, many predicted associations were seen in females only. For example, deeper streams yielded female fish with deeper bodies and longer fins, and warmer streams yielded larger fish overall (although this relationship was not strong, perhaps because warmer waters are associated with greater growth rate and not overall size). We also found higher amounts of red body colouration in populations inhabiting acidic streams among both sexes, corresponding to theoretically increased DOC levels. Only females showed the predicted

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relationship with pelvic fin length and to a lesser extent, pectoral fin length (deeper streams had longer pelvic fins and both pelvic and pectoral fins were longer in faster streams). Both sexes were larger in faster streams, perhaps also as a function of maintaining feeding positions (Riddell & Leggett 1981).

Although salmonids generally exhibit elaborate sexual dimorphism (Young 2005; Nitychoruk et al. 2013; Weir et al. 2016), population explained more variation than sex in Cape Race trout (Table 1). Natural and sexual selection occur concurrently in many vertebrates (Johnson 2001; Langerhans & Dewitt 2004; Crothers & Cummings 2013; Romano *et al.* 2016) and can also operate with different intensities between sexes to shape breeding behaviour and tactics (Fleming 1998; Dunn et al. 2015). Results from our study are consistent with these findings by showing that phenotypic traits in females are in general more a function of habitat characteristics than those of males. This may mean that females are under greater natural selection while mating competition continues to drive sexual selection in males. Both parallel and non-parallel evolution of the sexes has been shown to occur in vertebrates in response to habitat variables (Hendry et al. 2006; Butler et al. 2007; Berns & Adams 2013). In Cape Race brook trout, males and females exhibited similar trends in the direction that habitat variables acted upon traits, but the strength of phenotype-environment associations was greater in females.

Sexual selection may also differ in populations as a function of the environment, with ecological variation altering the context of sexual selection (Anderson & Langerhans 2015; Romano et al. 2016). This can be seen with body depth in male sockeye salmon; deep-bodied males in deep-water environments are dominant, while the dominant males in shallow-water are not significantly deeper-bodied (Hamon & Foote 2005). Of the 12 traits, those showing high sexual dimorphism (higher values for body depth, dorsal hump, and red colour saturation among

males) are typical of secondary sexual characteristics found in salmonids and are important indicators of sexual selection (Quinn & Foote 1994). However, each of these traits were still influenced by habitat and differed among populations. In the case of RW2 (dorsal hump), lower values also represent extended ventral areas which would be typical of mature females carrying eggs during their spawning season.

Traits known to be sexually selected in salmonids (e.g. body size and depth) were also influenced by the OSR. Male competition is highest at a male-biased OSR of 1-4, at which sexually selected traits should be the most exaggerated. A higher OSR could potentially decrease the rate of competition as the ratio of effort to outcome becomes more skewed (Quinn et al. 1996), including sperm competition (Pilastro et al. 2002). This can be seen with male (and to a lesser extent, female) body depth (RW1) in Cape Race populations; as the ratio of males to females increases (OSR ranging from 1.15 to 9.90), body depth decreases (Table 2). As for other secondary sexual characteristics, this relationship was also not seen with body size or redness in both sexes, perhaps as a result of stronger selection from habitat variables.

Although small and large populations did not consistently differ across most trait characteristics, two traits (red area over total body area and RW4) were shown to significantly change with population size, although the relationship with RW4 was not strong (Table 2). In the case of red colouration, this negative relationship may be a function of increased sexual selection in smaller populations, as competition remains high because of smaller or no spawning aggregates (OSR 1-4) (Quinn et al. 1996). Females may be more selective in choosing a more colourful male in those populations where there are no aggregates, as four of the five largest populations have a very high OSR (CC, FW, UO, WN) and three out of five have large aggregations during peak spawning period (BC, FW, UO; personal observations). We

hypothesize that the directional relationship seen between population size and red colouration is therefore a function of sexual selection, in lieu of accrued genetic drift.

Evolutionary ecology and conservation implications

The full genetic and evolutionary consequences of landscape modification remain understudied (Fischer & Lindenmayer 2007). Although habitat fragmentation and subsequent population size reduction can reduce genetic diversity within populations (Alò & Turner 2005; Blanchet et al. 2010), these changes can also cause changes to phenotypic selective pressures, with subsequent effects on population persistence before classic extinction vortexes might ensue (Fraser et al. 2014; Wood et al. 2014). We attempted to address this by comparing 14 naturally fragmented trout populations, finding that population size and genetic variation are less important indicators of morphological variability in body size, shape and colour in both sexes, compared to existing habitat characteristics within fragments. This suggests that Cape Race populations are able to maintain environmentally selected characteristics despite a potential lack of genetic diversity at small population size, until perhaps the populations become very small. Nevertheless, while selection appears to overcome drift in large to small Cape Race populations, it also appears to be highly contingent upon random starting conditions in each habitat fragment. Some populations appear to become fragmented in marginal, poorer quality habitats while others become isolated in fragments of high quality but that simply have a small finite size – and changing habitat characteristics with ongoing fragmentation can favour the maintenance of genetic diversity in some small populations rather than reducing it (Fraser et al. 2014; Wood et al. 2014, 2016).

Through similar studies to this one, it is becoming apparent that fine-scale local adaptation might play an important part in maintaining small, isolated populations. Forecasting traits and genetic makeup based on population size may not adequately predict the variation that is observed (Letcher et al. 2007; Giery et al. 2015; Wood et al. 2016). As anthropogenic disturbances escalate in scale and rate causing decreases in habitat and population sizes, it may therefore be difficult to predict trajectories of populations at a large geographic scale. Case-by-case consideration of each habitat fragment and the population inhabiting it may be critical. In the face of resource-limited conservation, a potentially effective method might be to prioritize those populations that (i) have experienced very small population sizes yet maintain relatively high genetic and phenotypic variation and (ii) are experiencing more similar environmental conditions to those presumably faced in the future with climate change.

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TABLES

Table 1. Overall p-values (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, NS $p > 0.05$) and *F*-values of all traits in relation to each tested variable, using linear models (or a beta regression model for red area).

