Evaluating the Relative Importance of Habitat and Genetic Predictors of Fitness Correlates and the Effectiveness of Genetic Monitoring Tools for Populations Experiencing Novel Environmental Change

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Abstract

Evaluating the relative importance of habitat and genetic predictors of fitness and the effectiveness of genetic monitoring tools for populations experiencing novel environmental change

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*Determining how organisms respond, at a population-level, to novel environmental conditions is an important area of research in the rapidly changing Anthropocene. Many factors have been theorized to affect population-level responses, including the nature of the habitat change, the genetic characteristics of exposed populations, and the levels of plasticity populations exhibit across environments. Using a combination of meta-analytical techniques and empirical experimentation, my thesis examined the relative influence of genetic and environmental factors on population-level responses to novel environments. For Chapter 1, I conduct a meta-analysis using reciprocal transplants and common garden experiments in novel environments with known census population sizes (N_c) to test the effect of Nc on survival in novel environments. I found that large populations exhibited stronger local adaptation, but that this comes with potential trade-offs in novel environments. For Chapter 2, I conducted translocations of brook trout (*Salvelinus fontinalis*) to novel pond environments to test the relative importance of habitat and genetic factors (genomic diversity (H_o) and effective number of breeders (N_b)) on fitness correlates. I found that habitat overwhelmingly predicted performance, with little influence of genetic factors on performance in novel environments. For Chapter 3, I tested if phenotypic plasticity in body morphology was released in the Chapter 2 transplants and evaluated if released plasticity was correlated with H_o. I found limited evidence that phenotypic plasticity was released, and no evidence that H_o affected phenotypic diversity in novel environments. In Chapter 4 I evaluated whether N_b can be used to effectively monitor N_c in salmonids and found that, overall, it could not. Collectively, my research demonstrates that habitat is the primary predictor of fitness correlates in novel environments, with N_c, N_b, and H_o explaining little variation in performance or plasticity across studies. Our results provide*

evidence that small, low-diversity populations may often be capable of persistence and potentially adaptation, and further highlight the importance of conserving habitat. Furthermore, my research demonstrates that there is no "free lunch" – N_b cannot be used to "cost-effectively" monitor N_c in populations of conservation concern, which require actual census operations to monitor changes in N_c.

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Contributions of Authors

As primary author I contributed to the conception, planning, data collection, data analyses, and writing for all four thesis chapters. Similarly, Dr. Fraser contributed to the conception, planning, data analyses, and writing/editing of all manuscripts. Dr. E. Bowles contributed to the set-up and analysis of the genomics pipeline used in Chapters 2 and 3, and T. Bernos contributed to the data collection and editing of the Chapter 4 manuscript.

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Chapter 3 is in preparation to be submitted to a TBD journal as:
Yates, M. C., D. J. Fraser. Release of plasticity in novel environments unaffected by genomic diversity in a vertebrate.

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General Introduction

In a world experiencing rapid human-induced change, the capacity to predict vulnerability to environmental change has become an increasingly important area of study for conservation biologists. At the species level, much research has focused on understanding intrinsic biological characteristics (e.g. age at maturity, fecundity, etc.) that confer vulnerability to extrinsic environmental risks (e.g. habitat degradation or loss, climate change, etc.) (Cardillo et al. 2005, Crooks et al. 2017). Comparatively less empirical and experimental research has focused on population level predictors of the capacity to tolerate environmental change. Several frameworks have been developed to assess sources of extinction risk for natural populations focusing on either preventing habitat loss/degradation (e.g. "Habitat quality" paradigm) or on genetic, environmental, and demographic stochasticity risks associated with small population size ("conservation genetics" and "small population" paradigms) (Caughley 1994, Ouborg et al. 2006).

Habitat loss and degradation have long been identified as a leading cause of population loss (Brooks et al. 2002) and habitat is a primary determinant of population fitness in novel and natural environments (Bowman et al. 2008, Lawrence and Kaye 2011). Yet source population evolutionary history can also affect fitness in novel environmental conditions. Several studies, for example, have demonstrated that transplanted populations have higher fitness in habitats that are ecologically similar to their native environments (Raabová et al. 2007, Lawrence and Kaye 2011). Habitat heterogeneity (both across and within environments) can also buffer against severe environmental change because, by chance, at least some individuals may be adapted to conditions closer to a "novel" change. Similarly, prior exposure to variable or stressful habitats can enhance a population's response to a novel environmental stressor (Reed et al. 2003, Gonzalez et al. 2013).

Extinction risk when exposed to novel environmental conditions can also be exacerbated by small census population size (N_c) and/or low genetic diversity. Small N_c can confer vulnerability to a variety of sources of risk, including environmental and demographic stochasticity (Lande 1988, 1993) and Allee effects (Courchamp et al. 1999). However, another mechanism through which small N_c has been theorized to affect long term viability is through its

effect on effective population size (N_e) (Frankham 2005), which is correlated with, and partially a function of, the N_c of a population (Bernos and Fraser 2016).

In populations with a small N_e, natural selection can be overwhelmed by genetic drift, resulting in both a loss of heterozygosity across loci and an accumulation of deleterious alleles (Lynch and Gabriel 1990). Small populations with low genetic diversity are thus expected to exhibit reduced fitness, and early meta-analyses appeared to confirm this tend (Reed and Frankham 2003, Leimu et al. 2006). However, these meta-analyses were based on studies that were largely observational; observed trends between fitness, genetic diversity, and N_c might reflect confounding correlations between habitat quality and population size (Vergeer et al. 2003) or population size and the strength of local adaptation (Leimu and Fischer 2008). Similarly, small populations may tend to inhabit more marginal, variable, and/or stressful environments (Vergeer et al. 2003, Wood et al. 2014), which could affect subsequent performance when exposed to novel change (Gonzalez et al. 2013). Follow-up reviews have found a positive, but very weak, association between genetic diversity and fitness (Chapman et al. 2009, Rodríguez-Quilón et al. 2015, Kardos et al. 2016). Additionally, the genetic markers used in many studies, such as microsatellite loci, have historically had small marker panel sets and may not accurately represent true genome-wide genetic diversity (DeWoody and DeWoody 2005, Chapman et al. 2009). The extent to which the very weak relationship between genetic diversity and fitness can be extended to estimates of genome-wide diversity is also a major outstanding question in the literature examining heterozygosity-fitness correlations.

Finally, plasticity has an important potential effect on how populations respond to novel environmental changes. Plasticity refers to the capacity of a genotype to express different phenotypes across multiple environments, and is characterized by reaction norms (Ghalambor et al. 2007). Reaction norms are themselves under selection (Moran 1992), but importantly selection cannot constrain reaction norms for novel environments that populations have not been exposed to, leading to the potential accumulation of neutral cryptic genetic variation in reaction norms (Ghalambor et al. 2007). In novel environmental conditions, this accumulated cryptic genetic variation could result in a release of phenotypic plasticity that manifests in increased phenotypic variability in that novel environment (Schlichting 2008, Ledón-Rettig et al. 2014). While the majority of this neutral cryptic variation is likely to be non-adaptive, increased phenotypic variation could result in an increased likelihood that an individual phenotype is closer

to the "optimal" phenotype within that novel environment (Ghalambor et al. 2007). The release of plasticity may therefore have a significant role to play in how populations adapt to novel environmental conditions, particularly if released variation has a heritable component (Schlichting 2008, Mcguigan et al. 2011). Yet comparatively few studies have examined the release of plasticity in novel natural environments, and to the authors' knowledge no study has examined population-level predictors of the release of cryptic genetic variation. Most genomic diversity, for example, is also functionally neutral (Nei et al. 2010); genomic estimates of diversity may therefore predict cryptic genetic variation and resulting plastic phenotypic release. As a result small, low-diversity populations may not exhibit the same degree of released phenotypic variation relative to large, genetically diverse populations, although other mechanisms (such as epigenetic release) may help maintain variation in small populations (Willi et al. 2006).

Collectively, empirical research has demonstrated that many different factors can affect a population's response to novel environmental conditions. Yet only a few studies have sought to simultaneously test the relative importance of habitat, genetics, and plasticity. By using meta-analytical techniques combined with experimental field studies, I hope to critically evaluate the relative important of population-level predictors of fitness in novel environmental conditions. I will also critically evaluate genetic tools to monitor N_c changes in populations of conservation concern.

Chapter one involved a meta-analytical review of published literature focused on determining the effect of source population size on performance in a novel transplant environment. By collating data on i) home vs. away transplants, ii) common garden experiments in "novel" environments, and iii) reciprocal transplants involving source populations of known census size (N_c), we will determine i) if source population N_c affects performance in novel environments; ii) if source population N_c affects local adaptation and, if it does, what trade-offs are associated with performance in novel environments; and iii) if N_c is correlated with the quality of source population habitat.

For chapter two, we conducted replicated translocations of juvenile brook trout (*Salvelinus fontinalis*) to novel, previously uninhabited ponds. Translocated individuals were sourced from populations descended from a (evolutionarily) recent common ancestor (Danzmann et al. 1998) that have experienced long-term isolation (Danzmann et al. 1998, Fraser et al. 2014).

Source populations also exhibit significant variability in stream habitat characteristics (Wood et al. 2014) and vary significantly in N_c and N_e (Bernos and Fraser 2016). Translocating these populations to novel natural environments that represented a gradient of ecologically important environment variables allowed us to assess the relative effect of different potential fitness correlates (e.g. habitat, N_e, genetic diversity, etc.) in settings representing differing yet realistic degrees of environmental change and stress. Specifically, we evaluated the relative importance of genome-wide H_o and N_e, translocation pond habitat, source stream habitat variability, and the degree of habitat change represented by the novel pond on two fitness correlates (survival and growth).

For chapter three, we assessed whether plasticity was released across novel environments in the stocking experiment conducted in chapter two. We assessed reaction norms for morphological traits (body size and four morphometric relative warps) across pond environmental gradients and evaluated the effect of genome-wide heterozygosity (H_o, from 4.6k single nucleotide polymorphisms (SNPs) on phenotypic variability within novel pond environments.

Finally, for chapter four we conducted another quantitative synthesis assessing the reliability of using genetic assessments of population size (e.g. effective number of breeders (N_b)) to predict N_c, and vice-versa. Chapter one and Chapter two findings indicated little effect of genetic variables on performance in novel environments; performance was primarily associated with habitat quality. However, small and moderately sized populations may still face additional risks due to environmental and demographic stochasticity. Developing tools to cost-effectively and reliably monitor N_b or N_c is therefore crucial for conservation biology. Efforts to develop genetic tools and techniques to monitor these population parameters are just emerging from their infancy, with several studies published in the last five years adding considerable data to the scientific literature on the subject. By collating associated N_b and N_c estimates for three salmonid species, we were able to model their relationship and critically assess the capacity and reliability of the generated model to predict N_b or N_c for novel, previously unsampled population.

Chapter 1: Does source population size affect performance in new environments?

Keywords: Population size, Adaptation, Reciprocal Transplant, Conservation Biology, Natural Selection and Contemporary Evolution, Population Dynamics, Translocation, Meta-Analysis.

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Abstract

Small populations are predicted to perform poorly relative to large populations when experiencing environmental change. To explore this prediction in nature, data from reciprocal transplant, common garden, and translocation studies were compared meta-analytically. We contrasted changes in performance resulting from transplantation to new environments among individuals originating from different sized source populations from plants and salmonids. We then evaluated the effect of source population size on performance in natural common garden environments and the relationship between population size and habitat quality. In 'home-away' contrasts, large populations exhibited reduced performance in new environments. In common garden experiments, the effect of source population size on performance was inconsistent across life-history stages and environments. When transplanted to the same set of new environments, small populations either performed equally well or better than large populations, depending on life stage. Conversely, large populations outperformed small populations within native environments, but only at later life stages. Population size was not associated with habitat quality. Several factors might explain the negative association between source population size and performance in new environments:(i) stronger local adaptation in large populations; (ii) the maintenance of genetic variation in small populations; and (iii) potential environmental differences between habitats inhabited by large and small populations.

Introduction

The management of small populations remains a major focus of conservation biology. Habitat fragmentation due to ongoing anthropogenic activities has resulted in the depletion of many species, such that many now exist only as small, isolated populations. Population size is thought to be associated with risk factors that impact the capacity of populations to persist in a changing environment (Willi et al. 2006, Frankham et al. 2014). In addition to an increased risk of extinction due to demographic and environmental stochasticity (Lande 1988, Frankham 2005), reduced genetic diversity and/or the exposure of accumulated deleterious alleles at small population size could diminish the capacity of small populations to persist under environmental change (Lynch and Lande 1993, Leimu et al. 2006, Willi et al. 2006, Bowman et al. 2008, Bijlsma and Loeschcke 2012).

Previous studies of natural populations have found positive relationships between population size and fitness components (Reed 2005, Leimu et al. 2006). However, these studies were largely based on observational measurements of populations in their local environments or artificial, common garden experiments (Oakley 2013). For several reasons, the extent to which the observed increased fitness in large populations might translate into enhanced persistence under changing or novel environmental conditions remains unclear. First, reciprocal transplants have demonstrated that the strength of local adaptation is positively associated with population size (Leimu and Fischer 2008), so observational studies that measure fitness solely within native environments could be confounded by this effect. Second, some forms of local adaptation involve antagonistic-pleiotropy, wherein alleles that are favoured in a population's local environment reduce fitness in other environments (Kawecki and Ebert 2004). Under such antagonistic pleiotropy, stronger local adaptation in large populations might actually reduce performance under changing environmental conditions. Third, small populations may inhabit marginal environments (Hoffman and Blows 1994, Kawecki 2008). Observational studies comparing fitness components between large and small populations may be confounded by a systematic bias in habitat quality (Oakley 2013). Fourth, a previous history of adaptation to marginal stressful environments may enhance performance in novel environmental conditions (Reed et al. 2003, Gonzalez et al. 2013). Finally, the relatively benign conditions in artificial common garden environments may not be representative of typical stresses found in nature.

Small populations might perform poorly in novel environmental conditions due to low levels of genetic variation and an increased number of fixed deleterious mutations as a result of inbreeding (Willi et al. 2006, Oakley 2013). However, while population size is positively correlated with neutral genetic diversity (Reed and Frankham 2003), neutral genetic diversity is weakly correlated with quantitative genetic variation (Reed and Frankham 2001, Ouborg et al. 2006). Existing empirical studies in nature rarely report strong correlations between population size and quantitative genetic variation or heritability in wild populations (Willi et al. 2006). Furthermore, under some forms of selection population size may not have a significant effect on genetic variation except at extremely small sizes (Willi et al. 2006). Finally, in plants, there is evidence that the magnitude of detrimental inbreeding effects is positively associated with population size, indicating that some small populations may evolve some resistance to inbreeding depression or purge deleterious alleles (Angeloni et al. 2011).

In the absence of information tracking how populations adapt to change within their native environment over successive generations, replicated common garden translocations to novel natural environments of subsamples of individuals from varying sized source populations represent an opportunity to discern the possible effect that source population size has on performance under environmental change. Such experiments control for environmental context by transplanting multiple individuals to the same (set of) environment(s), eliminating potential confounding effects associated with observational studies. Those few studies that have attempted such translocations have yielded inconsistent results. Small populations either (i) outperformed large populations (Hooftman et al. 2003), (ii) exhibited no loss of fitness or were outperformed by larger populations only in more benign environmental conditions (Oakley 2013) or (iii) exhibited reduced performance in increasingly dissimilar environments relative to their native environment (Bowman et al. 2008). Collectively, the effect of source population size on performance under natural environmental conditions merits further investigation before general inferences can be made.

Our meta-analysis is a first attempt on multiple taxa to directly test, in nature, the prediction that larger source population size improves the performance of individuals transplanted to novel environments, while simultaneously accounting for possible confounding relationships between population size and local adaptation or habitat quality.

We specifically conducted three separate analyses. The first evaluated how the performance of individuals from source populations of known size changed in novel environments. We performed a 'home-away' contrast analysis that compared the performance of individuals within a population's native environment to the performance of individuals translocated to a novel environment. Relevant data were obtained principally from reciprocal transplant studies and translocation experiments.

The second related 'common garden' analysis was conducted on data from common garden experiments in which randomly sampled individuals from source populations of known size were transplanted to the same set of natural novel environments (this included reciprocal transplants). By doing so, this analysis controlled for any potential confounding relationships between population size and the strength of local adaptation or habitat quality on performance not accounted for in the 'home-away' contrast above.

Finally, the third 'habitat quality' analysis used data exclusively from reciprocal transplants to determine whether large populations tended to inhabit better quality environments. By comparing the survival of individuals from the same set of populations within the same set of environments, this analysis could assess whether survival across these environments was associated with the size of the populations naturally inhabiting them, while controlling for the effect of local adaptation and source population size on survival.

Materials and Methods

Quantitative review of primary literature:

We conducted keyword searches on the academic search engine ISI Web of Science™. A complete keyword search of "local* adaptation*" + "reciprocal* Transplant*" was performed, as well as for the phrases "phenotyp*" + "plastic*" + "Transplant*". References within studies were then used to obtain studies missed by keyword searches, with emphasis on other reciprocal transplants and meta-analyses.

Survival was chosen as a relative fitness component for our three analyses due to its relatively unambiguous relationship with fitness and ease of standardization across studies. Only populations for which survival data and measurements of adult census population size could be found were included in the analysis. While suitable transplant experiments were quite common in plants, few of these experiments have been conducted on vertebrates outside of salmonid fishes; all suitable vertebrate studies found were conducted on salmonids.

Many transplant studies reported survival in both native and novel ('away') environments, but lacked data on source population size, whereas others reported population size but lacked survival data. For many studies source population size data were found using other resources (journal publications, government databases, etc.), particularly for well-studied salmonids. If relevant fitness or population size data were unobtainable in the original paper or through secondary sources, primary and secondary authors were directly contacted to obtain the information. When survival and/or population size information was contained in figures, the program ImageJ (Abràmoff et al. 2005) was used to extract relevant data. Finally, if multiple years of population size data existed for a population, the harmonic mean was used.

Testing performance in new environments using Home-Away contrasts

To test how source population size affects the performance of a population in a novel environment relative to its native environment, the survival of transplanted individuals from populations of known census size was compared in 'home' and 'away' environments. Although this only compares the performance of single populations across multiple environments, it is meant to assess the capacity of individual populations to respond to new environments regardless of the performance of other populations in those environments.

The effect size of the relative proportions of surviving individuals in the home- away contrast was calculated for each population using the log-odds ratio (Lipsey and Wilson 2001), represented by the following equation:

$$ES_{LOR} = \log_e [p_{home}/(1- p_{home})] – \log_e[p_{away}/(1- p_{away})],$$

where ES_{LOR} is the log odds ratio effect size, p_{home} was the proportion of individuals surviving in their home environment, and p_{away} was the proportion of individuals surviving in the transplant environment. A positive effect size value indicates better performance in the home relative to the novel environment, a negative effect size value the converse. For any comparisons with zero survival in either the home or transplant environment, a value of 0.5 was added to these cells; conversely, 0.5 was subtracted in any environment with 100% survival (Lipsey and Wilson 2001). This particular manipulation of the data tends to create a downward bias, and at worst will provide conservative estimates of the effect size statistic (Lipsey and Wilson 2001). Comparisons involving zero survival in both environments were excluded.

A formal, mixed-effects meta-analysis was conducted using a generalized linear mixed-effects (GLMM) model with ES_{LOR} as the dependent variable in the analysis, and weighted based on inverse variance weights. As genetic variation is non-linearly related to population size (Willi et al. 2006) and the detrimental effects of inbreeding are severe only at extremely small population sizes (Jamieson and Allendorf 2012), the $\log_{10}$ of the size of the source population was included as a fixed continuous covariate. To test how performance in novel environments could be affected by life history or evolutionary characteristics, two other categorical fixed effects were included: (i) the transplanted population's taxon (salmonid or plant) and (ii) the life-history stage of the transplanted organism (embryonic/post-embryonic stage vs. a later life-history stage, e.g. germination vs. seedling transplants for plants or fry vs. fingerling/smolt releases for salmonids), as this can affect subsequent performance (Raabová et al. 2007). All interactions between fixed effects were tested.

Species, population, and transplant site were included as random effects in all models to control for issues of non-independence (pseudoreplication) arising from multiple comparisons. Many species and populations included in our study were examined at multiple life-history stages, so random effects were conditioned on life-history stage. Although study is typically included in meta-analysis as a random effect, it was omitted here because of its almost complete

correlation with species (few studies examined the same species) and because most studies examining the same species were conducted by the same researchers.

To assess the effect of source population size on performance in novel environments, a formal meta-analysis was conducted using the MCMCglmm package (Hadfield 2010) in R 3.0.2 (R Development Core Team 2017). The analysis was initiated using a full model that included all fixed and random effects. Fixed effect parameters were removed in a stepwise fashion, using Deviance Information Criterion (DIC) to evaluate model fit (Spiegelhalter et al. 2002). All random effects were retained in each model, regardless of significance. The default (weakly informative) priors were used for each run, which had a burnin phase of 100 000, a thinning interval of 20, and 500 000 iterations. Alterations to priors (e.g. V = 1, nu = 0.002) did not significantly affect model conclusions.

Testing the effect of source population size on survival in natural common garden environments

If the previous statistic (ES_{LOR}) is solely used, it is possible that one population might exhibit greater performance in all environments relative to another transplanted population but exhibit a reduced effect size (i.e. worse survival in its home environment relative to the transplant environments). That is, comparing a population's performance in transplant environments relative to its performance in its home environment does not control for a population's overall performance relative to others. We therefore also collated and analyzed the survival of individuals from multiple source populations of known size that were transplanted to novel common garden natural environments, including reciprocal transplants.

Survival was assessed in relation to possible explanatory variables as a binomial variable using a GLMM with a logit-link function. The analysis was conducted using the function *glmer* in the statistical package *lme4* (Bates et al. 2015) in R 3.0.2. The $\log_{10}$ of population size was included as a continuous fixed covariate. Life-history stage was included as a categorical fixed effect, as was a 'local vs. foreign' contrast to account for differences in survival associated with local adaptation to home environments. All possible interactions were included as fixed effects. Taxon was not included in this analysis due to a lack of common-garden experiments amongst salmonids. Species, population, and transplant environment were included as random effects conditioned on life-history stage to account for any non-independence in the data. Observation

level random effects were fitted to the model to account for issues of overdispersion (Browne et al. 2005).

Model fit was evaluated using Akaike's Information Criterion (AIC) (Akaike 1974), corrected for small sample size bias (AIC_c) (Hurvich and Tsai 1989). Model selection was first conducted by stepwise reducing random effect terms, although intercept effects were retained regardless of fit. Fixed effects terms were then stepwise removed, eliminating interaction effects first. If an interaction was significant, all relevant lower order terms were retained. Once a best fit model was obtained, Wald χ^2 tests were used to evaluate the significance of fixed effect terms and Wald Z-tests were used to evaluate the significance of pairwise contrasts between term-levels.

Testing if large populations tend to inhabit better quality environments

To assess the potential relationship between habitat quality and population size that may have confounded previous estimates of population size and fitness (Oakley 2013), a third analysis was conducted on the subset of populations involved in reciprocal transplant experiments. In reciprocal transplants, every population is transplanted to every other population's native environment. The consistent use of multiple populations across environments provided an unbiased estimate of overall survival within each environment that could control for potential confounding effects of source population size and local adaptation on performance.

To test whether large populations tended to inhabit higher-quality environments, we assessed the correlation between overall survival in environments within reciprocal transplants and the size of the populations naturally inhabiting those environments. Survival was assessed as a binomial variable using a GLMM with a logit-link function. Analysis was conducted with the function glmer in the statistical package *lme4* (Bates et al. 2015) in R 3.0.2. Both the $\log_{10}$ of the size of the source population of the transplanted populations and the $\log_{10}$ of population size of the transplant site population were included as fixed continuous covariates. Life-history stage was also included as a categorical fixed effect, as was a 'local-foreign' contrast to account for differences in survival due to local adaptations. All possible interactions, with the exception of interactions involving the size of the population inhabiting the environment and source population size or a local-foreign contrast, were included in the initial model. Species, population, and transplant environment were included as random effects conditioned on life-

history stage to account for non-independence in the data. Observation level random effects were fitted to the model to account for issues of overdispersion (Browne et al. 2005). Model selection proceeded as described for the natural common garden analysis.

Results

Summary of meta-analysis data

Our meta-analysis contained 874 estimates of survival from 111 populations ranging in population size from 9 to 100 000 individuals (median = 400), of which 102 populations were from plants and 9 from salmonids (13 total species; Table 1); no suitable studies with population size data were found for other taxa. The first 'home-away' contrast dataset comprised 88 populations of plants and salmonids (Table 1). The second 'common garden' dataset included data on 100 plant populations (including reciprocal transplants; mean number of populations per experiment = 10; Table 1). The third 'habitat quality' dataset was constructed with 53 plant populations from reciprocal transplant studies (Table 1).

Effect of population size, life-history stage, and taxa on relative performance using Home-Away contrasts

The best fit model included only source population size as a fixed effect. The inclusion of other parameters did not improve model fit (Table 2) or change the significance of fixed effects terms. Although a simpler intercept only model had a close DIC value (ΔDIC = 1.08), population size was retained as a fixed effect due to its improved fit.

Source population size had a negative effect on relative performance in novel environments. As source population size increased, transplanted populations exhibited reduced performance in novel environments relative to their native environment (P_{mcmc} = 0.020, Figure 1).

The effect of population size on overall performance in natural common garden environments

The best fit model describing overall performance in natural common garden environments included all random effects, fixed effects, and two-way interactions (AICc = 4186.69, Table 3). There was some support for the removal of an interaction between the effect of source population size and local-foreign contrast (ΔAICc = 1.41) and the effect of source population size and life-history stage (ΔAICc = 1.49). However, both subsequent models had similar weights, the further removal of terms did not improve model fit, and both interaction terms exhibited statistical significance or marginal significance, so both interactions were

retained. As in previous studies of local adaptation (Hereford et al. 2009, Fraser et al. 2011), populations exhibited significantly better performance in their native environment relative to novel environments ($\chi^2 = 10.679$, $P = 0.001$, Table 4). However, this depended on the life-history stage of the transplanted organisms ($\chi^2 = 5.756$, $P = 0.016$). Evidence was also found that the effect of source population size depended upon the life-history stage of the transplants ($\chi^2 = 3.993$, $P = 0.046$, Table 4) and whether they were transplanted to a novel environment or their native environment ($\chi^2 = 3.580$, $P = 0.058$).

At early life-history stages, transplanted organisms exhibited improved performance in native habitats relative to novel environments. We found limited evidence that this was a result of a performance cost associated with source population size exhibited only in novel environments ($Z = 1.915$, $P = 0.055$, Table 5), although this trend was only marginally different relative to the effect of source population size on performance in native environments ($Z = 1.897$, $P = 0.058$). When transplanted to their native habitat at early life-history stages, all populations, regardless of source size, performed equally well ($Z = 0.158$, $P = 0.875$, Table 5).

The effect of source population size differed for organisms transplanted at later life-history stages relative to those transplanted at earlier stages ($Z = 1.998$, $P = 0.046$). When organisms were transplanted at later life-history stages to their native environments, source population size had a positive effect on performance that was significantly different from zero ($Z = 2.274$, $P = 0.023$, Table 5). Despite this association, no evidence was found that organisms at later life-history stages exhibited local adaptation due to a significantly lower intercept value in native environments relative to earlier life-history stages ($Z = 2.399$, $P = 0.016$). Although a trend was observed that large populations exhibited local adaptation at later life-history stages, neither large nor small populations exhibited significantly different overall performance in native relative to novel environments. In novel environments, the effect of source population size on performance at later life-history stages was small and not statistically different from zero ($Z = 0.674$, $P = 0.500$, Table 5), but was only marginally different relative to its effect on performance in native environments ($Z = 1.897$, $P = 0.058$).

The life-history stage of the transplanted organisms also had a significant overall influence on performance; plants transplanted at later life-history stages exhibited improved performance ($\chi^2 = 20.355$, $P < 0.001$, Table 4).

Do large populations tend to inhabit better quality habitat?

The best fit model evaluating habitat quality contained all random effects, all fixed effects except for transplant site population size, and all subsequent two-way interactions. (AICc = 2960.24, Table 6). There was some evidence for the removal of the interaction between source population size and the local-foreign contrast (AICc of 2960.48 vs. 2961.84, Table 6). However, for similar reasons as described in the common garden analysis the more complex model was retained. There was also some evidence to support the inclusion of the transplant site population size term (ΔAIC = 0.23). However, this term was not significant and was subsequently removed.

When only reciprocal transplants were examined, the relationships between performance and source population size, life-history stage, and local adaptation remained consistent with the previous analysis or increased in strength. Populations exhibited local adaptation ($\chi^2 = 10.584$, $P = 0.001$), but this was dependent upon the life-history stage of the transplant ($\chi^2 = 5.125$, $P = 0.024$). The effect of source population size also depended upon the life-history stage of the transplants ($\chi^2 = 4.740$, $P = 0.029$) and whether they were transplanted to a novel environment or their native environment ($\chi^2 = 4.492$, $P = 0.034$).

In reciprocal transplant experiments, only early life-history stage transplants exhibited local adaptation. Similar to the previous analysis, this was a due to a negative effect of source population size on performance in novel environments at early life-history stages ($Z = 2.493$, $P = 0.013$). The effect of source population size on transplanted organisms differed between native and novel environments ($Z = 2.115$, $P = 0.035$), with source population size having no effect on performance at early life-history stages within native environments ($Z = 0.475$, $P = 0.635$).

Source population size had a positive effect on performance in native environments at later life-history stages in reciprocal transplants ($Z = 2.253$, $P = 0.0243$). However, organisms transplanted at later life-history stages exhibited no effect of population size on performance in novel environments ($Z = 0.054$, $P = 0.957$). No evidence was also found that the performance of organisms transplanted at later life-history stages differed between native and novel environments.

Discussion

Effect of source population size on performance in novel environments

In home-away contrasts, individuals from large source populations experienced greater reductions in performance in novel environments than those from smaller populations. As ES_{lor} was based on the relative performance of a population in a novel environment compared to within its native environment, we cannot discern whether the decreased performance of large populations in novel environments is a result of stronger local adaptation in their native environments (Leimu and Fischer 2008), poor overall performance in novel environments, or a combination of the two. At the very least, our results indicate that large populations experienced greater declines in fitness relative to smaller populations when exposed to novel environmental change.

