Ecology, 96(4), 2015, pp. 1010–1019

Similar plastic responses to elevated temperature among different-sized brook trout populations

Jacquelyn L. A. Wood[1,2] and Dylan J. Fraser[1,2,3]

[1]*Department of Biology, Concordia University, 7141 Sherbrooke Street West, Montreal, Quebec H4B 2E5 Canada*
[2]*Group for Interuniversity Research in Limnology and Aquatic Environment (GRIL), Université du Québec à Trois-Rivières, Trois-Rivières, Quebec G9A 5H7 Canada*

Abstract. The potential influence of population size on the magnitude of phenotypic plasticity, a key factor in adaptation to environmental change, has rarely been studied. Conventionally, small populations might exhibit consistently lower plasticity than large populations if small population habitats are generally poor in quality and if genetic diversity underpinning plasticity is lost as population size is reduced. Alternatively, small populations might exhibit (1) consistently higher plasticity as a response to the increased environmental variation that can accompany habitat fragment size reduction or (2) greater variability in plasticity, as fragmentation can increase variability in habitat types. We explored these alternatives by investigating plasticity to increasing temperature in a common garden experiment using eight fragmented populations of brook trout varying nearly 50-fold in census size (179–8416) and 10-fold in effective number of breeders (18–135). Across six early-life-history traits and three temperatures, we found almost no evidence for differences in either the magnitude or variability of plasticity in relation to population size, despite that one temperature represented an extreme climate warming scenario. The documentation of similar plastic responses of small and large populations suggests that phenotypic plasticity is not reduced as population size decreases, and that even very small populations of some species might have the ability to respond to climate change.

Key words: adaptation; brook trout; climate change; common garden experiment; effective population size; fragmentation; phenotypic plasticity; reaction norm; salmonid.

Introduction

With accelerated climate change, habitat fragmentation, and diminishing population size, the degree to which wild populations can respond to environmental change is of growing concern (Berteaux et al. 2004, Willi et al. 2006). When dispersal is not possible, populations might respond adaptively to a changing environment via adaptive evolution or phenotypic plasticity, the latter reflecting the differential phenotypic expression of the same genotype depending on the environment (De Jong 1990). Of the two possibilities, less empirical attention has been paid to the role that plasticity might play in population responses to environmental change (see Chevin et al. 2010, Crispo et al. 2010, Reed et al. 2010), especially for populations of varying size (van Kleunan et al. 2000, Paschke et al. 2003, Berg et al. 2005).

This study presents a first investigation on a vertebrate into whether a relationship exists between population size and the expression of phenotypic plasticity. We specifically tested three hypotheses that provide a useful framework for relating population size, environmental variation, and phenotypic plasticity, as expressed by reaction norms. Reaction norms illustrate the pattern and magnitude of plasticity expressed by a population, where trait values in each environment correspond to the elevation and the strength of plasticity is given by the slope (De Jong 1990). Additive genetic variance underlies reaction norms suggesting that these can evolve in response to natural selection (De Jong 1990, Schlichting and Pigliucci 1998) and differently among populations due to genetic differences resulting from drift or habitat-specific adaptations (van Kleunan et al. 2000).

A first, "directional hypothesis" (Willi and Hoffmann 2012, Wood et al. 2014) posits that small populations might consistently exhibit reduced plasticity relative to large populations. For instance, habitat fragmentation results in populations becoming smaller, more isolated and often living under conditions that reduce recruitment (e.g., Ward and Johnson 2005). Alone or in tandem, these processes might reduce genetic diversity underpinning phenotypic trait plasticity due to restricted gene flow, drift, inbreeding, and/or overall increased environmental stress (Ouborg et al. 1991).

A second, opposing directional hypothesis is that small populations might exhibit consistently greater plasticity relative to large populations. Fragmentation into smaller habitats is often associated with increasing

Manuscript received 15 July 2014; accepted 5 September 2014. Corresponding Editor: D. E. Schindler.
[3] Corresponding author.
E-mail: dylan.fraser@concordia.ca

environmental variation (Marshall and Jain 1968). Therefore, although some small populations may have low genetic variation due to drift, fragmentation might also favor high levels of plasticity at key traits to cope with environmental fluctuations (van Kleunan et al. 2000, Paschke et al. 2003). For example, some very small founder populations have been shown to exhibit rapid plastic responses to novel environments (Haugen 2000).

A third, "variable hypothesis" is that habitat characteristics and resulting natural selection pressures—and by extension, phenotypic plasticity—become increasingly variable as habitat fragment size and population size decrease. Indeed, there is evidence that smaller population fragments can be simply random samples of larger fragments (Connor and McCoy 1979, Berteaux et al. 2004). In our study species, the brook trout (*Salvelinus fontinalis*), this fragmentation process is known to increase habitat variability within and among habitats as population size decreases (Wood et al. 2014), resulting in a greater diversity of selective pressures (Fraser et al. 2014) and perhaps plastic responses.

To test between these hypotheses, we conducted a common-garden experiment to investigate plasticity of early-life-history traits to increasing temperature on eight, differentially-abundant stream brook trout populations from Cape Race (CR), Newfoundland, Canada. In fishes, temperature is crucial for controlling metabolism and other life-history traits (Beacham and Murray 1985), and we have found temperature to be highly variable both within and among CR streams (Wood et al. 2014). Furthermore, CR trout populations are experiencing climate warming as the mean annual air temperature has increased by more than one degree Celsius over the past 100 years (Environment Canada, data *available online*);[4] temperature plasticity might thus play an important role in future population persistence (Shuter and Post 1990).

Our study represents a model for the investigation of phenotypic plasticity in vertebrate populations of varying size. Data linking plasticity and population size will be important for prioritizing populations for management and conservation, specifically for salmonids, a socioeconomically important group of fish species experiencing population declines in many regions (e.g., Parrish et al. 1998) as some small populations might have the ability to respond plastically to environmental change in the short term (e.g., Haugen 2000). Finally, we tested our hypotheses using both the adult census population size (N) as well as the effective number of breeders (N_b), an analogue of effective population size but for a single breeding event (Waples et al. 2013). Past studies used N only and assumed a correspondence between N and N_b, but N_b:N ratios can vary widely among intraspecific populations (Palstra and Fraser 2012) and it is N_b that is associated with selection and its effects on the evolution of plasticity.

