How does hybridization affect multiple metrics of fitness in fragmented populations of brook trout under moderate climate warming?

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GENERAL ABSTRACT

How does hybridization affect multiple metrics of fitness in fragmented populations of brook trout under moderate climate warming?

Zachery R. R. Wells

As a population's genetic makeup is often attributed to the combined fitness of its individuals, the adoption of deliberate hybridization practices is an area of interest for many hatchery and conservation programs. Using a common garden experimental design involving eight wild populations of *Salvelinus fontinalis* (brook trout), we studied how effective population size (N_e), divergence (Q_{ST}, km), and environmental dissimilarity (pH, temperature) may influence hybridization outcomes for fitness related traits under moderate climate warming. Additionally, we looked at the ability of six of these populations to tolerate acute thermal warming, and whether or not this tolerance could be altered by hybridizing populations. Critical thermal maximum (CT_{max}) assays were conducted on juveniles from each population to assess thermal tolerance, and agitation temperature (a behavioural metric quantifying temperature at the onset of refugia-seeking behaviour) was recorded for assessing behavioural changes to elevated temperatures. Gametes were collected from different-sized, isolated populations of brook trout, and crossed in the lab. Fitness-related traits were compared between pure and F1 hybrid crosses via common garden experimental design. We had the unique opportunity to jointly investigate how these factors influence multiple metrics of hybrid fitness in wild, isolated, and varyingly-sized populations of a vertebrate species inhabiting a relatively undisturbed environment. Although population size and environmental dissimilarity were found to significantly affect hybrid fitness, these relationships were biologically weak. Although significant differences in CT_{max} were found between populations, this difference was at most 0.68 °C (29.11-29.79 °C), and no effect of hybridization was seen despite varying thermal regimes between these populations' wild streams. These results will provide guidance to small population and captive-breeding conservation programs, as the lack of a strong relationship between hybridization and fitness encourages population-specific approach to genetic rescue projects. Additionally, this study highlights the level to which thermal tolerance is conserved between isolated populations of a vertebrate species, in the face of climate warming.

GENERAL SUMMARY

Chapter 1

To quantify the effects of hybridization on fitness, and potential factors influencing these outcomes, hybridization crosses were conducted on eight populations of brook trout from Cape Race, Newfoundland, Canada. Although hybridization significantly affected fitness (positively and negatively), and population size and pH dissimilarity were found to be statistically significant in determining these outcomes, there was little evidence for meaningful effects of hybridization on fitness, nor indication population size, divergence, or environmental dissimilarity influenced this outcome.

Chapter 2

To forecast the impact of climate warming on cold-water fishes, thermal tolerance trials were conducted on six populations of brook trout from Cape Race, Newfoundland, Canada. Of these, three were outcrossed to assess the effect of hybridization. There was little variation among populations in how they responded to thermal stress.

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CONTRIBUTION OF AUTHORS

Chapter 1

Study conception and design: Wells & Fraser

Acquisition of data: Wells

Analysis and interpretation of data: Wells

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Critical revision: Wells & Fraser

Chapter 2

Study conception and design: Wells

Acquisition of data: Wells & McDonnell

Analysis and interpretation of data: Wells

Drafting of manuscript: Wells

Critical revision: Wells, Fraser, McDonnell, Chapman

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ABBREVIATIONS

BC:	Bob's Cove
CC:	Cripple Cove
CO:	Coquita
CR:	Cape Race
CT_{max}:	Critical thermal maximum
F_{ST}:	Neutral genetic differentiation
FW:	Freshwater
ID:	Inbreeding depression
MVP:	Minimum viable population size
N:	Census population size
N_b:	Effective number of breeders
N_e:	Effective population size
OB:	Ouananiche Beck
OD:	Outbreeding depression
Q_{ST}:	Quantitative genetic differentiation
STBC:	Still There By Chance
WC:	Whale Cove
WN:	Watern Cove

GENERAL INTRODUCTION

How species and populations will adapt to climate change is of growing concern as forecasted temperatures are predicted to rise 1.8°C - 4.0°C by the year 2100 (IPCC 2007). For species and populations not bound by physical barriers, shifts to more favourable environmental conditions may allow for short-term relief from increasingly deleterious conditions in their historical native habitat range (Davis & Shaw 2001; Parmesan & Yohe 2003). However, for species and populations bound by physical (*e.g.* habitat fragmentation, drought) and physiological (*e.g.* pH, salinity) barriers, such as many freshwater species, their ability to adapt to environment change will determine their potential to persist, and as such, their genetic make-up is likely to influence survival.

Genetic diversity provides a means by which natural selection may act upon a population, selecting those individuals with the greatest fitness for a given set of environmental parameters. As such, it is thought that genetic diversity is positively correlated with individual fitness, as the greater amount of genetic material there is to work with, the greater the potential is for exhibiting a favourable set of fitness-related traits (Reed & Frankham 2003). Maintaining as much genetic diversity as possible in populations at risk or those soon to be experiencing changes in habitat composition is therefore important as climate change and other anthropogenic disturbances become ever-growing threats.

The future of many fish species and populations are of particularly concern due to their susceptibility to climate warming (Tonn 1990; Xenopoulos *et al.* 2005; Rijnisdorp *et al.* 2009; Pankhurst & Munday 2011), habitat fragmentation, history of non-random harvesting (Heino & Godø 2002; Kuparinen *et al.* 2016a, b), and the interplay of these threats (Brander 2007). As ectotherms, fish rely on external temperatures to dictate a number of life-history traits influencing fitness such as age at maturity, spawning season and growth rates. Salmonids in particular are a socioeconomically-important family of cold water fishes having traditional and commercial value. Although they are rich in populations and inhabit a diversity of landscapes, habitat fragmentation and fishing has depleted their numbers, and their viability is of growing concern as climate change warms northern regions (Walther *et al*. 2002; Alley *et al*. 2003; Hinzman *et al*. 2005).

As populations become smaller and isolated, so too does the level of genetic diversity. A potential solution to this is hybridization, which allows new genetic material to be introduced to a population to counteract inbreeding depression and increase adaptive potential (Pickup *et al*. 2012). However, hybridization can result in outbreeding depression (OD), where offspring exhibit reduced fitness. Quantification of heterosis (increased fitness) and OD has been the primary focus of outbreeding studies (Fraser *et al.* 2008; Stelkens & Seehausen 2008; Houde *et al.* 2011; Prill *et al.* 2014), and has offered mixed results. Increases, decreases, and neutral effects on fitness have been found depending on the hybrid generations considered, due to varying degrees of disruption of coadaptive gene complexes associated with local adaptation (Edmands 2007; Fraser *et al.* 2008; Clark *et al.* 2013). As hybridization studies have been somewhat contradictory, population size, divergence, and environmental dissimilarity are thought to jointly contribute to this variation.

Using eight genetically distinct and isolated populations of brook trout from Cape Race, Newfoundland, the first chapter of this thesis set out to investigate what factors may influence hybridization outcomes while looking at a number of early life-history traits related to fitness in salmonids. Chapter 2 looked at the effects of hybridization on upper thermal tolerance and a behavioural metric, agitation temperature, in six Cape Race populations of brook trout.

Predictions of our results are described in the following two chapters, as well as their implications for conservation management, and the use of hybridization as a potential tool for bolstering genetic diversity to increase fitness.

Chapter 1: What affects hybridization outcomes among fragmented populations? Testing the roles of population size, divergence and environmental dissimilarity in brook trout

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LAY SUMMARY

To quantify the effects of hybridization on fitness, and potential factors influencing these outcomes, hybridization crosses were conducted on eight populations of brook trout from Cape Race, Newfoundland, Canada. Although hybridization significantly affected fitness, and population size and pH dissimilarity were found to be statistically significant in determining these outcomes, there was little evidence for biological significance of hybridization and influencing factors.

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ABSTRACT

Conservation programs are increasingly used to offset the decline in abundance of certain species, and an area of concern with these programs is whether or not they can produce self-sustaining populations. As population viability is associated with the genetic makeup of its individuals, the adoption of deliberate hybridization practices is an area of interest for many conservation programs. We had the unique opportunity to investigate how multiple factors (effective population size, N_e; quantitative genetic divergence; Q_{ST}; environmental dissimilarity: pH, temperature) jointly influence hybridization outcomes for fitness-related traits in a vertebrate species under moderate climate change, using a common garden experimental design involving eight wild populations of brook trout (*Salvelinus fontinalis*). Gametes were collected from varyingly-sized, isolated populations and crossed; fitness-related traits were compared between pure and F1 hybrid crosses. Although several factors significantly influenced fitness, these relationships were biologically weak, with no consistent trends. Hybrid fitness was population-specific, with N_e, a factor often tied to individual fitness within a population, having a weak effect on fitness outcomes (range of population N_e = 44-588). The lack of a clear relationship between hybridization and fitness encourages a population-specific approach to small population, genetic rescue and captive breeding conservation programs.

Key words: Outbreeding depression, hybridization, heterosis, population size, divergence, stress

INTRODUCTION

It is generally accepted that increased genetic diversity allows populations to better adapt to environmental change. For smaller, isolated populations, pronounced genetic drift, inbreeding, and lack of gene flow should reduce genetic diversity, decreasing individual fitness and adaptive potential (Reed & Frankham 2003; Pickup *et al*. 2012). However, emerging evidence suggests that small populations may not show reduced adaptive potential or fitness in novel environments (Fraser *et al.* 2014; Wood *et al.* 2014; Yates & Fraser 2014; Labonne *et al.* 2016; Wood *et al.* 2016). Whether or not small, isolated populations have mechanisms in place to deal with unfavourable genetic constituency is of growing interest to researchers as human activities diminish population sizes. Herein, deliberate hybridization ('genetic rescue') has emerged as a potential tool to increase fitness in at-risk populations (Rhymer & Simberloff 1996; Tallmon *et al.* 2004; Edmands, 2007; Godefroid *et al.* 2011; Frankham 2015; Whiteley *et al*. 2015).

Hybridization allows new genetic material to be introduced to a population to counteract inbreeding depression (ID) and increase adaptive potential (Pickup *et al*. 2012). However, hybridization can result in outbreeding depression (OD), where offspring exhibit reduced fitness. Quantification of heterosis (increased fitness) and OD has been the primary focus of outbreeding studies (Fraser *et al.* 2008; Stelkens & Seehausen 2008; Houde *et al.* 2011; Prill *et al.* 2014), and has offered mixed results. Increases, decreases, and neutral effects on fitness have been found depending on the hybrid generations considered, due to varying degrees of the disruption of co-adaptive gene complexes associated with local adaptation (Edmands 2007; Fraser *et al.* 2008; Clark *et al.* 2013). As hybridization studies have been somewhat contradictory, a number of factors are thought to jointly contribute to this variation.

One factor thought to affect hybridization outcomes is effective population size (N_e). Small N_e populations are assumed to have reduced adaptive potential due low genetic diversity (Reed & Frankham 2003), so introducing new genetic material to them is thought to be beneficial. Conversely, larger N_e populations are hypothesized have greater genetic diversity, allowing them to respond quickly to changing environmental selective pressures (Willi *et al.* 2006). Nevertheless, these expectations do not always play out empirically, with small populations of certain species exhibiting more variable evolutionary responses to environmental change, similar levels of plasticity, or higher performance in new environments relative to large populations (Wood *et al.* 2014, 2015; Yates & Fraser 2014; Wood & Fraser 2015). These disparate results call into question whether or not hybridization is disproportionally beneficial to small populations.

The level of geographic and genetic divergence between populations has also been hypothesized to affect hybrid fitness. These distances between parental populations are hypothesized to affect F1 fitness negatively as distance becomes very large, if ecological dissimilarity between sites increases with spatial scale, and disruption of respective local adaptations occurs (OD) (Edmands 1999, 2002). Yet crossing between populations at very close geographic and genetic scales may result in ID, as similar environmental pressures likely dictate the genetic constituency of each population. It is thought that intermediate divergence is best for hybridization so as to retain useful co-adapted gene complexes whilst maintaining adequate differentiation to eliminate ID, but not so much as to result in OD (Lynch 1991; Fenster & Galloway 2001; Houde *et al.* 2011).

Finally, the degree of environmental dissimilarity experienced by offspring is hypothesized to affect outbreeding outcomes. Heterosis may be more easily observed when individuals are under more stressful conditions (Edmands & Deimler 2004; Edmands 2007). Yet recent work on plants has found that heterosis may be lost after the addition of an environmental stress (Prill *et al.* 2014). As temperature and pH control the metabolism and life-history traits (*e.g.* length, weight, growth rate, survival) of ectotherms (Krueger & Waters 1983; Beacham & Murray, 1985; Marten 1992; Baker *et al.* 1996), they are particularly relevant environmental parameters to study in fishes. How environmental dissimilarity may or may not affect hybridization outcomes in vertebrates and in combination with other factors is largely unknown (but see Waser *et al.* 2000; Edmands 2007).

In North America, salmonid fishes have been at the forefront of hatchery programs, due to having high socio-economic and cultural value. However, these programs have been documented to reduce fitness in relation to wild fish counterparts (Araki *et al.* 2007, 2008), via ID after extended rearing (Cross & King 1983; Eknath & Doyle 1990), resulting in the reduction of wild fish fitness after mixing (Houde *et al.* 2009; Fraser *et al.* 2010). As such, hybridization may counteract these negative effects by periodical supplementation of wild counterparts to hatchery stock. The evolutionary processes of salmonids have been studied extensively (Hendry & Stearns 2004), and their propensity to occupy diverse habitats and continually return to their natal streams to spawn (Stabell 1984), means they are highly plastic and often form genetically distinct, locally adapted populations (Hutchings 2011; Fraser *et al.* 2011). Brook trout (*Salvelinus fontinalis*) in particular are extremely diverse (Angers *et al.* 1995; Wood *et al.* 2015), highly plastic (Hutchings 1996; Imre *et al*. 2002), have a wide range of population sizes, and are a popular sportfish throughout North America. As with many cold-water fish, they are expected to suffer from changes in habitat structure resulting from climate warming and habitat fragmentation, and so induced inter-population hybridization may become crucial for their continued survival (Rouse *et al.* 1997; Walther *et al.* 2002; Alley *et al.* 2003; Hinzman *et al.* 2005; Heino *et al.* 2009; Qin *et al.* 2014).

Using eight populations of isolated brook trout from Cape Race, NL, Canada (Figure S1), we studied the effects that population size, geographic and genetic divergence, and environmental dissimilarity have on hybridization outcomes between F1 hybrids and pure crosses. Previous studies have collected multi-year habitat data, quantified population sizes (N_e=44-588; N=202-7801) and calculated genetic differentiation at quantitative traits (Q_{ST}) between these populations (Wood *et al.* 2015; Bernos & Fraser 2016), providing us the unique opportunity to assess their potential effects on hybrid fitness. In particular, these streams are variable in their temperature regimes (Figure S2; Wood *et al.* 2014) and pH (Table S1). Hence, we have the opportunity to combine our factors into a comprehensive analysis of how hybridization affects wild, isolated, and different-sized populations of a vertebrate species inhabiting a pristine environment.

Based on the theoretical predictions discussed, we expected that: (i) small population hybrids (i.e. a small pure population crossed with any other size of population) would show greater fitness compared to small, pure populations, and that large population hybrids would show equal or lesser fitness compared to their pures; (ii) hybrids generated between populations with smaller values of quantitative genetic divergence (Q_{ST}) and geographic divergence (km) would have greater fitness compared to their pures than at larger values; and (iii) hybrid fitness would be reduced as environmental dissimilarity (temperature, pH) between lab and wild conditions increases. Our study has implications for small population and captive-breeding conservation programs when managers are deciding whether to preserve or alter the genetic structure of wild or captive-bred populations at risk.

MATERIALS AND METHODS

Study site

Cape Race (CR) is a coastal region of land located on the Avalon Peninsula in Newfoundland and Labrador, Canada. It is dominated by low-lying shrubbery and a number of low-order streams inhabited primarily by *Salvelinus fontinalis* (brook trout). These streams range in length from 0.27 -8.10 *km*, allowing for thorough sampling and accurate estimates of population size. Fragmentation of CR streams occurred as a result of the late-Wisconsinan glaciation (10, 000-12, 000 ybp; Danzmann *et al*. 1998), and these populations are now isolated and genetically distinct (Wood *et al*. 2014). We focused on a total of eight different CR populations (Figure S1) for which demographic, habitat, and genetic data have been collected over a multi-year period.

Gamete collection

Wild

From October 13th - 26th, 2014, gametes were collected from seven CR populations: Bob's Cove (BC), Coquita (CO), Freshwater (FW), Ouananiche Beck (OB), Still There By Chance (STBC), Whale Cove (WC), and Watern Cove (WN). Individuals were collected from previously documented spawning sites, and from areas observed to have obvious redd formations and trout aggregates (Wood *et al.* 2014). Electrofishing was used to collect potential spawning individuals, which were then visually inspected for signs of "readiness": a release of sperm for males, and an elongated cloaca/soft belly for females. Readiness was assessed in the days leading up to the expected date of gamete collection and ready fish were held for 24-72 hours in flow-through cages before gamete collection.

Gamete collection took place between 19h00 and 1h00. Sperm was collected in 1.5ml micro-centrifuge tubes while eggs were collected in 60ml opaque plastic containers. Length (± 1 mm) and post-stripping weight (± 0.01 g) were collected for each fish, as well as an adipose fin clip for DNA identification. Gametes were kept on ice and insulated so as not to freeze, and transported to St. John's, NL immediately after collection. They were flown directly to Montreal, and crossed within 15 hours from the start of gamete collection. Shipments occurred on Oct 16th, 21st, and 26th.

Captive

From November 19th to December 12th, 2014, gametes were also collected from a large number of families within five CR populations maintained in captivity under common environmental conditions for one generation: BC, Cripple Cove (CC), FW, OB, and WN. These populations were used to bolster the number of crosses produced, and to include an additional CR population (CC) we were unable to obtain previously. Males and females from each population were sampled daily to check for signs of "readiness" (above) once the water temperature reached 7°C. If a sufficient proportion of males and females in two or more populations showed signs of readiness, gametes were immediately collected, and crossed on the same day. Individuals within populations were marked with unique individual tags to identify them for specific crosses and to avoid inbreeding of siblings. Crossing events occurred on November 19th, 26th, 28th, and December 4th and 12th. Gamete collection and general protocol followed as above.