By examining the performance of multiple populations in natural common gardens and reciprocal transplants, however, we were able to further clarify some aspects of the relationship between population size and performance. Common garden experiments allowed us to control for confounding effects if fitness is only examined observationally in each population's native environment (Oakley 2013) or through home-away comparisons. Similar to our home vs. away analysis, we found that large populations tended to exhibit improved performance in their native environments relative to novel environments. However, the effect of source population size on overall performance was inconsistent across life-history stages and transplant environments: in novel environments, large source population size was associated with a marginal performance cost at early life-history stages but had no effect at later life-history stages. Conversely, in native environments, large source population size had no effect on performance at early life-history stages but had a significant positive effect on performance at later life-history stages, although we found no overall evidence of local adaptation at this life-history stage. The finding that large source population size had either no effect or a negative effect on performance in novel environments runs counter to some theoretical predictions that small populations are expected to exhibit reduced performance in stressful conditions (Reed and Frankham 2003, Leimu et al. 2006, Bijlsma and Loeschcke 2012). Inbreeding, in particular, is thought to be exacerbated in stressful conditions (Fox and Reed 2010), but we found evidence that small populations either performed as well as or better than large populations when transplanted to the same set of novel natural environments.

Effect of taxa on performance in novel environments

Although comparative taxonomic data was limited to our home-away contrasts, we found no evidence that relative performance in novel environments differed between plants and salmonids. Data required for such taxonomic comparisons are still rare in the literature; despite being a well-studied species group, we found population size information for only nine salmonid transplants. Nevertheless, the extent of local adaptation in salmonids has been estimated to be similar to plants (Fraser et al. 2011), so a lack of differentiation between these two groups was not unexpected.

Is population size positively associated with habitat quality?

Previous studies examining the relationship between population size and fitness have largely relied on observational field studies (e.g. Leimu et al. 2006), which cannot account for potential differences in habitat quality and local adaptation. However, we found no evidence that overall survival differed in environments naturally harboring small or large populations. Our analysis was conducted on a subset of population data used in the common garden analysis (reciprocal transplants only). While the sample size for this analysis was the smallest of the three (only 53 populations), all other results were similar to those obtained from the analysis conducted on all common garden environments.

Potential caveats

When relating population size to genetic variation, the effective population size (N_e), not adult census population size, is the most appropriate measurement to use (Angeloni et al. 2011). Estimates of N_e were not available for any populations in our meta-analysis. Yet based on empirically estimated N_e/N ratios in nature (Frankham 1995, Palstra and Fraser 2012), we can infer that many of the small populations included in our meta-analysis had N_e well under 50 (minimum population size in our study = 9), below which populations should exhibit significantly reduced genetic variation and experience increased inbreeding (Willi et al. 2006, Frankham et al. 2014). In other words, if N_e was positively correlated with a population's

performance in new environments, survival reductions in small populations would still have been observed.

Our conclusions are also based on data from plants and salmonids; the extent to which they can be generalized to other taxa is unclear. Nevertheless, our meta-analysis included 874 estimates of survival from 111 populations across 13 species, and also covered a large range of census population sizes (between 9 and 100 000). Furthermore, the large number of populations sampled relative to the number of species may help control for variation in the response to novel environments.

Possible explanations for elevated performance of small populations

Why did we find evidence that small populations exhibited similar or better performance relative to large populations when transplanted to novel natural environments, when previous analyses based on observational studies or artificial common gardens have found significant positive relationships between source population size and fitness (e.g. Reed 2005, Leimu et al. 2006)? We propose three hypotheses. These raise a number of points meriting further discussion and empirical consideration, and they relate to: (i) the potential effect of population size on the strength of local adaptation and subsequent pleiotropic trade-offs; (ii) the maintenance of genetic variation in small populations; and (iii) other potential systemic differences in habitat between large and small populations.

Population size in relation to the strength of local adaptation

Previous research found that population size was positively associated with the strength of local adaptation (Leimu and Fischer 2008). We contend that results from our meta-analysis are consistent with this observation. In our natural common garden analysis, significant local adaptation was only exhibited at early life history stages, at which local adaptation is thought to be strong in plants (Raabová et al. 2007 and references therein). We found marginal evidence that this resulted from a negative correlation between source population size and performance in novel common garden environments. Antagonistic-pleiotropy can underlie local adaptations (Kawecki and Ebert 2004, Anderson et al. 2013), so if large populations exhibit stronger local adaptation, they may initially exhibit reduced performance in novel environmental conditions.

However, a concomitant increase in the association between population size and performance within native environments should also have been observed if the negative relationship between source population size and performance in novel environments resulted from antagonistic-pleiotropy. Instead, at early life-history stages, individuals from populations of all sizes exhibited similar performance within their native environments.

Due to the inherent design of the experiments used in the common garden analysis, our capacity to detect the effect of source population size on performance within native environments was limited relative to our capacity to detect trends in "novel" environments. The quantity of information available on the performance of a population in novel environments will exceed that available on their performance in their native environment in reciprocal transplants involving more than two populations. Additionally, due to the inclusion of non-reciprocal common garden transplants in our dataset, survival data for transplanted populations in their native environments were only available for 53 of the 100 populations analyzed, and of those only 29 populations had early life-history stage data available. Our capacity to detect benefits associated with local adaptation may have been reduced relative to our capacity to detect antagonistic-pleiotropic costs, particularly if the magnitude of those benefits is lower than the fitness costs exhibited in novel environments.

Despite these limitations, our data potentially suggest that the costs and benefits of local adaptation could be experienced during different life-history stages. Although we found no overall evidence of significant local adaptation at large source population sizes (or maladaptation at small source population sizes) during later life-history stages, we did find a statistically significant association between source population size and performance in later life-history stages that was exhibited within native environments. This finding is consistent with previous results from observational studies that found positive associations between population size, fitness, and local adaptation in wild populations in their native habitats (Reed 2005, Leimu et al. 2006, Leimu and Fischer 2008), and could suggest an improved capacity amongst large populations to locally adapt to their native environments.

Genetic variation and isolation in small populations

Small populations exhibited similar or better performance relative to large populations in novel common garden environments, providing no evidence of genetic Allee effects resulting

from reduced genetic diversity, increased inbreeding, and increased genetic load (Willi et al. 2006, Bowman et al. 2008). Although increased local adaptation in large populations and resulting antagonistic pleiotropy could account for some of this relationship, several processes might act to retain genetic variation in small natural populations, buffering them against the negative genetic effects of small population size. Purging may be more efficient in some smaller plant populations (Angeloni et al. 2011), resulting in a lower genetic load when faced with environmental change. Furthermore, gene flow may buffer some small populations against a loss of genetic diversity (Willi et al. 2006). The extent of migration in many of the study populations is relatively unknown. The potential for asymmetric gene flow between large and small populations could constrain local adaptation in small populations when selection is not strong or effective enough to eliminate non-local alleles (Ellstrand 1992) yet simultaneously alleviate the detrimental effects of inbreeding (Frankham 2005).

Systemic differences in environments between large and small populations

If large and small populations inhabit environments that vary systemically, previous observational studies examining the relationship between population size and fitness may potentially be confounded. While we did not find any association between habitat quality and population size, habitat may vary systematically between large and small populations in other ways. Habitats inhabited by small populations may tend to be more variable, for example (Wood et al. 2014), potentially resulting in an increased capacity to tolerate novel stressors (Reed et al. 2003, Gonzalez et al. 2013) or increased phenotypic plasticity that could confer tolerance to environmental change.

Conclusions and future research directions

Our meta-analysis raises important questions about the nature of commonly observed fitness trade-offs in local adaptation studies (Hereford 2009) and how they might relate to population size. Specifically, what is the magnitude of the cost of such trade-offs? Is a fitness increase in a population's native environment associated with an equal reduction in fitness in novel environments, or is it associated with a disproportionate fitness decline in novel

environments? How are the costs and benefits of fitness trade-offs distributed across life-history stages?

We found some evidence that source population size was associated with decreased performance in novel environments during life history stages at which local adaptation may be strong. However, because of limited data in the literature, we cannot presently conclude whether the performance of large populations in their native environments was compensated by increased local adaptation, although we postulate that it is likely based on related findings in previous studies (Reed 2005, Leimu et al. 2006, Leimu and Fischer 2008).

We also found no evidence for potential genetic Allee effects associated with small population size in novel environments. Under some novel selection regimes, small populations appear to cope with short-term environmental change as well as – or better than – large populations. Whether this also translates into enhanced long-term persistence is unknown: the potential for increased genetic diversity in larger populations may allow them to better adapt to novel change over subsequent generations than small populations, despite an initially larger demographic impact. Many organisms may be capable of responding to environmental change through adaptation, in which case large population size may play a significant and important role (i.e. Samani and Bell 2010). However, it is important to note that for species with long generation times, the capacity of individuals to tolerate environmental change may facilitate their persistence under novel environmental conditions.

Furthermore, the widespread distribution and/or generalist nature of most of the species in our study could affect the influence of population size on performance in new environments. Generalist species that are capable of tolerating a wide range of environments may be buffered against environmental change through phenotypic plasticity, and/or could be capable of persisting at small population sizes due to non-evolutionary responses. Conversely, specialist species that occupy narrow niches and limited geographical ranges are already vulnerable to environmental disturbance and prone to extinction (Kotiaho et al. 2005). While the small number of species in our study precluded our ability to test for the effect of common vs. rare distributions or generalist vs. specialist strategies, these may affect the relative importance of population size on performance.

Future research into the effect of source population size on the strength of local adaptation and performance in novel, natural environments should endeavor to focus on the

magnitude of trade-offs associated with local adaptation at multiple life-history stages. Additional research into the performance of subsequent generations in transplant environments could assess the long-term adaptive consequences of source population size and its effect on genetic variation, an issue of particular relevance for both the conservation of threatened species and invasive species biology (Theoharides and Dukes 2007, Frankham et al. 2014).

Reciprocal transplants represent the best research designs available to control for potential confounding effects that could influence estimates of the effect of source population size, and may also allow researchers to disentangle the magnitude of trade-offs associated with local adaptation. We would encourage future reciprocal transplant experiments to include, when possible, population size estimates.

Tables

Table 1: Summary of survival data for populations of known size transplanted to novel environments.

Species	Taxa	Populations	Transplant Type	Sub-analysis used	Life-History Stage	Total Transplants	Home vs. away[a]			References
							>	=	<	
Arabidopsis thaliana	Plant	2	Reciprocal	All	Late	8	2	-	2	Callahan and Pigliucci 2002
Hypochoeris radicata	Plant	10	Reciprocal	All	Late	34	6	15	3	Becker et al. 2008
Inula hirta	Plant	6	Reciprocal	All	Both	72	21	29	10	Raabová et al. 2011
Armeria elongate	Plant	24	Common Garden Translocation	Home vs. Away, Common Garden	Early	175	34	135	15	Seifert and Fischer 2010
Arabidopsis lyrata	Plant	8	Common Garden Translocation	Common Garden	Late	32	NA	NA	NA	Vergeer and Kunin 2013
Carlina vulgaris	Plant	23	Reciprocal	All	Both	108	17	41	22	Jakobsson and Dinnetz 2005, Becker et al. 2006
Aster amellus	Plant	12	Reciprocal	All	Both	351	48	184	29	Raabová et al. 2007, 2008
Purshia subintegra	Plant	1	Translocation	Home vs. Away	Late	4	-	-	3	Maschinski et al. 2004
Scorzonera humilis	Plant	1	Reciprocal	Home vs. Away	Early	12	1	5	5	Reckinger et al. 2010
Hypericum cumulicola	Plant	15	Common Garden Translocation	Common Garden	Late	30	NA	NA	NA	Oakley 2013
Salmo salar	Salmonid	3	Reciprocal, Translocation	Home vs. Away	Both	23	7	10	0	Ritter 1975[c], Houde et al. 2011[d]
Oncorhynchus kisutch	Salmonid	4	Translocation	Home vs. Away	Early	10	5	-	1	Bagatell et al. 1980, 1981[b], Fuss and Rasch 1981[b]
Oncorhynchus tshawytscha	Salmonid	2	Translocation	Home vs. Away	Both	15	2	1	5	Federenko and Shepherd 1986, Unwin et al. 2003

a: ‘>’ indicates statistically better performance in the home environment, ‘=’ indicates no statistical difference between performance in the ‘home’ and ‘away’ environments, and ‘<’ indicates when a population performed statistically better in the ‘away’ environment. Measurements where survival was zero in both home and away environment not included. NA refers to common garden experiments which lack a comparison in home environments, and were thus not used for the "home vs. away" meta-analysis.

b: Population size data obtained from Salmonscape, published by the Washington Department of Fisheries.

c: population size data obtained from Cameron et al. (in press) and Douglas et al. (2013)

d: population size data obtained from Gibson and Amiro (2003).

Table 2: Best fit MCMCglmm models (evaluated using Deviance Information Criteria (DIC)) predicting performance in novel environments relative to a population's native environment.

Model	DIC	ΔDIC
N	1476.218	0.0
N + LHS	1476.803	0.585
N + LHS + Taxon	1477.153	0.935
Intercept only	1477.302	1.084
N + Taxon	1477.420	1.202

*LHS refers to life-history stage, N refers to $\log_{10}$ source population size, and Taxon refers to whether the transplant was a salmonid or plant.

Table 3: The five best fit glmm models (evaluated using AIC_c) predicting overall performance in common garden experiments conducted in natural environments.

Model	AIC	AIC_c	ΔAIC	wAIC
N + LHS + Local + LHS:Local + N: LHS + N:Local	4185.9	4186.69	0	0.390
N + LHS + Local + LHS:Local + N: LHS	4187.4	4188.10	1.41	0.193
N + LHS + Local + LHS:Local + N:Local	4187.3	4188.19	1.49	0.185
Full Model	4187.7	4188.40	1.71	0.166
N + LHS + Local + LHS:Local	4189.5	4190.12	3.42	0.067

* LHS refers to life-history stage, N refers to $\log_{10}$ source population size, and Local refers to whether a population was transplanted to its native environment or a foreign environment.

Table 4: Analysis summaries of overall performance in common garden experiments performed in natural environments, and the relationship between population size and habitat quality. Survival, expressed as a binomial variable, was used as the response. Only results for the best fit models are presented.

	Overall performance		Habitat quality vs. *N*	
Predictor	χ^2	*P*-value	χ^2	*P*-value
N	0.040	0.841	0.200	0.655
LHS	20.355	< 0.001	8.157	0.004
Local	10.679	0.001	10.584	0.001
N:Local	3.580	0.058	4.492	0.034
N: LHS	3.993	0.046	4.740	0.029
LHS:Local	5.756	0.016	5.125	0.024

* LHS refers to life-history stage, N refers to $\log_{10}$ source population size, NTrans refers to the $\log_{10}$ size of the population naturally inhabiting a transplant site, and Local refers to whether a population was transplanted to its native environment or a foreign environment.

Table 5: Effect of $\log_{10}$ source population size (β) on performance in novel and native environments at different life-history stages (LHS). Units are in log-odds.

LHS and environment	Intercept	β	S.E. (β)	Z	*P*-value
Early LHS, novel	-2.999	-0.2727	0.1424	-1.915	0.055
Early LHS, native	-3.238	0.0310	0.1964	0.158	0.875
Later LHS, novel	0.383	0.0600	0.0889	0.674	0.500
Later LHS, native	-0.313	0.3578	0.1573	2.274	0.023

Table 6: The six best fit glmm models (evaluated using AIC_c) predicting the relationship between habitat quality and population size. Analysis was conducted using generalized linear mixed models in *lme4*.

Model	AIC	AIC_c	ΔAIC	wAIC
Local + LHS + N + N: LHS + N:Local + Local: LHS	2959.1	2960.24	0.0	0.339
NTrans + Local + LHS + N + N: LHS + N:Local + Local: LHS	2959.2	2960.48	0.23	0.301
NTrans + Local + LHS + N + N: LHS + Local: LHS	2960.7	2961.84	1.60	0.152
Full Model	2961.0	2962.43	2.18	0.114
NTrans + Local + LHS + N + N:Local + Local: LHS	2961.7	2962.84	2.60	0.093

* LHS refers to life-history stage, N refers to source population size, NTrans refers to the $\log_{10}$ size of the population naturally inhabiting a transplant site, and Local refers to whether a population was transplanted to its native environment or a foreign environment.

Figures

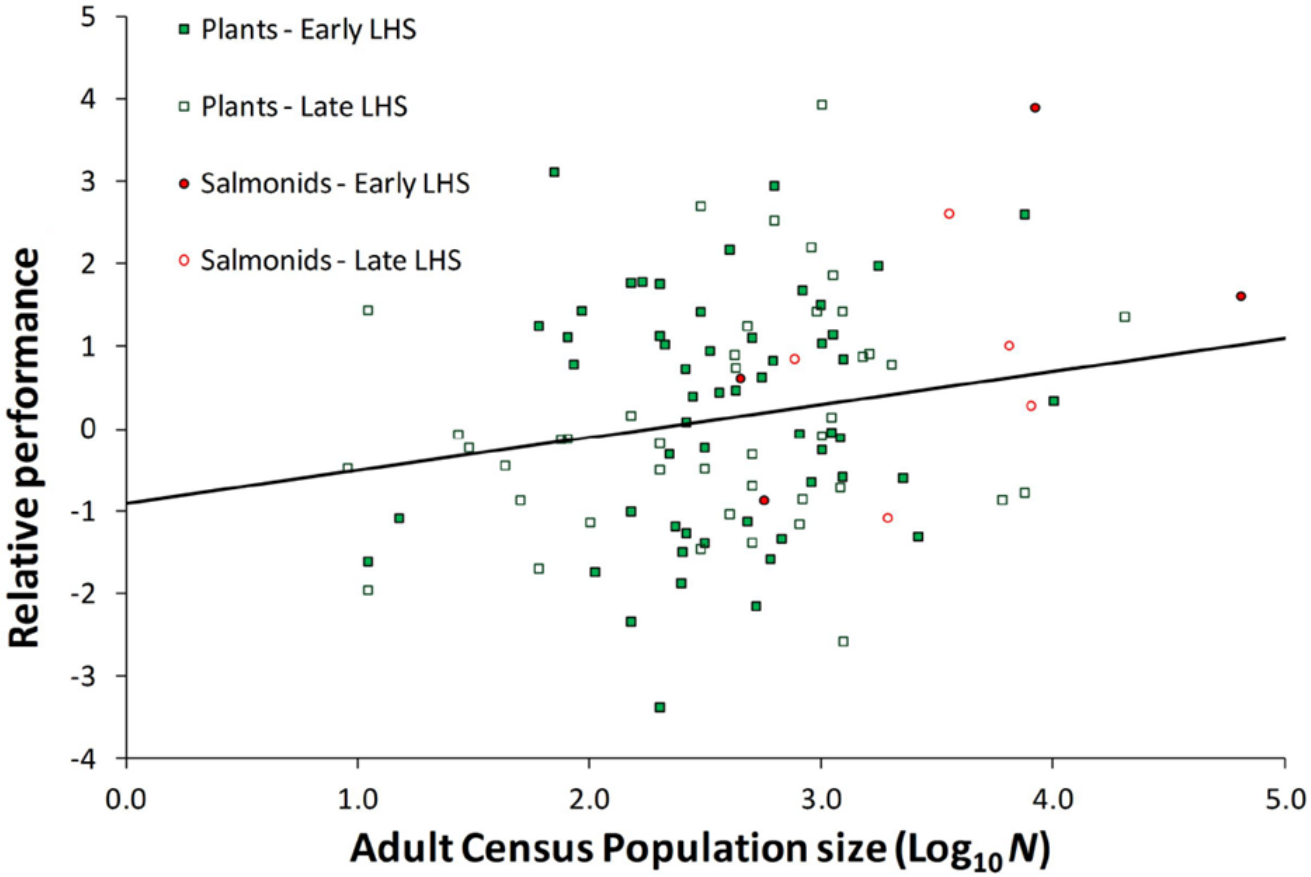

Figure 1: The effect of $\log_{10}$ census population size on the relative performance (Log-odds ratio) of a population in novel ('away') environments relative to its native environment. Solid squares = Plants, early life-history stages (LHS); Open squares = Plants, later LHS; Solid circles = Salmonids, early LHS; Open circles = Salmonids, later LHS.

Chapter 2: Experimental translocations of a vertebrate reveal the relative importance of habitat and population genetic risks to persistence under novel environmental change.

Abstract

*Little empirical work in nature has quantified the relative importance of habitat versus genetic risks (e.g. habitat degradation, low genetic diversity or small population size) to population persistence and adaptability. To test how populations vary in their response to novel environmental change, juvenile brook trout (*Salvelinus fontinalis*) from 12 isolated populations that differ in population size by orders of magnitude were transplanted to novel, fishless ponds that represent a wide gradient of ecologically important variables. We evaluated the effect of genome-wide variation, effective population size (N_e), pond habitat, pond and source population stream habitat differences, and initial body size on two fitness correlates (survival and growth). Genetic variables had little effect on either fitness correlate, which were instead determined primarily by habitat (pond temperature, depth, and pH). This suggests that some vertebrate populations with low genomic variation and Ne retain the capacity to tolerate novel environmental change despite being potentially isolated, in some cases, for thousands of years. Our results suggest that small, low-diversity populations can represent important sources of variation that may be capable of persistence and/or adaptation under novel change, and emphasize the importance of improving available habitat and slowing habitat degradation to species conservation.*

Introduction

Investigating the relative importance of sources of risk for populations of conservation concern remains an important area of research in conservation biology. Species and populations of conservation concern often face extrinsic threats (e.g. harvesting, habitat loss and degradation, etc.) that may be compounded by intrinsic characteristics that increase their vulnerability to those threats (long generation time, small population size, etc.). While much research has focused on understanding species-level intrinsic biological characteristics that confer vulnerability to extrinsic risks (Cardillo et al. 2005, Crooks et al. 2017) comparatively little empirical work in nature has comprehensively evaluated the relative importance of population-level sources of vulnerability. Intrinsic sources of extinction risk (such as long generation times, low fecundity, etc.) traditionally associated with cross-species comparisons of vulnerability do vary between populations within a species (e.g. Hutchings 1994), such traits are unlikely to exhibit levels of variation observed between species. Alternatively, the 'conservation genetics paradigm' represents a useful framework from which to evaluate between-population sources of intrinsic vulnerability; it posits that small and isolated populations, in exhibiting low effective population sizes (N_e) and reduced heterozygosity, are prone to heightened extinction risk from cumulative effects of increased genetic drift, inbreeding and resultant fitness reductions (Lynch et al. 1995, Frankham 2005, Hedrick and Garcia-Dorado 2016, Kardos et al. 2016).

Early studies of the conservation genetics paradigm found weak but positive correlations between neutral genetic diversity, population size, and fitness (Reed and Frankham 2003, Reed 2005, Leimu et al. 2006, Chapman et al. 2009). However, these studies were either observational (Oakley 2013), conducted in laboratory common garden settings (Oakley 2013), or based on limited genomic coverage (Chapman et al. 2009). Fitness differences observed between large and small populations might reflect systemic habitat differences (Vergeer et al. 2003, Oakley 2013, Yates and Fraser 2014) or stronger local adaptation in large populations (Leimu and Fischer 2008). Increased marginality, stress, and/or variability in small population environments (Vergeer et al. 2003, Wood et al. 2014) might also explain poor relative performance of small population in observational studies, yet conversely confer increased tolerance to novel change (Gonzalez et al. 2013). Experimental studies investigating how population-level genetic characteristics (N_e, heterozygosity) affect fitness in novel environments (Reed et al. 2003, Samani and Bell 2010) have also depended on model organisms over many generations in

simplified laboratory conditions that do not typify environmental heterogeneity observed in nature (Oakley 2013). Moreover, many populations of conservation concern are vertebrates with minimum generation times of several years (IUCN 2017); determining genetic correlates that predict fitness in changing environments are particularly important for vertebrate populations unable to adapt via natural selection over short evolutionary timescales.

Habitat degradation and loss represents a primary source of extrinsic risk for natural populations; the role of habitat quality in determining individual fitness and population/ species extinction is well supported (Brooks et al. 2002, Vergeer et al. 2003, Bowman et al. 2008, Lawrence and Kaye 2011). Yet characteristics of source population habitat conditions and evolutionary history may also affect fitness, population responses, and persistence in changing environments. For example, the rate of environmental change may impact performance in novel environmental conditions; translocated populations often exhibit improved performance in habitats ecologically similar to native ones (Raabová et al. 2007, Lawrence and Kaye 2011). Similarly, laboratory experiments have found that *Drosophila* and yeast populations adapted to chemically stressful environments enhance fitness when exposed to a novel stressor (Reed et al. 2003, Gonzalez et al. 2013). Across- and within-population habitat heterogeneity may therefore buffer against change by providing sources of individuals "pre-adapted" to potential future conditions (Nadeau et al. 2017).

Finally, genetics may have an interactive effect when populations are experiencing habitat loss and degradation (Ouborg et al. 2006). For example, as the ecological distance from a population's native habitat conditions increases it might exacerbate latent genetic issues such as inbreeding or accumulated genetic load (Fox and Reed 2010), and could be expected to translate into reduced fitness within low N_e or heterozygosity populations (Bowman et al. 2008).

Without the capacity to monitor long-term adaptation over multiple generations, replicated transplants in nature present an opportunity to test responses to novel environmental stressors over generationally short timespans (Oakley 2013). Furthermore, by translocating populations in natural environments the relative effect of different variables (e.g. habitat, N_e, genetic diversity, etc.) on fitness correlates can be elucidated in settings representing differing yet realistic degrees of environmental change. To this end, we conducted a large, replicated experimental translocation of a vertebrate in natural environments. Juveniles were collected from 12 naturally-fragmented brook trout (*Salvelinus fontinalis*) populations inhabiting Cape Race

(Newfoundland, Canada) and repeatedly translocated to isolated fishless ponds over four years (2012-2015) to generate 97 total translocation events. Cape Race trout represent an ideal system to test the relative importance of the two conservation paradigms because populations diverged from a common ancestor (Danzmann et al. 1998), have experienced long-term isolation (Danzmann et al. 1998, Fraser et al. 2014), exhibit significant variability in stream habitat (Wood et al. 2014), can be comprehensively sampled (Zastavniouk et al. 2017), have not been impacted by human activities (Hutchings 1993, Zastavniouk et al. 2017), and vary in N_e ranging from small to very large for vertebrates (Bernos and Fraser 2016).

We specifically evaluated the relative importance of genome-wide H_o and N_e, translocation habitat, source stream habitat variability, and the degree of habitat change represented by the novel pond on two fitness correlates (survival and growth). If genetic factors are important, fitness correlates should be determined by critical genetic variables (e.g. genetic diversity or N_e). If habitat is of primary importance, fitness correlates in novel environments should be primarily determined by key habitat variable values. If genetic factors affect fitness-correlates only in certain environmental contexts, significant interactions between genetic and habitat variables should be observed.

Materials and Methods

Study System

Brook trout are a socio-economically important salmonid species that, depending on the region, are a recreational/subsistence fishery resource, an invasive pest or of conservation concern (Korsu et al. 2007, Hudy et al. 2008). On Cape Race (46°39'31.43N, 53°04'22.27W) brook trout inhabit numerous small streams that are physically and genetically isolated from each other; most terminate over impassible water barriers on cliffs overlooking the ocean and have been isolated for thousands of years (Danzmann et al. 1998, Wood et al. 2014). Cape Race trout have no previous history of stocking and very little fishing pressure due to their small body size (100-150mm). Some population pairs exchange occasional gene flow (PN-FW; DY-UO-LO); only one population pair is accessible from the ocean (WN-BF) (Figure S1).

Translocations

Cape Race contains numerous isolated fishless ponds (commonly 20-100 m^2) that vary in habitat characteristics (e.g. temperature, pH, conductivity, etc.). In June 2012 and 2013, 36 ponds were identified which represented a gradient of ecologically important habitat parameters that could impact fitness of translocated trout (Table 1). Only ponds in watersheds uninhabited by trout populations were considered for translocations to eliminate any risk of potential escapees mixing. Ponds were prepared for translocations by identifying areas through which fish might escape during possible flood events. All potential outflows were blocked using chickenwire barriers embedded in the substrate and bank soil.

Juveniles (age 0+) from 12 populations were captured late June/early July using backpack electrofishing, conducted at random locations throughout each stream to eliminate potential non-random association of related individuals (Whiteley et al. 2012). Captured fish were transported in a backpack carrier with constant aeration and acclimated using a 50%-50% mixture of pond water and source stream water for 20 minutes. Ponds were stocked at a maximum of 2 fish/m^2; one pond was stocked at a density of 3 fish/m^2 due to an error measuring the surface area of that pond. Juveniles from small populations were translocated to fewer ponds (see Table 1) due to demographic concerns associated with over-harvesting; populations capable of demographically absorbing a larger loss of juveniles were translocated to more ponds (to a maximum of 14 across

the experimental period). Twelve pond replicates dried during drought years and were subsequently excluded from the final dataset. Only data from ponds with a minimum of two usable replicates were included in the final dataset. Cape Race populations exhibit significant behavioral differences (Wood et al. 2015) so a single population was translocated to each pond annually to avoid potential competition interactions. Populations were randomly assigned to ponds; however, due to limited pond availability no pond was stocked with fish from the same population in different years. Across four years, 2001 fish were translocated to 36 ponds over 97 translocations, with 20.6 fish per translocation event (mean density = 0.65 fish/m^2). Length measurements were obtained for all transplanted juveniles to account for potential effects of early initial growth and maternal investment on performance in novel environments (Hutchings 1991, Einum and Fleming 2004). Small tissues samples (small portion of caudal fin) were also collected for all transplanted juveniles each year.

In September, surviving trout were recaptured from ponds using a combination of electrofisher, beach seine, and/or gill nets; all ponds were fished repeatedly over multiple days until fish were no longer captured. Captured fish were euthanized using Tricaine Methanesulfonate (MS222); length, mass, and tissues samples were collected from all individuals.

Two fitness components were used to evaluate each population's performance in novel environments: survival and growth. The link between growth and fitness is more indirect than survival, but still significant for two reasons: i) in Cape Race trout, body size is linked to overwintering mortality, and ii) size-at-maturation is strongly correlated with fecundity, another trait strongly linked to fitness (Hutchings 1993, 1994). Survival was calculated based on complete recapture rates and growth was determined from length measurements taken prior to translocation and after recapture.

Habitat data collection

"Habitat quality" is often vaguely defined in the scientific literature, yet can importantly describe two different perspectives: that of the individual (the effect of habitat on fitness), and that of the population (the effect of habitat on carrying capacity) (Pidgeon et al. 2006). Although population-level considerations are of primary concern to conservationists, the transplant pond environments used were unable to sustain reproductive populations – our efforts to quantify

habitat quality therefore focused on the effect of habitat on fitness correlates in translocated individuals. We measured non-temperature habitat characteristics three times annually in all ponds: two separate occasions prior to fish translocation, and once immediately prior to fish removal (see Appendix 1 for details). Habitat data were also collected from 9-61 transects distributed uniformly across each source population stream, depending on stream length. Stream and pond temperatures were recorded every 90 minutes for the duration of the translocation period using waterproofed iButton^{TM} data loggers, one placed in each pond and two loggers (at separate locations) in each stream. The number of stream transects sampled occasionally differed between years; across-year stream means for all habitat variables were therefore calculated by bootstrap sampling values such that all years were weighted equally in final mean estimates.