Trait Category	Trait	Population (df = 13)	Sex (df = 1)	Centroid Size (df = 1)	Pop:Sex (df = 13)
Body size	Mass	***F = 64.491	**F = 8.939	N/A	NS
Body size	Length	***F = 64.743	**F = 11.616	N/A	NS
Body size	Condition factor	***F = 14.768	**F = 10.981	N/A	*F = 2.078
Body shape	RW1	***F = 14.768	***F = 1005.243	***F = 52.690	***F = 6.417
Body shape	RW2	***F = 30.285	***F = 64.836	***F = 29.011	NS
Body shape	RW3	***F = 55.660	***F = 59.529	***F = 16.942	NS
Body shape	RW4	***F = 65.044	*F = 5.296	***F = 65.533	*F = 1.958
Colouration	Red Area	***F = 50.899	***F = 15.335	***F = 50.646	***F = 3.284
Colouration	Red Saturation	***F = 34.839	***F = 382.549	*F = 4.795	***F = 5.330
Colouration	Spot number	***F = 64.772	NS	***F = 211.691	NS
Fin Length	Pectoral Fin	***F = 390.201	***F = 69.185	***F = 3501.326	**F = 3.087
Fin Length	Pelvic Fin	***F = 240.622	NS	***F = 2379.589	*F = 1.993

Table 2. *F*-values ($p < 0.05$) from linear mixed models with traits as dependent variables and with habitat characteristics, sex, number of breeders (N_b), and OSR as predictor variables in 14 brook trout populations in Cape Race, Newfoundland, Canada. Condition factor and spot number have overall results only as sex was not significant.

Trait		pH:Temp	Temp:Velocity	pH	Depth	Velocity	Temp	Sex	Nb	AvgOSR
Mass	O:		1.7425			(+) 2.9957	(+) 0.0088	(-) 9.5930		
	F:		3.6178			(+) 3.7648	(+) 0.2899		(-) 0.4007	
	M:		2.5496			(+) 0.7770	(+) 0.0107			
Length	O:		1.2518			(+) 1.9287	(+) 0.0235	(-) 10.8629		
	F:		1.2635			(+) 2.4362	(+) 0.0528		(-) 0.3460	
	M:		1.6757			(+) 1.1835	(+) 0.1321			
Condition	O:			(-) 13.25						
RW1	O:				(+) 2.4601		(+) 0.4205	(+) 1005.5837		(-) 6.1316
	F:				(+) 57.2616					
	M:									(-) 9.7182
RW2	O:					(+) 22.873	(+) 62.7150			
	F:						(+) 26.537			
	M:						(+) 14.4732			
RW3	O:						(-) 62.5886			
	F:									
	M:									
RW4	O:						(-) 5.4647		(+) 0.6477	(+) 12.2757
	F:								(+) 0.0051	
	M:								(+) 0.1555	(+) 8.3216
Red Area	O:			(-) 5.2457	(+) 7.1510		(+) 9.6239	(+) 4.6355	(-) 27.2992	
	F:			(-) 6.1497	(+) 8.5121	(+) 0.1145	(+) 37.7793		(-) 19.1734	
	M:			(-) 3.3276			(-) 9.2189		(-) 21.2761	
Red Saturation	O:				(-) 0.0810	(-) 1.3418		(+) 363.334		
	F:					(-) 4.2753				
	M:									
Spots	O:									(+) 8.3916
Pectoral Fin	O:						(+) 3.0219	(+) 58.9927		
	F:		0.8323			(+) 3.0351	(+) 3.9486			
	M:									
Pelvic Fin	O:		3.0472			(+) 20.2074	(+) 3.9688	(+) 0.8483		
	F:		10.3228		(+) 16.0433	(+) 2.5097	(+) 0.2002			(+) 85.5298
	M:									

Table 2 (con't).

Models were done for both sexes combined as well as separated. Overall results (with sexes combined) are indicated with "O", female model results are indicated with "F", and male model results are indicated with "M". "+" or "–" in front of values represents a positive or negative relationship (for sex, relationship is displayed in reference to males). Missing values represent insignificant results.

FIGURES

Figure 1. The directional (**A**) and variable (**B**) hypotheses.
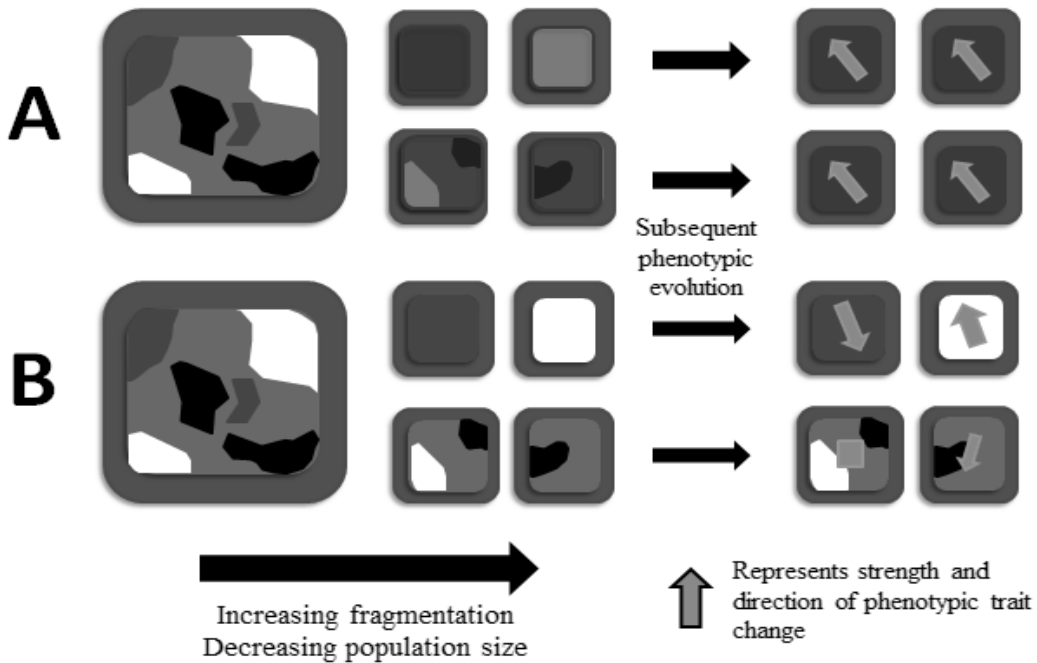
The different shades in the large squares on the left represent habitat types of different qualities and characteristics in an environment. As fragmentation occurs, the directional hypothesis (**A**) posits that the habitat parameters in each fragment change in a directional way, resulting in similar selection pressures across fragments, for example, through edge effects. When subsequent phenotypic evolution occurs, a directional change occurs in the phenotypic traits across all fragments and populations. This is the hypothesis that is most seen in traditional studies on habitat fragmentation (Willi & Hoffmann 2012; Fraser et al. 2014; Wood et al. 2014; 2016). The variable hypothesis (**B**) posits that habitat quality and characteristics are not changed in a directional way throughout fragmentation and are simply random samples of the habitats found in larger fragments; hence there are different selection pressures among the fragments. With subsequent phenotypic evolution, each fragment sees a different change in phenotypic trait, both in direction and extent. It is more difficult with the variable hypothesis to systematically predict what will further happen to fragmented populations once they experience large-scale environmental change (Fraser et al. 2014; Wood et al. 2014; 2016).