Materials and Methods

Study site

Cape Race is a region of coastal barren land traversed by a parallel series of low-order streams, many of which harbor resident brook trout populations. The small size of CR streams (0.27–8.10 km) permits comprehensive sampling and accurate and precise estimation of N and N_b. The populations are isolated and genetically distinct (Wood et al. 2014) and likely diverged from a common ancestor during the late-Wisconsinian glaciation (10 000–12 000 yr bp; Danzmann et al. 1998). CR populations also exhibit considerable differences in life histories likely due to changes to selective regimes following habitat fragmentation (Belmar-Lucero et al. 2012).

Gamete collection

From mid to late October 2011, eight CR populations were monitored for breeding individuals via electrofishing downstream and upstream of known spawning areas. The populations were Whale Cove (WC), Cripple Cove (CC), Watern Cove (WN), Lower Blackfly (BF), Upper Ouananiche Beck (UO), Freshwater River (FW), Ditchy (DY), and Still There By Chance (STBC) (for a map of population locations, see Wood et al. [2014]). Spawning sites were easily recognizable by dense aggregations of sexually mature trout and excavated redds. Breeding adults were gathered and placed in flow-through cages within the stream channel until gamete collection took place, between 21:00 and 02:00 of the same evening. Eggs were collected in 60-mL opaque plastic containers while sperm was collected in 1.5-mL microcentrifuge tubes on ice. Gametes were transported directly from CR to St. John's in refrigerated coolers then shipped to Montreal by air such that total transit time was approximately 10 hours from the start of gamete collection. Prior to subsequent fertilization, diameters of 10 randomly selected eggs were measured for each female using digital photographs. Gametes were transported in three separate shipments, on 19, 24, and 29 October; for most populations, all gametes were collected and transported on the same shipment date, but for three populations, (WN, STBC, and CC) gametes were shipped on two different dates.

Common garden experimental design

We investigated plasticity of six early-life-history traits (hatch time, length at hatch, yolk volume, emergence length, yolk-sac conversion efficiency, and survival) in relation to three temperature treatments; one temperature that mimicked naturally occurring temperatures during the incubation and early feeding phases for the eight CR populations (5.0° ± 0.2°C [mean ± SD]; hereafter the cold regime), a medium temperature that likely represents a climate change scenario for some

[4] www.climate.weatheroffice.gc.ca

CR populations (7.0° ± 0.3°C; medium regime), and one incubation temperature that could be experienced under more extreme climate warming in the future (9.2° ± 0.3°C; warm regime; Soloman et al. 2007; see Appendix A). Fertilization took place 10–14 hours after gamete collection, with eggs from each female being mixed with equal volumes of sperm from one randomly assigned male, yielding a total of 134 full-sib families or a mean of 18.2 families per population (range = 6–29). CR females are small in size (mean length = 138.3 ± 28.6 mm) and have low fecundity (mean number of eggs = 82.8 ± 53.9). Therefore, fertilized eggs were divided into three equal lots of 20.0 ± 8.0 (range = 3–50), which were incubated at the family level by being placed in 5.2 cm diameter individual egg containers within three 1000-L recirculating tanks. Egg containers were fitted with a mesh bottom to allow water circulation; pH was 6.9 ± 0.3 (mean ± SD) and dissolved oxygen was maintained at saturation throughout the experiment. Family placement among populations was assigned randomly within the first tank with families occupying the same location in the remaining tanks to minimize any effect of tank location on plasticity. To reduce potential mortality following fertilization, eggs were left undisturbed until they had reached the eyed stage, at which point dead individuals were counted and then removed daily.

The six early-life-history traits chosen are known to be related to individual fitness of salmonids under natural conditions (Einum and Fleming 2000). Each trait was measured and used to create reaction norms for each population over the three temperature treatments. (1) Hatch time was estimated as accumulated degree days from fertilization to hatch of all individuals within families. Once hatching began, numbers of hatched individuals in each family were counted at intervals that yielded the same number of accumulated degree days across all tanks; every 8 hours for the warm treatment, every 10 hours for the medium treatment and every 12 hours for the cold treatment. Hatch times were converted to degree days by summing the mean daily incubation temperatures over development (Beacham and Murray 1985). (2) Length at hatch (tip of the snout to the tip of the median rays of the tail; Koskinen et al. 2002) and (3) yolk-sac volume at hatch (estimated as $L \times H^2 \times [\pi/6]$, where L and H were the length and height of the yolk sac, respectively [Koskinen et al. 2002]) for each individual were measured by taking a standardized digital photograph using a mounted overhead camera. Photos were then imported into the program IMAGEJ (Rasband 2011) and traits were measured against a known size standard. Once yolk sacs had been absorbed, (4) emergence length (when the yolk sac is "buttoned-up" into the body cavity [Beacham and Murray 1985]) was measured for each individual similarly to length-at-hatch using IMAGEJ. (5) Yolk-sac conversion efficiencies ((length at yolk absorption − length at hatch)/yolk-sac volume) were then calculated using the family means in each population.

Finally, (6) we contrasted relative survival of each family for each treatment over the embryonic period (i.e., fertilization to hatch). If all individuals within a particular family died in one or more of the temperature treatments over the course of the experiment, measurements from that family were not taken for the remaining temperature treatments (with the exception of survival), but this only constituted a small number of families across all populations (3.5 ± 2.3). Our study was not designed to discern the difference between an egg that was fertilized and died during incubation vs. one that may not have been fertilized initially. Nevertheless, the proportion of unfertilized eggs was likely very low given the small family sizes and the large quantities of sperm used to ensure fertilization.