Genomic diversity and effective number of breeders

Whole-genome estimates of observed heterozygosity (H_o) were obtained using genotype-by-sequencing (GBS) conducted on a random subset of individuals from each transplanted trout population. Tissues samples were extracted using a modified Qiagen^{TM} DNeasy blood and tissue protocol. DNA quality and quantity were assayed using agarose gel electrophoresis and Qubit® dsDNA BR Assay Kit with a Qubit® Fluorometer. DNA concentration was normalized to 10ng/ul, with 10ul per sample (100ng DNA total). Library preparation and sequencing was performed on an Ion Torrent Proton Platform (IBIS, Laval University, Quebec, CA) following the protocol developed in Mascher et al 2013 (using enzymes *Pst*I and *Msp*I) as described in Perrault-Payette et al (2017).

Raw sequencing quality was assessed using FastQC (Andrews 2010) v. 0.11.4, and adapters were trimmed using cutadapt (Martin 2011); SNP filtering and discovery was conducted using the *de novo* assembly pipeline in *Stacks* v. 1.44 (Catchen et al. 2013). GBS was performed on 14 populations in total, but results for only the twelve populations used in this experiment are presented herein. *process_radtags* was used to demultiplex and filter reads based on quality; reads were trimmed to 80 base pairs to remove bases with low-quality scores on the 3' end. *ustacks* was then used to form loci, with the following parameters: a minimum stack depth (-m) of 5, a maximum distance allowed between stacks (-M) of 5, and a maximum distance allowed to align secondary reads (-N) of 7. The maximum number of mismatches allowed between sample tags when generating the catalogue (-n) in *cstacks* was 5. Individuals were then aligned to the

catalog using the *sstacks* module, and the *rxstacks* module was used to remove loci with a log-likelihood less than -30. The *populations* module was then used to export genotypes, with the minimum percentage of individuals in a population required to process a locus for that population ("r") set to 0.8 and the minimum number of populations a locus must be present in order to process a locus ("p") set to 11 (of 14).

Downstream filtering was conducted in the *radiator* package (Gosselin 2017) in R v. 3.3.3 (R Development Core Team 2017). Brook trout are residual tetraploids (Crete-Lafreniere et al. 2012); SNP identification is complicated by the occurrence of paralogues in such polyploid genetic codes (Paris et al. 2017). To remove potential paralogues, loci with more than 4 SNPs were removed; only the first SNP was used for all remaining loci with multiple SNPs. A strict H_o filtering criterion was also employed; loci with H_o greater than 0.5 in any sampled population were excluded. SNPs with a minor allele frequency (<0.01) were similarly excluded to remove potential sequencing errors and rare alleles. Individuals missing more than 40% of genotypes across all filtered loci were also removed. Genomic data were not mapped directly to a genome, so estimates of multi-locus H_o were used to represent an indirect measure of levels of individual inbreeding (Kardos et al. 2016). N_e for each translocated population was estimated from GBS samples obtained using age 0+ juveniles from a single cohort (either 2014 or 2015) and therefore reflect an estimate of the cohort effective number of breeders (N_b), rather than N_e, because genetic samples originated from a discrete age class (Waples and Do 2010). Point estimates of N_b were obtained using the linkage-disequilibrium (LD) method as implemented in LDNe (Waples and Do 2008) and corrected for bias associated with LD in large genomic datasets (Waples et al. 2016). Although confidence intervals are unreliable for genomic estimates of N_b (Waples et al. 2016), our estimates are broadly consistent with previous N_b estimates obtained for our study populations using microsatellite data, with an estimated Pearson's correlation coefficient of 0.819 (Figure S2).

Statistical Analysis

Survival

Survival across ponds was analyzed using generalized linear mixed-effects models with a binomial distribution (logit-link function). To avoid overfitting, survival trends were first modeled with a large suite of habitat variables; any variable that significantly impacted survival was subsequently interacted with genetic/phenotypic variables. Pond pH, mean temperature,

dissolved oxygen, conductivity, depth, percent silt substrate, percent aquatic vegetative cover, and (ln-transformed) initial density were included as fixed (mean-centered) continuous variables; year-of-translocation was included as a fixed categorical variable. Preliminary analyses found that survival exhibited a non-linear relationship with pond pH, so a second order polynomial for pH was also included. Source population and pond location were included as random effects to account for issues of non-independence across translocation replicates. Survival data also displayed signs of over-dispersion; an observation level random-effect was therefore added, significantly improving model fit (likelihood ratio test (LRT); $\chi^2 = 54.797$, $p < 0.001$) (Browne et al. 2005). After significant habitat variables were identified source population observed H_e, (ln-transformed) source population N_b, and mean initial body size were added as fixed continuous covariates. Two-way interactions were also included between remaining habitat and genetic/phenotypic variables.

Analysis was conducted using the glmer function from the package lme4 (Bates et al. 2015) in R 3.3.3 (R Development Core Team 2017). Backwards model selection was performed using LRTs to stepwise remove non-significant fixed-effects terms ($p > 0.05$), testing higher order terms first. Source population and pond were retained regardless of significance. The significance of pairwise contrasts among levels of different predictor variables were tested using *t* tests, with degrees of freedom calculations based on the number of pond translocations and *p*-values Bonferroni-corrected to adjust for type-1 error rates.

When translocated to a novel pond, the degree of habitat change from a source population's stream habitat might also influence performance. Two additional series of models were run in which habitat variables were expressed as the difference between pond habitat and a population's stream habitat. First, habitat variable differences were measured as the value of the pond habitat (pond_hab) subtracted from source population stream habitat (sourcepop_hab). Second, the degree of habitat differentiation between pond_hab and sourcepop_hab was calculated as:

SD_{unit} = (pond_hab mean – sourcepop_hab mean)/Standard deviation of sourcepop_hab

Further dividing the mean difference between pond_hab and sourcepop_hab by the standard deviation of sourcepop_hab additionally accounts for differences in habitat fluctuations source populations experience.

Survival data analysis and backwards model selection proceeded as described above. The two final best-fit models (after model selection) obtained using the pond/stream habitat differentiation data were then compared to the best-fit model obtained using exclusively pond habitat data. Akaike's Information Criterion (AIC) (Akaike 1974) was used to determine which habitat datasets (pond habitat vs. degrees of change) best described patterns of survival.

Growth

General linear mixed-effects models were used to determine the effect of population-level H_o, N_b and translocation habitat on growth. Initial and final body length were used to estimate growth rate. Similar to survival, habitat variables significantly associated with growth were first identified; pond pH, mean temperature, dissolved oxygen, conductivity, depth, percent silt substrate, percent aquatic vegetative cover, and (ln-transformed) initial and final density were included as fixed mean-centered continuous covariates; year-of-translocation was included as a fixed categorical covariate. Source population and pond were included as random effects. Time was included as a fixed continuous effect and interacted with all other fixed-effect variables; the slope of size-over-time represented overall growth rate and interactions between time and other variables represented their effect on growth. Preliminary analysis indicated that growth exhibited a non-linear relationship with pond temperature, so a second order polynomial for temperature (and its interaction with time) was included. Source-population-by-time and pond-location-by-time random terms were also included. After significant habitat variables were identified, source population observed H_o and ln-transformed source population N_b were included as fixed continuous covariates. Three-way interactions between time, habitat variables, and genetic variables were also included, except for three-way interactions between genetic variables, time, and the second-order polynomial for temperature. Exploratory analysis indicated that inclusion of a three-way interaction between H_o, time, and the second-order polynomial for temperature fitted a biologically impossible relationship between growth and temperature at lower H_o. A negative (approximately) parabolic relationship between temperature and growth has been well-established in salmonids, with growth peaking at "optimal" temperatures typically around 12-20°C (McCormick et al. 1972, Jonsson et al. 2001). However, the fitting of this term led to an inverse of this relationship at lower H_o: growth reached a minimum at moderate temperatures

and increased at extreme temperature values. This is indicative of an overfitted model and likely due to a relatively small amount of data from populations with low H_o.

Data analysis was conducted using the lmer function in the package lme4 (Bates et al. 2015) in R 3.3.3 (R Development Core Team 2017). Backwards model selection was conducted under maximum-likelihood using Wald *F* tests to remove nonsignificant fixed-effects terms ($p > 0.05$), testing higher order terms first. Denominator degrees of freedom estimates were obtained with the Kenward-Roger method (Kenward and Roger 1997) using the R package *pbkrtest* (Halekoh and Hojsgaard 2014). Pairwise contrast significance levels were evaluated using *t*-tests, with *p*-values adjusted as for generalized linear mixed-effects models.

Two additional series of models were run to evaluate the relative importance of the degree of difference a novel habitat represents relative to the habitat of the source population. Data analysis and final model comparisons were conducted as described for the survival analysis.

Results

Genomic diversity and effective number of breeders

We sequenced 327 individuals, with 58,126 SNPs (30,292 loci) identified after *stacks* processing; 4,614 SNPs were retained after further filtering for quality, putative paralogues, etc., (see Table S1 for filtering details). Prior to filtering in *radiator*, 44 individuals were removed due to missing >40% of genotypes, with 14 to 30 individuals remaining per population (283 total, see Table 1). Mean H_o values ranged from 0.016 to 0.119 (mean = 0.072); N_b estimates ranged from 25 to 608 (mean = 188) (Table 2).

Survival

Fish exhibited zero survival in all stocking events for nine ponds, establishing the baseline habitat conditions that Cape Race trout can tolerate. In remaining ponds, survival varied from 2.4% to 100% (mean 46%). Five habitat variables impacted trout survival in translocated ponds after initial model selection and were carried forward to further analyses (Table S2): year, pH, a second order polynomial term for pH, temperature, and percent aquatic vegetation cover.

After incorporating genetic/demographic variables, the best-fit model for survival included mean initial size at translocation, pH, a second order polynomial term for pH, temperature, and an interaction term between mean initial size and pH (Table 3). Neither the base effect of genetic variables (H_o and N_b) nor their interactions with environmental variables had an effect on survival. Temperature had a negative effect on survival; as temperature increased, survival decreased (Figure 1a). Survival exhibited a quadratic relationship with pH (Figure 2a). Depending on size at translocation, fish exhibited "maximal" survival (i.e. the pH at which survival was highest) at a pH of between 5.55 and 6.20. Larger fish were more capable of exploiting all habitats, exhibiting survival over a broader pH range and improved maximal survival outcomes relative to smaller fish ($t_{27} = 2.188$, $p = 0.038$).

The best-fit models that incorporated habitat difference data yielded similar results, with several minor differences (Tables S3 and S4). Despite similar final models, pond habitat predicted patterns of survival much better than habitat differentiation measures. AIC for models that used only pond habitat data (AIC = 463.5) indicated better fit relative to models using source population stream/pond habitat differences or habitat differences standardized by stream habitat

variability (AIC = 480.6 and 482.2, respectively). Notably, inclusion of two-way interactions involving the pH polynomial term led to model convergence errors when habitat differences were divided by native stream habitat standard deviation – these terms were dropped from model testing.

Growth

Five habitat variables and time had a significant effect on growth (i.e. significant interaction with time) after initial model selection (Table S5) and were carried forward to further analyses: year, temperature, a second-order polynomial term for temperature, depth, and initial density.

After incorporating genetic/demographic variables, the best-fit model for growth included a three-way interaction (and relevant lower-order terms) between time, H_o, and depth (Table 4). The final growth model also included two-way interactions between time and the second order polynomial for temperature, time and year of translocation, and time and initial density.

The effect of depth on growth was moderated by a weak effect of H_o (Figure 2b). Growth generally decreased with decreasing pond depth across all H_o levels; however, the magnitude of change in growth was greater for low H_o populations (H_o of 0.118 at depths of 5.42 vs 46.6, t_{315}= 2.73, p = 0.007; H_o of 0.016 at depths of 5.42 vs 46.6, t_{859}= 5.12, p = <0.001). However, the difference in growth across habitats for high and low diversity populations did not translate into significantly different growth within shallow (H_o of 0.118 vs. 0.016, $t_{52.8}$= 1.72, p = 0.079) or deep habitats ($t_{35.1}$ = 1.72, p = 0.094).

Growth exhibited a non-linear relationship with temperature; fish grew optimally in ponds with a mean temperature of 11°C, with growth rate decreasing as the temperature deviated from that optimum (Figure 1b). Growth differed between years, with fish growing faster in the final year of translocations (Table S6). Growth also exhibited signs of density dependence, declining with initial stocking density (Figure 1c). N_b had no discernable effect on growth rate.

Similar to the survival analysis, pond habitat predicted patterns of survival much better than habitat differentiation measures. Best-fit models that incorporated habitat difference data yielded similar results (Table S7 and S8). However, AIC values for the growth models that only incorporated pond habitat (AIC = 13934) indicated substantially better model fit relative to

models using stream/pond habitat differences or those differences standardized by stream habitat variability (AIC = 13953 and 13962, respectively).

Discussion

With 97 translocations of 12 different source populations across 36 unique environments, this study represents to our knowledge the largest replicated experimental translocation of a vertebrate. By integrating source population stream habitat data, translocation pond habitat data and genetic metrics, we were able to test the relative influence of genetic and habitat factors on fitness correlates in novel environments. Performance was primarily determined by the habitat characteristics of the translocation environment, with genetic variables explaining no variation in survival and little variation in growth.

Our study also represents one of the largest attempts to examine how genomic variation affects individual fitness in novel environments, with H_o estimates derived over 4.6k identified SNP markers located across the brook trout genome. Notably, we included populations exhibiting a ten-fold difference in genomic H_o, including a population with extremely low levels of polymorphism (i.e. $H_o < 0.017$). Many of the geographically isolated low-H_o populations likely possess fixed alleles due to descent from a shared common ancestor. Their correspondingly low N_b estimates also indicated vulnerability to the effects of genetic drift over time, yet H_o or N_b had little effect on the two fitness correlates examined. Despite using genome-wide markers derived from panel sets that were orders of magnitude larger (although note Rodríguez-Quilón et al. 2015), our results are consistent with other translocation experiments conducted in natural environments that have found little effect of population size or genetic diversity on subsequent performance in non-vertebrate populations (Hooftman et al. 2003, Lawrence and Kaye 2011, Yates and Fraser 2014, Rodríguez-Quilón et al. 2015, but see Bowman et al. 2008, Oakley 2013). Holistically, these results are inconsistent with the conservation genetics paradigm (Reed and Frankham 2003, Ouborg et al. 2006). Several explanations could account for the general lack of relationship between genetic variables and the fitness correlates examined.

Empirical evidence suggests that some small populations with low heterozygosity can respond effectively to selective pressures (Robinson et al. 2016, Benazzo et al. 2017). Natural selection acts on quantitative genetic variation (i.e. heritability) rather than genetic diversity per se; population size might have little effect on heritability estimates in natural populations until extremely small N_e (i.e. < 10) (Willi et al. 2006, Wood et al. 2016). None of the translocated trout populations reached that threshold, although several were below traditional minimum

viable N_e (e.g. 50/500, see Jamieson and Allendorf 2012). Stochasticity due to spatial and/or temporal environmental variability can also maintain selectively important additive genetic variation within populations (Huang et al. 2015). As population size decreases, populations can be subjected to increasingly variable and divergent selective pressures, including potential increases in balancing selection (Fraser et al. 2014) that can maintain polymorphism at selectively important loci (Bernatchez 2016). Natural selection can also favor the reproduction and recruitment of more heterozygous individuals, preserving genetic diversity in populations threatened with inbreeding (Bensch et al. 2006a). Some of our study populations have likely persisted at an (evolutionary) small N_e for thousands of years, yet exhibit fine-scale genetic and phenotypic differentiation suggestive of local adaptation (Hutchings 1993, Wood et al. 2014, 2015, Zastavniouk et al. 2017). Small populations or populations with low genetic-marker diversity may therefore represent reservoirs of important selective variation adapted to local environments that are capable of long-term persistence (Willi et al. 2007, Lawrence and Kaye 2011).

Purging and gene flow could have alleviated negative effects of small population size or low genetic diversity in some of the populations. No evidence for heterosis was observed when examining critical thermal maxima traits in a subset of these trout populations, including for hybrids of the most genetically depauperate (STBC) and 2nd-most diverse population (Freshwater River) (Wells et al. 2016). Purging occurs most effectively over long timeframes (Hedrick and Garcia-Dorado 2016) and could be more efficient in small populations (Angeloni et al. 2011); several of the study populations have likely been small and isolated for thousands of years. Isolated populations that have faced evolutionarily recent reductions in population size may be affected more by inbreeding (García-Dorado 2015). Similarly, low levels of immigration can alleviate inbreeding and increase genetic diversity (Vila et al. 2003, Willi et al. 2006). Seven of the study populations inhabit a meta-population structure with at least one other population and may have avoided long-term effects of inbreeding due to occasional immigration.

Overall, pond habitat was the primary driver of performance in the fitness correlates examined. While pH strongly affected survival, it had little effect upon growth. Conversely, temperature elicited an effect on both survival and growth. As a poikilothermic fish species adapted to cold headwater streams, brook trout metabolic activity, growth, and survival are inherently dependent upon external temperature (Baldigo and Lawrence 2000, Xu et al. 2010).

Yearly variation in growth rate (but not survival) was also detected, likely due to within-year weather patterns shared across ponds.

Despite significant variation among both source population habitat and transplant sites, adaptation to a population-specific suite of habitat conditions was not an important determinant of fitness correlate performance in novel environments. While geographic distance between transplant environment and source population habitat can affect transplant performance (Becker et al. 2006) it is typically used as a proxy for ecological distance (Raabová et al. 2007). Our transplant experiment occurred at a small geographic scale (up to 11.6 km), yet the novel pond environments represented a wide ecological gradient. Cape Race stream habitats were also highly variable across stream environments (Wood et al. 2014); novel pond environments therefore represented disparate levels of ecological distance for different populations (see Table), yet no major effect of habitat differentiation was detected. Even when the ecological similarity of transplant habitats was important in other transplant experiments, quality of the novel habitat is often the single-most important factor explaining fitness across environments (Vergeer et al. 2003, Bowman et al. 2008).

Our data also provided little evidence that genetic variables and pond habitat had an interactive effect on fitness correlates. We found no evidence that levels of genetic diversity affected survival only in more stressful low-pH ponds. Similarly, while high-H_o populations exhibited more consistent growth across varying depths, growth rates in high- H_o populations were only marginally greater in shallow habitats and were actually marginally lower in deep habitats. While previous laboratory experiments have found that the effects of inbreeding can be magnified in stressful environments (Reed et al. 2003, Fox and Reed 2010), the populations used are typically descended from captive-bred experimental lines that may not reflect genetic or phenotypic compositions of natural populations. Conditions in artificial environments may also not reflect typical stressors populations experience in natural environments, given that effects of genetic variation can be environment dependent (Agashe et al. 2011).

Much research has focused on how populations adapt based on genetic variation (Reed and Frankham 2003, Reed et al. 2003, Willi et al. 2006, Bernatchez 2016). While genetic components will always remain important to adaptive processes, non-genetic effects (e.g. maternal effects) are increasingly recognized as having important fitness consequences when individuals are exposed to environmental stressors (Chirgwin et al. 2017). We found evidence

that individuals from populations that were, on average, larger as juveniles tolerated harsher conditions and better exploited benign environments. Older (1 + or greater) trout are capable of inhabiting more acidic habitats relative to juveniles (Baldigo and Lawrence 2000), but we found evidence that body-size moderated acid tolerance even within the 0+ age-class.

Natural selection can favor increased maternal investment when juveniles are faced with stressful or unpredictable environments (Hutchings 1991, Einum and Fleming 2004, Rollinson and Hutchings 2013). We cannot disentangle whether initial size differences across populations in the wild were due to maternal effects, intrinsic differences in growth rates, or early environmental growth opportunity. However, levels of maternal investment and juvenile growth, under both natural and common garden conditions, differ for populations of Cape Race trout (Wood et al. 2015, Fraser et al. 2018). Populations with increased maternal investment or juvenile growth might exhibit improved relative fitness under some novel environmental conditions.

Conservation Implications

The rich literature examining the effect of genetics on adaptation, extinction risk and fitness leaves little doubt that genetics can play an important role in long-term population persistence (Jamieson and Allendorf 2012). The beneficial role of genetic rescue, for example, has been demonstrated often (Frankham 2015, Whiteley et al. 2015b, Weeks et al. 2017). However, little research has simultaneously experimentally evaluated the relative importance of both habitat and genetic risks to fitness in natural environments. While genetic effects are undoubtedly important, our results support the assertion that conservation triage decisions and the allocation of scarce resources should emphasize the protection and maintenance of natural habitat if population sizes are not below extremely low critical thresholds (Jamieson and Allendorf 2012). Although genetic issues should not be ignored, preserving habitat quality or slowing its degradation is likely to be the most important means through which many populations can be conserved.

Our study species, nevertheless, is a generalist fish species with a (relatively) wide fundamental ecological niche. Although of conservation concern in southern regions (Hudy et al. 2008), brook trout are widely distributed across northern temperate environments. Inferences drawn from this experiment should be extended to other taxonomic/ecological groups with

caution; rare endemic specialist species, for example, may respond differently to novel environmental stressors. Similarly, many of our study populations have likely been isolated for up to thousands of years, meaning that mechanisms such as purging may have been able to alleviate deleterious effects of small population size and low genetic diversity. The extent to which our results might be generalizable to populations that have experienced a recent rapid decline is unknown.

Previous transplant experiments that have examined fitness in novel environments in nature have typically focused on plant species, likely due to the ease with which experiments can be performed on them in the wild. Our study provides valuable data on fitness correlates in novel environments for a vertebrate species, which is sorely lacking in the scientific literature due to the difficult nature of working with mobile species. Our results add to the growing body of literature documenting that small and/or genetically depauperate natural populations still represent important sources of variation that are adapted to local environmental conditions, capable of long-term persistence and/or adaptation, and that warrant protection from threats, particularly in the form of habitat degradation (Willi et al. 2007, Lawrence and Kaye 2011, Benazzo et al. 2017).

Tables

Table 1: Range of environmental characteristics of streams and novel pond environments (mean, minimum, and maximum).

Variable	Stream	Pond
pH	6.2 (4.8-7.5)	5.5 (4.4-6.7)
Temp. (°C)	13.6 (8.1-17.3)	15.1 (7.6-19.5)
Depth (cm)	19.8 (10.4-38.2)	23.7 (5.4-46.6)
% Silt Substrate	22.2 (1.8-70.8)	72.7 (0-100)
Dissolved Oxygen (mg/L)	10.8 (8.3-11.9)	9.2 (5.8-11.7)
%Veg. Cover	38.8 (13.8-72.2)	23.2 (0.0-90.7)
Conductivity (µS)	80.2 (40.9-176.9)	264.0 (46.1, 3346.0)

Table 2: Summary of pond translocations and genetic analysis of transplanted populations.

Source Population	*No. of ponds*	*No. of individuals translocated over 4 years*	*No. of survivors recovered over 4 years*	*No. of individuals sequenced*	*Genomic Heterozygosity*	N_b
Bob's Cove	9	211	80	29	0.049	339.5
Blackfly	7	94	43	19	0.087	101.0
Cripple Cove	10	300	54	28	0.060	78.7
Ditchy Brook	5	35	17	18	0.069	25.2
Freshwater River	8	210	71	29	0.111	607.7
Lower Coquita	6	98	22	22	0.049	57.4
Lower Ouananiche Beck	7	106	41	19	0.068	68.6
Perdition	7	113	36	14	0.084	73.0
Still There By Chance	9	178	52	30	0.016	58.8
Upper Ouananiche Beck	10	170	81	20	0.063	229.3
Watern Cove	10	281	103	30	0.119	591.1
Whale Cove	9	205	71	25	0.083	26.4

Table 3: Results of model selection for survival analysis incorporating genetic, phenotypic, and habitat data, with statistically significant (retained) terms presented in bold. Model testing was conducted using LRTs.

Model No.	Description	Versus model No.	Term	χ^2	df	*p*
0	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:N_b + pH^2:IS + pH^2:Ho	-	-	-	-	-
1	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:N_b + pH^2:IS	0	pH^2:Ho	0.04	1	0.835
2	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:N_b	1	pH^2:IS	0.59	1	0.443
3	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho	2	pH^2:N_b	2.24	1	0.134
4	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + V:N_b + pH:Ho + T:Ho + V:Ho	3	T:N_b	0.00	1	0.979
5	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + V:N_b + pH:Ho + V:Ho	4	T:Ho	0.01	1	0.913
6	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + V:N_b + pH:Ho + V:Ho	5	V:IS	0.04	1	0.850
7	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + pH:Ho + V:Ho	6	V:N_b	0.44	1	0.508
8	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + pH:Ho	7	V:Ho	0.40	1	0.527
9	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + pH:N_b + pH:Ho	8	T:IS	0.80	1	0.370
10	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + pH:Ho	9	pH:N_b	0.95	1	0.330
11	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS	10	pH:Ho	0.49	1	0.483
12	IS + N_b + Ho + Y + pH + pH^2 + T + V	11	**pH:IS**	5.32	1	**0.021**
13	IS + N_b + Ho + Y + pH + T + V + pH:IS	11	**pH^2**	21.03	1	**<0.001**
14	IS + Ho + Y + pH + pH^2 + T + V + pH:IS	11	N_b	0.00	1	0.985
15	IS + Y + pH + pH^2 + T + V + pH:IS	14	Ho	0.23	1	0.630
16	IS + pH + pH^2 + T + V + pH:IS	15	Y	5.62	3	0.132
17*	**IS + pH + pH^2 + T + pH:IS**	16	V	3.50	1	0.062
18	IS + pH + pH^2 + pH:IS	17	**T**	5.65	1	**0.017**

Term abbreviations: IS – Initial Mean Size; ID – Initial Density; N_b – Effective Number of Breeders; Ho – Observed genomic heterozygosity; Y – Year; T – Temperature; V - % Aquatic Vegetative Cover.

* Selected Model

Table 4: Results of model selection for growth analysis incorporating genetic, phenotypic, and habitat data, with statistically significant (retained) terms presented in bold. Model testing was conducted using *F*-tests.

Parameter	df	*F*-value	*p*
Ti:N_b:D	1, 845.5	0.08	0.78
Ti:N_b:T	1, 1252.0	2.00	0.16
Ti:Ho:T	1, 902.4	0.59	0.44
Ti:Ho:D*	1, 1017.8	6.72	**0.010**
Ti:T^2*	1, 31.5	4.37	**0.037**
N_b:T	1, 109.0	1.45	0.230
N_b:D	1, 58.4	1.27	0.260
Ti:N_b	1, 9.44	3.20	0.110
Ho:T	1, 78.4	3.23	0.076
Ti:Y*	3, 722.2	9.28	<**0.001**
Ti:ID*	1, 65.6	24.1	**<0.001**
N_b	1, 9.1	1.23	0.300

Term abbreviations: Ti – Time; ID – Initial Density; Ho – Observed Genomic Heterozygosity; N_b – Effective Number of Breeders; Y – Year; T – Temperature; D – Depth.

* Retained terms

Table S1: Number of SNPs removed at each stage of filtering

Filtration Stage	SNPs remaining
After '*populations'* module	58,126
Maximum number of SNPs per loci ≤ 4	45,106
1st SNP per locus	28,228
Heterozygosity ≤ 0.5	25,953
Global Minor Allele Freq. ≥ 0.01	4,614

Table S2: Results of model selection for analysis identifying habitat variables significantly correlated with survival, with statistically significant (retained) terms presented in bold. Model testing was conducted using LRTs.

Model No.	Description	Versus model No.	Term	χ^2	df	*p*
0	ID + Y + pH + pH^2 + DO + T + C + D + S + V	-	-	-	-	-
1	ID + Y + pH + DO + T + C + D + S + V	0	**pH^2**	14.36	1	**<0.001**
2	Y + pH + pH^2 + DO + T + C + D + S + V	0	ID	0.03	1	0.874
3	Y + pH + pH^2 + T + C + D + S + V	2	DO	0.03	1	0.869
4	Y + pH + pH^2 + T + C + D + V	3	S	0.04	1	0.848
5	Y + pH + pH^2 + T + C + V	4	D	0.25	1	0.615
6*	**Y + pH + pH^2 + T + V**	5	C	0.27	1	0.604
7	pH + pH^2 + T + V	6	**Y**	17.45	3	**<0.001**
8	Y + pH + pH^2 + V	6	**T**	5.45	1	**0.020**
9	Y + pH + pH^2 + T	6	**V**	7.31	1	**0.007**

Term abbreviations: ID – Initial Density; Y – Year; DO – Dissolved Oxygen; T – Temperature; C – Conductivity; D – Depth; S - % Silt Substrate; V - % Aquatic Vegetative Cover.

* Selected Model

Table S3*:* Results of model selection for survival analysis incorporating genetic, phenotypic, and habitat data measured as the difference between the novel pond environment minus source population stream habitat mean, with statistically significant (retained) terms presented in bold. Model testing was conducted using LRTs.

Model No.	Description	Versus model No.	Term	χ^2	df	*p*
0	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:N_b + pH^2:IS + pH^2:Ho	-	-	-	-	-
1	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:IS + pH^2:Ho	0	pH^2:N_b	0.00	1	0.977
2	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho + pH^2:IS	1	pH^2:Ho	0.32	1	0.573
3	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho	2	pH^2:IS	1.03	1	0.311
4	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho	3	V:Ho	0.06	1	0.808
5	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + pH:Ho + T:Ho	4	V:N_b	0.22	1	0.638
6	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + pH:Ho	5	T:Ho	0.68	1	0.410
7	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + T:N_b + pH:Ho	6	V:IS	1.03	1	0.309
8	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + T:N_b + pH:Ho	7	pH:N_b	1.86	1	0.172
9	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + T:N_b	8	pH:Ho	1.05	1	0.304
10	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:IS + T:N_b	9	pH:IS	3.26	1	0.071
11	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:N_b	10	**T:IS**	4.69	1	**0.030**
12	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:IS	10	**T:N_b**	5.24	1	**0.022**
13	IS + N_b + Ho + Y + pH + T + V + T:IS + T:N_b	10	**pH^2**	11.26	1	**<0.001**
14	IS + N_b + Ho + pH + pH^2 + T + V + T:IS + T:N_b	13	Y	3.36	3	0.340
15*	IS + N_b + pH + pH^2 + T + V + T:IS + T:N_b	14	Ho	1.71	1	0.191
16	IS + N_b + pH + pH^2 + T + T:IS + T:N_b	15	**V**	10.73	1	**0.001**

Term abbreviations: IS – Initial Mean Size; ID – Initial Density; N_b – Effective Number of Breeders; Ho – Observed genomic heterozygosity; Y – Year; pH – pH difference between the novel pond environment and native stream habitat; T – Temperature

difference between the novel pond environment and native stream habitat; V - % Aquatic vegetative cover difference between the novel pond environment and native stream habitat.