Figure 2. Extreme positive and negative shapes for RW1-4, across 14 brook trout populations from Cape Race, Newfoundland, Canada.
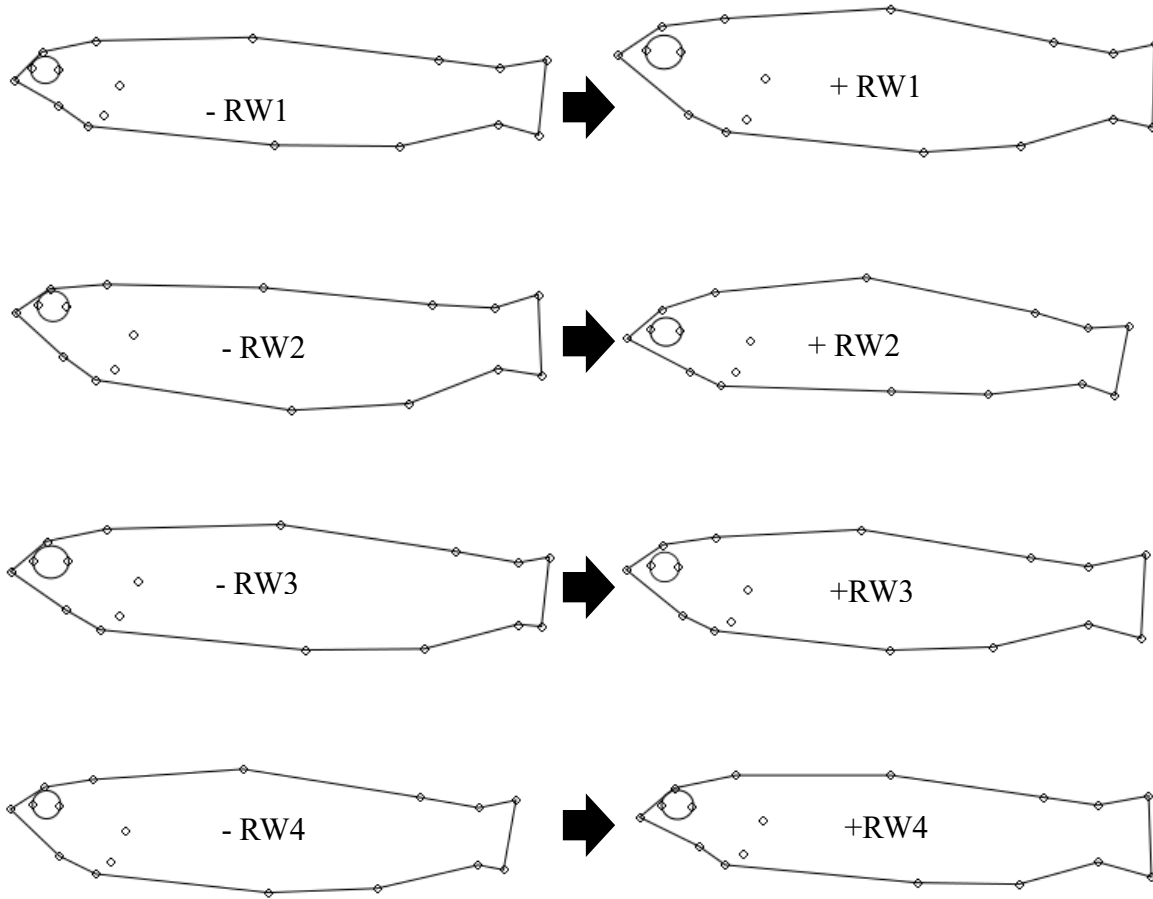
Variance explanation from each warp is as follows: RW1 29.32%, RW2 16.32%, RW3 10.93%, RW4 8.03%. From negatives values on the left to positive on the right: RW1 shows increase in body depth, RW2 shows horizontal alignment change going from extended ventral side to extended dorsal side, RW3 shows caudal peduncle increasing compared to torso length, and RW4 shows mouth angle increase, decrease body depth, and head narrowing.

Figure 3. Female and male means of traits that support the variable hypothesis (more variability in small populations), from left to right: RW1 (body depth), red area/total body area, RW2 (dorsal hump), red saturation, condition factor, and spot number across 14 brook trout populations in Cape Race, Newfoundland, Canada, increasing in population size (N_b) along the x-axes. Figure S3 shows remaining traits. Trait means depicted with 95% confidence intervals.

Figure 4. Examples of mean trait and abiotic habitat interactions in 14 brook trout populations in Cape Race, Newfoundland, Canada. From left to right: mass across stream temperatures, RW1 (body depth) across stream depth, RW2 (dorsal hump) across stream temperatures, red area/total body area across stream pH, red area/total body area across stream temperatures, pelvic fin length/total body length across stream velocities, pelvic fin length/total body length across stream velocities, RW1 (body depth) across stream OSRs, and spot number across steam OSRs. Trait means depicted with 95% confidence intervals.