Adult census population size (N) and effective number of breeders (N_b)

Multiyear estimates of population size for each population were estimated in a previous study based on N (two consecutive years) and N_b (three consecutive cohorts except for two in DY; Wood et al. 2014). These estimates included the same year (2011) in which this study's gametes were collected (Appendix B: Table B1). Either the Schnabel (1938) or Petersen (1896) method was used to estimate annual N; as a surrogate for generational N_e, we used the harmonic mean of N_b for three cohorts (2009–2011 estimated using the linkage disequilibrium method implemented in LDNe; Waples and Do 2008) weighted by the number of individuals sampled. N_e is the genetically effective population size and is defined as the number of breeding individuals in an idealized population that would show the same variation in allele frequencies under genetic drift or the same amount of inbreeding as the population being examined. Because generational N_e calculated for the five CR populations for which detailed life history data was available was strongly correlated with the weighted harmonic mean N_b (Waples et al. 2013; see Appendix B: Fig. B1), N_b was used for all analyses. For additional details on sampling and calculating N and N_b for Cape Race streams, see Wood et al. (2014).

Statistical analysis

Population size and mean trait values.—The effects of population size (either N or N_b) and temperature as well as the interactions between these factors on five of the six life-history traits (emergence length, hatch date, hatch length, yolk volume, and survival) were examined by generalized linear mixed models (GLMMs) using the lme4 package (Bates et al. 2012) of R version 2.14.1 (R Development Core Team 2011). GLMMs were used since the analysis of survival required a nonnormal error distribution, and to model random effects. A total of five GLMMs (one for each life-history trait) were run for each of the two population size metrics. Population size and temperature were treated as fixed effects with a random effect defined by family nested within popula-

tion (only population was included as a random effect for survival). Egg size and family size were included as additional fixed effects in order to investigate potential maternal effects and because family size differed across CR populations. Data were fitted with a normal error distribution, except for survival for which a binomial distribution was specified. Yolk-sac conversion efficiency is presented as a proportion but is not readily adapted to the binomial model where the response variable is based on the outcome of a series of trials. Therefore, to model yolk-sac conversion, we used the class of beta regression models implemented in the R package betareg (Cribari-Neto and Zeileis 2010), which are often used to model variables that assume values in the standard unit interval (0, 1). Prior to analysis, yolk-sac conversion was transformed ($[y \times (n-1)+0.5]/n$, where n is the sample size; Smithson and Verkuilen 2006) since, in a few cases, yolk conversion assumed a value of 1. In a preliminary analysis, shipment date was also tested but it had a significant effect in only 1 of 18 tests across the three populations with multiple shipments (Appendix C: Table C1) and was therefore omitted from further analyses.

Directional hypothesis: magnitude of plasticity.—To determine whether small populations consistently exhibited either greater or reduced plastic responses compared to large populations, we first calculated the absolute values of the family slopes for each trait within each population between the 5–7°C, 7–9°C, and 5–9°C temperature treatments. For each family, the mean trait values at each temperature were used to calculate the slopes for the 5–7°C, 7–9°C, and 5–9°C degree temperature treatments. These family slope values were then used as response variables in GLMMs (18 GLMMs for each population size metric: six traits × three temperature treatment family slopes). In each model, N or N_b, egg size, family size and two-way interactions with N or N_b were fixed effects, and population was set as a random effect. The absolute values of the slopes were used, as the strength of plasticity is directly proportional to the reaction norm slope irrespective of direction (positive or negative).

Variable hypothesis: variability in plasticity.—To test whether plastic responses were more variable at small relative to large population sizes, we included information on both the magnitude and directionality of the within-population family slopes from the 5–7°C, 7–9°C, and 5–9°C temperature treatments. We first used the residual variance of the slopes as response variables in GLMMs in order to test for significant effects of egg size, family size, and interactions of these two covariates with N and N_b (i.e., 18 GLMMs for each population size metric, calculated as in *Directional hypothesis: magnitude of plasticity*). We then used White's tests (White 1980) to establish whether the residual variance of the slopes for each temperature treatment against N and N_b was constant or exhibited heteroscedasticity, with the prediction that there would be increased variability in the residuals with decreasing population size.

Results

Population size and mean trait values

Trait plasticity in relation to temperature (i.e., nonzero slopes of the reaction norms) was evident in many cases based on statistically significant main effects of temperature regime on life history expression, but there was a significant main effect of population size in only one of the traits examined (yolk-sac conversion efficiency; Table 1 and Appendix D) in which yolk-sac conversion decreased with increasing N and N_b. Relative to the warm temperature treatment, the cold treatment generally resulted in significantly longer hatch times, longer lengths at hatch and emergence, reduced yolk-sac volume, and increased yolk-sac conversion efficiency. The medium temperature treatment was also associated with significantly longer hatch time compared with the warm treatment, increased length at emergence and increased survival for both N and N_b models, as well as greater hatch length, but only for models using N_b (Fig. 1; Table 1 and Appendix D).

Although there were population differences in mean trait values for the different temperature treatments (Table 1, Fig. 1, and Appendix D), across all traits, we only detected significant interactions between temperature and population size in three of six models with N_b and five of six models with N. The cold-treatment × population-size interaction reduced emergence length for both N and N_b models, but significantly reduced hatch length, hatch time, and yolk-sac conversion efficiency only with N. The medium-regime × population-size interaction significantly reduced survival for both N and N_b; the same interaction increased emergence length and decreased yolk volume for N_b models, but led to reduced hatch length for models using N.

Not surprisingly for a salmonid fish, egg size was associated with significantly increased hatch length, yolk-sac volume, and length at emergence regardless of whether N or N_b was used in the model, while yolk-sac conversion decreased with increasing egg size for N and N_b. However, there was rarely a main effect of family size, and only 12 of 36 interactions involving temperature regime × family size (cold or medium treatment × family size) or population size × family size were significant across the 12 N and N_b models; these showed no consistent trend in the effect of family size on the different life history traits.

Directional hypothesis: magnitude of plasticity

There was very little indication from GLMMs that the magnitude of plasticity differed significantly in relation to population size; small populations neither exhibited consistently greater or consistently lower plasticity relative to large populations (Fig. 2a, Table 2; see Appendix E: Fig. E1 and Tables E2 and E3 for detailed results of all traits analyzed). Exceptions were for yolk-

TABLE 1. GLMM regression coefficients (with SE in parentheses) to evaluate the effect of temperature treatment, number of breeders (N_b), egg size, family size, and interactions on trait mean values for two of six early life-history traits for eight Cape Race, Newfoundland, Canada, trout populations.