* Selected Model

Table S4: Results of model selection for survival analysis incorporating genetic, phenotypic, and habitat data measured as the difference between the novel pond environment minus source population stream mean, divided by stream habitat standard deviation. , Statistically significant (retained) terms presented in bold Model testing was conducted using LRTs.

Model No.	Description	Versus model No.	Term	χ^2	df	*p*
0	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + V:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho	-	-	-	-	-
1	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho + V:Ho	0	V:IS	0.02	1	0.878
2	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + T:N_b + V:N_b + pH:Ho + T:Ho	1	V:Ho	0.12	1	0.724
3	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + pH:N_b + T:N_b + V:N_b + pH:Ho	2	T:Ho	0.16	1	0.694
4	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:IS + T:IS + T:N_b + V:N_b + pH:Ho	3	pH:N_b	0.19	1	0.663
5	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:IS + T:N_b + V:N_b + pH:Ho	4	pH:IS	0.15	1	0.702
6	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:IS + T:N_b + pH:Ho	5	V:N_b	1.33	1	0.249
7	IS + N_b + Ho + Y + pH + pH^2 + T + V + T:N_b + pH:Ho	6	T:IS	3.78	1	0.052
8	IS + N_b + Ho + Y + pH + pH^2 + T + V + pH:Ho	7	T:N_b	3.28	1	0.070
9	IS + N_b + Ho + Y + pH + pH^2 + T + V	8	**pH:Ho**	4.67	1	**0.031**
10	N_b + Ho + Y + pH + pH^2 + T + V + pH:Ho	8	IS	0.26	1	0.608
11	N_b + Ho + Y + pH + pH^2 + V + pH:Ho	10	T	0.82	1	0.364
12*	Ho + Y + pH + pH^2 + V + pH:Ho	11	N_b	2.62	1	0.106
13	Ho + pH + pH^2 + V + pH:Ho	12	**Y**	14.86	1	**0.002**
14	Ho + Y + pH + pH^2 + pH:Ho	12	**V**	10.85	1	**0.001**

Term abbreviations: IS – Initial Mean Size; ID – Initial Density; N_b – Effective Number of Breeders; Ho – Observed genomic heterozygosity; Y – Year; pH – pH difference between the novel pond environment and native stream habitat, divided by stream pH standard deviation (SD); T – Temperature difference between the novel pond environment and native stream habitat, divided by stream temperature SD; V - % Aquatic vegetative cover difference between the novel pond environment and native stream habitat, divided by stream aquatic vegetative cover SD.

* Selected Model

Table S5: Results of model selection for analysis identifying habitat variables significantly correlated with growth, with statistically significant (retained) terms presented in bold. Model testing was conducted using *F*-tests.

Parameter	df	*F*-value	*p*
Ti:T²	1, 283.8	13.38	**<0.001**
Ti:C	1, 269.1	<0.01	0.993
Ti:V	1, 346.4	0.05	0.825
Ti:DO	1, 629.4	0.20	0.657
Ti:S	1, 74.8	0.21	0.646
Ti:FD	1, 841.7	0.78	0.377
Ti:pH	1, 143.7	1.83	0.179
Ti:Y*	3, 650.7	13.23	**<0.001**
Ti:ID*	1, 74.7	24.9	**<0.001**
Ti:D*	1, 280.0	21.1	**<0.001**
DO	1, 50.3	0.33	0.571
V	1, 40.3	0.18	0.670
S	1, 18.8	0.45	0.509
C	1, 39.9	0.10	0.753
pH	1, 18.1	1.04	0.322
FD	1, 20.9	1.48	0.237

Term abbreviations: Ti – Time; ID – Initial Density; FD – Final Density; Y – Year; DO – Dissolved Oxygen; T – Temperature; C – Conductivity; D – Depth; S - % Silt Substrate; V - % Aquatic Vegetative Cover.

* Retained terms

Table S6: Between-year growth (mm/day) differences in novel pond environments, with statistically significant comparisons presented in bold.

Years	Estimate	SE	df	*t*-value	*p**
2012 – 2013	0.0062	0.0163	1160.9	0.38	1.000
2012 – 2014	0.0002	0.0206	437.3	0.01	1.000
2012 – 2015	-0.0724	0.0217	645.3	3.34	**0.005**
2013 – 2014	-0.0030	0.0161	339.7	0.37	1.000
2013 – 2015	-0.0786	0.0145	710.0	5.42	**<0.001**
2014 - 2015	-0.0726	0.0180	850.1	4.03	**<0.001**

**p*-values are Bonferroni corrected to account for familywise error rate.

Table S7: Results of model selection for growth analysis incorporating genetic, phenotypic, and habitat data measured as the difference between the novel pond environment minus source population stream mean, divided by stream habitat standard deviation. Statistically significant (retained) terms presented in bold Model testing was conducted using *F*-tests.

Parameter	df	*F*-value	*p*
Ti:N_b:D	1, 852.4	0.10	0.760
Ti:Ho:T	1, 41.8	0.17	0.680
Ti:N_b:T	1, 1005.7	1.78	0.180
Ti:Ho:D*	1, 933.5	11.5	**0.001**
Ti:T^2	1, 92.7	1.20	0.280
N_b:D	1, 42.6	0.39	0.530
N_b:T	1, 82.0	1.89	0.170
Ho:T	1, 89.1	3.51	0.064
Ti:N_b	1, 8.86	4.88	0.055
Ti:Y*	3, 1270.2	9.99	**<0.001**
Ti:ID*	1, 44.1	14.4	**<0.001**
Ti:T*	1, 298.1	5.69	**0.018**
T^2	1, 35.7	0.51	0.480
N_b	1, 8.9	1.06	0.330

Term abbreviations: Ti – Time; ID – Initial Density; Ho – Observed Genomic Heterozygosity; N_b – Effective Number of Breeders; Y – Year; T – Temperature difference between the novel pond environment and native stream habitat, divided by stream temperature standard deviation (SD); D – Depth difference between the novel pond environment and native stream habitat, divided by stream depth standard deviation (SD).

* Retained terms

Table S8: Results of model selection for growth analysis incorporating genetic, phenotypic, and habitat data measured as the difference between the novel pond environment minus source population stream mean, divided by stream habitat standard deviation. Statistically significant (retained) terms presented in bold Model testing was conducted using *F*-tests.

Parameter	df	*F*-value	*p*
Ti:Ho:T	1, 279.0	<0.01	0.990
Ti:N_b:T	1, 842.4	0.36	0.550
Ti:N_b:D	1, 927.9	0.42	0.520
Ti:Ho:D*	1, 685.1	10.6	**0.001**
Ti:T^2	1, 83.5	0.08	0.780
N_b:D	1, 51.0	0.19	0.670
N_b:T	1, 78.8	1.42	0.240
Ti:N_b	1, 8.8	4.20	0.071
Ho:T	1, 83.3	3.88	0.052
Ti:Y*	3, 1158.5	10.1	**<0.001**
Ti:ID*	1, 43.3	15.3	**<0.001**
Ti:T*	1, 320.1	4.53	**0.034**
T^2	1, 42.1	0.49	0.49
N_b	1, 9.0	0.89	0.37

Term abbreviations: Ti – Time; ID – Initial Density; Ho – Observed Genomic Heterozygosity; N_b – Effective Number of Breeders; Y – Year; T – Temperature difference between novel pond environment and native stream habitat; D – Depth difference between novel pond environment and native stream habitat.

* Retained terms

Figures

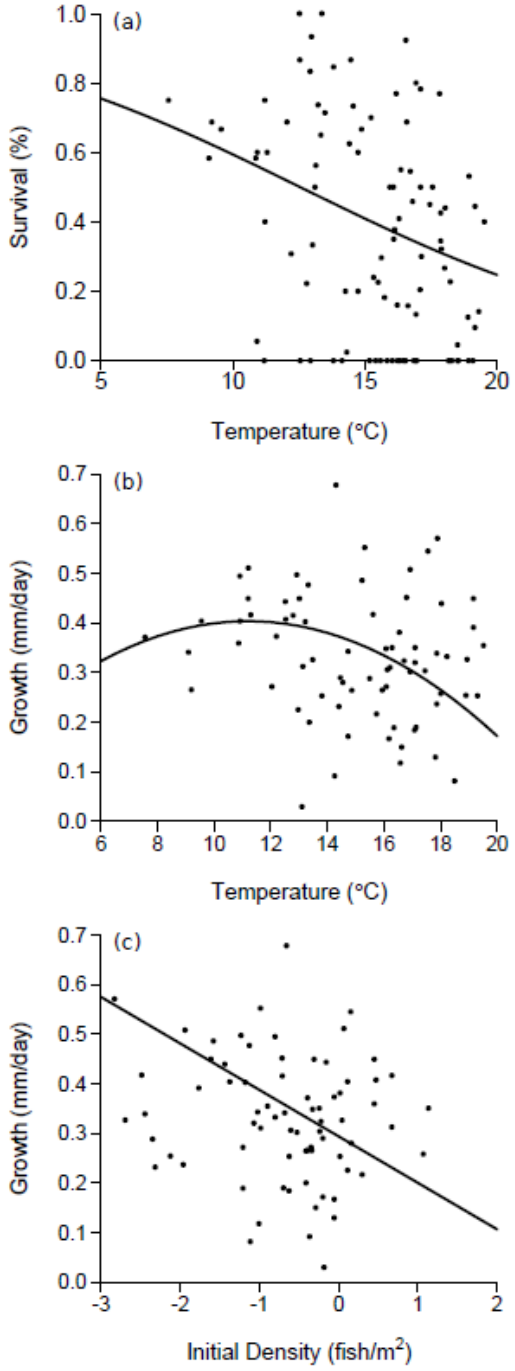

Figure 1: The effect of (a) temperature (°C) on survival (%); (b) temperature on growth rate (mm/day); and (c) initial stocking density (ln(fish/m^2) on growth rate (mm/day) for brook trout translocated to novel pond environments.

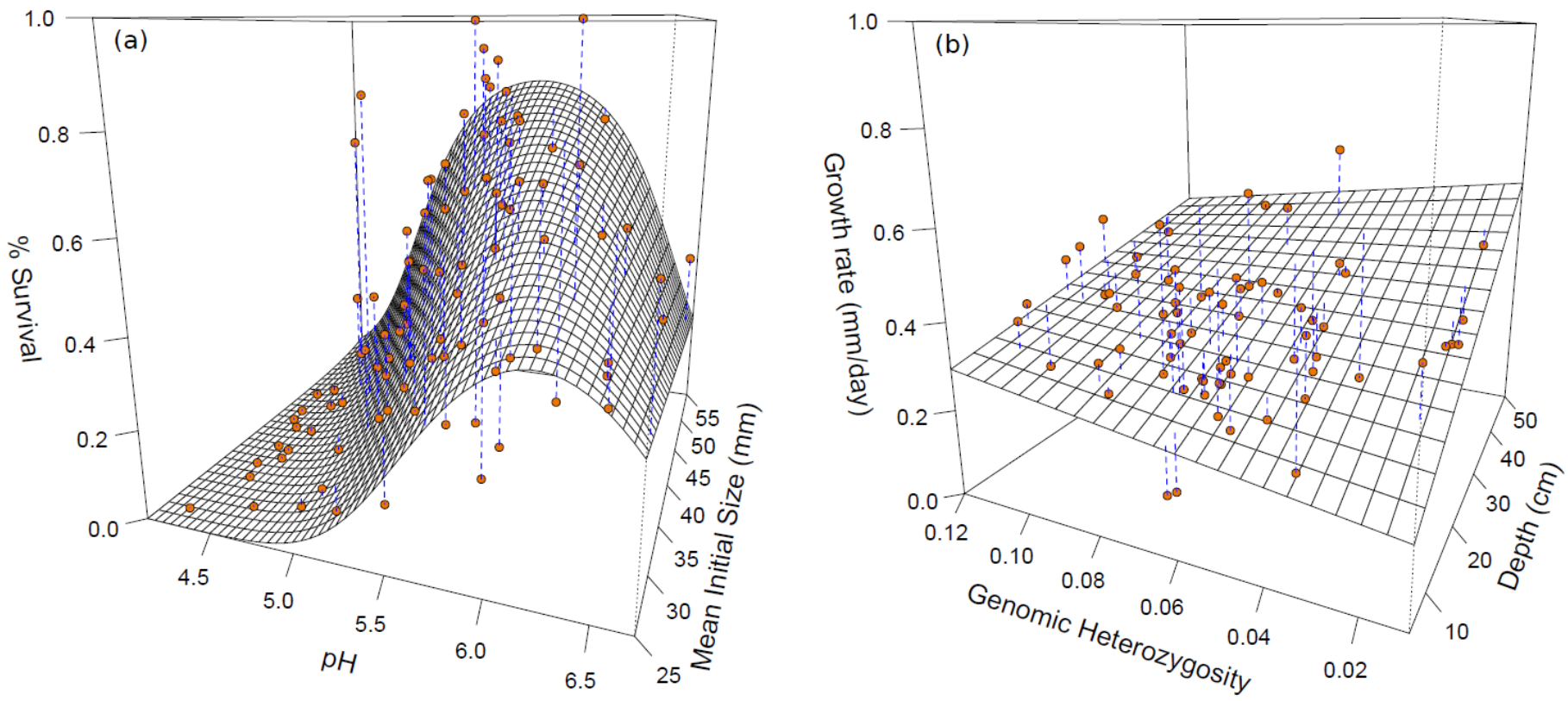

Figure 2: The effect of (a) pH and mean initial size at translocation on survival and (b) source population genomic heterozygosity and depth (cm) on growth rate (mm/day) for brook trout translocated to novel pond environments.

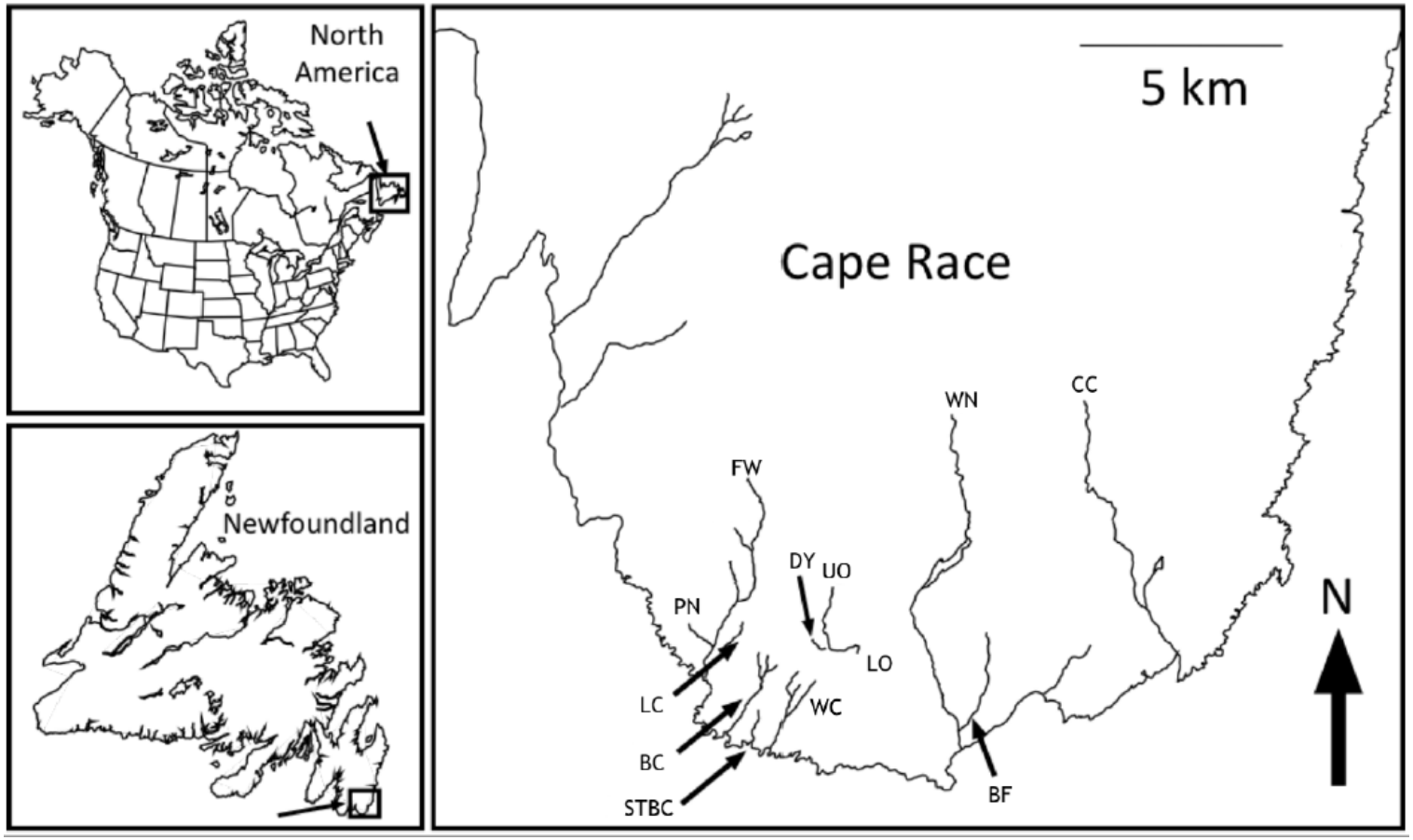

Figure S1: Brook trout populations located on Cape Race, Newfoundland, Canada. From west to east: Perdition (PD), Freshwater (FW), Lower Coquita (LC), Bob's Cove (BC), Still There By Chance (STBC), Whale Cove (WC), Ditchy (DY), Upper O. Beck (UO), Lower O. Beck (LO), Watern (WN), Lower Blackfly (LBF), Cripple Cove (CC).

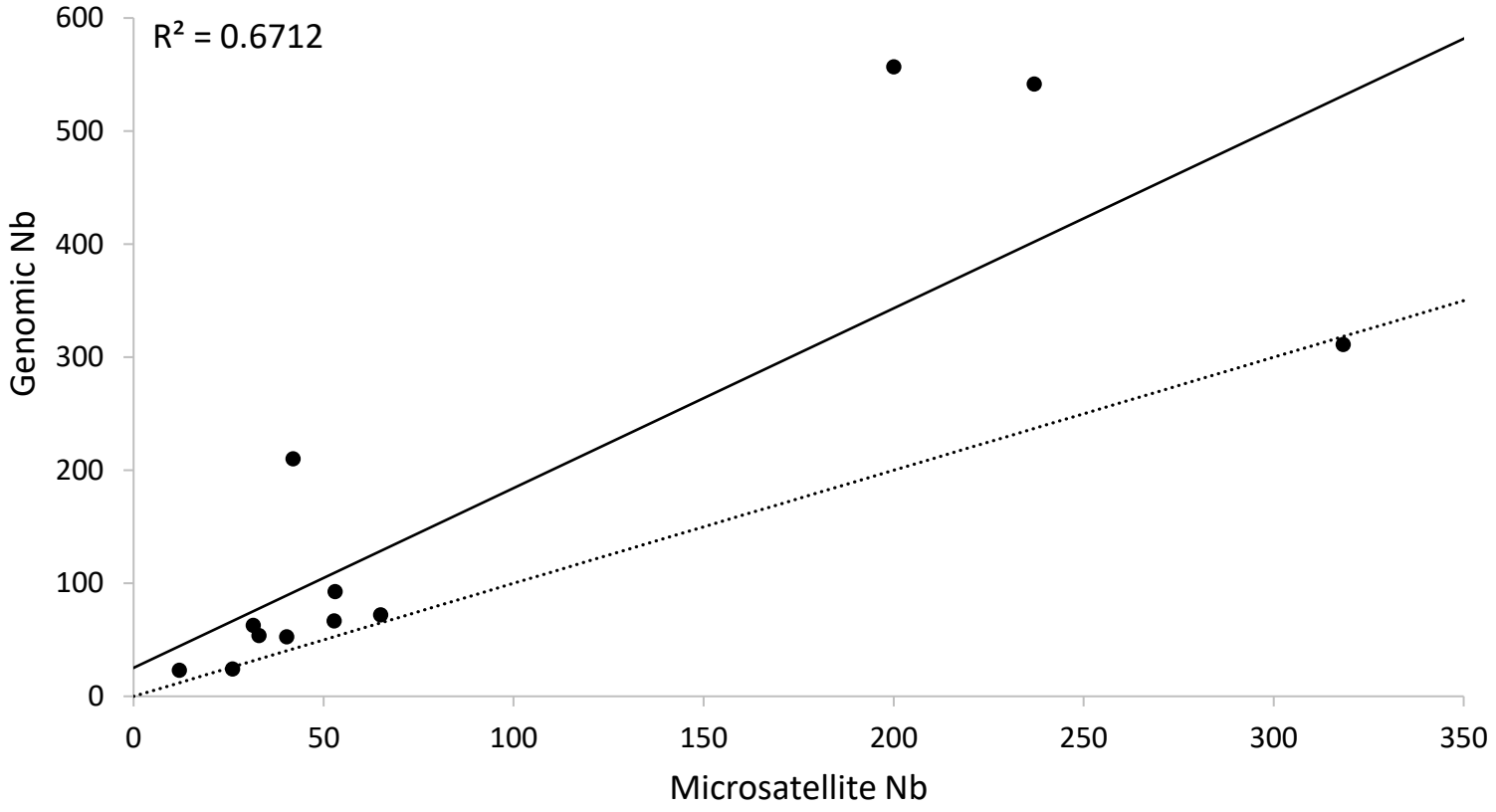

Figure S2: Relationship between N_b estimates obtained using microsatellite and genomic data. Solid line represents trendline (with corresponding R^2), dotted line represents 1:1 ratio.

Chapter 3: Release of plasticity in novel environments unaffected by genomic diversity in a vertebrate

Abstract

*Plastic reaction norms are often shaped and constrained by selection and are important mechanisms through which organisms respond to environmental change. However, selection cannot constrain reaction norms for environmental conditions that populations have never experienced, allowing neutral cryptic genetic variation for the reaction norm to potentially accumulate. When exposed to novel conditions, accumulated cryptic genetic variation may result in a release of phenotypic plasticity. Most genomic diversity is also functionally neutral; genomic estimates of diversity may be correlated with levels of neutral cryptic genetic variation and resulting plastic phenotypic release. To test how genomic diversity affects plastic phenotypic release in novel environments we conducted translocations of juvenile brook trout (*Salvelinus fontinalis*) from 12 populations to novel uninhabited ponds that represented a gradient of environmental conditions. We assessed reaction norms for morphological traits (body size and 4 morphometric relative warps) across pond environmental gradients and evaluated the effect of genome-wide heterozygosity on phenotypic variability. Despite all traits exhibiting plastic reaction norms, only one morphometric trait exhibited a release of phenotypic plasticity consistent with cryptic genetic variation. H_o had no effect on phenotypic variability across transplant environments. When the cost of maintaining plasticity is low, historical selection can constrain genetic variation in reaction norms that would otherwise be cryptic. Past conditions may have constrained reaction norms in the putatively novel environments despite significant deviations from contemporary source population habitat. Similarly, as a generalist colonizing species, brook trout may be capable of plastically altering their phenotype across a wide range of environmental conditions.*

Introduction

Plasticity (the capacity of a genotype to exhibit different phenotypes in different environments) or canalization (the capacity of a genotype to conserve phenotype across environments), can be important components of a population's response to environmental change, allowing individuals to maintain optimal phenotypes across a range of environments (Schlichting and Pigiucci 1998, Price et al. 2003). Selection typically shapes plastic responses, with organisms that are exposed to variable environments often exhibiting an increased capacity to adaptively alter their phenotypic response in response to environmental change (Cook and Johnson 1968, Moran 1992, Ghalambor et al. 2007). Selection, however, cannot shape reaction norms for environments to which populations are not naturally exposed (Ghalambor et al. 2007). As a result populations might accumulate cryptic genetic variation that controls trait expression in environmental conditions they do not typically experience (Paaby and Rockman 2014).

When exposed to novel conditions, the accumulation of cryptic genetic variation can result in a release of plastic phenotypic variation in reaction norms (Rutherford 2000, Schlichting 2008). Such a release should be particularly pronounced in increasingly stressful environments for environments a population is not originally adapted to (Ghalambor et al. 2007). While much of this plasticity might be non-adaptive, increased variation in phenotypes could result in a higher probability that some phenotypes lie closer to a potential theoretical optimum phenotype within that novel environment (Ghalambor et al. 2007). Furthermore, the genetic nature of this variation could allow selection to act upon reaction norms and allow subsequent generations of organisms to evolve towards a more optimal phenotype, particularly if such variation is heritable (Rutherford 2003). Cryptically released phenotypic variation could thus play an important role in how organisms adapt to novel conditions (Schlichting 2008, Mcguigan et al. 2011).

The cryptic genetic variation underlying the release of plasticity in novel environments can often be considered functionally neutral because selection has not shaped the reaction norms underlying trait expression in novel conditions (Paaby and Rockman 2014). In the absence of selection, the predominant force affecting the accumulation of genetic variation is drift, which is a function of effective population size (N_e) (Nei et al. 1975). Short-term N_e can vary significantly even at fine geographic scales (Bernos and Fraser 2016) and has been predicted to affect among-population variation in levels of cryptic variation (Ledón-Rettig et al. 2014). However, contemporary N_e estimates reflect current rates of genetic drift and may not reflect historical

events (e.g. bottlenecks, founder effects, etc.) that could affect genetic variation, cryptic or otherwise. Conversely, contemporary estimates of genomic diversity, which are affected both by historical and contemporary events, provide cost-effective yet comprehensive estimates of standing levels of genetic variation (Allendorf et al. 2010). Because most genomic variation may behave as if largely functionally neutral (Nei et al. 2010), genomic diversity may represent a better estimator of the pool of cryptic genetic variation present within populations than N_e.

Morphological characteristics represent useful phenotypic traits for the study of the release of cryptic genetic variation. Morphological traits can often exhibit plastic reaction norms that are adaptive responses to environmental conditions (e.g. Robinson and Parsons 2002, Hutchings 2011, Smith et al. 2017). Salmonid fishes, for example, can plastically alter body morphology in response to swimming demands (Peres-Neto and Magnan 2004). The release of cryptic genetic and subsequent phenotypic variation in morphological traits in novel or stressful environments has been well documented in controlled laboratory environments (Rutherford 2000, Mcguigan et al. 2011), and molecular/proteomic pathways responsible for the release of such variation have even been identified in some commonly studies species (e.g. heat-shock proteins in *Drosophila*) (Rutherford 2003). However, the release of plastic morphological variation in natural environments is considerably less studied. Replicated transplants in nature represent an opportunity to study how the release of plasticity in novel environments affects adaptation to those environments (Ghalambor et al. 2007). To our knowledge, no study has examined the influence of genomic diversity on the release of plastic phenotypic variation in natural environments.

To study how genomic diversity affects plastic phenotypic variation in novel environments, we conducted a large replicated translocation of brook trout (*Salvelinus fontinalis*) from multiple populations to previously uninhabited ponds. Brook trout are a socio-economically important salmonid species in the northern hemisphere that can tolerate a range of different abiotic characteristics (e.g. pH, temperature, sympatric fish species, etc. (Baldigo and Lawrence 2000, Xu et al. 2010). They are often an important recreational or subsistence fishery species, yet can also be invasive or of conservation concern depending on region (Korsu et al. 2007, Hudy et al. 2008). Furthermore, brook trout exhibit plasticity for many morphological and life-history characteristics (Peres-Neto and Magnan 2004, Rouleau et al. 2010, Wood and Fraser 2015).

Juvenile brook trout were collected from 12 naturally fragmented populations and translocated each spring to isolated uninhabited ponds that represented a wide range of environmental conditions for the species (e.g. pH of 4.4-6.7, temperature of 7.5-19.5℃) (Baldigo and Lawrence 2000, Xu et al. 2010). All survivors were recaptured each summer, and body size and morphological data were collected. Seventy-two independent translocations were conducted over a four-year period (2012-2015). If cryptic genetic variability results in a release of plasticity in novel environments, phenotypic variability of transplanted individuals should increase as novel transplant environments become increasingly marginal or extreme. Furthermore, if cryptic genetic variation is linked with genomic diversity, we should observe a positive relationship between source population genomic diversity (observed heterozygosity, H_o) and the phenotypic variability of transplanted individuals, particularly as environments become increasingly extreme.

Materials and Methods

Study System

Brook trout inhabit several small streams located on Cape Race, Newfoundland (46°39'31.43N, 53°04'22.27W); these populations are descended from a common ancestor (Danzmann et al. 1998) and are effectively free from anthropogenic influences, with no previous history of hatchery supplementation, little fishing pressure due to small body size (100-150mm average), and stream habitats largely free from human development impacts (Hutchings 1993, Zastavniouk et al. 2017). Cape Race trout populations are also typically isolated (both physically and genetically) from one another due to impassable waterfall barriers and have been isolated for several thousand years (Wood et al. 2014). However, some population pairs can occasionally exchange gene flow (PN-FW; DY-UO-LO) and one population pair (WN-BF) is accessible from the ocean (Figure S1).

Pond Translocations

Numerous isolated fishless ponds (typically 20-100 m^2) can be found on Cape Race that represent a wide gradient for several ecologically important environmental characteristics (e.g. temperature, pH, etc., see Table 1). To test how phenotypic plasticity is released in novel pond environments, surveyed ponds needed to represent a gradient of habitat parameters in which for brook trout survival was possible; 29 suitable ponds were identified between 2012-2015. Only ponds in watersheds uninhabited by natural trout populations were used to prevent accidental mixing from escapees. Any areas through which fish could escape during possible flood events were identified and blocked with chickenwire barriers embedded in the substrate and bank soil.

Backpack electrofishing was used to capture age 0+ juveniles from 12 source populations in late June/early July. In 2012 only 7 populations were translocated to novel pond environments; the project was expanded to include another 5 populations in later years. Juveniles were collected at random locations in each stream to eliminate potential non-random associations of related individuals (Hansen et al. 1997). A backpack transporter with constant aeration was used to transport fish from streams to novel pond environments, and fish were acclimated for 20 minutes using a 1:1 mixture of pond water and source stream water. Ponds were stocked at a maximum density of 2 fish/m^2, except for one pond stocked at a density of 3 fish/m^2 due to an

error measuring pond surface area. Juveniles from demographically small source populations were translocated to fewer ponds (see chapter 2) due to concerns associated with over-harvesting. Juveniles from larger populations capable of absorbing an increased sampling effort were translocated to an increased number of ponds. Behavior among study populations differs significantly (Wood et al. 2015) so a single population was translocated to each pond annually to avoid between-population competitive interactions. Populations were randomly assigned to pond environments; however, due to limited pond availability no pond was stocked with fish from the same source population in different years. Length measurements (± 1 mm) were taken on all stocked individuals and a small caudal fin tissue sample was collected for later genetic analyses.

Using a combination of gill nets, beach seine, and/or electrofisher all fish were recaptured in September each year. Ponds were fish repeatedly over multiple days until fish were no longer captured. Any individual captured was photographed (see below) and subsequently euthanized using Tricaine Methanesulfonate (MS222), after which and mass (± 0.1 g) data and a tissue sample were collected.

