Investigating the conservation genetics small population paradigm using the brook trout (*Salvelinus fontinalis*): do small populations have less adaptive potential?

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Abstract

Investigating the conservation genetics small population paradigm using the brook trout (*Salvelinus fontinalis*): do small populations have less adaptive potential?

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The conservation genetics small population paradigm predicts reduced quantitative genetic variation and consequently, adaptive potential, for populations which have become small and isolated due to habitat fragmentation. While these expectations are supported by laboratory studies, their generality in wild populations remain unresolved. In actuality, the evolutionary consequences of fragmentation may depend on whether environmental characteristics– and by extension, selective regimes are (1) shifted in a consistent directional manner as is widely assumed (Directional hypothesis), or (2) become more variable as population and fragment size decrease (Variable hypothesis); this latter possibility has so far received little empirical attention.

Implementing field techniques, I explore these two competing hypotheses by relating variability in habitat characteristics to population size in a series of differentially abundant brook trout (*Salvelinus fontinalis*) populations located at Cape Race, Newfoundland. I furthermore use these hypotheses as a foundation to test the assumptions of the conservation genetic small population paradigm by investigating the relationships of population size at Cape Race to additive genetic variation (V_A), trait differentiation (Q_{ST}), and phenotypic plasticity in common garden analyses, and to the extent of natural selection in a meta-analysis using a large number of natural populations and species.

Across two years and in relation to two population size metrics, patterns of habitat characteristics among small versus large Cape Race populations supported the Variable hypothesis. However, small brook trout populations did not significantly differ from large populations in either the magnitude or variability of V_A, Q_{ST}, or phenotypic plasticity. Results of the meta-analysis similarly revealed little support for differences in the strength, direction, and form of selection among wild populations differing in population size. The lack of differences might be explained by long term fluctuating environmental conditions which resulted in fluctuating selective pressures and similar outcomes among small compared to large Cape Race populations, and among the species included in the meta-analysis.

Overall, the results of this research contradict the assumption that small populations generally inhabit marginal environments and also dispute the major tenets of the conservation genetics small population paradigm. Taken together, they suggest that even very small populations of some species may retain the adaptive potential necessary to cope with future environmental change.

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Contribution of Authors

The chapters of this thesis were designed as manuscripts for submission to peer-reviewed journals. I contributed to the conception, planning, data collection, data analyses and preparation of all manuscripts. Dr. D. J. Fraser contributed to the conception, planning, data analyses and editing of all manuscripts. S. Belmar-Lucero contributed to data collection, population genetic analysis, and editing of the manuscript presented in Chapter 1, J. A. Hutchings contributed to the editing of the manuscript presented in Chapter 1, and M. Yates contributed to the data-analysis of the manuscript presented in Chapter 4.

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General Introduction

Human action is resulting in the rapid depletion of the planet's biological diversity. A suite of deterministic processes including habitat loss, overexploitation, and climate change have already caused the extinction of a large number of wild species, and many other species now exist only as small, isolated populations (Frankham et al. 2010). The current so-called 'sixth extinction' is comparable in magnitude with the other five mass extinctions in the geological record; the current rate of extinction also far exceeds background rates of extinction through history (Leakey and Lewin 1995; Pimm et al. 1995; Frankham et al. 2010).

In a concerted effort to protect the biodiversity that remains, the last several decades have seen the development of two overlapping paradigms which describe the breadth of the current biodiversity problem (Ouberg et al. 2006). The 'habitat quality' paradigm focuses on the inability of populations to cope with deteriorating environmental conditions within natural habitats where solutions involve the management and restoration of habitat quality (Ouberg et al. 2006). Conversely, the 'conservation genetics' paradigm (Caughley 1994; Frankham 1995; Ouberg et al. 2006), centers on population-level characteristics and how reductions in population size and increasing isolation can result in an increased risk of extinction. Here, solutions revolve around increasing abundance and connectivity between populations.

The potential influence of genetic factors on extinction risk were not widely appreciated prior to the publication of seminal papers by Frankel (1970; 1974) and the first treatise on conservation biology, 'Conservation and Evolution' by Frankel and Soulé (1981). These texts specifically drew attention to the increasing threats posed by

environmental, demographic, and genetic stochastic processes and catastrophic events with reductions in population size and increasing population isolation (also see Lande 1993; Chevin et al. 2010; Leimu et al. 2010). Today the importance of genetic factors in mitigating extinction risk are widely accepted such that genetic diversity is one of three levels of biodiversity recognized by the IUCN as deserving special concern (McNeely et al. 1990). This is largely due to the recognition that genetic diversity is required for adaptation to environmental change. There is now a vast literature linking genetic diversity with adaptive evolution, classic examples being the association of increasing genetic variation with increased resistance to infectious diseases and parasites (e.g., Spielman et al. 2004; Hale and Briskie 2007; Pederson et al. 2007), the rapid evolution of industrial melanism in moths (Kettlewell 1973; Majerus 1998), herbicide, insecticide, and antibiotic resistance in many pest species (Georghiou 1986; McKenzie 1996), and the evolution of heavy metal tolerance in some plants (Bradshaw 1991).

For isolated populations that are small or declining, once human-associated deterministic threats have taken their toll on population size, small populations may be prone to positive feedback loops of inbreeding and genetic drift that negatively affect population growth rate; as a result the small populations are drawn down a 'vortex' of continuously decreasing population size to a point where stochastic factors may deliver the final blow (Gilpin and Soule 1986). One key factor driving the extinction vortex is the loss of genetic variation necessary to enable a population to adapt to environmental change (e.g. Fagan and Holmes 2006; Hedrick et al. 2006).

Genetic diversity and population size

The disproportionately rapid loss of genetic diversity at small versus large population size is partly explained by genetic drift: the random sampling of alleles from one generation to the next (Lande 1976; Allendorf 1986). Genetic drift causes allele frequencies to fluctuate, which over time leads to random loss and fixation of alleles and subsequently to a reduction in the proportion of loci that are heterozygous (Lacy 1987).

For small populations, short-term population bottlenecks or long-term restriction of population size are predicted to cause significant reductions in neutral genetic variation (England et al. 2003; Garner et al. 2005; Frankham et al. 2010). Bottlenecks may occur because of intense harvesting or overexploitation (Hedrick 1992; Hoelzel et al. 1993; Groombridge et al. 2000) or during founder events (Wayne et al. 1991; Clegg et al. 2002; Koskinen et al. 2002) where the number of founders may be as few as a single pair, as was the case for the Isle Royale gray wolf population (Wayne et al. 1991). In general, the proportion of initial heterozygosity retained after a single generation bottleneck is:

$$H_t/H_0 = 1\text{-}(1/2N_e)$$

where H_t is the expected heterozygosity at time t, H_0 is the initial heterozygosity, and N_e is the effective population size (Falconer and Mackay 1996). It is clear from the equation that a single generation bottleneck has to be severe to have an appreciable impact on heterozygosity. For example, a bottleneck of N_e=25 reduces heterozygosity by 2%, while a 0.5% reduction is expected from a bottleneck of N_e=100 (Frankham et al. 2010). Theoretically, the impact of a bottleneck of a single pair is to reduce heterozygosity by

25%; reductions in genetic diversity and allelic diversity have been found to be close to these predictions in laboratory studies (e.g. England et al. 2003; Frankham et al. 2010).

Compared to single generation bottlenecks, the equation describing the decay of genetic diversity over successive generations is given by the equation:

$$H_t/H_0 = (1-[1/2N_e])^t$$

For example, *Drosophila* populations with an N_e of 100 lost 25% of its heterozygosity over 57 generations, the same loss as a single generation bottleneck of one pair (England et al. 1997). However, while a population bottleneck of $N_e = 25$ individuals reduces heterozygosity by only 2%, a population with a $N_e = 25$ will lose 64% of its initial heterozygosity over 50 generations (Foose 1986), suggesting that sustained reductions in population size lead to more extreme declines in genetic diversity than single generation bottlenecks (Frankham et al. 2010).

For both single generation bottlenecks and long term restriction of population size the rate of decay of neutral genetic diversity is intimately linked to the effective population size (N_e), which is defined as the number of individuals that would give rise to the calculated loss of heterozygosity, rate of inbreeding, or variance in allele frequencies, if they bred in the manner of the idealized population (Falconer and Mackay 1996). N_e is generally smaller than the total census population size (N; Wright 1938) with reported average N_e/N ratios across taxa ranging from 0.10-0.23 (Frankham 1995; Palstra and Ruzzante 2008; Palstra and Fraser 2012). In extreme cases, discrepancies between census population size and N_e can reach several orders of magnitude, as is the case for

some species of marine fish, oysters, shrimp and seaweed (see Coyer et al. 2008; Palstra and Ruzzante 2008). N_e may be reduced relative to N for a variety of reasons including unequal sex-ratios, variation in family size, and fluctuating population size (Crow and Kimura 1970). Over t generations, N_e in a fluctuating population is the harmonic mean of N_e and will be closest to the size of the generation with the smallest single generation N_e.

Predicted rates of decline of neutral genetic diversity with population size are well supported by observations from both experimental (Montgomery et al. 2000, 2010; Gilligan et al. 2005; Swindell and Bouzat 2005) and natural populations (Nei and Graur 1984; Nevo et al. 1984; Ellstrand and Elam 1993; Johnson et al. 2004; DiBattista 2008; Hoeck et al. 2010) in relation to both N (e.g. Soule 1976; Frankham 1996) and N_e (Palstra and Ruzzante 2008). Soule (1976) reported a highly significant correlation of 0.7 between heterozygosity and estimates of log N in animals and genetic diversity also increased significantly with population size in species of plants (Leimu et al. 2006). By definition, endangered species typically have smaller populations than related nonendangered species and should have lower levels of genetic variation. Genetic variation was indeed found to be significantly reduced in endangered compared to nonendangered species (Frankham 1995; Haig and Avise 1996; Spielman et al. 2004), including for birds (Haig and Avise 1996; Evans and Sheldon 2008), and for tetrapods in general (Flight 2010). Likewise island populations, which are often low in numbers, were found to have less allozyme genetic variation than mainland populations of the same species (165 of 203 comparisons; Frankham 1997) and neutral genetic diversity was also reduced in rare vs. common species of plants (Cole 2003).

Quantitative genetic variation in small populations

Models of genetic variation which quantify diversity at single loci by measuring heterozygosity (H) encompass only a small fraction of the naturally occurring variation within populations (Falconer and Mackay 1996). While traits governed by one or relatively few loci are generally discrete and only mildly susceptible to environmental effects, the characters of most interest in conservation biology, quantitative traits, are continuous, normally distributed, polygenic, and are strongly influenced by environmental factors such as food supply, density, temperature, and disease status (Lynch and Walsh 1998; Willi et al. 2006; Frankham et al. 2010). Quantitative traits and the genetic variation underlying them are critical to evolution and to the conservation of adaptive potential as they are frequently related to individual survival, reproductive rate, and mating ability (Willi et al. 2006).

Quantitative genetic parameters are typically estimated by comparing the phenotypes of related individuals, the idea being that the resemblance between relatives is determined by the degree to which phenotypic expression is determined by shared genes as opposed to random environmental influences (Lynch and Walsh 1998). One of the primary challenges in the study of quantitative genetics is in determining how much of the variation in trait value is due to genetics, and how much is due to environmental conditions (Falconer and Mackay 1996). The total phenotypic variance (V_P) within a population can be divided into contributions not only from genetic diversity (V_G) and environmental variation (V_E), but also from interactions between genotype and the environment:

$$V_P = V_G + V_E + 2\mathrm{Cov}_{GE}$$

where Cov_{GE} is the covariance between genetic and environmental effects. V_G can be further subdivided into contributions from additive genetic variance (V_A), dominance variance (V_D), and interaction variance (V_I). The additive component of the genetic variance is the main determinant of a population's response to selective pressures (Falconer and Mackay 1996) and is the most influential in determining the narrow-sense heritability (h^2) of a character, that is, the proportion of the total phenotypic variance that is due to genetic differences among individuals ($h^2 = V_A/V_P$; Frankham et al. 2010).

Heritability estimates are specific to particular populations living under specific environmental conditions (Hoffman and Parsons 1991; Hoffman and Merilä 1999); in general, populations in the least variable environment should have the greatest h^2, as higher environmental variances increase total V_P (Gebhardt-Henrich and Van Noordwijk 1991; Lynch and Walsh 1998; Frankham et al. 2010). Indeed, since the value of h^2 depends on the magnitude of phenotypic variance, which in turn is calculated by the sum of all the individual genetic and non-genetic variances, a change in any one of these values will cause a change in the heritability estimate. For example, genetic components are influenced by gene frequencies and may differ among populations based on genetic history (Falconer and Mackay 1996); in particular, small populations with considerable amounts of fixation are expected to show lower h^2 than larger populations.

Under selective neutrality and purely additive effects, the loss of quantitative genetic variation should occur linearly with loss of neutral genetic variation (Chakraborty and Nei 1982; Lynch and Hill 1986; Falconer and Mackay 1996).The connection

between quantitative and neutral genetic diversity is provided by the equation for V_A which is a function of the expected heterozygosity in a random mating population (Falconer and Mackay 1996):

$$V_A = \sum_{i=1,}^{\#loci} 2p_i q_i (a_i + d_i[q_i - p_i])^2$$

where p and q are the allele frequencies, a is twice the difference in mean phenotype between the two homozygotes and d is the deviation of the heterozygote phenotype from the mean of the two homozygotes. Although declines in V_A for traits strongly associated with fitness, such as life history traits, may exhibit nonlinear relationships with neutral variation due to nonadditive gene action (Crnokrak and Roff 1995; Roff and Emerson 2006; Van Buskirk and Willi 2006), for other types of traits decreases were consistent with neutral additive expectations (Briscoe et al. 1992; Wade et al. 1996; Whitlock and Fowler 1999; Gilligan et al. 2005; Kristensen et al. 2005; Swindell and Bouzat 2005). For example, h^2 for sternopleural bristle number decreased at a similar rate as allozyme genetic diversity in fruit flies (Briscoe et al. 1992). Similarly, Swindell and Bouzat (2005) compared observed and expected rates of decline of quantitative genetic variation in replicate lines of *Drosophila*; they reported observed reductions in h^2 that approached additive neutral predictions for all kinds of traits in populations that had been severely inbred or small for a long period of time. Fewer attempts have been made to compare quantitative genetic diversity for populations of different sizes in the field however Willi et al. (2007) estimated genetic variation in 13 populations of a plant species, and found

that small populations had significantly reduced h^2 averaged over a number of morphology and life history traits.

Loss of evolutionary potential in small populations

Loss of genetic diversity in small populations is expected to increase extinction risk by reducing a population's capacity to respond to natural selection in a changing environment. According to the breeder's equation, response to selection (R) is directly related to h^2 and the strength of natural selection acting on a trait (selection differential; Falconer and Mackay 1996):

$$R = Sh_2$$

A number of experimental tests using laboratory organisms have demonstrated that small populations show reduced response to selection (Frankham et al. 1968; Jones et al. 1968; Eisen 1975; Silvela et al.1989; Weber 1990; Weber and Diggins 1990; Wade et al. 1996; Weyhrich et al. 1998; Swindell and Bouzat 2005) and the effect of population size can be appreciable; selective response was 57% lower in *Drosophila* populations with an N_e of 8 compared with an N_e of 200 after 55 generations (Weber 1990). Likewise, population size affected the capacity of yeast to adapt to salt mediums (Samani and Bell 2010), and experiments on *Drosophila* demonstrated that adaptive response was larger for undivided or outbred populations versus fragmented or inbred populations populations exposed to stressful environments (Bakker et al. 2010).

Problems with the conservation genetics small population paradigm

Genetic diversity and population size

Theoretical models predict a positive correlation between genetic diversity and population size, and while there seems to be abundant evidence to support these expectations among laboratory organisms under experimental conditions (Willi et al. 2006) the actual relationship for wild populations of species is less clear. This uncertainty may in part be due to methodological issues which hampered previous attempts to empirically investigate the relationship between quantitative genetic diversity and population size. First, neutral marker diversity is often used as a surrogate for quantitative genetic variation even though neutral markers may lose genetic variation more rapidly than loci underlying traits closely associated fitness (Willi et al. 2006). Furthermore, the correlation between neutral and quantitative genetic variation was found to be weak (Reed and Frankham 2001) hence, loss of molecular variation may not necessarily also imply a loss of selectively relevant genetic variation.

Even where quantitative genetic diversity was compared with population size results have been unclear. For instance, two studies may have found no evidence for reduced h^2 at small population size because only a very small number of populations were compared and the studies therefore lacked statistical power (Widen and Andersson 1993; Waldmann 2001). Weak relationships were also observed between census size and broad sense genetic variation in studies using multiple populations (Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001) however, contemporary N in the populations may not have adequately reflected long term N_e since there was also no

relationship between population size and neutral genetic diversity (Meyer and Allen 1999; Podolsky 2001; Waldmann and Andersson 1998).

Some studies examined genetic diversity in relation to the census population size (N) instead of the effective population size (N_e) (Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001), when it is N_e that dictates rates of genetic drift and inbreeding. Average N_e/N ratios across taxa are often cited in the literature, and N and N_e are frequently assumed to be correlated (Willi et al. 2007). However, N_e/N ratios can vary widely among populations of closely related species or within the same species and may thus lead to incorrect conclusions if N is used to infer the magnitude of N_e or vice versa (Palstra and Fraser 2012). Finally, there is a paucity of empirical research investigating the relationship between genetic diversity and population size for wild populations of vertebrate species. Work has been almost entirely restricted to plants (Widen and Andersson 1993; Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001; Waldmann 2001; Leimu et al. 2006; Willi et al. 2007) but conclusions may not be easily extrapolated to vertebrates which can exhibit behaviours that might alter the relationship between genetic diversity and population size.

There are additional factors beyond methodological problems which might explain the lack of support for predicted declines in genetic diversity in natural populations. For example, populations that are small due to a recent bottleneck may experience a temporary boost to standing additive genetic variance from non-additive sources (Cheverud and Routman 1996; Goodnight 1988, 2000; Wade et al. 2002; Barton and Turelli 2004). The elapsed time since population decline is also an important consideration. According to theory, $1.4N_e$ generations are required for V_A to be reduced

by 50% (Foose 1986; Lynch and Hill 1986) therefore many decades may be needed before appreciable differences are observed among populations; this may be especially relevant for wild species with long generation times. Finally, field data might not support theory because V_A may only be sensitive to N_e at extremely small population size (N_e < 10; Willi et al. 2006) or if gene flow among populations results in a lack of relationship between V_A and local population size (Lande 1992; Whitlock 1999).

Selection at small population size

A second unsettled issue within the conservation genetics small population paradigm is related to predictions regarding the efficacy of natural selection as populations become small and isolated. A given locus under selection is predicted to approach neutrality when s ≤1/[2Ne] (Wright 1931), in other words, the influence of natural selection decreases relative to genetic drift with continuing reductions in population size. However, the extent of selection might differ among populations differing in abundance but there is virtually no work that has investigated this possibility for wild populations, despite the existence of a well-established standardized framework for quantifying and comparing patterns of natural selection in nature (Lande and Arnold 1983). The two notable exceptions that I was able to find found little supporting evidence that the magnitude of selection differs between small vs. large populations (Murua et al. 2010; Weber and Kolb 2013), although in one study total census population size was not reported (populations were from transformed and unaltered habitats and differed in density; Murua et al. 2010) and the second may have lacked statistical power (Weber and Kolb 2013).

The relative influence of selection versus genetic drift in small or fragmented populations can be inferred by comparing the magnitude of between-population neutral genetic differentiation (F_{ST}) to quantitative trait differentiation (Q_{ST}) (e.g. Merilä and Crnokrak 2001; Edelaar et al. 2011). Selection is more influential in differentiating populations when Q_{ST} is either significantly greater than or less than F_{ST}, whereas selection and drift cannot be distinguished if Q_{ST} and F_{ST} are similar (Mckay and Latta 2002; but see Ovaskainen et al. 2011). Results of two studies that contrasted these metrics for small populations were inconclusive because of the difficulty in interpreting the result of $Q_{ST} = F_{ST}$ (Johansson et al. 2007), and because the study populations may not have typified the situation of populations that have been size-restricted for a long period of time (Koskinen et al. 2002). In general, results of published Q_{ST}/F_{ST} analyses likely need to be interpreted with caution since there are now a variety of statistical and methodological issues recognized with such comparisons (Merilä and Crnokrak 2001; O'Hara and Merilä 2005; Leinonen et al. 2008; Whitlock 2008; Edelaar and Björklund 2011; Whitlock 2011).

Habitat quality and small population size

The assumption that selection is weak at small population size ignores the potential consequences of habitat fragmentation on the characteristics of environments that are occupied by small, fragmented populations. Small populations are frequently assumed to occur in habitats that are marginal, where conditions may be more stressful than those experienced by large populations in continuous habitats (Brown 1984; Hoffmann and Blows 1994). Ecological conditions do differ between habitat fragments

and continuous habitat in a variety of ways, including the impacts of physical disturbances (Lovejoy et al. 1986), predation (Andrén 1985), parasitism (Kruess and Tscharntke 1994), and abiotic variables (Lovejoy et al. 1986), and these can potentially result in reduced recruitment and increased mortality (Robinson et al. 1995; Ward and Johnson 2005). If environmental stress reduces h^2 through either reduced V_A or increased V_E then response to selection will also be reduced according to the breeder's equation (Blum 1988; Gebhardt-Henrich and Van Noordwijk 1991; Merilä 1997; Imasheva et al. 1998; Merilä and Sheldon 2001; Charmantier and Garant 2005). On the other hand, habitat fragmentation resulting in increased variability in environmental conditions might also cause selection regimes to differ in small fragments versus continuous habitat. If this is true then the strength of selection experienced by small populations in some small fragments might be greater than experienced by large populations in more benign environments. The effect of habitat fragmentation on selective regimes might furthermore depend on initial starting conditions within habitat fragments. Habitat complexity increases at larger scales (Kotliar et al. 1999) such that fragmentation might result in patches that become increasingly dissimilar as they are reduced in size and that may also vary in quality. As suggested by the habitat quality paradigm, habitat quality can be as important to the persistence of populations occurring in fragments as standing genetic variation (e.g. Lande 1988; Caro and Laurenson 1994; Delin and Andrén 1999; Thomas et al. 2001; Leimu et al. 2010). Thus, if small habitat fragments are random samples of larger, more spatially heterogeneous habitats (Connor and McCoy 1979, Haila 1983), this might result in more among-population variability in the types of habitats occupied by small versus large populations (e.g. Willi et al. 2007; but see Willi and Hoffman 2012).

Although this has potentially important implications for the persistence of small populations in small fragments there has been little theoretical development and no systematic investigation of the types of habitats occupied by small versus large populations of vertebrates, possibly because of the difficulty in collecting comprehensive habitat data for a large number of populations that differ in abundance.

Plasticity at small population size

Even if quantitative genetic variation declines with population size, small populations might be able to persist in the short term if they can respond plastically to changing environmental conditions (Schlichting 1986; Pigliucci 2005). Phenotypic plasticity is the expression of different phenotypes by the same genotype depending on the environment (De Jong 1990) and can include behavioral, morphological, physiological, demographic, and life history changes (Reed et al. 2010). Phenotypic plasticity can be important in allowing populations to track shifting selection pressures (Réale et al. 2003; Berteaux et al. 2004; Charmantier et al. 2008). For example, female red squirrels (*Tamiasciurus hudsonicus*) have maintained a high fitness by advancing their parturition dates to match the advancement in peak cone production of white spruce (Réale et al. 2003; Berteaux et al. 2004).

If there is additive genetic variance for phenotypic plasticity then plastic responses can evolve in response to natural selection (De Jong 1990, Schlichting and Pigliucci 1998). Plasticity will be favored when environments are spatially or temporally heterogeneous and when no one phenotype has greatest fitness across all environments (Via and Lande 1985; Scheiner 1993; Tollrian and Harvell 1999; Reed et al. 2010).

Plastic responses may also evolve differently among populations due to differences in genetic diversity resulting from drift or habitat-specific adaptations (van Kleunan et al. 2000), and therefore might vary among differentially abundant populations. For instance, small populations might consistently exhibit reduced plasticity relative to large populations if genetic diversity underpinning phenotypic trait plasticity is reduced due to restricted gene flow, drift, inbreeding, and/or overall increased environmental stress (Ouborg et al. 2010). Alternatively, the magnitude of plasticity exhibited by small populations might be greater than exhibited by large populations if small populations experience more widely fluctuating environmental conditions as a result of habitat fragmentation. Compared to adaptive evolution, little empirical research has focused on the role of plasticity in enabling population responses to environmental change for populations of varying size (van Kleunan et al. 2000, Paschke et al. 2003; Berg et al. 2005). In fact, only two studies have investigated the magnitude of plasticity in relation to population sizes (for plants) and these yielded equivocal results, possibly due to low statistical power (Paschke et al. 2003, Berg et al. 2005).

Thesis aims and study system

The future of small populations of wild species is uncertain. The conservation genetics small population paradigm predicts erosion of selectively relevant genetic diversity that occurs more rapidly with declining population size however, the results of the small number of field studies that have tested these predictions for natural populations have been inconclusive for the variety of reasons already discussed. Furthermore, it remains unclear whether small populations generally occupy habitats that differ in

selective regime compared to spatially contiguous habitats, or whether plasticity is a strategy adopted more intensely by small populations to cope with environmental variability. As remaining natural areas become increasingly fragmented, and as anthropogenic changes to the environment increase, such information may be key to answering several pressing questions in conservation biology. For example, what is the minimum size of viable populations? How large do nature reserves have to be to maintain viable populations of species? Should small populations receive high priority for conservation? And perhaps the most controversial question; will specific populations respond to future environmental change?

The overarching purpose of this thesis was to investigate the assumptions of the conservation genetics small population paradigm using a large number of differentially abundant populations of a salmonid fish, *Salvelinus fontinalis* from Cape Race, Newfoundland, Canada. First, in Chapter 1, I explored two alternative predictions which describe how habitat fragmentation might potentially alter environmental conditions differently among populations that vary size by measuring a large variety of physical and chemical habitat characteristics in streams occupied by nineteen Cape Race brook trout populations. Then, in Chapters 2 and 3, I explored whether these same alternative predictions might have influenced population-level characteristics known to affect a population's ability to cope with novel challenges. Specifically, in Chapter 2, I conducted common-garden experimentation of temperature plasticity for a variety of early life-history traits on a subset of eight Cape Race brook trout populations to test for differences in plastic response in relation to population size. In Chapter 3, I investigated the relationship between population size, quantitative genetic variation and the relative

role of drift vs. selection in population differentiation (Q_{ST} vs. F_{ST}) for the same subset of populations. Finally, in Chapter 4 I investigated the evidence for reduced efficacy of selection at small population size by conducting an extensive review of the primary literature and then using meta-analytic techniques to explore the relationship between population size and patterns of phenotypic selection in populations of wild species.

Salmonids are a popular model system for the study of evolutionary processes (Hendry and Stearns 2004), and are useful for examining questions relating to the link between population size, genetic diversity, adaptation, and plasticity. They occupy diverse aquatic habitats throughout North America, and frequently form genetically distinct populations, which is facilitated by the discrete nature of their rearing habitats, as well as their habit of returning to their natal streams to spawn (Stabell 1984). This strong degree of site fidelity is thought to lead to the development of local adaptation, culminating in the tremendous variability in morphology, behavioural, and life-history traits observed in nature (Taylor 1991; Klemetsen et al. 2003; Garcia de Leaniz et al. 2007). The brook trout, in particular, exhibits a high degree of phenotypic differentiation in response to localized selective pressures (Fraser and Bernatchez 2005; Perry et al. 2005), varying degrees of phenotypic plasticity (Hutchings 1996), and a wide range of population sizes (Power 1980). Furthermore, the brook trout is smaller and generally has a shorter generation time than most other salmonids, rendering it a more experimentally tractable species for in-lab common garden quantitative genetic studies involving a large number of families.

Salmonids in general are a socio-economically important group of fish species in the northern hemisphere, however, many natural populations are currently extinct or at

risk of extinction due to anthropogenic habitat loss and fragmentation, or by large-scale changes in community composition and habitat quality (Gustafson et al. 2007; McClure et al. 2008). Due to their cultural and economic significance, resources are routinely invested in studies of salmonid population genetic structuring, however, many of these studies focus on neutral rather than quantitative genetic variation. This is an obvious concern as management strategies often rely heavily on this information, including for small, fragmented, or at-risk populations (e.g. DFO 2005; Fraser 2008). In reality, solid empirical data regarding linkages between quantitative genetic variation, population size, and adaptation is lacking, but is imperative to improving the management and conservation of salmonids, as well as other taxa.

Chapter 1: Relationship of habitat variability to population size in a stream fish

Abstract

The relationship between habitat variability and population size in fragmented habitats is poorly understood, yet might have important evolutionary consequences. For instance, fragmentation could (1) shift habitat characteristics – and by extension, selective regimes – in a consistent direction as populations and the fragments they occupy are reduced in size (Directional Hypothesis), or (2) increase variability in habitats among similarly sized populations as fragment size decreases (Variable Hypothesis). I investigated these alternatives based on multi-year habitat, demographic, and genetic data from 19 fragmented populations of a stream fish varying in census size (N) and effective number of breeders (N_b). Mean habitat parameters were significantly related to N and N_b, but the forms of the relationships varied, and there was no evidence of consistent directional differences in habitat parameters from small to large population size. Small populations exhibited a wider range of variances in habitat parameters than large populations, and to a lesser extent, small populations also had greater variability in mean habitat parameters, possibly signaling more diverse selective regimes. These results suggest that many different environments are associated with small population size in nature, counter to the frequently cited assumption that small populations tend to occur only in marginal environments. In addition to well-documented demographic and genetic stochasticity operating within small populations, our work raises the possibility that small

populations exhibit more variable and potentially less predictable evolutionary responses to future environmental change.

Introduction

The study of habitat fragmentation has focused primarily on the ecological consequences of species-area relationships and colonization-extinction equilibria (MacArthur and Wilson 1967; Gilpin and Hanski 1991). Additional attention has also been paid to the heightened risk of extinction attributable to demographic, environmental, or genetic stochasticity and inbreeding for populations inhabiting fragments that are small and isolated (Soulé 1987; Gilpin and Hanski 1991). By comparison, how fragmentation alters habitat characteristics – and by extension, selection regimes – as fragment size and population size decrease, is poorly understood. Yet it might have important evolutionary implications if fragmentation affects the adaptive genetic composition of fragmented populations (Willi et al. 2007; Willi and Hoffman 2012) and their subsequent responses to environmental change, which will be largely influenced by population size (Lynch and Lande 1997). Herein, I consider two competing alternatives to explore the relationship between population size and habitat variability in a series of fragmented, isolated fish populations.

One alternative, hereafter the "Directional Hypothesis", is that habitat characteristics shift in a consistent manner during the habitat fragmentation process, resulting in directional relationships between these characteristics and population size (Willi and Hoffman 2012; Fig. 1.1). For example, small populations frequently occur in fragmented habitats that are generally assumed to be of poor quality and that provide consistently more stressful conditions (Brown 1984; Hoffmann and Blows 1994). Ecological conditions are indeed known to differ between habitat fragments and spatially contiguous habitat (Robinson et al. 1995), including impacts of physical disturbances

(Lovejoy et al. 1986), predation (Andrén 1985), parasitism (Kruess and Tscharntke 1994), and abiotic variables such as relative humidity and wind exposure (Lovejoy et al. 1986). In turn, such conditions can potentially lead to reduced recruitment (Ward and Johnson 2005) or increased mortality (Robinson et al. 1995) for small populations inhabiting these fragments.

A second possible alternative, the "Variable Hypothesis", is that habitat characteristics and resulting selective regimes become increasingly variable as fragment size and population size decrease (Willi and Hoffman 2012; Fig. 1.1). Under this hypothesis, the evolutionary effects of habitat fragmentation depend on initial starting conditions within habitat fragments. According to landscape ecology, for example, fragments inhabited by small populations are expected to be simply random samples of large population habitat fragments (Connor and McCoy 1979, Haila 1983). Complexity increases at larger scales, such that large populations' habitats are more heterogeneous (Kotliar et al. 1999). For instance, microclimate conditions or distribution of food within a large patch may not be spatially or temporally homogeneous, resulting in a variety of smaller patches that differ in their relative quality (e.g. Cartar and Real 1997; Vanwalleghem and Meentemeyer 2009). Habitat quality can be as important to the persistence of populations occurring in fragments as habitat area and isolation, as has been suggested in previous studies on diverse organisms (e.g. Delin and Andrén 1999; Thomas et al. 2001). Collectively, we might expect within-fragment habitat heterogeneity to be greater in larger vs. smaller population fragments, but greater between-fragment habitat heterogeneity among fragments occupied by smaller populations.

To my knowledge, no study of vertebrates has investigated these alternative hypotheses. To do so requires comprehensive data on the habitat characteristics and habitat variability in fragments occupied by a large number of populations that differ in census and effective population size. Such an investigation may contribute towards a better understanding for predicting population responses to future environmental change and for setting conservation priorities, if the intensity, form and direction of selection within habitat fragments differ or vary more in small relative to large populations. Indirect evidence for the Variable Hypothesis was provided by Willi et al. (2007) who found more variance in the ratio of quantitative to neutral genetic differentiation (Q_{ST} vs. F_{ST}) among small populations compared to large populations, suggesting distinct selection regimes within the small populations (but see Willi and Hoffman 2012).

The purpose of this study was to determine whether the habitats experienced by nineteen, differentially-abundant populations of a stream fish species (brook trout, *Salvelinus fontinalis*) from thirteen distinct stream drainages consistently differ in a number of physical and chemical characteristics that are related to individual fitness and population abundance. The Directional Hypothesis was tested by investigating whether the relationship between habitat parameters and population size differs from zero. The Variable Hypothesis was assessed by exploring whether (i) small populations exhibit greater among-fragment variability in mean habitat parameters than large populations; and (ii) whether small populations exhibit greater among-fragment variability in variance around the mean habitat parameters than large populations. The Variable Hypothesis essentially assumes a positive correlation between population size and fragment size, but it is possible that small fragments differing in habitat quality might support different

numbers of individuals, complicating the relationship between population size and fragment size. Therefore, as a corollary to the Variable Hypothesis, the relationship between population size and fragment size (drainage area) was quantified for our study populations.

The hypotheses were tested using estimates of both the adult census population size (N) and the effective number of breeders over multiple cohorts (N_b), a parameter which is related to the effective population size N_e (Waples et al. 2013). Indeed, it is N_e, not N, which will influence evolutionary responses of populations to habitat characteristics (Lynch and Lande 1997; Palstra and Fraser 2012). Moreover, previous studies have assumed a correspondence between N and N_e (e.g. Willi et al. 2007) but their relationship may be complex (Fraser et al. 2007) and N_e/N ratios can vary widely among populations of closely related species (Belmar et al. 2012; Palstra and Fraser 2012).

Materials and Methods

Study site

Cape Race, Newfoundland, Canada (Fig. 1.2) is a small region of coastal barren land characterized by extensive areas of heath moss interspersed with patches of stunted boreal forest. It is traversed by a parallel series of relatively short (0.27 – 8.10km), low-order streams, with most harbouring resident populations of brook trout (Ch. 1 Appendix A, Table A1). Several attributes make these populations excellent for investigating the relationship of habitat variability to population size in vertebrates: (i) the small size of Cape Race streams is amenable to thorough sampling and measuring of population and habitat characteristics; (ii) populations are pristine and largely unexploited due to the

small average size of individuals (typically < 15cm); (iii) most streams terminate in a 30-50 m waterfall emptying directly into the sea, effectively eliminating gene flow among populations (this study); and (iv) populations exhibit considerable differences in life histories which are apparently the result of changes to environmental selective regimes following habitat fragmentation at Cape Race (Hutchings 1993; Belmar-Lucero et al. 2012). Indeed, (v) phylogeographic work suggests that isolation of Cape Race populations occurred from a common ancestor since the late-Wisconsinan deglaciation (10-12000 ybp; Danzmann et al. 1998).

Habitat data

To quantify spatial and temporal habitat variability across trout streams, data was initially collected on 31 habitat parameters from 875 transects across 13 Cape Race stream drainages in 2010 and 2011. Highly correlated variables were excluded, yielding a reduced set of 17 transect-level habitat parameters per stream (Table A2; see Appendix A for a complete list of habitat parameters and data collection methodology). Several stream-level characteristics were measured in the lab, using Google Earth (Google 2012; Table A1), and drainage area was measured using Google Earth in conjunction with MapWindow GIS open source GIS software (MapWindow Open Source Team 2008). The use of Google Earth might have resulted in inaccurate estimates of some stream level characteristics (e.g. sinuosity, stream gradient), however these were not included in any formal analyses with the exception of drainage area and hence did not affect the conclusions of this study. Drainage area was estimated in the same manner for each stream and therefore inaccuracies are expected to be consistent across populations,

furthermore, inexact estimates of drainage area were likely not of a magnitude to greatly alter the relationship of fragment size and population size. Habitat data in most stream drainages (11 of 13) were collected in July for both years; remaining drainages were assessed in October 2011 (Table A1).

Beginning at the mouth of each stream drainage, data were collected from 18-32 transects (2010) and from 18-61 transects (2011) spaced 25-100m apart, depending on stream length (Table A1). I chose to space transects at regular intervals dependent on stream length, rather than a constant number of consistently spaced transects across all streams, in order to collect data in a logistically practical manner from the entire length of each stream and to sample as many potential habitat types as possible. In 2011, the number of transects was increased for several of the larger streams relative to 2010 to cover more detailed habitat surveys or to cover the entire length of these streams. GPS coordinates of each transect were recorded in 2010, and the same transects were sampled in 2011 (± 1-3m due to fine-scale GPS imprecision).

Number of trout populations

Following habitat data collection, an important next step was to determine the number of genetically distinct trout populations occupying the 13 stream drainages using DNA analyses based on 13 microsatellite loci. In 2010 and 2011, tissue samples from a total of 2647 individuals were obtained as adipose fin clips and stored in 95% ethanol until DNA was extracted, using a modified phenol-chloroform protocol. Samples were comprised of one to three age-based cohorts, or year-classes (depending on the drainage), that were readily distinguishable in all Cape Race streams (2009, age 1+; 2010 age 0+;

2011 age 0+; Table 1). Individuals were randomly sampled from a large number of locations within each stream to obtain a reliable genetic representation of different populations, using three-minute electrofishing surveys conducted at each 50m or 100m interval from the stream mouth, depending on stream length. Details of microsatellite PCR and polymorphism screening are found in Ch. 1 Appendix B.

STRUCTURE 2.1 (Pritchard et al. 2000) was used to evaluate if multiple populations existed within any drainage. Five independent runs per drainage were run under a model of admixture and correlated allele frequencies using *K* subpopulation values of 1 to 5, to estimate posterior probabilities (Ln P(D)) of the data (burn-in period, 50,000 replications; 100,000 Monte-Carlo Markov chain (MCMC) replicates per run). *K* was determined using a combination of the ΔK procedure of Evanno et al. (2005), by interpreting the Ln P(D) values themselves, and by assessing the strength of individual assignments within clusters, as recommended by Pritchard and Wen (2003). In 5 of 13 drainages, multiple populations were detected, being associated with clear geographic divisions such as waterfalls (populations coded 1-2, 4-5, 6-7) or fragmentation and isolation of the stream bed (populations 12-13-14, 16-17). Therefore, for all subsequent analyses these samples were treated as separate populations (Table 1.1). A suggestion of sub-population structure in population 5 (Freshwater River) was raised from higher posterior probabilities for $K = 2$ to 4 than for $K = 1$, and several heterozygote deficiencies (see below). Nevertheless, Freshwater River was treated as one population because individual assignments within clusters were ambiguous and no evident spatio-temporal clustering was observed among the three cohorts sampled (data not shown).