Effects	Emergence length	Survival
Fixed		
Cold treatment	0.5 (0.2)**	0.2 (0.3)
Medium treatment	0.6 (0.2)***	1.6 (0.3)***
N_b	−0.01 (0.01)	−0.0009 (0.004)
Egg size	0.1 (0.05)*	0.03 (0.02)
Family size	0.2 (0.1)	−0.3 (0.1)*
Cold treatment × N_b	−0.002 (0.0007)**	−0.001 (0.001)
Medium treatment × N_b	0.002 (0.0007)**	−0.005 (0.001)***
Cold treatment × egg size	0.05 (0.008)***	−0.03 (0.01)
Medium treatment × egg size	0.05 (0.008)***	−0.07 (0.01)***
N_b × egg size	0.0001 (0.0005)*	−0.0003 (0.0002)*
Cold treatment × family size	0.2 (0.09)	0.5 (0.1)***
Medium treatment × family size	−0.2 (0.08)	0.06 (0.1)
N_b × family size	−0.003 (0.001)*	0.0009 (0.001)
Random		
Family	0.4 [0.3]	†
Stream	0.3 [0.2]	0.4 [NA]

Notes: Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in square brackets (NA, not available). Results for remaining traits and for models with N are found in Appendix E.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

† The random effect of family was not included in the GLMM model for survival. This is because survival is already measured at the family level (the proportion of surviving offspring of the total number of offspring) compared with other traits (emergence length, yolk volume, etc.) which were measured for each individual within each family.

sac conversion efficiency (3 of 36 total models) for which the population-size × family-size interaction had a significant negative effect on the slopes from 5–7°C for both population size measures and a positive effect on the 7–9°C slopes for models using N_b, and hatch time (1 of 36 models) for which N_b × family size had a negative effect on the slopes from 5–7°C. Similarly, egg size and family size had little effect on the strength of plasticity across traits; there was a main effect of egg size in only 6 of 36 total models across both population size metrics, and a main effect of family size in only 4 of 36 models (Table 2, Appendix E: Tables E2 and E3). Moreover, Spearman's correlations for the relationship between the absolute values of slopes and either population size measure were not significant for any life-history trait, except yolk-sac conversion efficiency, which showed a negative correlation with N for the slopes from the 5–7°C temperature treatments (Appendix E: Table E1).

Variable hypothesis: variability in plasticity

The degree of variability in plasticity also was not influenced by population size (N, N_b): plastic trait responses were not more variable among small populations than large populations (Fig. 2b and Appendix F: Fig. F1). The few exceptions (9 of 36 total models) were a significant interaction of N_b × family size on the 7–9°C slopes for yolk-sac conversion efficiency as well as main effects of (1) family size on the 7–9°C slopes for yolk-sac conversion efficiency and (2) egg size, which aside from one instance, had a positive effect on residual variance of family slopes for several life-history traits for both N and N_b (Table 3 and Appendix F: Tables F1 and F2). Furthermore, only 2 of 36 and 1 of 36 White's tests that examined residual variance of family slopes for each trait relative to N and N_b, respectively, had significant heteroscedasticity to signal a difference in variability of slopes in relation to population size. Examination of the residual plots showed that in only one of these significant cases was the increased residual variance at small population sizes (N: hatch date, 5–9°C slopes; Appendix F: Table F3).

DISCUSSION

We found almost no evidence for differences in phenotypic plasticity in relation to population size among Cape Race brook trout populations despite a nearly 50-fold difference in N (179–8416), and a 10-fold difference in N_b (18–135). This result is particularly notable given the large number of families and populations used in comparison to analogous vertebrate studies (Haugen and Vøllestad 2000, Jensen et al. 2008) and that one of our incubation temperatures (9°C) represented an extreme condition that would not be experienced by these populations in a natural setting (Power 1980, Appendix A). In regard to the directional hypothesis, there was no evidence that the magnitude of plasticity was related to population size. Specifically, (1) small populations did not exhibit either consistently greater or consistently reduced plasticity relative to large populations, (2) correlations between population size and the absolute values of the slopes between the three temperature regimes were not significant for almost all