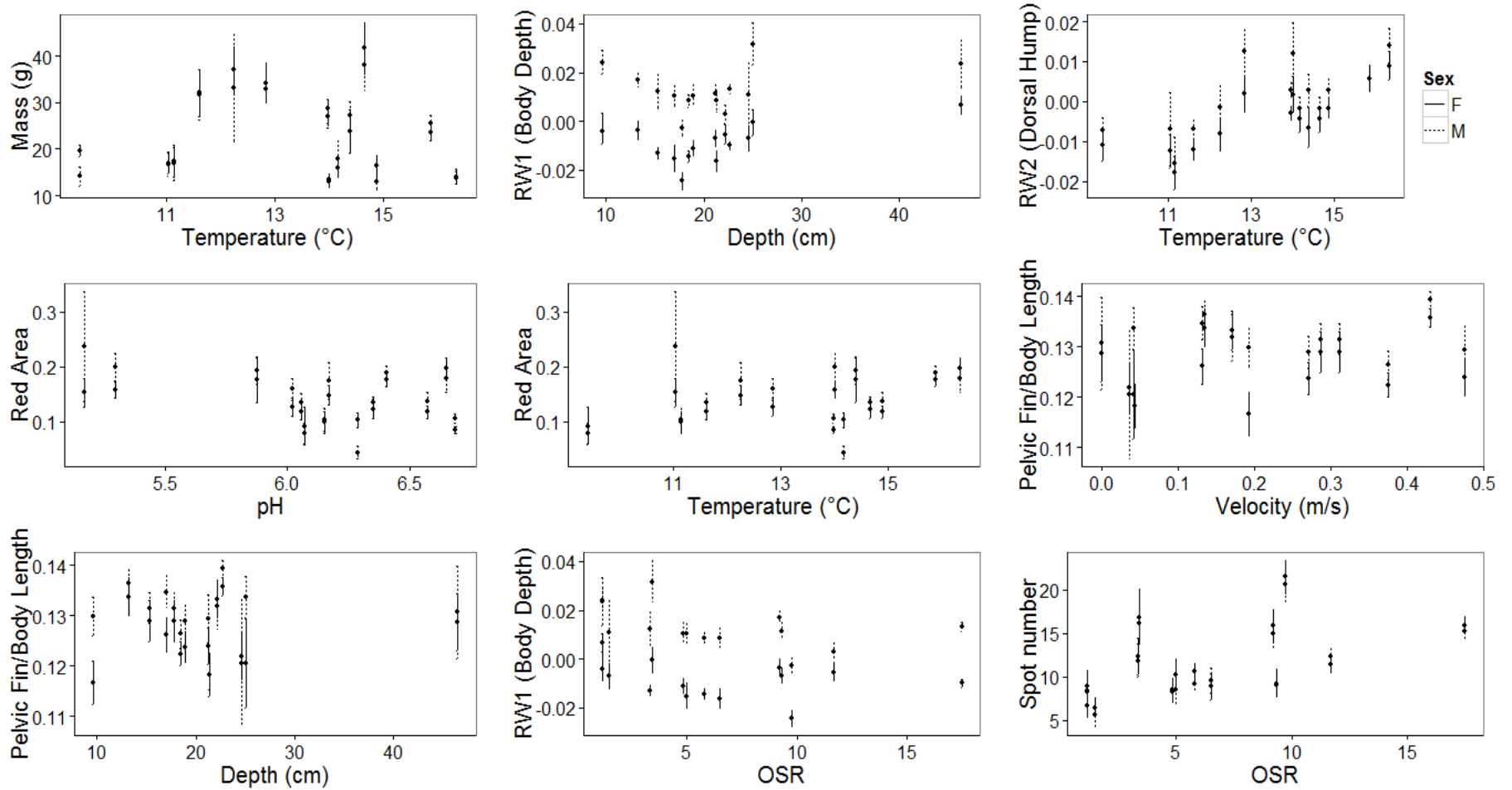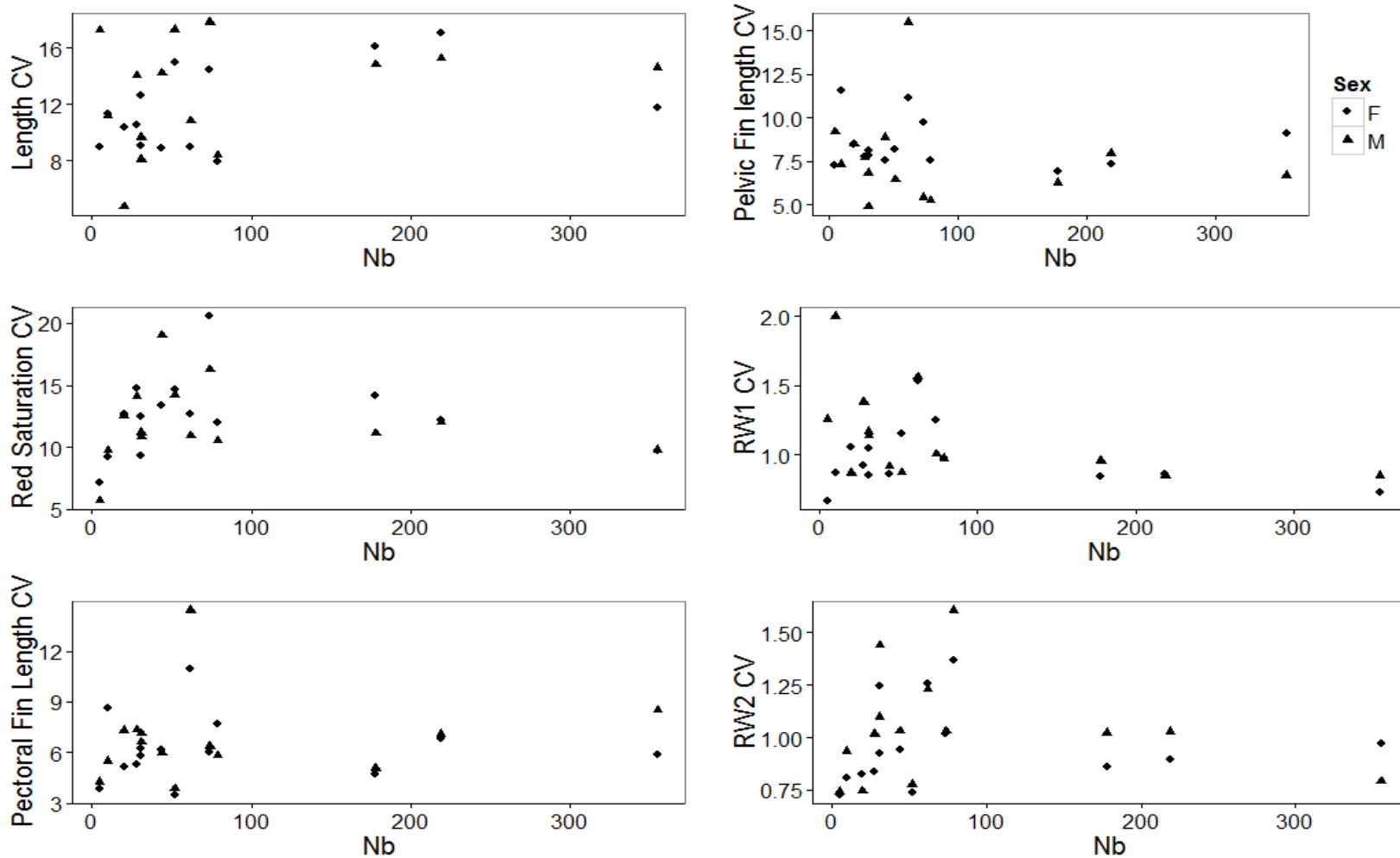**Figure 5.** Coefficient of variation (CV) of traits by sex against population size (N_b) in 14 brook trout populations in Cape Race, Newfoundland, Canada. From left to right: length, pelvic fin length/total body length, red saturation, RW1 (body depth), pectoral fin length/total body length, RW2 (dorsal hump). Out of twelve traits, these six showed semblance to the variable hypothesis (more variability in small populations).

APPENDIX A

Additional Tables and Figures Referred to In-text

Table S1. Overall means of traits across 14 brook trout populations in Cape Race, Newfoundland, Canada.