For each of the 19 demarcated populations (whether being from an isolated drainage or from within a drainage), GENEPOP 4.0 (Raymond and Rousset 1995) was then used to quantify alleles per locus and observed and expected heterozygosities, to verify Hardy–Weinberg equilibrium (HWE) expectations of genotypic frequencies (at each locus in each population), and to test for genotypic disequilibrium between all loci pairs. The degree of genetic differentiation separating each population pair (between and within drainages) and across years for the same population (where multiple cohort samples existed) was also estimated using Weir and Cockerham's (1984) θ_{ST} implemented in GENETIX 4.0 (Belkhir et al. 2004).

Census population size (N)

Either the Schnabel (1938) or Petersen (1896) method was used to estimate annual adult census population sizes (*N*) in Cape Race streams in 2010 and 2011 (see Table 1.1 for individual population details). Population size was estimated for the number of age 1+ yr and older individuals which, for most streams, roughly corresponds to adult population size since these constitute reproductive ages at Cape Race (Hutchings 1993; present study). However, *N* estimated in this way is likely to be higher than an *N* based on the number of breeding adults in Cape Race streams given that females typically do not reach maturity until 2+ yr in several streams. Multiple recapture events were performed in 4 of 15 populations in 2010 (Schnabel method applied), while a single recapture event was carried out in each stream in 2011 (Petersen method applied), with the exception of population 12 (3 recapture events; Schnabel method applied).

In 2010, individual fish were marked from each stream on at least two separate occasions, one each in the summer and fall (with the exception of population 13). In July, 2010, between 10 and 180 adult fish across reaches of each stream were marked by clipping the adipose fin; additional individuals (n = 17-133) in each stream were tagged by inserting individually numbered tags (FD-68B Fine Fabric Anchor Tags; Floy Tag and Manufacturing Inc.) for the purpose of collecting age-specific survival and life history data. In late September and October of 2010, tagged individuals were recovered by conducting electrofishing surveys of each stream; more individuals were simultaneously tagged (adipose fin clipped; n = 16-380, floy tagged; n = 5-123). In 2011, fish from most streams were tagged in July only (adipose fin clipped; n = 5-1736, floy tagged; n = 0-479) with subsequent recapture taking place approximately a week after tagging. The only exceptions were populations 1, 2, and 9 for which tagging and recapture took place in the fall sampling season. Where the Schnabel method was used to estimate N (populations 6-8, 10, 12 in 2010 and 13 in 2011), proportions of tagged recaptures were consistent across multiple passes. For population 12, the only stream with multiple tagging and recapture events, a regression plot of the proportion of tagged trout on the number of previously marked individuals was linear, suggesting that the assumptions of the Schnabel method had been met (N was constant across recapture events; sampling was random; individuals had equal recapture probabilities).

Effective number of breeders (N_b)

Habitat data was also related with population size data reflecting genetics. Specifically, the effective number of breeders (N_b) of each cohort was estimated for each

population, using the linkage disequilibrium method implemented in LDNe (Waples and Do 2008; Ch. 1 Appendix C). The principle behind this approach is that linkage disequilibrium should increase as the effective population size (N_e) decreases (i.e. as genetic drift increases) (Waples and Do 2008). Brook trout is an iteroparous species with overlapping generations, so the estimates obtained via LDNe correspond to the N_b for a specific cohort rather than a generational estimate of N_e. Because trout may reproduce in multiple years, an N_e estimate based on simple summing of N_bs across multiple cohorts (comprising the equivalent of one generation) could be biased. For simplicity, here the harmonic mean of N_b was used where multiple cohorts were available rather than N_e, with each cohort weighted based on the number of individuals sampled (Waples and Do 2010). This method appears to be justified since N_b has been found to be closely related to N_e within species with overlapping generations (Waples et al. 2013). As recommended by Waples and Do (2010), alleles were excluded with frequencies of either <0.02 (for small to intermediate samples) or <0.01 (for sample sizes of 80-100 or larger, and in populations suspected of having large N_b based on large N) to increase precision without generating too much bias in our N_b estimates.

Statistical Analysis

Directional Hypothesis: Relationships of habitat parameters to population size

Preliminary analyses using linear regressions revealed that the data violated the assumptions of the general linear model in many cases. For this reason, generalized additive models (GAMs; Hastie and Tibshirani 1990; Wood 2006) were adopted to test for non-zero relationships between all transect-level data for each habitat parameter with

N and N_b. Since smoothing models make no prior assumptions about the forms of the relationships between variables they are particularly useful for illustrating potential complexities in the relationships between habitat parameters and population size that could be missed by using linear models. Penalized cubic regression splines were used and errors were assumed to be normally distributed, except for habitat parameters presented as proportions for which a quasibinomial distribution was implemented to allow for overdispersion of the data. All of the models were fitted using the GAM functions in the mgcv package (Wood 2011) of R version 2.14.1 (R Development Core Team 2011). For each of the models fitted, the estimated degrees of freedom (edf) used to determine the optimal amount of smoothing were automatically selected using restricted maximum likelihood (REML). After fitting the model, plots were produced to highlight the shape of the relationships between each of the habitat parameters and N and N_b.

Variable Hypothesis: Variability of habitat means in relation to population size

To test for greater variability in habitat parameter means at smaller population size, all populations were divided into bins of 'small' N (76-1174, n=10 in 2010 and 79-1731, n=11 in 2011) and 'large' N (1683-6223, n=5 in 2010 and 2412-8416, n=8 in 2011) and 'small' N_b (15-49, n=10 in 2010 and 15-59, n=12 in 2011) and 'large' N_b (101-135, n=5 in 2010 and 93-249, n=7 in 2011), using the average N or N_b in each year. For streams that were sampled in both years, the populations in each size bin for N were concordant with the exception of Cripple Cove which was below the average N cut-off in 2010 and above the cut-off in 2011; Cripple Cove was included in the 'large' size bin in both years for consistency, and to increase sample size in 2010. Generalized linear mixed

models (GLMM) were implemented in the R package lme4 (Bates et al. 2012), using each parameter as a response variable with a random effect defined by population nested within bin to determine the relative amount of variation at the among-bin compared to the among-population level. Additionally, to determine if small populations experience increased variability in habitat parameters among years, relative proportions of variance in habitat parameters among years for 'small' and 'large' *N* bins were estimated and compared using GLMMs with year as a random effect.

Variable Hypothesis: Range of habitat variances in relation to population size

To investigate whether the range of variances surrounding habitat parameter means was related to population size, the coefficients of variation (CV; a normalized measure of dispersion where CV= σ/μ) of habitat parameters were plotted for each stream against *N* and N_b in each year. White's test was then used to establish whether the residual variance of each habitat parameter against *N* and N_b was constant or exhibited heteroscedasticity. White's test works by implementing an auxiliary regression analysis which regresses the squared residuals from the original regression model onto a set of regressors that contain the original regressors, the cross-products of the regressors, and the squared regressors (White 1980).

Relationship between population size and habitat size

Spearman's correlations were used to determine whether a positive relationship existed between *N* and N_b and habitat fragment size (drainage area) in each study year, as drainage area was not normally distributed. Furthermore, the CV of drainage area was

calculated for populations grouped in small vs. large size bins to determine if there was more variability in fragment size among small than large Cape Race populations.

Results

Habitat, genetic diversity, census population size and effective number of breeders

Collectively, the genetic data indicated random mating within Cape Race populations and independence of the loci examined. Mean (±1SE) allelic richness and observed heterozygosities across the thirteen loci screened within the 44 individual population cohorts of Table 1.1 were 5.2 ± 0.1 and 0.52 ± 0.01, respectively (see individual population data in Ch. 1 Appendix D, and θ_{ST} amongst populations in Ch. 1, Appendix E). Of the 572 individual HWE tests within cohorts (44 cohorts, 13 loci), 39 and 7 exhibited heterozygote deficiencies (6.8%) or homozygote excesses (1.2%), respectively, following Bonferroni correction ($\alpha = 0.05/13$) (Appendix D). These were distributed across 11 of 13 loci, 13 of 19 populations, and 27 different cohorts, though 9 of 39 heterozygote deficiencies originated from Freshwater River (Appendix D).The number of significant pair-wise linkage disequilibrium tests between loci was also low within populations and distributed across different loci pairs (52 of 3432 tests or 1.5%; based on the 44 cohorts, 78 comparisons per individual cohort).

Abundance varied about two orders of magnitude among populations. Estimates of N ranged from 76-6223 and 79-8416 in 2010 and 2011, respectively. Point estimates of N_b based on one to three cohorts (2009-2011) ranged from 15-249. N_b estimates of ∞ were not included in the weighted harmonic mean (see Table 1.1). Mean transect-level habitat characteristics for all Cape Race streams are found in Table A2.

Directional Hypothesis: Relationships of habitat parameters to population size

In both years, the GAMs revealed highly significant relationships with both estimates of population size for almost all habitat parameters investigated (Table 1.2). In 2010, for both N and N_b, 16 out of 17 relationships differed significantly from zero, 13 (N) and 12 (N_b) of which were significant at $p<0.001$, while in 2011, all relationships were significantly related to N (14 of 17 significant at $p<0.001$), and N_b (16 of 17 significant at $p<0.001$). There was a great deal of variability in the functional form of the relationships between almost all habitat parameters and population size not only among years but between N and N_b within years (Fig. 1.3; Ch. 1 Appendix F). The proportion of variance explained by the models (R^2-adj) was generally low to moderate (range= 0.001-0.864) likely because other relevant explanatory variables were omitted as we were interested in the relationship between habitat parameters and population size exclusively.

Variable Hypothesis: Variability of habitat means in relation to population size

Results of GLMMs for N in 2010 showed that there was more variation among bins of small vs. large population size for six habitat parameters (Table 1.3); for one additional case there was only marginally more variation at the among-bin versus the among-population level. In 2011 with an increased number of habitat transects sampled in each stream drainage, there was more variation associated with small vs. large population size bins for only 3 of 17 parameters. For N_b in 2010, 5 of 17 parameters exhibited more variation at the among-bin level, but only one parameter showed this trend for N_b in 2011. Relative to N in 2010, the amount of variation at the among-bin

level for N_b was reduced for two habitat parameters, but increased compared to N for two different parameters. Furthermore, three parameters showed greater variability at the among-bin level for N but more variability at the among population level for N_b. In 2011, compared to N, less variation was associated with the N_b size bins for two parameters (Table 1.3). GLMMs used to examine temporal variability in habitat parameters for small and large N bins showed that, for 5 of 17 and 8 of 17 parameters, respectively, there was more variability among years for both small N and large N populations (Table 1.3).

Visual examination of mean habitat parameters plotted against N showed evidence for increased variability at small N for 7 of 17 habitat parameters in both 2010 and 2011 though the parameters exhibiting trends differed between the two years (Fig.1.4 and Ch. 1 Appendix G). For the same means plotted against N_b, 4 of 17 parameters and 5 of 17 parameters showed increased variability at small N_b in 2010 and 2011, respectively. Means for 4 of 17 habitat parameters consistently exhibited increased variability at small population size across both N and N_b in both years. Two additional parameters were variable at small population size only for N in both years, and one other parameter was more variable at small population size for N and N_b in 2011 but exhibited no trend in 2010 (Fig.1.4 and Appendix G).

Variable Hypothesis: Range of habitat variances in relation to population size

White's test showed slightly different results for habitat variability in relation to N and N_b in both years (Table 1.4). A weighted z-test to combine the results of these tests across years found that, for 11 of 17 habitat parameters, variability was greater at small population size for N, while three parameters exhibited more variability at large

population size (assessed by visual examination of residual plots; Table 1.4). Similarly for N_b, spread was greater for small populations for 10 of 17 parameters, and was greater for large populations for two parameters (Table 1.4). Considering sampling years separately, 6 of 17 parameters consistently showed increased spread at small N and N_b (conductivity, temperature, mean depth, percent vegetation, percent fine gravel, and percent silt) in 2010 and 2011. Significant heteroscedasticity at small population size was also detected for one additional parameter (pH) for N and N_b in 2011 that was not significant in 2010. Only two parameters (channel width, current velocity) were consistently associated with increased spread at large population size for N and N_b in both years (Table 1.4).

Visual examination of habitat parameter CVs showed trends for increased variability at small N for 12 of 17 habitat parameters in both 2010 and 2011(Fig.1.5 and Ch. 1 Appendix H). Some trends were clear, but in a few cases, the trends might have been driven by one or perhaps two extreme values, specifically dissolved oxygen in 2010, and pH and conductivity in 2011. For the habitat parameter CVs plotted against N_b, increased variability was observed at small population sizes for 10 of 17 parameters in 2010 and 11 of 17 parameters in 2011(Fig.1.5 and Appendix H).

Relationship between population size and habitat size

Investigation of the relationship between fragment size and population size revealed strong positive relationships between drainage area and N and N_b in both 2010 ($r_s = 0.82$, $p < 0.001$ for N, and $r_s = 0.74$, $p = 0.002$ for N_b) and 2011 ($r_s = 0.82$, $p<0.001$ for N, and $r_s = 0.75$, $p<0.001$ for N_b) (Fig. 1.6). The CV of drainage area was higher for

populations in the small versus large population size bins in both years (CV = 1.33 vs. 0.51 in 2010, and 1.03 vs. 0.57 in 2011) but this variability did not affect the observation that small populations were associated with small fragment sizes (Fig.1.6).

Discussion

Relationship of habitat variability to population size

Comprehensive data on 19 fragmented populations of brook trout lend support to the Variable Hypothesis, that habitat fragmentation increases spatial habitat variability and, by extension, variability in selective regimes. Over a broad range of population sizes (N=76-8416; N_b=15-249), this study generally revealed a wider range of variances around habitat parameter means among small versus large populations, based on significant residual heteroscedasticity. To a lesser extent, increased variability in habitat parameter means was observed at smaller population size. Collectively, these trends were observed in both study years and when relating habitat characteristics to both N and N_b.

There was little clear evidence for the consistent directional habitat differences between small and large populations predicted by the Directional Hypothesis that might be associated with, for instance, more stressful conditions in small fragments due to increased edge effects (Brown 1984; Hoffmann and Blows 1994). For example, only 13 of 68 relationships across both study years and for N and N_b within years suggested a directional change from small to large population size. Furthermore, parameters showing directional relationships were infrequently consistent across years or between the two estimates of population size (Fig. 1.3; Appendix F). Taken together, although variability in habitat parameters was investigated as a function of population size when the reverse is

probably true (i.e. population size is a function of habitat variability), it appears that small populations are more often associated with more divergent habitats than large populations.

In the choice of habitat parameters, there was no attempt to determine which ones specifically drive selection within each study stream *per se*, but a variety of parameters were chosen that have been shown to be related to salmonid fish fitness and abundance in nature (Quinn 2005). However, at Cape Race, certain variables might be particularly important for adaptation such as temperature, pH, stream depth and velocity (e.g. Hutchings 1993; Belmar-Lucero et al. 2012). These factors are highly variable both within and among Cape Race streams (Table A2), and in both years results for the Variable Hypothesis confirmed a wider range of variances at small population size for most of them.

It is not surprising that N and N_b did not show identical relationships to population habitat variability. Most populations have larger N than N_b (Palstra and Fraser 2012), but aspects of the habitat and mating system of brook trout can result in different N_b/N ratios among populations (Belmar-Lucero et al. 2012). In the present study, given that habitats with greater environmental variability could generate more fluctuations in N, thus reducing the ratio between N_b and N (Waples et al. 2010), we might expect that smaller populations would exhibit more variability in N_b/N ratios than larger populations. A cursory inspection of N_b data in 2011 relative to N data in 2010 (Ch. 1 Appendix I; to properly associate N_b/N, see Palstra and Fraser 2012) supports this prediction. For example, the CV for N_b/N was 0.85 for populations in the 'small' N bin and 0.48 for populations in the 'large' N bin.

Possible study caveats

I set out to investigate whether habitat variability was related to population size, and thus substituted fragment size with population size under the assumption that the two were positively correlated. Yet same-sized habitats of differing quality might also differ in population size, invalidating this assumption. For example, a small fragment containing higher quality habitat could support a denser population than an equal sized fragment of low quality habitat, and thus population size would not be simply a function of fragment size. Investigating this issue, a strong positive correlation was found between population size (both N and N_b) and drainage area in both study years. There was more variability in the relationship at the lower end of the range of fragment sizes and population sizes, suggesting that the habitats resulting in small population size are on average less stable. Nevertheless, this variability was clearly low relative to the observed habitat variability at small population size. Replacing fragment size with population size thus appears to be justified for Cape Race populations, although this issue should be carefully considered for similar research in other systems.

The study system at CR was treated similarly to a terrestrial system, but there are important differences. First, the physical characteristics of large streams vary in a longitudinal fashion where low-order reaches have very different characteristics than higher-order reaches. Thus, from the perspective of fragmentation and its effects on habitat quality the covariance of stream position and fragmentation may be important. While the CR streams here represent a large range of sizes relative to one another, in a broader context all these streams are considered small; the largest CR streams examined

were third order streams, but most were of the first or second order (16 of 19 streams). As such, many of the systematic changes that are associated with increasing from low to much higher stream order are unlikely to be a major issue here.

Second, the disproportionate movement of individuals in a downstream direction could potentially affect the relationship of fragment size and abundance in a stream system. The relationship between abundance and fragment size for CR populations within the same drainage was compared and the correlation between N and drainage area was found to be similar for both upstream and downstream segments (downstream Pearson's r = 0.89, upstream r =0.81).

I also acknowledge that, in a few cases, the trend for increased habitat variability at small N and N_b might be driven by one or two outliers, such as the coefficients of variation for pH and temperature (Figure 1.4). But outliers cannot explain most cases of increased variability. Furthermore, the implicit assumption of the competing alternatives that habitat fragments support populations that are at their carrying capacities is likely satisfied. Thorough electrofishing surveys revealed trout inhabiting the entire length of most streams. Purchase and Hutchings (2008) also found evidence for ideal free distributions (*sensu* Fretwell and Lucas 1970) in one Cape Race stream.

Finally, because CR streams are the product of natural fragmentation, they might differ from habitats that have been the subject of anthropogenic habitat fragmentation. One possibility is that human induced fragmentation occurs and has subsequent affects on population size and habitat conditions, whereas in natural fragmentation conditions simply vary within a landscape, and conditions but not fragmentation determine population size. In reality, conditions likely vary within landscapes regardless of whether

fragmentation is naturally occurring or due to human interference. The process of fragmentation then alters conditions within fragments with the end result depending on the initial conditions. The primary difference between natural and anthropogenic fragmentation then is likely the time over which conditions are altered. Natural fragmentation resulting in relatively slower, incremental changes to environmental conditions might improve the ability of a small population to cope and increase chances for persistence in the long term. However, despite being a naturally fragmented system, there are lines of evidence that suggest that fragmentation for several CR populations may have occurred quite rapidly (Burdon and Fraser; unpublished data) and thus may not differ so greatly from a scenario of human-caused fragmentation.

Similarly, the predictions under the Variable Hypothesis will be most pronounced within habitats that are newly fragmented as opposed to habitats that have been separated for longer time periods. At Cape Race, the time since fragmentation among specific populations varies. Therefore, these observations might best apply to study populations within the same drainage (e.g. populations 6 and 7, 12-14), as these were likely more recently isolated from one another rather than populations inhabiting different drainages (Burdon and Fraser; unpublished data).

Conclusions and conservation implications

In a naturally fragmented system of populations of a vertebrate, evidence was found that small populations exhibited a wider range of variances in habitat parameters than large populations. There was also some evidence that small populations had greater variability in mean habitat parameters than large populations. Put another way, whereas

large populations commonly inhabit heterogeneous landscapes, there are many different environments that result in a small population size. This is a unique result that contrasts the frequently cited assumption that small populations tend to occur only in marginal environments where they are exposed to unfavourable conditions (Brown 1984; Hoffman and Blows 1994; Kawecki 2008).

The observed increase in habitat variability at smaller population size has potentially important conservation ramifications in the face of growing, worldwide habitat fragmentation of natural populations. Chiefly, this result raises the possibility that small populations might exhibit more varying selective regimes than large populations. A first implication, therefore, is that some small populations might represent distinct entities harbouring unique variation that, collectively, might be adaptive in a wide range of circumstances. Such knowledge could provide a more informed basis for setting biodiversity conservation priorities. Certainly, some small populations might indeed become extinct by succumbing to the mutually reinforcing and well-documented demographic and genetic stochasticity (extinction vortex; Gilpin and Soule 1986). Similarly, episodic catastrophic events may have an important influence on abundance and persistence in a particular fragment (Young 1994).

Other small populations might occupy habitats that are productive despite being small but might be dependent on conservation and management initiatives to persist in the long term. Developing criteria for distinguishing viable small populations from those that are likely to become extinct is critical, and depends on identifying factors that best predict the potential of a small population to persist. Such factors may include degree of habitat specialization (Andrén 1997), fragment characteristics (Ewers and Didham 2006),

and the rate at which the environment changes or the fragment is reduced in size (Lynch and Lande 1993).

A second conservation implication of more varying selective regimes in small than large populations is the possibility that small populations might exhibit more variable and potentially less predictable evolutionary responses to future environmental change. This "evolutionary stochasticity" would represent an under-appreciated process affecting the probability of small population persistence. Though selection is generally assumed to become less effective in small populations as genetic drift becomes more important, this might only affect the overall implication in the very smallest populations (see Koskinen et al. 2002; Willi et al. 2006). Ultimately, the evolutionary responses of small populations likely depend on how the magnitude and rate of environmental change interacts with prevailing conditions within habitat fragments.

Table 1.1: Cape Race trout population census sizes for 2010 and 2011 as well as the effective number of breeders (N_b).

Population	2010 *N* (95% CI)	2011 *N* (95% CI)	N_b	C	Sample size
Lower Whelan's	NS	4421 (3883-5190)	93 (45-1363)[b]	1	48
Upper Whelan's	NS	3588 (3206-4107)	249 (114-∞)[b]	1	74
Cotton	1174 (692-2042)	2871 (2016-4240)	41 (29-59)	2	56, 47
Perdition	NS	726 (636-853)	33 (17-115)[b]	1	24
Freshwater	4550 (4171-5028)	5385 (5076-5743)	101 (81-452)	3	45, 95, 114
Lower Coquita	316 (229-452)[a]	278 (173-483)	30 (26-36)	3	48, 59, 42
Upper Coquita	76 (50-99)[a]	79 (43-196)	15 (15-16)	2	19[c], 25[c]
Bella's Brook	NS	510 (309-1169)	59 (39-105)[b]	1	48
Bob's Cove	6132 (4500-9739)[a]	4527 (4052-5167)	117 (69-423)	3	62, 95, 105
Still There By Chance	1081 (696-1600)	1405 (1211-1696)	18 (7-72)	3	93, 42, 40
Whale Cove	1101 (857-1539)[a]	735 (626-936)	44 (35-87)	3	66, 48, 108
Ditchy	107 (76-161)	179 (132-265)[a]	29 (26-34)	2	26[d], 35[d]
Upper OuananicheBeck	2233 (1651-3247)	3835 (3355-6269)	135 (93-231)	3	67, 36, 93
Lower Ouananiche Beck	461 (292-859)	372 (244-610)	43 (39-45)	2	39[d], 25[d]
Watern Cove	6223 (5049-8434)	8416 (7225-10255)	130 (119-137)	3	59, 96, 133
Upper Blackfly	235 (164-418)	317 (185-1055)	49 (14-138)	2	41[c], 28[c]
Lower Blackfly	966 (806-1237)	1731 (1148-2238)	45 (30-64)	3	46, 54, 52
Tannin Brook	769 (452-1284)	965 (814-1209)	104 (51-882)[e]	3	90, 53, 44
Cripple Cove	1683 (992-2927)	2412 (2231-2632)	46 (28-101)	3	80, 76, 71

Notes: N_b reported is the weighted harmonic mean of point estimates across cohorts within a population. The range of point estimates are in parentheses. A supplementary Table C1 includes the 95% CI for each individual cohort.

NS = not sampled.

C = number of cohorts sampled. Unless otherwise stated; cohort sample sizes screened at microsatellite loci are listed in this order (3 = 2009, 2010, 2011; 2 = 2010, 2011; 1 = 2011).

[a]Schnabel method used for *N* estimation.

[b](95%CI reported if only one cohort sampled).

[c]2009 and 2010 cohorts and [d]2010 and 2011 cohorts, respectively.

[e]Two cohort estimates of ∞ not included.

Table 1.2: GAM results for habitat parameters vs. N and N_b for Cape Race trout populations.

	2010 N		2011 N		2010 N_b		2011 N_b	
Parameter	R^2-adj	edf	R^2-adj	edf	R^2-adj	edf	R^2-adj	edf
pH	0.585***	7.71	0.371***	7.06	0.00795	1.00	0.0407*	2.33
DO	0.180**	2.96	0.190***	5.22	0.129**	2.66	0.200***	3.27
Conductivity	0.864***	8.92	0.430***	5.01	0.560***	8.08	0.632***	8.23
Temperature	0.421***	8.83	0.178***	6.66	0.536***	8.15	0.396***	8.74
Width	0.242***	4.61	0.267***	8.92	0.256***	4.78	0.303***	8.06
Depth	0.179***	7.00	0.172***	8.84	0.160***	7.86	0.216***	8.75
Undercut depth	0.102***	4.36	0.138***	5.21	0.153***	8.03	0.075***	4.89
Velocity	0.272***	6.73	0.224***	6.82	0.372***	8.26	0.282***	8.26
% riparian	0.101***	6.23	0.0420***	5.29	0.0179**	2.02	0.0741***	6.76
% vegetation	0.0359**	2.57	0.0579***	2.93	0.147***	5.90	0.183***	7.79
No. of species	0.195***	4.46	0.0948***	4.01	0.148***	3.29	0.0616***	4.33
% large boulder	0.00106	1.70	0.0841***	3.63	0.0298**	3.64	0.135***	7.02
% small boulder	0.0205*	1.58	0.0257**	2.16	0.0682**	3.11	0.0961***	6.41
% cobble	0.240***	6.62	0.0428**	4.27	0.215***	3.39	0.173***	6.24
% coarse gravel	0.248***	6.81	0.0168**	3.90	0.245***	3.28	0.0316***	6.29
% fine gravel	0.202***	6.27	0.0701***	8.15	0.187***	3.27	0.0517***	7.92
% silt	0.608***	7.70	0.234***	6.82	0.527***	5.33	0.439***	7.62

Notes: Results are *p*-values to assess the null hypothesis that each smooth term is constant where (edf) = estimated degrees of freedom and (R^2-adj) = the proportion of variance explained by the model.

*<0.1, **<0.05,***<0.001.

Table 1.3: GLMM results for the percent of total variation in habitat parameter values explained at the among group vs. among population level for N and N_b grouped into bins of small vs. large size for two years, and the percentage of variation in habitat parameters associated with year for Cape Race trout populations divided into 'small' or 'large' N bin size.

	2010 N		2010 N_b		2011 N		2011 N_b		Across years	
Variable	bin	population	bin	population	bin	population	bin	population	small N	large
pH	13.50	60.95	0	72.29	0	83.00	0	83.00	4.82	28.59
DO	0	57.60	0	57.60	0	57.59	0	57.59	0	8.28
Conductivity	0	88.80	0	88.80	41.86	51.44	20.53	71.95	10.58	8.41
Temperature	0	79.08	0	79.08	0	77.76	0	77.76	0	3.42
Width	35.54	10.39	14.45	24.11	0	44.67	0	44.67	3.67	0
Depth	0	25.04	4.27	22.48	0	33.15	0	33.15	0.17	5.84
Undercut depth	0	23.63	0	23.63	0	18.12	0	18.12	0	0
Velocity	5.46	44.69	4.02	45.75	17.16	22.46	13.04	25.53	0	0
% riparian	100	0	65.10	34.90	0	100	0	100	0	0
% vegetation	0	100	0	100	0	100	0	100	0.56	0
No.of species	72.62	27.38	0	100	21.16	78.84	0	100	1.50	0.33
% large boulder	100	0	59.24	40.76	70.65	29.35	14.05	85.95	0	4.85
% small boulder	0	100	0	100	100	0	0	100	0	0
% cobble	65.34	34.66	71.11	28.89	0	100	11.98	88.02	15.91	0
% coarse gravel	16.42	83.58	100	0	100	0	100	0	0	56.01
% fine gravel	100	0	100	0	0	100	0	100	0	5.65
% silt	50.84	49.16	0	100	7.83	92.17	0	100	0	11.97

Notes: Populations were divided into bins of 'small' N (76-1174, n=10 in 2010 and 79-1731, n=11 in 2011) and 'large' N (1683-6223, n=5 in 2010 and 2412-8416, n=8 in 2011) and 'small' N_b (15-49, n=10 in 2010 and 15-59, n=12 in 2011) and 'large' N_b (101-135, n=5 in 2010 and 93-249, n=7 in 2011) using the average N or N_b in each year as a cut off, with the exception of Cripple Cove in 2010, which was included in the 'large' size bin for consistency among years.

Table 1.4: Results of White's test for residual heteroscedasticity in habitat parameter values in relation to N and N_b in two years of sampling at Cape Race, NL.

	N			N_b		
Variable	2010	2011	Combined p	2010	2011	Combined p
pH	2.46	12.49**	†0.00206	4.02	8.26**	†0.00807
DO	3.23	2.85	0.154	10.90**	8.94**	†<0.001
Conductivity	16.54***	10.66**	†<0.001	20.21***	20.82***	†<0.001
Temperature	25.12***	17.19***	†<0.001	16.18***	18.24***	†<0.001
Width	7.84**	10.70**	<0.001	10.81**	1.45	0.0946
Depth	12.25**	15.44***	†<0.001	10.00**	12.84**	†<0.001
Undercut depth	0.75	1.26	0.622	3.50	0.84	0.458
Velocity	4.88*	11.15**	0.00139	3.41	6.64* *	0.0222
% riparian	3.22	4.28	†0.0739	3.36	0.85	0.463
% vegetation	13.53**	19.76***	†<0.001	32.21***	26.90 ***	†<0.001
No. of species	3.92	4.72*	0.0469	3.52	2.12	0.208
% large boulder	1.71	5.63*	†0.0771	1.50	1.09	0.553
% small boulder	3.94	5.20*	†0.0361	6.50**	2.24	†0.0958
% cobble	1.40	13.47**	†0.00502	1.81	2.89	0.230
% coarse gravel	2.04	2.71	0.230	21.34***	4.48	†<0.001
% fine gravel	8.43**	5.47*	†0.00748	24.08***	5.23*	†<0.001
% silt	57.30***	66.25***	†<0.001	123.78***	70.06***	†<0.001

Notes: P-values for both years of data combined (Combined p) were calculated using a z-transform test weighted by the sample size in each year. Parameters for which significant heteroscedasticity was at small N or N_b are indicated by (†).

*<0.1,**<0.05,***<0.001.

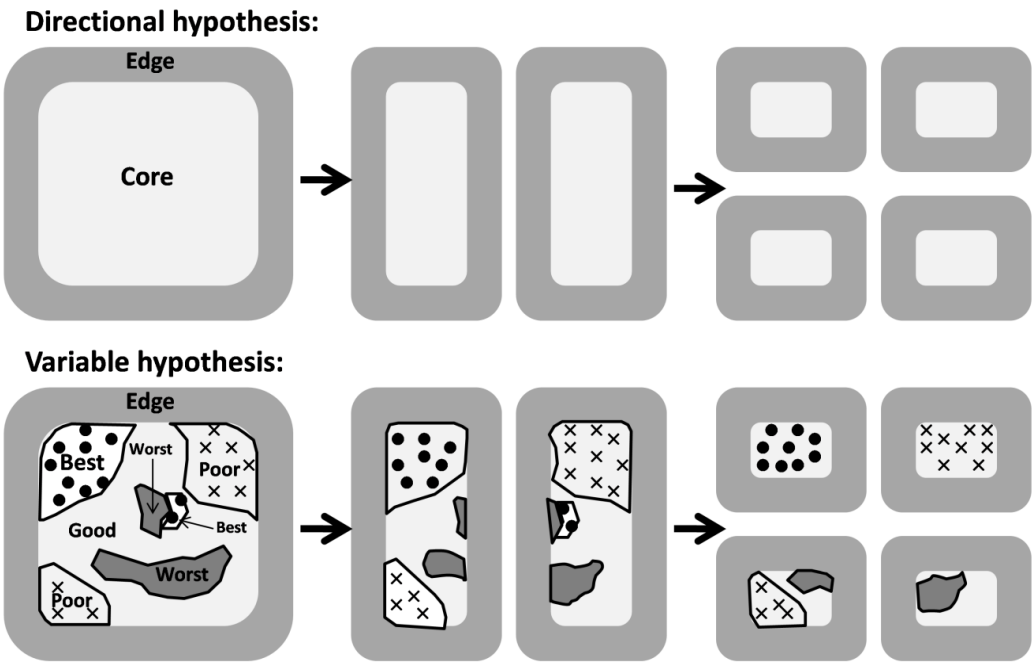

Figure 1.1: Two alternatives for the affect of habitat fragmentation on selection regimes within fragments occupied by populations of varying size. Habitat fragmentation may shift selection regimes in a consistent direction within fragments occupied by similarly sized populations if the proportion of edge habitat increases relative to core habitat as fragment size decreases (Directional Hypothesis). Alternatively, if small population fragments are random samples of larger, heterogeneous landscapes, this might result in greater variability in selection regimes among fragments occupied by similar sized populations as habitat fragmentation progresses (Variable Hypothesis).

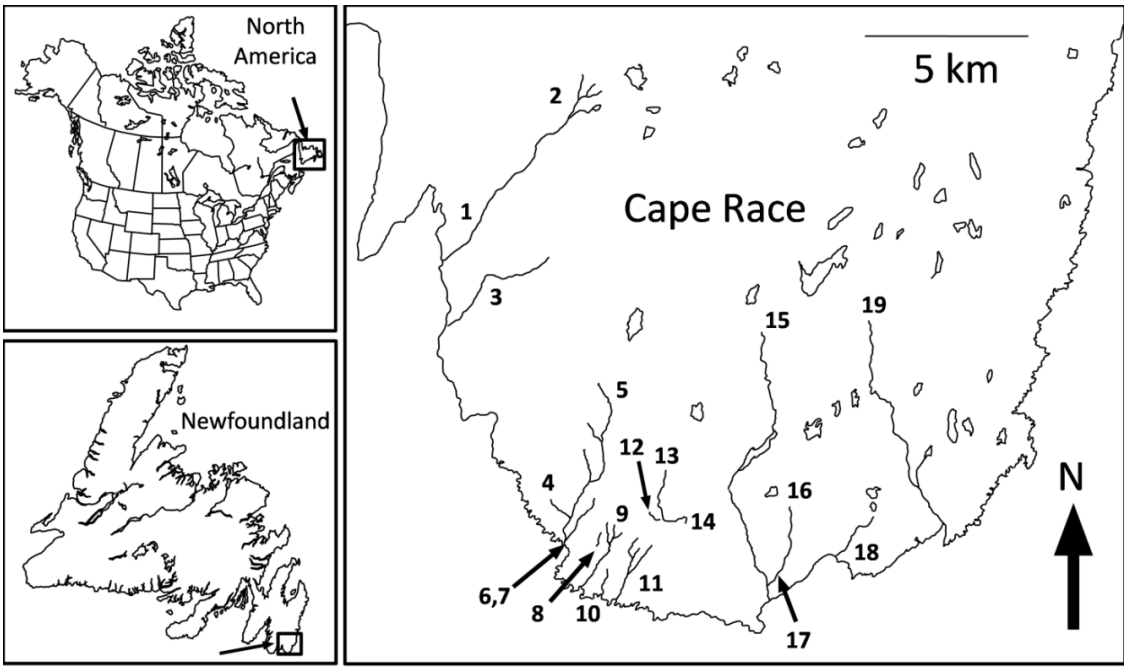

Figure 1.2: The geographic locations of study streams on Cape Race, Newfoundland. Numbers on inset correspond to streams situated from east to west: 1) Lower Whelan's, 2) Upper Whelan's, 3) Cotton 4) Perdition 5) Freshwater, 6) Lower Coquita, 7) Upper Coquita, 8) Bella's Brook,9) Bob's Cove, 10) Still There By Chance, 11) Whale Cove, 12) Ditchy, 13) Upper Ouananiche Beck, 14) Lower Ouananiche Beck, 15) Watern Cove, 16) Upper Blackfly, 17) Lower Blackfly, 18) Tannin Brook, and 19) Cripple Cove. GPS coordinates of each stream can be found in Table A1 of Ch. 1 Appendix A.

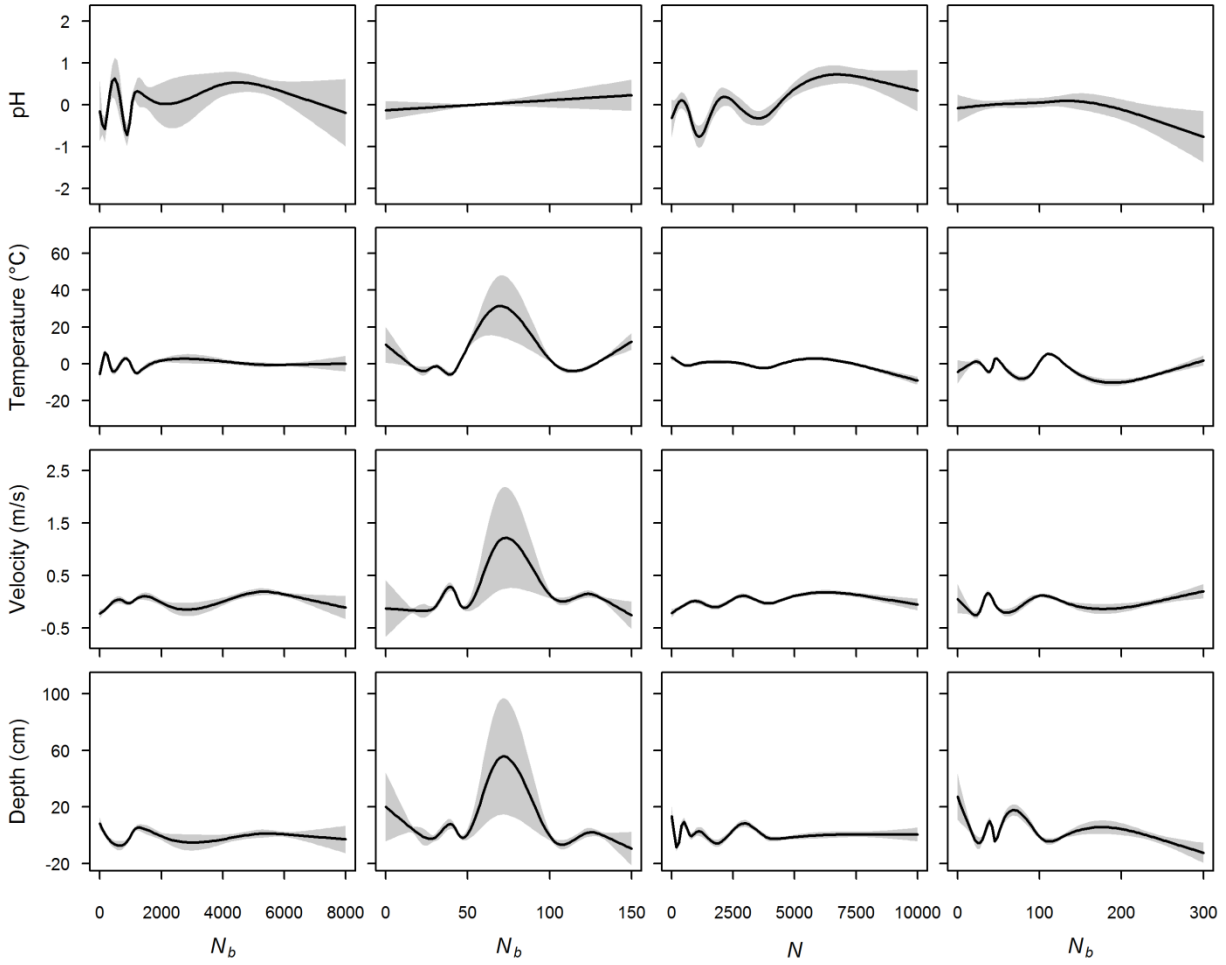

Figure 1.3: Directional hypothesis GAM plots of habitat parameters vs. N and N_b in 2010 and 2011 for 4 of 17 parameters that might be related to the fitness and abundance of trout populations at Cape Race, NL. Plots for the remaining 13 habitat parameters are found in Ch. 1 Appendix F.