Common garden experimental design

Crosses were generated using the gametes of 1+ to 4+ wild and 2+ aged captive fish and experienced a constant, warm temperature regime (7°C) from October, 2014 – April, 2015. Each cross was randomly assigned to a 5.2 cm diameter, mesh-bottomed, flow-through tube within a 1000L recirculating tank, and replicated in the same position in a second tank to account for tank and location effects. Each tank was maintained under a common temperature regime mimicking forecasted climate warming in these populations' native streams (6.9°C; Figure S2) and pH (7.0; Table S1). Eggs were left undisturbed until the eyed stage at which time dead eggs were removed daily to reduce fungal infections. A total of 9 full reciprocal inter-population hybrids (243 families × 2) and 2 half reciprocal inter-population hybrids (5 families × 2) were made, with 265 (× 2) pure families across all populations (Table 1).

Four fitness-related, early life history traits were measured on all individuals from all families generated: (i) survival to the eyed egg stage, (ii) survival to hatch, (iii) total length at hatch (± 0.01 mm), and (iv) total length at full absorption of yolk sac (± 0.01 mm; Beacham & Murray, 1985; Araki *et al.* 2008). Preliminary analyses revealed no differences in survival until the eyed egg stage and to hatching, so only three traits are discussed further – survival to hatch (hereafter *survival_hatch*), length at hatch (*length_hatch*), and length at yolk-absorption (*length_absorbance*).

Quantifying the effects of different factors on hybridization outcomes

The effects of population size, quantitative genetic divergence (Q_{ST}), geographic divergence (*km*), environmental dissimilarity (temperature and pH), cross type (pure or hybrid) and tank on fitness were assessed by generalized linear mixed models (GLMMs; logit transformed) for *survival_hatch*, and linear mixed models (LMMs) for length traits, using the lme4 package (Bates *et al*. 2012; all analyses were conducted using R (R Core Team 2016), and all packages retrieved from its open-source directory). These factors were fixed effects in the initial models, while mother was included as a random effect in order to elucidate any potential differences in gamete quality and allow quantification of maternal effects. Additionally, factors identifying the origin of families (wild or captive), specific population cross (i.e. FW × OB, OB × FW, etc.), and family I.D. were also included as random effects to control for variability in fitness between wild and captive trout, specific population crosses, and at the family-level. Models were simplified using reverse model selection via ANOVA, where the most complex model was compared to more simple models; the resulting significant factors were examined *post hoc* using the lsmeans package (Lenth, 2013). Likelihood ratio tests were used instead of AICc due to limited sample size at the population level, which may have been over-penalized by AICc.

To assess the effects of population size on fitness, two initial models were created for each of the three traits, which varied only by metrics of population size; either maternal number of breeders ($N_{b,}$ see below) or a binned factor of population size based on N_b. All other factors were identical between models. Population size can be quantified as census population size (N), N_e, or the effective number of breeders (N_b), the latter two relating to the number of individuals actively contributing to a sample of offspring and therefore influencing evolutionary trajectories (Lynch & Lande, 1998; Palstra & Fraser, 2012; Waples *et al*. 2013). As such, N_b and N_e are more likely to relate to hybridization outcomes. N_e and N_b differ in that N_e is a parameter describing the number of reproducing individuals in one generation, whereas N_b represents the total effective number of breeders in a given year (Palstra & Fraser, 2012). Although a relationship exists between these parameters (Waples *et al.* 2013), evolutionary studies on iteroparous organisms such as our brook trout, often rely on N_b as a meaningful descriptor of population size. N_b was used in this study as an analogue of N_e, as they are tightly linked in CR populations (Bernos & Fraser 2016). Maternal N_b was selected to provide insight on and be more closely related to maternal influences on fitness in salmonids (Einum & Fleming 1999; Heath *et al.* 1999). Binning allowed us to disentangle the effects of crossing a small and large population versus two medium ones, and consider the effects of maternal vs paternal population size, which would not be apparent if a mean value of N_b was generated. Binned population size was determined by labelling populations with $N_b < 51$ as "small", $51 < N_b < 125$ as "medium", and $N_b > 125$ as "large" due to the observed population sizes of our study populations. Next, for each population cross, these codes were combined: a FW female × OB male would be coded "LM" for large X medium, and a FW male × OB female would be "ML". Remaining factors were the same throughout all initial models. As pure families would either be coded "LL", "MM", or "SS", an interaction between binned population size and a factor of whether or not a family was hybrid or pure is also included in order to see the specific effect of population size on hybrid fitness alone.

To assess the effects of divergence on fitness, genetic divergence was measured by quantitative genetic differentiation between population pairs (Q_{ST}), as calculated and described in Wood et al. (2015). Q_{ST} was chosen over genetic differentiation at neutral markers (F_{st}) as quantitative traits are more strongly tied to fitness and influenced by environmental selective pressures. Geographic divergence was calculated using geographical coordinate data of CR stream mouths which, once entered into the "distVincentyEllipsoid" function in the "geosphere" package, calculated the shortest pairwise distances between locations (Hijmans 2015).

To assess the effects of environmental dissimilarity on fitness, we measured the degree of change between wild stream and in-lab temperature and pH (temperature=7.0℃, pH=6.9). For all populations, 7℃ was an environmental stress mimicking moderate climate warming at the life stages being investigated (Wood & Fraser 2015), while a pH of 6.9 was higher than all mean spawning-period pH measurements (Figure S1, Table S1). Each population's peak spawning date was calculated using data acquired in that year's spawning ground assays (Wood & Fraser 2015). From these unique starting dates, using previously documented data on the number of degree days to hatch for each population (Wood & Fraser 2015), the average temperature and pH was taken for each stream over the incubation stage in the wild and compared to the constant temperature and pH experienced by crosses in the lab setting. For hybrid crosses, mean temperature and pH values between parental streams were used for comparison to lab conditions. The absolute difference between wild and in-lab temperature and pH was taken as the degree of environmental dissimilarity experienced by the crosses.

Whether a family was hybrid or pure was accounted for via a two-level metric of "hybrid" or "pure" designated for each family. Interactions between this factor and each of population size and environmental dissimilarity were accounted for to assess whether hybrids performed better or worse than pures at different population sizes and degrees of environmental dissimilarity.

Hybrid metric of fitness in relation to factors thought to influence hybridization

To assess the relative difference in fitness between hybrids and their maternal pures while accounting for factors thought to influence this relationship, a standardized, average outbreeding response was calculated for each reciprocal hybrid in an inter-population cross. For *survival_hatch*, the log odds ratio was used to weigh the differences in fitness between pures and maternal hybrids using the follow formulas:

$$\ln((X_{AB}/(1-X_{AB}))/((X_{damA}/(1-X_{damA}))))$$

&

$$\ln((X_{BA}/(1-X_{BA}))/((X_{damB}/(1-X_{damB}))))$$

For the two length traits, the two following adaptations of an equation from Edmands (2007) were used:

$$\mathrm{X}_{\mathrm{AB}}/\mathrm{X}_{\mathrm{damA}} \quad \& \quad \mathrm{X}_{\mathrm{BA}}/\mathrm{X}_{\mathrm{damB}}$$

For all equations X_{AB} and X_{BA} are the average of all family fitness values for each reciprocal hybrid cross in an inter-population comparison, and X_{damA} and X_{damB} are the average of all family fitness values for each pure cross using dam *A* and dam *B* respectively. Although hybrid fitness is often a single metric compared to midpoint parental fitness (Edmands, 2007; Fraser *et al.* 2010), preliminary results suggested an effect of asymmetric sexual recombination on reciprocal hybrid fitness, as is commonly found in salmonids (Sakamoto *et al.* 2000; Moen *et al.* 2004; Woram *et al.* 2004). Reciprocal hybrids were therefore treated individually and compared to pure crosses using the same dam, as maternal effects have a significant effect on fitness. Per inter-population comparison, this resulted in two metrics of hybrid fitness in relation to pure fitness, which were then regressed with N_b, Q_{ST}, geographic divergence, temperature dissimilarity, and pH dissimilarity for each trait, in order to quantify the effects of these factors on the comparative fitness of pure and hybrid families.

Comparative hybrid to midpoint fitness

For each inter-population comparison, means and confidence intervals were also calculated and plotted with hybrids in between pures and next to their maternal pure. Comparisons were visually inspected to quantify the number of hybrids with mean fitness falling above or below the midpoint fitness value between the two pures; those above indicating heterosis and those below indicating OD. This was quantified per trait, and per maternal population to see if outbreeding was more or less beneficial depending on the trait, and whether certain populations exhibited heterosis or OD more than others.

RESULTS

Effects of different factors on hybridization outcomes

Population size and environmental dissimilarity had a statistically significant influence on trout fitness across the three measured traits as they were included in final models using reverse model selection (Table 2). However, there was little to no agreement across the six final models (two models per trait, three traits), across or within traits, and in most cases, statistical differences were difficult to interpret biologically. No consistent trends were found between final models using the same factor of population size, and so terms in final models were summed to assess proportional representation across all models.

The influence of incubation tank was included in all six final models (P=3.8e-14 - 1.05e-14); all other fixed effects were found in at most four of six models run (Table 3). Of the factors found in over half of the models, the two metrics of population size and pH dissimilarity were the only fixed effects. Temperature dissimilarity, geographic divergence, and genetic divergence were only present in half, one-third, and one-sixth of the final models respectively (Table 3).

All three traits decreased with increasing maternal N_b, and maternal N_b was included in the final model for *length_hatch* (t=0.73) and *length_absorbance* (t=0.70, Figures 1, S3). Binned population size was included in final models for *survival_hatch* (P=0.0047-0.94) and *length_absorbance* (t=0.98-1.04). Pairwise comparisons for *survival_hatch* showed significant differences where, in all cases, relatively smaller families had greater survival than larger ones (Table 4, Figure 2); only one pairwise comparison was significant for *length_hatch*, with a similar trend (Table 4). However, the interaction term of binned population size and whether or not a family was hybrid or pure had no significant effect. Therefore, it is possible that pure families designated “LL”, “MM”, or “SS” may be driving some significant pairwise differences.

Although pH dissimilarity was positively correlated with overall survival ($P=0.003$) and negatively correlated with the two length traits ($t=-2.50-1.63$), all relationships were visually weak (Figures 3, S4). Similarly, when regressing pH dissimilarity and each metric of population size with metrics of relative hybrid to maternal pure fitness, there was no indication of a meaningful biological relationship (Figures 4-6). There was, however, a weak indication that as pH dissimilarity increased, hybrids exhibited greater heterotic size relative to their maternal pures (Figure 4).

Comparative hybrid to midpoint fitness

OD and heterosis were common among all hybridized population pairs in that hybrids routinely had survival that fell both above and below midpoint pure fitness, suggesting heterosis and OD, respectively (Figure 7). Similar results were found for both length traits (Figure S5a, b).

For *survival_hatch*, one third of hybrids exhibited heterosis (i.e. means and confidence intervals fell above the midpoint fitness), one third exhibited OD, and one third were of similar midpoint fitness (Figure 7).

For *length_hatch*, two of eighteen hybrids exhibited heterosis, one of eighteen exhibited OD, and fifteen were of similar midpoint fitness (Figure S5a). Finally, for *length_absorbance* one third of comparisons showed heterosis while the remainder of crosses were of similar midpoint fitness (Figure S5b).

A general analysis of hybrid performance by specific population revealed no trend relating certain populations to consistently exhibit heterosis or OD. A chi squared test was performed to evaluate whether the distributions of above and below the midpoint deviated from the null hypothesis of 50:50, and was not rejected ($P=0.99$, $x^2=0.35$, $df=7$; Table S2).

DISCUSSION

We quantified how hybridization affected the offspring fitness of eight isolated, genetically distinct populations of a vertebrate species (brook trout), and if factors related to the populations' demographics and environments were able to explain these outcomes. We specifically investigated four candidate predictors of hybridization outcomes, due to their generally-accepted, theoretical relationships with fitness. In contrast to prevalent theories, we found that neither contemporary N_e (analogue: N_b), genetic divergence (Q_{ST}), geographic divergence (km), nor environmental dissimilarity (in temperature, pH) had a readily interpretable biological relationship with fitness, although population size and pH dissimilarity were included as statistically significant factors in over half of the models generated. This lack of a meaningful biological relationship was found even though the fitness-related traits measured are important for our study species and related ones (e.g. Einum & Fleming 2000), and fitness was assessed under conditions of moderate climate warming so as to elicit challenges to survival and growth. Further investigation found limited evidence for relationships between relative hybrid fitness and population size or environmental dissimilarity, though increased hybrid performance was seen at the latest developmental stage assessed (*length_absorbance*). Finally, population-specific hybrid performance was common, however, no specific population consistently exhibited heterosis or OD. This was despite the variety of population sizes of our study populations, including some populations falling at or below $N_e = 50$ (e.g. STBC=44, CO=45, WC=50; Bernos & Fraser 2016), often considered a critical threshold for maintaining short-term adaptive potential (Franklin 1980; Frankham *et al.* 2013; see below for more on minimum viable population size, hereafter MVP).

Population size

A large body of literature has identified higher N_e and concomitant higher genetic diversity (at least neutral genetic diversity) as being paramount to a population's potential fitness (Soulé 1976; Frankham 1996; Hitchings & BeeBee 1998; Reed & Frankham 2003; Mattila & Seeley 2007), and so it is generally expected that larger populations will have greater fitness than small ones (Lande 1988; Ellstrand & Elam 1993; Vrijenhoek 1994; Frankham 1996; Reed *et al.* 2003; Reed 2005; Hoffman & Sgrò 2011). However, we found limited evidence for this, with overall fitness having a weak negative relationship with N_e (analogue: N_b) and only 1/3, 1/9, and 1/3 of crosses experiencing heterosis for each of *survival_hatch*, *length_hatch*, and *length_absorbance* respectively. These results are in agreement with recent findings on the populations in our study, among others, suggesting that in relation to large populations, small populations (i) retain similar quantitative genetic variation and trait differentiation (V_A, Q_{ST}; Wood *et al.* 2015); (ii) exhibit no differences in magnitude of variability of plasticity (Wood & Fraser 2015); (iii) perform as well or better in novel environments (Yates & Fraser 2014); (iv) are not disproportionately impacted by genetic drift (Wood *et al.* 2016); and (v) may have lower neutral genetic diversity, but not fitness (Lammi *et al.* 1999). Yet, we did find that small populations showed weak signs of increased heterosis compared to large ones, consistent with the hypothesis that small populations would benefit more from hybridization. Combined, these results indicate that smaller populations do not necessarily mean less fit ones, but that hybridization may provide them with minor increases in fitness if experiencing ID. As such, it may be that individual fitness is not as strongly influenced by population size as previously thought. No declines in CR population sizes have been observed in nearly a decade of observation (Bernos & Fraser 2016), further supporting the notion that these populations may be at least somewhat tolerant of ID.

Genetic divergence, geographic divergence and environmental dissimilarity

Of the remaining factors assessed, only pH dissimilarity was found in over 50% of the final models generated. Having not found either metric of divergence in most final models, our second hypothesis that hybrid fitness would be negatively correlated with genetic and geographic divergence was not supported. However, as pH dissimilarity increased, length at yolk-absorption was weakly negatively affected. Interestingly, pH dissimilarity was positively correlated with *survival_hatch* when looking at relative hybrid fitness. Other research suggests that increased stress promotes F1 hybrid performance, but severe to moderate decreases in fitness may occur in the F2 generation (Edmands & Deimler 2004; Willett 2012). Although the F2 generation was not explored in our study, our results support the notion that F1 hybrids have greater relative fitness in more stressful conditions. This is likely due to our populations suffering somewhat from ID due to their isolation over the past 10, 000-12, 000 (Danzmann *et al.* 1998). We might speculate that fitness would decrease in the F2 and F3 generations, as continued disruption of useful gene complexes might be lost. It is possible that the increase in relative hybrid fitness is in fact being driven by a decrease in pure fitness at greater levels of environmental dissimilarity, as it has been found that inbred populations take on a greater inbreeding load as stress increases (Reed *et al.* 2002; Fox & Reed 2011). These results highlight a relatively understudied area of the influence of stress on genetics, whose importance has predominantly been placed on inbreeding (Kristensen *et al.* 2003).

Minimum viable population size

Although we tested populations ranging in size from N_e=44-588 and N=202-7801, and exposed them to environmental conditions different from their native habitat, our small populations might have been insufficiently small to detect significant effects of ID. However, as MVPs are theorized to be near $N_e = 50$ for short term and $N_e = 500$ for long-term adaptive potential (Franklin 1980; however, see Jamieson & Allendorf 2012 concerning their skepticism of this range, and rebuttal by Frankham *et al.* 2013) and MVP of N = 3577 - 7316 (Reed *et al.* 2003; Traill *et al.* 2007), we would expect to see increases in fitness for hybridized populations whose size falls well below these recommended minimums (e.g. STBC $N_e = 44$, CO $N_e = 45$, WC $N_e = 50$). With no indication of such gains from hybridization, nor any recent indication of decreasing size in these isolated populations that have persisted over 10,000 years, it is unclear that hybridization always benefits very small populations with more limited genetic diversity.

Two chief aspects of brook trout biology may explain why we did not large effects of hybridization, nor biologically meaningful influences of population size, divergence and environmental dissimilarity: (i) high levels of plasticity found in this species (compared to other freshwater fish) may allow it to respond to novel environmental conditions (Wood & Fraser 2015), and (ii) its residual tetraploidy (Thorgaard *et al.* 1983) may moderate or combat the effects of both inbreeding depression and benefits from outbreeding. In combination, these characteristics have been shown to reduce the effects of ID in many plants (Husband & Schemske 1997; Grineland 2008) and reduce the benefits of outcrossing (Pickup & Young 2008), and may have resulted in the dampening of fitness benefits in even our smallest hybridized populations. Consequently, only much smaller populations of brook trout may respond to hybridization as a means of genetic rescue.

Reciprocal recombination

We also found evidence for variable population-specific hybrid performance and maternal influences on fitness between reciprocal hybrids. Despite using eight genetically-distinct populations varying greatly in N_e, none exhibited consistent directional changes in fitness when hybridized. This is consistent with our conclusions that overall fitness and hybrid fitness are not readily explained by a number of demographic, genetic, and environmental factors. Although it is common for studies on hybridization to treat reciprocal hybrids as one measurement of fitness, or make no clear indication of their delineation (Edmands 1999; Clark *et al.* 2013), our results demonstrate the propensity for salmonid fishes to exhibit maternal-dependent variability in fitness, likely due to asymmetrical sexual recombination rates (Sakamoto *et al.* 2000; Moen *et al.* 2004; Woram *et al.* 2004).