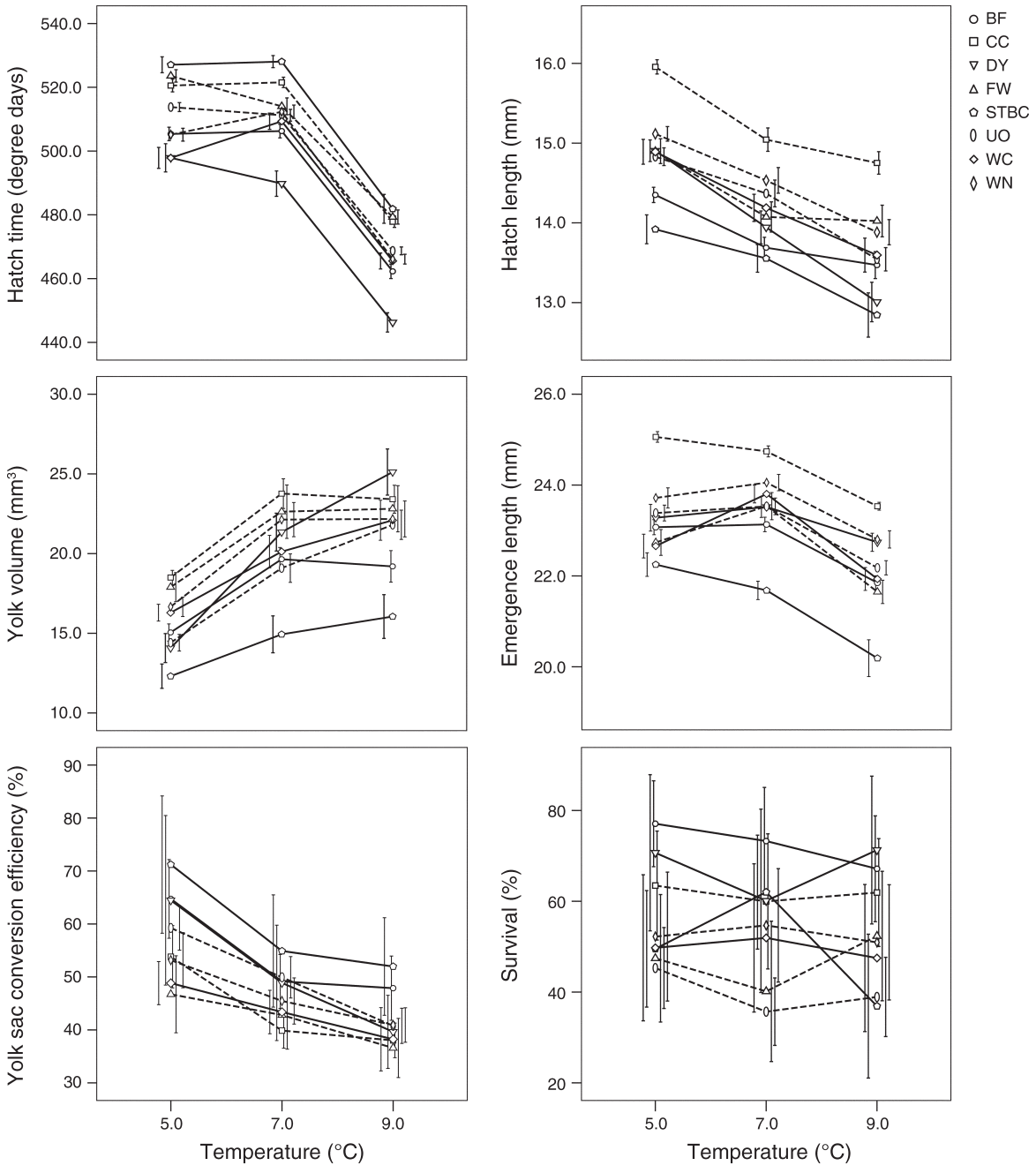

FIG. 1. Reaction norms to assess phenotypic plasticity in six early life-history traits across three different temperature treatments for eight Cape Race, Newfoundland, Canada, brook trout populations. Small Cape Race populations ($N = 179$–1731) are denoted by the solid lines, with large populations ($N = 2412$–8416) as dashed lines.

traits, and (3) only 4 of 32 total models across the six studied traits revealed a significant interaction effect with population size. Similarly, there was no evidence that small populations might express a greater variety of plastic responses than large populations (Variable Hypothesis): only 1 of 32 White's tests exhibited significant heteroscedasticity of slope residuals at small population size, there were no significant main effects of population size, and in only one instance did a significant interaction involve population size.

While there was a significant main effect of population size on mean trait values for only one of the six traits (yolk-sac conversion efficiency), significant interactions between temperature and population size were observed for about half of the comparisons, indicating differences in the way different sized populations altered

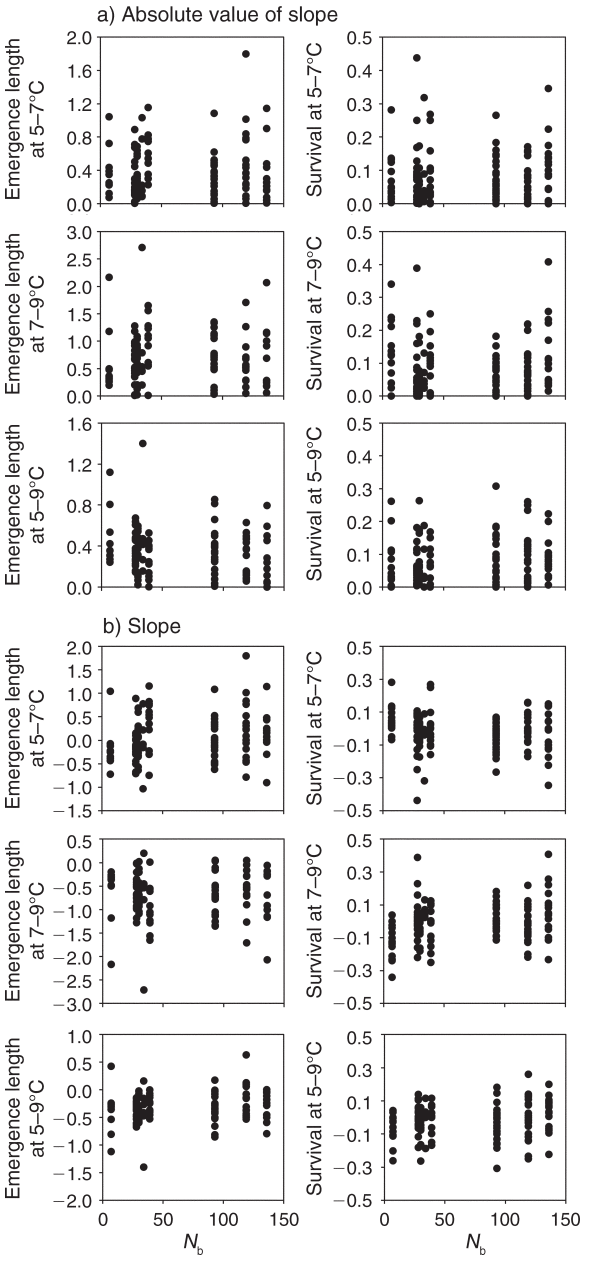

FIG. 2. (a) Under the directional hypothesis, absolute values of slopes were used to assess the magnitude of plasticity between three temperature treatments in relation to the number of breeders, N_b, and (b) under the variable hypothesis, values of slopes were used to assess the variability of plasticity between three temperature treatments in relation to N_b for two of six early life-history traits in relation to population size for eight book trout populations at Cape Race, Newfoundland. Plots for the remaining four traits, and for the six traits in relation to N are found in Appendices F and G: Figs. F1 and G1.

their mean phenotype with changing temperature. Individuals from larger populations tended to have the largest body size at early stages of development and larger yolk-sac volumes at hatch. This result is not unexpected because trout from more abundant Cape Race populations also come from larger, deeper streams where space may not be limiting, and where females from large populations also have larger egg sizes. Conversely, smaller populations had significantly higher yolk-sac conversion efficiencies according to a beta regression model, and also, on average, higher survival across all temperatures (Appendix G). Small Cape Race populations, therefore, might be capable of maintaining their fitness under suboptimal or potentially even the more extreme temperature conditions expected under future climate change.