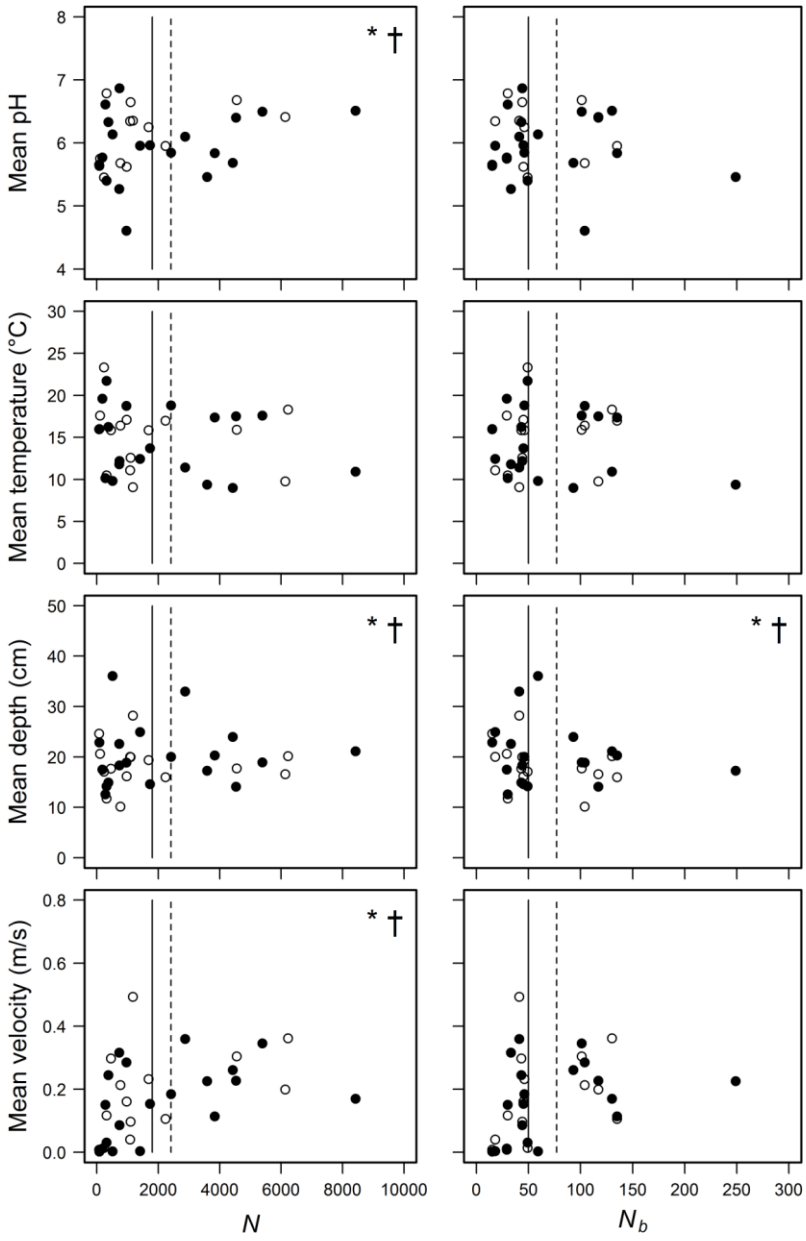

Figure 1.4: Variable hypothesis plots of habitat parameter means vs. N and N_b in 2010 (○) and 2011(●) for 4 of 17 parameters that might be related to the fitness and abundance of trout populations at Cape Race, NL. Trends for increased variability at small populations size in 2010 and 2011 are indicated by (*) and (†), respectively. Cut-offs for population size bins are represented by solid lines for 2010 and dashed lines for 2011. Plots for the remaining 13 habitat parameters are found in Ch. 1 Appendix G.

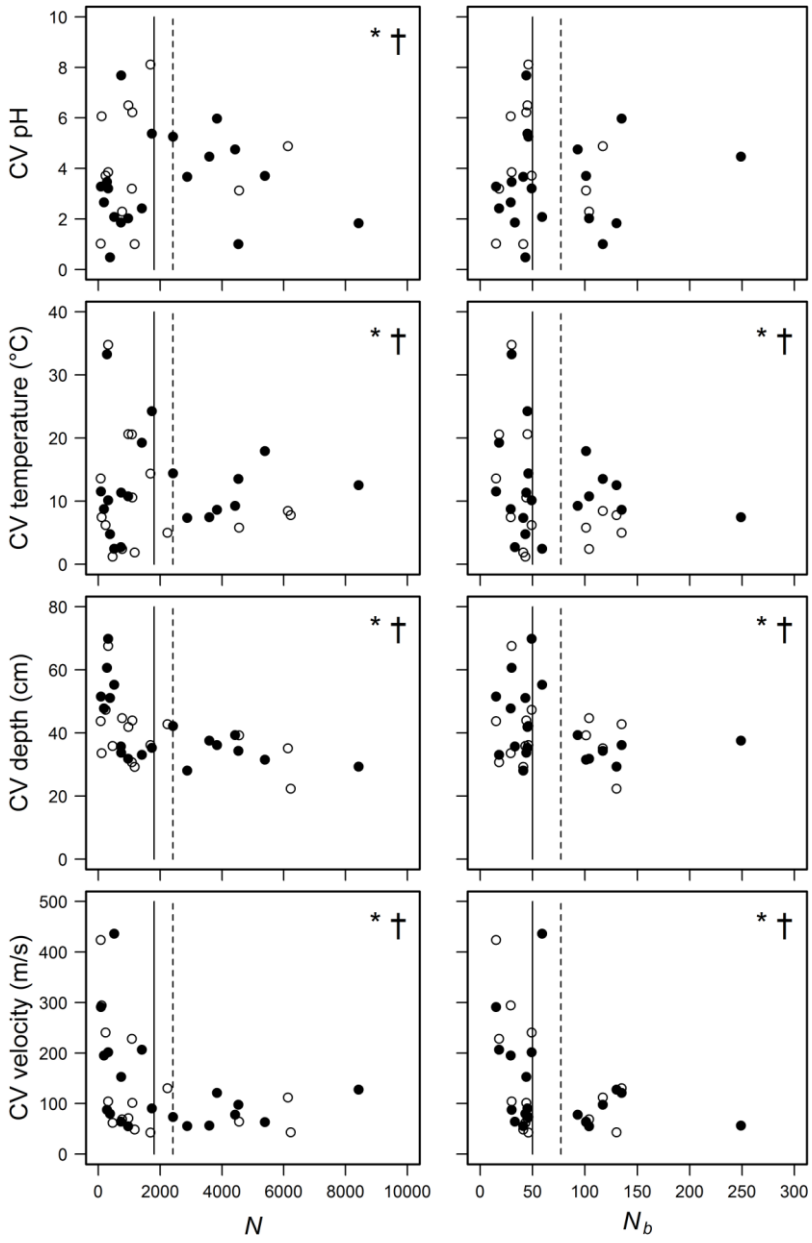

Figure 1.5: Variable hypothesis plots of habitat parameter CVs vs. N and N_b in 2010 (○) and 2011(●) for 4 of 17 parameters that might be related to the fitness and abundance of trout populations at Cape Race, NL. Trends for increased variability at small populations size in 2010 and 2011 are indicated by (*) and (†), respectively. Cut-offs for population size bins are represented by solid lines for 2010 and dashed lines for 2011. Plots for the remaining 13 habitat parameters are found in Ch. 1 Appendix H.

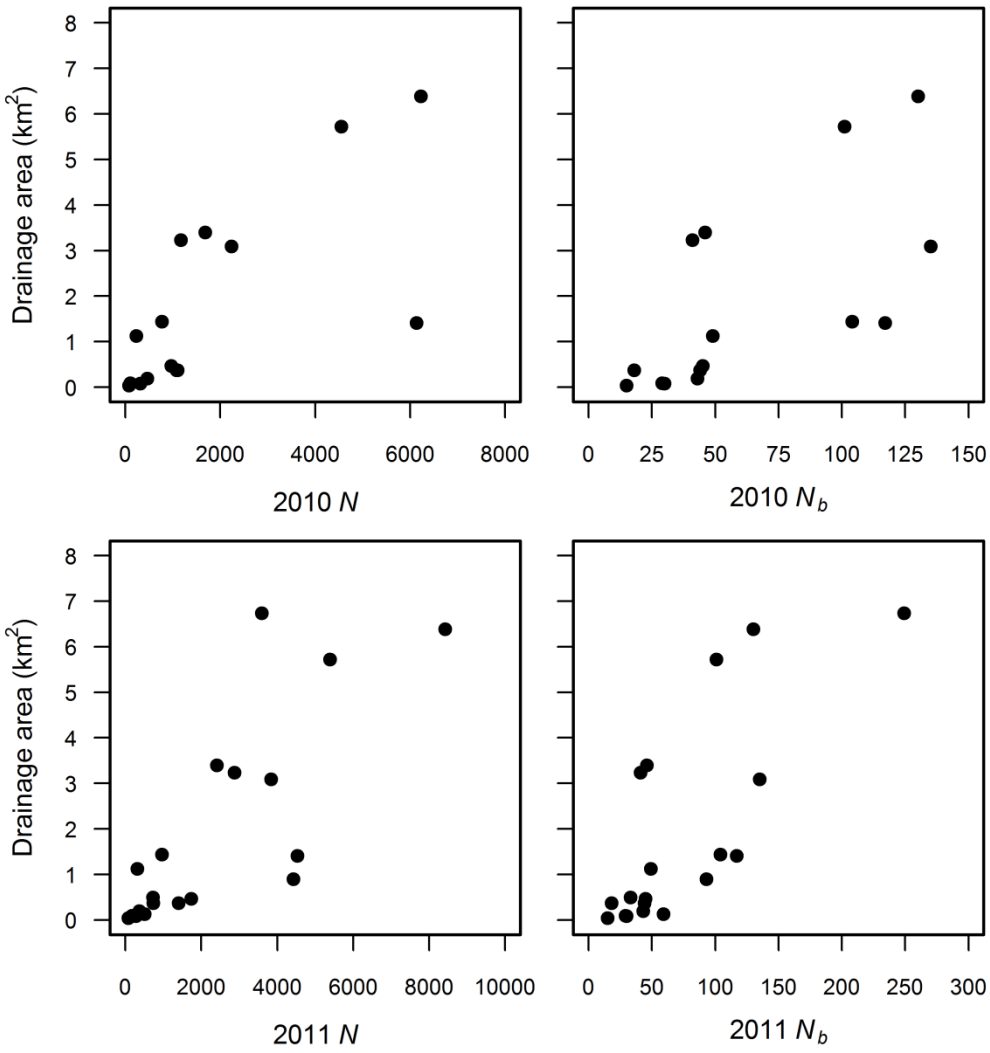

Figure 1.6: The relationship between drainage area and N and N_b in 2010 and 2011 for 19 fragmented populations of brook trout at Cape Race, Newfoundland.

Chapter 2: The extent of phenotypic plasticity to increasing temperature relative to population size in a fish

Abstract

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

Introduction

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

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

A first, "Directional hypothesis" (Willi and Hoffman 2012, Wood et al. 2014) posits that small populations might consistently exhibit reduced plasticity relative to large populations. For instance, habitat fragmentation results in populations becoming smaller, more isolated and often living under conditions that reduce recruitment (e.g. Ward and Johnson 2005). Alone or in tandem, these processes might reduce genetic diversity underpinning phenotypic trait plasticity due to restricted gene flow, drift, inbreeding, and/or overall increased environmental stress (Ouborg et al. 2010).

A second, opposing directional hypothesis is that small populations might exhibit consistently greater plasticity relative to large populations. Fragmentation into smaller habitats is often associated with increasing environmental variation (Marshall and Jain 1968). Therefore, although some small populations may have low genetic variation due to drift, fragmentation might also favour high levels of plasticity at key traits to cope with environmental fluctuations (van Kleunan et al. 2000, Paschke et al. 2003), or plasticity might become canalized, resulting in low additive genetic variation but high plasticity. Indeed, some very small founder populations have been shown to exhibit rapid plastic responses to novel environments (Haugen 2000).

A third, "Variable hypothesis" is that habitat characteristics and resulting natural selection pressures – and by extension, phenotypic plasticity – become increasingly variable as habitat fragment size and population size decrease. Indeed, there is evidence that smaller population fragments can be simply random samples of larger fragments (Connor and McCoy 1979, Berteaux et al. 2004). In this study species, the brook trout (*Salvelinus fontinalis*), the fragmentation process is known to increase habitat variability

within and among habitats as population size decreases (Wood et al. 2014), perhaps resulting in a greater diversity of selective pressures and plastic responses.

To distinguish between these hypotheses, common-garden experimentation of temperature plasticity was conducted on eight, differentially-abundant stream brook trout populations from Cape Race (CR), Newfoundland. In fishes, temperature is crucial for controlling metabolism and other life-history traits (Beacham and Murray 1985), and temperature has been found to be highly variable both within and among CR streams (Wood et al. 2014). Furthermore, CR trout populations may currently be experiencing climate warming as the mean annual air temperature has increased by more than one degree Celsius over the past 100 years (Environment Canada 2012); temperature plasticity might thus play an important role in future population persistence (Shuter and Post 1990).

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

intraspecific populations (Palstra and Fraser 2012) and it is N_b that is associated with selection and its effects on the evolution of plasticity.

Materials and Methods

Study site

Cape Race is a region of coastal barren land traversed by a parallel series of low-order streams, many of which harbour resident brook trout populations. The small size of CR streams (0.27-8.10km) permits comprehensive sampling and accurate and precise estimation of N and N_b. The populations are isolated and genetically distinct (Wood et al. 2014) and likely diverged from a common ancestor during the late-Wisconsinan glaciation (10-12000 ybp; Danzmann et al. 1998). CR populations also exhibit considerable differences in life histories likely due to changes to selective regimes following habitat fragmentation (Belmar-Lucero et al. 2012).

Gamete collection

From mid to late October 2011, eight CR populations were monitored for breeding individuals via electrofishing downstream and upstream of known spawning areas. The populations were Whale Cove (WC), Cripple Cove (CC), Watern Cove (WN), Lower Blackfly (BF), Upper Ouananiche Beck (UO), Freshwater River (FW), Ditchy (DY), and Still There By Chance (STBC) (For a map of population locations, see Wood et al. 2014). Spawning sites were easily recognizable by dense aggregations of sexually mature trout and excavated redds. Breeding adults were gathered and placed in flow-through cages within the stream channel until gamete collection took place, between

21h00 and 2h00 of the same evening. Eggs were collected in 60 ml opaque plastic containers while sperm was collected in 1.5ml microcentrifuge tubes on ice. Gametes were transported directly from CR to St. John's in refrigerated coolers then shipped to Montreal by air such that total transit time was approximately 10 hours from the start of gamete collection. Prior to subsequent fertilization, diameters of 10 randomly selected eggs were measured for each female using digital photographs. Gametes were transported in three separate shipments, on Oct 19th, 24th, and 29th; for most populations, all gametes were collected and transported on the same shipment date, but for three populations, (WN, STBC, and CC) gametes were shipped on two different dates.

Common garden experimental design

Trait plasticity in CR populations was investigated in relation to three temperature treatments; one temperature which mimicked naturally occurring temperatures during the incubation and early feeding phases for the eight CR populations (5.0 ± 0.2 SD °C; hereafter the cold regime), a medium temperature that likely represents a climate change scenario for some CR populations (7.0 ± 0.3 °C; medium regime) and one incubation temperature that could be experienced under more extreme climate warming in the future (9.2 ± 0.3 °C; warm regime) (IPCC 2007; see Ch. 2 Appendix A). Fertilization took place 10 to 14 hours after gamete collection, with eggs from each female being mixed with equal volumes of sperm from one male, yielding a total of 134 full-sib families or a mean of 18.2 families per population (range=6-29). CR females are small in size (mean length = 138.3 ± 28.6mm) and have low fecundity (mean egg number = 82.8 ± 53.9 SD). Therefore, fertilized eggs were divided into three equal lots of 20.0 ± 8.0 SD (range = 3-

50) which were incubated at the family level by being placed in 5.2 cm diameter individual egg containers within three 1000L recirculating tanks. Egg containers were fitted with a mesh bottom to allow water circulation; pH was 6.9 ± 0.3 and dissolved oxygen was maintained at saturation throughout the experiment. Family placement among populations was assigned randomly within the first tank with families occupying the same location in the remaining tanks to minimize any effect of tank location on plasticity. To reduce potential mortality following fertilization, eggs were left undisturbed until they had reached the eyed stage, at which point dead individuals were counted and then removed daily.

Six early-life history traits related to individual fitness of salmonids under natural conditions were measured (Einum and Fleming 2000) and used to create reaction norms for each population over the three temperature treatments. (i) Hatch time was estimated as accumulated degree days from fertilization to hatch of all individuals within families. Once hatching began, numbers of hatched individuals in each family were counted at intervals that yielded the same number of accumulated degree days across all tanks; every 8 hours for the warm treatment, every 10 hours for the medium treatment and every 12 hours for the cold treatment. Hatch times were converted to degree-days by summing the mean daily incubation temperatures over development (Beacham and Murray 1985). (ii) Length at hatch (tip of the snout to the tip of the median rays of the tail; Koskinen et al. 2002) and (iii) yolk sac volume at hatch (estimated as $LH^2(\pi/6)$, where L and H were the length and height of the yolk sac, respectively; Koskinen et al. 2002) for each individual were measured by taking a standardized digital photograph using a mounted overhead camera. Photos were then imported into the program IMAGEJ (Rasband 2011) and traits

were measured against a known size standard. Once yolk sacs had been absorbed, (iv) emergence length (when the yolk sac is 'buttoned-up' into the body cavity; Beacham and Murray 1985) was measured for each individual similarly to length-at-hatch using IMAGEJ. (v) Yolk sac conversion efficiencies ((length at yolk absorption – length at hatch)/yolk sac volume) were then calculated using the family means in each population.

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

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

Multi-year estimates of population size for each population were estimated in a previous study based on N (two consecutive years) and N_b (three consecutive cohorts except for two in DY; Wood et al. 2014). These estimates included the same year (2011) in which this study's gametes were collected (Ch. 2 Appendix B, Table B1). Either the Schnabel (1938) or Petersen (1896) method was used to estimate annual N; as a surrogate for generational N_e the harmonic mean of N_b for three cohorts was used (2009-2011

estimated using LDNe; Waples and Do 2008) weighted by the number of individuals sampled. Because generational N_e calculated for the five CR populations for which detailed life history data was available was strongly correlated with the weighted harmonic mean N_b (Waples et al. 2013; see Ch. 2 Appendix C, Fig. C1) N_b was used for all analyses. For additional details on sampling and calculating N and N_b for Cape Race streams see Wood et al. (2014).

Statistical analysis

Population size and mean trait values

The effects of population size (either N or N_b) and temperature as well as the interactions between these factors on each life-history trait were examined by generalized linear mixed models (GLMMs) using the lme4 package (Bates et al. 2012) of R version 2.14.1 (R Development Core Team 2011). GLMMs were used since two of six of the life history traits analyzed required non-normal error distributions, and to model random effects. A total of 6 GLMMs (one for each life history trait) were run for each of the two population size metrics. Population size and temperature were treated as fixed effects with a random effect defined by family nested within population. Egg size and family size were included as additional fixed effects in order to investigate potential maternal effects and because family size differed across CR populations. Data were fitted with a normal error distribution, except for proportion data for yolk-sac conversion efficiency and survival for which a binomial distribution was specified. In a preliminary analysis, shipment date was also tested but it had no effect on any of the traits (Ch. 2 Appendix D) and was therefore omitted from further analyses.

Directional hypothesis: magnitude of plasticity

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

Variable hypothesis: variability in plasticity

To test whether plastic responses were more variable at small relative to large population sizes, information on both the magnitude and directionality of the within-population family slopes from the 5-7, 7-9, and 5-9 temperature treatments were included. First, the residual variance of the slopes were used as response variables in GLMMs in order to test for significant effects of egg size, family size, and interactions of these two covariates with N and N_b (i.e. 18 GLMMs for each population size metric, calculated as above). White's tests (White 1980) were then used to establish whether the residual variance of the slopes for each temperature treatment against N and N_b was

constant or exhibited heteroscedasticity, with the prediction that there would be increased variability in the residuals with decreasing population size.

Results

Population size and mean trait values

Trait plasticity in relation to temperature (i.e. non-zero slopes of the reaction norms) was evident in many cases based on statistically significant main effects of temperature regime on life history expression, but no significant main effect of population size (N or N_b) on any of the traits examined was detected (Table 2.1 and Ch. 2 Appendix E). For example, relative to the warm temperature treatment, the cold treatment generally resulted in significantly longer hatch times, longer lengths at hatch and emergence, and reduced yolk-sac volume. The medium temperature treatment was also associated with significantly longer hatch time compared with the warm treatment, increased length at emergence for both N and N_b models, as well as greater hatch length, but only for models using N_b. There was no significant main effect of temperature on either yolk sac conversion efficiency or survival (Fig. 2.1; Table 2.1 and Appendix E).

Although there were population differences in mean trait values for the different temperature treatments (Table 2.1, Fig. 2.1, and Appendix E) across all traits, significant interactions between temperature and population size were detected in only 3 of 6 models with N_b and 4 of 6 models with N. The cold treatment × population size interaction reduced emergence length for both N and N_b models but significantly reduced hatch length and hatch time only with N. The medium regime × population size interaction

significantly increased emergence length and decreased yolk volume for N_b models, while the same interaction led to reduced hatch length for models using N.

Not surprisingly for a salmonid fish, egg size was associated with significantly increased hatch length, yolk-sac volume, and length at emergence regardless of whether N or N_b was used in the model. However, there was rarely a main effect of family size, and only 10 of 36 interactions involving temperature regime × family size (cold or medium treatment × family size) or population size × family size were significant across the 12 N and N_b models; these showed no consistent trend in the effect of family size on the different life history traits.

Directional Hypothesis: magnitude of plasticity

There was very little indication from GLMMs that the magnitude of plasticity differed significantly in relation to population size; small populations neither exhibited consistently greater or consistently lower plasticity relative to large populations (Fig. 2.2, Table 2.2; see Ch. 2 Appendix F, Fig. F1 and Tables F1-F3 for detailed results of all traits analyzed). Exceptions were for yolk-sac conversion efficiency (3 of 36 total models) for which the population size × family size interaction had a significant negative effect on the slopes from 5-7 degrees for both population size measures and a positive effect on the 7-9 degree slopes for models using N_b, and hatch time (1 of 36 models) for which N_b × family size had a negative effect on the slopes from 5-7 degrees. Similarly, egg size and family size had little effect on the strength of plasticity across traits; there was a main effect of egg size in only 5 of 36 total models across both population size metrics, and a main effect of family size in only 4 of 36 models (Table 2.2, Appendix F, Tables F2 and F3).

Moreover, Spearman's correlations for the relationship between the absolute values of slopes and either population size measure were not significant for any life-history trait, except yolk-sac conversion efficiency which showed a negative correlation with *N* for the slopes from the 5-7 degree temperature treatments (Appendix F, Table F1).

Variable Hypothesis: variability in plasticity

The degree of variability in plasticity also was not influenced by population size (*N, N_b*): plastic trait responses were not more variable among small populations than large populations (Fig. 2.3 and Ch. 2 Appendix G, Fig. G1). The few exceptions (8 of 36 total models) were main effects of (i) family size on the 7-9 degree slopes for yolk-sac conversion efficiency and (ii) egg size, which aside from one instance, had a positive effect on residual variance of family slopes for several life-history traits for both *N* and N_b (Table 2.3 and Appendix G, Tables G1, G2). Furthermore, only 2 of 36 and 1 of 36 White's tests that examined residual variance of family slopes for each trait relative to *N* and N_b, respectively, had significant heteroscedasticity to signal a difference in variability of slopes in relation to population size. Examination of the residual plots showed that in only one of these significant cases was the increased residual variance at small population sizes (*N*: hatch date, 5-9 degree slopes; Appendix G, Table G3).

Discussion

There was almost no evidence for differences in phenotypic plasticity in relation to population size among Cape Race brook trout populations despite a nearly 50-fold difference in *N* (179-8416), and a 10-fold difference in N_b (18-135). This result is

particularly notable given the large number of families and populations used in comparison to analogous vertebrate studies (Haugen and Vollestad 2000, Jensen et al. 2008) and that one of the incubation temperatures (9°C) represented an extreme condition that would not be experienced by these populations in a natural setting (Power 1980, Appendix A). In regards to the Directional Hypothesis, there was no evidence that the magnitude of plasticity was related to population size. Specifically, (i) small populations did not exhibit either consistently greater or consistently reduced plasticity relative to large populations, (ii) correlations between population size and the absolute values of the slopes between the three temperature regimes were not significant for almost all traits, and (iii) only 3 of 32 total models across the six studied traits revealed a significant interaction effect with population size. Similarly, there was no evidence that small populations might express a greater variety of plastic responses than large populations (Variable Hypothesis): only 1 of 32 White's tests exhibited significant heteroscedasticity of slope residuals at small population size, there were no significant main effects of population size, and in only one instance did a significant interaction involve population size.

While there was no main effect of population size on mean trait values, there were significant interactions between temperature and population size for about half of the comparisons, indicating differences in the way different sized populations altered their mean phenotype with changing temperature. Individuals from larger populations tended to have the largest body size at early stages of development and larger yolk-sac volumes at hatch. This result is not unexpected because trout from more abundant Cape Race populations also come from larger, deeper streams where space may not be limiting, and

where females from large populations also have larger egg sizes. Conversely, smaller populations had, on average, higher yolk-sac conversion efficiencies and also higher survival across all temperatures. Small Cape Race populations, therefore, might be capable of maintaining their fitness under suboptimal or potentially even the more extreme temperature conditions expected under future climate change.

A previous study at Cape Race found evidence for increased among-population spatial variability in habitat parameters at small population size, a sign that small populations might be subject to a greater diversity of selective pressures (Wood et al. 2014). Correspondingly in this study, small populations on average exhibited greater CVs for mean trait values for 4 of 6 life history traits than did large populations. Mean plastic responses did not differ between small versus large populations, but long term data on environmental conditions is currently unavailable for Cape Race streams. Temporal variability in environmental conditions might be generally higher among small versus large populations at Cape Race, even though there is evidence for more variability in the types of habitats occupied by small populations spatially. Therefore, one possible explanation for the lack of difference in the plastic responses of populations of varying size is that plasticity at key traits might be favoured among small populations to cope with increased temporal environmental variability (van Kleunan et al. 2000, Paschke et al. 2003), but in large populations that occupy large habitats with greater within-habitat spatial environmental heterogeneity, genotypes with varying patterns of plastic response may occur simultaneously (Sultan 1995).

Plasticity was compared across populations in relation to both N and N_b. We might have expected stronger relationships with N_b vs N since N_b represents the

proportion of individuals in the population that are contributing to the next generation, and this will ultimately be dictated by the specific features of each habitat. There was no support for this expectation, as very few significant relationships for plasticity with either population size measure was found.

Possible caveats

Plasticity was compared among different sized populations for early life-history traits. Traits associated with adult phenology could be equally important for responding to climate variability through their effect on embryonic traits (Hebert et al. 1998; Bradshaw and Holzapfel 2008). This possibility could not be investigated due to the logistical constraints of rearing large numbers of salmonids to later life stages. That said, the traits investigated here are associated with fitness in salmonids at a life stage that has a critical impact on recruitment, since mortality to the early fry stage is usually very high (Einum and Fleming 2000). Such traits are therefore expected to be important for the persistence of these populations.

It should be acknowledged that temperature likely interacts with other habitat characters in a natural setting to generate more stressful conditions than would be experienced in most common garden laboratory experiments. Using three constant temperatures treatments, for example, did not account for potential fluctuations in temperature over the incubation period in Cape Race streams (Fig. A1). Nevertheless, this variability would be difficult to incorporate into a common garden experiment as CR populations likely all experience somewhat different temperature regimes in nature. In this study, I have attempted to choose an incubation temperature that is likely

experienced by all populations (5°C), one that might be high for many populations (7°C), and one that would be considered extreme for Cape Race trout (9°C, Appendix A).

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

The magnitude and extent of plasticity did not differ between small and large CR populations, but one important unanswered question is whether the plasticity that was observed is adaptive. Plasticity is maladaptive if it reduces fitness in a novel environment (Ghalambor et al. 2007; Crispo et al. 2010) for example, if smaller body size at higher temperatures is maladaptive, then plasticity might actually decrease a population's ability to persist under climate change. Data regarding the fitness consequences of plasticity are unavailable for the CR populations in this study, however because plastic responses were in the same direction across all population sizes, it suggests that even if plasticity in this study is not adaptive that increasing temperature at least impacted the small and large populations in a similar manner.

Finally, one question is whether this study had sufficient statistical power to reject the null hypothesis of no plasticity differences among populations. A power analysis revealed that here, the capacity to detect Type II error was low. However, generating larger numbers of families in the small CR populations was not possible for ethical reasons, and the low fecundity of females precluded the generation of larger numbers of families in large populations (despite the large numbers of males and females sampled). It seems likely that low statistical power is typical for common garden studies of vertebrates considering that this study used a larger number of populations and mean number of families per population than most analogous studies involving fish (see Hutchings 2011).

Conclusions

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

The results of this study furthermore suggest that, encouragingly, some small populations may have the ability to respond plastically to climate change even at extremely low population sizes. For example, five of the CR populations have N_b of less than 50 and at least one (DY) likely also has an effective population size of less than 50. These values are frequently cited as critical minimum sizes below which populations are predicted to suffer disproportionately higher reductions in fitness owing to inbreeding

depression and also experience more rapid reductions in genetic diversity required for adaptive evolution (Franklin 1980). However, some caution is warranted in applying the results of this study to other taxa. Brook trout are a generalist, colonizing species and exhibit residual tetraploidy (Allendorf and Thorgaard 1984) which might allow them to deal with small population size more effectively than other species. Certainly, not all populations that become small will be able to adapt to climate change. Nevertheless, to demonstrate similar plasticity in relation to population size in this case is important given the scarcity of such research on salmonids, a socio-economically important group of fish species, and vertebrates in general. With climate change occurring so rapidly, phenotypic plasticity rather than adaptive evolution may be the quickest way that populations will deal with future environmental change.

Table 2.1: GLMM Regression coefficients (±SE) to evaluate the effect of temperature treatment, N_b, egg size, family size, and interactions on trait mean values for two of six early life-history traits for eight Cape Race trout populations. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets. Results for remaining traits and for models with N are found in Ch. 2 Appendix E.

Fixed effects	Emergence length (mm)	Survival
Cold treatment	0.5(0.2)**	0.22 (1.20)
Medium treatment	0.6(0.2)***	1.34 (1.21)
N_b	-0.01(0.01)	-7.0×10^{-3} (0.014)
Egg size	0.1(0.05)*	0.075 (0.076)
Family size	0.2(0.1)	0.19 (0.80)
Cold treatment × N_b	$-2\times10^{-3}(7\times10^{-4})$**	-1.3×10^{-3} (5.7×10^{-3})
Medium treatment × N_b	$2\times10^{-3}(7\times10^{-4})$**	-3.4×10^{-3} (5.6×10^{-3})
Cold treatment × egg size	$0.05(8\times10^{-3})$***	-0.021 (0.061)
Medium treatment × egg size	$0.05(8\times10^{-3})$***	-0.068 (0.061)
N_b × egg size	$1\times10^{-4}(5\times10^{-4})$*	-6.1×10^{-4} (7.5×10^{-4})
Cold treatment × family size	0.2(0.09)	0.41 (0.73)
Medium treatment × family size	-0.2(0.08)	0.11 (0.71)
N_b × family size	$-3\times10^{-3}(1\times10^{-3})$*	-2.7×10^{-3} (7.9×10^{-3})
Random effects		
Family	0.4(0.3)	0.9(0.8)
Stream	0.3(0.2)	0.2(0.2)

*<0.05, **<0.01, ***<0.001

Table 2.2: GLMM regression coefficients (±SE) for the effect of N_b, egg size, family size, and interactions on magnitude of plasticity for 5-7, 7-9, and 5-9°C treatments for two of six early life-history traits at Cape Race. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets. Results for remaining traits and for models with N are found in Ch. 2 Appendix F, Tables F2 and F3.

	Emergence length			Survival		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
N_b	$4\times10^{-3}(4\times10^{-3})$	$-3\times10^{-3}(5\times10^{-3})$	$-4\times10^{-3}(3\times10^{-3})$	$-6\times10^{-4}(7\times10^{-4})$	$-1\times10^{-3}(8\times10^{-4})$	$-2\times10^{-4}(6\times10^{-4})$
Egg size	0.02(0.02)	0.02(0.02)	-7×10^{-5}(0.01)	$4\times10^{-3}(3\times10^{-3})$	$-2\times10^{-3}(4\times10^{-3})$	$-1\times10^{-3}(3\times10^{-3})$
Family size	0.09(0.2)	-0.2(0.3)	-7×10^{-4}(0.1)	-0.04(0.04)	-0.05(0.04)	-0.02(0.04)
N_b × egg size	$-2\times10^{-4}(2\times10^{-4})$	$-9\times10^{-6}(2\times10^{-4})$	$2\times10^{-4}(1\times10^{-4})$	$1\times10^{-5}(4\times10^{-5})$	$4\times10^{-5}(4\times10^{-5})$	$3\times10^{-5}(3\times10^{-5})$
N_b× family size	$-1\times10^{-3}(2\times10^{-3})$	$3\times10^{-3}(3\times10^{-3})$	$-8\times10^{-5}(1\times10^{-3})$	$4\times10^{-4}(4\times10^{-4})$	$5\times10^{-4}(4\times10^{-4})$	$-8\times10^{-5}(4\times10^{-4})$
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	0.09(0.2)	0.0(0.0)	0.04(0.2)	$2\times10^{-7}(2\times10^{-6})$	0.02(0.2)	0.0(0.0)

*<0.05, **<0.01, ***<0.001

Table 2.3: GLMM regression coefficients (±SE) for the effect of N_b, egg size, family size, and interactions on variability of plasticity for 5-7, 7-9, and 5-9°C treatments for two of six early life-history traits at Cape Race. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets. Results for remaining traits and for models with N are found in Ch. 2 Appendix G, Tables G1 and G2.

	Emergence length			Survival		
Fixed effects	5-7°C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
N_b	$7\times10^{-3}(6\times10^{-3})$	$2\times10^{-3}(5\times10^{-3})$	$4\times10^{-3}(3\times10^{-3})$	$-4\times10^{-4}(1\times10^{-3})$	$1\times10^{-3}(1\times10^{-3})$	$1\times10^{-3}(9\times10^{-4})$
Egg size	0.04(0.02)	-0.02(0.02)	-4×10^{-3}(0.01)	$-8\times10^{-3}(5\times10^{-3})$	0.01(5×10^{-3})*	$6\times10^{-3}(4.0\times10^{-3})$
Family size	-0.2(0.3)	0.2(0.3)	-0.02(0.2)	0.03(0.06)	-0.03(0.06)	-7×10^{-3}(0.05)
N_b × egg size	$-4\times10^{-4}(3\times10^{-4})$	$4\times10^{-5}(3\times10^{-4})$	$-2\times10^{-4}(2\times10^{-4})$	$-3\times10^{-5}(5\times10^{-5})$	$-6\times10^{-5}(6\times10^{-5})$	$-4\times10^{-5}(5\times10^{-5})$
N_b × family size	$9\times10^{-4}(3\times10^{-3})$	$-3\times10^{-3}(3\times10^{-3})$	$-1\times10^{-3}(2\times10^{-3})$	$-5\times10^{-4}(6\times10^{-4})$	$2\times10^{-4}(6\times10^{-4})$	$-3\times10^{-4}(6\times10^{-4})$
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	0.1(0.2)	0.0(0.0)	$1\times10^{-5}(1\times10^{-6})$	0.03(0.2)	0.04(0.3)	1×10^{-7} (8×10^{-6})

*<0.05, **<0.01, ***<0.001

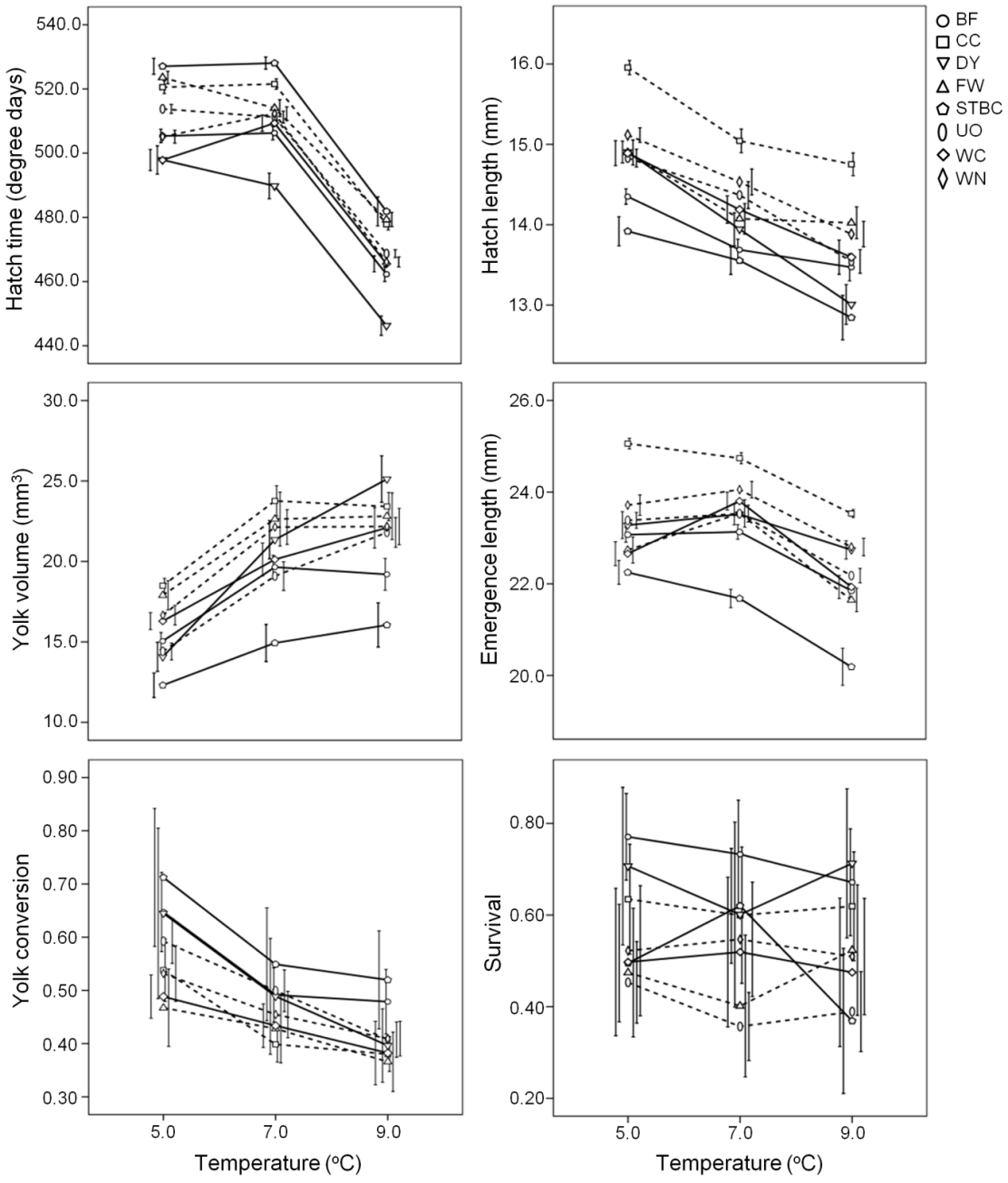

Figure 2.1: Reaction norms to assess phenotypic plasticity in six early life-history traits across three different temperature treatments for eight Cape Race brook trout populations. Small Cape Race populations (N = 179-1731) are denoted by the solid lines, with large populations (N = 2412-8416) as dashed lines.