Table S2. Overall trait means for females and males in 14 brook trout populations in Cape Race, Newfoundland, Canada.

Table S3. Environmental and demographic variables in 14 brook trout populations in Cape Race, Newfoundland, Canada, collected from 2012-2015 (Bernos & Fraser 2016).

Table S4. *F*-values (p < 0.05) from linear mixed models with traits as dependent variables and with habitat characteristics, sex, census population size (N_c), and OSR as predictor variables for 14 brook trout populations in Cape Race, Newfoundland, Canada. Condition factor and spot number have overall results only as sex was not significant.

Figure S1. Cape Race, Newfoundland, Canada streams. From west to east: 1) Perdition (PD), 2) Freshwater (FW), 3) Lower Coquita (LC), 4) Upper Coquita (UC), 5) Hermitage (HM), 6) Bob's Cove (BC), 7) Still There By Chance (STBC), 8) Whale Cove (WC), 9) Ditchy (DY), 10) Upper O'Beck (UO), 11) Lower O'Beck (LO), 12) Watern (WN), 13) Lower Blackfly (LBF), 14) Cripple Cove (CC).

Figure S2. Landmarks for geometric morphometric analysis on brook trout.

Figure S3. Female and male means of (left to right) mass, RW4 (head size), length, pectoral fin length/total body length, RW3, and pelvic fin length/total body length across 14 brook trout populations in Cape Race, Newfoundland, Canada, increasing in population size (N_b) along the x-axes. Trait means depicted with 95% confidence intervals.

Figure S4. Examples of mean trait and habitat interactions 14 brook trout populations in Cape Race, Newfoundland, Canada. From left to right: mass across stream velocities, length across stream velocities, condition factor across stream pH, RW4 across stream OSRs, red area/total body area across stream depths, red saturation across stream velocities, pectoral fin length/total body length across stream temperatures, pelvic fin length/total body length across stream velocities, and pelvic fin length/total body length across stream OSRs. Trait means depicted with 95% confidence intervals.

Table S1. Overall means of traits across 14 brook trout populations in Cape Race, Newfoundland, Canada.

Population	Mass (g)	Length (mm)	Condition Factor	Pectoral Fin/Body Length (%)	Pelvic Fin/Body Length (%)
BC	16.32	110.87	1.14	17.28	13.02
CC	39.51	149.38	1.09	16.71	12.72
DY	26.12	129.38	1.18	17.19	12.93
FW	14.68	104.74	1.19	16.64	12.43
HM	35.41	137.64	1.31	16.66	12.95
LBF	32.28	135.90	1.21	17.69	13.51
LC	17.14	109.44	1.27	15.71	12.17
LO	13.93	105.94	1.13	16.68	12.64
PD	13.35	101.13	1.29	14.87	12.56
STBC	16.00	109.78	1.15	15.28	11.82
UC	16.90	110.22	1.25	15.40	12.19
UO	24.56	127.16	1.16	16.95	13.23
WC	33.60	141.05	1.70	17.92	13.78
WN	27.46	130.63	1.16	16.79	13.03

Table S1 (cont.)

Population	RW1 (body depth)	RW2 (dorsal hump)	RW3 (caudal peduncle)	RW4 (head size)	Red Area/ Body Area (%)	Red Saturation	Spot Number
BC	-4.98E-04	-3.05E-03	1.09E-02	8.61E-03	7.33	106.73	12
CC	-1.16E-02	1.34E-03	1.26E-02	-2.67E-03	12.95	112.02	21
DY	2.10E-02	-2.65E-04	6.77E-03	6.96E-03	18.76	141.49	16
FW	-3.52E-03	5.24E-04	-3.82E-03	5.99E-03	12.74	134.45	10
HM	1.32E-02	-5.55E-03	-5.12E-03	6.87E-03	15.80	119.26	9
LBF	6.98E-03	-9.49E-03	9.51E-04	-5.52E-03	12.61	139.16	15
LC	6.54E-03	-1.70E-02	-2.80E-03	1.97E-03	10.07	123.12	7
LO	1.51E-03	1.13E-02	-8.64E-03	4.76E-03	18.83	133.72	9
PD	-3.52E-03	5.46E-03	-8.43E-03	1.98E-03	17.21	132.17	8
STBC	4.73E-05	-8.57E-03	-6.47E-03	4.17E-03	8.33	114.38	9
UC	-3.27E-03	-1.13E-02	-2.12E-03	9.69E-03	17.08	119.65	6
UO	-8.91E-04	5.84E-03	-3.30E-03	-1.66E-03	18.35	138.70	12
WC	-2.56E-03	7.27E-03	-2.87E-03	2.12E-03	9.52	132.01	9
WN	1.84E-03	1.89E-02	6.04E-04	-8.06E-03	14.25	139.68	16

Table S2. Overall trait means for females and males in 14 brook trout populations in Cape Race, Newfoundland, Canada.

Trait Category	Trait	Female Mean	Male Mean
Body size	Mass (g)	24.96	24.58
Body size	Length (mm)	125.00	123.64
Body size	Condition factor	1.18	1.18
Body shape	RW1 (body depth)	-1.05E-02	1.01E-02
Body shape	RW2 (dorsal hump)	-2.64E-03	2.51E-03
Body shape	RW3 (caudal peduncle)	-2.28E-03	2.19E-03
Body shape	RW4 (head size)	6.68E-04	-6.44E-04
Colouration	Red Area/Body Area (%)	12.64	13.57
Colouration	Red Saturation	118.89	138.30
Colouration	Spot number	12.68	13.02
Fin Length	Pectoral Fin/Body Length (%)	20.34	21.50
Fin Length	Pelvic Fin/Body Length (%)	16.00	16.33

Table S3. Environmental and demographic variables in 14 brook trout populations in Cape Race, Newfoundland, Canada, collected from 2012-2015 (Bernos & Fraser 2016).