Morphological data collection

Phenotypic variability in body size and shape was evaluated for all recaptured individuals from photographs. Prior to photography, all fish were anaesthetized using Tricaine Methanesulfonate. Photographs were taken on a levelled wooden platform and standardized such that the location of the tripod and height of the (levelled) camera were identical for all photographs. A size reference (ruler) was also placed in a standardized location along with an individual identifier in each photograph. Approximately 20-50 juveniles (depending on abundance) from each source population at random stream locations distributed throughout the entire stream length every year in September using a backpack electrofisher . Captured individuals were photographed using the same methodology as described for pond recaptures, with the exception that captured individuals were returned alive to their native stream habitat after sampling was completed.

A geometric morphometric analysis was performed to quantify the body shape of all (re)captured individuals. Size was first calibrated for each photograph and fourteen digitized landmarks were then placed on each individual using the program tpsDig2 (v. 2.30, Rohlf 2014, see Figure S2). Digitized landmarks were then used to calculate a consensus shape; relative

warps (RWs), a multivariate description of shape variation, were then calculated from the digitized landmarks using the program tpsRelW (v. 1.68, Rohlf 2014). The broken stick method (Borcard et al. 2011) was used to identify relative warps that explained a significant proportion of morphological shape variation; significant warps were retained for subsequent analyses.

Habitat data collection

All ponds were surveyed three times annually to quantify non-temperature habitat characteristics; two times prior to translocation, and once immediately prior to recapture (see Appendix 1 for details). Habitat data for all source population stream environments were simultaneously obtained from 9-61 uniformly distributed transects. Source population stream and pond temperatures were recorded every 90 minutes for the duration of the stocking period using waterproofed iButton^{TM} data loggers; a single logger was placed in each pond and two loggers (at separate locations) were placed in each stream. The number of stream habitat transects sampled occasionally differed between years; across-year stream means for all environmental variables were therefore calculated by bootstrap sampling values such that all years were weighted equally in final mean estimates.

Estimation of genomic diversity

Whole-genome estimates of observed heterozygosity (H_o) were obtained using genotype-by-sequencing (GBS) conducted on a random subset of individuals from each transplanted trout population. Tissues samples were extracted using a modified Qiagen^{TM} DNeasy blood and tissue protocol. DNA quality and quantity were assayed using agarose gel electrophoresis and Qubit® dsDNA BR Assay Kit with a Qubit® Fluorometer. DNA concentration was normalized to 10ng/ul, with 10ul per sample (100ng DNA total). Library preparation and sequencing was performed on an Ion Torrent Proton Platform (IBIS, Laval University, Quebec, CA) following the protocol developed in Mascher et al 2013 (using enzymes *PstI* and *MspI*) as described in Perrault-Payette et al (2017).

Raw sequencing quality was assessed using FastQC (Andrews 2010) v. 0.11.4, and adapters were trimmed using cutadapt (Martin 2011); SNP filtering and discovery was conducted using the *de novo* assembly pipeline in *Stacks* v. 1.44 (Catchen et al. 2013). GBS was performed

on 14 populations in total, but the two non-translocated populations were excluded from post-*stacks* downstream analyses. *process_radtags* was used to demultiplex and filter reads based on quality; reads were trimmed to 80 base pairs to remove bases with low-quality scores on the 3' end. *ustacks* was then used to form loci, with the following parameters: a minimum stack depth (-m) of 5, a maximum distance allowed between stacks (-M) of 5, and a maximum distance allowed to align secondary reads (-N) of 7. The maximum number of mismatches allowed between sample tags when generating the catalogue (-n) in *cstacks* was 5. Individuals were then aligned to the catalog using the *sstacks* module, and the *rxstacks* module was used to remove loci with a log-likelihood less than -30. The *populations* module was then used to export genotypes, with the minimum percentage of individuals in a population required to process a locus for that population ("r") set to 0.8 and the minimum number of populations a locus must be present in order to process a locus ("p") set to 11 (of 14).

Downstream filtering was conducted in the *radiator* package (Gosselin 2017) in R v. 3.3.3 (R Development Core Team 2017). Brook trout are residual tetraploids (Crete-Lafreniere et al. 2012); SNP identification is complicated by the occurrence of paralogues in such polyploid genetic codes (Paris et al. 2017). To remove potential paralogues, loci with more than 4 SNPs were removed; only the first SNP was used for all remaining loci with multiple SNPs. A strict H_o filtering criterion was also employed; loci with H_o greater than 0.5 in any sampled population were excluded. SNPs with a minor allele frequency (<0.01) were similarly excluded to remove potential sequencing errors and rare alleles. Individuals missing more than 40% of genotypes across all filtered loci were also removed.

Statistical Analysis

Effect of environment and genetic parameters on body size and shape

General linear mixed-effects models were used to assess the effect of environmental variables on body size and shape in novel pond environments. Only relative warp values obtained from fish translocated to pond environments were used for this analysis. Centroid size, which is a geomorphometric estimate of total body size, was used as the dependent variable when analyzing plastic reaction norms in body size. For the analysis of body size, mean dissolved oxygen content, pH, temperature, conductivity, depth, substrate silt content (%), and aquatic vegetation coverage (%) were included as continuous fixed covariates; translocation year

was included as fixed environmental categorical covariates. Source population observed SNP H_o was included as a fixed genetic covariate, and *ln*-transformed initial translocation density was included to control for the effect of density on growth. Source population and transplant pond location were included as random effects in all models regardless of significance to account for issues of non-independence across translocation replicates. Backwards model selection was conducted on models estimated under maximum-likelihood; backwards model selection was performed by using Wald *F* tests to remove nonsignificant fixed-effects terms ($p > 0.05$). If body size was found to exhibit significant plastic variation with an environmental variable after model selection, a random population slope for that variable was subsequently added and tested using likelihood ratio tests (LRTs) to determine if a genotype-by-environment (GxE) interaction existed.

Data analysis was conducted using the lmer function in the package lme4 (Bates et al. 2015) in R 3.3.3 (R Development Core Team 2017). Denominator degrees of freedom estimates were obtained with the Kenward-Roger method (Kenward and Roger 1997). Pairwise contrast significance levels were evaluated using *t*-tests, with *p*-values Bonferroni corrected to account for potential type-1 errors.

Similar mixed-effects models were run for each significant relative warp, except centroid size was included in all models as a fixed continuous covariate to account for potential allometric changes in body morphology. Independent fixed covariates and random effects included in all models and all analytical stages were otherwise identical to the initial body size analysis.

Release of phenotypic plasticity

If exposure to novel environmental conditions releases cryptic genetic variation that results in a release of phenotypic plasticity, phenotypic variability should increase as environmental conditions become increasingly extreme in novel pond environments. To assess if phenotypic plasticity is released in novel environments, the within-year coefficient of variation (CV) for centroid size values for each pond replicate was calculated; CV values were then used as the dependent variable in a linear mixed effects model analysis. A bias corrected estimate of the standard deviation was used to calculate the CV of centroid size in all ponds (Brugger 1969) because of small sample sizes resulting from low survival. Due to the replicate-level nature of this analysis, the number of datapoints available was equal to the total number of pond replicates

(72); including all environmental parameters from the previous analysis (including potential interactions with genetic variables) would result in model overfitting. Environmental variables were therefore selected based on satisfying at least one of two criteria: i) a significant association with body size in the previous analysis; or ii) a known effect on growth (see chapter 2). Mean-centered and scaled source population H_o was included as a continuous fixed effect. Translocation year was included as a fixed categorical variable. In salmonids, variance in body mass can increase with the mean (Yates et al. 2015); mean-centered and scaled centroid size was also therefore included as a continuous fixed covariate. The CV of centroid size values for source population samples were also included as fixed continuous covariates to test whether populations that naturally exhibit more phenotypic diversity in their natural environments continue to do so in novel pond habitats. All environmental variables were interacted with H_o. Source population and transplant pond location were included as random effects in all analyses regardless of significance level.

Similar mixed-effects models were again run for each relative warp; however, the within-pond standard deviation (bias corrected for small sample size) for each relative warp was used as a dependent variable instead of the CV due to the interval-scale nature of the data. Centroid size and the CV of centroid size for each pond was also included in all models as fixed continuous covariates; environmental variables with a known effect on growth were only included if the relative warp displayed an allometric relationship with centroid size as determined from previous analysis. The selection process for fixed covariates and the random effects included in all models was otherwise identical to the body size analysis.

Data analysis and model selection were conducted as previously described for the analysis examining how body size and shape changes in novel environments.

Results

Total samples

The final dataset included 2357 fish sampled from 28 ponds and 12 streams across 4 years. In total, fish were sampled from 72 pond replicates and 41 stream cohorts (Table 2).

Genomic diversity and effective number of breeders

We sequenced 327 individuals, with 58126 SNPs (30292 loci) identified after *stacks* processing; 4614 SNPs were retained after further filtering for quality, putative paralogues, etc., (see Table for details). Prior to filtering in *radiator*, 44 individuals were removed due to missing >40% of genotypes, with 14 to 30 individuals remaining per population (283 total, see Table 3). Mean H_o values ranged from 0.016 to 0.119 (mean = 0.072) (Table 2).

Relative Warps

The first four (of 24) significant relative warps explained 61.56% of the variation in observed body shape; these relative warps were used for subsequent analyses (Figure S3). From negative to positive values, i) RW1 corresponded to extension of the ventral belly; ii) RW2 corresponded to an increase in head size as a proportion of total body length; iii) RW3 corresponded to horizontal alignment change from extended dorsal side to extended ventral side; and iv) RW4 corresponded to an increase in body depth (Figure S4).

Effect of environment and genetic parameters on body size and shape

Body size was significantly correlated with temperature, with trout exhibiting decreased size in warm ponds (Figure S5a, Table S1). Similarly, body size was positively correlated with pond depth (Figure S5b). Significant inter-annual variation in size was also observed, with fish exhibiting smaller sizes in 2014 compared to other years (Table S2). Finally, body size was negatively correlated with the initial translocation density of trout in a pond, suggesting a density-dependent effect upon growth (Figure S5c).

All four RWs exhibited an allometric relationship with body size (Table S3). RW 1 exhibited a positive allometric relationship with body size, with the extension of the ventral belly

increasing with centroid size. RW2 exhibited a strong negative allometric relationship, with head size as a proportion of body length decreasing with increasing centroid size. RW3 also exhibited a negative allometric relationship, with larger trout possessing an extended dorsal body relative to smaller fish. Finally, RW4 exhibited a positive allometric relationship, with body depth increasing in larger fish.

Of the environmental variables tested, the percentage of silt as a component of pond substrate had the most consistent effect on body shape, significantly affecting all RWs examined (Figure 1, Table S3). For RW1, extension of the ventral belly decreased with increasing silt substrate (Figure 1a). For RW2, trout from ponds with a higher proportion of silt substrate exhibited smaller head size as a proportion of total body length (Figure 1b). For RW3, trout from silty ponds tended to exhibit a flatter dorsal body shape (Figure 1c). For RW4, trout from silty ponds tended to exhibit a narrower body shape (Figure 1d). RW4 was the only morphological trait to exhibit a significant relationship with any other environmental variables, with fish from warmer ponds or ponds high in dissolved oxygen exhibiting a dorso-ventrally broader body shape (Figure S6).

Finally, RW2 and RW3 exhibited significant inter-annual variation, with trout from 2015 exhibiting a smaller head size as a proportion of body length (RW2) and trout from 2013 exhibiting flatter dorsal body shape (RW3).

Although transplanted trout exhibited plastic reaction norms for body size and each relative warp, a significant G*E interaction (i.e. reaction norms depended upon population) was only observed with percentage silt substrate with RW4 and with mean temperature and depth for body size (Table S4).

Release of phenotypic plasticity with environmental gradients or heterozygosity

No evidence was found that phenotypic variability in body size was correlated with habitat variables, mean centroid size, H_o, or native stream centroid size variability (Table S5). Limited evidence wasfound that variability in plasticity increased with habitat gradients for the four RW examined (RW1-4, Table S6). Only RW1 exhibited such a relationship, with within-pond variability increasing with increasing temperature (Figure 2). RW4 exhibited significant inter-annual variability (Table S7), but variability was not correlated with any specific habitat variables. H_o was not correlated with phenotypic variability in any RWs.

Discussion

With 72 experimental translocations sourced from 12 populations to 29 natural novel environments and H_o estimates derived over 4.6k SNPs located across the brook trout genome, our study represents (to our knowledge) the largest replicated experimental translocation of a vertebrate and the only attempt to examine how genomic variation affects the release of plasticity in novel environments. Translocated individuals were sourced from populations that varied 10-fold in levels of genomic diversity. Yet limited evidence was found that cryptic phenotypic/genetic variation was released, despite translocating individuals to novel natural environments that represented a relatively extreme gradient of several ecologically important environmental variables (Table 1).

Of the five traits examined, only RW1 (extension of ventral belly) exhibited a pattern associated with a release of cryptic genetic variation as pond temperature increased. A lack of significant G*E interaction across populations for this morphological trait indicated that populations exhibited similar plastic responses. However, as mean pond temperature increased, within-pond phenotypic variability for RW1 also increased, indicating a within population G*E interaction. Due to the experimental design, it was not possible to specifically quantify additive genetic variation underlying the morphological traits; this would have required the release half-sibling families bred under controlled conditions (e.g. Dammerman et al. 2016), whereas translocated fish represented random samples of fish obtained from source populations. Nevertheless, previous research conducted on 9 of 14 of the same populations in this study demonstrated significant additive genetic variation for size and shape at early life-history stages (Wood et al. 2015). So while not a definitive confirmation, the results for this RW1 are consistent with a release of cryptic phenotypic/genetic variability (Ghalambor et al. 2007). No other morphological traits exhibited a pattern of phenotypic release consistent with a release of cryptic genetic variation. Furthermore, despite evidence of plastic reaction norms for all morphological traits and significant G*E interactions observed for several of them, within-pond phenotypic variability generally did not increase as environments became increasingly marginal or extreme (e.g. as pH decreased, temperatures increased, silt substrate increased, etc.).

H_o was not correlated with a release of phenotypic variation, even when the morphological trait examined suggested a release of cryptic additive genetic variation. These results are broadly consistent with other studies performed on 8 or 9 of the studied populations

that found no relationship between effective number of breeders (N_b) and variability in plastic response to incubation temperature and little among-population differences in additive genetic variance for a variety of traits (including morphology) (Wood and Fraser 2015, Wood et al. 2015). Overall, these results are inconsistent with the hypothesis that cryptic genetic variation is correlated with levels of genomic diversity, but broadly consistent with previous studies that Cape Race brook trout populations exhibit similar levels of genetic variation underlying studied traits.

Both the frequency and strength of historical selective forces can affect the pool of cryptic genetic variation available in natural populations (Ghalambor et al. 2007, Paaby and Rockman 2014). Transplant ponds represented contemporary novel (and in some cases, quite extreme) environments previously uninhabited by trout. Furthermore, some novel ponds were also undoubtedly stressful, as they elicited significant differences in survival and growth (see chapter 2). However, historical data on conditions experienced within source population streams for the previous few millennia were unavailable. Translocations were conducted on a small micro-geographic scale (11.6 km maximum distance between ponds/streams), with novel pond environments located in close proximity to source population stream watersheds. It is plausible that historical environments experienced by some source populations were more "extreme" and/or similar to the novel pond environments relative to contemporary conditions, given that such conditions continue to be present in close physical proximity at a small geographic scale. Similarly, rare natural events (i.e. 50- or 100-year storms or severe droughts) or extreme seasonal variations might cause source populations to temporary experience extreme conditions similar to some ponds, resulting in strong selective events that could act on otherwise cryptic reaction norms (Ledón-Rettig et al. 2014). Excessive rainwater on Cape Race, for example, often lowers stream pH due to acidic runoff from bordering bog environments (M. Yates, personal observation). If historical conditions or rare strong selective events consistently exposed ancestral populations to environmental conditions similar to the novel pond environments, historical selection could limit the release of phenotypic variation in the putatively cryptic reaction norms (Ledón-Rettig et al. 2014, Paaby and Rockman 2014), especially when the costs of maintaining phenotypic plasticity are low (e.g. Sultan and Bazzaz 1993) as is suspected for many morphological traits in salmonids (Marin et al. 2016).

Finally, the reaction norms for the morphological traits examined may also have been highly conserved across populations and environments. Salmonids are residual tetraploids (Allendorf and Thorgaard 1984), and brook trout are a generalist colonizing species that greatly expanded their range post-North American deglaciation (Danzmann et al. 1998). As a generalist colonizing species, brook trout may retain the capacity to plastically alter some phenotypic traits across a range of environments while canalizing important fitness-related traits. In a controlled laboratory setting, for example, the studied populations exhibited similar levels of plasticity despite novel and (likely) stressful thermal rearing conditions (Wood and Fraser 2015). Similarly, populations exhibited similar critical thermal maxima, despite experiencing variable natural thermal regimes (Wells et al. 2016). Although many of the morphological traits examined did display at least a weak plastic association with some environmental variables, physiological or developmental processes can also buffer trait expression even in relatively extreme conditions (Rutherford 2003). Several of the specific morphological traits examined could be largely canalized across environments. The extent to which these results are generalizable to species with a narrower fundamental niche for some of the environmental conditions examined (e.g. artic charr) are unknown; such species might exhibit comparatively less canalization.

Transplanted brook trout populations exhibited significantly plastic reaction norms across a number of environmental variables. Body size exhibited the most plastic reaction norm, probably as a result of variable growth opportunities associated with depth and temperature (see chapter 2). Substrate composition also significantly affected plastic changes in body shape for all relative warps, with fish in ponds with a large proportion of silt substrate exhibiting consistently narrower body shape (RW 1, 3, and 4) and smaller heads as a total proportion of body size (RW 2). Narrower body forms and/or smaller heads could be a response to or a result of feeding opportunities available to salmonids in environments dominated by zoobenthic prey. Benthic morphs of arctic char, for example, can have smaller bodies with a comparatively blunted snout and smaller head, and these characteristics are thought to be adaptations to benthic feeding strategies (Hindar and Jonsson 1982, Malmquist 1992, Snorrason et al. 1994). However, benthic char forms typically have broader, less fusiform body morphologies (Kristjánsson et al. 2011), whereas brook trout transplanted to ponds with a high proportion of silt substrate exhibited narrower body forms. Substrate in source population streams is primarily rocky/gravel, with streams exhibiting a mean stream silt substrate composition of 25%. Given mean pond silt

substrate composition was 73% and many novel pond environments had substrate composed of 100% silt, it is likely that invertebrate prey communities differed between streams and ponds, although we lack the empirical data to confirm such a speculation. Switching diets can be associated with significant physiological costs (Hooker et al. 2017). Brook trout are primarily drift feeders in streams (McNicol et al. 1985), so a forced shift to a feeding strategy focused on zoobenthic foraging could have imposed additional costs. Alternatively, trout may have been forced to switch to planktonic and/or surface foraging strategies in silt dominated ponds. Such a strategy requires prolonged swimming which energetically favors narrower fusiform body forms (Peres-Neto and Magnan 2004). In either case, plastic variation in body forms associated with silt environments may not be adaptive, but instead result from reduced condition factor due to changing prey species, foraging strategies, or limited resource availability.

Implications for future research

We found limited evidence that phenotypic plasticity was released in novel environments despite the observation of plastic reaction norms for all traits examined, a wide gradient of H_o across previously isolated study populations, and a wide range of novel environmental conditions. Phenotypic variation increased with one environmental variable for only one morphological trait; due to the experimental design, we are unable to assess if additive genetic variance increased for that trait. However, significant additive variation underlies several morphological traits when these populations were reared in a common-garden setting (Wood et al. 2015), suggesting that the pattern of phenotypic release observed is consistent with, and likely due to, a release of cryptic variation. No other traits exhibited a pattern of phenotypic release in any environment. Reaction norms for brook trout morphological traits appear to be relatively conserved across environments, regardless of genomic diversity, environmental extremity, or effective population size (see Wood and Fraser 2015). As a generalist colonizing species, brook trout may retain the capacity to plastically alter or canalize functionally important phenotypes in response to a broad range of environments, regardless of contemporary conditions. Perhaps the release of cryptic genetic variation may play little role in the process of adaptation for brook trout; however, the extent to which these results can be applied to other taxonomic groups or species with different ecological niches may be limited. Future research on the effect of genomic diversity on the release of phenotypic plasticity should focus on study species known to harbor

cryptic variation and, although costly and difficult, emphasize a controlled breeding regime to directly quantify additive genetic variance underlying studied traits.

Tables

Table 1: Range of environmental characteristics of streams and novel pond environments (mean, minimum, and maximum).

Variable	Stream	Pond
pH	6.2 (4.8-7.5)	5.5 (4.4-6.7)
Temp. (°C)	13.6 (8.1-17.3)	15.1 (7.6-19.5)
Depth (cm)	19.8 (10.4-38.2)	23.7 (5.4-46.6)
% Silt Substrate	22.2 (1.8-70.8)	72.7 (0-100)
Dissolved Oxygen (mg/L)	10.8 (8.3-11.9)	9.2 (5.8-11.7)
%Veg. Cover	38.8 (13.8-72.2)	23.2 (0.0-90.7)
Conductivity (µS)	80.2 (40.9-176.9)	264.0 (46.1, 3346.0)

Table 2: Summary of pond translocations and genetic analysis of transplanted populations.

Population	*No. of ponds*	*No. of individuals translocated over 4 years*	*No. of recaptures recovered over 4 years*	*No. of individuals sequenced*	*Genomic Heterozygosity*
Bob's Cove	6	149	63	29	0.049
Blackfly	6	86	40	19	0.087
Cripple Cove	8	301	62	28	0.060
Ditchy Brook	4	31	14	18	0.069
Freshwater River	6	271	140	29	0.111
Lower Coquita	3	38	20	22	0.049
Lower Ouananiche Beck	5	83	37	19	0.068
Perdition	4	79	31	14	0.084
Still There By Chance	6	118	50	30	0.016
Upper Ouananiche Beck	8	129	74	20	0.063
Watern Cove	8	238	75	30	0.119
Whale Cove	8	185	54	25	0.083

Table 3: Number of SNPs removed at each stage of filtering

Filtration Stage	SNPs remaining
After '*populations'* module	58,126
Maximum number of SNPs per loci ≤ 4	45,106
1st SNP per locus	28,228
Heterozygosity ≤ 0.5	25,953
Global Minor Allele Freq. ≥ 0.01	4,614

Table S1: Results of model selection for genetic and habitat variables significantly associated with body size, with statistically significant (retained) terms presented in bold. Model testing was conducted using F-tests.

Parameter	df	*F*-value	*p*
V	1, 180.09	<0.001	0.986
C	1, 140.72	0.012	0.915
S	1, 53.52	0.062	0.804
H_o	1, 10.49	0.759	0.403
DO	1, 439.14	1.942	0.164
pH	1, 100.27	2.831	0.096
ID*	1, 53.67	11.235	**0.001**
Y*	3, 535.49	23.444	**< 0.001**
T*	1, 104.36	8.043	**0.005**
D*	1, 147.46	24.901	**< 0.001**

Term abbreviations: ID – Initial Density; Y – Year; DO – Dissolved Oxygen; T – Temperature; C – Conductivity; D – Depth; S - % Silt Substrate; V - % Aquatic Vegetative Cover; H_o – Observed genomic heterozygosity.

*Retained terms

Table S2: Between-year body size differences, *with statistically significant comparisons presented in bold*.

Years	Estimate	SE	df	*t*-value	*p**
2012 – 2013	-0.4074	0.1928	235.63	-2.11	0.214
2012 – 2014	0.8929	0.2210	196.14	4.04	**0.001**
2012 – 2015	0.0545	0.2322	209.72	0.24	1.000
2013 – 2014	1.3003	0.1509	69.54	8.62	<**0.001**
2013 – 2015	0.4619	0.1471	71.83	3.14	**0.015**
2014 - 2015	-0.8385	0.1662	145.87	5.05	**<0.001**

**p*-values are Bonferroni corrected to account for familywise error rat

Table S3: Results of model selection for genetic and habitat variables significantly associated with relative warps (RW) 1-4, with statistically significant (retained) terms presented in bold. Model testing was conducted using F-tests.

	RW1			RW2			RW3			RW4		
Variable	df	*F*-value	*p*	df	*F*-value	*p*	df	*F*-value	*p*	df	*F*-value	*p*
D	1, 32.58	<0.001	0.987	1, 41.54	0.006	0.937	1, 43.25	0.325	0.572	1, 34.27	0.924	0.343
V	1, 29.93	0.024	0.878	1, 39.40	1.543	0.222	1, 37.34	0.300	0.587	1, 36.90	0.086	0.771
pH	1, 28.69	0.055	0.817	1, 33.56	2.153	0.152	1, 34.67	1.765	0.193	1, 27.75	0.067	0.797
Y	3, 312.59	0.539	0.656	3, 430.16	7.234	**< 0.001***	3, 484.17	12.329	**<0.001***	3, 298.58	1.553	0.201
C	1, 27.71	0.965	0.334	1, 40.83	0.064	0.802	1, 35.58	1.502	0.229	1, 40.39	0.024	0.877
H_o	1, 10.20	1.048	0.330	1, 10.32	2.741	0.128	1, 10.67	0.069	0.797	1, 9.41	1.327	0.278
DO	1, 72.97	2.261	0.137	1, 98.36	1.630	0.205	1, 111.37	2.227	0.138	1, 110.84	4.247	**0.042***
ID	1, 22.55	2.816	0.107	1, 26.30	0.706	0.408	1, 29.11	0.218	0.644	1, 23.93	2.478	0.129
T	1, 29.86	1.927	0.175	1, 26.95	0.303	0.587	1, 31.42	0.750	0.393	1, 31.76	9.195	**0.005***
BS	1, 412.39	15.964	**< 0.001***	1, 498.46	505.950	**< 0.001***	1, 552.80	67.870	**<0.001***	1, 456.59	55.218	**<0.001***
S	1, 20.36	9.681	**0.005***	1, 25.08	6.900	**0.014***	1, 27.76	4.625	**0.040***	1, 22.35	7.448	**0.012***

Term abbreviations: BS – body Size; ID – Initial Density; Y – Year; DO – Dissolved Oxygen; T – Temperature; C – Conductivity; D – Depth; S - % Silt Substrate; V - % Aquatic Vegetative Cover; H_o – Observed genomic heterozygosity.

*Retained terms

Table S4: Results of tests to determine presence of genotype-by-environment interactions (i.e. a random environmental variable slope-by-population term), with statistically significant (retained) terms presented in bold. Model testing was conducted using likelihood ratio tests (LRTs). RW = Relative Warp.

Trait	Slope-By-Population Term	χ^2	df	p
RW 1	% Silt Substrate	2.494	1	0.114
RW 2	% Silt Substrate	0.010	1	0.922
RW 3	% Silt Substrate	0.004	1	0.952
RW4	**Dissolved Oxygen**	5.685	1	**0.017**
RW4	% Silt Substrate	0.315	1	0.575
RW4	Temperature	0.826	1	0.364
Body Size	**Temperature**	23.582	1	**<0.001**
Body Size	**Depth**	10.062	1	**0.002**

Table S5: Results of model selection for genetic and habitat variables significantly associated with phenotypic variability in body size. Model testing was conducted using F-tests.

Parameter	df	F-value	p
Ho:T	1, 52.47	2.909	0.094
Ho:D	1, 59.71	3.260	0.076
H_o	1, 9.76	0.003	0.957
Y	3, 52.48	0.203	0.894
BS	1, 65.98	0.086	0.770
St_{SD}	1, 20.04	0.917	0.350
ID	1, 29.68	1.217	0.279
T	1, 27.41	1.504	0.231
D	1, 31.17	3.005	0.093

Term abbreviations: BS – Body Size; Y – Year; T – Temperature; D – Depth; H_o – Observed genomic heterozygosity; St_{SD} – standard deviation of centroid size from native stream sample.

Table S6: Results of model selection for genetic and habitat variables significantly associated with phenotypic variability in relative warps (RW) 1-4, with statistically significant (retained) terms presented in bold. Model testing was conducted using F-tests.

	RW1			RW2			RW3			RW4		
Variable	df	F-value	*p*	df	F-value	*p*	df	*F*-value	*p*	df	*F*-value	*p*
H_o:T	1, 52.76	0.329	0.569	1, 54.40	0.094	0.760	1, 58.27	0.008	0.928	1, 56.91	0.156	0.694
H_o:S	1, 49.16	1.056	0.309	1, 56.75	0.622	0.434	1, 54.55	2.126	0.151	1, 55.21	0.178	0.675
H_o:D	1, 54.94	0.743	0.392	1, 54.31	1.065	0.307	1, 56.16	0.003	0.957	1, 58.75	0.507	0.479
H_o	1, 8.82	0.003	0.960	1, 9.19	0.018	0.896	1, 9.29	0.003	0.961	1, 9.61	2.666	0.135
D	1, 35.53	0.190	0.666	1, 35.36	0.206	0.653	1, 38.40	0.052	0.820	1, 26.25	0.046	0.832
S	1, 24.98	0.151	0.701	1, 26.89	0.002	0.964	1, 25.22	3.461	0.075	1, 23.95	0.032	0.860
BS_{CV}	1, 59.97	0.451	0.504	1, 68.11	1.568	0.215	1, 68.55	1.841	0.179	1, 59.11	0.104	0.748
Y	3, 52.63	1.057	0.375	3, 52.64	0.340	0.796	3, 54.70	0.302	0.824	3, 53.78	2.859	**0.045***
BS	1, 67.14	0.934	0.337	1, 67.93	1.491	0.226	1, 61.28	0.554	0.459	1, 62.69	0.096	0.758
St_{SD}	1, 49.52	1.679	0.201	1, 37.75	0.005	0.942	1, 52.57	0.614	0.437	1, 41.02	0.003	0.959
T	1, 32.73	6.329	**0.017***	1, 26.06	0.850	0.365	1, 19.52	0.648	0.430	1, 21.10	1.132	0.299
DO	-	-	-	-	-	-	-	-	-	1, 52.87	3.623	0.062
H_o:DO	-	-	-	-	-	-	-	-	-	1, 55.15	0.499	0.483

Term abbreviations: BS – Body Size; BS_{CV} – Coefficient of variation of body size; Y – Year; T – Temperature; D – Depth; S - % Silt Substrate; DO – Dissolved Oxygen; H_o – Observed genomic heterozygosity; St_{SD} – standard deviation of centroid size from native stream sample.

*Retained terms

Table S7: Between-year differences in body morphology for relative warp 4 (body depth), with statistically significant comparisons presented in bold.