A previous study at Cape Race found evidence for increased spatial variability in the mean and CVs of many habitat parameters at small population size, a sign that small populations might be subject to a greater diversity of selective pressures (Wood et al. 2014; see also Fraser et al. 2014). Correspondingly in this study, small populations on average exhibited greater CVs for mean trait values at 11 of 18 comparisons across the life history traits than did large populations (Appendix G). Mean plastic responses did not differ between small vs. large populations, but long term data on environmental conditions is currently unavailable for Cape Race streams. Temporal variability in environmental conditions might be generally higher among small vs. large populations at Cape Race, even though there is evidence for more variability in the types of habitats occupied by small populations spatially. Therefore, one possible explanation for the lack of difference in the plastic responses of populations of varying size is that plasticity at key traits might be favored among small populations to cope with increased temporal environmental variability (van Kleunan et al. 2000, Paschke et al. 2003), but in large populations that occupy large habitats with greater within-habitat spatial environmental heterogeneity, genotypes with varying patterns of plastic response may occur simultaneously (Sultan 1995).

We compared plasticity across populations in relation to both N and N_b. We might have expected stronger relationships with N_b vs. N since N_b represents the proportion of individuals in the population that are contributing to the next generation, and this will ultimately be dictated by the specific features of each habitat. Our research did not support this expectation, as we found very few significant relationships for plasticity with either population size measure.

Caveats

We compared plasticity among different sized populations for early life-history traits. Traits associated with adult phenology could be equally important for responding to climate variability through their effect on embryonic traits (Hebert et al. 1998, Bradshaw and Holzapfel 2008). We could not investigate this possibility due to the logistical constraints of rearing large numbers of salmonids to later life stages. That said, our study's traits are associated with fitness in salmonids at a life stage that has a critical impact on recruitment, since

TABLE 2. GLMM regression coefficients for the effect of N_b, egg size, family size, and interactions on magnitude of plasticity for 5–7, 7–9, and 5–9°C treatments for two of six early life-history traits at Cape Race.

Effects	Emergence length			Survival		
	5–7°C	7–9°C	5–9°C	5–7°C	7–9°C	5–9°C
Fixed						
N_b	0.004 (0.004)	−0.003 (0.005)	−0.004 (0.003)	−0.0006 (0.0007)	−0.001 (0.0008)	−0.0002 (0.0006)
Egg size	0.02 (0.02)	0.02 (0.02)	−0.00007 (0.01)	0.004 (0.003)	−0.002 (0.004)	−0.001 (0.003)
Family size	0.09 (0.2)	−0.2 (0.3)	−0.0007 (0.1)	−0.04 (0.04)	−0.05 (0.04)	−0.02 (0.04)
N_b × egg size	−0.0002 (0.0002)	−0.000009 (0.0002)	0.0002 (0.0001)	0.00001 (0.00004)	0.00004 (0.00004)	0.00003 (0.00003)
N_b× family size	−0.001 (0.002)	0.003 (0.003)	−0.00008 (0.001)	0.0004 (0.0004)	0.0005 (0.0004)	−0.00008 (0.0004)
Random Stream	0.09 [0.2]	0.0 [0.0]	0.04 [0.2]	0.0000002 [0.000002]	0.02 [0.2]	0.0 [0.0]

Notes: Error measurements are SE. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in square brackets. Results for remaining traits and for models with N are found in Appendix F: Tables F2 and F3.

mortality to the early fry stage is usually very high (Einum and Fleming 2000). Such traits are therefore expected to be important for the persistence of these populations.

We also acknowledge that temperature likely interacts with other habitat characters in a natural setting to generate more stressful conditions than would be experienced in most common garden laboratory experiments. In using three constant temperature treatments, for example, we did not account for potential fluctuations in temperature over the incubation period in Cape Race streams (Fig. A1). Nevertheless, this variability would be difficult to incorporate into a common garden experiment as our populations likely all experience somewhat different temperature regimes in nature. In our study, we have attempted to choose an incubation temperature that is likely experienced by all populations (5°C), one that might be high for many populations (7°C), and one that would be considered extreme for Cape Race trout (9°C, Appendix A).

One factor that might affect our overall conclusions is if contemporary population sizes are not representative of long term ones at Cape Race. Long term population size data are not available but two lines of reasoning suggest that populations have been at their current size for extended time periods. First, small populations inhabit streams that are a great deal smaller than those inhabited by large populations (Wood et al. 2014), placing an upper limit on the former's abundance. Second, neutral genetic diversity should be positively correlated with population size in isolated populations. As large Cape Race populations indeed have high levels of neutral genetic diversity, at the very least these populations may have not experienced any major historical reductions in population size.

TABLE 3. GLMM regression coefficients for the effect of N_b, egg size, family size, and interactions on variability of plasticity for 5–7°, 7–9°, and 5–9°C treatments for two of six early life-history traits at Cape Race.

Effects	Emergence length			Survival		
	5–7°C	7–9°C	5–9°C	5–7°C	7–9°C	5–9°C
Fixed						
N_b	0.006 (0.006)	0.002 (0.005)	0.004 (0.003)	−0.00001 (0.001)	0.0008 (0.001)	0.0008 (0.0009)
Egg size	0.04 (0.02)	−0.02 (0.02)	0.004 (0.01)	−0.008 (0.005)	0.01 (0.005)*	0.005 (0.004)
Family size	−0.2 (0.3)	0.2 (0.3)	−0.03 (0.2)	0.03 (0.06)	−0.03 (0.06)	−0.006 (0.05)
N_b × egg size	−0.0004 (0.0003)	0.00004 (0.0003)	−0.0002 (0.0002)	−0.00003 (0.00005)	−0.00006 (0.00006)	−0.00004 (0.00005)
N_b × family size	0.0009 (0.003)	−0.003 (0.003)	−0.001 (0.002)	−0.0005 (0.0006)	0.0002 (0.0006)	−0.0003 (0.0005)
Random Stream	0.1 [0.2]	0.0 [0.0]	0.03 [0.0]	0.03 [0.2]	0.04 [0.3]	0.01 [0.01]

Notes: Error measurements are SE. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in square brackets. Results for remaining traits and for models with N are found in Appendix G: Tables G1 and G2.

* $P < 0.05$.