Caveats

The scope of our experimental design was limited to early life-history traits related to fitness. As our results seemed to suggest increasing hybrid fitness from *length_hatch* to *length_absorbance*, it is possible that relative hybrid fitness may increase at later life stages. However, early life-history stages are often critical in a fish's life, and growth at this stage is correlated with later recruitment (Houde 1987). Additionally, while our experiment was large for a vertebrate species in encompassing eight populations, it was limited to one generation of hybridization and two environmental factors. To perform a multi-generational study on eight populations of a large-bodied vertebrate species would require extensive resources and time that would likely not be feasible over the long-term; even F1 studies on related species to brook trout commonly only encompass two to five populations (Clark *et al.* 2013; Stelkens *et al.* 2014).

Lastly, other environmental factors such as pH, salinity, or dissolved oxygen might influence fitness.

Conclusions

Although hybridization had population specific influences on fitness-related traits in our trout species, these outcomes were not consistent across populations, nor readily predictable using a number of metrics hypothesized to influence hybrid outcomes. These results give credence to the precautionary approach when dealing with the question of genetic rescue, as it is not always clear whether small populations are (i) at any particular risk, or (ii) likely to benefit from inter-population hybridization (also see Waller 2015). As our study populations have been isolated for approximately12,000 years and vary greatly in population sizes, some falling well below theoretical MVPs; it is likely that the benefits of hybridization will be Family, species, and population specific. For example, salmonids have high levels of local adaptation: rapid habitat fragmentation, climate change and supplementation of hatchery-reared individuals makes them an ideal target for hybridization work, as these factors combined have potential to seriously decrease their long-term viability. For brook trout specifically, their naturally high levels of plasticity and tetraploidy may be sufficient to dampen hybridization influences on fitness in a number of situations. Lastly, at the population level, knowing historical episodic events such as bottlenecks, stochastic environmental events, or human-induced changes to population composition are likely to influence hybridization outcomes via their effects on genetic composition (Nason *et al.* 2002). As such, we suggest using hybridization as a tool for genetic rescue only when a great deal of information is known about the organisms in question, and only when there are obvious signs of rapid fitness declines within a population. Such rapid declines may be more common as temperatures continue to rise due to climate change. As such, the following chapter explores the ability of isolated brook trout populations and hybrids created between them to tolerate rapid climate warming.

FUNDING

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TABLES

Table 1: Number of families created for all pures and hybrids generated using eight populations of brook trout from Cape Race, Newfoundland, Canada, in fall/winter of 2014.

Cross type	# families
BC	10
CC	29
CO	13
FW	65
OB	68
STBC	11
WC	18
WN	51
BC × FW	3*
BC × OB	2*
CC × OB	19
CC × WN	18
CO × OB	10
FW × OB	60
FW × STBC	14
FW × WC	23
FW × WN	20
OB × WN	65
STBC × FW	14

Total pures:	265**
Total hybrids:	248**

*semi reciprocal
**values do not include replication

Table 2: Initial models used for each of three fitness-related traits, with final models determined by reverse model selection using ANOVA. Models within traits differed by factors of population size, with: a = maternal N_b, b = temperature dissimilarity, c = pH dissimilarity, d = Q_{ST}, e = geographic divergence, f = pure or hybrid, g = tank, h = binned population size, 1 = mother I.D., 2 = origin, 3 = population cross, 4 = family I.D.

Trait	Model	Fixed Effects	Random Effects	Interactions	Final Model
Survival to Hatch	$GLMM_1$	a,b,c,d,e,f,g	1,2,3,4	a:f,b:f,c:f	b,g,1,2,4
	$GLMM_2$	h,b,c,d,e,f,g	1,2,3,4	h:f,b:f,c:f	h,b,c,g,1,2,4
Length at Hatch	LMM_1	a,b,c,d,e,f,g	1,2,3,4	a:f,b:f,c:f	a,b,c,f,g,a:f,b:f,c:f,1,3,4
	LMM_2	h,b,c,d,e,f,g	1,2,3,4	h:f,b:f,c:f	g,1,3,4
Length at Yolk Absorption	LMM_1	a,b,c,d,e,f,g	1,2,3,4	a:f,b:f,c:f	a,c,d,e,f,g,a:f,c:f,1,3,4
	LMM_2	h,b,c,d,e,f,g	1,2,3,4	h:f,b:f,c:f	h,c,e,f,g,1,3,4

Table 3: Summary of proportional representation of model factors within and across fitness-related traits.. Three factors were included in the final models for all three traits, one of which was a fixed effect (tank).

Factors	Survival to Hatch	Length at Hatch	Length at Yolk Absorption	All (actual)	All (possible)
Tank	2	2	2	6	6
Mom I.D.	2	2	2	6	6
Family I.D.	2	2	2	6	6
Maternal N_b	0	1	1	2	3
pH diss.	1	1	2	4	6
Binned population size	1	0	1	2	3
Population cross	0	2	2	4	6
Maternal N_b:Pure or hybrid	0	1	1	2	3
Temp. diss.	2	1	0	3	6
Pure or hybrid	0	1	2	3	6
Geographic div.	0	0	2	2	6
Origin	2	0	0	2	6
pH diss.:Pure or hybrid	0	1	1	2	6
Q_{ST}	0	0	1	1	6
Temp. diss.:Pure or Hybrid	0	1	0	1	6
Binned population size:Pure or hybrid	0	0	0	0	3
All (actual)	12	15	19		
All (possible)	28	28	28		

Table 4: Pairwise comparison of binned population sizes, with standard errors and p-values for survival to hatch and length at hatch. Binned population size was determined by labelling

populations with $N_b < 50$ as “small”, $50 < N_b < 150$ as “medium”, and $N_b > 150$ as “large”. Next, for each population cross, these codes were combined: a FW female × OB male would be coded “LM” and a FW male × OB female would be “ML”.

Comparison [†]	Hatch Survival SE	Hatch Length SE	Hatch Survival p-value	Hatch Length p-value
ll-lm	0.34	0.026	0.11	0.78
ll-ls	0.38	0.044	0.01*	0.67
ll-ml	0.37	0.028	0.17	0.78
ll-mm	0.64	0.019	0.07	0.005**
ll-sl	0.43	0.049	0.07	0.57
ll-ss	0.57	0.047	0.04*	0.54
lm-ls	0.46	0.031	0.003**	0.54
lm-ml	0.22	0.018	0.83	0.57
lm-mm	0.38	0.030	0.10	0.09
lm-sl	0.48	0.037	0.01*	0.54
lm-ss	0.55	0.050	0.003**	0.54
ls-ml	0.49	0.033	0.004**	0.68
ls-mm	0.69	0.043	0.003**	0.57
ls-sl	0.41	0.034	0.65	0.12
ls-ss	0.44	0.049	0.65	0.72
ml-mm	0.35	0.028	0.06	0.12
ml-sl	0.50	0.038	0.01*	0.42
ml-ss	0.57	0.052	0.004**	0.57
mm-sl	0.68	0.047	0.005**	0.09
mm-ss	0.67	0.039	0.002**	0.72
sl-ss	0.40	0.049	0.31	0.15

[†] Where letters indicate the size of populations crossed: “l” is large, “m” is medium, and “s” is small.

FIGURES

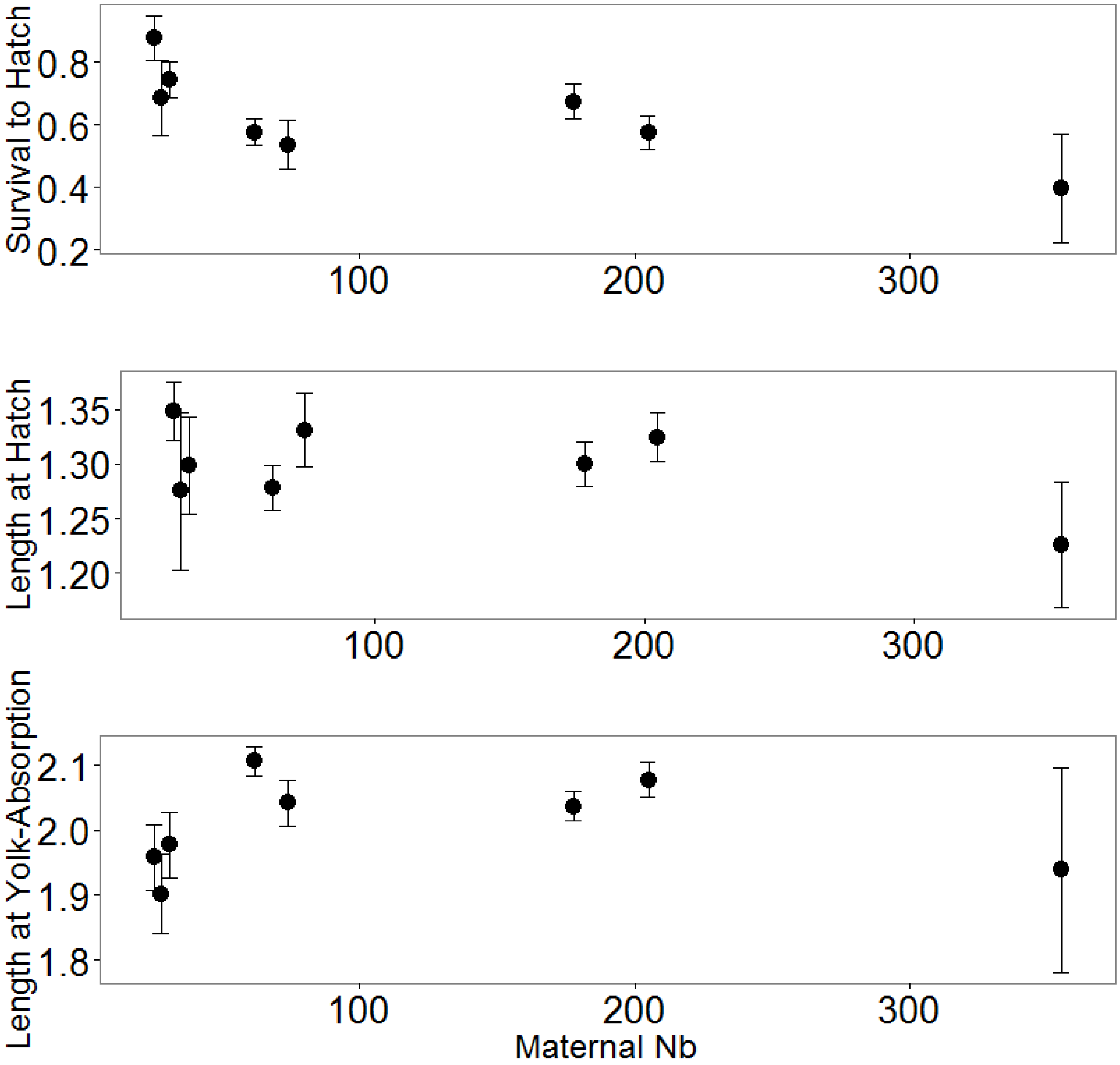

Figure 1: The relationship between maternal number of breeders (N_b) and each of the three fitness-related traits across all populations of brook trout from Cape Race, Newfoundland, Canada. Means and 95% confidence intervals are shown.

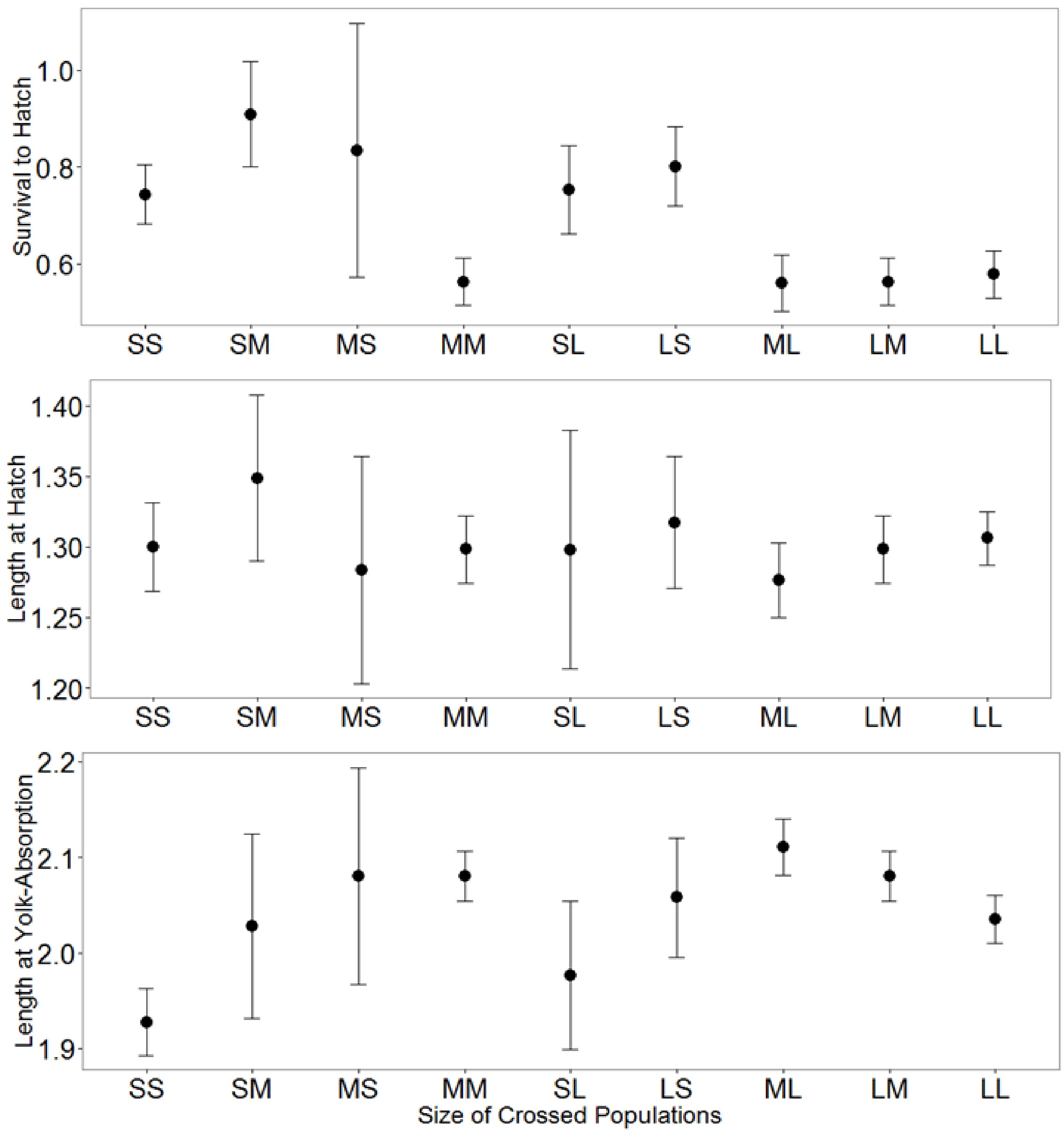

Figure 2: The relationship between binned population size and three fitness-related traits. Binned population size was determined by labelling populations with $N_b < 51$ as "small", $51 < N_b < 125$ as "medium", and $N_b > 125$ as "large". Next, for each population cross, these codes were combined: a FW female × OB male would be coded "LM" and a FW male × OB female would be "ML". Means and 95% confidence intervals are shown.

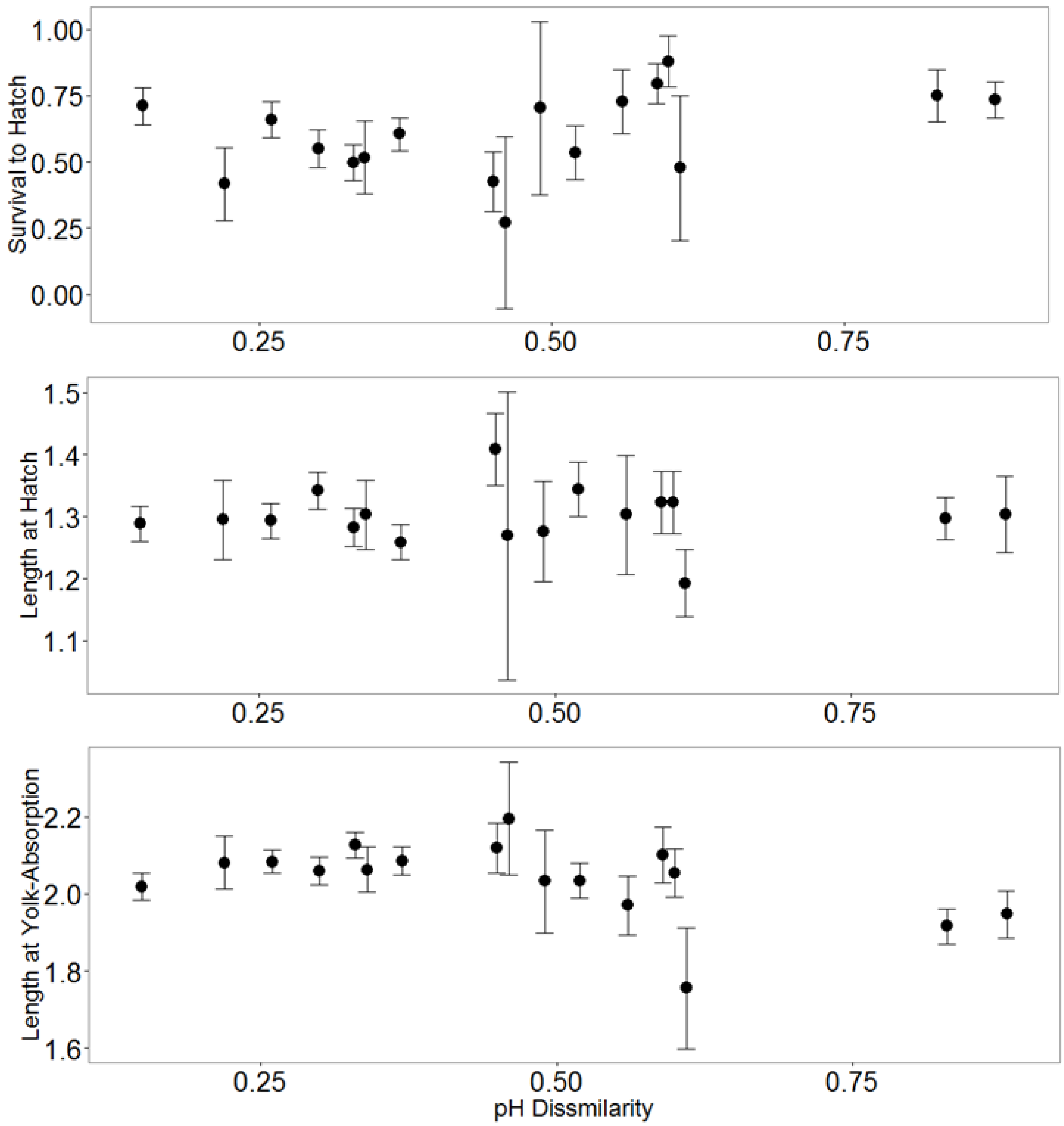

Figure 3: The relationship between pH dissimilarity of wild Cape Race streams and in-lab conditions and each of the three fitness-related traits across all populations of brook trout from Cape Race, Newfoundland, Canada. Means and 95% confidence intervals are shown.