Years	Estimate	SE	df	*t*-value	*p**
2012 – 2013	0.000297	0.000978	66.58	0.304	1.000
2012 – 2014	-0.000398	0.001013	72.19	0.393	1.000
2012 – 2015	-0.002264	0.001023	70.91	2.212	0.181
2013 – 2014	-0.000695	0.000852	69.27	0.815	1.000
2013 – 2015	-0.002561	0.000865	71.60	2.963	**0.025**
2014 - 2015	-0.001866	0.000904	71.73	2.065	0.255

**p*-values are Bonferroni corrected to account for familywise error rate.

Figures

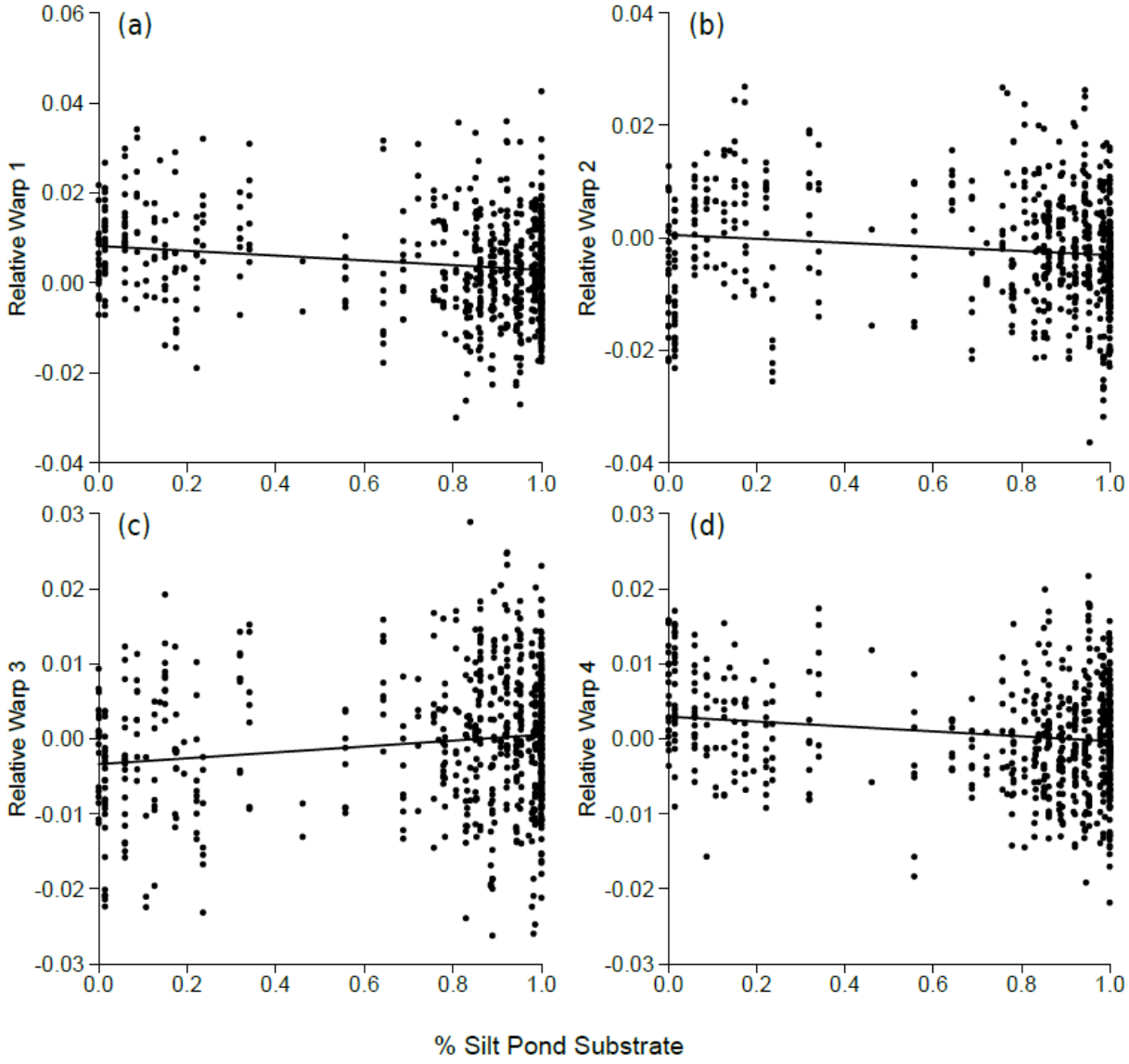

Figure 1: Effect of % silt substrate in novel pond environment on relative warps (RW) 1-4 (panels a-d, respectively). RW1 corresponds to ventral extension of the belly, RW2 corresponds to head size as a proportion of body length, RW3 corresponds to dorsal extension of the body, and RW4 corresponds to dorso-ventral body depth.

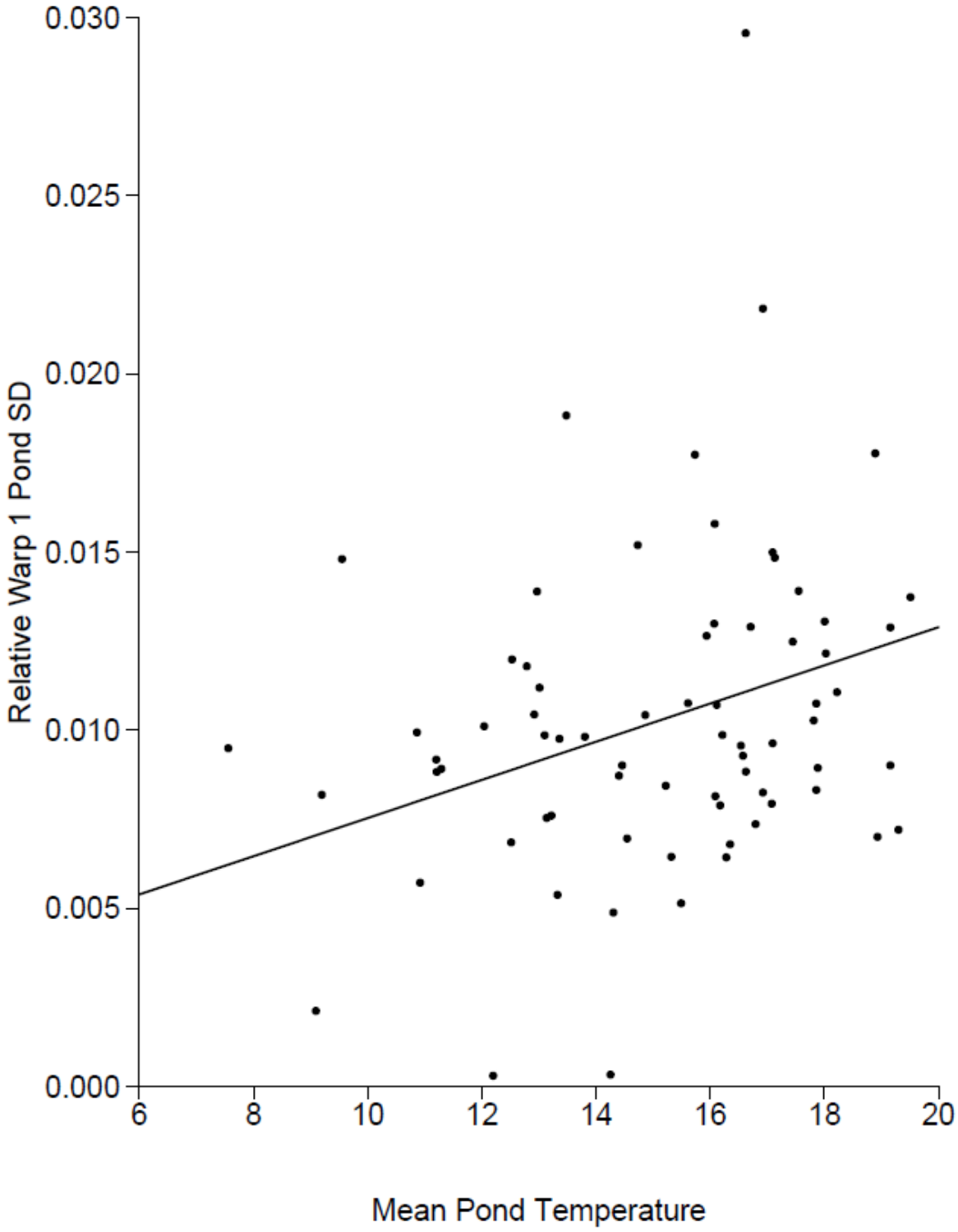

Figure 2: Effect of temperature (°C) on the release of phenotypic plasticity (within-pond standard deviation) in relative warp 1.

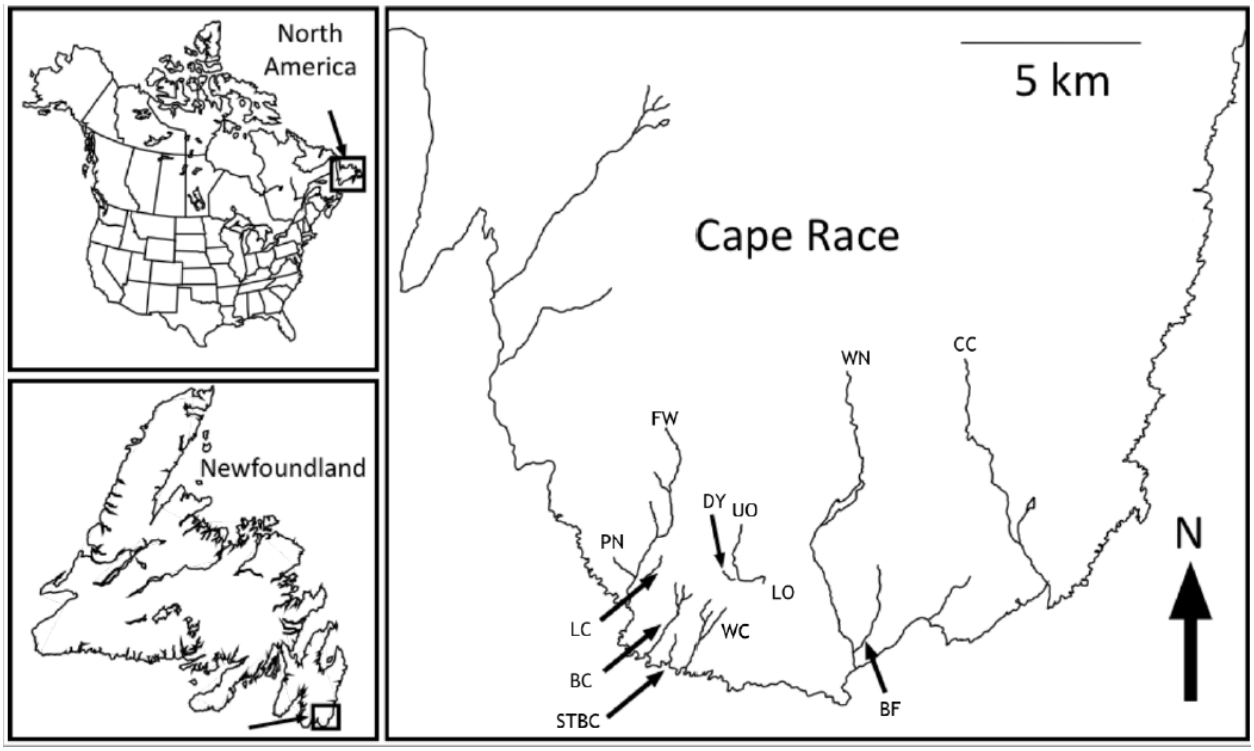

Figure S1: Brook trout populations located on Cape Race, Newfoundland, Canada. From west to east: Perdition (PD), Freshwater (FW), Lower Coquita (LC), Bob's Cove (BC), Still There By Chance (STBC), Whale Cove (WC), Ditchy (DY), Upper O. Beck (UO), Lower O. Beck (LO), Watern (WN), Lower Blackfly (LBF), Cripple Cove (CC).

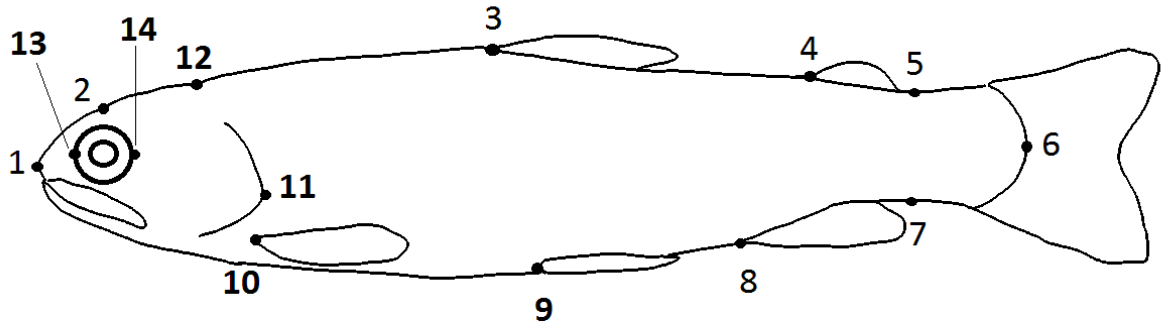

Figure S2: Landmarks for geometric morphometric analysis on brook trout. 1) anterior point of body; 2) the head directly above midpoint of the eye; 3) anterior insertion of the dorsal fin; 4) anterior limit of adipose fin; 5) dorsal position above narrowest part of the caudal peduncle; 6) posterior terminus of the caudal peduncle; 7) ventral position below the narrowest part of the caudal peduncle; 8) anterior insertion of the anal fin; 9) anterior insertion of the left pelvic fin; 10) anterior insertion of the left pectoral fin; 11) posterior point of the operculum; 12) posterior aspect of the neurocranium; 13) anterior point of left eye; 14) posterior point of left eye.

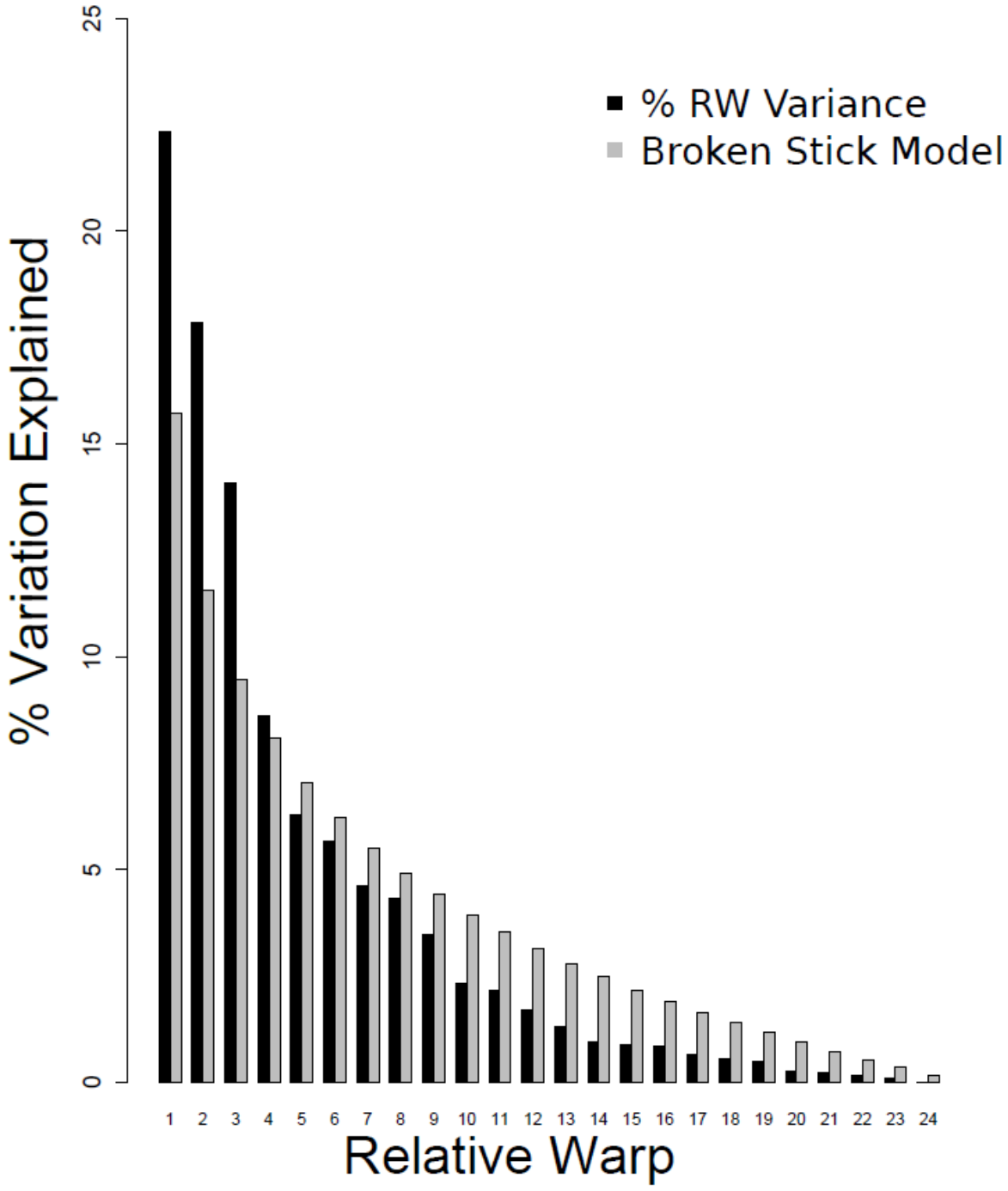

Figure S3: Proportion of total variance explained by each relative warp, compared to "broken stick" method.

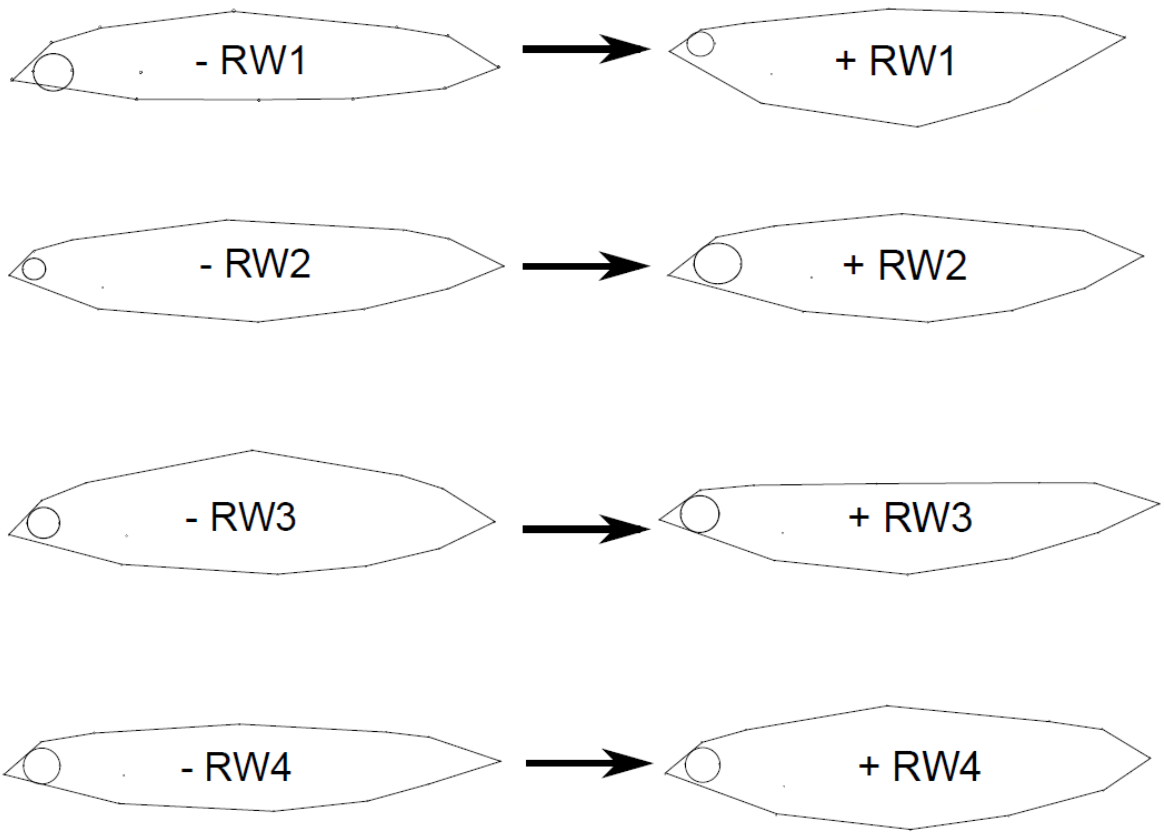

Figure S4: Body shapes associated with each relative warp. From negative to positive values, shapes represent extreme body forms for each warp.

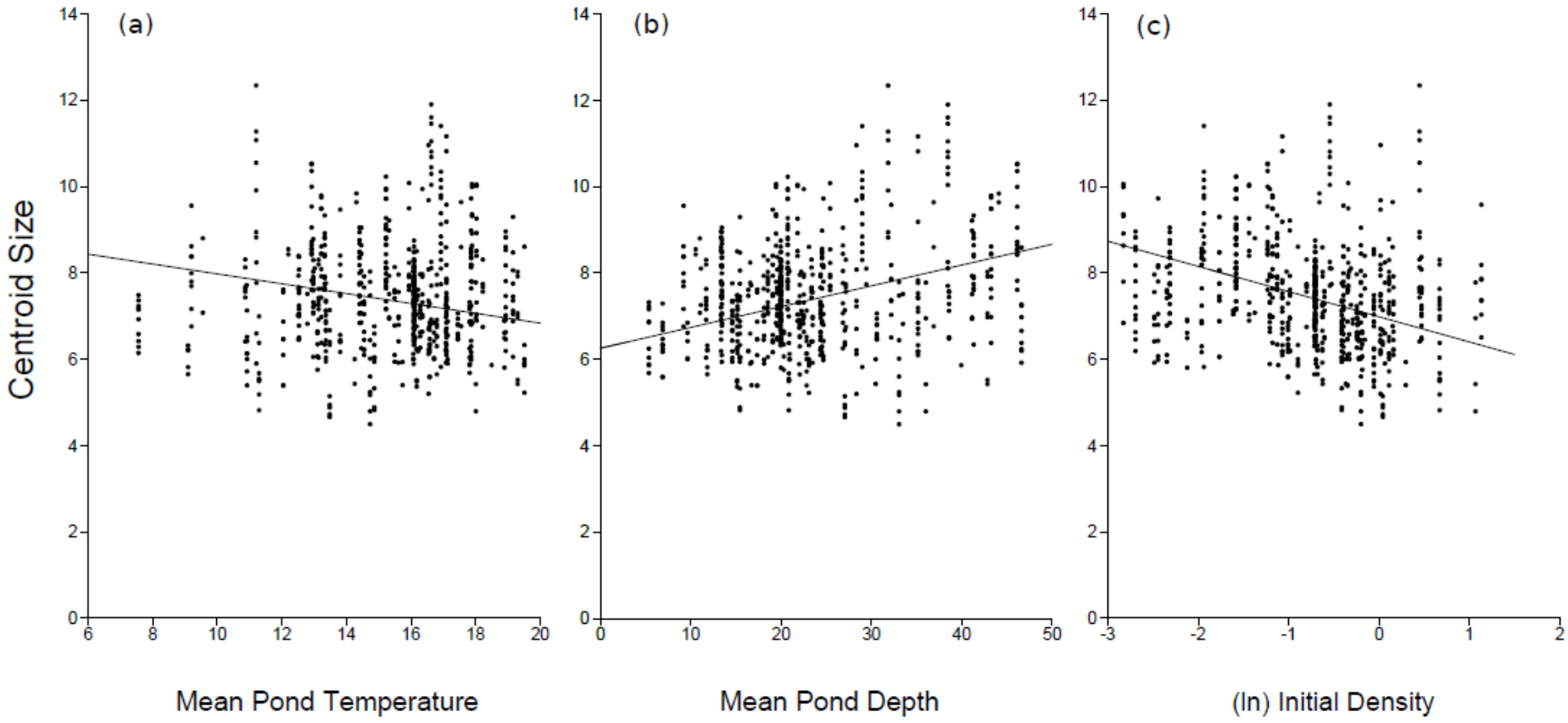

Figure S5: Effect of mean pond temperature (°C) (panel a), depth (cm) (panel b), and initial transplant density (fish/m^2) (panel c) on centroid size.

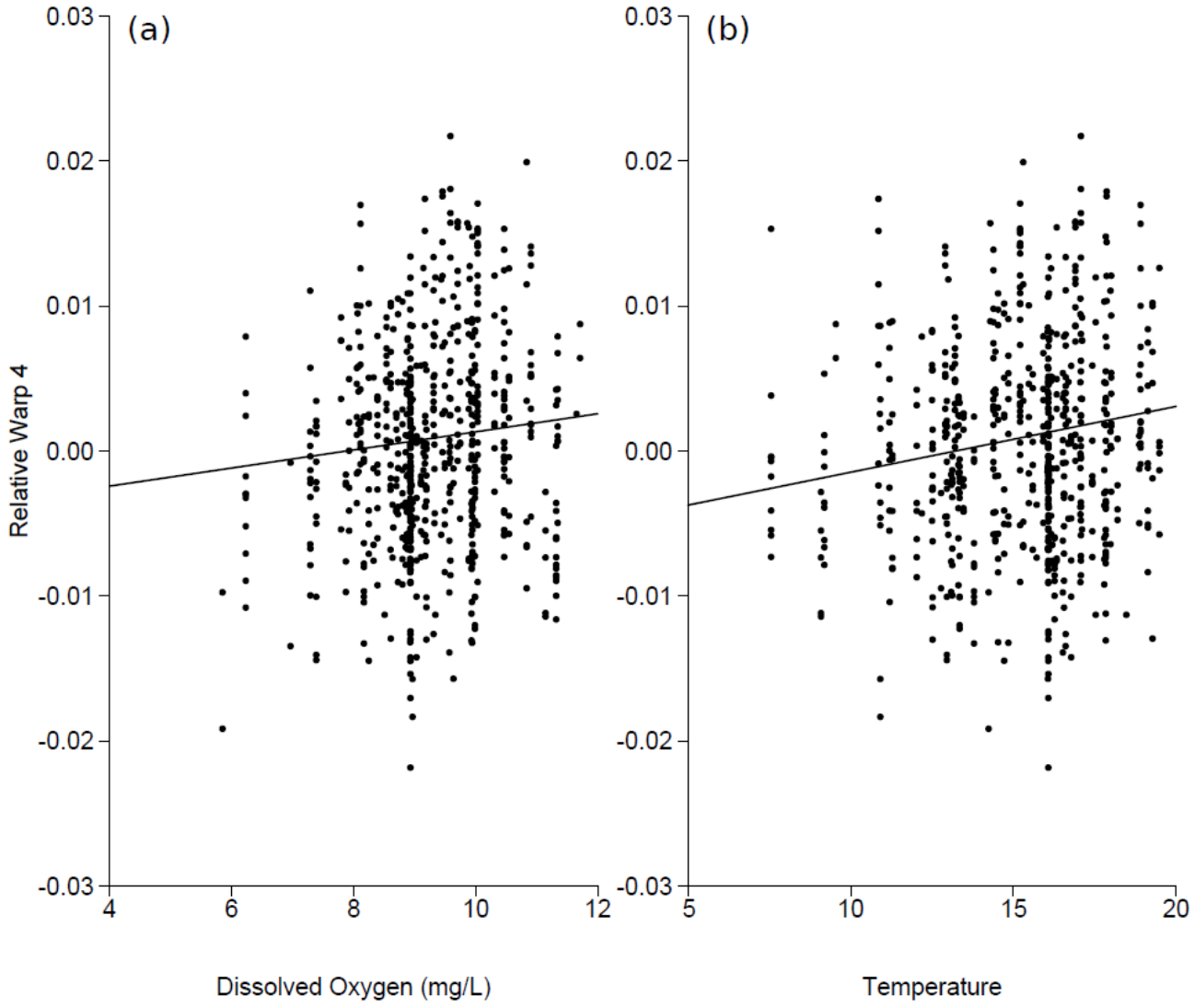

Figure S6: Effect of dissolved oxygen (mg/L) (panel a) and temperature (°C) (panel b) on relative warp 4.

Chapter 4: A critical assessment of estimating census population size from genetic population size (or vice versa) in three fishes

Keywords: Conservation Biology; Conservation Genetics; Fisheries Management; Wildlife Management; Inventory and Monitoring; Effective Population Size

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ABSTRACT

Technological and methodological advances have facilitated the use of genetic data to infer census population size (N_c) in natural populations, particularly where traditional mark-and-recapture is challenging. The effective number of breeders (N_b) describes how many adults effectively contribute to a cohort and is often correlated with N_c. Predicting N_c from N_b or vice-versa in species with overlapping generations has important implications for conservation by permitting (i) estimation of the more difficult to quantify variable and (ii) inferences of N_b/N_c relationships in related species lacking data. We quantitatively synthesized N_b/N_c relationships in three salmonid fishes where sufficient data has recently accumulated. Mixed-effects models were analyzed in which each variable was included as a dependent variable or predictor term (N_b from N_c and vice versa). Species-dependent N_b/N_c slope estimates were significantly positive in two of three species; variation in species slopes were likely due to varying life histories and reinforce caution when inferring N_b/N_c from taxonomically-related species. Models provided maximum probable estimates for N_b and N_c for two species. However, study, population, and year effects explained substantial amounts of variation (39-57%). Consequently, prediction intervals were wide and included or were close to zero for all population sizes and species; model predictive utility was limited. Cost-benefit trade-offs when estimating N_b and/or N_c were also discussed using a real-world system example. Our findings based on salmonids suggest that no short-cuts currently exist when estimating population size; researchers should focus on quantifying the variable of interest or be aware of caveats when inferring the desired variable because of cost or logistics. We caution that the salmonid species examined share life-history traits that may obscure relationships between N_b and N_c. Sufficient data on other taxa were unavailable; additional research examining N_b/N_c relationships in species with potentially relevant life-history trait differences (e.g. differing survival curves) are needed.

INTRODUCTION

Rapid technological and methodological advances in molecular genetics have increased interest in using genetic data to estimate or infer census population size (N_c), especially where counting individuals is challenging (e.g. in large populations, elusive species, or extremely remote locations) (Guschanski et al. 2009, Luikart et al. 2010, Fraser et al. 2013, Ovenden et al. 2016, Baldigo et al. 2017). While direct individual counts could be obtained from comprehensive genetic surveys (e.g. Guschanski et al. 2009), methodologies that indirectly estimate N_c from environmental DNA (eDNA) or subsamples of individuals from a population represent potentially cost-effective means through which census sizes could be estimated. Although eDNA is emerging as a potential method through which N_c could be inferred (Lacoursière-Roussel et al. 2016, Baldigo et al. 2017), its application for this purpose remains relatively novel. In comparison, the scientific literature examining methodologies for estimating the contemporary effective population size (N_e) of natural populations is relatively well-developed.