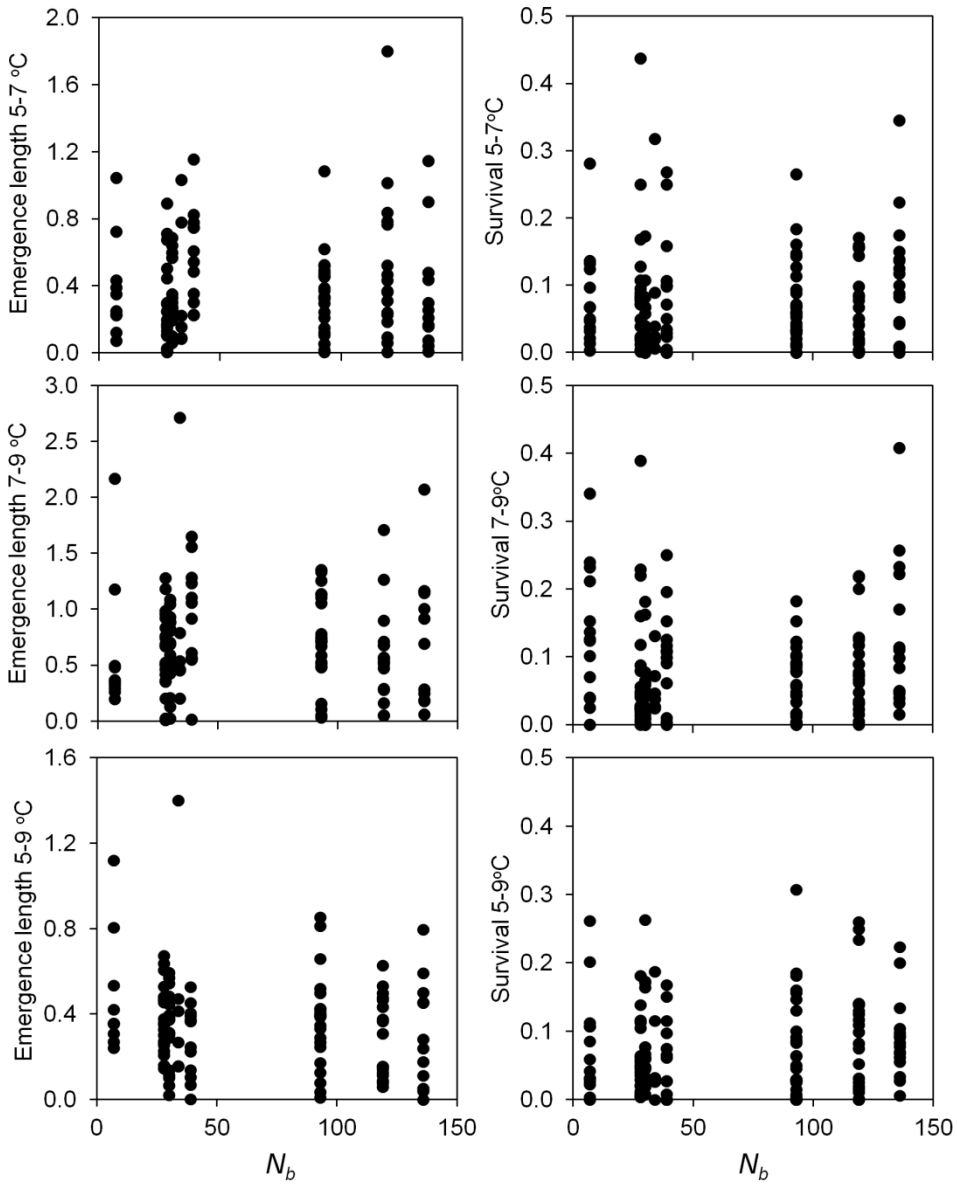

Figure 2.2: Directional Hypothesis: Absolute values of slopes to assess the magnitude of plasticity between three temperature treatments in relation to N_b for two of six early life-history traits in relation to population size for eight book trout populations at Cape Race, NL. Plots for the remaining four traits, and for the six traits in relation to N are found in Ch. 2 Appendix F, Fig. F1.

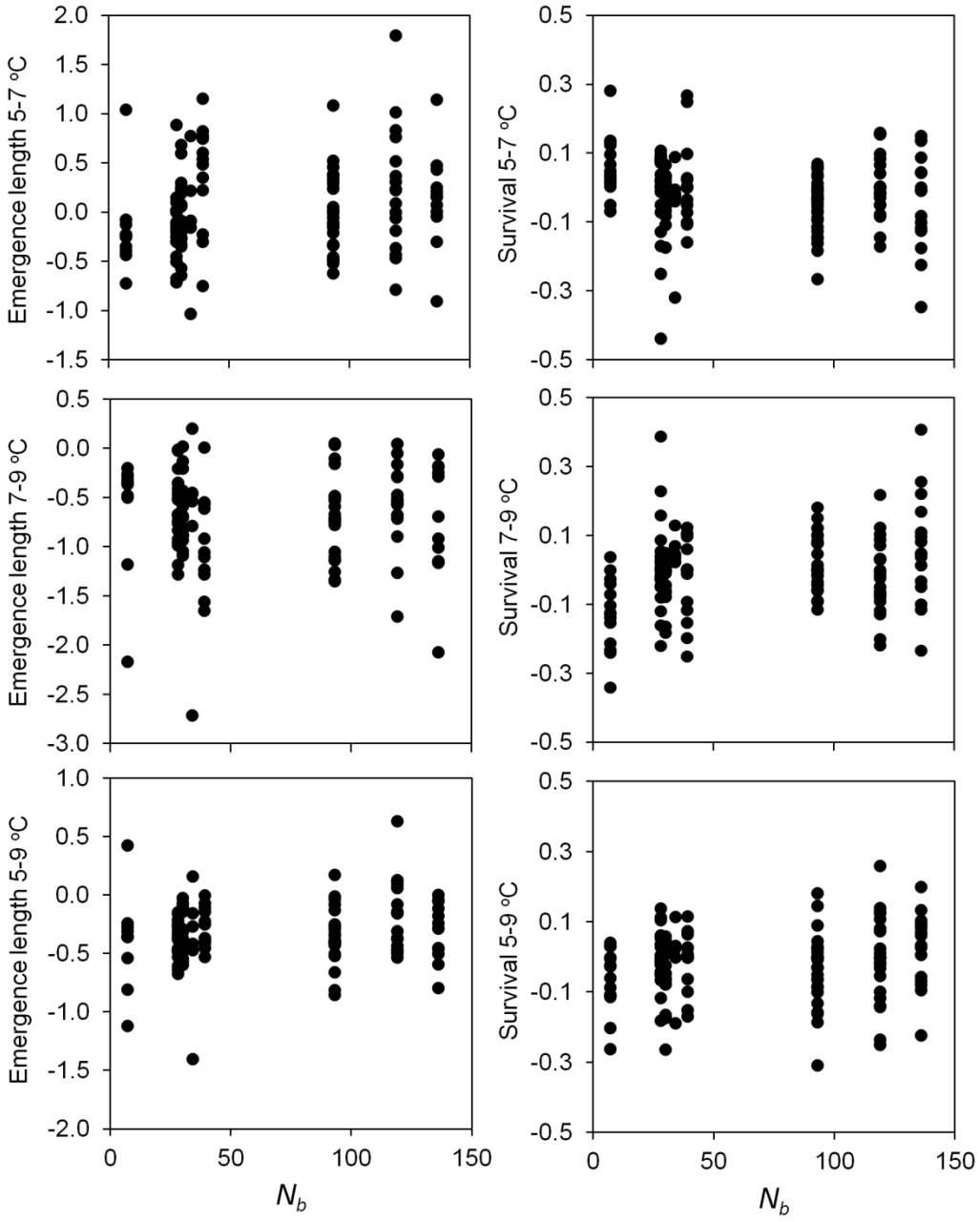

Fig. 2.3: Variable Hypothesis: Values of slopes to assess the variability of plasticity between three temperature treatments in relation to N_b for two of six early life-history traits in relation to population size for eight book trout populations at Cape Race, NL. Plots for the remaining four traits, and for the six traits in relation to N are found in Ch. 2 Appendix G, Fig. G1.

Chapter 3: Population size is weakly related to quantitative genetic variation and differentiation in a stream fish

Abstract

How population size might influence quantitative genetic variation and differentiation among natural populations remains largely unresolved. If small population habitats are generally poor in quality and if genetic drift erodes genetic variation and overcomes selection as population size is reduced, then small populations may harbor consistently reduced additive genetic variation (V_A) relative to large populations and metrics of pairwise genetic differentiation (quantitative trait differentiation, Q_{ST}, and neutral genetic differentiation, F_{ST}) might exhibit consistent directional changes from small to large population size. Alternatively, small populations might exhibit larger variation in V_A and greater differentiation if habitat fragmentation increases variability in habitat types. I explored these alternatives by investigating V_A, Q_{ST}, and F_{ST} in a common garden experiment using nine fragmented populations of brook trout varying nearly 50-fold in census size N (179-8416) and 10-fold in effective number of breeders, N_b (18-135). Across 21 early life history, morphological, and behavioural traits, no evidence was found for consistent differences in V_A and Q_{ST} in relation to population size and almost no evidence for increased variability of V_A, Q_{ST} or F_{ST} estimates at small population size. The finding of similar quantitative genetic variation and Q_{ST} between small and large populations suggests that small populations of some species may retain the ability to respond to environmental change via adaptive evolution and also that selection can potentially overcome genetic drift even at very small population size.

Introduction

The expectation that small populations of species will have a reduced capacity to respond to environmental change relative to large populations is based on the common assumption that genetic variation is positively related to adaptive potential (Lande 1988; Reed and Frankham 2003). Conventionally, populations that have become small and isolated due to habitat fragmentation lose genetic variation through genetic drift more rapidly than large populations (Ellstrand and Elam 1993; Frankham 1996; Spielman et al. 2004) and this will result in a decreased ability to respond to environmental change (Willi et al. 2006). One potentially important factor that is rarely considered however, is how habitat fragmentation might alter habitat conditions and hence also the selective pressures and adaptive genetic characteristics of fragmented populations (Willi et al. 2007; Willi and Hoffman 2012; Wood et al. 2014).

While theoretical models predict a positive correlation between genetic diversity and population size (Willi et al. 2006), the actual relationship in nature remains largely unresolved. I suggest that this is mainly because previous attempts to empirically investigate how genetic diversity relates to population size have been inhibited by several methodological issues. For instance, previous studies either compared only a very small number of populations (Widen and Andersson 1993; Waldmann 2001), or used neutral marker diversity as a surrogate for quantitative genetic variation even though the relationship between these metrics is weak (Reed and Frankham 2001). Other studies examined genetic diversity in relation to the census population size (N) instead of the effective population size (N_e) (Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001), when it is N_e that represents the proportion of individuals contributing

genetically to the next generation and that consequently dictates rates of genetic drift and inbreeding. Moreover, N and N_e are frequently assumed to be correlated (Willi et al. 2007), but N_e/N ratios can vary widely among populations of closely related species, which may lead to erroneous conclusions when using N to infer the magnitude of N_e or vice versa (Palstra and Fraser 2012). Finally, empirical research in this subject area has been restricted to plants (Widen and Andersson 1993; Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001; Waldmann 2001; Willi et al. 2006); virtually no work exists that explores the relationship between quantitative genetic diversity and population size among vertebrate species. Conclusions based solely on plant studies may not be easily extrapolated to vertebrates which, unlike plants, exhibit substantial behaviour (e.g. dispersal, complex mate choice, inbreeding avoidance) that might alter the relationship between genetic diversity and population size.

Likewise, the influence of genetic drift versus natural selection in relation to population size is unclear. The relative importance of drift and selection within populations is often assessed by comparing neutral marker differentiation (F_{ST}) to quantitative trait differentiation (Q_{ST}) (e.g. Merilä and Crnokrak 2001; Edelaar et al. 2011).When Q_{ST} deviates significantly from F_{ST}, selection is credited as the primary force causing differentiation among populations, whereas if Q_{ST} and F_{ST} do not differ, genetic drift and selection cannot be disentangled (Mckay and Latta 2002, but see Ovaskainen et al. 2011). Q_{ST} frequently exceeds F_{ST} in analyses, yielding the conclusion that directional selection is pervasive (Merilä and Crnokrak 2001; Leinonen et al. 2008; Lamy et al. 2012; De Kort et al. 2013). Yet there are potential caveats with these comparisons including estimates of Q_{ST} based on small numbers of populations, a focus

on small numbers of traits or specific traits types (Merilä and Crnokrak 2001; Leinonen et al. 2008), and improper statistical methods used to estimate Q_{ST} and its confidence intervals (O'Hara and Merilä 2005; Whitlock 2008). Furthermore, the choice of marker for F_{ST} estimation can affect the outcome of Q_{ST}/F_{ST} comparisons. For example, the high mutation rates of microsatellite loci which are often used to estimate F_{ST} can drastically deflate F_{ST} and potentially result in the observation that Q_{ST} is greater than F_{ST} (Edelaar and Björklund 2011; Whitlock 2011).

Here, I investigate two alternative hypotheses regarding the relationship between population size, quantitative genetic variation (measured as additive genetic variation, V_A) and the relative role of drift vs. selection in population differentiation (Q_{ST} vs. F_{ST}). We compare V_A rather than narrow-sense heritability (h^2) to population size even though h^2 is frequently reported in quantitative genetic analyses since h^2 can be a poor predictor of evolutionary response in natural populations (Merilä et al. 2001; Morrissey et al. 2010) and because predictions about the role of selection and drift relate directly to V_A rather than h^2 (Houle 1992; Hansen 2011). The model system for this work is nine differentially abundant and fragmented populations of a vertebrate fish (brook trout, *Salvelinus fontinalis*).

Under a first, "Directional hypothesis" (Willi and Hoffman 2012; Wood et al. 2014) small populations are predicted to have consistently reduced V_A and thus also reduced adaptive potential relative to large populations. For instance, habitat fragmentation decreases population size while simultaneously increasing isolation and environmental stress (e.g. Ward and Johnson 2005), and hence genetic diversity may be

reduced due to the combined effects of restricted gene flow, drift, and inbreeding (e.g. Menges 1991; Ouborg et al. 1991).

While genetic drift imposes a directional element to the comparison of Q_{ST} and F_{ST} in relation to population size, the form of the relationship of Q_{ST}/F_{ST} with population size is dependent on assumptions regarding the characteristics of selection regimes (direction and form in addition to magnitude) acting on differentially abundant populations. For instance, genetic drift might result in similarly high Q_{ST} and F_{ST} values at small population size (Willi et al. 2006) and decrease as population size increases and selection becomes more effective, with two possible outcomes. One is that the ratio of Q_{ST}/F_{ST} might simultaneously increase and also become more variable with increasing population size (Fig. 3.1a). This might occur if selection regimes and consequently Q_{ST} estimates are more variable among large compared with small populations, and if Q_{ST} is increasingly greater than F_{ST} as population size increases (Fig. 3.1a). Conversely, a second outcome is that the ratio of Q_{ST}/F_{ST} will be similar among the smallest and largest populations but more variable at medium population size (Fig. 3.1b). This might occur if genetic drift results in $Q_{ST} = F_{ST}$ at small population size and if large populations contain similar complements of habitat types such that Q_{ST} is consistently low and similar to F_{ST}.

Alternatively, under the "Variable hypothesis" (Willi and Hoffman 2012; Wood et al. 2014), small population fragments are expected to be random samples of larger, more complex fragments. Habitat fragmentation might thus result in increased variability in environmental conditions and consequently, selection regimes, as population size decreases, and hence V_A might also be more variable at small population size (e.g. Connor and McCoy 1979; Kotliar et al. 1999; Wood et al. 2014).

Two potential outcomes in regards to Q_{ST} and Q_{ST}/F_{ST} are plausible based on the prediction of increased variability in selection regimes at small population size. First, Q_{ST} and Q_{ST}/F_{ST} might also be more variable at small than large population size (Fig. 3.1c). Concurrently, Q_{ST}/F_{ST} might also increase with increasing population size if Q_{ST} is more frequently reduced relative to F_{ST} as population size is reduced (Fig. 3.1c). Or, Q_{ST} and Q_{ST}/F_{ST} might be equally variable among both small and large populations, but with Q_{ST}/F_{ST} generally increasing overall with increasing population size (Fig. 3.1d). This might be the case, for example, if fluctuating environmental conditions over long time periods result in complex, fluctuating selective regimes that ultimately yield a similar spread of Q_{ST} at all population sizes (Fig. 3.1d).

This study is the first to explore, for a large number of populations of a vertebrate species, the relationship between V_A and the relative effects of genetic drift and natural selection with population size (measured as adult census population size, N and the effective number of breeders, N_b a parameter which is closely linked with N_e; Waples et al. 2013). Moreover, V_A and Q_{ST} were examined for a large number of different traits across several trait categories including one that is rarely investigated (behavioural traits, e.g. see Carlson and Seamons 2008). Finally, F_{ST} was estimated using both microsatellite loci and single nucleotide polymorphisms (SNPs) to account for the potential downward bias of F_{ST} due to the polymorphic nature of microsatellites (Edelaar and Björklund 2011; Whitlock 2011).

Materials and Methods

Study site

Cape Race, Newfoundland, Canada, is a region of coastal barren land traversed by a parallel series of low-order streams, many of which harbour resident, pristine populations of brook trout. The streams are small in size (0.27-8.10km) enabling thorough sampling for N and N_b estimation. CR populations likely diverged from a common ancestor (10-12000 ybp; Danzmann et al. 1998); all populations are genetically distinct and almost all are also completely isolated (Wood et al. 2014). Exceptions in this study are the population pairs BF x WN and DY x UO for which occasional gene flow may occur (see Ch. 3 Appendix A for population codes).

Gamete collection and common garden experimental design

Nine CR populations were monitored for spawning individuals via electrofishing from mid to late October 2011(for a map of CR populations see Wood et al. 2014). Breeding adults were gathered and placed in flow-through cages within the stream channel until gamete collection took place, between 21h00 and 2h00 of the same evening. Following collection, gametes were transported directly from CR to St. John's, Newfoundland in refrigerated coolers, and then shipped to Montreal, Quebec by air such that total transit time was approximately 10 hours.

Fertilization of gametes took place between ten and fourteen hours after initial collection, with eggs from each female being mixed with equal volumes of sperm from 2-7 males, yielding a total of 389 half-sib families or an average of 43.1 families per population (range =17-64). CR females are relatively small in size (mean length = 138.3 ± 28.6 mm) and have low fecundity (mean number of eggs = 82.8 ± 53.9 SD) such that mean family size was 20.0 eggs ± 8.0 SD (range = 3-50). Families were incubated

separately within 5.2 cm diameter mesh-bottom egg containers placed randomly with respect to population within a single 1000L recirculating tank and maintained at 7.0 ± 0.3 °C throughout the course of the experiment. Eggs were left undisturbed until the eyed stage to reduce potential mortality following fertilization, at which point dead individuals were counted and then removed daily. Dissolved oxygen and pH did not differ in different tank locations and were maintained at consistent levels throughout the experiment. Across-population family mortality was generally low (mean = 3.8 families ± 4.4 SD) with the exception of WC for which 14 families had zero survival. However, almost all the mortality was in a small number of females indicating an issue with egg quality such that these eggs likely would not have survived under natural conditions. Across population family mortality without WC was 2.5families ± 2.2 SD.

Traits

Early life-history traits

Six early-life history traits known to be related to individual fitness of salmonids under natural conditions were measured (Hutchings 1993; Einum and Fleming 2000): (i) hatch time, estimated as accumulated degree days from fertilization to hatch of all individuals within families (Kinnison et al. 1998); (ii) length at hatch (tip of the snout to the tip of the median rays of the tail; Koskinen et al. 2002); (iii) yolk sac volume at hatch (estimated as $LH^2(\pi/6)$, where L and H were the length and height of the yolk sac, respectively; Koskinen et al. 2002); (iv) emergence length (when the yolk sac is 'buttoned-up' into the body cavity: Beacham and Murray 1985); (v) yolk sac conversion efficiencies ((length at yolk absorption – length at hatch)/yolk sac volume), calculated

using the family means in each population and (vi) relative survival of each family for each treatment over the embryonic period (i.e., fertilization to hatch).

Behavioural traits

Three traits relating to anti-predator behaviour were assessed from 301 behavioural trials (mean number of trials per population = 33.4 ± 9.9SD) carried out from March 5th- 27th, 2012. All traits (pre-stimulus foraging, latency, post-stimulus foraging) were scored using video footage of individual behavioural observations taken with digital cameras. An average of 17.3 families (range 10-24, 159 total) from each CR population were evaluated and each family was represented by 3-16 individuals (depending on family size), selected randomly from holding containers and divided between one or two 30 L tanks in groups of 3-5. Prior to observations a small amount of food was added to each tank and fish were left to acclimate for a period of 4 hours. Each observation consisted of a 5 minute pre-stimulus period during which the number of foraging attempts made by each focal fish was recorded. At the end of the 5 minute period, a predation attempt was simulated by introducing a plastic duck head to each tank for 5 seconds, after which the amount of time that elapsed until foraging resumed (latency) was calculated for each fish (Brown et al. 2011). This was followed by a second 5 minute post-stimulus period in which we recorded the number of foraging attempts. Foraging rates for the pre-and post-stimulus periods were estimated as the total number of forages attempted by each focal fish, divided by the observation time (5 minutes).

Morphology

Landmark based morphometrics were used to acquire data on body morphology for individuals post yolk absorption. Fourteen landmarks were measured (Ch. 3 Appendix B) corresponding to 12 different morphological traits that might reasonably differ among CR populations due to differences in environmental conditions such as prey regimes, flow characteristics, or predation pressure (Taylor and McPhail 1985; Fraser and Bernatchez 2005; Keeley et al. 2007). An average of 6.0 individuals (range 2-14) per family per population (2107 individuals total from 15-51 families per population) were randomly sampled and anaesthetized non-lethally using MS-222. The number of families used to measure morphology is lower than the number initially generated since some families had an insufficient number of surviving individuals at this stage for meaningful trait data for h^2 or Q_{ST} estimation. After being anaesthetized, each fish was positioned on its right side beneath a ruler with the caudal fin extended and subsequently photographed using a secured overhead digital camera. Morphological traits were then measured from digital photos imported into ImageJ (Rasband 2011).

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

Estimates of population size for each population in 2011 were generated in a previous study based on N estimated using either the Schnabel (1938) or Peterson (1896) method and weighted harmonic N_b (three consecutive cohorts except for two in DY; Appendix A) estimated using LDNe (Waples and Do 2008). Weighted harmonic N_b was strongly correlated with generational N_e for the five CR populations for which detailed life history data was available (Wood et al. 2014; Waples et al. 2013) and therefore N_b was used for all analyses.

Molecular genetic variation

Details on population genetic analysis of CR populations (microsatellite data, genotyping, electrophoresis etc.) can be found in Wood et al. (2014). This data has been used here to calculate F_{ST} for comparison against Q_{ST} and F_{ST} calculated from single nucleotide polymorphism (SNP) data. For each population, 28-38 individuals were screened (mean =34.9 ± 3.3SD) using 237 SNPs developed for brook trout. All SNPs analyzed were located in coding regions, positioned on a genetic map, tested for association with QTL at a large number physiological traits, and annotated when possible (Sauvage et al. 2012a, b). Excluding monomorphic SNPs yielded a total of 164 polymorphic SNPs which amplified in over 85% of individuals and conformed to HWE equilibrium expectations. Details of SNP development, validation and sequencing at the Genome Quebec Innovation Center (McGill University, Montreal, QC, Canada) are found in Sauvage et al. (2012a, b).

F_{ST} estimation with microsatellites and SNPs

Neutral genetic differentiation across all populations and between population pairs at microsatellite loci and SNPs was quantified by estimating F_{ST} following Weir and Cockerham (1984), and the associated 95% CI was estimated by bootstrapping over loci using FSTAT 2.9.3.2 (Goudet 2001). F_{ST} outliers were detected using the FDIST2 approach of Beaumont and Nichols (1996) implemented in LOSITAN (100000 simulations; Antao et al. 2008). LOSITAN simulates the expected distribution of F_{ST} in an island model with 100 islands across a wide range of heterozygosities, and the

observed F_{ST} and heterozygosity values are compared with their expected distribution in order to detect outliers that are potentially under selection. All outlier SNPs were removed from further analyses. For microsatellite loci, F_{ST} calculated using all 13 loci or excluding loci potentially under selection for any population pairs generated similar results and were strongly correlated (Spearman's $r = 0.98$ p = <0.001). For these reasons, the inclusion of the few outlier loci among certain population pairs likely did not greatly influence overall F_{ST} estimates using microsatellites, and therefore all 13 loci were retained in the analyses.

Quantitative genetic analysis

Heritability and Q_{ST}

Additive genetic variation and Q_{ST} were estimated from pedigree data and fitted with generalized linear mixed models (GLMM) with a Gaussian error distribution using MCMC techniques implemented in the R package MCMCglmm (Hadfield 2010). Specifically, animal models (Kruuk 2004), a form of mixed model that allows the specification of both fixed and random effects and which is relatively insensitive to unbalanced experimental design and pedigree structure was used (O'Hara et al. 2008). Variance components were estimated according to the model:

$$y_{ij} = \eta + Dam_i + Animal_j + \varepsilon_{ij}$$

where η is the population mean; *Dam* is the random effect due to dams; *Animal* is the component of V_A; and ε is the residual error term. Dam was included as a random effect

since the influence of maternal effects can potentially result in inflated estimates of V_A (Falconer and MacKay 1996; Wade 1998). For morphological traits, total length was also included as a covariate to account for the potential effects of body size on morphology (Fraser et al. 2010). For all models, proper priors were used which partitioned the total variance equally among the random effects and we specified a low degree of belief (n=1) such that little weight was placed on these values. MCMC chains were run for 1000000 iterations with a burn- period of 300000 and thinning interval of 50, hence parameters and associated confidence intervals were based on sampling the posterior distribution 14000 times.

To estimate Q_{ST} among populations, a similar model was used as for V_A with the exception that population was included as an additional random effect in order to obtain an estimate of the between population component of V_A. MCMC chains for Q_{ST} were run for 1000000 iterations such that estimates and confidence intervals were based on 1400 samples of the posterior distribution. Q_{ST} was estimated as $\sigma^2_{GB}/ (\sigma^2_{GB} + 2\sigma^2_{GW})$, where σ^2_{GB} and σ^2_{GW} represent the between- and within-population components of V_A, respectively (Merilä and Crnokrak 2001).

Statistical analysis

Directional hypothesis

As some traits included in the analysis were not normally distributed, Spearman's correlations were used to determine whether a directional relationship existed between population size (N or N_b) and V_A for individual traits. Because Q_{ST} and F_{ST} are presented as matrices of genetic distances between pairs of populations, individual estimates are not

independent of each other, therefore simple (Mantel 1967) and partial (Smouse et al. 1986) Mantel tests were implemented to determine the relationship of F_{ST}, Q_{ST}, and Q_{ST}/F_{ST} with pairwise harmonic mean N and N_b. The harmonic mean of population size was used rather than the arithmetic mean since the harmonic mean scales more closely with the effects of genetic drift (Crow and Kimura 1970). Simple Mantel tests were used to examine the correlation between F_{ST} and population size whereas partial Mantel tests were use to determine if Q_{ST} for traits was related to population size after controlling for F_{ST}.

Variable hypothesis

To investigate whether there was increased variability in V_A, Q_{ST}, and the ratio of Q_{ST}/F_{ST} at small population size, White's test was used (p-value of the corresponding test statistic = W-p below) to determine whether the residual variance of each parameter was constant or exhibited heteroscedasticity in relation to N or N_b. White's test implements an auxiliary regression analysis which regresses the squared residuals from the original regression model onto a set of regressors that contain the original regressors, the cross-products of the regressors, and the squared regressors (White 1980).

Results

Additive genetic variation

Across the nine populations, V_A for specific traits differed significantly between two or more of the populations in several cases (e.g. hatch time, emergence length), but for the majority of comparisons the confidence intervals were wide and overlapping such

that there were no statistically significant differences in V_A among populations (Ch. 3 Appendix C).

Additive genetic variation: Directional hypothesis

There were no consistent directional trends between point estimates of V_A and population size across the different trait categories (Table 3.1 and Appendix C). Relationships for 11 of 21 traits with N_b and 10 of 21 traits with N were in the opposite direction as that predicted by conservation genetics theory with V_A actually increasing with population size reductions. Results of Spearman's correlations however, revealed no significant correlations between V_A and population size for any of the traits investigated. Furthermore, there was no evidence for directional differences in maternal variation (V_M) with increasing population size (Table 3.1).

Additive genetic variation: Variable hypothesis

There was also little evidence for increased variation in V_A at small population size. Only one of 21 single trait comparisons showed significant heteroscedasticity of V_A in relation to N_b, and examination of the residual plots revealed that the significant heteroscedasticity was at small population size. None of the traits exhibited significant heteroscedasticity with N (Table 3.1).

F_{ST}

Estimates of neutral genetic differentiation across the nine populations were large and significant for F_{ST} calculated with both microsatellites and SNPs. Mean F_{ST} for SNPs

was significantly greater than for microsatellite loci (0.38 vs. 0.25, Fig. 3.2), however the correlation between F_{ST} estimates from the two sources among all pairwise population comparisons was high (Spearman's $r = 0.91$, $p<0.001$). F_{ST} for both microsatellites and SNPs decreased with increasing population size but the relationships were not significant (N_b; $r_M = -0.18$, $p = 0.90$ for microsatellites, and $r_M = -0.33$, $p = 0.87$ for SNPs and N; $r_M = -0.25$, $p = 0.79$ for microsatellites and $r_M = -0.10$, $p = 0.65$ for SNPs). There was also no evidence of increased variation in F_{ST} at small population size for SNPs (N_b; W-p= 0.72, and N; W-p = 0.64) or microsatellites (N_b; W-p = 0.89, and N; W-p = 0.50).

Q_{ST}: All populations

Q_{ST} estimated for all nine populations revealed significant quantitative trait differentiation for all traits analyzed (Fig. 3.2). Morphological traits tended to be the most differentiated among populations (mean Q_{ST} = 0.44, range 0.040-0.87) followed by life history traits (mean Q_{ST} = 0.29, range 0.11-0.56), while behavioural traits showed the lowest levels of among population differentiation (mean Q_{ST} = 0.15, range 0.037-0.27). Among the 21 traits investigated only two (pre-stimulus foraging, and head depth behind the eye) had Q_{ST} values that were significantly different from F_{ST} for both SNPs and microsatellites, and in both instances Q_{ST} was significantly lower than F_{ST}.

Q_{ST}: Pairwise comparisons

General trends

Q_{ST} estimates among populations pairs were also higher for morphological traits (Mean Q_{ST} = 0.56, range 0.27-0.72) than for life history traits (Q_{ST} = 0.31, range 0.12-

0.53) or behavioural traits (Q_{ST} = 0.18, range 0.10-0.27). Confidence intervals however, were extremely wide and overlapping for all pairs and all traits, and there was also no difference between Q_{ST} and pairwise F_{ST} estimated using microsatellites or SNPs since CIs were overlapping in nearly all cases.

Pairwise Q_{ST}: Directional hypothesis

After correcting for F_{ST}, mean Q_{ST} was not significantly related to population size for life history traits, (N_b; r_M= 0.31, p = 0.11, and N; r_M= 0.35, p = 0.22), behavioural traits (N_b; r_M= -0.27, p = 0.88, and N; r_M= -0.37, p = 0.95) or for morphological traits (N_b; r_M=0.080, p = 0.43, and N; r_M= 0.17, p = 0.29) (Fig. 3.3 and Ch. 3Appendix D and E). For traits considered individually, there was no evidence that Q_{ST} was related to population size as there were no traits in any of the three trait classes that exhibited a significant correlation with either N or N_b (Table 3.2).

Pairwise Q_{ST}: Variable hypothesis

White test results for Q_{ST} vs. N_b and N revealed little evidence of increased variation at small population size. Mean Q_{ST} for life history traits did not exhibit significant heteroscedasticity with either N_b (W-p = 0.67) or N (W-p = 0.53). Likewise the spread of residuals of mean Q_{ST} for behavioural traits was homogenous across population sizes (N_b; W-p= 0.31, and N; W-p = 0.082). For morphological traits, there was significantly more variation in residuals of mean Q_{ST} values at small N_b (W-p = 0.043) but not at small N (W-p = 0.74). Across individual traits, four of 21 traits exhibited significant heteroscedasticity with N_b (Table 3.2), and examination of residual plots

showed that for two of the traits (hatch time and transect 9) the increased variation was at small population size. No traits were significantly heteroscedastic with *N*.

Q_{ST}/F_{ST} microsatellites and SNPs

Q_{ST}/F_{ST}: Directional hypothesis

Where F_{ST} was calculated using microsatellites, mean Q_{ST}/F_{ST} for life history traits was not significantly related to either N_b or N (N_b; $r_M = 0.21$, $p = 0.18$, and N; $r_M = 0.060$, $p = 0.37$). Likewise, mean Q_{ST}/F_{ST} was not significantly related to population size for morphological traits (N_b; $r_M = 0.12$, $p = 0.22$, and N; $r_M = 0.12$, $p = 0.17$) or behavioural traits (N_b: $r_M = 0.059$, $p = 0.36$, and N: $r_M = -0.043$, $p = 0.56$) (Fig. 3.4 and Ch. 3 Appendix F and G). Q_{ST}/F_{ST} was also not significantly related to either N or N_b for any of the traits individually (Table 3.3).

The relationship between mean Q_{ST}/F_{ST} SNPs was the same as for F_{ST} estimated from microsatellite data; Q_{ST}/F_{ST} SNPs and N_b or N was not significant for life history traits (N_b: $r_M = 0.054$, $p = 0.036$, and N; $r_M = -0.023$, $p = 0.51$), morphological traits (N_b; $r_M = 0.056$, $p = 0.25$, and N; $r_M = 0.050$, $p = 0.33$) or for behavioural traits (N_b; $r_M = -0.00057$, $p = 0.45$, and N; $r_M = -0.10$, $p = 0.69$; Fig 3.4 and Ch. 3 Appendix F and G) and Q_{ST}/F_{ST} SNPs was also not significantly related to either population size metric for any of the 21 traits examined (Table 3.3 and Appendix G).

Q_{ST}/F_{ST}: Variable hypothesis

There was no statistically significant heteroscedasticity between mean Q_{ST}/F_{ST} microsatellites and population size either for life history traits (N_b; *W*-$p = 0.10$, and N; *W*-

p = 0.72) or morphological traits (N_b; W-p = 0.48, and N: W-p = 0.29). White's test results for mean behavioural Q_{ST}/F_{ST} was significant, but only for N_b (W-p = 0.0083, and N: W-p = 0.15) and the increased heteroscedasticity was at large rather than small population size. Likewise, increased heteroscedasticity was observed at large population size for the only significant result among 21 White tests with N_b and for four significant tests out of 21 total with N (Table 3.3).

The spread of residuals for mean Q_{ST}/F_{ST} SNPs was also similar across population sizes for the three trait categories (N_b; all W-p > 0.065, and N; all W-p = 0.22). There were furthermore only three significant White tests out of 42 total across the two measures of population size (Table 3.3) but in none of these cases was the increased spread at small population size.

Discussion

There was no evidence for consistent differences in quantitative genetic variation and trait differentiation in relation to population size among natural brook trout populations over a nearly 50-fold difference in N (179-8416), a 10-fold difference in N_b (18-135) and despite a large number of traits investigated over three different trait classes for a relatively large number of families and populations. In regards to the Directional hypothesis analysis of V_A for individual traits revealed no evidence for persistent trends in the direction of the relationships with population size. Small populations did not exhibit consistently reduced V_A relative to large populations, and in fact, V_A for approximately half the traits actually increased with decreasing population size, though none of the relationships were statistically significant. Similarly, there was no evidence to suggest

that small populations exhibited more variability in V_A as predicted by the Variable hypothesis: only 1 of 42 tests across the 21 traits and two population size measures demonstrated significant heteroscedasticity in relation to population size. There were also no differences observed in maternal variation for the different traits between small and large populations suggesting that maternal effects contributed roughly equally to the resemblance between related individuals among both small and large CR populations.

The relationship between F_{ST} and population size was in the direction predicted by theory (i.e. F_{ST} decreased with increasing population size) but the correlation was not significant for either N or N_b. Similarly, the relationship of Q_{ST} and also Q_{ST}/F_{ST} with population size was weak and nonsignificant for all of the traits investigated although Q_{ST}/F_{ST} did tend to increase with increasing population size as expected in all but one of the initial predictions (Fig. 3.1a, c and d). F_{ST} estimates for SNPs or microsatellites were not more variable at small population size and evidence for increased spread in Q_{ST} at smaller population size was found in only 2 of 21 tests with N_b and none with N. Taken together, these results support the prediction that populations at varying levels of N and N_e might experience a variety of environmental conditions (Fig. 3.1d).

This study is perhaps one of the first to simultaneously investigate Q_{ST} and F_{ST} for a large number of traits from several trait categories on the same populations. Although confidence intervals were wide, morphological traits tended to have higher Q_{ST} estimates relative to F_{ST}, possibly signaling divergent selective regimes acting on morphology in CR brook trout populations while conversely, Q_{ST} for behavioural traits tended to be lower than F_{ST} values suggesting that the behavioural responses favored across the

populations are similar. This latter result is particularly notable given the general paucity of data regarding behavioural traits for natural populations.

A previous study on the physical habitat of Cape Race trout populations found evidence to support the Variable hypothesis; there was greater spatial habitat variability among small than large populations, suggesting the former may be subject to a greater diversity of selective pressures (Wood et al. 2014). Yet, intriguingly, this did not translate into more variable V_A and Q_{ST} among small than large populations in the present study. I propose three hypotheses for the apparent disparity in the spatial habitat and quantitative trait data on these populations. First, the habitat assessment was based on two years of data whereas contemporary genetic structuring among Cape Race brook trout populations is the product of a long evolutionary history. Similarly, as predicted for Q_{ST} and Q_{ST}/F_{ST}, long term fluctuating environmental conditions may have resulted in complex, fluctuating selective pressures and similar levels of genetic variation among both small and large Cape Race populations (Blanckenhorn et al. 1999; Siepielski et al. 2009; 2013). Second, environmental heterogeneity may induce a negative correlation between selection and V_A in small populations wherein little genetic variance is available for strong selection to act upon when conditions are harsh, but genetic variance is abundant when selection is weak under favourable conditions (Merilä et al. 2001; Wilson et al. 2006). Third, there were also similar levels of phenotypic plasticity observed among small and large Cape Race populations. If plasticity is favoured to cope with increased environmental variability (Sultan 1995; Paschke et al. 2003), this might buffer the loss of adaptive genetic variation similarly between small and large populations (Schlichting 1986; Sultan 1987).

Quantitative genetic variation and differentiation were compared across Cape Race populations in relation to both N and N_b with the finding that tests of heteroscedasticity were more often significant using N_b as a population size measure (though there were few significant tests overall). Correlations for Q_{ST} and Q_{ST}/F_{ST} were similar for both N and N_b but this may have been due to the preponderance of negative results in the study in general.

Finally, F_{ST} estimated with SNPs was found to be 1.53 times higher than F_{ST} estimated using microsatellites. This suggests that some previous studies which have used microsatellite based F_{ST} estimates and found that Q_{ST} was greater than F_{ST} might have reached incorrect conclusions. However, this does not mean that F_{ST} should always be estimated using SNPs rather than microsatellites since the appropriate choice of marker depends on mutational inputs to Q_{ST} as well (Hendry 2002) and hence merely illustrates the challenges in Q_{ST} /F_{ST} comparisons in general.