The magnitude and extent of plasticity did not differ between small and large CR populations, but one important unanswered question is whether the plasticity that we observed is adaptive. Plasticity is maladaptive if it reduces fitness in a novel environment (Ghalambor et al. 2007, Crispo et al. 2010), for example, if decreasing body size at higher temperatures is maladaptive, then plasticity might actually reduce a population's ability to persist under climate change. Whether plasticity is adaptive will also depend on the reliability of environmental cues that trigger the plastic response. Data regarding the reliability of environmental cues and the fitness consequences of plasticity are unavailable for the populations in this study however, because the patterns of plastic response for most traits were similar across all population sizes, it suggests that even if plasticity in this study is not adaptive that increasing temperature at least impacted the small and large populations in a similar manner.

Finally, although our study used a larger number of populations and mean number of families per population (18.2) than most analogous studies involving fish (see Hutchings 2011) one question is whether our sample size was adequate to reject the null hypothesis of similar plasticity among populations. This is a particularly important consideration given the sensitive nature of our main finding (equal plasticity in small and large Cape Race trout populations). We calculated effect size 95% confidence intervals (CIs) for the terms in our plasticity GLMMs and found that the upper CIs are consistently low for main effects and interactions with population size (Appendix H). For this reason, our results are consistent with our conclusion that plasticity does not differ among the study populations as they are inconsistent with large effect size values for population size.

Conclusions

We found no evidence that small populations consistently differed from large populations either in the magnitude or extent of plastic responses to changing temperature regimes. This suggests that small populations may not always occur in marginal environments where they are exposed to unfavorable conditions that adversely affect their ability to respond adaptively to environmental change (Kawecki 2008).

Our results furthermore suggest that, encouragingly, some small populations may have the ability to respond to climate change even at low population sizes. For example, five of our populations have N_b of less than 50 and at least one (DY) likely also has an effective population size of less than 50. These values are frequently cited as critical minimum sizes below which populations are predicted to suffer disproportionately higher reductions in fitness owing to inbreeding depression and also experience more rapid reductions in genetic diversity required for adaptive evolution (Franklin 1980), which might include genetic variation underlying plasticity. However, some caution is warranted in applying the results of this study to other taxa. Brook trout are a colonizing species and exhibit residual tetraploidy (Allendorf and Thorgaard 1984), which might allow them to deal with small population size more effectively than other species. We certainly do not advocate that all populations that become small will be able to adapt to climate change. Nevertheless, to demonstrate similar plasticity in relation to population size in this case is important given the scarcity of such research on salmonids, a socio-economically important group of fish species, and vertebrates in general. With climate change occurring so rapidly, phenotypic plasticity rather than adaptive evolution may be the quickest way that populations will deal with future environmental change.

Acknowledgments

We thank M. Yates, S. Belmar, A. Harbicht, A. Meli, C. Desjardins, M. Bonamy, and D. Joyal for lab or field assistance, the Mistaken Point Ecological Preserve and the Department of Fisheries and Oceans, Newfoundland, for providing permits allowing this work, the Aquatron Laboratory at Dalhousie University, Halifax, for their custom designing of the infrastructure to complete this work, and J. Grant, M. Yates, A. Harbicht, K. Marin, and R. Carson for providing feedback on a draft of this paper. This research was supported by an NSERC Discovery Grant to D. Fraser and by a NSERC PGS Scholarship to J. Wood. Research undertaken in this study complies with the requirements of the Canadian Council on Animal Care (CCAC).

Literature Cited

Allendorf, F. W., and G. H. Thorgaard. 1984. Tetraploidy and evolution of salmonid fishes. Pages 1–53 *in* B. J. Turner, editor. Evolutionary genetics of fishes. Plenum Press, New York, New York, USA.

Bates, D., M. Maechler, and B. Bolker. 2012. lme4: linear mixed-effects models using S4 classes. R package version 2.14.1. http://CRAN.R-project.org/package=lme4

Beacham, T. D., and C. B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Canadian Journal of Fisheries and Aquatic Sciences 42:1755–1765.

Belmar-Lucero, S., J. L. A. Wood, S. Scott, A. B. Harbicht, J. A. Hutchings, and D. J. Fraser. 2012. Concurrent habitat and life history influences on effective/census population size ratios in stream-dwelling trout. Ecology and Evolution 2: 562–573.

Berg, H., U. Becker, and D. Matthies. 2005. Phenotypic plasticity in *Carlina vulgaris*: effects of geographical origin, population size, and population isolation. Oecologia 143: 220–231.

Berteaux, D., D. Reale, A. G. McAdam, and S. Boutin. 2004. Keeping pace with fast climate change: can Arctic life count on evolution? Integrative and Comparative Biology 44:140–151.

Bradshaw, W. E., and C. M. Holzapfel. 2008. Genetic response to rapid climate change: it's seasonal timing that matters. Molecular Ecology 17:157–166.

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

Connor, E. F., and E. D. McCoy. 1979. The statistics and biology of the species–area relationship. American Naturalist 113:791–833.

Cribari-Neto, F., and A. Zeileis. 2010. Beta regression in R. Journal of Statistical Software 34(2):1–24.

Crispo, E., J. D. DiBattista, C. Correa, X. Thibert-Plante, A. E. McKellar, A. K. Schwartz, D. Berner, L. F. De León, and

A. P. Hendry. 2010. The evolution of phenotypic plasticity in response to anthropogenic disturbance. Evolutionary Ecology Research 12:47–66.

Danzmann, R. G., R. Morgan, M. Jones, and L. Bernatchez. 1998. A major sextet of mitochondrial DNA phylogenetic assemblages extant in eastern North American brook charr (*Salvelinus fontinalis*): distribution and postglacial dispersal patterns. Canadian Journal of Zoology 76:1300–1318.

De Jong, G. 1990. Quantitative genetics of reaction norms. Journal of Evolutionary Biology 3:447–468.

Einum, S., and I. A. Fleming. 2000. Selection against late emergence and small offspring in Atlantic salmon (*Salmo salar*). Evolution 54:628–639.

Franklin, I. R. 1980. Evolutionary change in small populations. Pages 135–149 *in* M. E. Soule and B. A. Wilcox, editors. Conservation biology: an evolutionary-ecological perspective. Sinauer Associates, Sunderland, Massachusetts, USA.