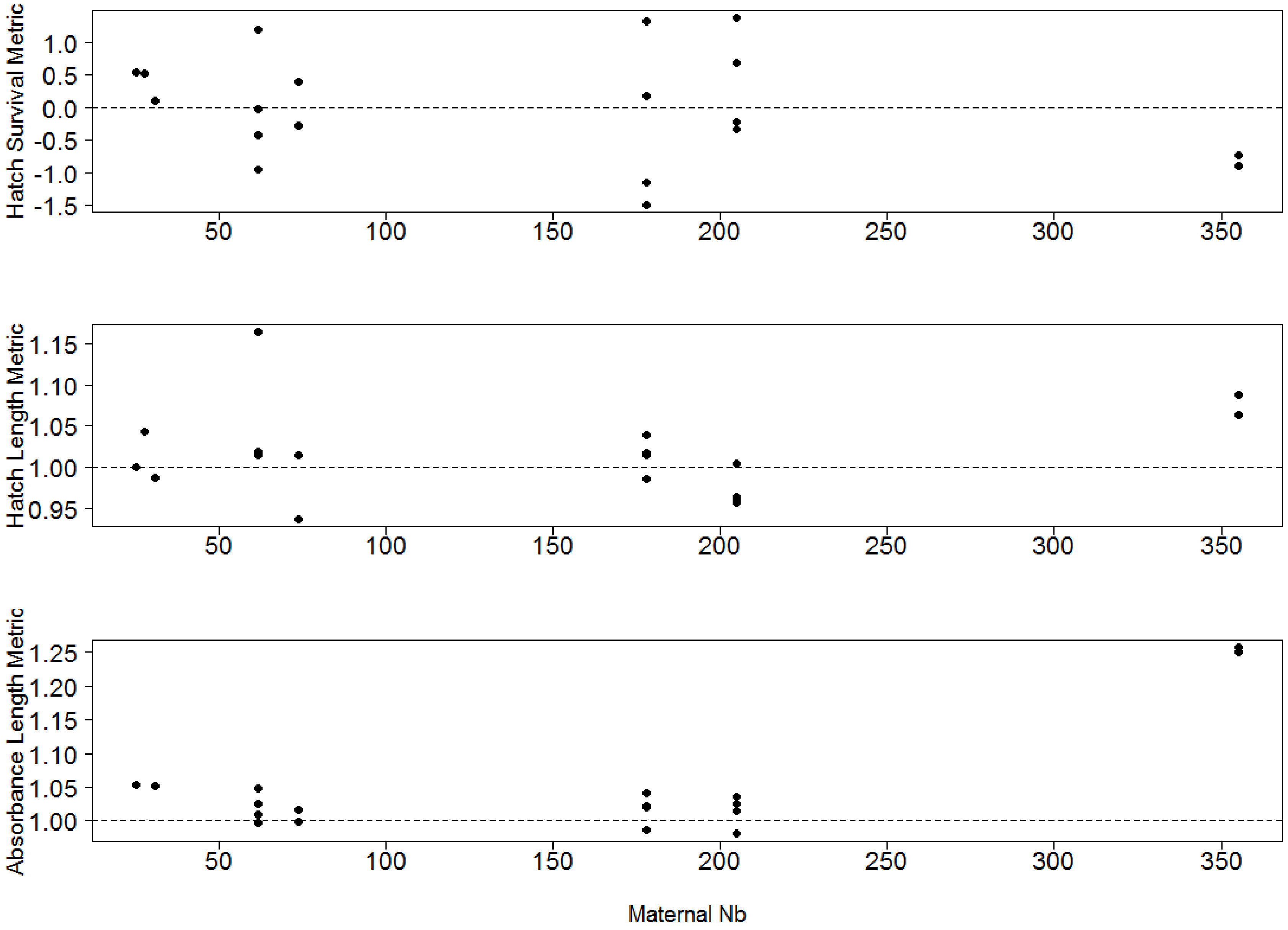
Hatch Survival Metric
1.0
0.5
0.0
-0.5
-1.0
-1.5
Hatch Length Metric
1.15
1.10
1.05
1.00
0.95
Absorbance Length Metric
1.25
1.20
1.15
1.10
1.05
1.00
50
100
150
200
250
300
350
Maternal Nb

Figure 4: Three fitness-related metrics of hybrid relative to pure differences in fitness versus maternal number of breeders (N_b). For survival to hatch, values above ‘0’ (dashed line) show hybrids performing proportionally better than their maternal pure. For each length trait, values greater than ‘1’ (dashed line) on the y-axis show hybrids performing proportionally better than their maternal pure.

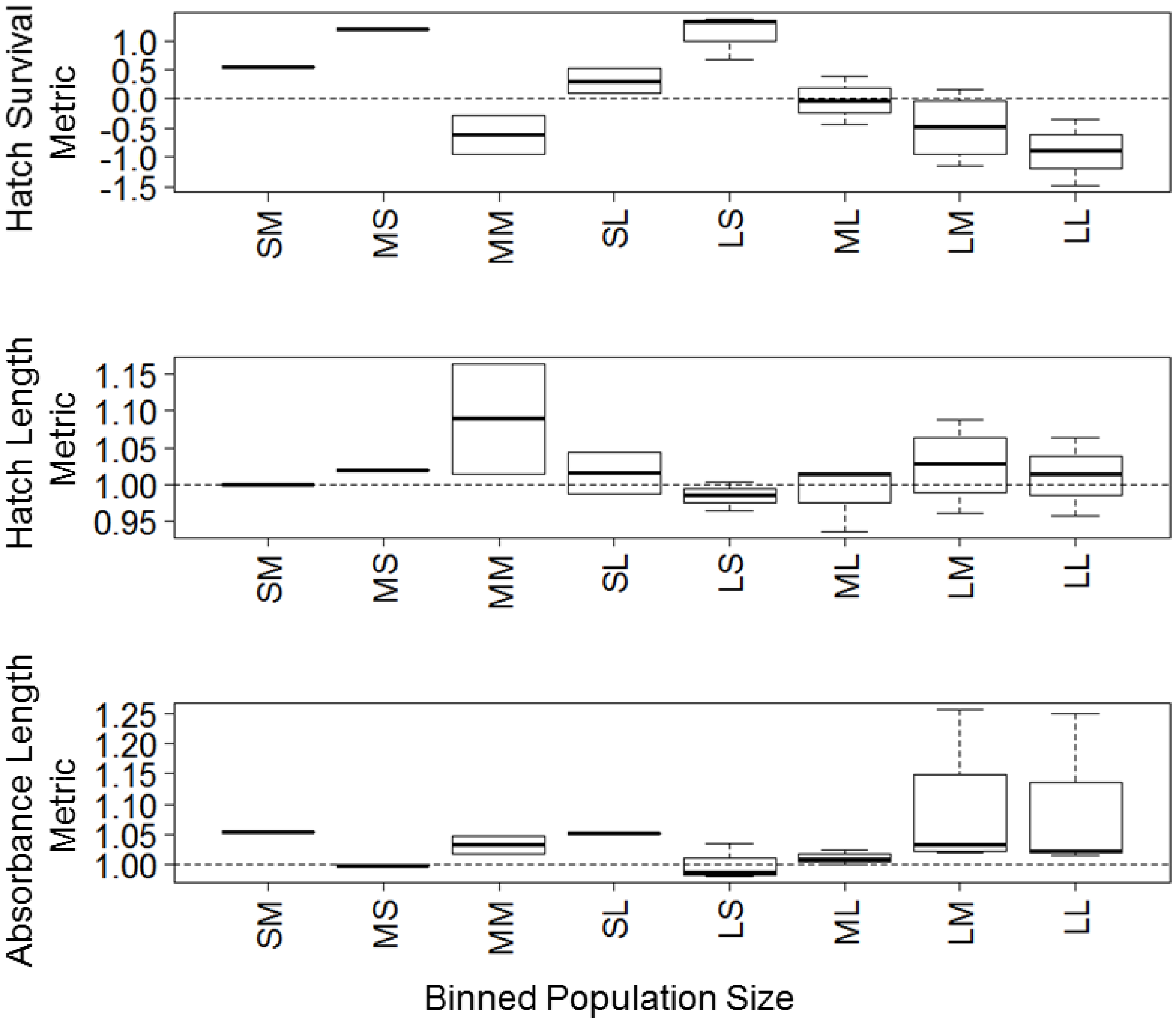
Hatch Survival Metric
1.0
0.5
0.0
-0.5
-1.0
-1.5
SM
MS
MM
SL
LS
ML
LM
LL
Hatch Length Metric
1.15
1.10
1.05
1.00
0.95
SM
MS
MM
SL
LS
ML
LM
LL
Absorbance Length Metric
1.25
1.20
1.15
1.10
1.05
1.00
SM
MS
MM
SL
LS
ML
LM
LL
Binned Population Size

Figure 5: Three fitness-related metrics of hybrid relative to pure differences in fitness versus binned population size. For survival to hatch, values above ‘0’ (dashed line) show hybrids performing proportionally better than their maternal pure. For each length trait, values greater than ‘1’ (dashed line) on the y-axis show hybrids performing proportionally better than their maternal pure.

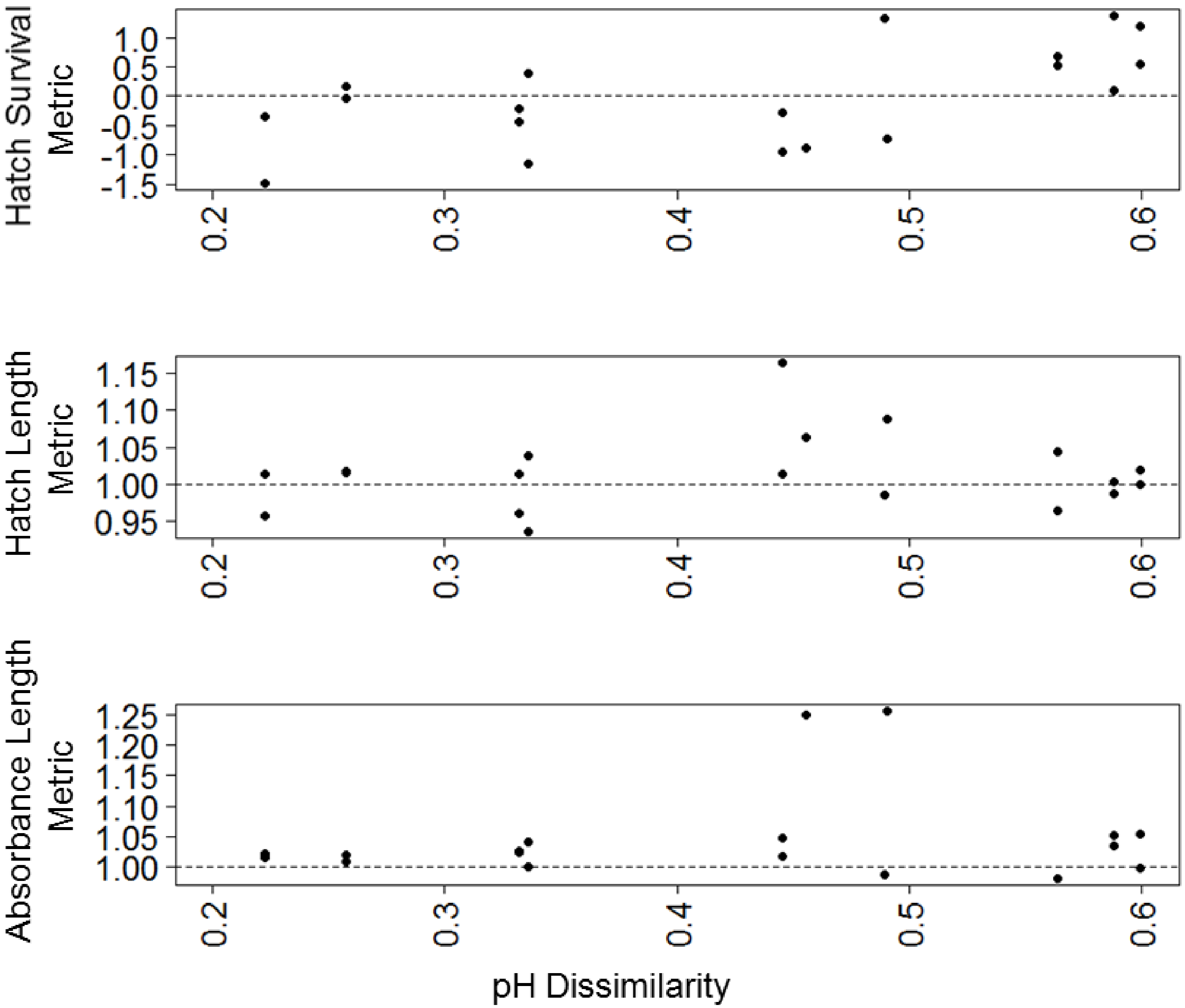
Hatch Survival Metric
1.0
0.5
0.0
-0.5
-1.0
-1.5
0.2
0.3
0.4
0.5
0.6
Hatch Length Metric
1.15
1.10
1.05
1.00
0.95
0.2
0.3
0.4
0.5
0.6
Absorbance Length Metric
1.25
1.20
1.15
1.10
1.05
1.00
0.2
0.3
0.4
0.5
0.6
pH Dissimilarity

Figure 6: Three fitness-related metrics of hybrid relative to pure differences in fitness versus wild stream and in-lab pH dissimilarity. For survival to hatch, values above '0' (dashed line) show hybrids performing proportionally better than their maternal pure. For each length trait, values greater than '1' (dashed line) on the y-axis show hybrids performing proportionally better than their maternal pure.

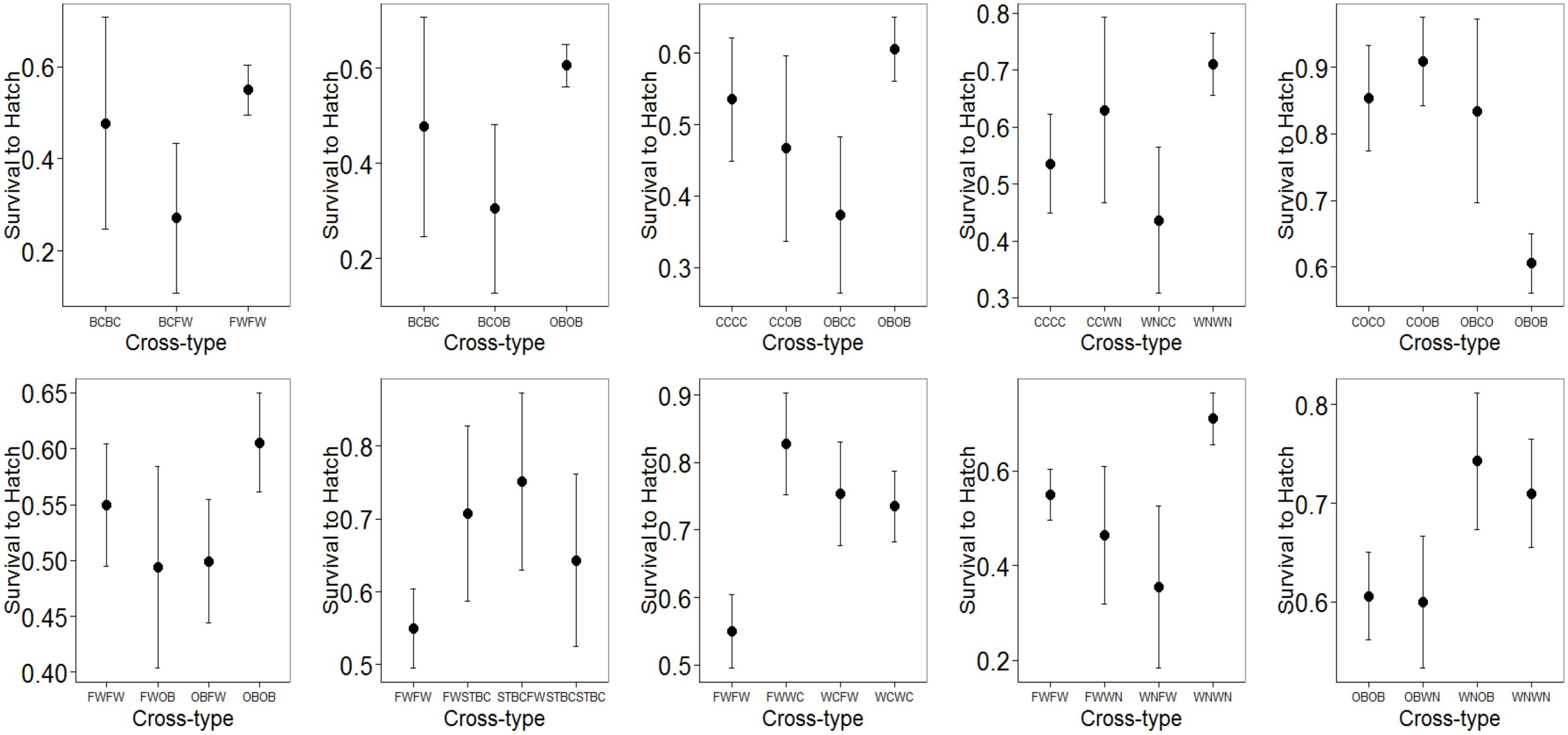

Figure 7: Plots of means and 95% confidence intervals (CI) of survival to hatch by inter-population hybrid comparison. Reciprocal hybrids are between each pure, and adjacent to their maternal pure. Where a hybrid mean and CI fall above a straight line drawn between its pures, heterosis has occurred, below represents OD. Six hybrids resulted in heterosis, six resulted in outbreeding depression, and six had their CIs overlapping the midpoint fitness line. Identical plots for *length_hatch* and *length_absorbance* in supplementary figures.