Caveats

Family crosses were generated from a subset of all Cape Race populations, so one possibility is that the small streams that were investigated might not be representative of all regional small populations. If by chance the subset of small populations chosen were those with the most similar environmental characteristics, or with the highest quality habitat, this might explain why no support was observed for the Directional or the Variable hypotheses. However, habitat character means and CVs for the populations included in this study were not different from other small Cape Race populations. Moreover, the variability around the means and CVs were equal in these two groups

(small populations included/excluded), suggesting that the populations included in this study represented the full range of habitat types occupied by small Cape Race populations.

Additive genetic variation and Q_{ST} were compared for traits at the early life-history phase but traits at later life stages could not be investigated due to the logistical constraints of rearing large numbers of salmonid fishes. Whether similar patterns would be observed in older juvenile or adult individuals is uncertain. However, this study included a large number of traits across several different trait categories including traits that are known to be associated with fitness in salmonid fishes at a life stage that has a critical impact on recruitment (Einum and Fleming 2000). I therefore expect that these traits are important for the persistence of CR populations.

Finally, to investigate the two alternative hypotheses, point estimates of V_A and Q_{ST} were examined in relation to population size, but it should be noted that confidence intervals calculated for V_A and pairwise Q_{ST} in this study were large and overlapping across populations for all traits. Even calculating Q_{ST} using all nine populations produced confidence intervals that were as large as or larger than the point estimates of Q_{ST} themselves. This underscores the point that extremely large numbers of families and populations may be required to make firm conclusions regarding quantitative genetic characteristics of vertebrate populations in nature. O'Hara and Merilä (2005) suggested >20 populations are required to achieve reasonable precision in Q_{ST} estimates, however an experiment of that magnitude would be difficult to carry out for most species. As this study is the largest thus far performed in a vertebrate species in terms of numbers of

populations and families, it suggests that conclusions derived from studies using a smaller sample size than was included here should be interpreted with caution.

Evolutionary and conservation implications

There was scant evidence that quantitative genetic variation and trait differentiation consistently differed between small and large trout populations. The results thus do not support the frequently cited assumption that the environments occupied by small populations tend to be marginal and that small populations may experience disproportionate reductions in adaptive potential relative to large populations (Frankham 1996; Kawecki 2008). While genetic drift may indeed become more important as population size decreases, selection may also be stronger in some fragments if conditions become more extreme or variable as fragment size decreases. Overall, these findings suggest that while the mechanisms might differ from small to large population size, these have led to a similar result in regards to V_A and Q_{ST}.

Regarding whether small populations are capable of evolving, the results suggest that some small populations might retain the adaptive potential necessary to respond to future environmental changes even at very small population size. Reductions in fitness due to inbreeding and loss of quantitative genetic variation are expected to be disproportionately greater at $N_e < 50$ (Franklin 1980). Five of the populations included in this study have an N_b of less than 50 and at least one (DY) most likely also has an N_e of less than 50; these populations have also been isolated for some time and yet have retained similar levels of V_A as the larger populations. As brook trout are a colonizing species that exhibit residual tetraploidy, they might have an enhanced capacity to deal

with small population size relative to other species (Allendorf and Thorgaard 1984), therefore how these results might apply to other taxa is an open question. Still, these findings are relevant given the paucity of similar research among salmonids, and vertebrates in general. Indeed, they suggest that demographic and environmental stochasticity rather than genetics might pose the most immediate threat to persistence for some small populations (e.g. Lande 1988; Caro and Laurenson 1994). Finally, as suggested in previous works (e.g O'Hara and Merilä 2005; Edelaar et al. 2011), I recommend that future studies in this area exercise care in choosing molecular markers for estimating F_{ST} and that they also attempt to maximize the number of populations and families used in calculating quantitative genetic parameters.

Table 3.1: Spearman's correlations (Directional hypothesis) and White's test results (Variable hypothesis) for V_A vs. N_b and N and Spearman's correlations for V_M vs. population size for 21 traits measured using 9 brook trout populations at Cape Race, Newfoundland. Estimates of V_A and V_M across populations for the 21 traits are found in Ch. 3 Appendix C.

		V_A				V_M	
		N_b		N		N_b	N
Trait class	Trait	r_S	White's p	r_S	White's p	r_S	r_S
Life history	Hatch time	-0.15	0.17	-0.28	0.46	0.22	-0.083
	Hatch length	0.27	0.71	0.083	0.47	0.75*	0.60
	Yolk volume	0.067	0.66	-0.033	0.87	0.27	0.28
	Emergence length	0.40	0.86	0.45	0.67	0.30	0.48
	Yolk conversion	0.067	0.034	0.050	0.63	0.49	0.41
	Survival	-0.050	0.33	0.067	0.12	0.53	0.53
Morphology	Head length	-0.17	0.52	-0.23	0.57	-0.067	-0.17
	Head width	-0.23	0.42	-0.050	0.45	-0.17	0.050
	Eye diameter	0.22	0.15	0.075	0.060	0.40	0.30
	Head depth behind eye	-0.42	0.052	-0.29	0.65	-0.32	0.00
	Body depth	-0.31	0.34	-0.075	0.41	-0.28	0.00
	BD: ADP	-0.56	0.37	-0.58	0.70	-0.10	-0.067
	ANA: ADP	-0.27	0.046†	-0.017	0.62	-0.44	-0.43
	ANA: CPD	-0.37	0.41	-0.22	0.40	-0.12	-0.12
	ANA: CPV	-0.27	0.88	0.017	0.47	0.050	0.38
	ADP: CPD	-0.18	0.37	-0.30	0.39	-0.28	-0.20
	ADP: CPV	0.27	0.21	0.23	0.40	-0.12	0.15
	CPD: CPV	0.19	0.34	0.025	0.35	-0.29	-0.31
Behaviour	Pre-stimulus foraging	0.17	0.28	0.20	0.11	-0.20	-0.45
	Latency	0.57	0.25	0.28	0.21	0.58	0.67
	Post-stimulus foraging	0.017	0.25	0.47	0.30	-0.47	-0.40

*<0.05, **<0.01, ***<0.001

†Significant heteroscedasticity located at small population size

Table 3.2: Partial Mantel test (Directional hypothesis) and White's test results (Variable hypothesis) for Q_{ST} vs. harmonic mean N_b and N for 21 traits measured using 9 brook trout populations at Cape Race, Newfoundland.

		N_b		N	
Trait class	Trait	r_M	White's	r_M	White's p
Life history	Hatch time	-0.067	0.028†	0.044	0.13
	Hatch length	0.39	0.42	0.37	0.66
	Yolk volume	0.23	0.52	0.23	0.46
	Emergence length	0.23	0.57	0.31	0.73
	Yolk conversion	0.30	0.54	0.15	0.79
	Survival	0.11	0.42	0.032	0.76
Morphology	Head length	-0.14	0.046	-0.038	0.90
	Head width	-0.24	0.092	-0.070	0.46
	Eye diameter	0.22	0.11	0.33	0.17
	Head depth behind eye	0.26	0.10	0.090	0.095
	Body depth	0.029	0.17	0.11	0.52
	BD: ADP	0.16	0.26	0.22	0.77
	ANA: ADP	0.083	0.23	0.086	0.85
	ANA: CPD	0.068	0.17	0.17	0.86
	ANA: CPV	0.14	0.14	0.18	0.71
	ADP: CPD	0.083	0.15	0.33	0.33
	ADP: CPV	-0.069	<0.001†	0.15	0.31
	CPD: CPV	0.077	0.66	0.031	0.54
Behaviour	Pre-stimulus foraging	-0.10	0.85	-0.15	0.26
	Latency	-0.16	0.0022	0.010	0.20
	Post-stimulus foraging	-0.39	0.51	-0.30	0.27

*<0.05, **<0.01, ***<0.001

†Significant heteroscedasticity located at small population size

Table 3.3: Partial Mantel test (Directional hypothesis) and White's test results (Variable hypothesis) for Q_{ST}/F_{ST} vs. harmonic mean N_b and N for 21 traits measured using 9 brook trout populations at Cape Race, Newfoundland.

		F_{ST} microsatellites				F_{ST} SNPs			
		N_b		N		N_b		N	
Trait class	Trait	r_M	White's	r_M	White's	r_M	White's	r_M	White's
Life history	Hatch time	-0.11	0.98	-0.14	0.035	-0.14	0.40	-0.21	0.062
	Hatch length	0.27	0.22	0.21	0.87	0.18	0.19	0.12	0.83
	Yolk volume	0.097	0.11	0.12	0.30	0.042	0.68	0.053	0.12
	Emergence length	0.23	0.75	0.28	0.21	0.11	0.75	0.15	0.31
	Yolk conversion	0.21	0.13	0.016	0.86	0.060	0.54	-0.094	0.60
	Survival	0.089	0.32	-0.012	0.43	0.018	0.38	-0.090	0.50
Morphology	Head length	0.099	0.084	0.11	0.0024	0.053	0.42	0.059	0.17
	Head width	0.072	0.084	0.10	0.032	0.026	0.96	0.048	0.059
	Eye diameter	0.23	0.13	0.26	0.072	0.13	0.56	0.15	0.16
	Head depth behind eye	0.17	0.13	0.15	0.14	0.10	0.28	0.082	0.23
	Body depth	0.083	0.38	0.058	0.45	0.034	0.93	0.0027	0.63
	BD: ADP	0.24	0.33	0.17	0.29	0.10	0.60	0.032	0.38
	ANA: ADP	0.11	0.24	0.098	0.42	0.045	0.49	0.012	0.59
	ANA: CPD	0.15	0.31	0.14	0.12	0.064	0.79	0.038	0.24
	ANA: CPV	0.11	0.26	0.10	0.48	0.044	0.40	0.030	0.50
	ADP: CPD	0.033	0.36	0.092	0.14	0.015	0.088	0.062	0.28
	ADP: CPV	0.021	0.12	0.079	0.35	-0.026	0.0076	0.0009	0.43
	CPD: CPV	0.10	0.15	0.12	0.26	0.052	0.11	0.070	0.34
Behaviour	Pre-stimulus foraging	-0.018	0.56	-0.17	0.85	-0.049	0.58	-0.18	0.83
	Latency	0.20	<0.001	0.13	0.033	0.099	<0.001	0.026	0.043
	Post-stimulus foraging	-0.011	0.74	-0.068	0.31	-0.028	0.65	-0.088	0.15

*<0.05, **<0.01, ***<0.001

†Significant heteroscedasticity located at small population size.

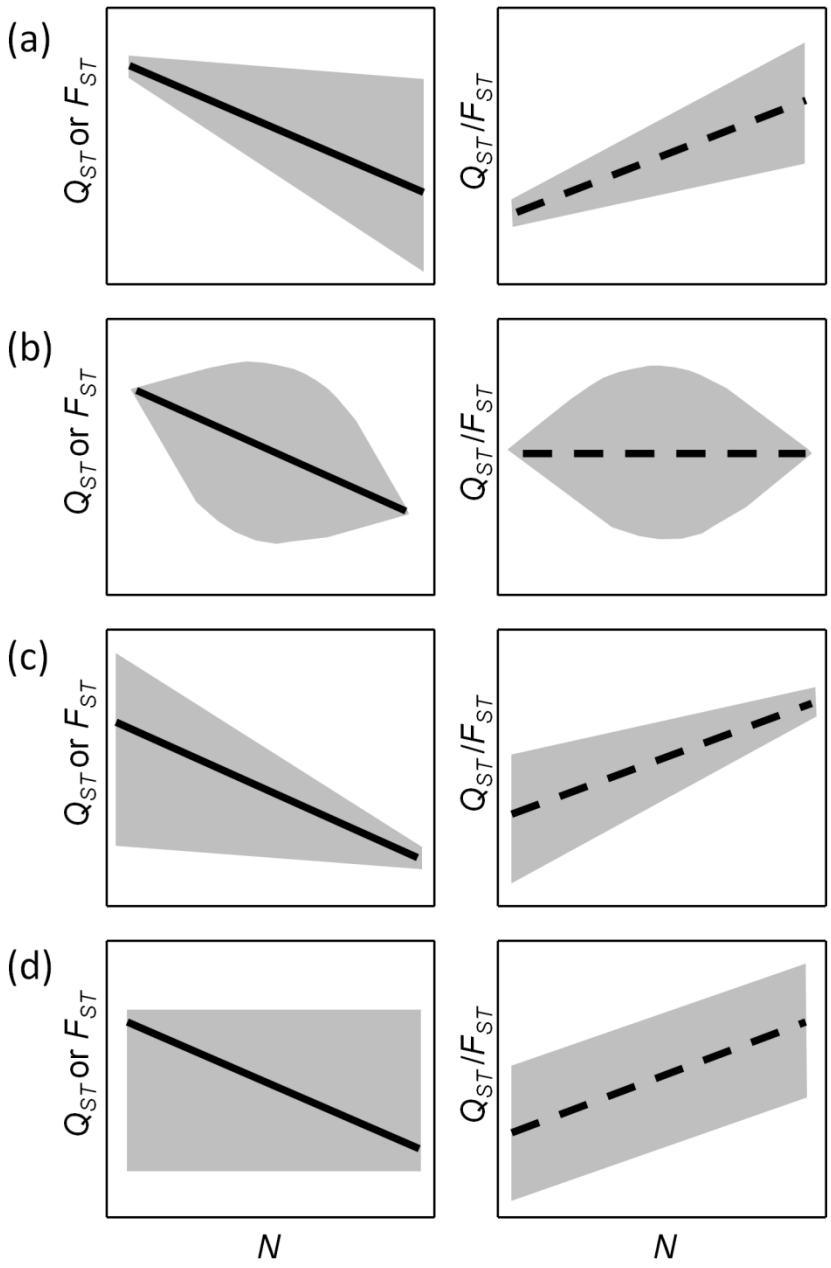

Figure 3.1: Four hypotheses for the relationship of Q_{ST}, F_{ST}, and Q_{ST}/F_{ST} with population size. The solid line represents the mean relationship of F_{ST} with population size, and the dashed line is the mean relationship of Q_{ST}/F_{ST} with population size. The shaded areas represent the expected spread of Q_{ST} values (left column) and of Q_{ST}/F_{ST} values (right column) for each hypothesis.

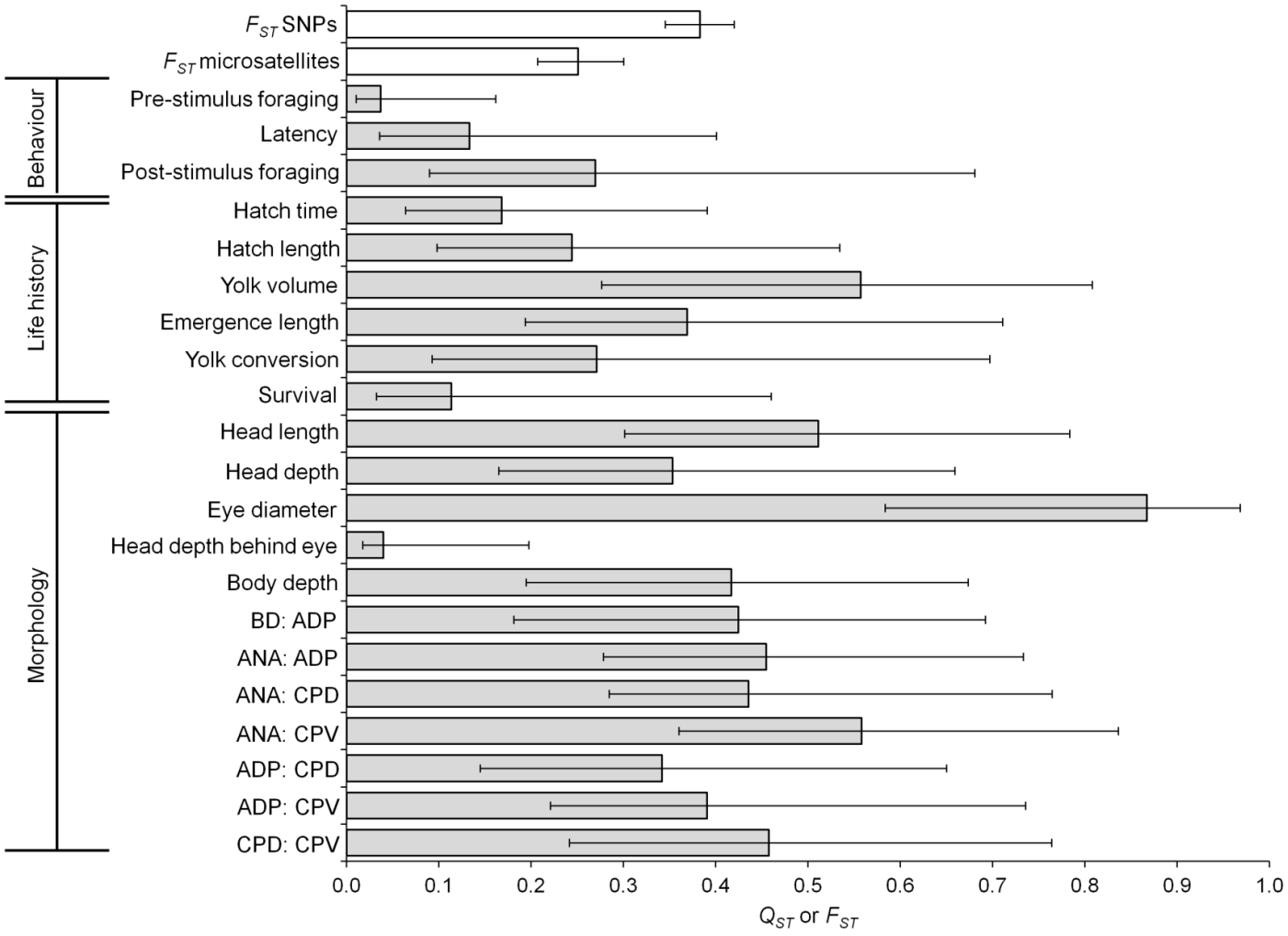

Figure 3.2: F_{ST} and Q_{ST} estimated across nine Cape Race brook trout populations. Descriptions for coded morphological traits are found in Ch. 3 Appendix B.

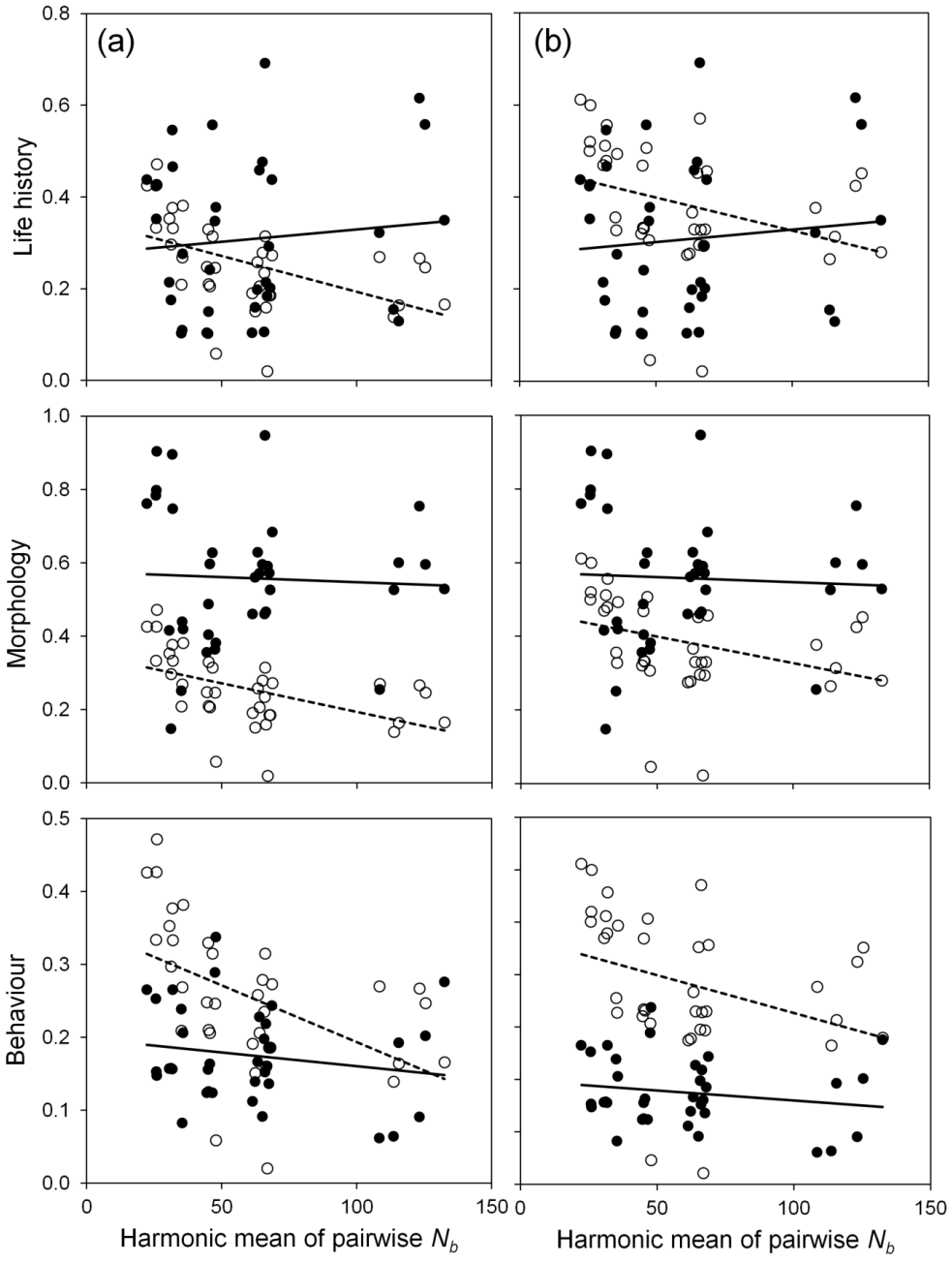

Figure 3.3: Mean Q_{ST} (●) and F_{ST} (○) vs. N_b across traits in each of three trait categories. F_{ST} values among trout populations pairs were estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$ Relationships for mean Q_{ST} and F_{ST} with N are found in Ch. 3 Appendix D and for individual traits in Ch. 3 Appendix E.

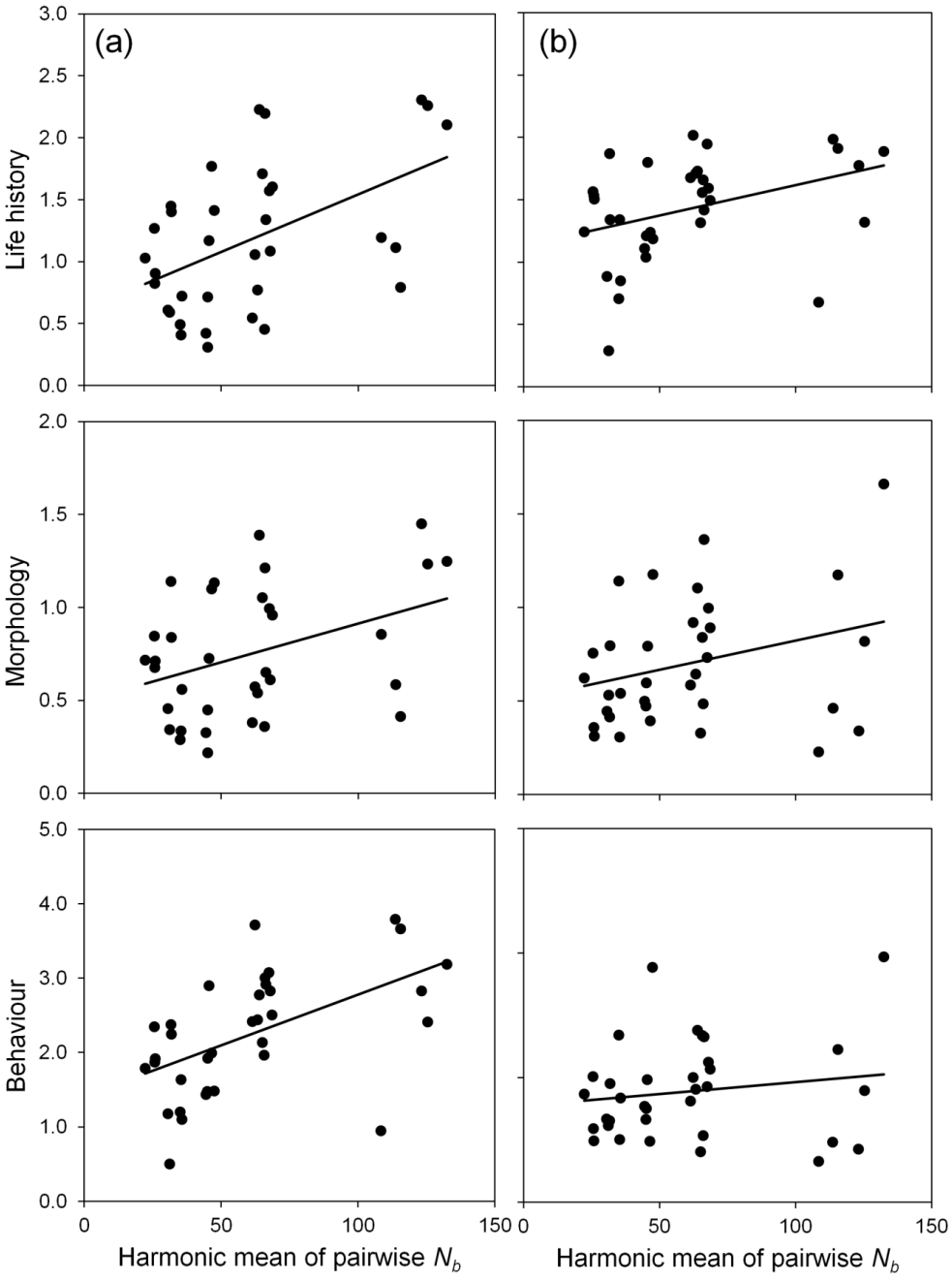

Figure 3.4: Mean Q_{ST} /F_{ST} vs. N_b across traits in each of three trait categories. F_{ST} values among trout populations pairs were estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions. Relationships for mean Q_{ST}/F_{ST} with N are found in Ch. 3 Appendix F and for individual traits in Ch. 3 Appendix G.

Chapter 4: Across taxa meta-analysis of the relationship between population size and strength of selection in nature

Abstract

The relationship between the extent of natural selection and population size for populations of wild species is uncertain. Selection may become less effective as populations become smaller and more isolated due to the increasing influence of genetic drift, or selection might be stronger at small population size if fragmentation generates more extreme environmental conditions. Alternatively, there might be more variability in selection coefficients at small population size if habitat fragmentation results in a greater variety of habitat types among small versus large populations. I surveyed the literature for studies which estimated selection in natural populations for which population size data was also available and conducted a formal meta-analysis to explore the relationship between selection pressures and population size. Across populations ranging in harmonic mean census size from four to one million, there was no evidence for significant differences in the strength, direction, or form of selection in the smallest populations (N <100) relative to populations with N = 100-1000. There was also no difference in selection pressures between the smallest and largest populations however statistical power was low due to insufficient data at population sizes of N > 1000. This lack of difference relative to population size was consistently observed across different trait types (morphology versus life history) and taxa (plants versus vertebrates). This study represents an important first attempt to investigate the relationship between natural selective pressures and population size in nature. The results suggest that some small

populations in nature may retain their capacity to adapt to future environmental change; however, there are several important information gaps that need to be addressed in future studies before solid conclusions can be reached.

Introduction

Natural selection plays a key role in shaping the observed phenotypic diversity among populations of wild species (e.g. Darwin 1859; Endler 1986; Siepielski et al. 2013) and in allowing populations to adapt to changing environmental conditions (Reed and Frankham 2003; Hoffman and Sgrò 2011). The number of studies estimating natural selection for quantitative traits in wild populations has increased dramatically since Lande and Arnold (1983) developed a standardized framework for quantifying selection that also permitted direct comparisons across different taxa, traits, and components of fitness. Several syntheses and meta-analyses have now considered some of the major patterns of phenotypic selection in the wild including the strength and direction of selection in nature (Endler 1986; Hoekstra 2001; Kingsolver 2001; Hereford et al. 2004; Siepielski et al. 2011; Kingsolver et al. 2012), temporal variability in selection within populations (Hoekstra 2001; Siepielski et al. 2009, 2011; Morrissey and Hadfield 2012), and spatial variability between populations (Siepielski et al. 2013). None however, have attempted to link patterns of selection with population size for natural populations, a surprising result given the widespread assumption that selection is less effective in small populations of wild species (Willi et al. 2006; Frankham et al. 2010).

In the absence of gene flow, the efficacy of natural selection is expected to be reduced when populations become small, as the relative influence of genetic drift increases until it overcomes selection as the primary force influencing differentiation at small population size (Willi et al. 2006). Hence, if the strength of selection systematically differs among populations of varying size, the overall response to selection according to the breeder's equation of conservation biology ($R=h^2S$; Falconer and Mackay 1996)

might also vary. While such theoretical predictions seem like logical subject material for empirical work, research that has attempted to link patterns in selective regimes with population size for natural populations is extremely sparse. Such research would be critical to determining the ability of populations of different sizes to respond adaptively to environmental change and also for prioritizing populations for conservation measures.

Only a few empirical studies have directly investigated the relationship between the extent of selection on quantitative traits and population size (Murua et al. 2010; Weber and Kolb 2013). These found little supporting evidence that the magnitude of selection differs between small vs. large populations, but suffered from methodological issues. For example, in one study the magnitude of selection was similar between small and large plant populations, but statistical power was low (Weber and Kolb 2013); in another, pollinator-mediated selection did not differ between plant populations differing in density but total census population size was not reported (Murua et al. 2010). Additional studies have explored the relative influence of selection versus genetic drift in small or fragmented populations by comparing the magnitude of between-population neutral genetic differentiation (F_{ST}) to quantitative trait differentiation (Q_{ST}). For example, Q_{ST} differed from F_{ST} only in continuous habitat in a species of frog (Johansson et al. 2007), however a lack of difference between Q_{ST} and F_{ST} does not imply that selection is not occurring, only that it is indistinguishable from genetic drift (see also Chapter 3). Additionally, most of the "large" populations in the study had an abundance of approximately 100 individuals, an N which is still quite small. Q_{ST} exceeded F_{ST} among small populations of a fish (Koskinen et al. 2002) but the study populations may

not have typified the situation of populations that have been size-restricted for extended time periods.

The overall lack of empirical attention given to understanding the relationship between population size and selection's extent may in part be due to the absence of a clear theoretical framework for linking habitat fragmentation effects on natural selection as populations are reduced in size. To address this, I conducted an extensive review of the primary literature and used meta-analytic techniques to explore two competing alternative hypotheses regarding the potential relationship between population size and patterns of phenotypic selection in populations of wild species.

First, selection might systematically differ from small to large population size. As predicted by the conservation genetics small population paradigm, the effectiveness of selection might be consistently reduced due to the increasing influence of genetic drift as population size decreases (Willi et al. 2006). Conversely, selection might be stronger at small population size for some populations if fragmentation results in environmental conditions that become more extreme as fragment size decreases. We might furthermore observe consistent differences in the direction and form of selection with population size if habitat fragmentation shifts habitat conditions in a persistent manner as population and fragment size are reduced ("Directional hypothesis"; Willi and Hoffman 2012; Wood et al. 2014).

Alternatively, there might be more variability in selective regimes among small versus large populations if environmental conditions become increasingly variable as fragment size and population size are reduced (Willi et al. 2006, 2007; Wood et al. 2014). This "Variable Hypothesis" predicts that evolutionary consequences of habitat

fragmentation depend on initial conditions within habitats and that fragments inhabited by small populations are simply random samples of larger, more complex fragments (e.g. Connor and McCoy 1979; Kotliar et al. 1999). Evidence to support this latter prediction so far has been mixed. Q_{ST}/F_{ST} was more variable at small than large population size in populations of a plant species (Variable hypothesis; Willi et al. 2007), but not among rainforest populations of *Drosophila* (Willi and Hoffman 2012). Environmental conditions were more variable among small vs. large populations of brook trout (Wood et al. 2014), but this did not translate into similar differences in V_A, Q_{ST}, or plasticity (Chapters 2 and 3).

Materials and Methods

Quantitative review of primary literature

To assemble the database, the peer-reviewed literature was surveyed from 1984 to December 2013 to identify studies that estimated selection acting on populations for which population size data was also available. The online search engine Google Scholar was used to search within studies citing Lande and Arnold (1983) using one or more of the following key terms: *natural population*, *wild population*, *population size*, *effective population size*, and *breeding pairs*. Keyword searches of all Google Scholar articles were also conducted using various combinations of the terms: *phenotypic selection*, *natural selection*, *sexual selection*, *natural population*, *wild population*, *population size*, *effective population size*, *breeding pairs*, *selection coefficient*, *selection differential*, and *selection gradient*. In assembling the databases selection studies included in earlier syntheses by Kingsolver et al. (2001) and Siepielski et al. (2009) were also reviewed and

included where they met the necessary requirements. Finally, population size databases provided in Leimu et al. (2006) and Palstra and Fraser (2012) were reviewed to determine whether any of the populations therein had also been investigated for selection.

Selection gradient and differential data

The criteria for inclusion in the selection database were similar to those of previous database compilations (Kingsolver 2001; Siepielski et al. 2009). Specifically, studies were only included that: 1) examined selection on quantitative traits in the study population(s), 2) performed measurements using wild populations living under natural conditions (i.e. studies of selection that involved experimentally or genetically manipulated populations were excluded), and 3) estimated selection using either standardized linear selection gradients (β), standardized quadratic selection gradients, standardized linear selection differentials (*s*), standardized quadratic selection differentials, or any combination thereof (Lande and Arnold 1983; Arnold and Wade 1984a, 1984b). These metrics estimate selection on a trait as the effect on relative fitness in units of phenotypic standard deviations thereby allowing cross-study comparisons of different populations, species, and traits. Linear selection gradients estimate the strength of selection directly on a trait by removing the effects of selection from correlated traits included in the analysis, whereas selection differentials estimate total selection on the trait including indirect selection on other, correlated traits (Lande and Arnold 1983). Quadratic selection gradients and differentials estimate the curvature of the selection function. Stabilizing selection implies negative quadratic gradients and differentials, while disruptive selection implies positive values, though the observation of negative and

positive values do not necessarily prove stabilizing or disruptive selection (Kingsolver et al. 2001).

A number of authors estimated selection in multiple years, but presented their data averaged over the time period of their study. I attempted to contact these authors directly to obtain year-specific selection coefficients, but these studies were included in the database irrespective of whether annual data was available. I also contacted authors where data were presented in figure format, and in several cases I extracted selection coefficients using the Figure calibration digitizing plugin available for ImageJ (Rasband 2011).

Population size data

Adult census population size, N, was used as the measure of population size in the analyses because only a small proportion of the total number of articles reviewed reported N_e , a caveat that is treated in the Discussion. Very few selection studies also reported estimates of N. For some of those studies lacking N data, N estimates were able to be obtained from other sources conducting work on the same population (other peer-reviewed publications, government technical reports, etc.). Where N data could not be obtained from the original article or related sources, authors were contacted directly to obtain the information. There were also seven papers in the selection database where N information was contained in figures; in these instances ImageJ was used to extract the relevant data digitally. Where selection was estimated in multiple years but only a range of N across all years was provided (annual data were not available), the harmonic mean of the range was used for each year. The harmonic mean rather than the arithmetic mean

was used since, over t generations, N_e in a fluctuating population is the harmonic mean of N_e and will be closest to the size of the generation with the smallest single generation N_e (Frankham 1995; Frankham et al. 2010), hence the harmonic mean will be more closely related to the genetic characteristics of the study populations.

The database included a large number of studies of colonially breeding or cavity nesting species of wild birds. For most of these studies the available population size metric was the total number of breeding pairs, therefore, the number of breeding pairs in a given year was multiplied by two in order to provide an approximation of N. For a few studies, (16 of 153 total populations) population size was reported only as being greater than a certain value (more specific N data could not be obtained); here the value itself was used as the estimate of N. For example, if N was estimated to be greater than 100000 individuals, 100000 was reported as the population size in the database. Where N was very large (7 cases) this was likely justified since it only constituted a small number of cases in the entire database and given that genetic diversity is sigmoidally related to N_e (Willi et al. 2006) I did not expect much difference between populations that were 10000 vs 20-30000 individuals for example, or between those above a certain threshold population size, genetically speaking. For eight additional populations N was specified as >500 and for one population N was >1000. Though a disproportionately larger difference in genetic diversity is expected with incremental changes in N among small or medium sized populations compared with very large populations, the exclusion of these populations did not affect the results, therefore these population were included in the analysis.

Selection-population size meta-analysis data

For each study that met the criteria, the species name and taxonomic grouping (vertebrate, plant, or invertebrate), common grouping (mammal, bird, fish, etc.), *N*, trait class (morphological vs. life-history), selection estimates with standard errors and *p*-values, and the sample size were recorded. Each individual row in the database corresponded to a single estimate of linear and/or quadratic selection gradient or differentials on an individual trait for a single selection episode in a study; therefore some studies contributed many data points to the analysis.

Statistical analysis

Standard errors for selection estimates were only available for 50% of the estimates in the database. Therefore, a formal meta-analysis for the subset of selection estimates with associated standard errors was conducted, and a qualitative sub-analysis using the entire dataset was also performed. It should be noted that relationships under the qualitative analysis assume all data points are independent, though this is not the case for studies which contribute multiple selection estimates or where the same population was assessed in multiple studies. In addition, standard errors for selection coefficients were negatively related to population size (Ch. 4 Appendix A), but the relationships were positive between sample size and population size (Ch. 4 Appendix B), together suggesting greater uncertainty in selection estimates for small populations. For these reasons, this sub-analysis was used only to investigate relatively whether similar trends to the meta-analysis might be observed with a larger number of populations, but the results should be interpreted cautiously.

Meta-analysis

For the subset of data that included standard errors we conducted random-effects meta-analyses using Bayesian techniques implemented in the package MCMCglmm (Hadfield 2010) in R (version 2.13.0; R Development Core Team 2011). The benefit of using this method is that it allowed for the effects of sampling error to be accounted for as well as for study and population-level autocorrelation. Species was also initially included as a random effect, however this did not result in a significantly improved model fit (models compared using Deviance Information Criterion, DIC; Spiegelhalter et al. 2002) therefore it was excluded from the analysis. The posterior modes of selection coefficients were calculated from models in which each selection coefficient was the response variable, and fixed effects were specified for population size (divided into four population size categories; <100 individuals, 100-499, 500-999, and $\geq$ 1000 individuals), trait class (morphology versus life history traits), taxa (plants versus vertebrates) and two-way interactions with population size. It was not possible to evaluate more specific taxonomic groupings (mammal, fish, plants, etc.) since there was not sufficient data at this level. Since using alternative priors produced similar results, the default (weakly informative) priors were used for all models. MCMC chains were run for 300000 iterations with a burn- period of 100000 and thinning interval of 50, hence parameters and associated confidence intervals were based on sampling the posterior distribution 4000 times. Since not only the direction of selection but also the magnitude (absolute value) was of interest, a second set of models were run which incorporated the folded

normal distribution (Hereford et al. 2004; Kingsolver et al. 2012; Morrissey and Hadfield 2012).

Populations with N close to 1000 individuals (which were included in the largest population size bin) could still potentially have a small N_e and hence might be genetically similar to populations in the smallest size bins (Frankham 1995; Palstra and Ruzzante 2008; Palstra and Fraser 2012). Therefore, a second set of analyses were conducted where the largest bin consisted of populations with an N greater than 4000 individuals which is close to the median across species minimum viable population size that was found in one meta-analysis (median = 4169 individuals; Traill et al. 2007).