The effective size of a population is a central evolutionary parameter influencing the extent of genetic drift, inbreeding and response to natural selection in isolated populations. Contemporary N_e (as opposed to long-term N_e, see Wang 2005) represents a potentially useful tool to infer N_c because it can be linked specifically to recent cohorts and can be estimated from a (relatively) small number of genetic samples collected during a single collection event or over multiple temporal periods (Waples and Do 2008, Palstra and Fraser 2012). Understanding the conditions under which contemporary N_e (or its analogues) and N_c are associated with each other is highly valuable for conservation: it may be possible to use N_e to predict or monitor N_c (or vice-versa) provided that relationships between N_e and N_c exist among or within populations and/or taxonomic groups (Tallmon et al. 2010, Whiteley et al. 2015a, Bernos and Fraser 2016, Ovenden et al. 2016).

For species with overlapping generations, the comparison of genetic and census population size can be made by comparing N_c to how many of those adults effectively contribute their genes to a single cohort, termed the effective number of breeders (N_b) (it should be noted, however, that this is dependent on the capacity to assign individuals to specific cohorts) (Waples and Do 2010). With minimal life history information, N_b can be used to infer contemporary N_e (Waples et al. 2013) and substitute for N_e when attempting to predict N_c. N_b also provides valuable insights into the eco-evolutionary dynamics of a population because inter-annual variation in N_b may be

attributable to differences in individual adult reproductive success, family survival, and the overall number of families comprising the cohort (Waples and Antao 2014, Whiteley et al. 2015a).

Several recent studies have estimated N_b and N_c within multiple populations of the same species (e.g. Beebee 2009; Hoehn et al. 2012; Whiteley et al. 2015; Bernos and Fraser 2016; Ferchaud et al. 2016; Perrier et al. 2016). They identified important biological factors shaping N_b/N_c within species, such as habitat limitations, life history traits, or density-dependence (Belmar-Lucero et al. 2012; Whiteley et al. 2013; Bernos & Fraser 2016). Time-series of N_b and N_c revealed that the two variables were positively correlated but that N_b/N_c was variable among populations and across years. Those results provided mixed support for the usefulness of one variable to infer the other in a monitoring context (Whiteley et al. 2015a, Bernos and Fraser 2016, Ferchaud et al. 2016). By comparison, few empirical investigations of the relationship between N_b and N_c among species have been conducted (Osborne et al. 2010, Gomez-Uchida et al. 2013). Such comparisons would be extremely practical for determining how concordant N_b/N_c ratios are among taxonomically related species, an especially pertinent issue for rare species that lack data. It would also allow researchers to better understand factors contributing to variation in N_b/N_c in natural populations with contrasting biology or life history.

Most taxa still have little N_b/N_c data emerging, but sufficient data have become recently available in three fishes from the *Salmoninae* subfamily (Chinook salmon, Atlantic salmon, and brook trout); these species form the basis of our quantitative analysis of N_b/N_c relationships. The studies examining these species have largely found positive relationships between N_b and N_c (e.g. Van Doornik et al. 2011; Bernos and Fraser 2016; Ferchaud et al. 2016; Perrier et al. 2016, etc.); we collated data across studies to produced models for converting N_b to N_c (and vice versa) in each species. We then and evaluated the efficacy of using one population variable to infer the other by generating population size parameter prediction intervals under which novel previously unsampled populations with only a single known population size variable (either N_b or N_c) are likely to fall. We also explored whether N_b/N_c curves differed for taxonomically related species and if they could be used to infer population size parameters across species. Lastly, the monetary cost-benefit trade-offs of estimating N_b or N_c are discussed using a real-world example system in which N_b/N_c estimates were obtained across twelve populations.

MATERIALS AND METHODS

Primary Literature review

To find articles in which both N_b and N_c estimates were obtained for the same populations, keyword searches were conducted on the academic search engine ISI Web of Science™. A complete keyword search for "Effective number of breeders" was performed. Relevant references within retrieved studies were also searched for usable data. The goal of the analysis was to derive linear relationships between N_b and N_c across multiple species. A particular species was therefore only included in the final dataset if a minimum of 10 total N_b/N_c estimates from at least three different populations were found; the only taxa with species for which this data requirement was satisfied were salmonids. A subsequent search of "Effective population size salmon*" was subsequently conducted to find any additional salmonid references missed by the initial search; this search found a single additional article. In most cases, N_c was "correctly linked" with brood year; that is, each N_b estimate generated from a cohort was matched with the N_c estimate of the parental generation. Only in two cases were N_b and N_c incorrectly linked; these were estimates taken from populations in which N_c and N_b were estimated for the same year (i.e. researchers only sampled the population once). Both estimates were still included in the final dataset.

Multiple methods exist to estimate N_b which make use of either linkage disequilibrium, heterozygote excess, molecular coancestry, or sibship frequency information obtained from genetic markers (Wang 2016). Although there is currently debate regarding which estimators perform best under a variety of scenarios (e.g. the violation of assumptions necessary for the linkage disequilibrium method such as random mating, no migration, etc.) (see Gilbert and Whitlock 2015; Wang 2016; Waples 2016) the most commonly used estimator in our literature survey was the program LDNe (Waples and Do 2008). This program makes use of linkage disequilibrium information to estimate N_e/N_b and is one of the most accurate programs currently available (Gilbert and Whitlock 2015). Furthermore, Bernos and Fraser 2016 included a comparison between Colony (which uses the sibship method) and LDNe and found a stronger link between N_b and N when the LDNe method was used. To reduce potential bias associated with different estimators and/or methods, only N_b estimates obtained from LDNe were therefore used.

One potential issue that emerged with using data obtained from LDNe was the inconsistent use across studies of critical p-value thresholds, which are used to exclude alleles with low frequencies from N_b estimation; low frequency alleles can cause bias in N_b estimates (Waples and

Do 2010). This problem is most apparent at low sample sizes, which require higher critical p-values (Waples and Do 2010); only N_b estimates derived from ≥ 30 samples were therefore retained (as in Johnstone et al. 2013). Similarly, N_b estimates which included "infinity" as an upper confidence limit were excluded from the primary dataset. When N_b and N_c estimates were contained in figures the ImageJ program (Abràmoff et al. 2005) was used to extract the data.

Quantitative analysis

The efficacy of predicting both N_c from N_b and N_b from N_c was assessed using generalized linear mixed effect models (GLMMs). To generate models from which we could derive predictions, we evaluated the effect of N_b on N_c (and vice versa) across multiple species using the MCMCglmm package (Hadfield 2010) in R (version 2.13.0; R Development Core Team 2011) with a Poisson distribution and a log-link function given that census data represent counts of discrete individuals (as in Bernos and Fraser 2016). MCMC chains were run for 1 000 000 iterations with a "burn in" period of 100 000 and thinning intervals of 50; the posterior distribution was therefore sampled > 10 000 times to obtain model parameters and associated 95% posterior density credible intervals (CI). Posterior traces and autocorrelation values were examined visually to evaluate and verify model convergence and mixing. The default (weakly informative) priors were used for all models.

Posterior modes for N_c or N_b were calculated from models in which N_b or N_c (respectively) were included as a continuous fixed effect and 'species' was included as a categorical fixed effect. An interaction between both fixed effects terms was also included. Population, study and year-within-study terms were included as random effects to account for issues of non-independence in the data. The year random effect was nested within study except when studies were conducted by the same group of researchers on the same populations, in which case year was nested across the relevant studies. Heterogeneous variances for the residuals were specified using the *idh* function; residual error variance was allowed to differ for each level of the species variable.

As population size becomes large it becomes increasingly difficult to confidently estimate N_b or N_e (Waples and Do 2010). Models were also fitted that allowed us to explore whether residual variance changed with the fixed effect population size (N_b or N_c) variable. This was accomplished by fitting an observation-level random effect of the form "idh(species:sqrt(1/ln(N_x))):units" (when testing if variance decreased with the relevant population

size variable i.e. N_c or N_b) or "idh(species:sqrt(N_x)):units" (when testing if variance increased with the relevant population size variable) (as in Wood et al. 2016). Significance of this term was evaluated by comparing 95% CIs of N_x-related residual error estimates at five population sizes representative of the gradients present in our dataset: 20, 50, 100, 300, and 600 for models predicting N_c from N_b, and 50, 100, 500, 1 000, and 10 000 for models predicting N_b from N_c. If CIs for the N_x-related residual variances overlapped between all representative population sizes, the heteroscedastic error term was subsequently removed.

Model performance was evaluated by calculating both marginal and conditional R^2 (Nakagawa and Schielzeth 2013); slight modifications to the code described in the paper had to be made to accommodate the modelling of heterogeneous residual variances at the species level. Multiple R^2 values were computed for each model at the species level.

The efficacy and practicality of predicting N_c or N_b from a novel population (i.e. with random effects marginalised) was evaluated by examining 95% prediction intervals generated for each model across a gradient of N_c (when predicting N_b) or N_b (when predicting N_c). For most natural populations, N_b (or N_e) is almost always less than N_c (Kalinowski and Waples 2002, Waples et al. 2013). Hence, when predicting N_b from N_c for natural populations, biologically meaningful and informative predicted values should typically fall within the predictor N_c value and 0. If upper N_b prediction interval values were greater than the predictor N_c values used to obtain them, the upper prediction intervals were considered fundamentally uninformative. When predicting N_b from N_c, lower 95% prediction interval values were considered "informative" only when they did not include (or were extremely close to) zero. When predicting N_c from N_b, meaningful predicted values could include any value greater than the predictor N_b value; both upper and lower prediction interval values were considered "informative" at a given size only when they were greater than the predictor N_b value used to obtain them.

A supplementary analysis was conducted that predicted N_c from the lower N_b CI reported in each paper as these are relevant for many conservation situations. Namely, when populations are difficult to sample effectively (i.e. populations are too large or individual samples are difficult to obtain) it can be challenging to obtain bounded N_b point estimates, in which case lower CI may be more informative (Waples and Do 2010). Using exclusively lower CI estimates allowed us to incorporate N_b estimates that had infinite upper CI, which increased the number of estimates in the

dataset by 42. However, prediction intervals calculated from N_b lower CI were always wider than models generated from point estimates (Figure S1); these models were therefore not reported.

RESULTS

Literature review

Of the 209 papers reviewed on N_b/N_c across taxa, 11 contained data that met inclusion criteria. The final dataset contained 144 individual N_b/N_c estimates from 40 populations of three species: brook trout (15 populations), Atlantic salmon (14 populations), and Chinook salmon (11 populations) (Table 1). Any duplicate N_b/N_c estimates across studies on the same populations were removed from the dataset. No other species had 3 or more populations for which N_b and N_c data had been estimated in adequate quantities (i.e. ≥ 10 data points total). N_b/N_c estimates for the three salmonid species included in this analysis were typically obtained across multiple years of sampling involving the genotyping of thousands of individuals; they represent the best data presently available in the literature for examining the predictive capacity of N_b to predict N_c (or vice versa).

Predicting N_b from N_c

No evidence was found that residual error exhibited heteroscedasticity associated with N_c. Estimates of residual variance did not change with N_c; 95% CIs for residual error estimates overlapped for all population size ranges compared (Appendix 3). The heteroscedastic error term was therefore dropped from all subsequent analyses.

The slope of the relationship predicting N_b from N_c differed significantly between some species. Slope estimates were significantly or marginally lower for Atlantic salmon relative to Chinook salmon ($P_{mcmc} = 0.0413$, Table 2) and brook trout ($P_{mcmc} = 0.076$, Table 2), respectively; 95% CIs for estimated differences barely overlapped zero. The slope of the relationship predicting N_b from N_c differed marginally from zero for Atlantic salmon ($P_{mcmc} = 0.078$, Table 3, Fig. 1), whereas posterior mode slope estimates for brook trout and Chinook salmon were significant and positive with CIs not overlapping zero ($P_{mcmc} < 0.001$ and $P_{mcmc} < 0.001$, Table 3, Fig. 1). Slope estimates did not differ between brook trout and Chinook salmon ($P_{mcmc} = 0.563$, Table 2).

The N_c and species terms accounted for 34% to 42% of the variation present in the data, depending on species; the population, study, and year random effects terms accounted for 39% to 47% of the variation (Table 3).

Prediction intervals for Atlantic salmon were uninformative as a result of a lack of a significant relationship predicting N_b from N_c (i.e. slope estimate CIs overlapped zero). Lower 95% prediction intervals for brook trout and Chinook salmon were uninformative; they included (or were extremely close to) zero for both species (Fig. 1). Upper 95% prediction interval values were informative for most N_c values for brook trout: upper N_b prediction intervals were lower than predictor N_c values for census sizes greater than approximately 100 individuals. Chinook salmon upper prediction interval values were meaningful only for large N_c values; upper 95% prediction interval values were lower than predictor N_c values for census sizes greater than approximately 650 individuals.

Predicting N_c from N_b

No evidence was found that residual error exhibited heteroscedasticity associated with N_b. The CIs for residual error estimates also overlapped for all population size ranges compared (Appendix 3). The heteroscedastic error term was therefore dropped from all subsequent analyses.

The slope of the relationship predicting N_c from N_b also differed significantly between some species. The slope estimates for Atlantic salmon were significantly lower than for Chinook salmon (P_{mcmc} = 0.011, Table 2) and brook trout (P_{mcmc} = 0.031, Table 2). The slope of the relationship predicting N_c from N_b did not differ from zero for Atlantic salmon (P_{mcmc} = 0.550, Table 3, Fig. 2). Posterior mode slope estimates for both brook trout and Chinook salmon were again significantly positive and CIs did not overlap zero (P_{mcmc} = 0.009 and P_{mcmc} = 0.001, Table 3, Fig. 2); slope estimates also did not differ between brook trout and Chinook salmon (P_{mcmc} = 0.941, Table 2).

The N_b and species terms accounted for 32% to 38% of the variation present in the data, depending on species; the population, study, and year random effects terms accounted for 53% to 57% of the variation.

Prediction intervals for Atlantic salmon were uninformative as a result of a lack of a significant relationship predicting N_c from N_b (i.e. slope estimate CIs overlapped zero). Lower 95% prediction intervals for brook trout and Chinook salmon were uninformative; they included (or were extremely close to) zero for both species (Fig. 2). Upper 95% prediction interval values were meaningful for all N_c values for both species: upper prediction intervals for N_c were always greater than predictor N_b values for all population sizes.

DISCUSSION

Using N_c to predict N_b or using N_b to predict N_c

Recent studies have suggested that N_b and N_c were correlated among intraspecific populations and one could be used to predict the other if N_b/N_c relationships were well characterized for a particular species (Osborne et al. 2010, Bernos and Fraser 2016, Ferchaud et al. 2016). To formally test this hypothesis, we modelled the relationship between N_b and N_c using a database of 40 populations from three salmonid fishes and generated prediction intervals using those models to determine efficacy of predicting one population size variable from the other. The 95% prediction intervals for some species provided potential maximum thresholds for some population size variables. For example, a brook trout population with an N_c of approximately 1000 is not likely to have an N_b higher than 300. However, the practical usefulness of this upper threshold varies depending on the species and the estimated variable.

Brook trout and Chinook salmon had potentially informative and biologically meaningful upper prediction intervals for N_c when predicted from N_b. Upper prediction thresholds, however, were up to almost two orders of magnitude larger than the predictor N_b values, placing them on the extreme end of N_b/N_c ratios documented in wild salmonid populations (Palstra and Fraser 2012). Furthermore, while "informative" upper thresholds for N_b can be predicted from moderate and large N_c values for brook trout and Chinook salmon, these thresholds may not be informative from a practical management standpoint because, from a conservation genetics standpoint, N_e is often the variable of more interest. Both N_e and N_b are likely to be less than N_c in natural populations (Waples et al. 2013); the criteria for biologically meaningful predicted N_b values would, however, be even more stringent when translating predicted N_b values to N_e values given that N_b is typically less than N_e. N_b in brook trout, for example, can range from 0.34 to 0.68 of N_e, depending on the conversion methodology used (Bernos and Fraser 2016).

It is also unsurprising that N_b upper prediction interval values overlapped with predictor N_c values at small N_c in brook trout and small-to-moderate N_c in Chinook salmon; as N_c increases, the N_b/N_c ratio tends to decrease because of density dependent effects on reproduction (Whiteley et al. 2015a, Bernos and Fraser 2016, Ferchaud et al. 2016). The models also did not accurately provide minimum prediction thresholds for both population size variables; for all species, lower prediction intervals at all N_b or N_c sizes either included or were extremely close to zero across all population sizes examined.

Recent empirical studies have shown that changes in N_b do not always track changes in N_c within a population over time (Bernos and Fraser 2016). Primary studies also reported that spatial variation among populations in N_b/N_c ratios was approximately two-fold greater than temporal variation within populations for two of the three species in our synthesis (Bernos and Fraser 2016, Ferchaud et al. 2016). Similarly, study, population, and year level random effects accounted for substantial amounts of variation in our analysis (between 39-57%). This variability in N_b/N_c is likely a result of several biological processes acting differentially and simultaneously within and among populations, including the degree of population connectivity (Fraser et al. 2004, Lamy et al. 2012, Gomez-Uchida et al. 2013), environmental conditions (Whiteley et al. 2015a, Bernos and Fraser 2016), or ecological differences (Belmar-Lucero et al. 2012, Waples et al. 2013). Such population variability present in both N_b and N_c measurements probably affected the accuracy and precision of predictions, limiting the utility of the models for predicting N_b or N_c for novel, non-sampled populations.

Relationship between N_b and N_c among three salmonid species

Another primary study objective was to assess whether the trajectory and magnitude of the relationship between N_b and N_c differed among taxonomically related species; our results provide mixed support for this at the Salmoninae subfamily level. A general positive correlation between N_b and N_c was observed in brook trout and Chinook salmon: larger populations tend to have larger N_b. However, the slope estimates for Atlantic salmon predicting N_c from N_b or N_b from N_c were either not significantly different from zero or only marginally different from zero. Therefore, i) taxonomically related species should not be assumed to exhibit similar N_b/N_c ratios; and ii) ecological and life history characteristics of naturally spawning Atlantic salmon could buffer small populations against a loss of genetic diversity.

Reproductive life histories differ markedly amongst salmonids. While male brook trout exhibit variable ages at maturity (Hutchings 1993) and male Chinook salmon exhibit alternative reproductive phenotypes (Berejikian et al. 2010), male Atlantic salmon exhibit one of two extreme reproductive phenotypes: an early maturing freshwater phenotype (1-2 years of age) or an anadromous phenotype (typically 4-6 years of age) (Myers et al. 1986, Hutchings and Jones 1998). In some populations, up to 80% of males delay or forgo oceanic migration to mature in freshwater (Myers et al. 1986) at a size much smaller than their anadromous conspecifics (Hutchings 1988).

The presence of the early maturation phenotype is well known to have a positive influence on N_e by balancing sex-ratios, decreasing variance in reproductive success, and increasing outbreeding between cohorts within a population (Jones and Hutchings 2001; Saura et al. 2008; Johnstone et al. 2013; Perrier et al. 2014).

The primary literature N_c estimates excluded early maturation phenotypes ('parr') in all but two (landlocked) Atlantic salmon populations. Most N_c estimates are actually estimates of anadromous adults only and therefore underestimate the number of reproductive individuals within a population (Myers 1984, Perrier et al. 2014). This very likely explains the lack of relationship between N_c and N_b for Atlantic salmon; observed N_b/N_c ratios are probably upwardly biased because the male alternative phenotype may buffer N_b estimates when male anadromous numbers are small (Johnstone et al. 2012, Ferchaud et al. 2016). Future research on any species should include all reproductive phenotypes when estimating N_c.

N_b/N_c relationships were similar in brook trout and Chinook salmon, with N_b tending to increase at a similar rate with N_c. These species have substantial differences in life-histories (e.g. semelparity vs. iteroparity, obligate vs. facultative anadromy, etc.) but their spawning behavior can be similar. Both, for example, prefer spawning habitat with hypoheic upwelling (Curry and Noakes 1995, Geist and Dauble 1998, Geist 2000) and spawn at high densities; brook trout have been observed to exhibit aggregate spawning (Blanchfield and Ridgway 1997, Belmar-Lucero et al. 2012) and Chinook salmon spawn in clusters at densities much higher than Atlantic salmon (Fleming 1998, Geist and Dauble 1998). As N_c increases within populations in both species similar density-dependent issues may emerge (i.e. mate competition, nest superimposition, etc.) and affect N_b.

Overall, the among species comparisons suggest that extrapolating N_b or N_c estimates from N_b/N_c curves for species related at the family/subfamily taxonomic level may, in some cases, over- or underestimate population size estimates; mixed evidence was found that N_b/N_c relationships differed between these species, with observed differences likely a result of different species-level life-history characteristics. If N_b/N_c relationships for a taxonomically related species are used as "proxy" population parameters for another "data-deficient" species, careful consideration should be taken to evaluate life-history and behavioral similarities to determine if such an extrapolation is valid or meaningful.

Cost-benefit consideration in quantifying N_b or N_c to infer the other

Conservation resources are often limited; it is therefore important to consider the relative costs of quantifying N_c and N_b in wild populations given the degree of uncertainty in converting one to the other. To help other researchers considering similar research projects, we provided an example of the comparative costs of estimating N_c and N_b in a series of stream brook trout populations of varying size from Cape Race, Newfoundland, Canada (Table 4). This was based on one of the largest empirical studies of N_b/N_c to date (Bernos and Fraser 2016; see Table 1). Intriguingly, the relative costs of quantifying N_c and N_b were very similar. Costs unsurprisingly increased with increasing population size: in general, more labour resources were required to estimate N_c and more consumables were required to estimate N_b using molecular markers. Given this, the choice of estimating N_c or N_b may depend largely on how much confidence one desires in estimating either variable specifically while balancing other considerations. For example, at Cape Race estimating N_c with accuracy and precision is feasible but can be invasive, requiring the tagging of many adults within streams (especially for large populations). Conversely, estimating N_b is arguably less invasive in relying on sampling juvenile cohorts that naturally experience density dependence, but these N_b estimates may only translate into maximum estimates of N_c.

Future research

The number of populations with data available for each species in our models was modest (11-15 per species, limited to the *Salmoninae* subfamily). The species examined in this study (salmonids) may share life-history traits that could potentially obscure the relationship between N_b and N_c. Salmonids, for example, are characterized by type-III survival curves; species with high fecundity and juvenile mortality typically exhibit low N_e/N_c ratios (Palstra and Ruzzante 2008). The salmonid species examined also exhibit high variance in reproductive success (Blanchfield et al. 2003, Tentelier et al. 2016). Relationships between N_b and N_c for species with higher N_b/N_c ratios or lower variance in N_b over time could be stronger. This review examined data for all taxa, but sufficient data was available only for species from the *Salmoninae* subfamily; unfortunately, the data necessary to examine N_b/N_c relationships among other taxonomic groups with differing life-history characteristics are not available in the scientific literature at this time.

While several other species (both salmonid and non-salmonid) did have studies in which both N_b and N_c were estimated (see Appendix 4) they were excluded from our final dataset for

three reasons: i) population size variables were only estimated for one or two populations across studies in each species; ii) adequate data did not exist to generate robust species curves (i.e. ≥ 10 datapoints); or iii) LDNe was not used to obtain N_b estimates. As further studies examine N_b/N_c relationships within a variety of taxa, it may be possible to generate more robust predictive models and increase their practical utility for conservation purposes.

This study focused partially on the practicality of predicting N_b or N_c for novel, previously unsampled populations based on relationships generated from recently published data. While these predictive models were somewhat limited in their practical applications, it may still be possible to use these models to reliably infer one population size variable from the other for populations with well-established baseline data. Population and temporal model terms often account for a significant component of the variation observed in population size terms (Ferchaud et al. 2016); with enough temporal data, it may be possible to reliably track changes in one population size variable through the other (but see Bernos and Fraser (2016)). Although outside the scope of this study, future research could examine under what conditions this could be reliably carried out. For example, how many years of historical data are necessary to reliably track a given population? Are N_b/N_c relationships in some species more variable over time than others? Are certain ecological or life-history traits among populations associated with more stable N_b/N_c relationships?

The extent to which the N_b/N_c relationships explored herein apply to differing ecotypes of the explored species is also unknown. Salmonids are an extremely plastic taxon; many species have multiple life-histories and/or inhabit a wide range of habitats. The brook trout populations represented in this study, for example, are largely lentic; whether the modelled N_b/N_c relationships could be extrapolated to lotic or anadromous populations remains undetermined.

Finally, we found no evidence for heteroscedasticity in any of the modelled N_b/N_c relationships, although there have been indications of this across population size gradients in studies with a large number of populations (Bernos and Fraser 2016). Therefore, future studies are encouraged to continue to account for this potential heteroscedasticity, particularly given that it becomes increasingly difficult to estimate genetic population size variables (N_b, N_e) for large populations (Waples and Do 2010).

Conclusions

Although estimating the maximum number of adults present in a given population could help guide management and conservation decisions, the upper prediction intervals determined herein generally represented documented taxonomic extremes for N_b/N_c ratios in salmonids and lower prediction intervals were largely uninformative; predicting a precise N_b or N_c estimate for a novel population based off of a single population size variable is, with current data available, not realistically possible. While N_c prediction intervals generated from N_b estimates were marginally worse than prediction intervals in salmonids generated from other molecular data (e.g. eDNA in Baldigo et al. 2017), realizing the full potential of the anticipated conservation applications of genetic techniques to predict and estimate N_c (e.g. Luikart et al. 2010) will realistically require the accumulation of more data.

Molecular technologies and methods are rapidly advancing and could represent a practical means of estimating N_c in the future. However, researchers should be cognizant of the limitations of using one population size variable to infer the other; researchers and/or managers should, whenever possible, focus efforts on quantifying the population size variable of interest except when the costs/logistics of measuring that variable are prohibitive. Further research is also necessary to determine whether less variable relationships exist between N_b and N_c for other taxonomic groups with differing life-history characteristics.

Supporting Information

Unpublished N_b/N_c estimates for Atlantic salmon populations at Cape Race, Newfoundland (Appendix S2), heteroscedastic residual error estimates for N_b and N_c relationships (Appendix S3), species for which limited Nb/Nc data are published (Appendix S4), and N_c prediction intervals generated from N_b lower CIs (Figure S1) are available online.

Tables

Table 1: Published studies examining N_b/N_c relationships amongst the three study species.

Authors	Year	Species	Number of Populations	Total N_b/N_c estimates
Johnstone et al.	2012	*Salmo salar*	1	8
Palstra et al.	2009	*Salmo salar*	2	2
Perrier et al.	2014	*Salmo salar*	1	1
Perrier et al.	2015	*Salmo salar*	9	23
Bernos et al.	Submitted*	*Salmo salar*	2	4
Ferchaud et al.	2016	*Salmo salar*	9	19
Whiteley et al.	2015	*Salvelinus fontinalis*	2	12
Bernos and Fraser	2016	*Salvelinus fontinalis*	11	31
Ruzzante et al.	2016	*Salvelinus fontinalis*	2	2
Van Doornik et al.	2011	*Oncorhynchus tshawytscha*	5	15
Van Doornik et al.	2013	*Oncorhynchus tshawytscha*	6	27
		Overall totals	40**	144

*See Supporting Information (Appendix 2)

**Some populations were examined more than once across studies

Table 2: Between-species slope estimate contrasts and 95% credible intervals when predicting N_b from N_c and N_c from N_b. AS = Atlantic salmon, CS = chinook salmon, BT = brook trout.

N_b from N_c		N_c from N_b	
Contrast	Estimate	Contrast	Estimate
AS vs CS	-0.336 (-0.658, -0.016)	AS vs CS	-0.518 (-0.895, -0.102)
AS vs BT	-0.269 (-0.510, 0.031)	AS vs BT	-0.488 (-0.945, -0.037)
BT vs CS	-0.066 (-0.377, 0.189)	BT vs CS	-0.127 (-0.557, 0.519)

Table 3: Slope and intercept estimates with 95% credible intervals for models predicting N_b from N_c and N_c from N_b for three salmonid species.

N_b from N_c				
Species	Intercept	Slope	Marginal R^2	Conditional R^2
Atlantic salmon	3.705 (2.335, 5.266)	0.195 (-0.029, 0.429)	0.424	0.857
Brook trout	0.932 (-0.329, 2.480)	0.449 (0.278, 0.611)	0.394	0.865
Chinook salmon	1.200 (-0.411, 2.761)	0.528 (0.303, 0.765)	0.343	0.737
N_c from N_b				
Species	Intercept	Slope	Marginal R^2	Conditional R^2
Atlantic salmon	5.821 (4.655, 7.013)	0.067 (-0.141, 0.272)	0.376	0.941
Brook trout	4.932 (2.824, 6.449)	0.590 (0.133, 0.976)	0.376	0.902
Chinook salmon	2.992 (1.537, 4.527)	0.558 (0.236, 0.902)	0.321	0.856

Table 4: Example cost-benefit trade-offs associated with estimating N_c and N_b in wild populations, based on one of the largest N_b/N_c studies to date conducted on brook trout occupying small streams in Cape Race, Newfoundland, Canada (Bernos and Fraser 2016). Expenses are approximate and in CDN dollars.

Expense	Small population N_c = 50-500	Medium population N_c = 500-1500	Large population N_c = 1500-10000
N_c estimation from mark-recapture			
Field labour (person days)	$180 (1.2)	$360 (2.4)	$600 (4.0)
Equipment use and maintenance demands	$35	$50	$95
Office labour (person days)	$20 (0.13)	$20 (0.13)	$20 (0.13)
Miscellaneous field expenses*	$200	$225	$715
Total cost, N_c estimation	*$435*	*$655*	*$1460*
N_b estimation using molecular markers			
Field labour (person days)	$75 (0.5)	$150 (1.0)	$225 (1.5)
Equipment use and maintenance demands	$65	$110	$150
Molecular lab and office labour (person days)	$180 (1.20)	$255 (1.70)	$330 (2.20)
Molecular consumables†	$240	$440	$640
Total cost, N_b estimation	*$560*	*$955*	*$1345*

*Does not include travel expenses to/from field site (gas/food/accommodation), nor travel expenses for the marking event (these would be equivalent for N_b and N_c estimation).

†Based on 10-15 microsatellite loci, and sample sizes of n=35, 65, and 95 for small, medium and large populations, respectively.