SUPPLEMENTARY

Tables

Table S1: Yearly mean pH and overall mean pH by stream for eight populations of brook trout in Cape Race, Newfoundland, Canada.

Stream	2012	2013	2014	2015	**Mean**
Bob's Cove	6.26	5.73	6.58	6.14	6.18
Coquita	6.18	5.59	6.37	6.14	6.07
Cripple Cove	6.14	NA	6.82	6.17	6.38
Freshwater	NA	NA	6.7	6.51	6.61
Ouananiche Beck	NA	NA	7	6.06	6.53
Still There By Chance	6.12	5.66	6.27	6.22	6.07
Whale Cove	6.25	5.56	6.21	6.06	6.02
Watern Cove	NA	NA	6.67	6.83	6.75

Table S2: The number of times the mean of a hybrid, designated by its maternal population, fell above or below midpoint pure fitness, regardless of confidence intervals.

Maternal Population	Above	Below
BC	4	2
CC	4	2
OB	7	5
FW	7	5
STBC	2	1
WC	2	1
WN	7	2
CO	3	0

Figures

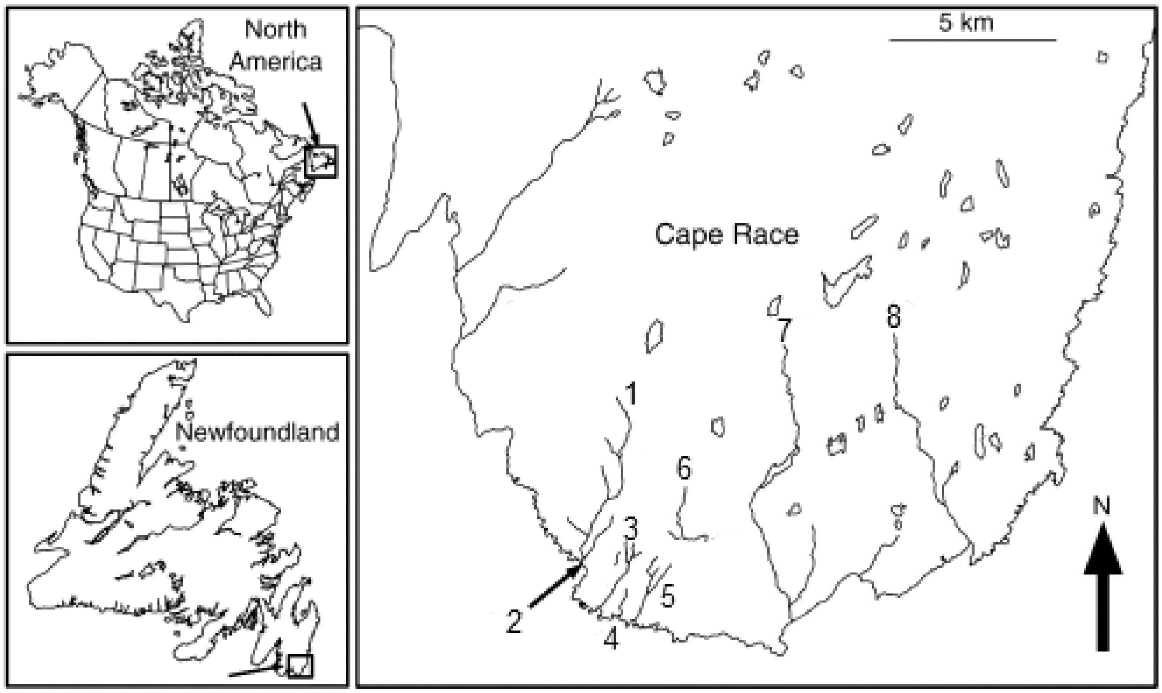

Figure S1: The locations of streams where gamete collection took place: 1) Freshwater (FW), 2) Coquita (CO), 3) Bob's Cove (BC), 4) Still There By Chance (STBC), 5) Whale Cove (WC), 6) Ouananiche Beck (OB), 7) Watern Cove (WN), 8) Cripple Cove (CC).

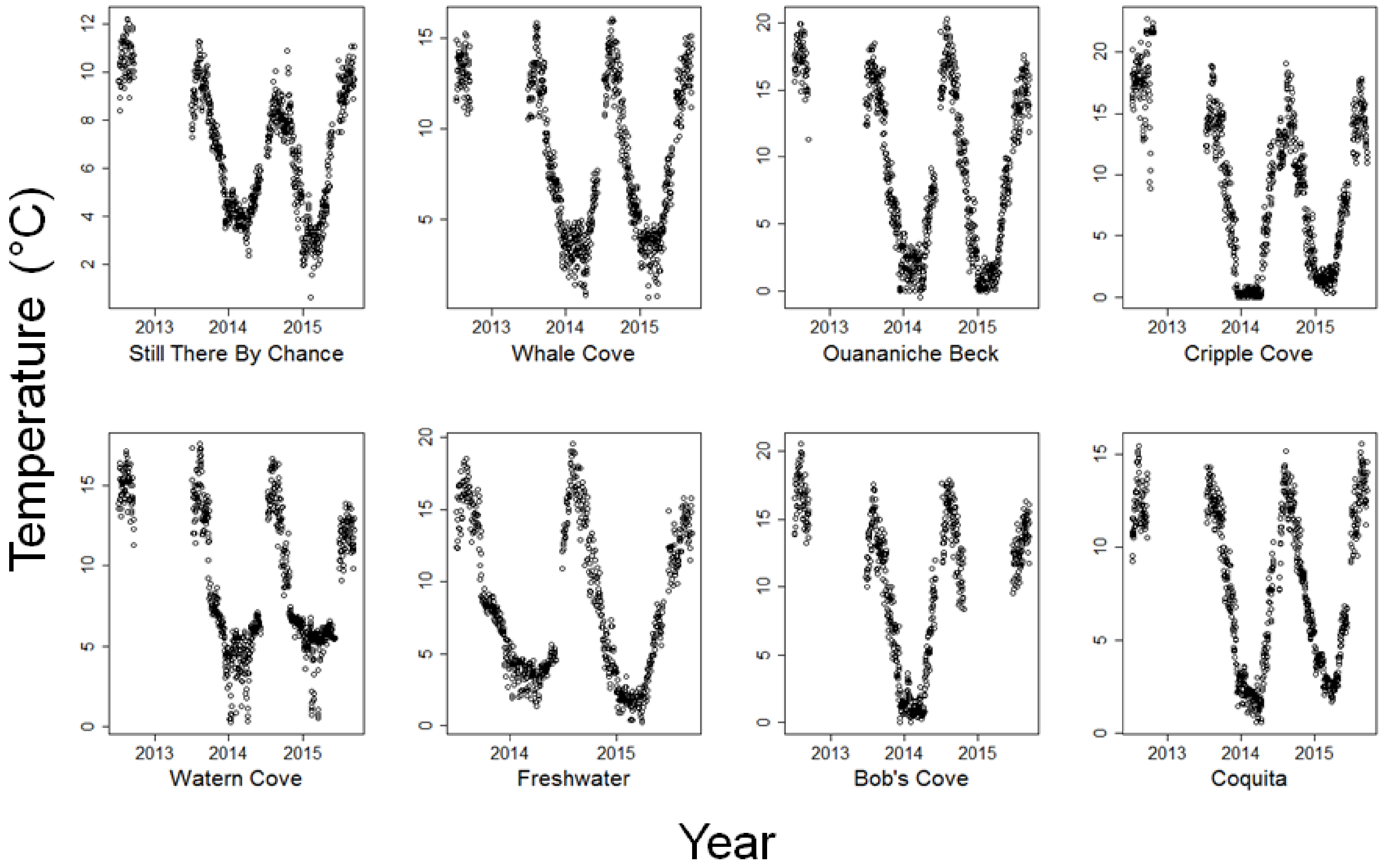
Temperature (°C)
Year
Still There By Chance
2
4
6
8
10
12
Whale Cove
5
10
15
Ouananiche Beck
0
5
10
15
20
Cripple Cove
0
5
10
15
20
Watern Cove
0
5
10
15
Freshwater
0
5
10
15
20
Bob's Cove
0
5
10
15
20
Coquita
0
5
10
15
2013
2014
2015

Figure S2: Thermal regimes of eight streams in Cape Race, Newfoundland, Canada, bearing isolated and genetically distinct populations of brook trout, where data are available from 2012-2015. Each dot representing the mean daily temperature of a stream, as recorded by 1 to 4 temperature data loggers.

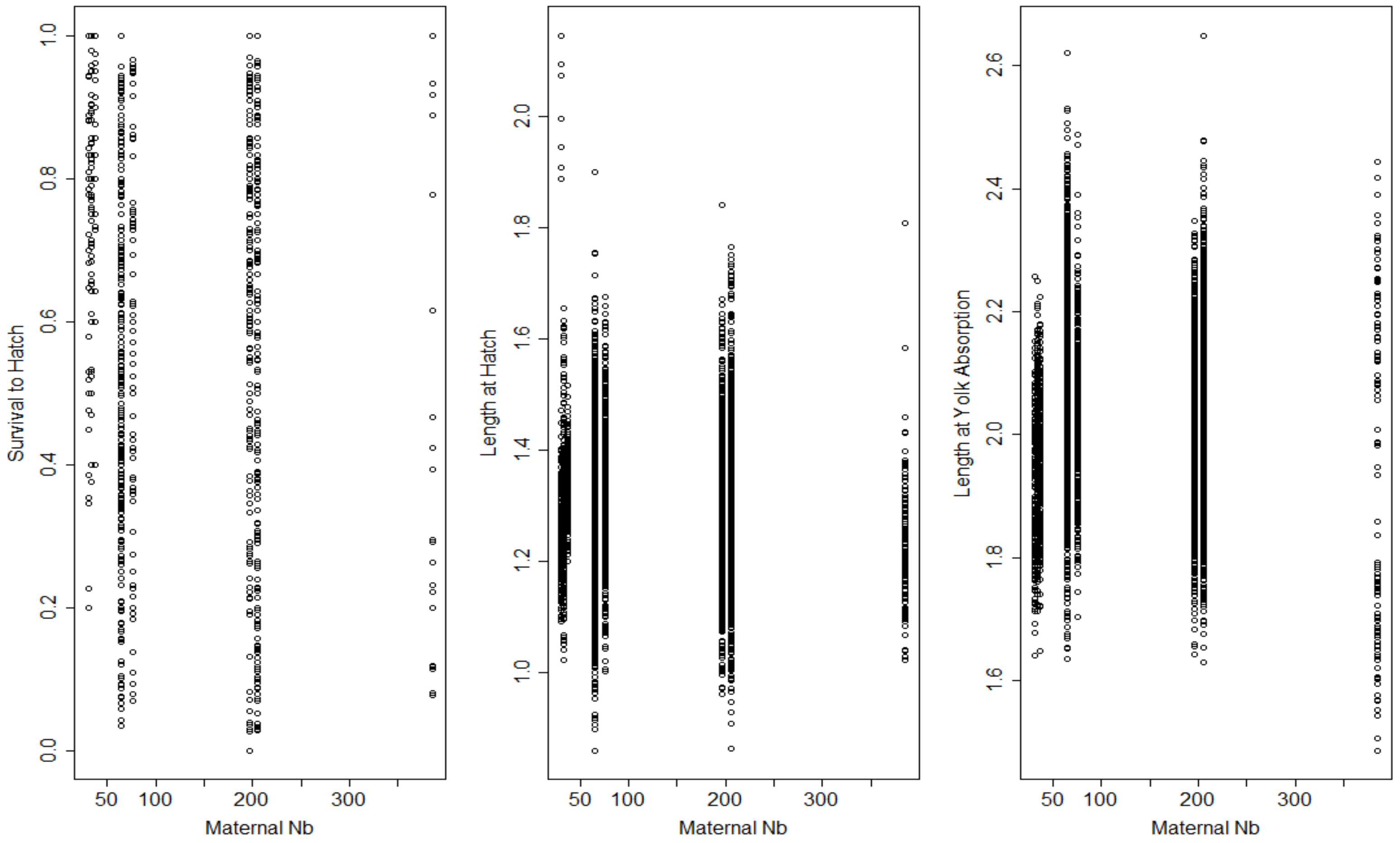

Figure S3: The relationship between maternal number of breeders (N_b) and each of the three fitness-related traits across all populations of brook trout from Cape Race, Newfoundland, Canada.

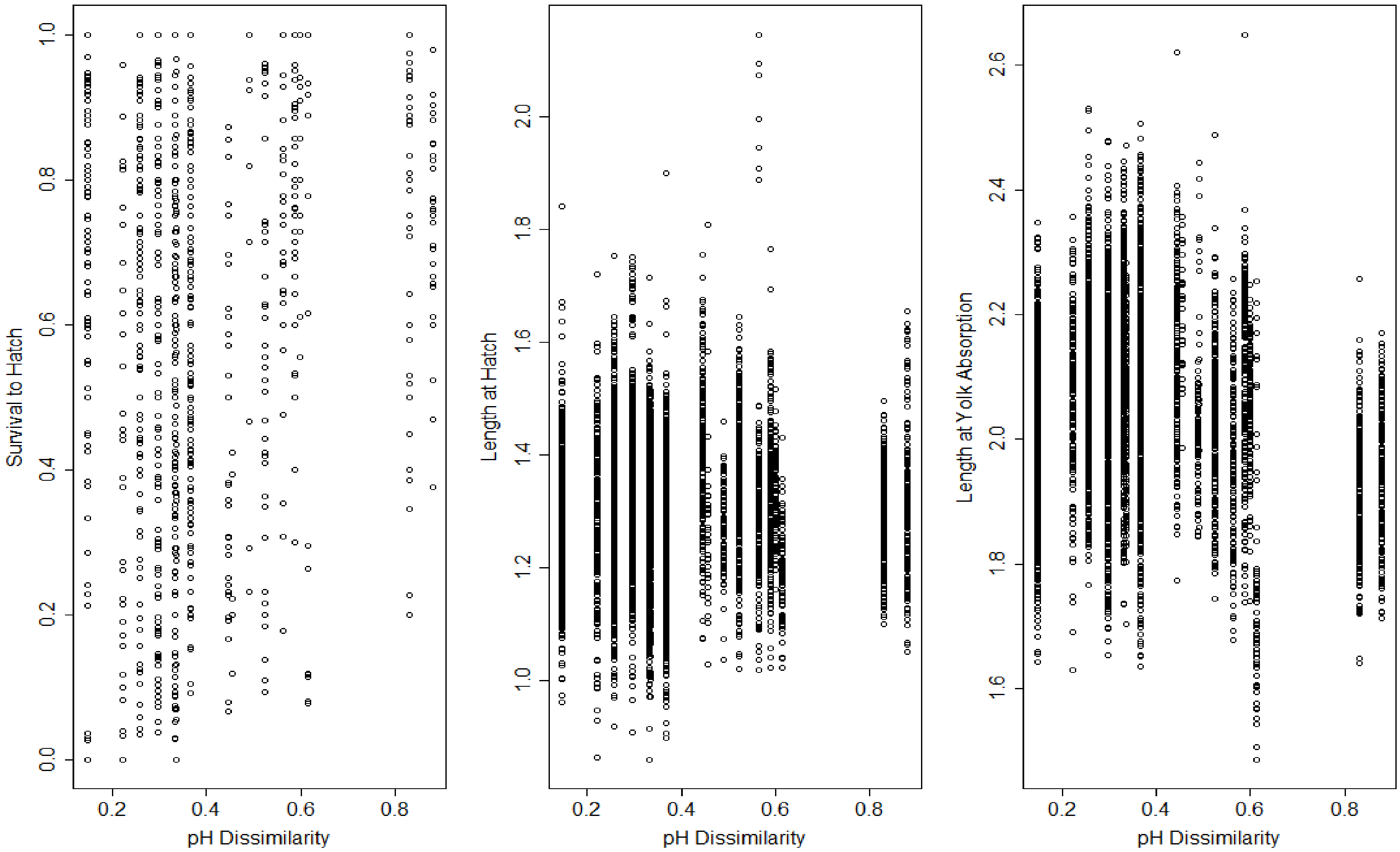

Figure S4: The relationship between *pH* dissimilarity of wild Cape Race streams and in-lab conditions and each of the three fitness-related traits across all populations of brook trout from Cape Race, Newfoundland, Canada.