Qualitative analysis: Directional and Variable hypotheses

Pearson's correlations were used to determine whether a directional relationship existed between population size and linear or quadratic selection gradients and differentials (direction and form of selection) and also the absolute values of linear selection coefficients (strength of selection). Because patterns may differ among different taxa, groups, or trait classes Pearson's correlations were also used to test for consistent directional trends between selection estimates and population size for morphological and life history traits, for vertebrates and plants, as well as for mammals, birds, and fish within the vertebrate class. Invertebrates and reptiles were excluded from the taxa and group specific analyses since only a few populations represented these categories.

To investigate whether there was increased variability in selection estimates at small population size White's test was used to determine whether the residual variance of selection coefficients was constant or heteroscedastic in relation to population size.

White's test implements an auxiliary regression analysis which regresses the squared residuals from the original regression model onto a set of regressors that contain the original regressors, the cross-products of the regressors, and the squared regressors (White 1980).

Results

Over 2000 studies were reviewed, 115 of which met the criteria for inclusion in the selection database. Eighteen of the studies were also included in a previous synthesis by Kingsolver et al. (2001) and an additional 47 studies overlapped with those included in Siepielski et al. (2009); thus, the meta-analysis found 50 additional studies with selection estimates (43% of the total). The database included 4097 records and 6963 individual estimates of selection across the four types of selection coefficients. The database represented 153 different populations across 73 species in six different taxonomic groups (see Tables 4.1 and 4.2 for summaries of the database). Most of the species included were widespread (88% of the total), generalist (89%) and diploid (81%). Overall, there were 59% more estimates of linear versus quadratic selection and 15% more estimates of selection gradients than selection differentials. The full database was biased towards estimates of selection for vertebrates (specifically for birds) than for plants or invertebrates, and there were also more estimates for morphology (3912 total selection estimates) than for other trait types (3047 individual estimates; Table 4.2)

Meta-analysis

Since meta-analysis accounts for the influence of sampling error, it should therefore provide a powerful means of detecting potential differences related to population size. Unfortunately, standard errors were not reported in a large proportion of the studies included in the database and were available for 62% of linear selection gradients, 29% of linear selection differentials, and 69% of quadratic selection gradients (meta-analysis using quadratic selection differentials was not attempted because of the small number of estimates relative to the other types of selection coefficients).

Meta-analysis: linear selection gradients

Results of the meta-analysis for data with associated standard errors showed little evidence for a relationship between population size and the direction or magnitude of linear selection gradients, the selection coefficient for which there was the largest amount of data. HPD confidence intervals were overlapping for all population size bins suggesting no statistically significant difference in the direction or strength of selection. The point estimates were also similar, although there was a trend for decreasing linear gradient values as population size increased (Fig. 4.1). The same trend was observed in the supplementary analysis where the largest population size bin contained only populations of $N > 4000$ (Fig. 4.1). There was also no difference in linear gradient values or strength of linear gradients among trait types or taxa between the different population size bins (Figs. 4.2 and 4.3). Linear gradients for morphological traits tended to be shifted towards more positive values than life history traits but not significantly so (Fig. 4.2). Likewise, the posterior modes of linear gradient values were slightly higher for plants than for vertebrates, but the magnitude of selection was similar when the largest bin was

N >1000 (Fig. 4.3). Conversely, the magnitude of selection appeared to be significantly greater for plants than vertebrates in the two smallest bins when the largest bin was N > 4000, but this may be because of the greater degree of uncertainty around the selection estimate in the smallest bins for plants.

Meta-analysis: linear selection differentials

Although there was less data available for meta-analysis using linear selection differentials than gradients, largely similar trends were observed: there was no difference in linear differential values in relation to population size (Ch. 4 Appendix C, Fig. C1). Furthermore, although the mean magnitude of selection was greater in the largest size bin (*N* > 1000), this bin also had larger confidence intervals and confidence intervals for all three *N* bins overlapped. Similarly the *N* > 4000 bin was significantly greater than for the two smaller bins but again, this was likely due to the large degree of uncertainty around this estimate due to the relatively small number of data points (Fig. C1). Selection on different traits and taxa did not differ significantly among the different population size bins. The posterior modes were also similar, although morphological traits tended to have slightly greater modes of linear differentials than life history traits, and likewise plants had higher posterior modes than vertebrates (Appendix C, Fig. C2).

Meta-analysis: quadratic selection gradients

No significant differences were observed in the form of selection in relation to population size, although there was a trend for mean quadratic selection estimates in the smallest population size to be shifted towards negative values, possibly suggesting weak

stabilizing selection (Ch. 4 Appendix D, Fig. D1). Quadratic gradient values for traits and taxa were similar across the different population size bins. There was also no difference in the form of selection acting on life history compared to morphological traits (Appendix D, Fig. D2) or for vertebrates compared to plants (Appendix D, Fig. D3). Overall, the posterior mode of quadratic selection estimates was near zero, which was also found in a previous meta-analysis (Kingsolver et al. 2012).

Qualitative analysis

Directional hypothesis: strength, direction, and form of selection

Results of Pearson's correlations largely were consistent with the results of the meta-analysis, revealing predominantly weak, non-significant relationships between the absolute value of linear selection coefficients with population size (Table 4.3 and Figs 4.4 and Ch. 4 Appendix E, Fig. E1). The direction of the relationships differed between linear gradients and differentials but this may have been due to the larger amount of data available for the linear selection gradients compared to linear differentials, specifically at large population size. In regards to the direction of selection, almost all correlations for linear selection coefficients with population size were negative and significant (Table 4.3, Fig. 4.5, and Ch. 4 Appendix F, Fig. F1) yet the relationships were still relatively weak (all $r < 0.281$). Likewise, relationships for quadratic selection gradients also were consistent with the meta-analysis in that nearly all relationships for quadratic selection gradients with population size were positive but weak and none were statistically significant (Table 4.3 and Appendix F, Fig. F2). The opposite was true for the

relationships between population size and quadratic selection differentials, probably due to the small number of selection estimates (Table 4.3 and Appendix F, Fig. F3).

Variable hypothesis

There was statistically significant heteroscedasticity in 10 of 16 and 8 of 16 data subsets for linear and quadratic coefficients, respectively calculated from White's tests (Table 4.4 and Appendix F). Examination of the residual plots however, showed that in only two instances was the increased variability at the smallest population sizes (N <100; Table 4.4) while for most of the remainder of significant tests the increased heteroscedasticity was located between N = 100-1000.

Discussion

There was little evidence for differences in the extent of natural selection as populations are reduced in size. This result is especially notable given the general lack of research investigating patterns of selection in relation to population size in wild species (but see Murua et al. 2010; Weber and Kolb 2013). No evidence was observed for a consistent directional relationship between the magnitude of selection coefficients and populations size suggesting that the strength of selection did not differ among populations differing in N. Meta-analysis results revealed HPD 95% confidence intervals of the posterior modes of the magnitude of linear coefficients that overlapped for all population size bins and also correlations for the absolute values of linear selection coefficients were weak. Although the direction of linear selection consistently decreased with increasing population size, the posterior modes of linear selection coefficients were not significantly

different among size bins such that the mean direction of selection was also similar. There was also no evidence for significant differences in the form of quadratic selection with population size or in the direction or magnitude of selection coefficients acting on different trait classes or taxonomic groups among the different population size bins.

The Variable hypothesis predicts increasing variability in selection coefficients as population size is reduced; while 18 of 32 total White's tests were significant for heteroscedasticity, in only two cases were selection coefficients more variable in the smallest population size bin ($N > 100$). Examination of residual plots revealed that for most of the significant tests, selection coefficient values were more variable at $N = 100$-1000, a range of population sizes that likely still includes many populations with small N_e (Frankham 1995; Palstra and Ruzzante 2008; Palstra and Fraser 2012). Still, the White's tests did not account for the potential influence of increased sampling error at small population sizes, and there was also less data available at the largest population size, therefore these results should be interpreted with caution.

Two prior empirical field studies found evidence to support the Variable hypothesis (Willi et al. 2007; Wood et al. 2014). For the latter study however, this did not translate into more variable phenotypic plasticity, V_A or Q_{ST} among the small versus the large populations (Chapters 2 and 3). The explanation was suggested to be due to a lack of long term data on temporal and spatial environmental conditions. Long term fluctuating environmental conditions may have resulted in complex fluctuating selective pressures and similar levels of genetic variation, differentiation, and plasticity among both the small and large populations (e.g. Blanckenhorn et al. 1999; Siepielski et al. 2009; 2013). I postulate that similar processes might account for the results of this study

and therefore extend to a wide variety of taxa. According to the classic breeder's equation, the strength of selection is calculated as h^2 divided by the selective response ($S= h^2/R$; Falconer and Mackay 1996). A lack of relationship between selection pressures and population size could thus be partly accounted for by variability in the amount of quantitative genetic variation retained among populations of all sizes owing to fluctuating environmental conditions over time.

Meta-analysis limitations and caveats

Harmonic mean N was used as the estimate of population size, but it is the effective population size N_e which dictates rates of genetic drift and inbreeding. Very few studies were found that estimated selection on populations for which N_e data was also available so relationships with N_e could not be examined in this analysis. However, point estimates of population size in this study ranged from four to one million such that the database likely also adequately captured a large range of N_e. Specifically, there were 28 populations in the study (18% of the total) with N estimates less than 50, well below the minimum population size at which populations are expected to suffer disproportionately from inbreeding and reduced adaptive potential (Willi et al. 2006; Frankham et al. 2014).

One potentially influential factor that could not be accounted for was the degree of isolation of the populations included in the database. An unknown proportion of the populations might experience some level of gene flow from conspecific populations, however most studies lacked information to fully evaluate this possibility (e.g. analysis of neutral genetic markers). Gene flow might help to retain genetic variation in small populations that would otherwise be lost by genetic drift and inbreeding and thus might

have affected the observations regarding patterns of selection in relation to population size (Jamieson and Allendorf 2012). Nevertheless, the results herein are still relevant given the extreme paucity of data linking selective regimes to population size, but research investigating selection in natural populations that are known to be isolated is clearly needed. Isolated populations specifically are predicted to lose genetic diversity and adaptive potential over time and therefore merit greater concern from a conservation perspective.

Although the database included a large number of selection estimates across a large number of populations and species, the analysis may have suffered from a lack of statistical power particularly at the largest population sizes. While selection is frequently measured for large populations, there are fewer attempts to formally quantify *N* likely because such populations might occupy a large range, or because of uncertainty in demarcating the boundaries of large, continuous populations. Still, for the selection coefficients for which the most data was available (linear selection gradients) results of the meta-analysis showed that the strength and direction of selection did not differ between populations of $N > 100$ versus $N = 100$ -1000. This represents an important finding again given the rate of loss of genetic diversity is predicted to increase (and strength of selection decrease) as population size is reduced (Willi et al. 2006).

Finally, there is the question of whether the populations included in this database are representative of all populations in nature, i.e. there might be a systematic bias in the types of populations/taxa that are chosen for selection analyses. For example, populations with temporally replicated estimates of selection might be more likely to inhabit stable environments and experience stable selective regimes since this permits the repeat

application of standardized methods of data collection and analysis (Morrissey and Hadfield 2012). The selection database was heavily biased in favor of longitudinal studies however, the range of population sizes was large and there also a large number of populations with $N < 500$ ($N_e < 50$ applying a conservative average N_e/N ratio of 0.1; Frankham 1995). Hence, even if the database was biased towards populations living under more stable environmental conditions, it implies that small populations may not always inhabit suboptimal habitats where they lose genetic variation and adaptive potential more rapidly than large populations.

Future research needs

This study revealed various lacunae that need to be addressed before solid conclusions can be reached regarding the nature of selective regimes in relation to population size. First, empirical research which focuses on populations that are truly isolated will be critical to elucidating the actual link between selection and population size in nature. A related point, given the importance placed on population size in conservation biology but the general lack of data that relates population size to population-level processes in nature, is that greater effort should be made to formally estimate population size in future selection studies. Specifically, given the increasing accessibility of methods for estimating N_e (Waples 2013; Palstra and Fraser 2012) future studies are encouraged to include estimates of this important metric in their work as it corresponds more closely to genetic characteristics of natural populations (Frankham 1995; Willi et al. 2006; Palstra and Fraser 2012).

A call for additional studies which estimate selection and population size for taxonomic groups which were underrepresented in this dataset is also warranted. For example, no instances where estimates of population size and selection coefficients overlapped for species of amphibians were found, yet this is one of the planet's most threatened taxonomic groups (IUCN 2013). Likewise, there were only a handful of populations of reptiles and invertebrates where *N* was also estimated, and even for mammals the data was restricted to a relatively small number of well-studied systems. In the same vein, a truly representative database would require data on populations that are rare as well as widespread, habitat specialists and generalists, etc.; this database was biased towards widespread, generalist, diploid species, such that the capacity to extrapolate this data to other systems is limited. Additionally, as the database was weighted towards population sizes of $N < 100$ to 1000, more studies are required which measure selection and population size for very large populations; these will provide the most important contrast for comparison with selection at small population sizes.

As a last point, recommendations by Kingsolver et al. (2012) that researchers report standard errors for selection coefficients in their studies are echoed here; this will facilitate and drastically improve the statistical power of future syntheses regarding patterns of natural selection in wild populations.

Conservation implications

This study represents an important first attempt to investigate the relationship between natural selective pressures and population size across a large number of populations and species in nature. Similarities in patterns and the extent of selection across a wide range of population sizes were observed suggesting that populations at various levels of *N* and

N_e may experience a variety of environmental conditions. If selection is strong in some habitats occupied by small populations, and if these populations also have adequate quantitative genetic variation, it would counter one of the primary assertions of conservation biology, namely that response to selection is reduced at small versus large population size. Quantitative genetic variation was found to be similar for small and large brook trout population (Chapter 3) while a separate study that compiled data from a large number of experimental studies found evidence to suggest that h^2 might only be reduced at extremely small population sizes ($N < 10$; Willi et al. 2006). If these results are not exceptional, response to selection at small population size might be more extensive than previously assumed; more data is clearly needed to determine the relationship between quantitative genetic variation and population size among wild populations of a large variety of taxa.

Encouragingly, these results also imply that small populations may not always occupy suboptimal habitats resulting in more rapid loss of quantitative genetic variation and adaptive potential (Frankham 1996; Willi et al. 2006; Kawecki 2008). Thus, even though genetic drift may indeed become more important as population size decreases, selection may also be stronger in some fragments if conditions become more extreme or variable as fragment size decreases. If true, some small populations in nature may retain their ability to adapt to future environmental change.

Table 4.1: Summary of selection database characteristics. The database includes studies of phenotypic selection in natural populations from 1984-2013 for which population size data was also available.

	Number of items
Studies	115
Species	73
Populations	153
Records (total)	4097
Linear gradients	2693
Linear differentials	2364
Quadratic gradients	1174
Quadratic differentials	956
Taxon:	
Vertebrates (all)	4734
Mammals	897
Birds	2641
Fish	1087
Reptiles	109
Plants	1840
Invertebrates	385

Table 4.2: Number of estimates of linear and quadratic selection as a function of taxon and trait type.

Taxon		Trait	
Linear selection gradients:			
Mammals	213	Morphology	1079
Birds	1177	Life history	1457
Fish	289	PC	80
Reptiles	56	Behaviour	11
Plants	765	Other	9
Invertebrates	136		
Linear selection differentials:			
Mammals	573	Morphology	1612
Birds	591	Life history	534
Fish	395	PC	142
Reptiles	29	Behaviour	7
Plants	623	Other	12
Invertebrates	96		
Quadratic selection gradients:			
Mammals	60	Morphology	478
Birds	579	Life history	596
Fish	157	PC	35
Reptiles	17	Behaviour	0
Plants	231	Other	8
Invertebrates	73		
Quadratic selection differentials:			
Mammals	51	Morphology	743
Birds	294	Life history	129
Fish	246	PC	24
Reptiles	7	Behaviour	0
Plants	221	Other	3
Invertebrates	80		

Table 4.3: Pearson's correlations, for the relationship between selection coefficients and population size for different subsets of the selection database.

	Magnitude		Direction			
	Linear gradients	Linear differentials	Linear gradients	Linear differentials	Quadratic gradients	Quadratic differentials
All	-0.035	0.048*	-0.136***	-0.055**	0.036	-0.130***
Morphology	-0.035	0.0011	-0.011	-0.037	0.053	-0.148***
Life history	-0.032	0.139**	-0.204***	-0.096***	0.033	0.208*
Plants	-0.015	0.046	-0.232***	-0.281***	0.13	-0.163*
Vertebrates	-0.019	0.107***	-0.203***	0.192***	0.011	-0.216***
Birds	-0.0075	0.096*	-0.160***	-0.060	0.013	0.174**
Fish	-0.114	0.098	-0.188***	-0.208***	-0.014	-0.337***
Mammals	-0.048	0.021	-0.049	0.0052	0.14	-0.054

*<0.1,**<0.05,***<0.001

Table 4.4: White's test *p*-values to test for increased heteroscedasticity of selection coefficients in relation to population size for different subsets of the selection database. Significant *p*-values in bold indicate were increased variability in selection coefficients was located at $N < 100$.

	Linear gradients	Linear differentials	Quadratic gradients	Quadratic differentials
	W p-value	*W p*-value	*W p*-value	*W p*-value
All	0.99	<0.001	0.084	0.002
Morphology	0.26	0.0089	0.14	0.0028
Life history	**0.042**	<0.001	0.066	0.059
Plants	0.15	<0.001	0.99	0.027
Vertebrates	0.068	<0.001	**0.019**	<0.001
Birds	0.22	0.16	0.39	0.011
Fish	0.045	0.020	0.16	<0.001
Mammals	0.20	0.014	0.23	<0.001

*<0.1,**<0.05,***<0.001

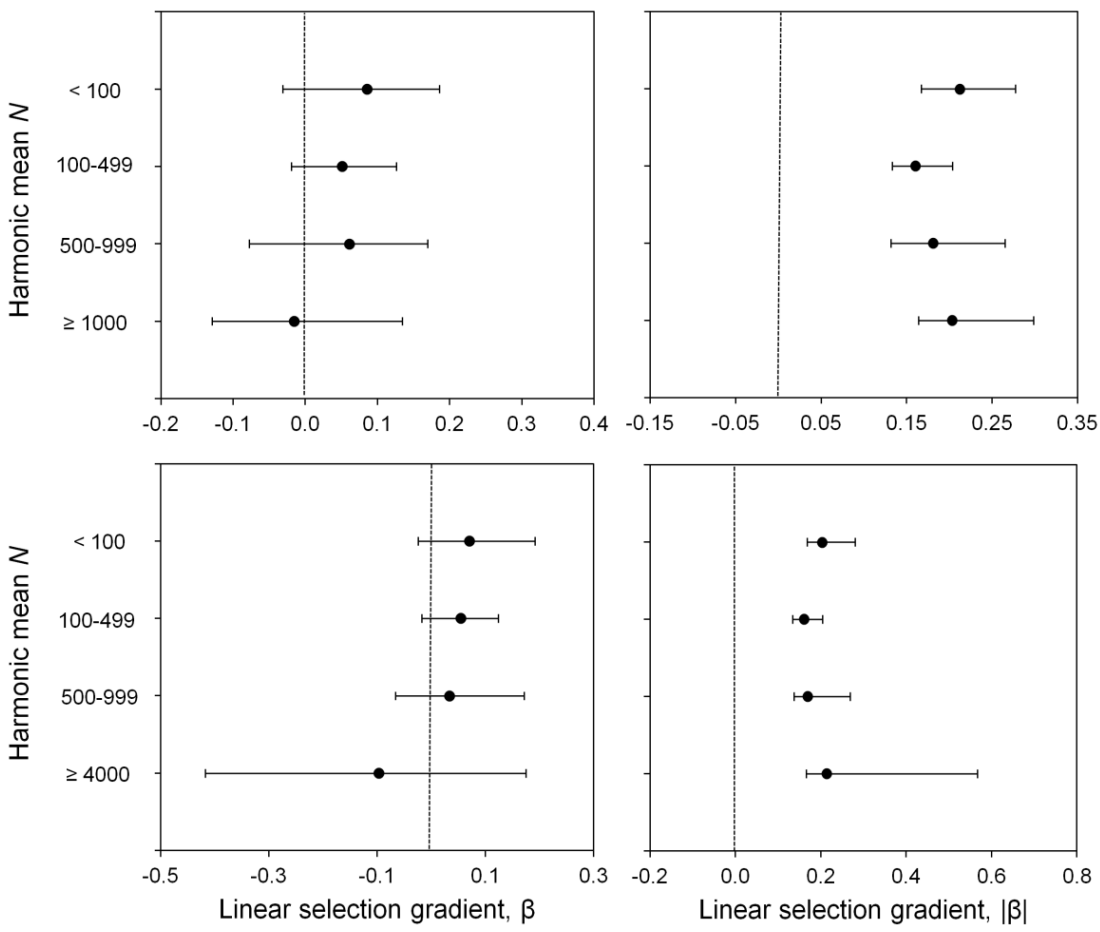

Figure 4.1: Posterior modes for linear gradient values, β and the magnitude of linear selection gradients, |β| in each of four different population size bins. The magnitude of selection was calculated using the folded binomial distribution. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm.

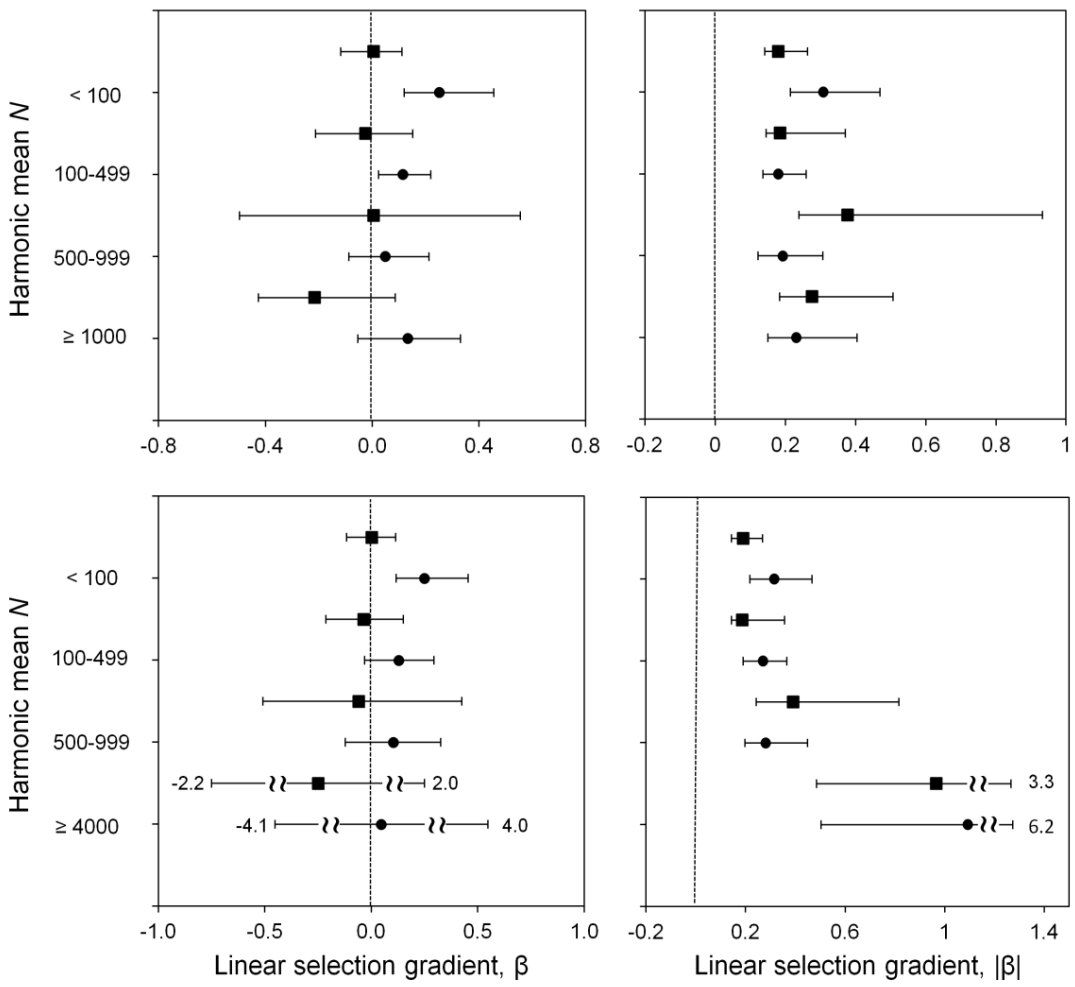

Figure 4.2: Posterior modes for linear gradient values, β and the mean magnitude of linear selection gradients, |β| for morphological traits (●) and life history traits (■) in each of four different population size bins. The magnitude of selection was calculated using the folded binomial distribution. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large.

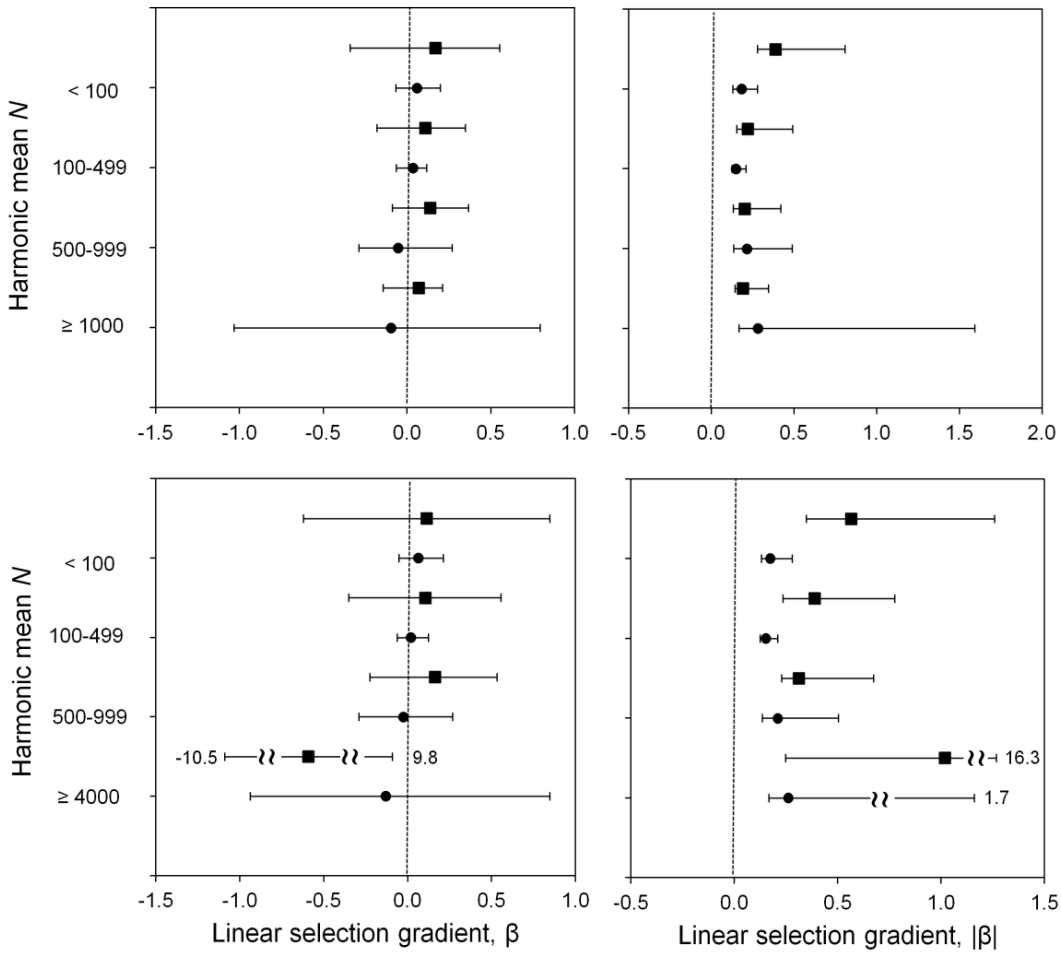

Figure 4.3: Posterior modes of linear gradient values, β and the mean magnitude of linear selection gradients, |β| for vertebrates (●) and plants (■) in each of four different population size bins. The magnitude of selection was calculated using the folded binomial distribution. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large.

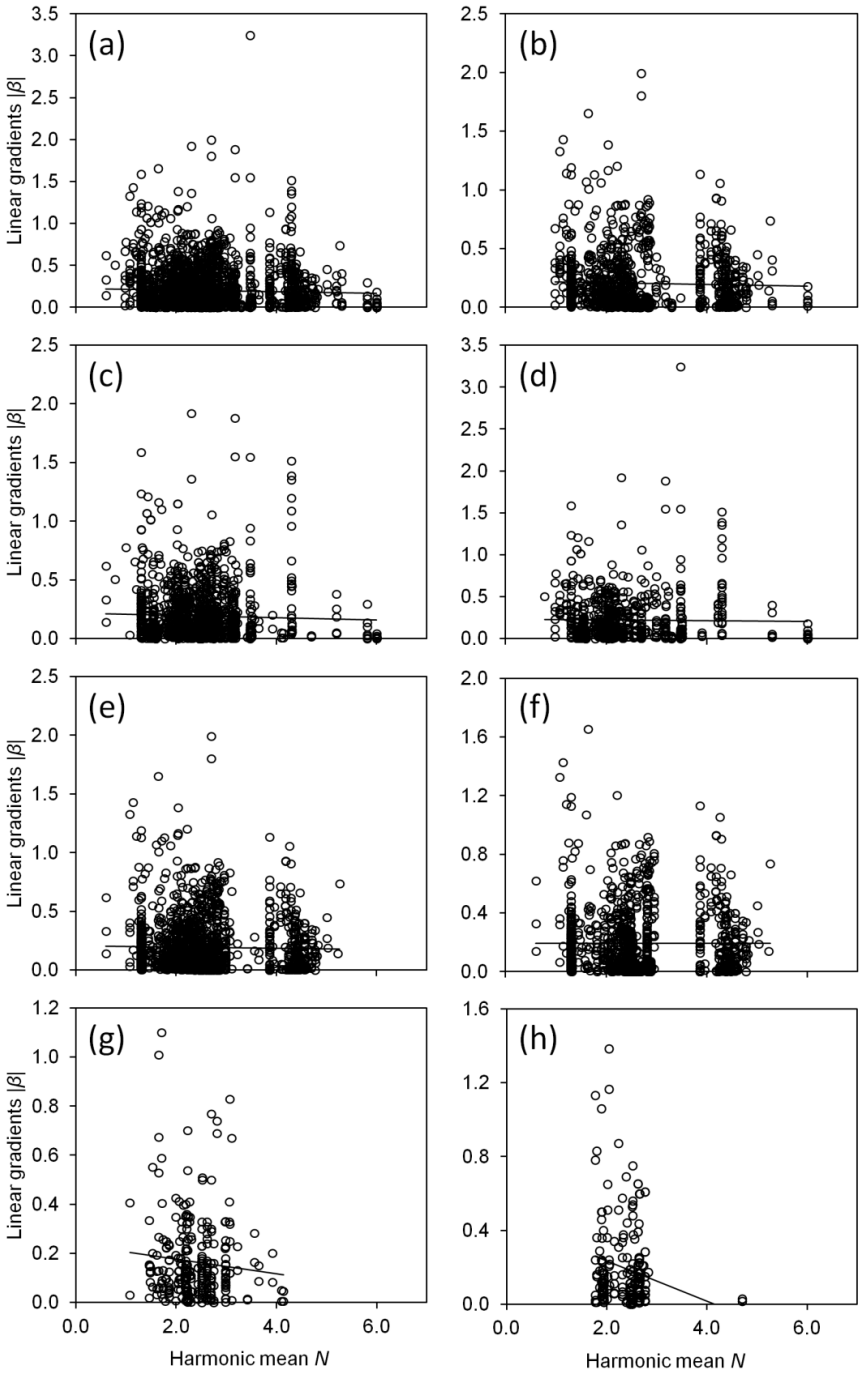

Figure 4.4: Absolute values of linear selection gradients to assess the strength of directional selection in relation to population size across a) all taxa and trait types, b) life-history traits c) morphological traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.

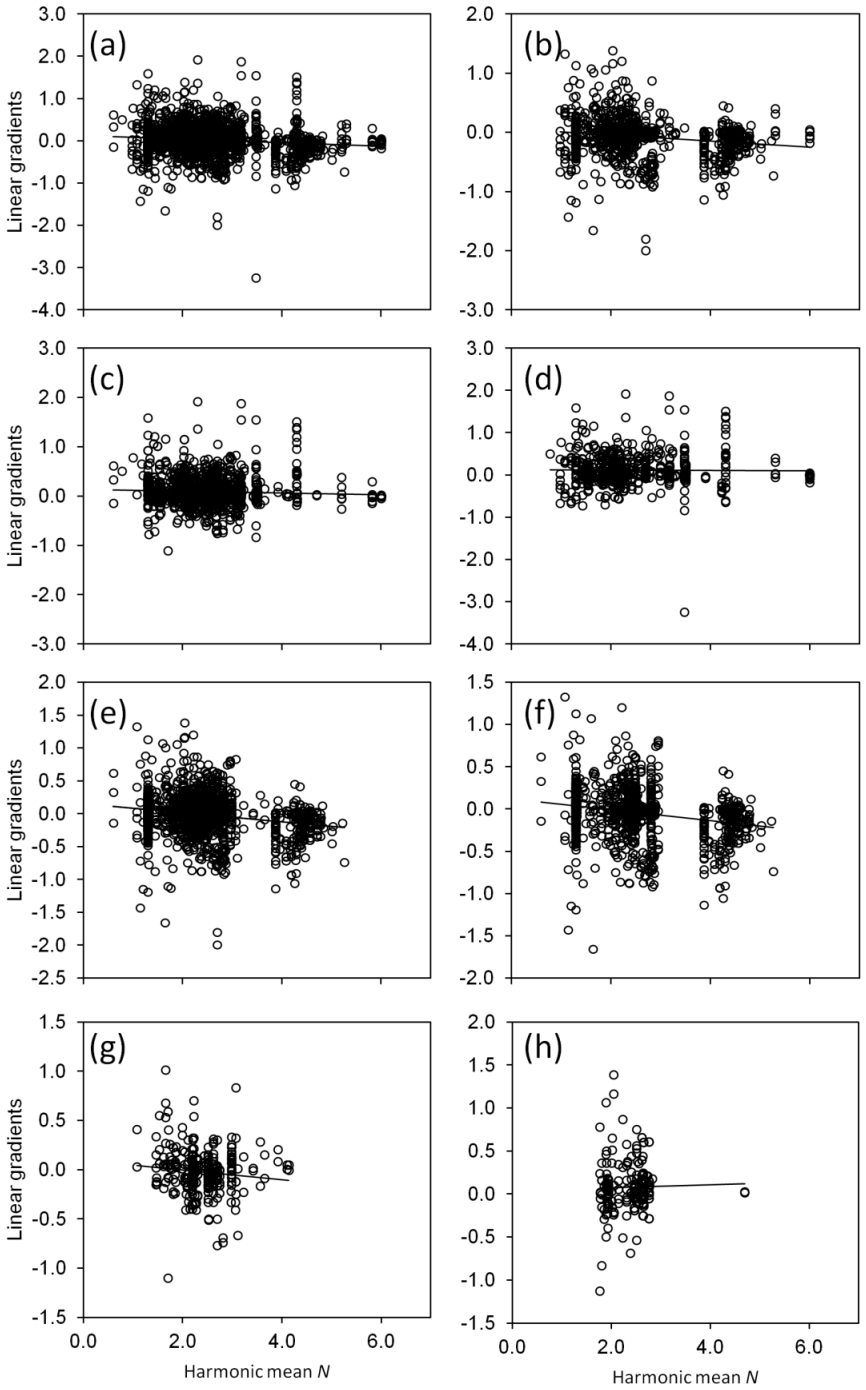

Figure 4.5: Linear selection gradient estimates to assess the direction of selection in relation to population size across a) all taxa and trait types, b) life history traits, c) morphology traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.

General Discussion

As wild species are divided into increasingly smaller and more isolated populations due to habitat fragmentation, conservation genetics predicts that small populations will suffer disproportionate reductions in genetic diversity and adaptive potential relative to large populations (Willi et al. 2006; Frankham et al. 2010). While there is a wealth of theoretical and empirical work using laboratory populations that support these expectations (Willi et al. 2006; Frankham et al. 2010), results of the few field studies using natural populations have been inconclusive (Widen and Andersson 1993; Waldmann and Andersson 1998; Meyer and Allen 1999; Podolsky 2001; Waldmann 2001; Willi et al. 2006). A second issue is the general lack of consideration in conservation biology, both theoretical and empirical, that addresses how habitat fragmentation might alter selection regimes as fragment size and population size decrease. This last point has potentially important implications for evolution and conservation if fragmentation affects the adaptive genetic characteristics of populations and their subsequent responses to environmental change (Willi et al. 2007; Willi and Hoffman 2012).

Two alternative hypotheses for how habitat fragmentation might affect environmental conditions have been suggested in previous population genetic field studies (Willi et al. 2007; Willi and Hoffman 2012). First, habitat characteristics might shift in a consistent directional manner as habitat fragments and populations are reduced in size (Directional hypothesis; Willi and Hoffman 2012) resulting in similar directional relationships between population size and quantitative genetic variation, trait differentiation, plasticity and selective pressures. Alternatively, fragmentation might

result in increased between-fragment variability in habitat conditions among small versus large population fragments (Variable hypothesis: Willi and Hoffman 2012), and consequently, increased variability in selection regimes, quantitative genetic variation, trait differentiation, and phenotypic plasticity.

Directional versus Variable hypothesis

Habitat characteristics for 19 fragmented populations of brook trout at Cape Race, Newfoundland, varying widely in population size supported the Variable Hypothesis (Chapter 1). Across two years and in relation to two population size metrics (N and N_b) there was a wider range of variances around habitat parameter means among small versus large populations; increased variability in habitat parameter means at smaller population size was also observed, although to a lesser extent. These observations included variables that might be particularly important for adaptation of Cape Race populations such as temperature, pH, stream depth and velocity (e.g. Hutchings 1993; Belmar-Lucero et al. 2012). There was comparatively little support for the consistent directional differences predicted under the Directional Hypothesis as evidenced by the small proportion of GAM models which suggested a directional change from small to large population size (Chapter 1).

While the assessment of habitat characteristics among Cape Race brook trout populations supported the Variable hypothesis, this apparently didn't translate into increased variability in phenotypic plasticity (Chapter 2), quantitative genetic variation (V_A; Chapter 3), trait differentiation (Q_{ST}; Chapter 3) among the small versus the large populations. There was almost no evidence for differences in phenotypic plasticity in

relation to population size (Chapter 2). This included at incubation temperatures that would be considered extreme at Cape Race (9°C; Ch. 2 Appendix A). Small populations did not exhibit either consistently greater or consistently reduced plasticity relative to large populations and there was no evidence that small populations might express a greater variety of plastic responses than large populations. Because of the inability to generate larger numbers of families in the small Cape Race populations for ethical reasons statistical power of the analysis was low, but this is likely typical for common garden studies of vertebrates and the mean number of families used per population here (18.2) was more than most analogous studies involving fish (see Hutchings 2011).

Likewise, there were no consistent differences in V_A and Q_{ST} in relation to population size (Chapter 3) despite a large number of traits investigated over three trait classes, and using a larger number of families and populations than other previous similar studies (Koskinen et al. 2002, Jensen et al. 2008). Confidence intervals calculated for V_A and pairwise Q_{ST} however, were large suggesting that even larger numbers of families and populations (>20; O'Hara and Merilä 2005) may be required to make firm conclusions regarding quantitative genetic characteristics of vertebrate populations in nature; this would be difficult to carry out for most species.