Population	pH	Depth (cm)	Temperature (°C)	Velocity (m/s)	Mean N_c	Mean N_b	OSR (M/F)
BC	6.29	15.31	14.17	0.29	4693 (4404-6132)	355 (267-567)	3.34
CC	6.35	17.82	14.66	0.31	1862 (1471-5246)	74 (65-99)	9.75
DY	5.88	25.02	14.39	0.04	116 (84-179)	10 (3-34)	3.43
FW	6.57	18.45	14.88	0.38	5367	219	5.80
HM	6.17	46.40	12.25	0	66 (52-80)	5 (3-5)	1.15
LBF	6.06	13.26	11.61	0.14	1184 (877-1383)	52 (34-83)	9.20
LC	6.15	9.60	11.15	0.19	338 (250-798)	31 (11-117)	1.17
LO	6.65	21.24	16.34	0.48	470 (372-625)	44 (23-188)	9.33
PD	5.30	18.91	14.01	0.27	992	79	4.85
STBC	6.07	21.34	9.40	0.04	917 (587-1405)	28 (14-54)	6.50
UC	5.17	24.62	11.03	0.04	65 (49-79)	20 (13-48)	1.50
UO	6.41	22.16	15.88	0.17	2569 (1949-3835)	62 (41-87)	9.90
WC	6.02	17.01	12.84	0.13	783 (530-1148)	31 (21-52)	3.73
WN	6.69	22.68	13.98	0.43	7801 (6713-10032)	178 (110-267)	7.96

Table S4. *F*-values ($p < 0.05$) from linear mixed models with traits as dependent variables and with habitat characteristics, sex, census population size (N_c), and OSR as predictor variables for 14 brook trout populations in Cape Race, Newfoundland, Canada. Condition factor and spot number have overall results only as sex was not significant.

Trait		pH:Temp	Temp:Velocity	pH	Depth	Velocity	Temperature	Sex	Nc	OSR
Mass	O:		8.7682		(+) 2.6631	(+) 2.6631	(+) 0.0125	(-) 9.3712		(+) 3.5964
	F:		2.0334		(+) 1.4409	(+) 2.2839	(+) 0.3075			(+) 3.1184
	M:		8.3551			(+) 4.0397	(+) 0.0124			(+) 2.4573
Length	O:		6.8088		(+) 1.4910	(+) 1.8530	(+) 0.0400	(-) 10.6381		(+) 4.5924
	F:		6.0924		(+) 1.7494	(+) 0.7131	(+) 0.0910			(+) 5.8272
	M:		3.7325			(+) 3.2263	(+) 0.1715			(+) 3.2598
Condition	O:			(-) 13.2500						
RW1	O:				(+) 2.4601		(+) 0.4205	(+) 1005.5837		(-) 6.1316
	F:				(+) 0.5760					
	M:									(-) 9.7182
RW2	O:						(+) 22.7100	(+) 63.6710		
	F:						(+) 26.5370			
	M:						(+) 14.4732			
RW3	O:							(-) 62.3040		
	F:									
	M:									
RW4	O:							(-) 5.4647	(+) 0.6477	(-) 12.2757
	F:									(-) 8.4886
	M:								(+) 2.8942	(-) 8.3216
Red Area	O:	0.4013	1.5680	(-) 1.7070	(+) 4.4557	(+) 0.0716	(-) 8.4862	(-) 4.9084	(-) 3.1182	(-) 4.5186
	F:	1.9321	4.2067	(+) 4.5885	(+) 9.2137	(+) 0.0337	(-) 45.2201		(-) 21.3188	
	M:	0.3187		(-) 2.9769	(+) 3.5581		(-) 3.6279		(-) 1.7603	(-) 1.4698
Red Saturation	O:				(-) 0.0810			(+) 363.3344		
	F:									
	M:									
Spots	O:									
Pectoral Fin	O:						(+) 3.0219	(+) 58.9927		
	F:		0.8323			(+) 6.6123	(+) 0.8745			
	M:									
Pelvic Fin	O:		5.6512		(+) 11.8457	(+) 12.9409	(+) 4.3748	(+) 0.9906		
	F:		10.3228		(+) 16.0433	(+) 2.5097	(+) 0.2002			(+) 85.5298
	M:									

Figure S1. Cape Race, Newfoundland, Canada streams. From west to east: 1) Perdition (PD), 2) Freshwater (FW), 3) Lower Coquita (LC), 4) Upper Coquita (UC), 5) Hermitage (HM), 6) Bob's Cove (BC), 7) Still There By Chance (STBC), 8) Whale Cove (WC), 9) Ditchy (DY), 10) Upper O'Beck (UO), 11) Lower O'Beck (LO), 12) Watern (WN), 13) Lower Blackfly (LBF), 14) Cripple Cove (CC).
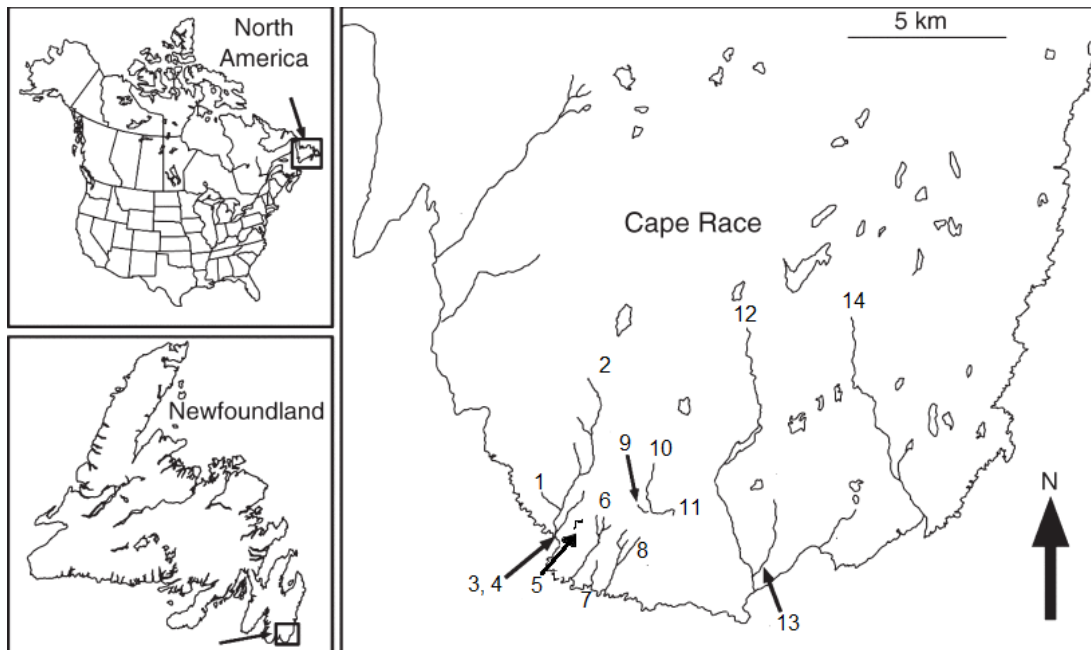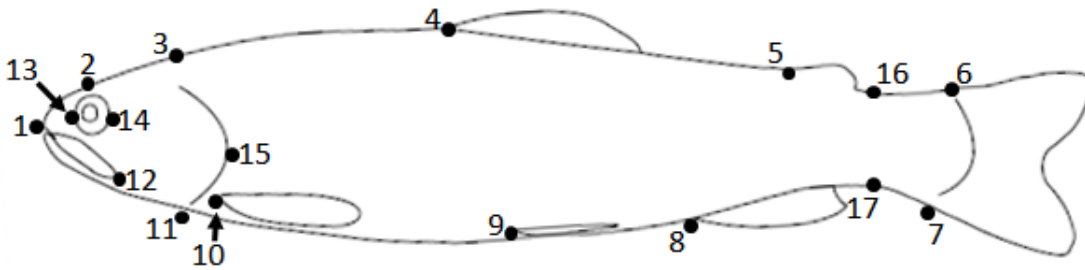
Figure S2. Landmarks for geometric morphometric analysis on brook trout.