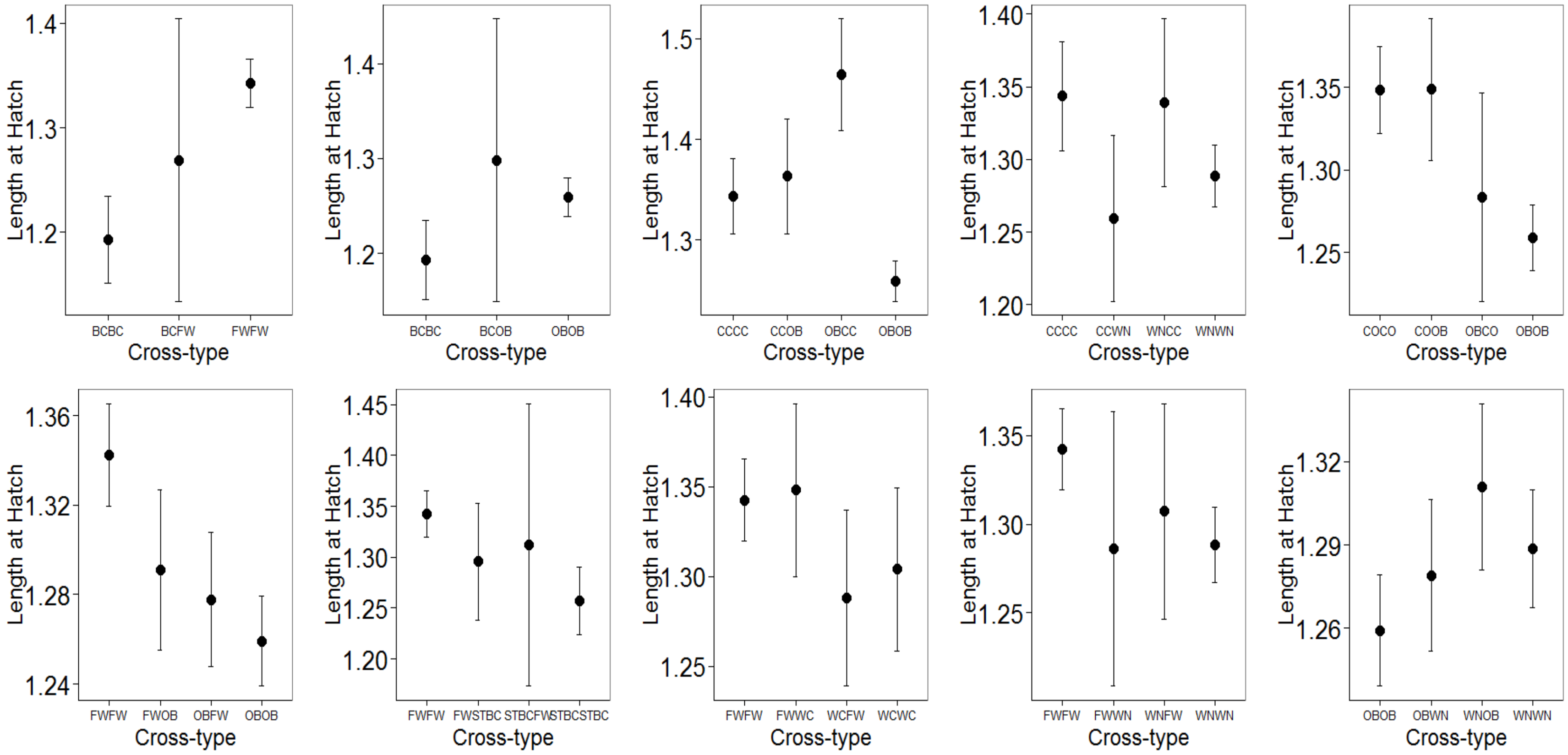

Figure S5a: Plots of means and 95% confidence intervals of length at hatch by each pair of populations outbred. Reciprocal hybrids are between each pure, and adjacent to their maternal pure. Where a hybrid's mean and its confidence intervals fall above a straight line drawn between its pures, heterosis has occurred. Where means and confidence intervals fall below this line, outbreeding depression has occurred. For this trait, two hybrids resulted in heterosis, one resulted in outbreeding depression, and fifteen had their confidence intervals overlapping the midpoint fitness line.

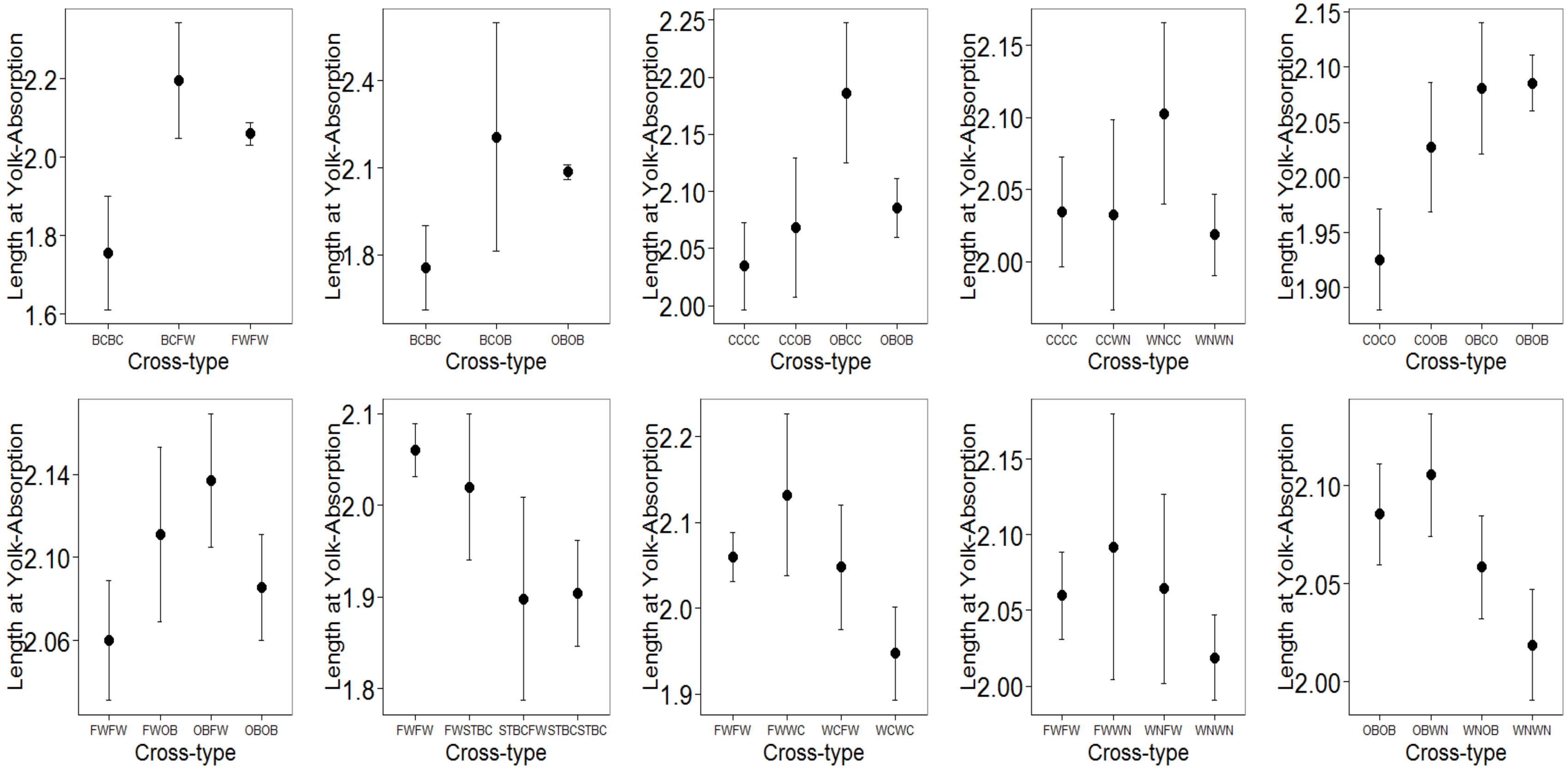

Figure S5b: Plots of means and 95% confidence intervals of survival to hatch by each pair of populations outbred. Reciprocal hybrids are between each pure, and adjacent to their maternal pure. Where a hybrid's mean and its confidence intervals fall above a straight line drawn between its pures, heterosis has occurred. Where means and confidence intervals fall below this line, outbreeding depression has occurred. For this trait, six hybrids resulted in heterosis, zero resulted in outbreeding depression, and twelve had their confidence intervals overlapping the midpoint fitness line.

Chapter 2: Limited variability in upper thermal tolerance among pure and hybrid populations of a cold water fish

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LAY SUMMARY

To forecast the impact of climate warming on cold-water fishes, thermal tolerance trials were conducted on six populations of brook trout from Cape Race, Newfoundland, Canada. Of these, three were outcrossed to assess the effect of hybridization. Although differences were found, there was little variation among populations in how they responded to thermal stress.

Total word count: 4527

ABSTRACT

As climate warming threatens the persistence of many species and populations, it is important to forecast their responses to warming thermal regimes. Climate warming often traps populations in smaller habitat fragments, not only changing biotic parameters, but potentially decreasing adaptive potential by decreasing genetic variability. We examined the ability of six genetically-distinct and different-sized populations of a cold water fish (brook trout, *Salvelinus fontinalis*) to tolerate acute thermal warming, and whether or not this tolerance could be altered by hybridizing populations. Critical thermal maximum (CT_{max}) assays were conducted on juveniles from each population to assess thermal tolerance, and agitation temperature was recorded for assessing behavioural changes to elevated temperatures. CT_{max} differed among four populations, though the maximum CT_{max} difference was only 0.68 °C (29.11-29.79 °C). Hybridization between one large population and two small populations yielded no significant differences in CT_{max}, and no differences in agitation temperature were detected among pure populations nor hybrids. Despite being one of the most phenotypically divergent and plastic north temperate freshwater fishes, our results suggest that limited variability exists in CT_{max} among populations of brook trout, regardless of their population size, standing genetic variation, and differing natural thermal regimes (temperature range, minimum, and maximum). This study highlights the level to which thermal tolerance is conserved between isolated populations of a vertebrate species, in the face of climate warming.

INTRODUCTION

Human-induced climate change may be the single greatest threat to global biodiversity (Sala *et al.* 2000). Climate change can interact with habitat fragmentation by creating physical (*i.e.* drought) or physiological (*i.e.* temperature) barriers (Hughes 2000; Walther *et al.* 2002; Pearson & Dawson 2003; Travis 2003). As fragments become smaller, so too do the populations within them, resulting in a loss of genetic diversity via an increased likelihood of inbreeding, genetic drift, and reduced gene flow (Young *et al.* 1996; Keller & Largiader 2003; Andersen *et al.* 2004; Ezard & Travis 2006). In general, this process reduces adaptive potential, the ability of a population to tolerate environmental change, and it further decreases population size. This paper aims to investigate the ability of isolated and different-sized populations to deal with climate warming, and the extent to which outbreeding might enhance responses to such warming.

By increasing genetic variability within a population upon which natural selection can act, hybridization may improve population responses to climate warming, such as upper thermal tolerance (Stockwell *et al.* 2003; Pickup *et al.* 2012). Nevertheless, a number of factors influence the outcomes of hybridization, which may also decrease or have no effect on average fitness within populations (Edmands 2007; Fraser *et al.* 2008). In part, such outcomes depend on population size: larger populations are expected to provide more genetic material than small ones, and small populations are expected to greatly benefit due to having lower genetic variability and possibly reduced phenotypic plasticity (Lande 1988; Ellstrand & Elam 1993; Frankham 1996; Reed & Frankham 2003; but see Wood *et al.* 2015; Wood & Fraser 2015). Therefore, when studying population responses to climate warming, it is important to consider both the relative benefits of population size and hybridization concurrently.

A practical metric for assessing the thermal tolerance of individuals from different, fragmented populations of a species is the critical thermal maximum (CT_{max}), defined as the temperature at which an organism can no longer maintain coordinated movement or equilibrium control (Becker & Genoway 1979). Subjecting an organism to a linear increase in temperature, the onset of spasms and loss of equilibrium are used as markers for CT_{max}, with loss of equilibrium being the most commonly used (Lutterschmidt & Hutchison 1997). In nature, a loss of equilibrium affects an organism's ability to forage or avoid predation, which may ultimately affect individual fitness. Since its introduction (Cowles and Bogert 1944), CT_{max} studies have evolved to account for a number of factors influencing CT_{max} results. A low rate of temperature increase (RTI) allows organisms to acclimate to rising temperatures, while a high RTI results in core body temperature lag, skewing CT_{max} results (Cox *et al.* 1974; Becker & Genoway 1979; Galbreath *et al.* 2004). Acclimation temperature (T_a) has been found to be positively correlated with CT_{max} (Cox *et al.* 1974; Zhang & Kieffer 2014; McDonnell & Chapman 2015, although see Galbreath *et al.* 2004; Recsetar *et al.* 2012), and as such, the ancestral history and origin of a population are thought to be linked to an organism's ability to tolerate temperature increases (Stockwell *et al.* 2003; McDermid *et al.* 2012).

Salmonids are a socioeconomically-important family of cold water fishes having traditional and commercial value. Although they are rich in populations and diversity, habitat fragmentation has depleted their numbers, and their viability is of growing concern as climate change warms northern regions (Walther *et al.* 2002; Alley *et al.* 2003; Hinzman *et al.* 2005). Recently, studies on the ability of salmonids to tolerate climate warming have been variable - some finding evidence of population-level variation in thermal physiology (Eliason *et al.* 2011) and others finding little to none (Elliott & Klemetsen 2002; Kelly *et al.* 2014). In particular, brook trout (*Salvelinus fontinalis*) is an extremely diverse (Angers *et* al. 1995; Wood *et al.* 2015) and highly plastic (Hutchings 1996; Imre *et al.* 2002) stenotherm, inhabiting a thermal window of 1-22°C (Xu *et al.* 2010) to maintain both an internal body temperature below 20°C (Scott & Crossman 1973) and physiological pathways affecting individual growth, reproductive timing, foraging, and predator avoidance (De Staso III & Rahel 1994; Magoulick & Wilzbach 1998). With such a low thermal window, cold water species like brook trout might be strongly affected by climate change, as global temperature is expected to rise from 0.7 -7.4°C over the course of the 21st century (Rouse *et al.* 1997; Heino *et al.* 2009; Qin *et al.* 2014). Additionally, northern brook trout populations may be at an adaptive disadvantage due to being genetically depauperate as a result of isolation in glacial refugia and historical bottlenecks (Bernatchez & Wilson 1998). Only a small number of studies have examined the thermal performance of brook trout; these have focused on the effects of ploidy, heating rate, or interspecific differences with other salmonids; measured thermal tolerance at a static upper thermal limit; or compared only a few populations with long histories of hatchery manipulation (McCauley 1958; Benfey *et al.* 1997; Galbreath *et al.* 2004; McDermid *et al.* 2012; Stitt *et al.* 2014). More research is needed in order to prepare for, and adequately address, the effects of climate change on this socioeconomically important species at the intraspecific scale in taking into account population size and outbreeding/hybridization.

Our study used six fragmented, genetically distinct populations of brook trout occupying streams on Cape Race (CR), Newfoundland, Canada (Figure 1), to explore effects of hybridization and population size on upper thermal tolerance. Fragmentation of CR streams occurred as a result of the late-Wisconsinan glaciation (10-12,000 ybp; Danzmann *et al.* 1998), and these populations have been studied extensively (Hutchings 1991; Fraser *et al.* 2014; Wood *et al*. 2015; Wood & Fraser 2015). Besides having a common ancestry, CR trout populations have a number of additional attributes for such a study. First, the small size of CR streams (ranging in length from 0.27 to 8.10 km) allows for thorough sampling and accurate estimates of population size. Second, CR populations range greatly in size from avg. N=840-8000 and avg. N_b=30-205 (N being the census population size, and N_b being the effective number of individuals breeding in one spawning season; an analogue of effective population size that is positively related to genetic diversity of a population; Bernos & Fraser 2016). Third, consistent with theory, small CR populations have less neutral genetic variation than large CR populations (Fraser et al. 2014), yet genetic variation underlying quantitative traits does not vary with population size (Wood et al. 2015); such apparent discrepancies make this population system an intriguing one for investigating what genetic metrics best predict responses to environmental change. Finally, CR streams vary in their thermal regimes (Figure 2), with those inhabited by the two smallest populations in the present study having the coldest overall mean monthly temperatures (Table 1).

The upper thermal tolerance of pure and hybrid individuals were compared in terms of CT_{max} and a behavioural metric, agitation temperature, described by McDonnell & Chapman (2015), which is defined as the temperature at which a fish first begins to exhibit refugia-seeking behaviour (circling of the chamber, seeking refuge in substrate). This behavioural metric is hypothesized to be related to individual fitness in that fish that perceive an increase in temperature sooner than others will be able to find adequate habitat more quickly. We hypothesized that large populations would have a higher CT_{max} due to more genetic variation and inhabiting generally warmer streams, while small populations would be constricted within a smaller thermal window as a result of both lower genetic diversity and colder thermal regimes in the wild (Table 1; Figure 2). Similar to chapter 1, we also hypothesized that the magnitude of population size difference would affect that relationship; specifically, hybridizing small populations with a large one would benefit the small populations, as they may have reduced fitness due to inbreeding depression.

MATERIALS AND METHODS

Procuration of brook trout

From October 13th to October 26th, 2014, gametes were collected from six CR populations: Cripple Cove (CC), Freshwater (FW), Ouananiche Beck (OB), Still There By Chance (STBC), Whale Cove (WC), and Watern Cove (WN). For larger streams, individuals were collected from previously documented spawning sites, and from areas observed to have obvious redd formations and large brook trout aggregates (Wood *et al.* 2014). For smaller streams wherein fish densities were lower in the spawning grounds, individuals were collected throughout the entire stream.

Potential spawning individuals were collected via electrofishing surveys and checked for "readiness": a release of sperm for males, and an elongated cloaca/soft belly for females. Readiness was assessed in the days leading up to the expected date of gamete collection, and ready fish were held for 24-72 hours in flow-through cages before collection.

Gamete collection took place between 19h00 and 1h00. Sperm was collected in 1.5-ml microcentrifuge tubes, while eggs were collected in 60-ml opaque plastic containers. Gametes were kept on ice and insulated so as not to freeze, and transported to St. John's, NL, immediately after collection. They were then flown directly to Montreal, and crossed within 15 hours from the beginning of gamete collection. Crosses were conducted to produce pure population offspring as well as full-reciprocal F1 hybrids (Table 2). Families were incubated separately within 5.2 cm diameter mesh-bottom containers placed randomly with respect to population within a single 1000 L recirculating tank and maintained at 7.0 ± 0.3°C. Eggs were left undisturbed until the eyed stage to reduce potential mortality following fertilization, at which point dead individuals were counted and removed daily. Dissolved oxygen (DO) and pH did not differ in different tank locations and were consistently maintained throughout the experiment.

After reaching yolk-absorption, separate brook trout families were kept in flow-through bins within two larger, identical, 3000L tanks prior to thermal tolerance trials. Water temperature was maintained between 15.5 and 16.5 °C (+/- 0.2°C), and multiple air stones ensured DO saturation. pH was 7.5 across all tanks (+/- 0.2), and artificial light was set at a natural daylight cycle (corresponding to St. John's, Newfoundland). Tanks were cleaned daily, fish were fed *ad libatum* two times daily, feeding time was constant, and all fish were kept under the same thermal conditions from fertilization to the end of the thermal tolerance experiments. At the time of the experiment fish were approximately two to four months post-yolk absorption. Although this is not the age at which they would experience maximum temperatures in the wild, the design of the project limited our experimental timeline.