Patterns of selection and population size

A widespread assumption in the conservation biology literature suggests a link between the efficacy of natural selection and population size, but there is a notable lack of empirical research that has investigated the extent of selection in relation to population size to substantiate this prediction (but see Murua et al. 2010; Weber and Kolb 2013). In

a meta-analysis including populations ranging in size from four to one million there was little reinforcing evidence for systematic differences in the extent of selection in relation to population size (Chapter 4). Neither the strength, direction, nor the form of selection differed among populations differing in *N* including for different trait types (morphology versus life-history traits) and taxa (plants versus vertebrates). There was evidence for increased variability in selection coefficients between $N = 100\text{-}1000$ which might include many populations with small N_e (Frankham 1995; Palstra and Ruzzante 2008; Palstra and Fraser 2012). However, these tests did not account for the potential influence of increased sampling error at small population sizes, and there was also less data available at the largest population size, therefore these results should be interpreted with caution.

Although the finding of no difference in selection in relation to population size is a novel result, there are several gaps that will need to be addressed in future works. Specifically, more data is needed on populations known to be isolated, and across different taxonomic groups and types of species (rare versus common, generalist versus specialist, etc.). Future studies estimating selection in large populations should attempt to overlap this data with formal estimates of population size. Regular reporting of standard errors will also greatly improve the efficacy of future syntheses, as would greater overlap of studies estimating selection and N_e which corresponds more closely to genetic characteristics of natural populations (Frankham 1995; Willi et al. 2006; Palstra and Fraser 2012).

Total census population size (N) versus effective number of breeders (N_b)

The relationships observed in Chapters 1 and 3 were not always similar between N and N_b which is not surprising since aspects of the habitat and mating system of brook trout can result in different N_b/N ratios among populations (Belmar-Lucero et al. 2012). Although the use of N versus N_b did not produce differing results in Chapter 2, overall several lines of evidence suggest that using N to infer the magnitude of N_b or vice versa might lead to erroneous conclusions. In Chapter 1, relationships of habitat variability to population size exhibited different patterns depending on whether N or N_b was used, and in Chapter 3, heteroscedasticity tests of V_A were more often significant using N_b than N.

Why the lack of relationship between population-level parameters and population size?

The lack of support for both hypotheses in Chapters 2 and 3, despite support for the Variable hypothesis in Chapter 1 may have been due to the lack of long term data on environmental characteristics at Cape Race. Long term fluctuating environmental conditions may have resulted in complex fluctuating selective pressures and similar levels of plasticity (Chapter 2), quantitative genetic variation (Chapter 3), and trait differentiation (Chapter 3) among both small and large Cape Race populations (Blanckenhorn et al. 1999; Siepielski et al. 2009; 2013). Additionally, in Chapter 3, negative correlations between V_A and selection generated due to environmental heterogeneity (Merilä et al. 2001; Wilson et al. 2006), and phenotypic plasticity (Chapter 3) may have helped to buffer the loss of adaptive genetic variation at small population size for some populations (Schlichting 1986; Sultan 1987). A similar process might account for the results of the meta-analysis of selection (Chapter 4) and therefore it might extend to a wide variety of taxa.

General Conclusion

Though small Cape Race brook trout populations inhabited a larger variety of habitats than large populations, the small populations did not significantly differ from large populations either in the magnitude or variability of quantitative genetic variation, trait differentiation, or phenotypic plasticity. The lack of difference in V_A in relation to population size is especially notable since standing levels of neutral genetic variation in Cape Race populations is positively correlated with population size; this point provides additional evidence against the assumption that neutral and quantitative genetic variation are correlated (see also Reed and Frankham 2001). There was also little support for differences in selection pressures in relation to population size for populations of wild species in general. Though isolation could not be confirmed in the meta-analysis, the results are in agreement with the findings for small versus large populations at Cape Race which have been isolated for some time, and therefore might apply to isolated populations of other species as well.

Overall, the results of these studies are notable in that they (i) contradict the frequently cited assumption that the environments occupied by small populations tend to be marginal and (ii) dispute the major tenets of the conservation genetics small population paradigm. Specifically, they oppose the prediction that small, isolated populations lose quantitative genetic variation related to adaptive potential more rapidly than large populations (Frankham 1996; Kawecki 2008), and that selection becomes less effective owing to the increasing influence of genetic drift. Although genetic drift may indeed become more important as population size decreases, selection may also be

stronger in some fragments if conditions become more extreme or variable as fragment size decreases. Regarding the capacity of small populations to evolve, the results imply that even at small population size, populations of some species might retain the adaptive potential necessary to cope with future environmental change. Several Cape Race populations included in this study had N_b estimates of less than 50 and at least one (DY) most likely also had N_e of less than 50. An N_e of 50 is often cited as a lower threshold size below which the effects of inbreeding depression and loss of genetic diversity are expected to be greatest (Franklin 1980). Many populations in the meta-analysis also had $N < 50$; even with genetic compensation the N_e of these populations would still be below the lower threshold of conservation genetic MVP guidelines. Even if these populations are not completely isolated, the results of the meta-analysis provide an encouraging signal for conservation biology that as long as there is a small amount of gene flow, even extremely small populations may be able to respond to selection.

Certainly, not all small populations will be able to adapt to future environmental change. Minimum viable population sizes for brook trout might be shifted downwards relative to many other species since brook trout are a colonizing species able to occupy diverse aquatic habitats and potentially deal more effectively with environmental stress. Brook trout furthermore exhibit residual tetraploidy and hence might lose genetic diversity at slower rates than diploids possibly resulting in an enhanced capacity to deal with small population size relative to other species (Allendorf and Thorgaard 1984; Frankham et al. 2010). Still, the results herein are important given the scarcity of such research on salmonids, a socio-economically important group of fish species, and vertebrates in general.

Future avenues of research should involve developing criteria for distinguishing viable small populations from those that are likely to become extinct, though ultimately, the evolutionary responses of small populations likely depend on how the magnitude and rate of environmental change interacts with prevailing conditions within habitat fragments over long time periods. A larger number of comprehensive, long term studies of populations from different taxa and types of species are needed, but may not always be possible, or timely enough to aid small populations that might face a threat of extinction in the near the future. However, integrative studies of wild populations such as the ones described herein that combine environmental, demographic, molecular genetic, and quantitative genetic data can contribute crucial knowledge to conservation and management programs that may improve the chances of some small populations to persist into the future.

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Chapter 1 Appendices

Appendix A: Detailed description of habitat data collection methodology and habitat character estimates for 2010 and 2011.

A.1 Data collection methodology for stream-scale characteristics.

A.1.1 Drainage area

Drainage area dictates stream size; larger streams can support larger populations, which may be less vulnerable to environmental and demographic stochasticity (Lande 1993), and are more likely to contain the diverse range of habitat types needed by salmonids at different stages of life history. For example, juveniles are typically found in shallow, riffle areas, whereas adult fish prefer large, deep pools (Gibson and Cutting 1993). In one study, watershed area was the one basin-scale habitat attribute found to be useful as a coarse filter for predicting translocation success of cutthroat trout (Harig and Fausch 2002). Large watersheds are also likely to have sufficient input of large woody debris and boulders to create physical structure in pools. Drainage area was measured in this study using MapWindow open source GIS software (http://www.mapwindow.org/ downloads /index.php?show_details=1, MapWindow Open Source Team 2008) in conjunction with the MSWAT plug-in (SWAT Development Team 2009). Digital elevation maps (90 m resolution) for Cape Race were obtained at http://srtm.csi.cgiar.org /SELECTION /inputCoord.asp (Jarvis *et al*. 2008). Once the drainage basins had been delineated using MSWAT, an additional plug-in was used to convert the shape file to a KML formatted overlay which was then imported into Google Earth (Google 2011). Drainage areas were then calculated using ImageJ (Rasband 2011). Details on data pre-

processing, and the use of MSWAT can be found at the WaterBase website: http://www.waterbase.org/ (Briley 2010; Leon 2007, 2010).

A.1.2 Stream order

Stream order can be a useful indicator of stream size as well as the types of habitat that can be expected to be present within a stream. Gradient and elevation decrease and discharge increases with stream order. The stream channel also becomes wider, deeper, and slower, water temperature becomes warmer with less variation, substrate size and canopy cover decreases, with associated changes in invertebrate communities (Quinn 2005). Stream order may thus dictate the abundance and diversity of salmonid species found within a particular stream. Stream order in this study was determined from satellite images using Google Earth (Google 2011).

A.1.3 Stream length

Stream length is another metric related to stream size and the quantity of habitat that could potentially be used by salmonids. Stream length was measured using the path tool of Google Earth; length was measured along the thalweg from the mouth to the end of each stream.

A.1.4 Sinuosity

Sinuosity controls the pool-riffle sequence and therefore is responsible for providing the variety of flow conditions required by salmonids for cover and foraging. Fast water habitat is maximized at low sinuosity values, whereas a high degree of

sinuosity provides diverse habitat and fauna (Gordon, McMahon and Finlayson 1992). A higher density of redds has also been found to be associated with increased sinuosity (Ono 1995). Stream sinuosity was measured using the path tool of Google Earth; sinuosity was calculated as the stream -length along the thalweg divided by the valley length.

A.1. 5 Gradient

Gradient is a stream-level character that determines the shape of a stream channel. For example, at steep gradients, the channel is often narrow, confined, has a high current velocity and is characterized by boulders and large woody debris. At lower gradients the stream changes shape, adopting step-pool, plane bed, and pool-riffle morphologies (Montgomery and Buffington 1997). High stream gradient can act as a barrier to movement for salmonid populations and can result in population substructure (e.g., Latterell 2001). Different species of salmonids utilize habitats at differing gradients; for brook trout, abundance has been found to decrease with increasing gradient (Chisolm and Hubert 1986). Percent gradient of CR streams was estimated using Google Earth by dividing the change in elevation from the mouth to the top of each stream by the total stream length, multiplied by 100.

A.2 Data collection methodology for transect-level characteristics

A.2.1 DO

Reduced concentrations of dissolved oxygen (DO) can adversely affect the swimming performance of salmonids. For example, maximum sustained swimming

speeds of coho salmon and brook trout at temperatures of 10-20°C were reduced when DO dropped below air-saturation levels, and performance declined sharply when DO fell to 6.5-7.0 mg/L (Graham 1949). Dissolved oxygen levels can also affect the survival and rate of development of embryos (Alderice, Wickett and Brett 1958; Garside 1966). Dissolved oxygen was measured at every fourth transect using a WTW Multiline P4 universal meter by submerging the probe in the stream and gently stirring it back and forth until the reading stabilized.

A.2.2 Conductivity

Conductivity in water is affected by the presence of inorganic dissolved solids such as chloride, nitrate, calcium, and iron, and is primarily driven by the geology of the area through which the water flows. Conductivity is related to stream productivity and as such can affect vegetative and inverterbrate community productivity (Northcote and Larkin 1956; Johnson 1974). Conductivity was measured using a WTW Multiline P4 universal meter by submerging the probe in the stream until the reading stabilized.

A.2.3 Substrate

The composition of the substrate determines the roughness of stream channels, and roughness has a large influence on channel hydraulics (depth, width, and current velocity) of stream habitat. Furthermore, many species require specific substrates for spawning, for example, among salmonids ideal substrate size for spawning maintains high interstitial flow of oxygen around buried eggs (Chapman 1988). Substrate can also provide important refuge from stream currents among drift feeding fish as well as thermal

refuge and cover from predation. Substrate complexity may also affect density of territorial salmonids during certain life history stages by controlling territory boundaries through visual isolation of neighbours (Dolinsek, Grant and Biron 2007). In this study, substrate characterization followed a modified Wentworth classification. The categories were as follows; vegetation, silt, fine gravel (2-15 mm), coarse gravel (16-63 mm), cobble (64-256 mm), small boulder (256-1024 mm), large boulder (>1024 mm). Proportions of each substrate type were visually estimated for each transect.

A.2.4 pH

Water pH can have a large effect on numbers of species and individuals in a stream as well as ecosystem processes. At very low pH levels (less 3) coagulation of mucous on gill surfaces and subsequent anoxia may be the primary cause of acid induced mortality of fish. At pH 4-5, disturbance of normal ion and acid-base balance is the likely cause of mortality. In one study, fertilization success of Atlantic salmon eggs by large sea-run males and precocious male parr and recruitment of juveniles was found to decline at pH values of less than 5.0 due to decreasing spermatozoan motility; no eggs were fertilized below pH 4.0 (Daye and Glebe 1984). For brook trout, the selection of underground spring areas for spawning reduces the risk that low pH will affect the survival of early life stages, however, the critical time for young brook trout may be when they emerge from the redd. As alevins emerge to begin feeding exogenously, they may be forced to cross a chemical gradient representing a more than 100 fold increase in H+. Though brook trout have been found to be very tolerant of low pH (Grande, Muniz and Anderson 1978), the shock of crossing from alkaline to very acidic water may cause

high mortality (Gunn 1986). pH was measured using a WTW Multiline P4 universal meter. At each transect the probe was submerged in the stream and held in place until the reading stabilized.

A.2.5 Mean temperature

Temperature is a critical environmental variable determining the metabolic rates of organisms, as well as their distribution along a river's length. Because species compositions and metabolic rates are temperature dependent, ecosystem processes including leaf breakdown, nutrient uptake, and biological production are also affected (Allan and Castillo 2009). Among fish, temperature is an important factor controlling not only metabolism and growth, but also the timing of spawning and emergence (Brett, Shelbourn and Shoop 1969). Stream temperature at Cape Race was measured in conjunction with pH using the WTW Multiline P4 universal meter pH probe. Stream temperature at each transect was measured mid-channel and away from large objects projecting above the water surface, to avoid elevated readings. The probe was held just below the water surface for the amount of time required for pH readings to stabilize, approximately 10 minutes.

A.2.6 Mean velocity

Current velocity influences both channel shape and substrate composition and strongly affects ecological interactions, rates of energy transfer, and resource distribution within the stream environment (Hart and Finelli 1999). Flow conditions are important to ecosystem processes through the delivery of nutrients and gasses and removal of wastes,

and by influencing which age classes or even species occur at a site. For drift feeding fish such as brook trout, capture rate is a function of visual reaction distance, depth, and velocity; swimming costs also depend on velocity (Hughes and Dill 1990). Velocity in this study was measured using a ball attached to a 1 meter string. Holding one end of the string the ball was released from an upstream direction by one person and allowed to reach the end of the string. A second person recorded the time required for the ball to travel one meter using a stopwatch. The velocity was calculated as one meter divided by the time required for the ball to travel the length of the string to obtain a velocity estimate in m/s. Mean velocity of the transect was calculate as the average of three measurements spaced equally across the width of the stream channel.

A.2.7 Percent riparian cover

Riparian vegetation can help to moderate stream temperature by reducing the amount of solar radiation reaching the water surface (Beschta *et al.* 1986). For salmonids, riparian vegetation provides important thermal refuges as well as cover from avian predators. Allochthonous inputs of riparian vegetation also provide important resource subsidies to aquatic environments affecting microbial, invertebrate, as well as fish communities (Allan and Castillo 2009). Percent riparian cover was visually estimated as the percentage of the stream channel that was shaded by overhanging stream side vegetation.

A.2.8 Number of plant species per transect/percent transect with vegetation

As an indirect indicator of stream productivity, the number of different species of aquatic submerged and partially submerged vegetation present in each transect was recorded, and the proportion of substrate covered by aquatic vegetation for each stream transect was visually estimated. The diversity and abundance of aquatic vegetation dictates the diversity and abundance of stream invertebrates, which are a primary food source for stream dwelling salmonids.

A.2.9 Wetted width

Bank width is a character that provides a measure of stream size and therefore the quantity of habitat available for salmonids (Kaufmann 1993). If space is limiting in small habitats, then mortality and reproduction may occur in a density-dependent manner, and individual success will be determined by relative competitive ability. Previous studies have found positive relationships between stream width and trout presence or abundance (e.g. Clarkson and Wilson 1995, Kruse, Hubert and Rahel 1997). Channel width may also affect in stream temperature regimes, since wider channels will have less riparian shading and more surface area exposed to direct sunlight (Allan and Castillo 2009). Wetted width was measured as the horizontal distance along a transect, from bank to bank at the existing water surface using a measuring tape.

A.2.10 Mean depth

Stream depth is an important characteristic for stream dwelling salmonids since water temperature is directly influenced by stream depth; the shallower the stream, the more temperature will fluctuate in accordance with air temperature changes. One

manipulative experiment that increased the number of deep pools caused increased abundance of adult trout in six northern Colorado streams (Gowan and Fausch 1996*a*). Deep, low velocity pool habitats with undercut banks are likely to be especially critical refuges for fish in Cape Race streams since there is a noted absence of riparian shading in many streams; pools also provide critical overwintering habitat. Depth was measured as the mean of 5 equally spaced points along the transect using a meter stick.

Table A1: Study streams coded from east to west, location, and stream-level habitat characters estimated for each stream at Cape Race in southeastern Newfoundland, Canada.

Population	Code	Location	Order	Drainage (km^2)	Length (m)	Sinuosity	Gradient (%)	No. transects 2010/2011
LW*	1	46° 42.450' N, 53° 15.516' W	2	0.90	2418	1.08	2.36	NS/32
UW*	2	46° 42.257' N, 53° 14.359' W	2	6.74	5332	1.17	1.43	NS/32
CN	3	46° 41.522' N, 53° 15.450' W	2	3.23	3494	1.18	2.65	18/32
PD	4	46° 39.039' N, 53° 13.191' W	2	0.50	912	1.36	2.74	NS/21
FW	5	46° 38.760' N, 53° 13.304' W	2	5.72	4882	1.31	2.05	30/61
LC	6	46° 38.740' N, 53° 13.290' W	2	0.084	698	1.16	5.11	18/18
UC	7	46° 39.163' N, 53° 12.754' W	1	0.041	262	1.20	2.21	18/18
BB*	8	46° 38.668' N, 53° 12.697' W	1	0.13	337	1.20	0.30	NS/19
BC	9	46° 38.197' N, 53° 12.983' W	2	1.41	2050	1.29	2.44	21/21
STBC	10	46° 38.098' N, 53° 12.627' W	1	0.38	774	1.33	3.82	18/19
WC	11	46° 38.020' N, 53° 12.351' W	2	0.37	1822	1.23	2.38	18/20
DY	12	46° 38.940' N, 53° 11.424' W	1	0.09	441	1.37	2.77	18/18
UO	13	46° 38.944' N, 53° 11.137' W	2	3.09	1977	1.05	1.06	13/32
LO	14	46° 38.971' N, 53° 10.992' W	1	0.19	208	1.07	1.06	11/11
WN	15	46° 37.942' N, 53° 09.546' W	3	6.38	8062	1.33	1.03	26/49
UB	16	46° 38.678' N, 53° 09.090' W	1	1.12	884	1.27	1.59	18/18
LB	17	46° 38.034' N, 53° 09.545' W	1	0.47	1605	1.23	1.04	32/34
TB	18	46° 38.424' N, 53° 08.303' W	3	1.44	2993	1.32	1.43	18/29
CC	19	46° 38.759' N, 53° 06.164' W	3	3.40	6318	1.26	1.37	18/42

*Sampled in October 2011.

Population codes: LW=Lower Whelan's, UW=Upper Whelan's, CN=Cotton River, PD= Perdition Brook, FW=Freshwater River, LC=Lower Coquita, UC=Upper Coquita, BB=Bella's Brook, BC=Bob's Cove, STBC=Still There By Chance River, WC=Whale Cove, DY=Ditchy Brook, UO=Upper Ouananiche Beck, LO=Lower Ouananiche Beck, WN=Watern Cove, UB=Upper Blackfly River, LB=Lower Blackfly River, TB=Tannin Brook, CC=Cripple Cove River.

NS = not sampled.

Table A2: Mean transect-level habitat characteristics for Cape Race streams coded from east to west in 2010 and 2011.

	Population									
Parameter	1	2	3	4	5	6	7	8	9	10
2010										
pH	NS	NS	6.35	NS	6.68	6.79	5.66	NS	6.41	6.35
DO (mg/l)	NS	NS	9.62	NS	9.39	11.64	8.48	NS	9.68	11.72
Conductivity (µS/cm)	NS	NS	55.40	NS	na	na	64.33	NS	69.10	77.67
Temperature (^{o}C)	NS	NS	9.07	NS	15.90	10.48	15.97	NS	9.77	11.09
Width (cm)	NS	NS	162.17	NS	270.55	97.78	169.44	NS	196.05	175.83
Depth (cm)	NS	NS	28.20	NS	17.72	11.76	24.65	NS	16.57	20.02
Undercut depth (cm)	NS	NS	5.60	NS	7.57	17.39	7.35	NS	17.35	15.56
Velocity (m/s)	NS	NS	0.49	NS	0.30	0.12	0.01	NS	0.20	0.04
% riparian	NS	NS	0.00	NS	0.05	0.08	0.08	NS	0.01	0.07
% vegetation	NS	NS	0.95	NS	0.49	0.59	0.20	NS	0.34	0.21
No. of species	NS	NS	1.29	NS	1.80	1.33	0.83	NS	1.30	1.25
% large boulder	NS	NS	0.00	NS	0.03	0.08	0.01	NS	0.01	0.04
% small boulder	NS	NS	0.00	NS	0.27	0.19	0.03	NS	0.31	0.15
% cobble	NS	NS	0.00	NS	0.48	0.22	0.08	NS	0.26	0.08
% course gravel	NS	NS	0.00	NS	0.17	0.06	0.00	NS	0.26	0.00
% fine gravel	NS	NS	0.00	NS	0.03	0.00	0.00	NS	0.08	0.00
% silt	NS	NS	0.00	NS	0.03	0.05	0.88	NS	0.00	0.73
2011										
pH	5.69	5.46	6.10	5.27	6.50	6.61	5.64	6.14	6.40	5.96
DO (mg/l)	10.10	11.79	8.79	9.47	8.55	11.24	8.37	10.34	9.98	11.13
Conductivity (µS/cm)	42.63	36.89	50.13	94.67	58.73	103.40	70.00	71.60	69.60	79.60
Temperature (^{o}C)	9.00	9.39	11.42	11.80	17.58	10.14	15.99	9.81	17.51	12.43
Width (cm)	350.84	233.91	177.72	203.62	259.85	99.89	167.89	1643.32	201.62	255.00
Depth (cm)	24.01	17.27	32.99	22.60	18.94	12.62	22.89	36.08	14.12	24.97
Undercut depth (cm)	9.41	9.00	13.24	12.03	7.12	18.95	8.94	10.54	15.80	20.64
Velocity (m/s)	0.26	0.23	0.36	0.32	0.35	0.15	0.00	0.00	0.23	0.00
% riparian	0.16	0.13	0.09	0.18	0.04	0.06	0.05	0.09	0.08	0.02
% vegetation	0.42	0.39	0.91	0.68	0.42	0.60	0.03	0.15	0.52	0.58

No. of species	2.13	1.63	2.47	1.45	1.85	1.44	0.72	1.53	2.67	2.74
% large boulder	0.27	0.14	0.23	0.04	0.04	0.00	0.01	0.02	0.05	0.03
% small boulder	0.28	0.29	0.17	0.43	0.29	0.36	0.01	0.15	0.16	0.06
% cobble	0.27	0.43	0.24	0.27	0.60	0.56	0.18	0.18	0.48	0.06
% course gravel	0.00	0.01	na	0.00	0.04	0.09	0.05	0.07	0.12	0.05
% fine gravel	0.01	0.05	na	0.00	0.00	0.00	0.07	0.00	0.02	0.01
% silt	0.00	0.02	na	0.16	0.00	0.00	0.68	0.58	0.09	0.78

	Population								
Parameter	11	12	13	14	15	16	17	18	19
2010									
pH	6.65	5.75	5.96	NS	NS	5.46	5.62	5.68	6.25
DO (mg/l)	10.62	7.95	8.47	8.74	13.76	8.56	8.98	9.47	10.46
Conductivity (µS/cm)	76.65	58.83	43.00	44.00	45.85	na	52.76	na	52.28
Temperature (^{o}C)	12.57	17.58	16.97	15.84	18.31	23.32	17.10	16.42	15.87
Width (cm)	212.67	84.06	411.13	250.67	448.31	203.11	128.97	136.72	330.17
Depth (cm)	20.00	20.65	16.00	17.72	20.17	17.08	16.18	10.15	19.41
Undercut depth (cm)	21.68	8.88	13.15	7.57	15.38	13.11	20.20	14.23	12.85
Velocity (m/s)	0.10	0.01	0.11	0.30	0.36	0.01	0.16	0.21	0.23
% riparian	0.08	0.12	0.04	0.12	0.04	0.08	0.18	0.13	0.02
% vegetation	0.41	0.47	0.50	0.64	0.45	0.20	0.43	0.28	0.74
No. of species	1.39	1.28	2.40	2.22	2.31	1.00	1.03	0.89	2.17
% large boulder	0.12	0.09	0.06	0.24	0.14	0.05	0.04	0.00	0.09
% small boulder	0.29	0.13	0.28	0.33	0.24	0.26	0.41	0.12	0.21
% cobble	0.24	0.07	0.39	0.27	0.35	0.31	0.30	0.29	0.27
% course gravel	0.16	0.02	0.21	0.12	0.13	0.08	0.09	0.35	0.17
% fine gravel	0.04	0.00	0.04	0.00	0.04	0.01	0.01	0.12	0.00
% silt	0.09	0.69	0.00	0.00	0.00	0.29	0.14	0.12	0.00
2011									
pH	6.87	5.77	5.84	6.33	6.51	5.40	5.96	4.61	5.85
DO (mg/l)	10.98	8.95	9.58	8.43	10.45	7.94	10.17	8.88	9.92
Conductivity (µS/cm)	72.20	56.80	47.44	55.33	49.58	49.40	53.78	59.88	43.73

Temperature (^{o}C)	12.18	19.60	17.38	16.26	10.93	21.73	13.71	18.74	18.81
Width (cm)	175.70	93.59	336.56	276.11	527.14	210.61	149.06	140.83	494.60
Depth (cm)	18.32	17.50	20.31	14.94	21.12	14.19	14.60	18.86	20.06
Undercut depth (cm)	16.35	6.78	15.26	10.81	19.37	10.15	18.95	17.84	15.94
Velocity (m/s)	0.09	0.01	0.11	0.24	0.17	0.03	0.15	0.29	0.18
% riparian	0.08	0.09	0.06	0.06	0.02	0.05	0.13	0.05	0.01
% vegetation	0.38	0.58	0.40	0.40	0.19	0.08	0.41	0.17	0.28
No. of species	1.35	1.94	3.58	3.56	1.94	1.50	1.18	1.24	2.83
% large boulder	0.00	0.00	0.12	0.36	0.17	0.00	0.03	0.09	0.16
% small boulder	0.21	0.13	0.36	0.38	0.27	0.16	0.28	0.18	0.31
% cobble	0.32	0.03	0.27	0.23	0.37	0.42	0.52	0.65	0.38
% course gravel	0.18	0.00	0.01	0.01	0.07	0.08	0.12	0.07	0.05
% fine gravel	0.19	0.00	0.01	0.00	0.01	0.01	0.01	0.00	0.00
% silt	0.08	0.89	0.22	0.00	0.06	0.32	0.04	0.01	0.07

NS = not sampled.

na = data not available.

Chapter 1 Appendix A: References

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Appendix B: Detailed description of PCR conditions for microsatellite DNA analyses for samples collected in 2010 and 2011.

Microsatellite polymorphism was analysed using fluorescently labelled primers (*SfoC28*, *SfoC113*, *SfoC129, SfoD100*; *SfoD91*, T. L. King, US Geological Survey, unpublished: *Sco204, Sco212, Sco216, Sco218*, *Sco220*, DeHaan and Ardren 2005: *Ssa408uos*, *Ssa407uos*, Cairney, Taggart and Hoyheim 2000; *Sfo262*, Perry *et al*. 2005). PCR amplification of the loci was performed in a total of 10-μL reaction volume, containing 1μL of 10X TSG buffer, 1 μL of 20-mm MgSO4, 1 μL of 2-mm dNTPs, 0.1-0.3 μL of each of 10-mm forward/reverse primers, 0.1 μL of TSG polymerase, 4.6–4.8 μL of ddH2O, and 2μL of genomic DNA. The PCR conditions were an initial denaturation at 94◦C for 3 min, followed by 30 cycles of denaturation for 30 sec at 94◦C, annealing at 57◦C for 30 sec and elongation at 72◦C for 1 min, and a final elongation at 72◦C for 15 min. Amplified fragments were separated electrophoretically using a Life Technologies Inc. Genetic Analyzer 3500, and allele sizes were scored based on a fluorescently labeled size standard.

Chapter 1 Appendix B: References

DeHaan, P. W., and Arden, W. R. (2005) Characterization of 20 highly variable tetranucleotide microsatellite loci for bull trout (*Salvelinus confluentus*) and cross-amplification in other Salvelinus species. Molecular Ecology Notes 5:582–585.

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Appendix C (Table C1): Number of breeders (N_b) estimated for each sampled cohort of each study brook trout population at Cape Race, Newfoundland using LDNe (Waples and Do 2008).

Population	2009 N_b (95% CI)	2010 N_b (95% CI)	2011 N_b (95% CI)	n
Lower Whelan's	NS	NS	93 (45-1363)	48
Upper Whelan's	NS	NS	249 (114-∞)	74
Cotton	59 (42-98)	na	29 (24-36)	56, 47
Perdition	NS	NS	33 (17-115)	24
Freshwater	452 (129-∞)	136 (100-200)	81 (68-100)	45, 95, 114
Lower Coquita	29 (18-55)	26 (17-42)	36 (20-95)	48, 59, 42
Upper Coquita	15 (7-41)	16 (9-38)	na	19, 25
Bella's Brook	NS	NS	59 (39-105)	48
Bob's Cove	423 (98-∞)	69 (48-108)	148 (91-315)	62, 95, 105
Still There By Chance	72 (41-186)	7 (3-13)	15 (8-33)	93, 42, 40
Whale Cove	87 (58-158)	39 (28-56)	35 (29-41)	66, 48, 108
Ditchy	na	34 (17-161)	26 (15-52)	26, 35
Upper Ouananiche Beck	231 (106-∞)	93 (48-444)	116 (85-174)	67, 36, 93
Lower Ouananiche Beck	na	45 (27-95)	39 (19-285)	39, 25
Watern Cove	126 (84-237)	119 (89-165)	137 (109-180)	59, 96, 133
Upper Blackfly	138 (47-∞)	14 (9-23)	na	41, 28
Lower Blackfly	63 (39-132)	30 (23-40)	64 (42-116)	46, 54, 52
Tannin Brook	∞ (∞-∞)	∞ (∞-∞)	104 (51-882)	90, 53, 44
Cripple Cove	101 (55-293)	28 (20-39)	44 (32-65)	80, 76, 71

NS = not sampled.

na = estimate not available because of low sample size.

Chapter 1 Appendix C: References

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

Appendix D (Table D1): Within-population genetic diversity characteristics based on thirteen microsatellite loci for all sampled cohorts of each study brook trout population at Cape Race, Newfoundland.

Population	Cohort	n	Na (SE)	Ho (SE)	He (SE)	Loci with heterozygote deficiencies	Loci with heterozygote excesses
Lower Whelan's	2011	48	5.462 (0.713)	0.574 (0.0728)	0.595 (0.0689)	*Sco216*	
Upper Whelan's	2011	74	6.308 (0.728)	0.471 (0.0695)	0.552 (0.0768)	*Ssa407* *Sco212*	
Cotton	2009	56	6.308 (0.858)	0.625 (0.0644)	0.642 (0.0639)	*Ssa408*	
Cotton	2011	47	6.231 (0.769)	0.655 (0.0487)	0.657 (0.0511)	*SSa407*	
Perdition	2011	24	5.308 (0.603)	0.638 (0.0325)	0.654 (0.0292)	*Ssa408*	
Freshwater	2009	45	7.462 (1.158)	0.578 (0.0450)	0.708 (0.0389)	*Sco220* *SSa408* *Sco212* *Sco216*	
Freshwater	2010	95	7.615 (1.444)	0.628 (0.0426)	0.708 (0.0364)	*Sco212* *Sco216*	
Freshwater	2011	114	8.769 (1.236)	0.636 (0.0482)	0.702 (0.0418)	*Ssa407* *Sco212* *Sfo262*	
Lower Coquita	2009	48	5.538 (0.813)	0.499 (0.0416)	0.595 (0.0497)	*Ssa407*	
Lower Coquita	2010	59	4.385 (0.813)	0.421 (0.0431)	0.534 (0.0418)	*Sco204* *Sco212* *Sco216*	
Lower Coquita	2011	42	5.000 (0.760)	0.474 (0.0716)	0.524 (0.0652)		

Upper Coquita	2009	19	4.385	0.550	0.536		
			(0.656)	(0.0569)	(0.0511)		
Upper Coquita	2010	25	3.846	0.444	0.496		
			(0.465)	(0.0441)	(0.0414)		
Bella's Brook	2011	48	5.615	0.618	0.584		
			(0.694)	(0.0802)	(0.0670)		
Bob's Cove	2009	62	4.231	0.481	0.460	*SSa408*	*Ssa408*
			(0.533)	(0.0729)	(0.0699)		*Sco204*
Bob's Cove	2010	95	4.538	0.483	0.461	*Ssa407*	
			(0.704)	(0.0758)	(0.0698)		
Bob's Cove	2011	105	5.462	0.468	0.447		
			(0.756)	(0.0729)	(0.0681)		
Still There By Chance	2009	93	3.538	0.279	0.324	*Sco218*	
			(0.386)	(0.0555)	(0.0626)	*Sco220*	
Still There By Chance	2010	42	3.385	0.321	0.333	*Sco212*	
			(0.385)	(0.0750)	(0.0613)	*SfoC129*	
Still There By Chance	2011	40	2.615	0.304	0.319	*SfoC28*	
			(0.290)	(0.0756)	(0.0690)		
Whale Cove	2009	66	6.385	0.541	0.605	*Sco220*	
			(0.805)	(0.0633)	(0.0620)		
Whale Cove	2010	48	5.231	0.553	0.575		*Ssa407*
			(0.533)	(0.0639)	(0.0514)		
Whale Cove	2011	108	6.308	0.575	0.590	*Sco220*	
			(0.771)	(0.0601)	(0.0543)	*Sco216*	
Ditchy	2010	26	4.385	0.593	0.566		
			(0.446)	(0.0533)	(0.0431)		
Ditchy	2011	35	4.462	0.514	0.513		
			(0.514)	(0.0474)	(0.0463)		
Upper Ouananiche Beck	2009	67	6.538	0.623	0.649	*SfoC129*	
			(0.722)	(0.0264)	(0.0282)		
Upper Ouananiche Beck	2010	36	5.231	0.642	0.609		
			(0.690)	(0.0497)	(0.0459)		
Upper Ouananiche Beck	2011	93	6.923	0.618	0.637	*Ssa407*	

			(0.873)	(0.0459)	(0.0433)		
Lower Ouananiche Beck	2010	39	4.769	0.589	0.571		
			(0.642)	(0.0439)	(0.0445)		
Lower Ouananiche Beck	2011	25	4.615	0.619	0.592		
			(0.626)	(0.0343)	(0.0355)		
Watern Cove	2009	59	7.538	0.616	0.616		
			(1.107)	(0.0728)	(0.0677)		
Watern Cove	2010	96	7.154	0.591	0.597		*Sco218*
			(0.980)	(0.0766)	(0.0702)		*Sco220*
Watern Cove	2011	133	8.154	0.585	0.601		
			(1.061)	(0.0762)	(0.0779)		
Upper Blackfly	2009	41	5.769	0.465	0.541		
			(0.778)	(0.0605)	(0.0742)		
Upper Blackfly	2010	28	4.154	0.512	0.516		
			(0.421)	(0.0690)	(0.0646)		
Lower Blackfly	2009	46	5.308	0.550	0.534		*Sco220*
			(0.754)	(0.0824)	(0.0773)		
Lower Blackfly	2010	54	5.462	0.549	0.551		
			(0.748)	(0.0798)	(0.0734)		
Lower Blackfly	2011	52	5.615	0.574	0.580		*Sco220*
			(0.712)	(0.0820)	(0.0680)		
Tannin Brook	2009	90	7.385	0.548	0.616	*Sco218*	
			(0.836)	(0.0597)	(0.0635)	*Sco220*	
						Ssa407	
Tannin Brook	2010	53	5.385	0.603	0.607	*Sco212*	
			(0.646)	(0.0650)	(0.0615)	*Sco216*	
Tannin Brook	2011	44	5.000	0.519	0.555	*Sco220*	
			(0.689)	(0.0696)	(0.0609)		
Cripple Cove	2009	80	4.154	0.404	0.414		
			(0.553)	(0.0702)	(0.0674)		
Cripple Cove	2010	76	3.923	0.431	0.421		
			(0.525)	(0.0644)	(0.0617)		
Cripple Cove	2011	71	4.615	0.483	0.464		

	(0.525)	(0.0681)	(0.0616)

n=sample size.

Na=mean number of alleles.

Ho=mean observed heterozygosity.

He=mean expected heterozygosity.

Appendix E (Table E1): Pairwise θ_{ST} estimates between each cohort from all study populations at Cape Race, Newfoundland estimated using GENETIX 4.0 (Belkhir et al. 2004).