Figures

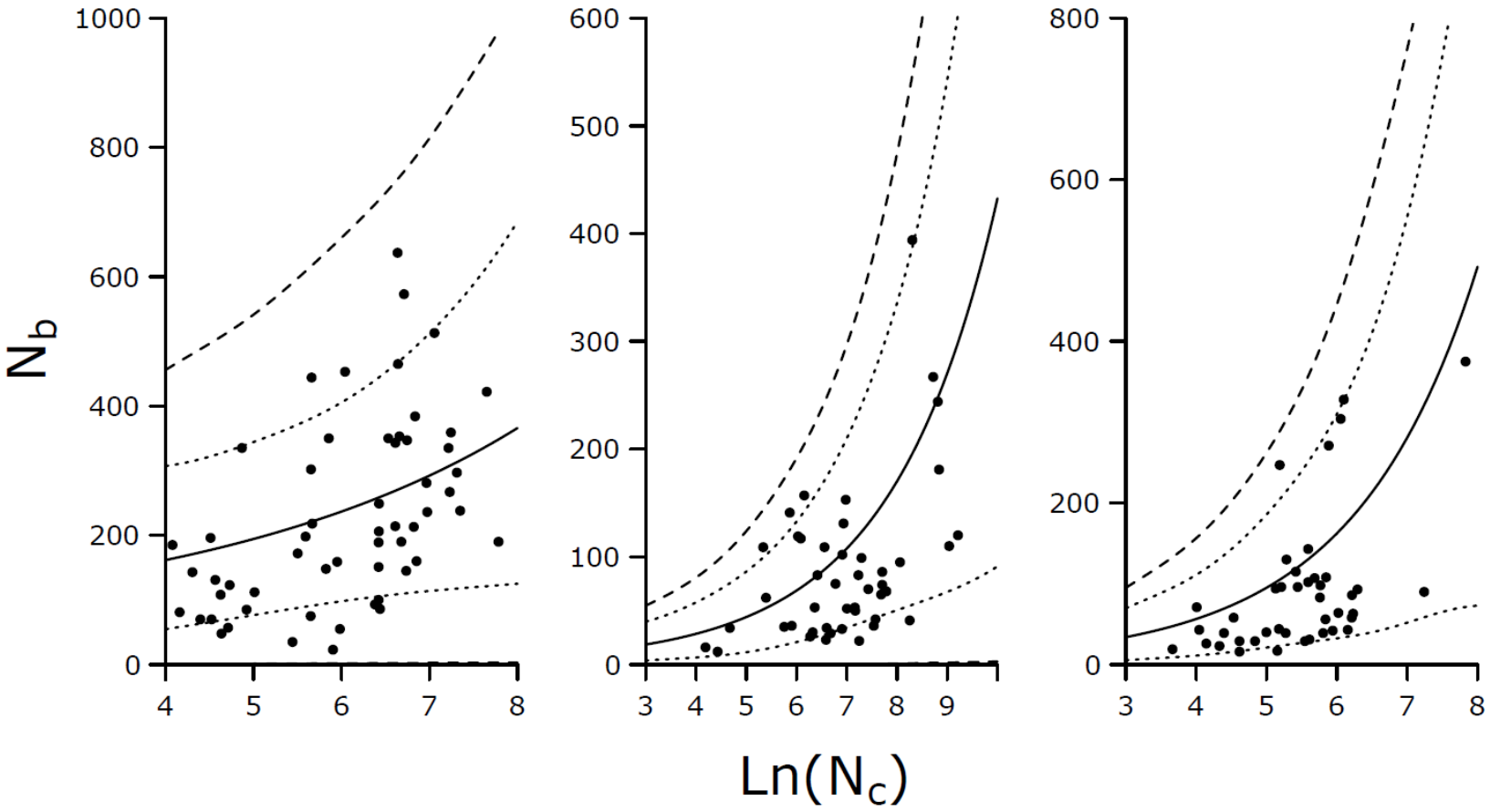

Figure 1: Relationship predicting N_b from N_c in Atlantic salmon (a), brook trout (b), and Chinook salmon (c). Dotted lines represent 95% credible intervals; dashed lines represent 95% prediction intervals.

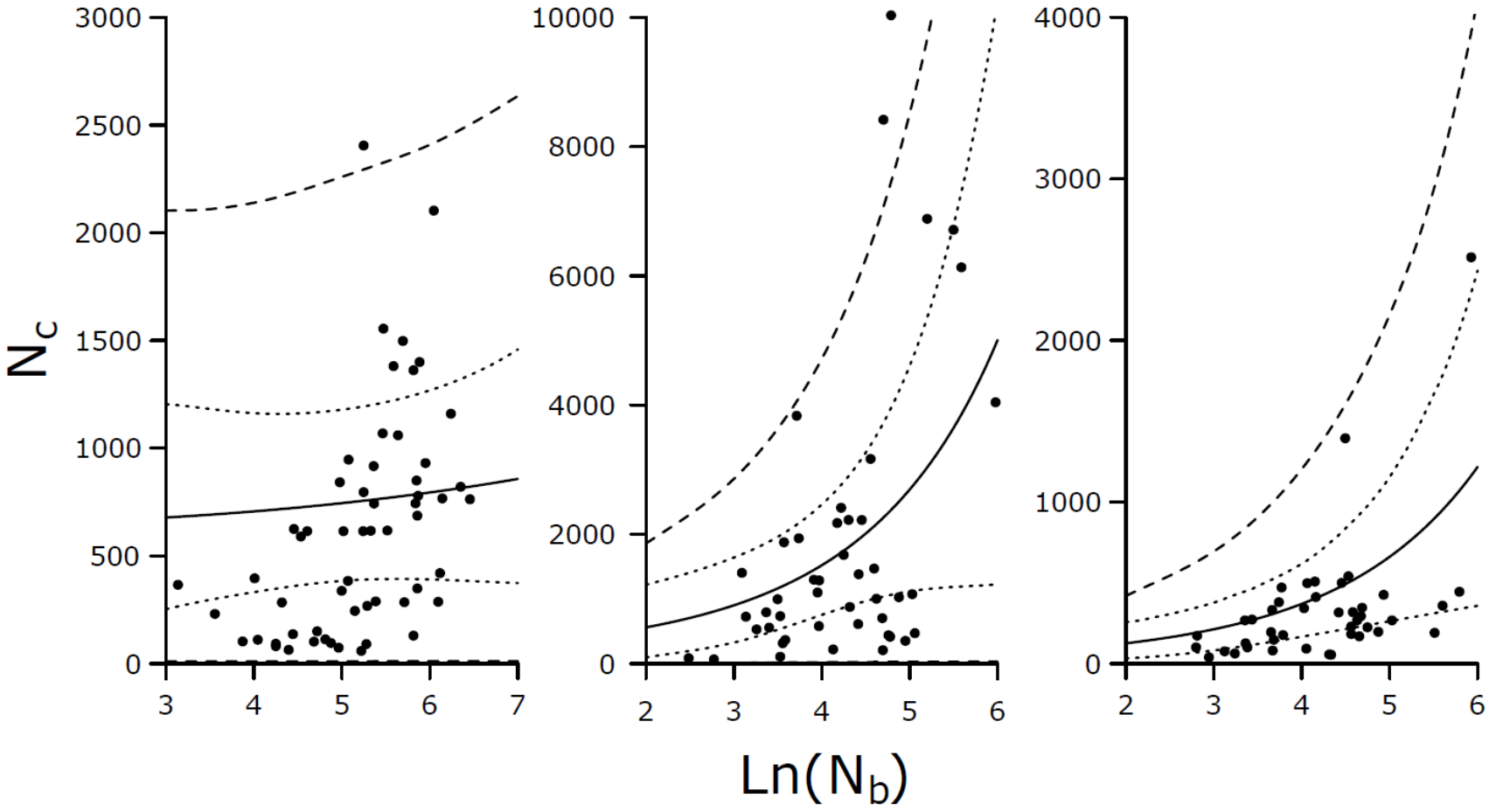

Figure 2: Relationship predicting Nc from Nb in Atlantic salmon (a), brook trout (b), and Chinook salmon (c). Dotted lines represent 95% credible intervals; dashed lines represent 95% prediction intervals.

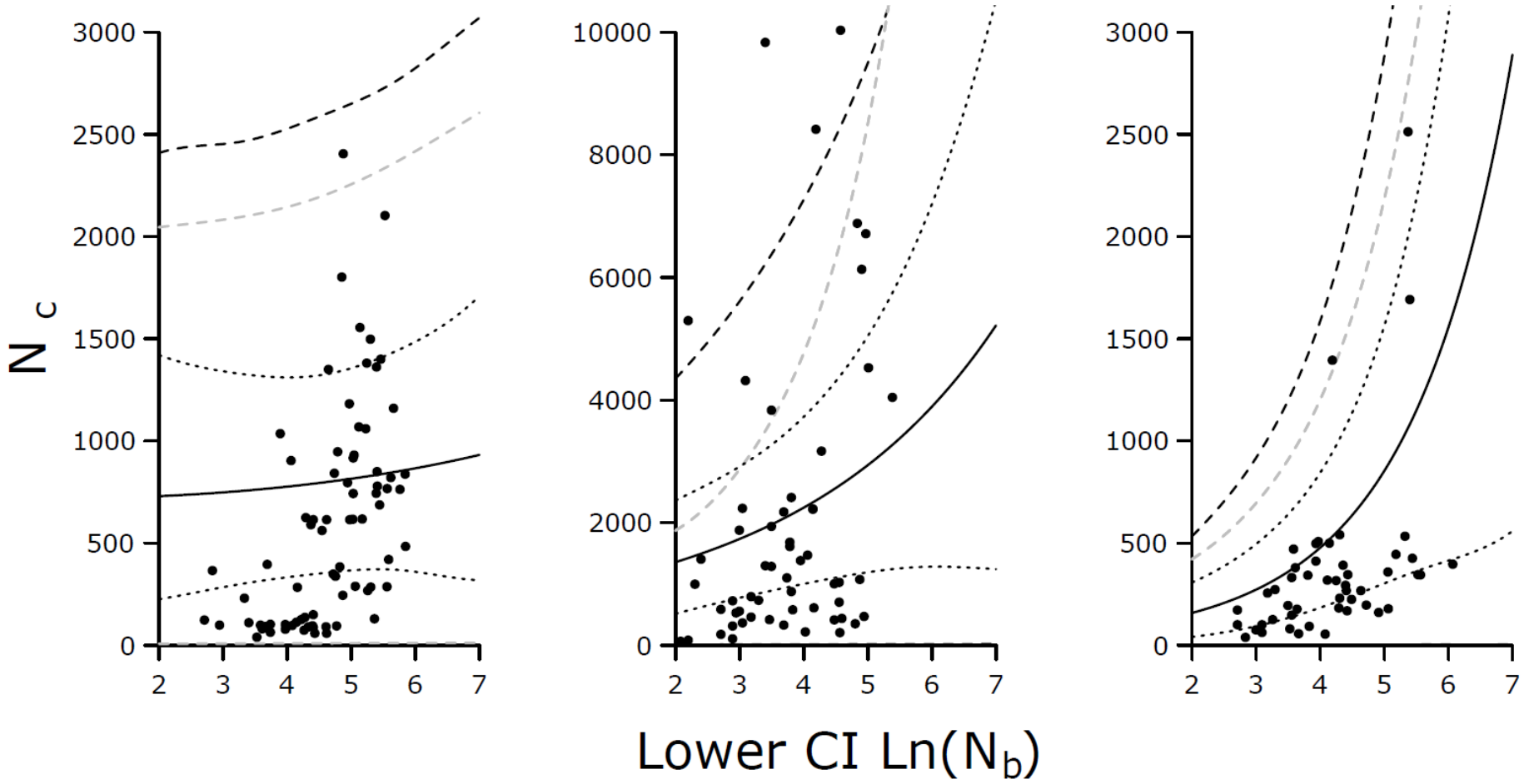

Figure S1: Relationship predicting N_c from N_b lower confidence intervals in Atlantic salmon (a), brook trout (b), and Chinook salmon (c). Dotted lines represent 95% credible intervals; dashed black lines represent 95% prediction intervals generated from N_b lower confidence interval estimates, dashed grey lines represent 95% prediction intervals generated from mean N_b estimates.

General Discussion

As environments across the globe undergo rapid changes in the Anthropocene, significant empirical research has focused on identifying sources of extinction risk for population of conservation concern and the capacity of populations to adapt and persist in the face of environmental change (Ouborg et al. 2006). Many theoretical frameworks, such as the "conservation genetics" and "habitat quality" paradigms, provide useful tools for evaluating risks to endangered natural populations. The "conservation genetics" paradigm posits that small population size and associated loss of genetic diversity, accumulated genetic load, and inbreeding are significant drivers of species and population extinction, whereas the habitat quality paradigm posits that habitat degradation and loss are the primary drivers of biodiversity loss in natural populations (Ouborg et al. 2006). While habitat degradation is recognized as a primary source of population vulnerability and decline (Brooks et al. 2002, Lawrence and Kaye 2011), evaluating the effect of genetic factors on population persistence has been substantially more controversial. Laboratory studies (e.g. Reed et al. 2003, Bakker et al. 2010, Samani and Bell 2010) and genetic rescue experiments in natural populations (Frankham 2015, Whiteley et al. 2015b, Weeks et al. 2017) provide evidence that genetics can influence fitness and persistence. However, comparatively little empirical research in nature has sought to comprehensively test the relative importance of, and potential interaction between, habitat and genetic risks in populations exposed to novel change in natural environments.

Evaluating the importance of habitat and genetic risks to persistence

Collectively, the results of my thesis do not support predictions associated with the conservation genetics paradigm; genetic factors having little effect on performance in novel environments. Small population size (or small N_b) and low genetic diversity had little overall effect on survival or growth in transplanted organisms. In the experimental systems examined herein habitat characteristics of the novel environments were the primary driver of the fitness correlates examined, identifying habitat degradation and loss as an important source of risk for populations of conservation concern.

In chapter one, my meta-analysis found no evidence that small populations exhibited reduced performance when transplanted to novel environments. Contrary to the expectations of

the conservation genetics paradigm large populations actually exhibited reduced performance in novel environments. Large populations did, however, exhibit increased performance in native environments, indicating stronger local adaptation consistent with previous studies (Leimu and Fischer 2008). This stronger local adaptation may be the cause of their reduced performance in novel environments, with large populations potentially exhibiting increased trade-offs in novel environments as a result of adaptation to the specific suite of characteristics present in their native habitats.

In chapter two, I directly tested the relative importance of genetic and habitat risks in natural environments using replicated translocations of brook char to novel pond environments. Similar to chapter one, I found little evidence to support predictions associated with the conservation genetics paradigm. Genetic variables (genomic H_o, N_b) had little to no effect on survival and subsequent growth of the transplanted brook char despite 10-fold differences across populations and, in some cases, effective population sizes likely well below typical conservation targets (Frankham 1995, Jamieson and Allendorf 2012). Conversely, our results demonstrated that survival and growth exhibited significant and strong relationships with the habitat characteristics of the novel pond environments.

In chapter 3, I tested how phenotypic variation changed across environmental gradients in the translocations used in chapter two. The release of phenotypic variation in novel conditions has important implications for how populations adapt to changing environments (Ghalambor et al. 2007). Although fish exhibited plastic reaction norms across habitat gradients, we only found limited evidence that phenotypic variation was released in extreme environments. Furthermore, we again found no evidence to support predictions associated with the conservation genetics paradigm, as levels of phenotypic variation (and potential underlying cryptic genetic variation) across populations did not differ despite 10-fold differences in genomic heterozygosity.

Why did we find no evidence to support the conservation genetics paradigm?

Contrary to the predictions formulated from the conservation genetics paradigm, I found no evidence that small, genetically depauperate populations exhibited reduced performance in fitness correlates or phenotypic variability in novel environments. There are several factors that could account for the lack of observed relationship:

(i) Only the "toughest" small populations remain

It is possible that the small populations surveyed herein represent a biased subsample from a historical perspective; that is, we only sample the small populations that have persisted to the present day and are unable to test putative small populations that have already gone extinct. Our study populations therefore represent a sample of the "hardiest" small populations and may not accurately reflect the effect of low genetic diversity and N_b on performance. While we cannot rule this possibility out for the study populations in the meta-analysis from chapter one, it is unlikely that this is the case for the populations of brook trout on Cape Race studied in chapters two and three. Cape Race is small geographically, yet extremely densely populated by brook trout; extensive explorations of the region have demonstrated that almost any habitat on Cape Race that seems capable of sustaining brook trout populations (i.e. circum-neutral pH, seeps for spawning, etc.) is inhabited by them.

Additionally, it is difficult to collect evidence to support or reject such a hypothesis. Although beyond the scope of this thesis, paleolimnology may reveal what habitats on Cape Race may or may not have historically been inhabited by brook trout; however, it is unlikely that such tools could, if they were previously inhabited, explain why they may have gone extinct. The scope of that argument is outside of what is reasonably demonstrable from an empirical perspective; all we can do is utilize what data are currently available by studying the process of extinction/persistence in known small and/or low diversity populations in the present day.

(ii) Additive genetic variation not linked to neutral genetic variation

There is a general lack of relationship between additive genetic variance (heritability) and genetic diversity at neutral markers, both across taxa and among the populations examined herein (Reed and Frankham 2001, Wood et al. 2015). As a result, neutral genetic markers or genomic diversity might represent poor predictors of the capacity of populations to respond to selection or of the release of phenotypic variability in novel environments. Selective forces (i.e. balancing selection) might allow populations to retain genetic diversity at important loci (Bensch et al. 2006b, Fraser et al. 2014), despite small population size or overall levels of low genetic/genomic diversity.

(iii) Purging and migration in natural populations

The genetic load of a population can be masked and relieved by purging and/or immigration. Purging may be more efficient in small populations (Angeloni et al. 2011), so many of the small populations observed in chapters one, two, and three could have at least partially purged deleterious alleles contributing to inbreeding and genetic load. Similarly, even low levels of immigration can alleviate inbreeding and increase population-level genetic diversity (Vila et al. 2003, Frankham 2005). The majority of the study populations examined in chapter one were plants, and cross-population pollination is common in plant populations (Ellstrand 1992). Additionally, although two of the small study populations in chapters two and three were isolated (WC and STBC), several small and medium-sized populations inhabited meta-population structures. It is possible that gene flow may have alleviated the loss of genetic diversity in the many of the small wild populations studied herein.

(iv) Historical selection can constrain plastic reaction norms and limit associated phenotypic diversity

Finally, historical selection may have constrained reaction norms governing body morphology in the populations studied in chapter 3. If the costs associated with maintaining phenotypic plasticity in body morphology traits are low (as may be the case in salmonids, see Marin et al. 2016), putatively cryptic reaction norms may exhibit constrained phenotypic variation as a result of historical selective forces (Ledón-Rettig et al. 2014, Paaby and Rockman 2014).

Evaluating the effectiveness of genetic tools to monitor abundance

Although we found little evidence supporting predictions typical of the conservation genetics paradigm, small populations still face significant demographic and environmental threats (Lande 1993). Ironically, it might be possible to use genetic tools to monitor changes in abundance in small, threatened natural populations (Ovenden et al. 2016). However, no empirical research has actually evaluated the reliability and accuracy of using genetic tools to monitor changes in abundance.

My meta-analysis examining the utility of using N_b to infer N_c (or vice versa) found that neither population parameter reliably inferred the other. Although upper prediction intervals with

"meaningful" information could be generated for some species/parameter combinations, variability in N_b/N_c ratios across populations and species ultimately resulted in predictive models with little conservation or monitoring utility.

General conclusions and future research directions

Overall, the results of my thesis dispute several key predictions of the conservation genetics paradigm. More specifically, my experimental results did not find that small populations with low genetic diversity and high potential genetic load exhibited reduced overall fitness or have a limited capacity to adapt or respond to novel environmental change. Overall, genetic parameters or population size (both N_b or N_c) failed to predict subsequent performance in novel environments or were not associated with a release of phenotypic plasticity. My thesis contributes to an emerging literature that demonstrates that many small populations with low levels of genetic diversity may be fully capable of long-term persistence and adaptation (e.g. Willi et al. 2007, Robinson et al. 2016, Benazzo et al. 2017). Although still susceptible to other risks associated with small population size (e.g. environmental and demographic stochasticity), such populations warrant protection from threats.

My thesis further highlights the relative importance of maintaining habitat integrity for populations of conservation concern. Environmental variables, regardless of levels of N_b or H_o, strongly predicted performance in controlled replicated transplants of brook char at Cape Race, and similarly dictated their plastic phenotypic responses in novel environmental conditions. My thesis similarly contributes to a strong body of literature emphasizing the primary importance of slowing or reversing habitat loss and degradation for species of conservation concern (Brooks et al. 2002, Lawrence and Kaye 2011). At-risk species or populations are likely to benefit most from efforts targeting habitat restoration and loss unless population sizes reach extremely low levels.

Finally, my thesis emphasizes that no shortcuts exist to estimating abundance for natural populations of salmonids – monitoring efforts for small populations of conservation concern will still require resource-intensive “boots-on-the-ground” sampling techniques (e.g. mark-recapture field surveys) when abundance data is required for conservation efforts.

These results prompt several novel questions which future studies could explore. First, brook char represent a generalist colonizing species well known for exhibiting plastic reaction norms and with a fairly broad fundamental niche. Similarly, most of the species examined in the chapter one meta-analysis represent fairly generalist taxa. As a result, the extent to which the inferences drawn from these studies can be extended to rare, specialist, or endemic species is unknown, although results from chapters one, two, and three are at least likely to be broadly

extendable to other salmonids. Although conducting similar experiments on endangered taxon is admittedly more difficult, future research should explore if the relationships observed herein are similar in rarer species.

Second, chapters two and three only explored the effect of overall levels of genomic diversity on fitness correlates in novel environments. I examined how the quantity of the genomic diversity present among populations influenced performance. However, the "qualities" of the genomic diversity may have more of a significant impact. Genomics researchers are developing novel techniques to identify putative deleterious SNP mutations (e.g. Perrier et al. 2017); the relative proportion of putative deleterious mutations may have more of an impact on performance in novel environments compared to overall levels of genomic diversity. Future studies examining could explore how the presence of deleterious mutations affects fitness in novel environments.

Third, chapters one, two, and three only examined fitness correlates for a single generation. Although outside of the scope of the timeframe of a typical PhD thesis, future studies could explore how populations *adapt* to novel environments; this would require examining how fitness changes across multiple generations in translocated organisms. A notable difficulty with conducting multi-generational studies is the requirement that translocation environments must be capable of sustaining reproduction of translocated organisms; this further limits the number of potential environments usable for such empirical studies, but does not diminish the potential importance of long-term studies to our understanding of the process of adaptation.

Finally, although N_e estimates from microsatellites proved poor predictors of abundance in salmonids, N_e estimates obtained from other genetic data might prove more effective. Microsatellite loci often generate relatively wide confidence intervals (e.g. Bernos and Fraser 2016), implying uncertainty in N_e that may contribute significant "noise" to predictive models. Although currently linkage disequilibrium methodologies for estimating N_e overestimate precision when using thousands of SNP loci (Waples et al. 2016) these technical limitations may be overcome in the future. N_e generated from thousands of loci may prove more precise than microsatellite-based estimates, in which case better predictive models may be obtained. Similarly, other relatively untested DNA methods may prove to be more effective tools to predict abundance. Environmental DNA, for example, may prove to be an effective tool to monitor census size once predictive models are further refined (Goldberg et al. 2015, Wilcox et al. 2016).

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Appendices

Appendix 1

Table A1.1: Instruments and methodology used to estimate environmental variables measured in stocked pond and stream environments.

Environmental Variable	Instrument	Methodology
pH	Oakton™ PCSTestr 35	Stream estimates obtained from one reading per transect, from a minimum of 18 transects per stream, obtained in the Spring/early Summer. Pond estimates were obtained from readings taken at stocking and recapture.
DO	WTW™ Multiline P4 Universal Meter	Stream estimates obtained from one reading per transect, from a minimum of 18 transects per stream, obtained in the Spring/early Summer. Pond estimates were obtained from readings taken at stocking and recapture.
Temperature	iButton™ thermochron data loggers	Data loggers were placed in in two locations per stream and in one location per pond. Data loggers recorded temperatures every 90 minutes throughout translocation experimental period.
Conductivity	Oakton PCSTestr 35	Stream estimates obtained from one reading per transect, from a minimum of 18 transects per stream, obtained in the Spring/early Summer. Pond estimates were obtained from readings taken at stocking and recapture.
Depth	Meter stick	5 equidistant depth measurements were taken along a transect (at the 25th, 50th, and 75th, percentile of transect length and at each transect end). Stream estimates obtained from an average of a minimum of 18 random transects, pond estimates obtained from seven transects equally distributed across an arbitrary pond axis. Stream measurements were collected in the Spring/Early Summer, pond measurements were collected at stocking and recapture.
% Silt Substrate	N/A	Visual observation of substrate along a transect approximately 1 m in width. Stream estimates obtained from an average of a minimum of 18 random transects, pond estimates obtained from seven transects equally distributed across an arbitrary pond axis. Stream measurements were collected in the Spring/Early Summer, pond measurements were collected at stocking and recapture.

% Aquatic Vegetative Cover	N/A	Visual observation of substrate along a transect approximately 1 m in width. Stream estimates obtained from an average of a minimum of 18 random transects, pond estimates obtained from seven transects equally distributed across an arbitrary pond axis. Stream measurements were collected in the Spring/Early Summer, pond measurements were collected at stocking and recapture.

Appendix 2

Table A2.1: Sample size (S), number of marked individuals (m), proportion of marked individuals during recapture in % (M), effective number of breeders calculated from LDNe (N_b), adult census population sizes (N), and Nb/N ratios for Atlantic salmon populations coexisting within two Cape Race streams: Upper Ouananiche Beck (UO) and Watern (WN). See Bernos et al (submitted) for methods and complete analysis.

Cohort	*S*	*m*	*M*	$N_{b(LD)}$	N	$N_{b(LD)}$ /N	$N_{b(Sib)}$ /N
UO11	5	NA	NA	100 (67-	NA	NA	NA
UO12	2	11	39	23 (17-	366 (295-	0.06	0.10
UO13	4	49	29	35 (28-	220 (274-	0.16	0.25
UO14	3	82	18	57 (30-	440 (336-	0.13	0.10
WN12	4	NA	NA	55 (40-	NA	0.14	0.12
WN13	3	43	10	80 (44-	405 (240-	0.20	0.09
WN14	5	27	22	62 (42-	111 (67-	0.56	0.50

Appendix 3

Table A3.1: Point estimates and 95% credible intervals (from posterior distribution) for heteroscedastic residual variance in a model predicting N_c from N_b at a gradient of population sizes representative of N_b estimates contained within the dataset. This model assumed decreasing residual variance with increasing N_b (term = "(idh(species:sqrt(1/ln(N_b))):units)").

Species	N_b = 20	N_b = 50	N_b = 100	N_b = 300	N_b = 600
Chinook salmon	0.271 (0.114, 0.612)	0.234 (0.113, 0.545)	0.239 (0.097, 0.509)	0.198 (0.083, 0.483)	0.214 (0.080, 0.478)
Atlantic salmon	0.092 (0.046, 0.187)	0.078 (0.046, 0.151)	0.074 (0.046, 0.137)	0.074 (0.042, 0.124)	0.068 (0.038, 0.119)
Brook trout	0.160 (0.072, 0.354)	0.157 (0.071, 0.304)	0.121 (0.069, 0.286)	0.126 (0.060, 0.267)	0.121 (0.058, 0.265)

Table A3.2: Point estimates and 95% credible intervals (from posterior distribution) for heteroscedastic residual variance (term = "(idh(species:sqrt(ln(N_x))):units)",) in a model predicting N_c from N_b at a gradient of population sizes representative of N_b estimates contained within the dataset. This model assumed increasing residual variance with increasing N_b (term = "(idh(species:sqrt(1/ln(N_b))):units)").

Species	N_b = 20	N_b = 50	N_b = 100	N_b = 300	N_b = 600
Chinook salmon	0.208 (0.089, 0.470)	0.257 (0.111, 0.525)	0.266 (0.128, 0.584)	0.287 (0.134, 0.676)	0.269 (0.135, 0.738)
Atlantic salmon	0.051 (0.028, 0.108)	0.060 (0.036, 0.116)	0.074 (0.041, 0.123)	0.078 (0.046, 0.137)	0.083 (0.051, 0.151)
Brook trout	0.113 (0.051, 0.252)	0.124 (0.062, 0.280)	0.149 (0.072, 0.310)	0.163 (0.081, 0.361)	0.167 (0.083, 0.391)

Table A3.3: Point estimates and 95% credible intervals (from posterior distribution) for heteroscedastic residual variance (term = "(idh(species:sqrt(1/ln(N_x))):units)") in a model predicting N_b from N_c at a gradient of population sizes representative of N_c estimates contained within the dataset. This model assumed decreasing residual variance with increasing N_c (term = "(idh(species:sqrt(1/ln(N_c))):units)").

Species	$N_c = 50$	$N_c = 100$	$N_c = 500$	$N_c = 1\ 000$	$N_c = 10\ 000$
Chinook salmon	0.335 (0.154, 0.588)	0.301 (0.151, 0.535)	0.265 (0.133, 0.463)	0.242 (0.124, 0.450)	0.185 (0.097, 0.430)
Atlantic salmon	0.166 (0.114, 0.366)	0.162 (0.111, 0.319)	0.165 (0.103, 0.269)	0.142 (0.094, 0.257)	0.118 (0.062, 0.243)
Brook trout	0.119 (0.058, 0.296)	0.116 (0.058, 0.264)	0.110 (0.057, 0.225)	0.109 (0.055, 0.218)	0.092 (0.049, 0.206)

Table A3.4: Point estimates and 95% credible intervals (from posterior distribution) for heteroscedastic residual variance (term = "(idh(species:sqrt(ln(N_x))):units)") in a model predicting N_b from N_c at a gradient of population sizes representative of N_c estimates contained within the dataset. This model assumed increasing residual variance with increasing N_c (term = "(idh(species:sqrt(1/ln(Nc))):units)").

Species	$N_c = 50$	$N_c = 100$	$N_c = 500$	$N_c = 1\ 000$	$N_c = 10\ 000$
Chinook salmon	0.203 (0.117, 0.421)	0.259 (0.132, 0.444)	0.285 (0.162, 0.528)	0.308 (0.171, 0.572)	0.354 (0.185, 0.731)
Atlantic salmon	0.123 (0.073, 0.235)	0.129 (0.088, 0.248)	0.168 (0.108, 0.282)	0.176 (0.110, 0.299)	0.210 (0.118, 0.373)
Brook trout	0.075 (0.037, 0.175)	0.085, 0.042, 0.183)	0.113 (0.056, 0.217)	0.118 (0.055, 0.227)	0.137 (0.065, 0.280)

Appendix 4

Table A4.1: Published studies examining N_b/N_c relationships in species for which limited data are available. Note that some studies did not use LDNe to estimate N_b.

Authors	Year	Species	Number of Populations	Total usable N_b/N_c estimates
Scribner et al	1997	*Bufo bufo*	3	3
Ardren and Kapuscinski	2003	*Oncorhynchus mykiss*	1	3
Brede and BeeBee	2006	*Rana temporaria*	2	2
Schmeller and Merila	2007	*Rana temporaria*	2	2
Brede and BeeBee	2006	*Bufo bufo*	2	2
BeeBee	2009	*Bufo calamita*	6	6
Ficetola et al	2010	*Rana latastei*	9	9
Hoehn et al	2012	*Oedura reticulata*	4	7
Christie et al	2012	*Oncorhynchus mykiss*	1	11