Upper thermal tolerance trials

Experimental fish were starved for 24 h prior to trials, and trials were performed at the same time daily to ensure similar metabolic rates (Clark *et al*. 2013). Fish were given 1 h of acclimation time after being moved to a rectangular experimental test tank (60 cm x 32 cm x 30 cm; *L* x *W* x *H*) to reduce stress associated with handling and to acclimate to minute changes in water temperature. Significant efforts were made to standardize starting water temperature, which ranged from 16.20 to 17.97 °C over 34 trials. Each trial consisted of two pure trout from the same family and two maternal hybrids, with a total of n=122 trout tested across 61 families from 6 populations (Table 3). Within the larger rectangular test tank, four smaller, tapered circular flow-through chambers (14 cm top diameter x 10 cm bottom diameter x 11.5 cm deep) were used to hold each experimental fish. Rock substrate was provided in each chamber to act as potential fish refuge. During the trial, individuals were subjected to a constant (0.3°C/min) increase in water temperature that was controlled, monitored, and recorded by a temperature-control unit and software (TMP-REG, AutoResp; Loligo Systems) (McDonnell & Chapman 2015). Agitation temperatures were recorded for each fish as the point where an obvious shift in behaviour first occurred. For brook trout in this study, fish generally remained relatively still as temperatures increased until a point (agitation temperature), after which they either began to hurriedly circle the chamber, sought refuge in substrate, or a combination of both behaviours. This agitation temperature, along with temperature at CT_{max}, were both confirmed after each experiment using time-stamped video footage taken via a webcam. Immediately after loss of equilibrium, fish were removed and placed in an aerated recovery chamber until regaining equilibrium and normal opercular movement; total length (mm) was then recorded, and no mortalities recorded in the proceeding 24h period.

Statistical analysis

Linear mixed models (LMMs) were used to determine whether CT_{max} and agitation temperature differed across populations and between pure fish and their corresponding hybrids. Length and population were included as fixed effects in the pure LMM, as size is known to affect CT_{max} (Cox *et al.* 1974) and population is hypothesized to affect CT_{max}. Pure vs. hybrid LMMs replaced the "population" fixed effect with "cross-type", and one analysis was conducted for each of the two pure-hybrid comparisons, each of which crossed the same large population with a different small population. Mother I.D. was included as a random effect to account for maternal effects on thermal tolerance, and to incorporate the replication of trials (2 trials/family). For each model, data were normally distributed, and p-values were calculated and FDR corrected (Benjamini & Hochberg 1995) using the R package, lsmeans (Lenth 2015).

RESULTS

CT_{max} was significantly different between certain pure population comparisons after accounting for fish length. Plots of 95% confidence intervals for CT_{max} by population (Figure 3A) showed a maximum mean difference in CT_{max} of 0.68°C: WN had significantly higher CT_{max} than STBC and FW, and OB had significantly higher CT_{max} than STBC (see Table 3). Mean agitation temperatures did not differ between all other pure populations, and mean agitation temperature did not exceed a difference greater than 0.5°C between all populations; 95% CI overlapped in all cases (Figures 3B).

Within each comparison of pure populations vs. reciprocal F1 hybrids, there were no significant differences in CT_{max}; mean CT_{max} differed by a maximum of 0.3°C between different cross-types and 95% CI overlapped in all cases (Figure 4A, B; Table 4). Mean agitation temperatures were not significantly different between pure populations and associated hybrids (Table 4), and 95% confidence intervals overlapped in all cases (Figure 4C, D). A supplementary analysis was conducted to determine if any relationship existed between dependent variables CT_{max} and agitation temperature, and across-month thermal range and maximum monthly mean temperature experienced by these populations in nature. No significant difference was found between either dependent variable and temperature range or maximum temperature (see Figures S1 & S2; see Table S1).

DISCUSSION

The aim of this study was to investigate how varyingly-sized and genetically distinct populations of a cold water species respond to acute thermal warming, and how hybrids created with these populations respond in comparison. Our study on brook trout found significant differences in CT_{max} between some populations, but no population differences in agitation temperature, and no general effect of population size or hybridization on either trait. Although some studies have found similar evidence for intraspecific variation in upper thermal tolerance in salmonids (Eliason *et al*. 2011), including brook trout (McDermid *et al*. 2012; Stitt *et al*. 2014), others have found no differences between populations (Elliott & Klemetsen 2002), differences between hybrid and pure crosses (Fields *et al*. 1987), evidence for heterosis in thermal tolerance of copepods (Willett 2011), and evidence for increased survival of heterozygotes at near-lethal temperatures in Eastern mosquitofish (*Gambusia holbrooki*) (Meffe *et al*. 1995). To our knowledge, agitation temperature has only been assessed in fishes once before (McDonnell & Chapman, 2015) but was studied in relation to sex and acclimation temperature.

Our study examined six genetically distinct populations of brook trout that have been isolated, without gene flow or human disturbances, for potentially 12,000 years (Danzmann *et al*. 1998). Previous studies have found that although CR brook trout populations differ nearly 50-fold in census size *N* and 10-fold in effective number of breeders *Nb* (Bernos & Fraser 2016), there is no evidence for differences in (i) quantitative genetic variation and trait differentiation in relation to population size, nor (ii) phenotypic plasticity in relation to population size (Wood *et al*. 2015; Wood & Fraser 2015). Therefore, our study provides further evidence that population size may not be tightly related to the ability of a population to respond to environmental change, and that thermal tolerance in particular (physiologically and behaviourally), may be highly conserved even in such a plastic species as *S. fontinalis*. A key factor supporting this is that some of our populations fall below what many deem minimum viable population (MVP) size for long-term persistence, which is hypothesized to range from N = 4100-7300 (Table 1; Reed *et al*. 2003; Traill *et al*. 2007). It might be expected then, that populations exceeding this size would show greater CT_{max} or earlier refugia-seeking behaviour, but we found little supporting evidence for either. Namely, while the second largest population (WN: avg. N_b = 196.3, avg. N = 8011.0) had the highest CT_{max} and one of the two smallest populations had the lowest (STBC: avg. N_b = 30.8, avg. N = 989.2), the largest population (FW: avg. N_b = 205.0, avg. N = 5118.3) also had the second lowest *CTmax*. Another expected outcome might be that hybridizing between populations above and below the MVP threshold would benefit smaller populations disproportionally. In our outbred crosses, one large population (FW: avg. N_b=205.0, avg. N = 5118.3) was hybridized with two, smaller populations (STBC: avg. N_b=30.8, avg. N = 989.2; WC: avg. N_b=33.3, avg. N = 839.2, (Bernos & Fraser 2016) with no significant effect on CT_{max} or agitation temperature.

Owing to the considerable number of populations we compared, and hybridizing between populations vastly different in population size and annual thermal regimes (Figure 2), our results suggest that thermal tolerance (in terms of temperature tolerance and behavioural responses to temperature increases) seems to be highly conserved in *S. fontinalis* and remains relatively unchanged across isolated populations and in their hybrid offspring; this being in contrast to previous studies and the large amount of phenotypic plasticity found in salmonids. Even the significant mean differences found between our populations support this theory, as their magnitude is likely not biologically meaningful (0.68°C; see Fields *et al*. 1987 for a similar discussion), with many of these populations experiencing such fluctuations by the hour. Allowing our populations to acclimate at a temperature regularly experienced during summer months in the wild (16°C; Table 1; Figure 2), measuring CT_{max} in a fluctuating thermal environment (Ketola & Saarinen 2015), increasing temperature at a rate previously found to be optimal for fish studies on thermal tolerance (0.3°C/minute) (Becker & Genoway 1979), and measuring both CT_{max} and a behavioural metric of agitation temperature, has provided new evidence for less variability in thermal tolerance than previously thought. Reasons for this may be the scale at which other studies were conducted (see McDermid *et al*. 2012; Stitt *et al*. 2014) as well as the historical genetic or environmentally-driven (dis)similarities in the populations being assessed. In our case, although populations experience different thermal regimes (Table 1, Figure 2) and have been isolated for thousands of years, the relative geographic proximity of the populations may have resulted in similar environmental pressures shaping their ability to cope with climate warming. It is possible that at larger scales, between-population and pure vs. hybrid differences in thermal tolerance may have been larger. Additionally, similar thermal performance may have to do with the highly conserved natures of heat shock proteins (HSP) (example in Molina *et al*. 2000) (Basu *et al.* 2002). As these proteins have increased expression in thermal crises, their naturally high level of conservation may correlate to a highly conserved CT_{max}. A next research step could be to acclimate these trout populations to different temperatures and then measure CTmax, to determine if acclimation potential is reduced with population size.

Our study is one of only a few to have examined upper thermal tolerance in a large number of populations of cold water fishes (see also Eliason *et al*. 2011; McDermid *et al*. 2012; Stitt *et al*. 2014), and we have additionally accounted for genetic population size, family-level variation, and population mixing; factors that may affect the degree of tolerance. We found little population differentiation in upper thermal tolerance, and no indication that population size or hybridization (enhancing genetic variability) affects thermal tolerance. It is, however, difficult to disentangle the effects of temperature regime from population size, as the two are weakly positively correlated. We have therefore highlighted the potential for a highly plastic and divergent species to have lower than expected resilience in the face of climate warming; large or mixed populations are not necessarily conferred any greater resilience to climate warming than small, isolated populations, nor do they provide increased resilience to small populations via hybridization. Although we cannot completely disentangle the relative roles of historical genetic vs. environmentally-driven similarities in the populations being assessed, our results are a cause for concern for the general conservation of this and related cold water species as the climate warms.

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TABLES

Table 1: Monthly mean annual temperatures and standard deviations of six streams in Cape Race, Newfoundland across years 2012-2015, by increasing genetic population size (N_b); mean N_b and adult census population size (N) based on data from 2012 to 2015 (range of annual point estimates in parentheses).

	STBC	WC	OB	CC	WN	FW
January	4.02 (1.59)	3.47 (1.98)	1.14 (1.26)	0.91 (0.71)	4.33 (1.83)	3.19 (2.14)
February	3.66 (1.49)	3.54 (1.92)	1.18 (1.22)	0.91 (0.71)	4.38 (1.74)	2.86 (1.91)
March	3.55 (1.29)	3.42 (1.71)	1.44 (1.25)	1.04 (0.92)	4.4 (1.59)	2.71 (1.74)
April	4.07 (1.30)	3.76 (1.7)	3.04 (2.12)	3.16 (2.34)	5.11 (1.43)	3.3 (1.57)
May	5.48 (1.61)	6.39 (2.23)	7.47 (2.44)	7.71 (2.63)	6.2 (0.80)	5.31 (2.06)
June	7.56 (2.73)	8.84 (3.41)	10.43 (3.45)	10.26 (3.3)	6.59 (1.88)	8.94 (3.79)
July	9.18 (2.68)	12.40 (2.58)	15.57 (3.10)	13.63 (3.1)	14.01 (2.64)	14.04 (3.32)
August	9.94 (2.44)	13.58 (2.37)	16.69 (2.6)	15.78 (2.94)	14.76 (2.38)	15.49 (2.75)
September	9.44 (2.27)	12.25 (2.48)	14.52 (2.59)	14.18 (3.17)	12.84 (2.34)	13.69 (2.84)
October	7.67 (1.13)	8.89 (1.99)	10.15 (2.5)	12.12 (4.99)	9.28 (1.80)	9.80 (2.15)
November	6.51 (0.96)	6.63 (1.37)	5.86 (2.22)	5.94 (2.44)	6.72 (0.64)	7.26 (1.47)
December	4.78 (1.87)	4.74 (2.09)	3.19 (1.93)	2.22 (2.00)	5.42 (1.20)	5.04 (2.09)
N_b	30.8 (14-54)	33.3 (21-52)	64.8 (41-87)	75.6 (65-99)	196.3 (110-267)	205.0 (173-237)
N	989.2 (587-1405)	839.2 (530-1148)	2715.8 (1940-3835)	2597.6 (1471-5246)	8011.0 (6713-10032)	5118.3 (4024-6514)

Table 2: Full-reciprocal cross design for pure versus hybrid comparisons: one large population (FW) with two smaller populations (STBC and WC).

FW	**FW**
♀	♂
1	**A**
2	**b**
3	**c**
4	**d**
5	**e**
6	**f**
7	**g**

FW	**STBC**
♀	♂
3	**a**
7	**b**

STBC	**FW**
♀	♂
1	**h**
2	**g**
3	**d**
4	**c**

STBC	**STBC**
♀	♂
1	**c**
2	**d**
3	**e**
4	**f**

FW	FW
♀	♂
1	s
4	e
7	f
10	j
19	t
20	n
25	p

FW	WC
♀	♂
1	15
4	26
10	29
19	14
20	20

WC	FW
♀	♂
3	q
5	s
6	k
7	o
8	l
10	r
12	e
13	j

WC	WC
♀	♂
3	15
5	14
6	13
7	26
8	27
10	24
12	30
13	29

Table 3: Number of families used per population (pures) and per cross-type (hybrids) in the experiment. Two unique individuals were used from each family across the six populations, and each reciprocal hybrid cross-type.

Pure or Hybrid	Maternal Population	Paternal Population	# families	# individuals
Pure	Cripple Cove	Cripple Cove	4	8
Pure	Freshwater	Freshwater	7	14
Pure	Ouananiche Beck	Ouananiche Beck	9	18
Pure	Still There By Chance	Still There By Chance	4	8
Pure	Whale Cove	Whale Cove	8	16
Pure	Watern	Watern	10	20
Hybrid	Freshwater	Still There By Chance	2	4
Hybrid	Still There By Chance	Freshwater	4	8
Hybrid	Freshwater	Whale Cove	5	10
Hybrid	Whale Cove	Freshwater	8	16

Table 4: Summary of LMMs results for two thermal tolerance traits. A total of six LMMs were conducted (three per trait, one comparing all pure populations and for each of the two pure-hybrid comparisons).

Measured trait	Model	Pairwise comparison	Standard error	Degrees of freedom	Pairwise p-value
CT_{max}	Pure	CC - FW	0.19	29.58	0.37
		CC - OB	0.18	29.54	0.37
		CC - STBC	0.23	38.48	0.27
		CC - WC	0.19	30.49	0.82
		CC - WN	0.18	28.53	0.19
		FW - OB	0.15	39.31	0.06
		FW - STBC	0.22	49.43	0.47
		FW - WC	0.14	34.70	0.37
		FW - WN	0.15	37.37	0.009**
		OB - STBC	0.18	41.05	0.03*
		OB - WC	0.15	42.81	0.29
		OB - WN	0.13	32.82	0.37
		STBC - WC	0.23	52.57	0.29
		STBC - WN	0.19	40.85	0.009**
		WC - WN	0.15	40.76	0.06
	Hybrid 1	FW - FWSTBC	0.18	28.98	1
		STBC - STBCFW	0.25	27.91	1
	Hybrid 2	FW - FWWC	0.13	41.12	0.35
		WC - WCFW	0.11	39.31	0.75
Agitation Temperature	Pure	CC - FW	0.53	33.42	0.96
		CC - OB	0.51	33.11	0.96
		CC - STBC	0.62	38.33	0.96
		CC - WC	0.53	34.26	0.96
		CC - WN	0.51	32.76	0.96
		FW - OB	0.40	37.48	0.96
		FW - STBC	0.55	46.82	0.96
		FW - WC	0.40	34.14	0.96
		FW - WN	0.40	36.28	0.96
		OB - STBC	0.49	38.87	0.96
		OB - WC	0.40	40.35	0.96
		OB - WN	0.37	33.66	0.96

	STBC - WC	0.55	50.50	0.96
	STBC - WN	0.49	39.78	0.96
	WC - WN	0.39	38.77	0.96
Hybrid 1	FW - FWSTBC	0.44	25.78	0.85
	STBC - STBCFW	0.57	24.52	0.85
Hybrid 2	FW - FWWC	0.24	39.66	0.76
	WC - WCFW	0.20	38.75	0.76

FIGURES

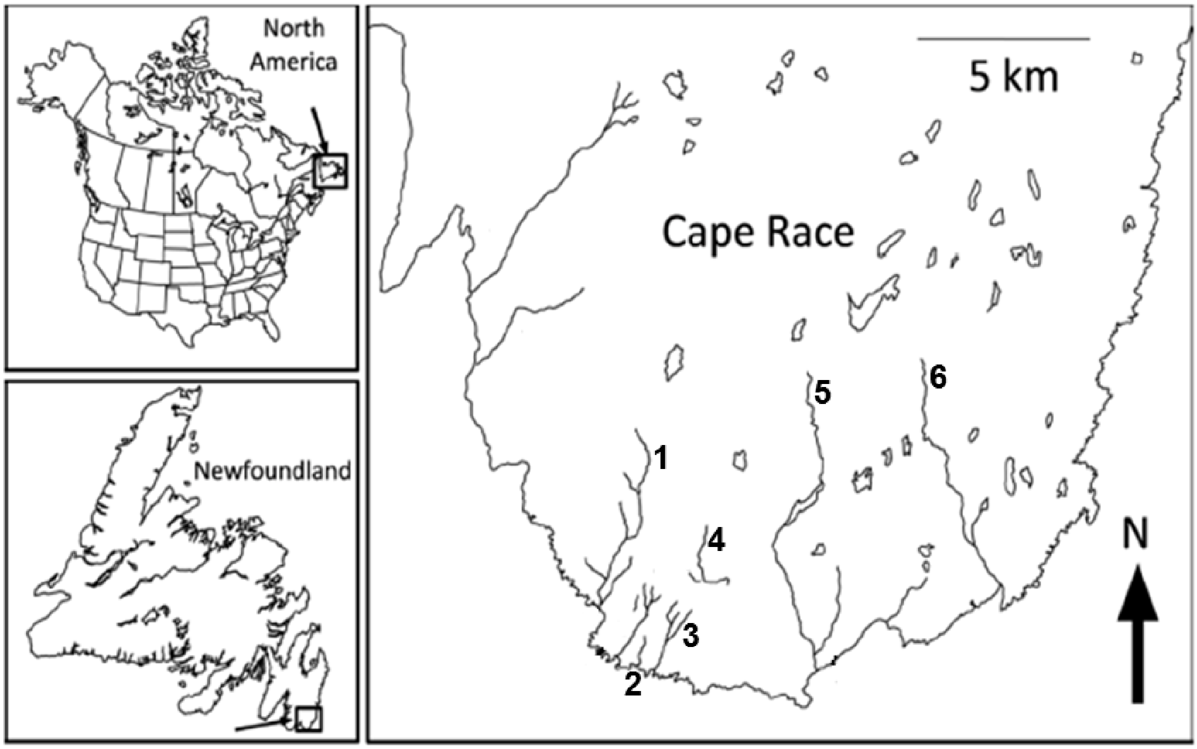

Figure 1. The geographic locations of study streams in Cape Race, Newfoundland, Canada: (1) Freshwater, (2) Still There By Chance, (3) Whale Cove, (4) Ouananiche Beck, (5) Watern Cove, and (6) Cripple Cove.