1, the most anterior part of body; 2, the head directly above midpoint of the eye; 3, the head directly above dorsal limit of operculum; 4, the anterior insertion point for dorsal fin; 5, the anterior limit of adipose fin; 6, the dorsal terminus of the caudal peduncle; 7, the ventral terminus of the caudal peduncle; 8, the anterior insertion point of the anal fin; 9, the anterior insertion point for the left pelvic fin; 10, the anterior insertion point for the left pectoral fin; 11, the meeting point of the gill plate and the ventral midline; 12, the most posterior point on upper mandible; 13, the most anterior point on the eye; 14, the most posterior point on the eye; 15, the most posterior point on the operculum; 16, the dorsal position above the thinnest part of the caudal peduncle; 17, the ventral position below the thinnest part of the caudal peduncle.

Figure S3. Female and male means of (left to right) mass, RW4 (head size), length, pectoral fin length/total body length, RW3, and pelvic fin length/total body length across 14 brook trout populations in Cape Race, Newfoundland, Canada, increasing in population size (N_b) along the x-axes. Trait means depicted with 95% confidence intervals.
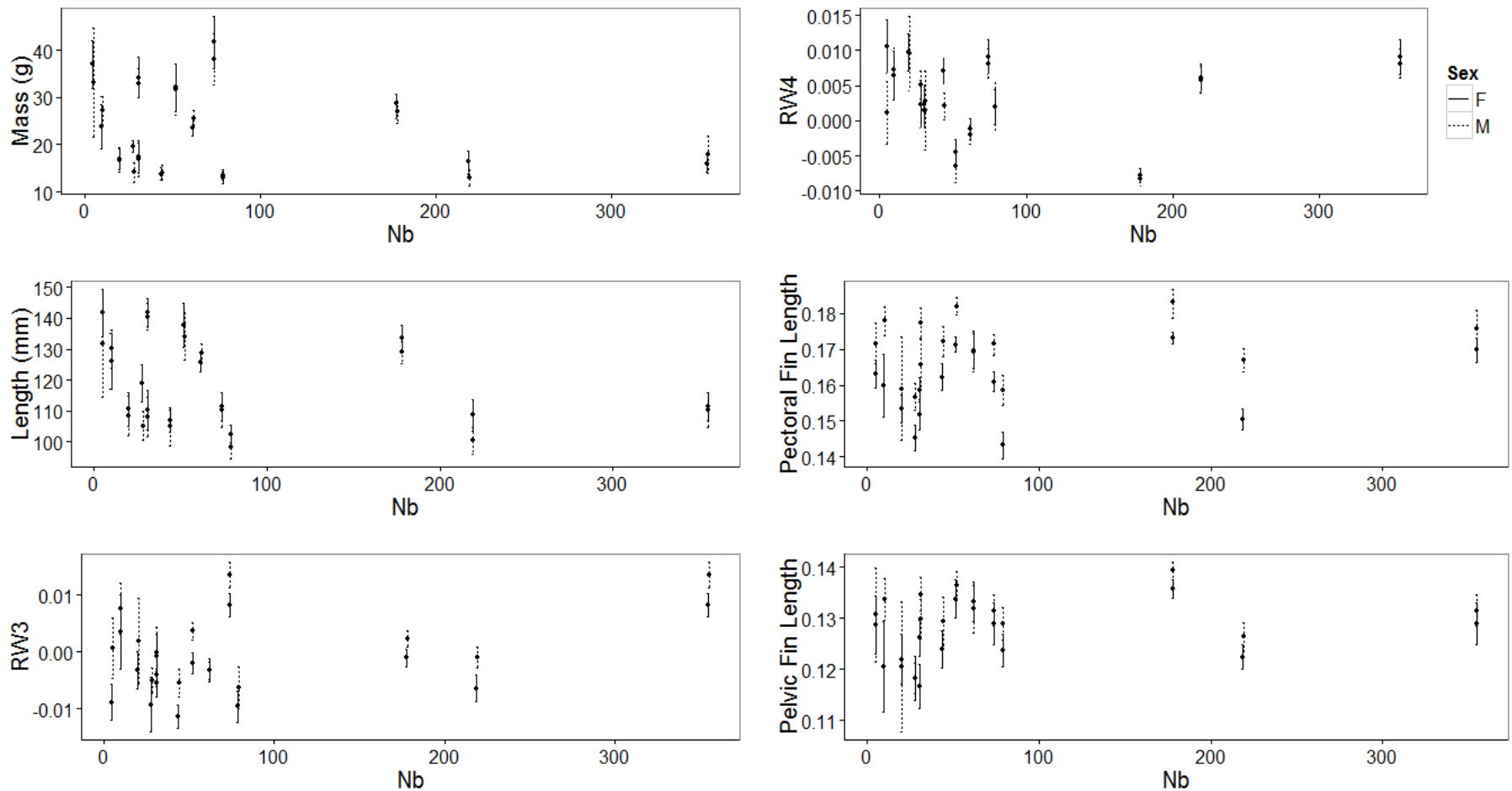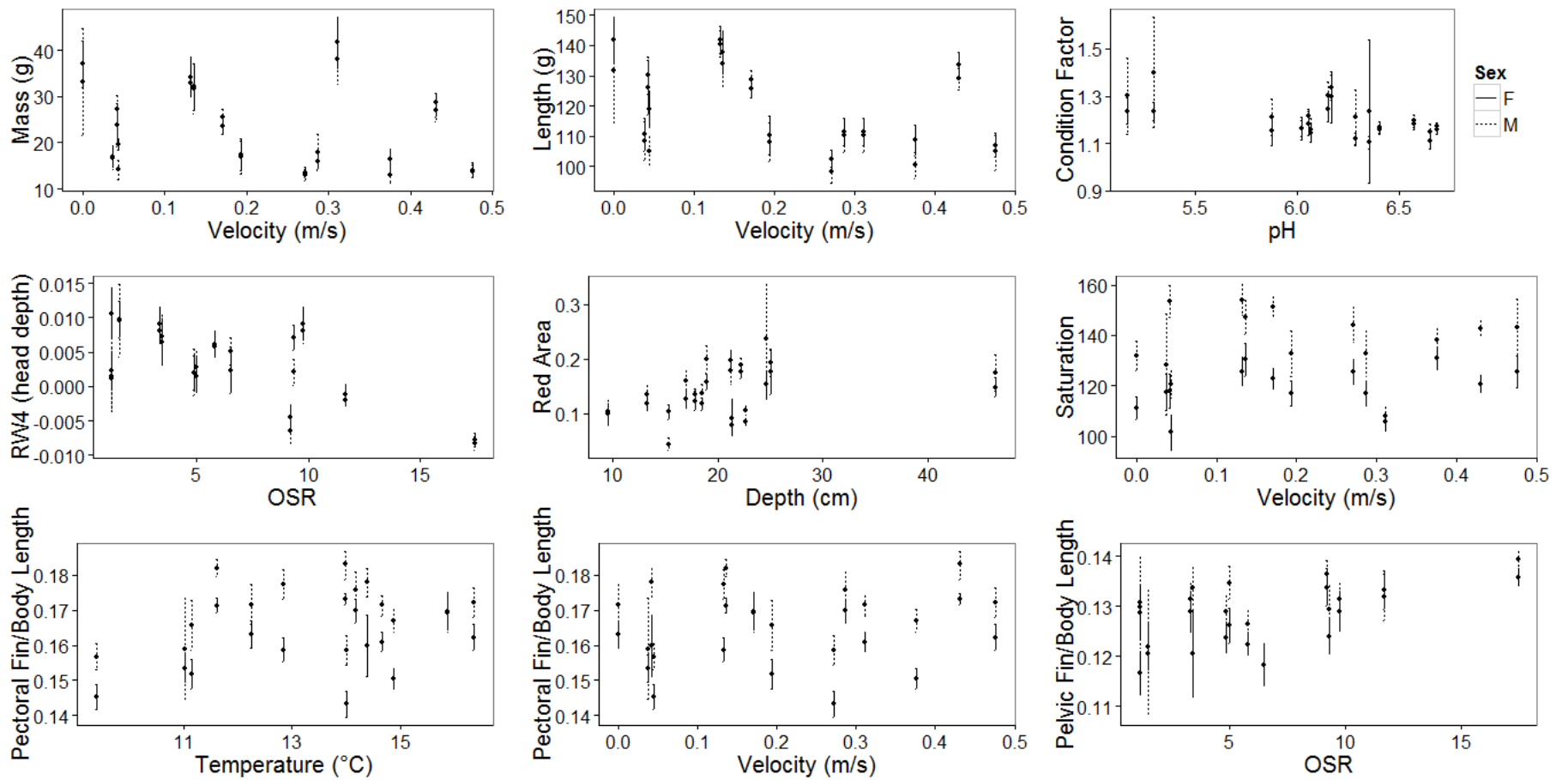