Fraser, D. J., P. V. Debes, L. Bernatchez, and J. A. Hutchings. 2014. Population size, habitat fragmentation, and the nature of adaptive variation in a stream fish. Proceedings of the Royal Society B. http://dx.doi.org/10.1098/rspb.2014.0370

Ghalambor, C. K., J. K. McKay, S. P. Carroll, and D. N. Reznick. 2007. Adaptive versus non-adaptive phenotypic plasticity and the potential for contemporary adaptation in new environments. Functional Ecology 21(3):394–407.

Haugen, T. O. 2000. Early survival and growth in populations of grayling with recent common ancestors—field experiments. Journal of Fish Biology 56:1173–1191.

Haugen, T. O., and L. A. Vøllestad. 2000. Population differences in early life-history traits in grayling. Journal of Evolutionary Biology 13:897–905.

Hebert, K. P., P. L. Goddard, W. W. Smoker, and A. J. Gharrett. 1998. Quantitative genetic variation and genotype by environment interaction of embryo development rate in pink salmon (*Oncorhynchus gorbuscha*). Canadian Journal of Fisheries and Aquatic Sciences 55:2048–2057.

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

Jensen, L. F., M. M. Hansen, C. Pertoldi, G. Holdensgaard, K. L. D. Mensberg, and V. Loeschcke. 2008. Local adaptation in brown trout early life-history traits: implications for climate change adaptability. Proceeding of the Royal Society B 275:2859–2868.

Kawecki, T. J. 2008. Adaptation to marginal habitats. Annual Review of Ecology, Evolution, and Systematics 39:321–342.

Koskinen, M. T., T. O. Haugen, and C. R. Primmer. 2002. Contemporary Fisherian life-history evolution in small salmonid populations. Nature 419:826–830.

Marshall, D. R., and S. K. Jain. 1968. Phenotypic plasticity of *Avena fatua* and *A. barbata*. American Naturalist 102:457–467.

Ouborg, N. J., R. Van Treuren, and J. M. M. Van Damme. 1991. The significance of genetic erosion in the process of extinction. Oecologia 86:359–367.

Palstra, F. P., and D. J. Fraser. 2012. Effective/census population size ratio estimation: a compendium and appraisal. Ecology and Evolution 2:2357–2365.

Parrish, D. L., R. J. Behnke, S. R. Gephard, S. D. McCormick, and G. H. Reeves. 1998. Why aren't there more Atlantic salmon (*Salmo salar*)? Canadian Journal of Fisheries and Aquatic Sciences 55:281–287.

Paschke, M., G. Bernasconi, and B. Schmid. 2003. Population size and identity influence the reaction norm of the rare, endemic plant *Cochlearia bavarica* across a gradient of environmental stress. Evolution 57:496–508.

Petersen, C. G. J. 1896. The yearly immigration of young plaice into the Limfjord from the German Sea. Report of the Danish Biological Station 1895 6:1–77.

Power, G. 1980. The brook charr, *Salvelinus fontinalis*. Pages 141–203 *in* E. K. Balon, editor. Charrs: salmonid fishes of the genus *Salvelinus*. Dr. W. Junk Publishers, The Hague, The Netherlands.

R Development Core Team. 2011. R: a language and environment for statistical computing. R Foundation of Statistical Computing, Vienna, Austria. http://www.R-project.org/

Rasband, W. S. 2011. ImageJ. U.S. National Institutes of Health, Bethesda, Maryland, USA.

Reed, T. E., R. S. Waples, D. E. Schindler, J. J. Hard, and M. T. Kinnison. 2010. Phenotypic plasticity and population viability: the importance of environmental predictability. Proceedings of the Royal Society B 277:3391–3400.

Schlichting, C. D., and M. Pigliucci. 1998. Phenotypic evolution: a reaction norm perspective. Sinauer, Sunderland, Massachusetts, USA.

Schnabel, Z. E. 1938. Estimation of the total fish population in a lake. American Mathematical Monthly 45:348–352.

Shuter, B. J., and J. R. Post. 1990. Climate, population viability, and the zoogeography of temperate fishes. Transactions of the American Fisheries Society 119:314–336.

Smithson, M., and J. Verkuilen. 2006. A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables. Psychological Methods 11(1):54–71.

Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, editors. 2007. Climate change 2007: the physical science basis. Intergovernmental Panel on Climate Change Fourth Assessment Report. Cambridge University Press, Cambridge, UK.

Sultan, S. E. 1995. Phenotypic plasticity and plant adaptation. Acta Botanica Neerlandica 44:363–384.

van Kleunan, M., M. Fischer, and B. Schmid. 2000. Costs of plasticity in foraging characteristics of the clonal plant *Ranunculus reptans*. Evolution 54:1947–1955.

Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilbrium. Molecular Ecology Resources 8:753–756.

Waples, R. S., G. Luikart, J. Faulkner, and D. Tallmon. 2013. Simple life history traits explain key effective population size ratios across diverse taxa. Proceedings of the Royal Society B 280. http://dx/doi.org/10.1098/rspb.2013.1339

Ward, M., and S. D. Johnson. 2005. Pollen limitation and demographic structure in small fragmented populations of *Brunsvigia radulosa* (Amaryllidaceae). Oikos 108:253–262.

White, H. 1980. A heteroskedasticity-consistent covariance matrix estimator and a direct test for heteroskedasticity. Econometrica 48:817–838.

Willi, Y., and A. A. Hoffmann. 2012. Microgeographic adaptation linked to forest fragmentation and habitat quality in the tropical fruit fly *Drosophila birchii*. Oikos 121:1627–1637.

Willi, Y., J. Van Buskirk, and A. A. Hoffmann. 2006. Limits to the adaptive potential of small populations. Annual Review of Ecology, Evolution, and Systematics 37:433–458.

Wood, J. L. A., S. Belmar-Lucero, J. A. Hutchings, and D. J. Fraser. 2014. Relationship of habitat variability to population size in a stream fish. Ecological Applications 24:1085–1100.

Supplemental Material

Ecological Archives

Appendices A–H are available online: http://dx.doi.org/10.1890/14-1378.1.sm