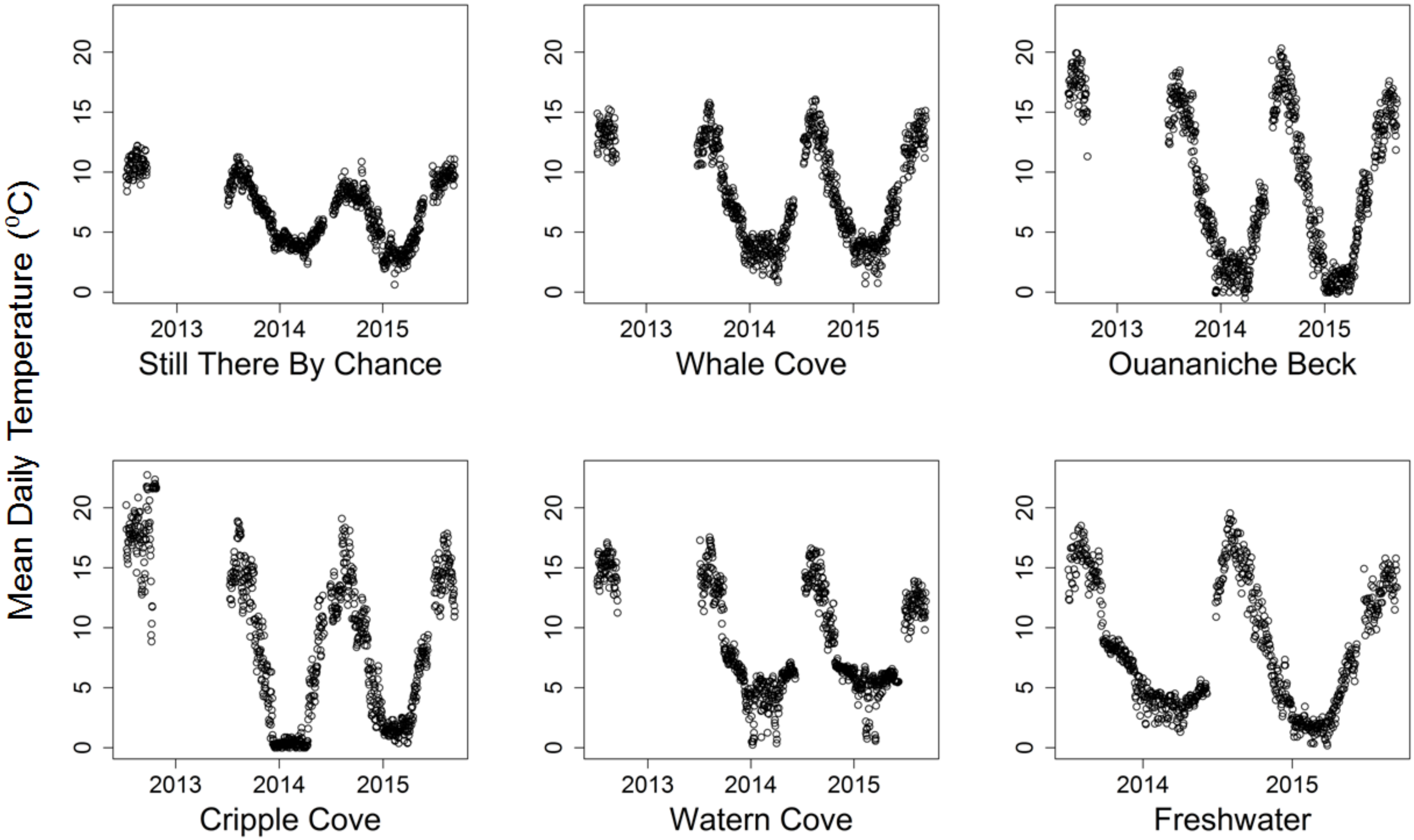

Figure 2. Thermal regimes of the six brook trout-containing streams (Cape Race, Newfoundland, Canada) where data are available from 2012 to 2015. Each dot being the mean daily temperature recorded across 1 to 4 temperature data loggers.

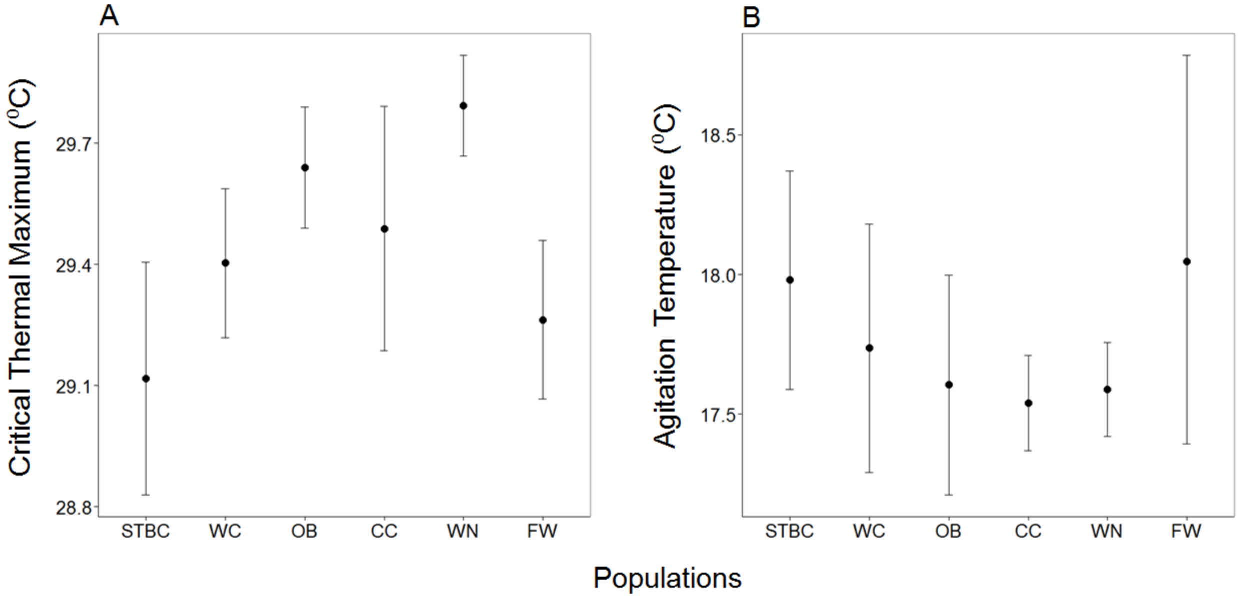

Figure 3. A: Mean CT_{max} and 95% confidence intervals (CI) for six brook trout populations from Cape Race, Newfoundland, Canada, in order of ascending N_b; the maximum difference in mean CT_{max} was 0.68°C. **B:** Mean agitation temperature and 95% CI for the six populations in order of ascending N_b; the maximum difference in mean agitation temperature was 0.51°C.

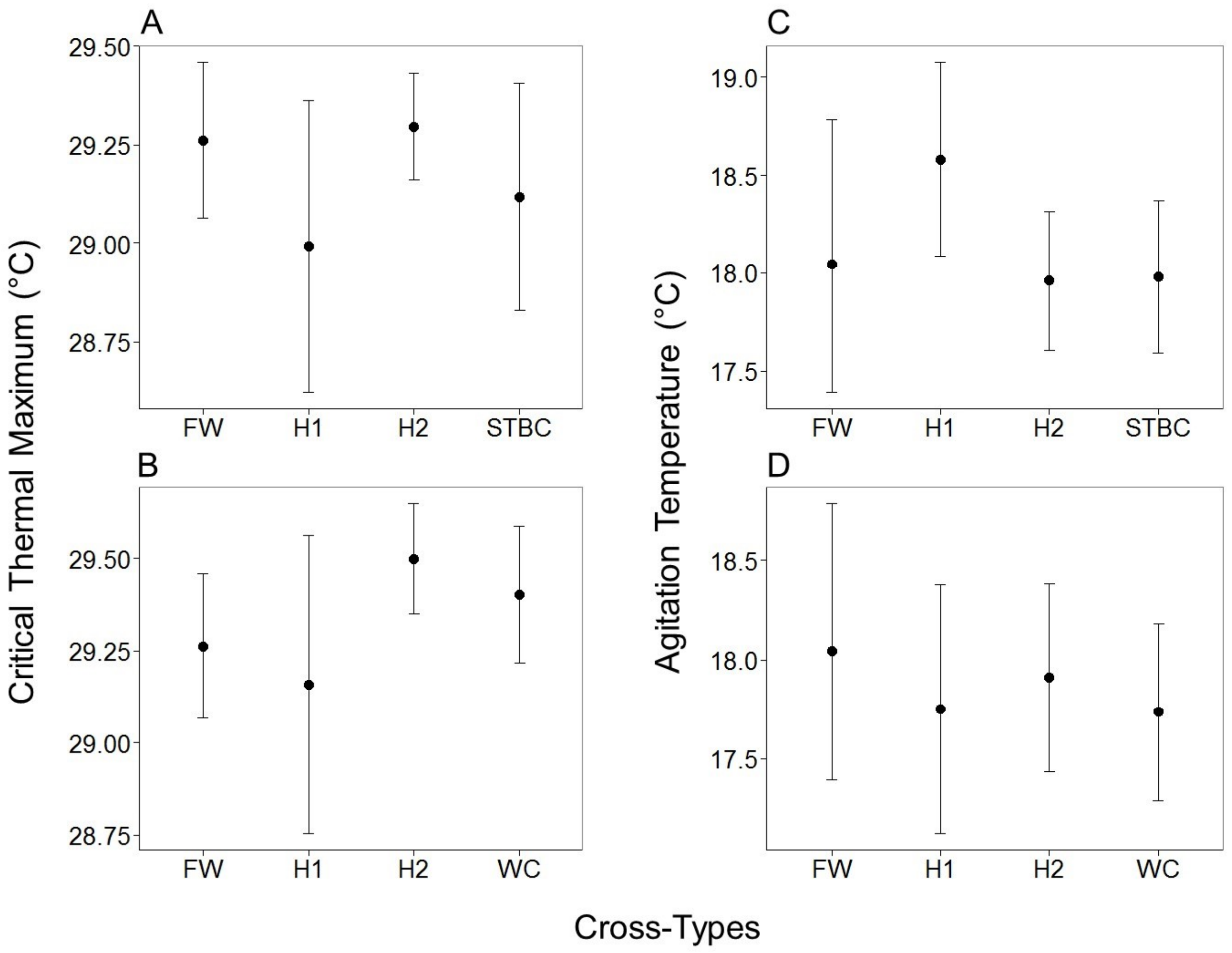

Figure 4. A & B: Mean CT_{max} and 95% confidence intervals (CI) for each pure-hybrid comparison. Freshwater (large population) crossed with Still There By Chance and Whale Cove (two small populations). H1 and H2 represent reciprocal F1 hybrid crosses. **C & D:** Mean agitation temperature and 95% CI for each pure-hybrid comparison. Freshwater (large population) crossed with Still There By Chance and Whale Cove (two small populations). H1 and H2 represent reciprocal F1 hybrid crosses.

SUPPLEMENTARY

Tables

Table S1: Summary of linear model results for two thermal tolerance traits. Dependent variables are mean CT_{max} and agitation temperature by pure population, and the independent variables are mean fish length by population, and either the range between mean monthly temperatures by stream or maximum monthly temperature by stream.

Measured trait	Temperature range p-value	Adjusted R^2	Maximum temperature p-value	Adjusted R^2
CT_{max}	0.98	-0.27	0.22	0.12
Agitation temperature	0.84	-0.20	0.34	-0.13

Figures

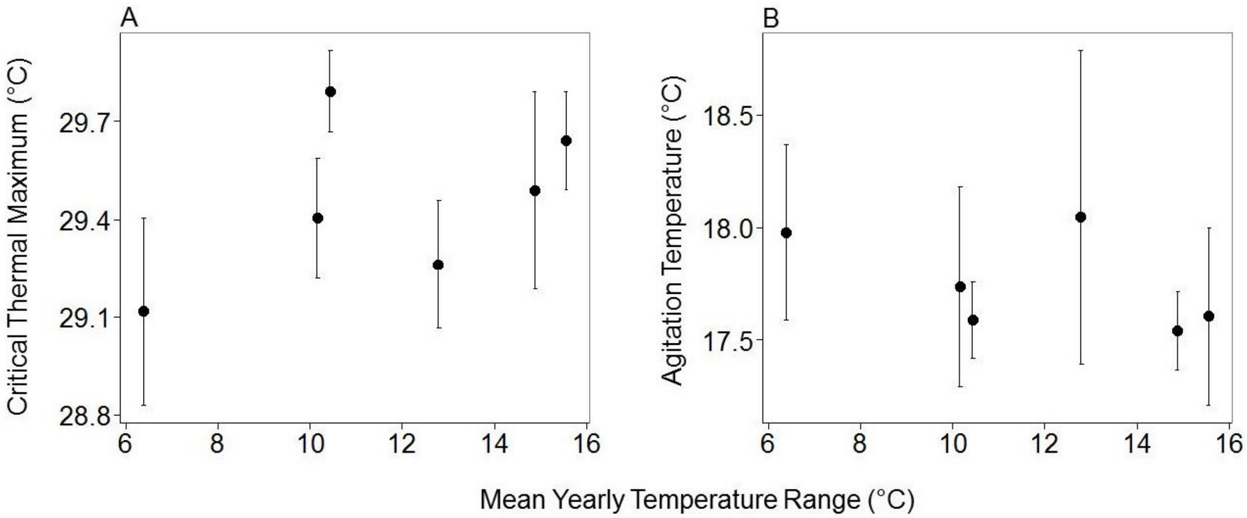

Figure S1. A: Mean CT_{max} and 95% confidence intervals for six brook trout populations from Cape Race, Newfoundland, by the range between mean monthly temperatures (calculated using temperature data from 2012-2015 where available). **B:** Mean agitation temperature and 95% confidence intervals for the six populations, by the range between mean monthly temperatures (calculated using temperature data from 2012-2015 where available).

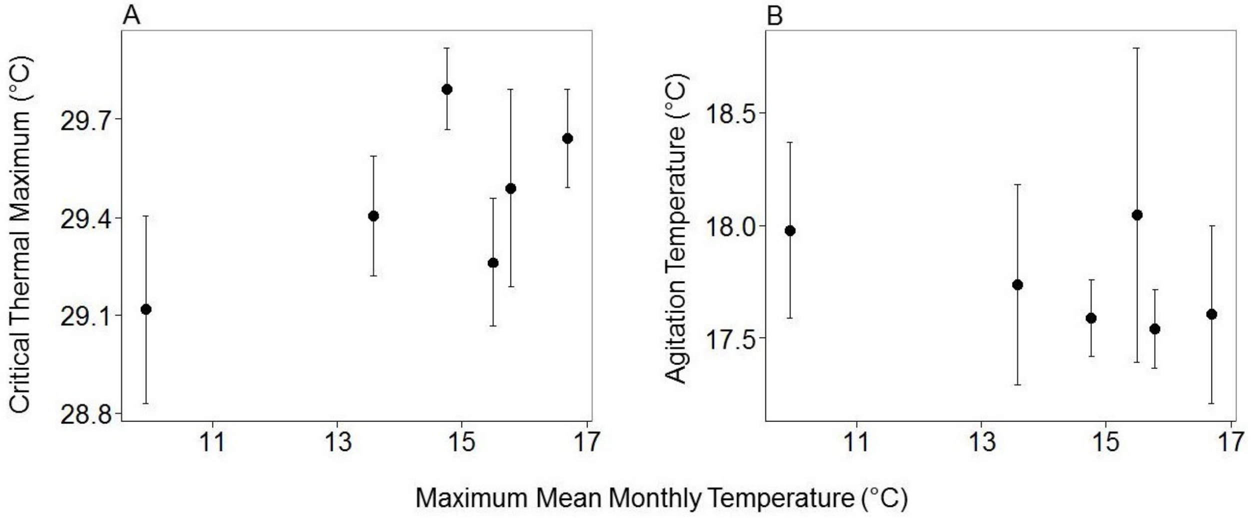

Figure S2. A: Mean CT_{max} and 95% confidence intervals for six brook trout populations from Cape Race, Newfoundland by maximum mean monthly temperature (calculated using temperature data from 2012-2015 where available). **B:** Mean agitation temperature and 95% confidence intervals for the six populations by maximum mean monthly temperature (calculated using temperature data from 2012-2015 where available).

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GENERAL CONCLUSIONS

Although hybridization had population specific influences on fitness-related traits, these outcomes were not consistent across populations, nor readily predictable using a number of metrics hypothesized to influence hybrid outcomes. Additionally, having found little population differentiation in upper thermal tolerance, and no indication that population size or hybridization (enhancing genetic variability) affects thermal tolerance, our results do not support the notion that hybridization may be used as a tool for genetic rescue.

These results give credence to the precautionary approach when dealing with the question of genetic rescue, as it is not always clear whether small populations are (i) at any particular risk, or (ii) likely to benefit from inter-population hybridization (also see Waller 2015). We have also highlighted the potential for a highly plastic and divergent species to have lower than expected resilience in the face of climate warming; with large or mixed populations not necessarily conferred any greater resilience to climate warming than small, isolated populations, nor any effect of hybridization. Although we cannot completely disentangle the relative roles of historical genetic vs. environmentally-driven similarities in the populations being assessed, as knowing historical episodic events such as bottlenecks, stochastic environmental events, or human-induced changes to population composition are likely to influence hybridization outcomes via their effects on genetic composition (Nason *et al.* 2002), our results are a cause for concern for the general conservation of this and related cold water species as the climate warms. We suggest using hybridization as a tool for genetic rescue only when a great deal of information is known about the organisms in question, and only when there are obvious signs of rapid fitness declines within a population.