Figure S4. Examples of mean trait and habitat interactions 14 brook trout populations in Cape Race, Newfoundland, Canada. From left to right: mass across stream velocities, length across stream velocities, condition factor across stream pH, RW4 across stream

OSRs, red area/total body area across stream depths, red saturation across stream velocities, pectoral fin length/total body length across stream temperatures, pelvic fin length/total body length across stream velocities, and pelvic fin length/total body length across stream OSRs. Trait means depicted with 95% confidence intervals.

APPENDIX B

White's Tests for Heteroscedasticity

White's Test with N_b and Trait Means

Traits	Female	Female P value	Male	Male P value
Mass Mean	1.71	0.42	2.52	0.28
Length Mean	1.99	0.37	2.82	0.24
Condition Factor Mean	4.15	0.13	1.18	0.55
RW1 Mean	1.07	0.59	1.42	0.49
RW2 Mean	1.50	0.47	1.70	0.43
RW3 Mean	0.40	0.82	0.65	0.72
RW4 Mean	2.10	0.35	2.41	0.30
Red Area Mean	1.23	0.54	1.22	0.54
Saturation Mean	0.17	0.92	1.00	0.61
Spot Number Mean	0.77	0.68	0.79	0.67
Pectoral Fin Mean	4.72	0.09	1.38	0.50
Pelvic Fin Mean	2.18	0.34	0.33	0.85

White's Test with N_b and Trait CV

Traits	Female	Female P value	Male	Male P value
Mass CV	4.85	0.09	1.86	0.40
Length CV	8.85	0.01*	2.50	0.29
Condition Factor CV	3.99	0.14	0.66	0.72
RW1 CV	0.56	0.76	2.10	0.35
RW2 CV	1.29	0.53	0.52	0.77
RW3 CV	3.15	0.21	2.12	0.35
RW4 CV	1.12	0.57	1.27	0.53
Red Area CV	0.46	0.79	0.32	0.85
Saturation CV	0.23	0.89	1.64	0.44
Spot Number CV	0.39	0.82	1.96	0.38
Pectoral Fin CV	0.63	0.73	0.48	0.79
Pelvic Fin CV	0.38	0.83	0.63	0.73

APPENDIX C

Variation Tests

Linear mixed models: Mean of traits (dependent variable) vs habitat CVs

F values for those that are p<0.05. "-" for negative relationships.

Traits	pH CV	Depth CV	Velocity CV	Temp CV	Sex	N_b
Mass mean						
Length mean	4.6077	(-) 10.6450		(-) 5.7088	(-) 7.5149	
Condition mean						
RW1 mean					220.22	
RW2 mean	7.4285				43.4488	
RW3 mean					74.371	
RW4 mean	2.9193		2.9697	2.2399		
Red area mean			8.1434	10.6119		
Saturation mean	3.805				68.866	
Spot number mean					66.809	
Pectoral fin mean					12.9	
Pelvic fin mean						

Linear mixed models: CV of traits (dependent variable) vs habitat CVs

F values for those that are p<0.05. "-" for negative relationships.

Traits	pH CV	Depth CV	Velocity CV	Temp CV	Sex	N_b
Mass CV						7.6886
Length CV					5.1713	5.8261
Condition CV						
RW1 CV						
RW2 CV	10.4175		(-) 1.5805		6.1401	
RW3 CV						
RW4 CV						
Red area CV						
Saturation CV						
Spot number CV		11.7107	(-) 5.3947	(-) 0.2922		(-) 3.3884
Pectoral fin CV						
Pelvic fin CV						

As a consistent trend was not seen between habitat variation (represented by coefficient of variation; CV) and phenotypic trait means and CVs, the impact of habitat variation on results was not considered in the main body of text.