Population	2 (2011)	3 (2009)	3 (2011)	4 (2011)	5 (2009)	5 (2010)	5 (2011)	6 (2009)	6 (2010)	6 (2011)
1 (2011)	0.0569	0.1421	0.1500	0.2441	0.1748	0.1984	0.1922	0.2741	0.3196	0.2983
2 (2011)		0.1851	0.1884	0.2864	0.2116	0.2321	0.2274	0.3056	0.3471	0.3185
3 (2009)			***0.0040****	0.2003	0.1392	0.1733	0.1499	0.2093	0.2629	0.2497
3 (2011)				0.1927	0.1422	0.1727	0.1514	0.2189	0.2722	0.2566
4 (2011)					0.0858	0.0574	0.0715	0.2042	0.2317	0.2249
5 (2009)						***0.0271***	***0.0285***	0.1112	0.1385	0.1056
5 (2010)							***0.0174***	0.1358	0.1430	0.13830
5 (2011)								0.1370	0.1570	0.1467
6 (2009)									***0.0468***	***0.0808***
6 (2010)										***0.0643***
6 (2011)										

Population	7 (2009)	7 (2010)	8 (2011)	9 (2009)	9 (2010)	9 (2011)	10 (2009)	10 (2010)	10 (2011)	11 (2009)
1 (2011)	0.3122	0.3296	0.1082	0.2956	0.3049	0.3181	0.4534	0.4064	0.4122	0.2084
2 (2011)	0.3431	0.3641	0.1870	0.3426	0.3547	0.3632	0.4677	0.4241	0.4248	0.2349
3 (2009)	0.2400	0.2571	0.1710	0.2428	0.2501	0.2572	0.3752	0.3333	0.3514	0.1966
3 (2011)	0.2497	0.2635	0.1800	0.2500	0.2572	0.2649	0.3721	0.3275	0.3440	0.2068
4 (2011)	0.2172	0.2404	0.2481	0.3477	0.3629	0.3670	0.5022	0.4590	0.4669	0.2456
5 (2009)	0.1184	0.1428	0.1751	0.2700	0.2804	0.2911	0.4009	0.3544	0.3678	0.1408
5 (2010)	0.1520	0.1606	0.2019	0.2878	0.3017	0.3061	0.3955	0.3576	0.3628	0.1977
5 (2011)	0.1560	0.1704	0.1925	0.2461	0.2540	0.2591	0.3549	0.3277	0.3313	0.1771
6 (2009)	0.0402	0.0813	0.2852	0.3561	0.3596	0.3746	0.4683	0.4385	0.4491	0.2417
6 (2010)	0.0565	0.0586	0.3288	0.4058	0.4059	0.4207	0.5050	0.4784	0.4893	0.2903
6 (2011)	0.1052	0.1217	0.3061	0.3921	0.3920	0.4072	0.5206	0.4884	0.4984	0.2798

Population	11 (2010)	11 (2011)	12 (2010)	12 (2011)	13 (2009)	13 (2010)	13 (2011)	14 (2010)	14 (2011)	15 (2009)
1 (2011)	0.2473	0.2377	0.2705	0.3081	0.2101	0.2390	0.2236	0.2758	0.2641	0.1939
2 (2011)	0.2716	0.2607	0.3068	0.3429	0.2488	0.2724	0.2527	0.2992	0.2913	0.2156
3 (2009)	0.2313	0.2223	0.2237	0.2592	0.1602	0.2018	0.1874	0.2390	0.2232	0.1701
3 (2011)	0.2402	0.2302	0.2090	0.2452	0.1578	0.1901	0.1784	0.2298	0.2147	0.1664
4 (2011)	0.2664	0.2701	0.2792	0.3204	0.2167	0.2534	0.2414	0.2811	0.2625	0.2054
5 (2009)	0.1694	0.1704	0.2015	0.2347	0.1419	0.1826	0.1719	0.2024	0.1746	0.1325
5 (2010)	0.2303	0.2329	0.2136	0.2435	0.1686	0.1997	0.1940	0.2147	0.1955	0.1466
5 (2011)	0.2086	0.2073	0.1964	0.2292	0.1505	0.1832	0.1840	0.2010	0.1820	0.1301
6 (2009)	0.2626	0.2571	0.2892	0.3148	0.2294	0.2785	0.2687	0.2943	0.2680	0.2472
6 (2010)	0.3062	0.2955	0.3422	0.3630	0.2766	0.3235	0.3112	0.3359	0.3148	0.2920
6 (2011)	0.2984	0.2826	0.3439	0.3711	0.2690	0.3142	0.3036	0.3256	0.3033	0.2441

Population	15 (2010)	15 (2011)	16 (2009)	16 (2010)	17 (2009)	17 (2010)	17 (2011)	18 (2009)	18 (2010)	18 (2011)
1 (2011)	0.2069	0.1991	0.2185	0.2223	0.2298	0.2039	0.2086	0.1955	0.2356	0.2454
2 (2011)	0.2221	0.2174	0.2443	0.2539	0.2457	0.2219	0.2217	0.2361	0.2749	0.2845
3 (2009)	0.1859	0.1774	0.1995	0.1911	0.2183	0.2005	0.1815	0.1677	0.1837	0.2267
3 (2011)	0.1848	0.1783	0.1952	0.1926	0.2149	0.2028	0.1835	0.1646	0.1826	0.2182
4 (2011)	0.2258	0.2168	0.2415	0.2513	0.2591	0.2421	0.2229	0.2188	0.1943	0.2690
5 (2009)	0.1539	0.1448	0.1565	0.1703	0.1769	0.1661	0.1482	0.1402	0.1322	0.1881
5 (2010)	0.1630	0.1575	0.1699	0.1745	0.1827	0.1727	0.1605	0.1571	0.132	0.1932
5 (2011)	0.1496	0.1403	0.1472	0.1544	0.1582	0.1492	0.1371	0.1584	0.1152	0.1935
6 (2009)	0.2748	0.2578	0.2966	0.3078	0.3100	0.2963	0.2666	0.2432	0.2355	0.2970
6 (2010)	0.3084	0.2932	0.3368	0.3457	0.3467	0.3295	0.2992	0.2824	0.2767	0.3376
6 (2011)	0.2577	0.2430	0.2915	0.3028	0.3009	0.2778	0.2656	0.2663	0.2558	0.3282

Population	19 (2009)	19 (2010)	19 (2011)
1 (2011)	0.3248	0.3142	0.2921

2 (2011)	0.3541	0.3452	0.3229
3 (2009)	0.2887	0.2887	0.2571
3 (2011)	0.2700	0.2711	0.2394
4 (2011)	0.3833	0.3777	0.3496
5 (2009)	0.2899	0.2900	0.2601
5 (2010)	0.2765	0.2726	0.2520
5 (2011)	0.2595	0.2587	0.2399
6 (2009)	0.4073	0.4018	0.3710
6 (2010)	0.4357	0.4298	0.4005
6 (2011)	0.4091	0.4088	0.3799

Population	7 (2010)	8 (2011)	9 (2009)	9 (2010)	9 (2011)	10 (2009)	10 (2010)	10 (2011)	11 (2009)	11 (2010)
7 (2009)	*0.0285*	0.3290	0.4063	0.4080	0.4227	0.5311	0.5061	0.5215	0.2564	0.2699
7 (2010)		0.3577	0.4320	0.4279	0.4436	0.5445	0.5232	0.5404	0.2915	0.3040
8 (2011)			0.2277	0.2409	0.2540	0.4114	0.3654	0.3738	0.1746	0.2207
9 (2009)				***0.0105***	***0.001****	0.3251	0.2990	0.3039	0.1834	0.2460
9 (2010)					***0.0114***	0.3073	0.2905	0.3023	0.1940	0.2489
9 (2011)						0.3080	0.2898	0.2967	0.2052	0.2682
10 (2009)							***0.0079***	***0.0439***	0.3489	0.4111
10 (2010)								***0.0240***	0.3113	0.3747
10 (2011)									0.3303	0.3962

Population	11 (2011)	12 (2010)	12 (2011)	13 (2009)	13 (2010)	13 (2011)	14 (2010)	14 (2011)	15 (2009)	15 (2010)
7 (2009)	0.2666	0.3263	0.3492	0.2476	0.3102	0.2897	0.3244	0.2950	0.2796	0.3070
7 (2010)	0.2968	0.3399	0.3528	0.2654	0.3227	0.2990	0.333	0.3048	0.2997	0.3233
8 (2011)	0.2007	0.2666	0.3117	0.2098	0.2325	0.2137	0.2715	0.2633	0.1877	0.1922
9 (2009)	0.1987	0.2746	0.3354	0.2485	0.2445	0.2364	0.3059	0.3072	0.2558	0.2608
9 (2010)	0.2024	0.2886	0.3429	0.2667	0.2589	0.2513	0.3187	0.3181	0.2749	0.2793
9 (2011)	0.2181	0.291	0.3495	0.2697	0.2644	0.2563	0.3250	0.3276	0.2776	0.2804

10 (2009)	0.3386	0.4151	0.4536	0.3753	0.3843	0.3462	0.4294	0.4351	0.4069	0.4068
10 (2010)	0.3089	0.3846	0.4284	0.3372	0.3455	0.3117	0.3924	0.3987	0.3618	0.3660
10 (2011)	0.3259	0.3856	0.439	0.3413	0.3444	0.3137	0.3932	0.4029	0.3610	0.3654

Population	15 (2011)	16 (2009)	16 (2010)	17 (2009)	17 (2010)	17 (2011)	18 (2009)	18 (2010)	18 (2011)	19 (2009)
7 (2009)	0.2869	0.3301	0.349	0.3455	0.3335	0.2917	0.2835	0.2696	0.3420	0.4683
7 (2010)	0.3017	0.3519	0.362	0.3671	0.3506	0.3167	0.2918	0.2762	0.3464	0.4709
8 (2011)	0.1920	0.2121	0.2443	0.2272	0.2176	0.2161	0.1864	0.2258	0.2322	0.3090
9 (2009)	0.2516	0.2784	0.3009	0.2761	0.2711	0.2664	0.2849	0.2644	0.3295	0.3331
9 (2010)	0.2720	0.2992	0.3104	0.2943	0.2877	0.2875	0.2948	0.2699	0.3311	0.3197
9 (2011)	0.2712	0.2969	0.3156	0.2941	0.2886	0.2862	0.2997	0.2739	0.3392	0.3329
10 (2009)	0.3982	0.4724	0.5104	0.4644	0.4633	0.442	0.4295	0.4221	0.4729	0.5031
10 (2010)	0.3621	0.4296	0.4733	0.4235	0.4222	0.3980	0.3889	0.3859	0.4364	0.4769
10 (2011)	0.3575	0.4286	0.4765	0.421	0.4196	0.3941	0.3952	0.3899	0.4437	0.4851

Population	19 (2010)	19 (2011)
7 (2009)	0.4624	0.4269
7 (2010)	0.4645	0.4296
8 (2011)	0.3009	0.2797
9 (2009)	0.3302	0.3092
9 (2010)	0.3160	0.2976
9 (2011)	0.3315	0.3129
10 (2009)	0.4979	0.4774
10 (2010)	0.4692	0.4460
10 (2011)	0.4768	0.4551

Population	11 (2010)	11 (2011)	12 (2010)	12 (2011)	13 (2009)	13 (2010)	13 (2011)	14 (2010)	14 (2011)	15 (2009)

11 (2009)	***0.0406***	***0.0301***	0.1826	0.2109	0.1428	0.1612	0.1428	0.1979	0.1830	0.2010
11 (2010)		***0.0165***	0.2421	0.2746	0.2013	0.2288	0.2030	0.2635	0.2474	0.2654
11 (2011)			0.2042	0.2322	0.1748	0.1883	0.1730	0.2281	0.2096	0.2430
12 (2010)				***0.0286***	0.0702	0.0536	0.0626	0.1084	0.0924	0.2170
12 (2011)					0.0806	0.0841	0.0811	0.1186	0.0908	0.2572
13 (2009)						***0.0356***	***0.0354***	0.0380	0.0225	0.1497
13 (2010)							***0.0132***	0.0405	0.0347	0.1732
13 (2011)								0.0536	0.0416	0.1708
14 (2010)									***0.0013****	0.1943
14 (2011)										0.1774

Population	15 (2010)	15 (2011)	16 (2009)	16 (2010)	17 (2009)	17 (2010)	17 (2011)	18 (2009)	18 (2010)	18 (2011)
11 (2009)	0.2166	0.2132	0.2253	0.2581	0.2297	0.2401	0.2099	0.2128	0.2273	0.2506
11 (2010)	0.2777	0.2703	0.2893	0.3123	0.2962	0.3009	0.2668	0.25207	0.2635	0.2847
11 (2011)	0.2526	0.2495	0.2663	0.2886	0.2688	0.2748	0.2463	0.23672	0.2478	0.2701
12 (2010)	0.2357	0.2289	0.2517	0.2677	0.2538	0.2665	0.2330	0.20288	0.2305	0.2684
12 (2011)	0.2750	0.2706	0.2983	0.3078	0.2967	0.3101	0.2803	0.24721	0.2700	0.3207
13 (2009)	0.1707	0.1607	0.1693	0.1887	0.1819	0.1936	0.167	0.17296	0.1877	0.2312
13 (2010)	0.1877	0.1852	0.2002	0.2173	0.202	0.2180	0.1950	0.1883	0.2030	0.2379
13 (2011)	0.1860	0.1853	0.1968	0.2099	0.2026	0.2172	0.1953	0.18415	0.1944	0.2292
14 (2010)	0.2107	0.2069	0.2306	0.2575	0.2312	0.2504	0.2266	0.23529	0.2312	0.2868
14 (2011)	0.1968	0.1904	0.2167	0.2437	0.2190	0.2368	0.2140	0.21676	0.2138	0.2698

Population	19 (2009)	19 (2010)	19 (2011)
11 (2009)	0.3256	0.3214	0.2908
11 (2010)	0.3932	0.3887	0.3528
11 (2011)	0.3445	0.3417	0.3125
12 (2010)	0.3784	0.3688	0.3381
12 (2011)	0.4136	0.4091	0.38

13 (2009)	0.3014	0.2982	0.2750
13(2010)	0.2967	0.2884	0.2632
13 (2011)	0.2870	0.2791	0.2592
14 (2010)	0.3366	0.3348	0.3105
14 (2011)	0.3396	0.3375	0.3069

Population	15 (2010)	15 (2011)	16 (2009)	16 (2010)	17 (2009)	17 (2010)	17 (2011)	18 (2009)	18 (2010)	18 (2011)
15 (2009)	***0.0146***	***0.0054***	0.0198	0.0590	0.0246	0.0366	0.0342	0.1419	0.1568	0.2146
15 (2010)		***0.0141***	0.0294	0.0582	0.0292	0.0357	0.0435	0.1608	0.1693	0.2303
15 (2011)			0.0139	0.0487	0.0196	0.0232	0.0292	0.1428	0.1565	0.2114
16 (2009)				***0.0342***	0.0092	0.0184	0.0239	0.1468	0.1781	0.2227
16 (2010)					0.0553	0.0240	0.0524	0.1379	0.1695	0.2261
17 (2009)						***0.0186***	***0.0122***	0.1774	0.1920	0.2484
17 (2010)							***0.0266***	0.1568	0.1735	0.2308
17 (2011)								0.1552	0.1802	0.2326
18 (2009)									***0.1030***	***0.0514***
18 (2010)										***0.1339***
18 (2011)										

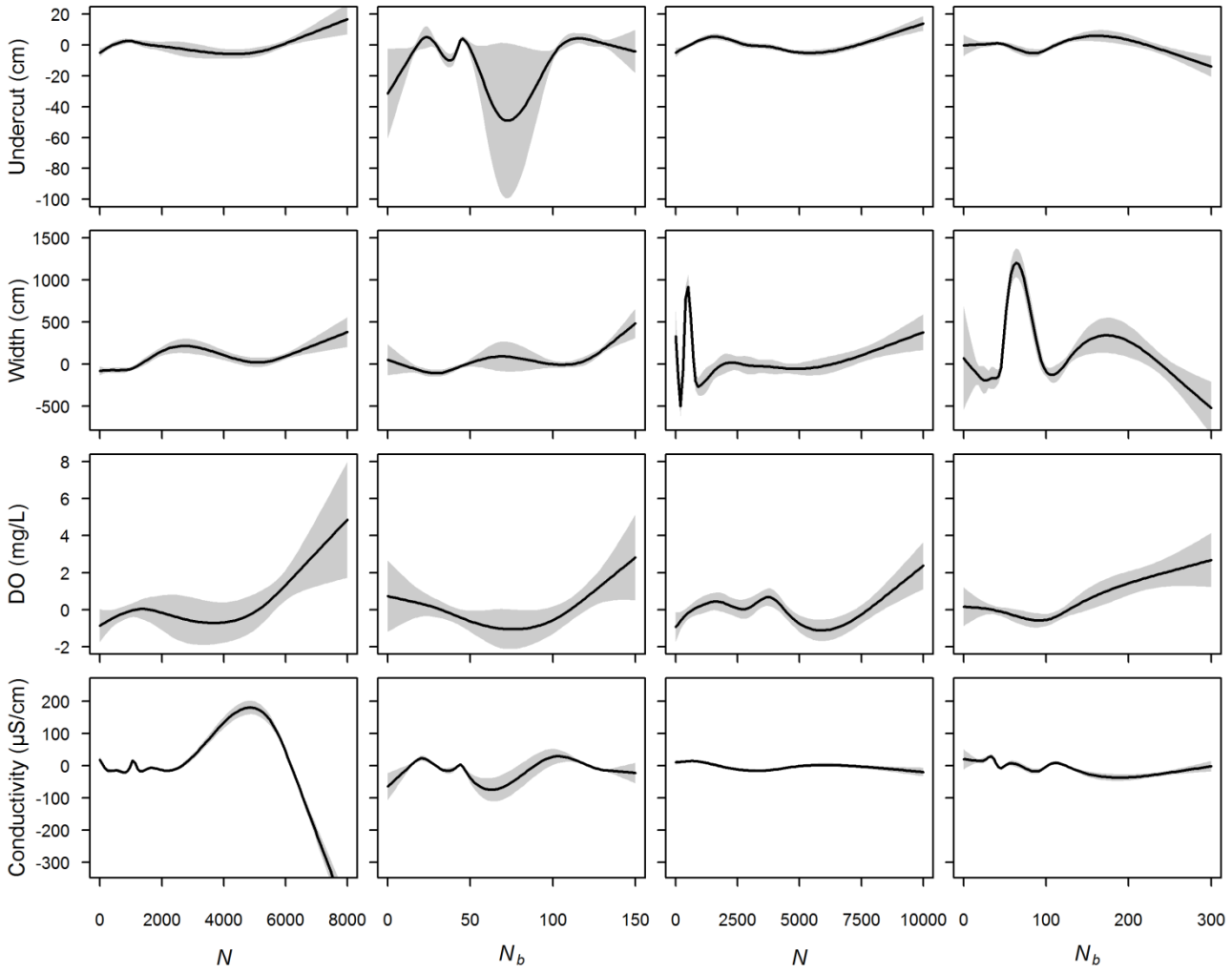
Population	19 (2009)	19 (2010)	19 (2011)
15 (2009)	0.1960	0.1943	0.1875
15 (2010)	0.1829	0.1824	0.1764
15 (2011)	0.1967	0.1982	0.1914
16 (2009)	0.1951	0.1999	0.1929
16(2010)	0.1950	0.1934	0.1902
17 (2009)	0.2072	0.2080	0.2034
17 (2010)	0.2016	0.2034	0.1982
17 (2011)	0.2374	0.2356	0.2242
18 (2009)	0.2322	0.2284	0.2093

18 (2010)	0.2676	0.2649	0.2404
18 (2011)	0.2533	0.2502	0.2137
19 (2009)		***0.0061***	***0.0157***
19 (2010)			***0.0116***
19 (2011)			

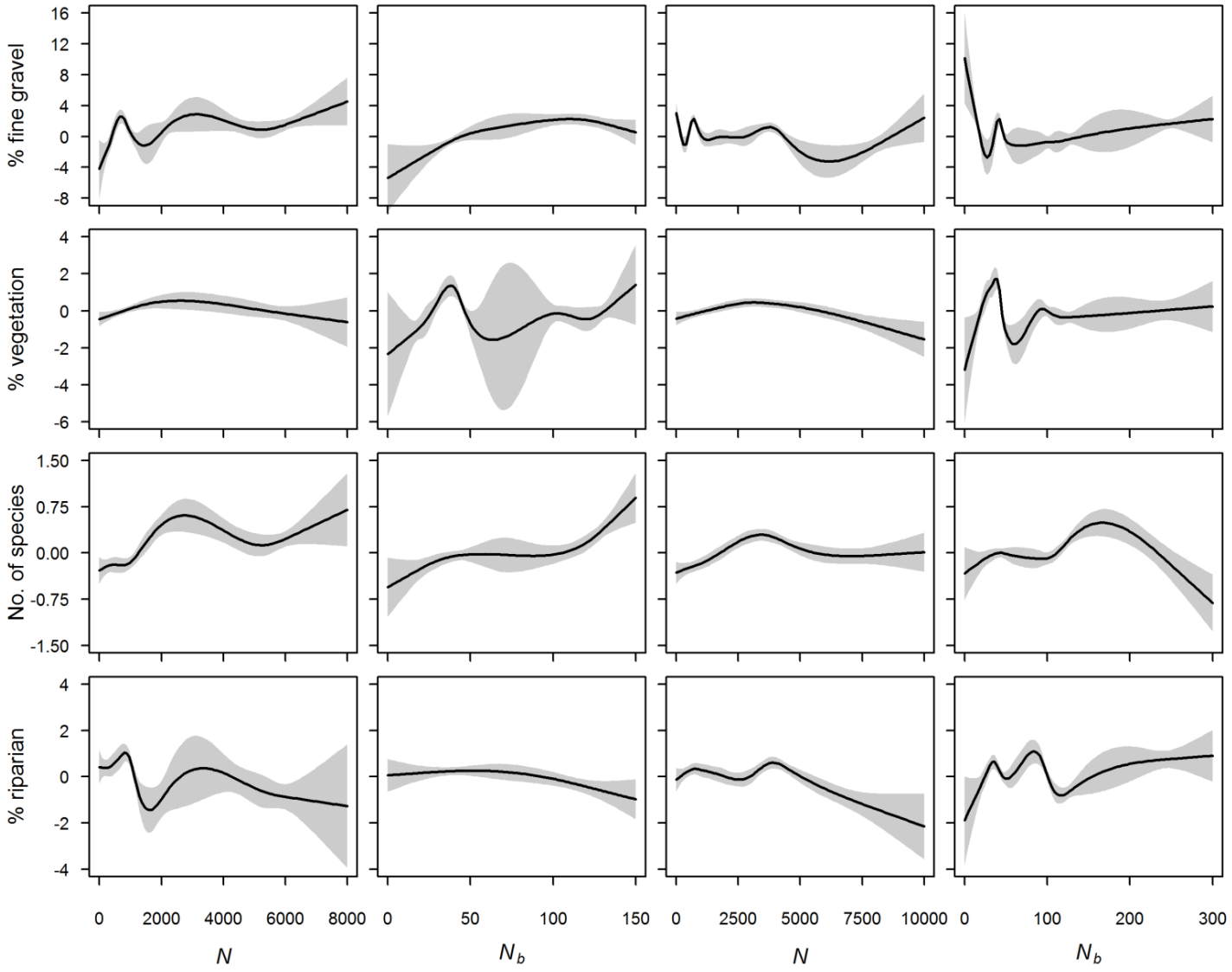
Notes: Significance was based on the percentage of 500 permutations where θ_{ST} was greater than the observed data; non-significant values are indicated by an asterisk. Populations are coded from east to west, followed by the cohort year in brackets. Within-population comparisons between cohorts are in bold and italics while inter-population comparisons within the same drainages are underlined.

Chapter 1 Appendix E: References

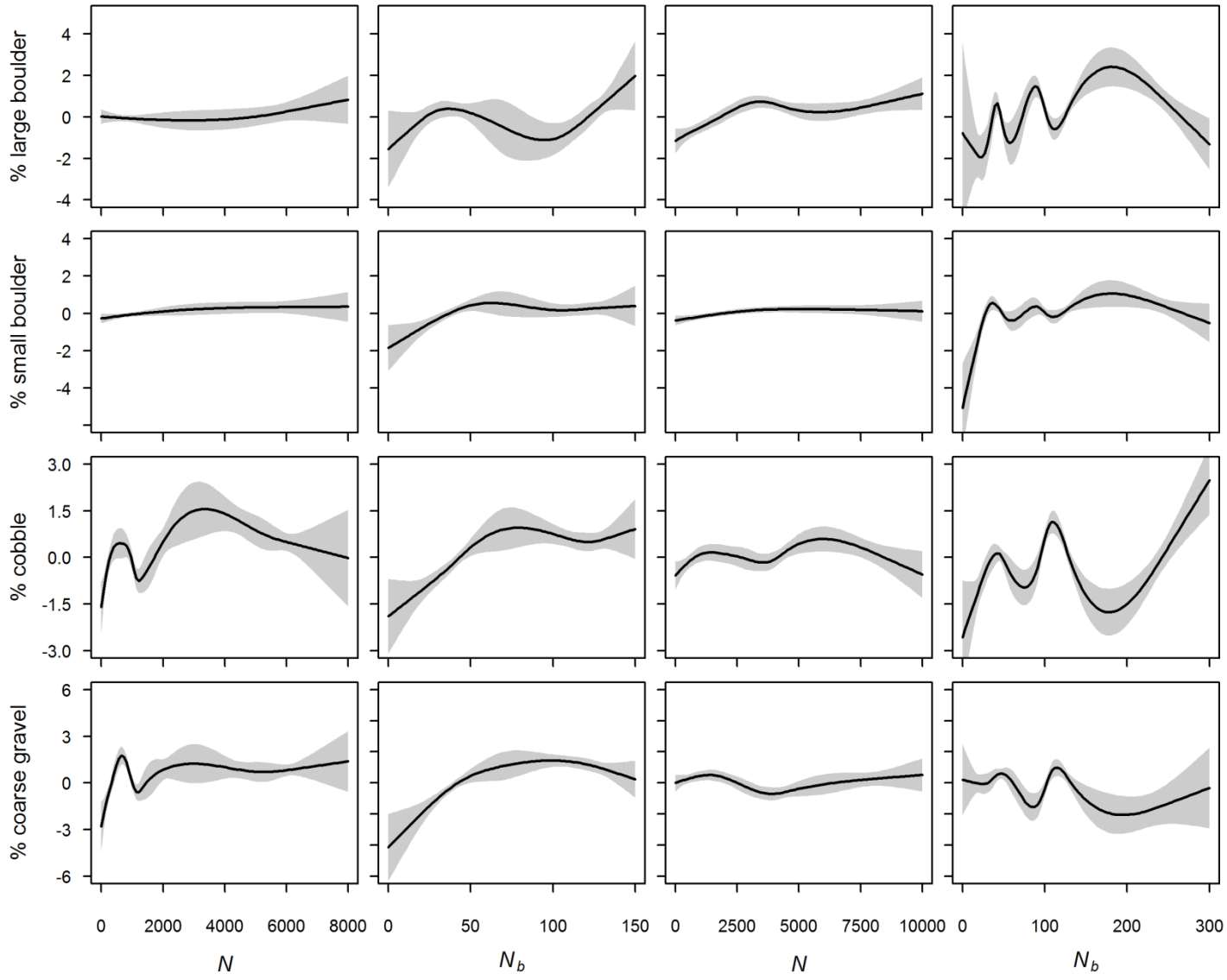
Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., and Bonhomme, F. (2004) *GENETIX 4.05, logiciel sous Windows pour la génétique des populations*. Laboratoire Génome, Populations, Interactions; CNRS UMR 5000. Université Montpellier II, Montpellier, France.



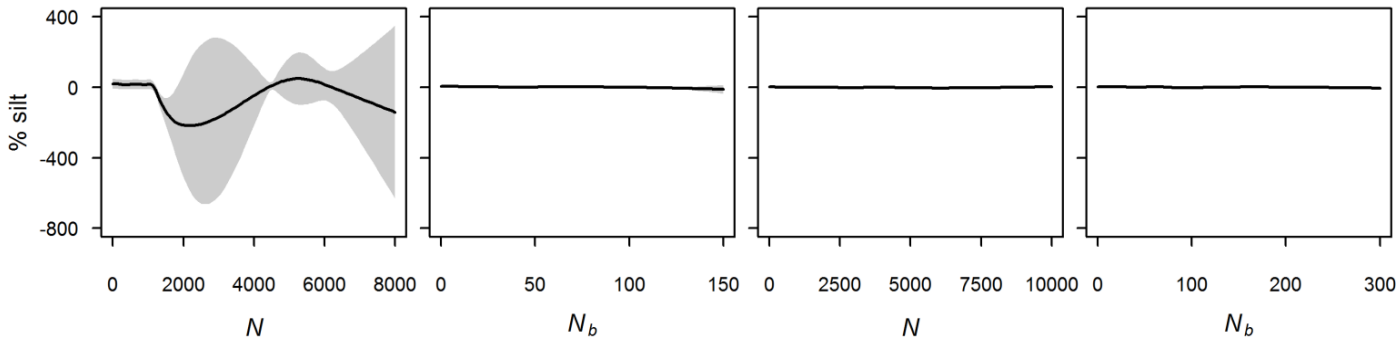
Appendix F (Fig. F1): Directional Hypothesis: GAM plots of habitat parameters vs. *N* and N_b in two years of sampling at Cape Race, Newfoundland.

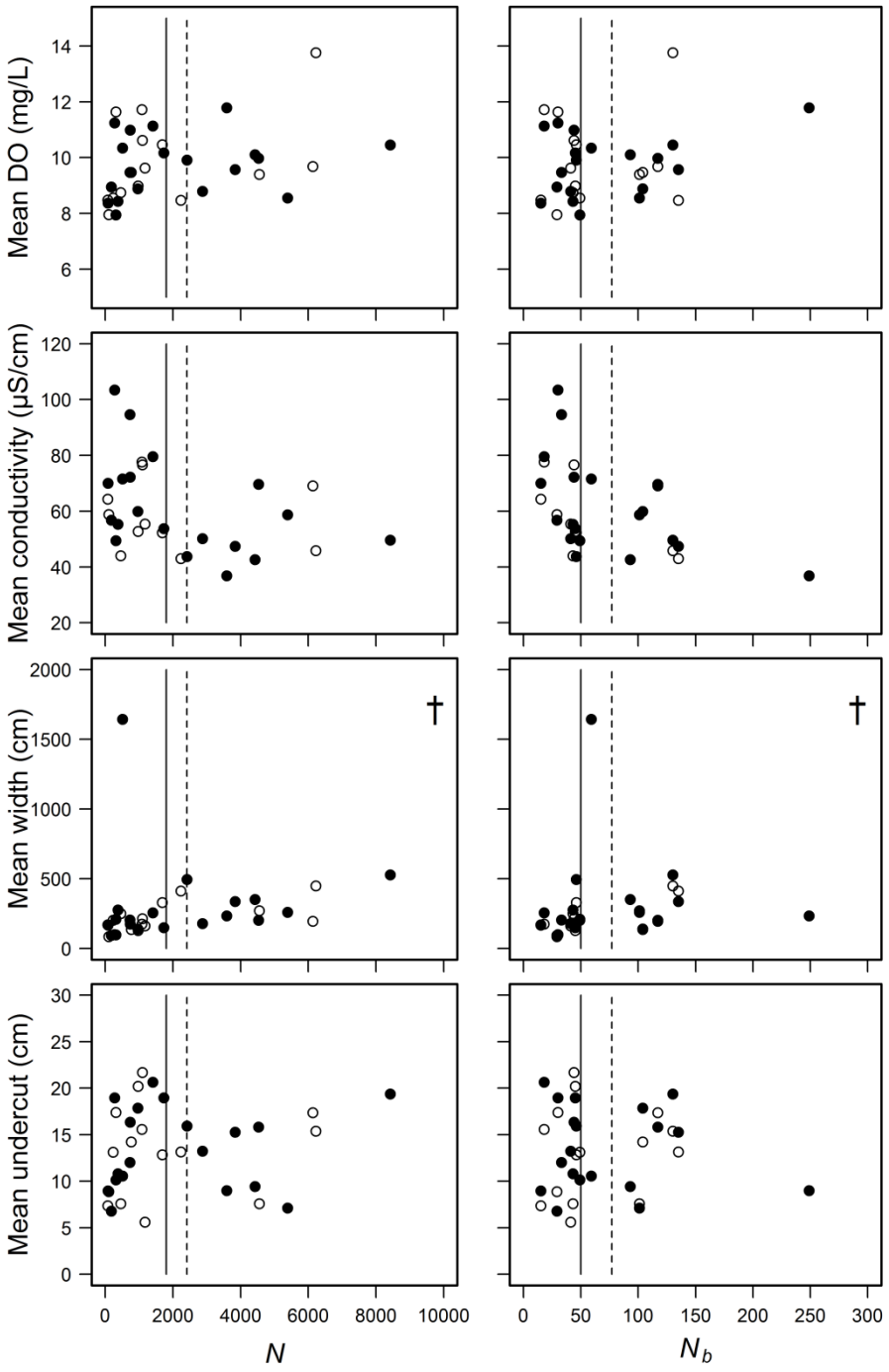

2010
2011
% fine gravel
% vegetation
No. of species
% riparian
N
N_b
N
N_b

2010	2011

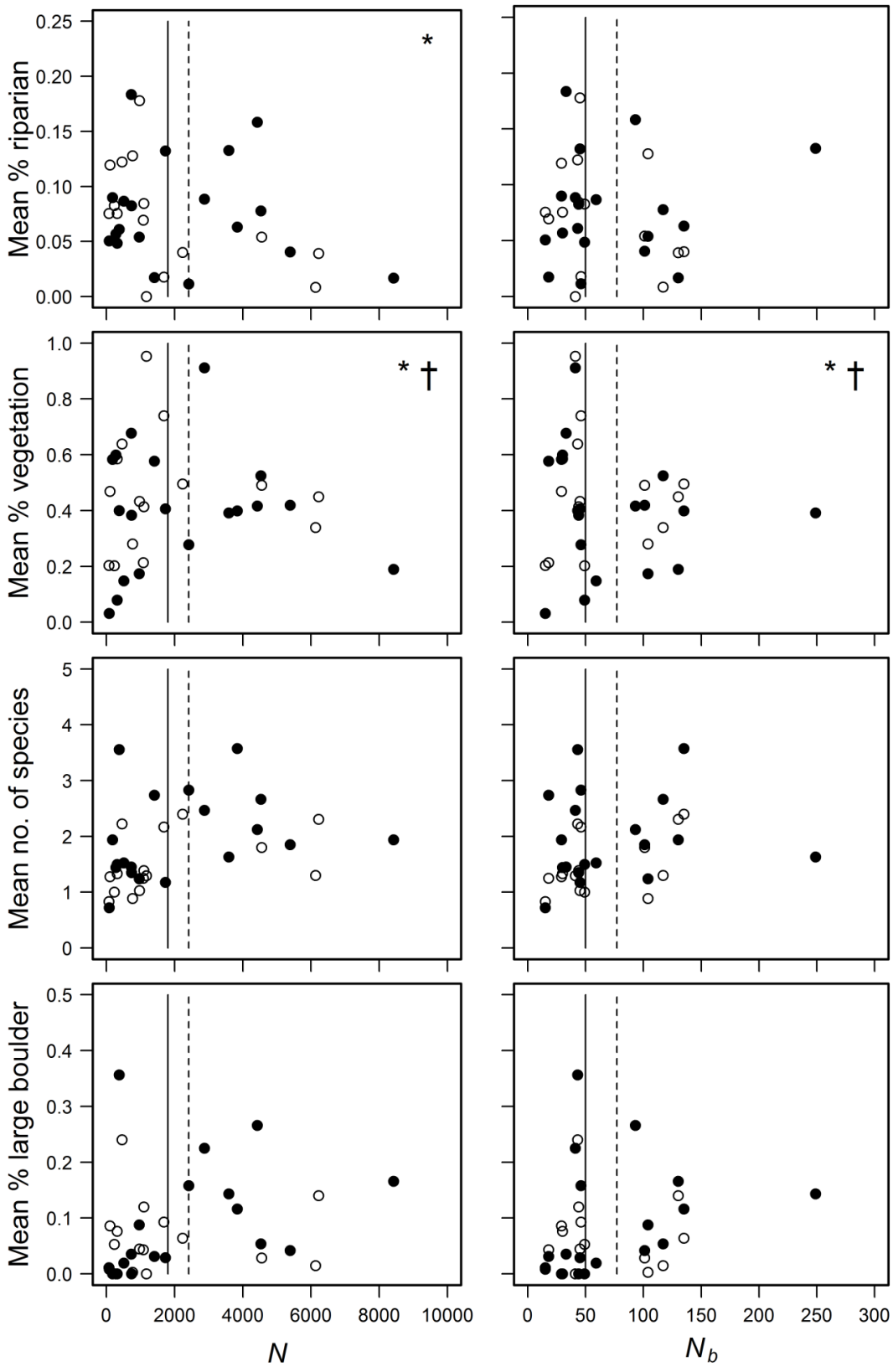


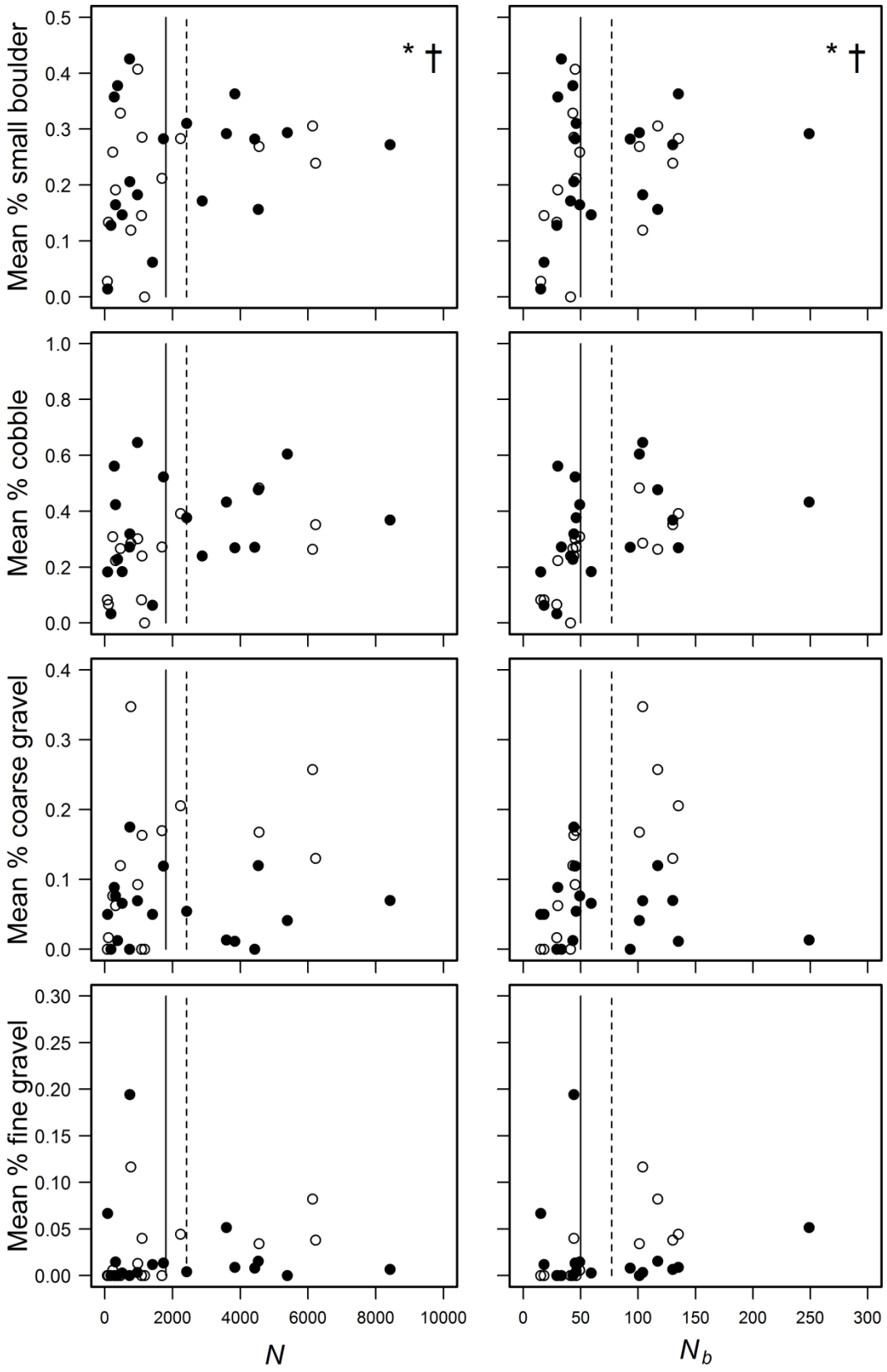
2010	2011

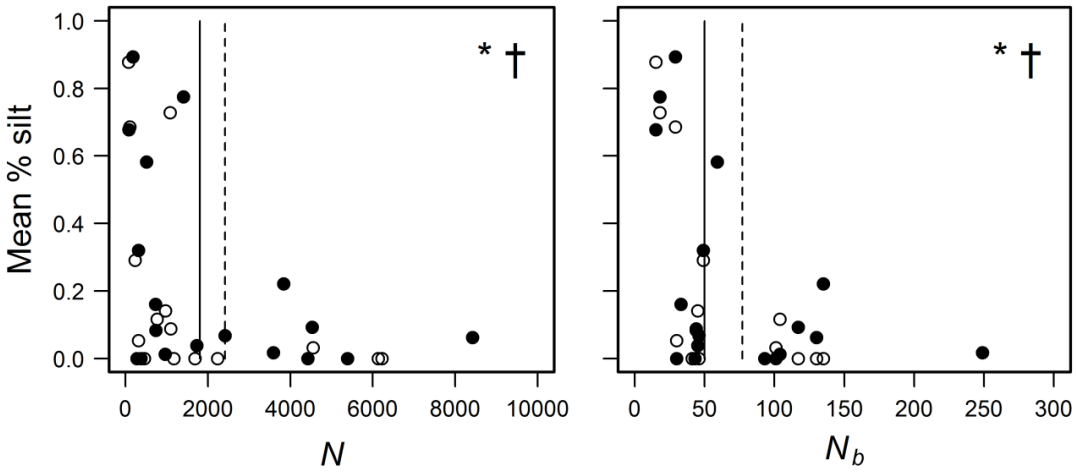

400
0
-400
-800
% silt
0
2000
4000
6000
8000
N
0
50
100
150
N_b
0
2500
5000
7500
10000
N
0
100
200
300
N_b

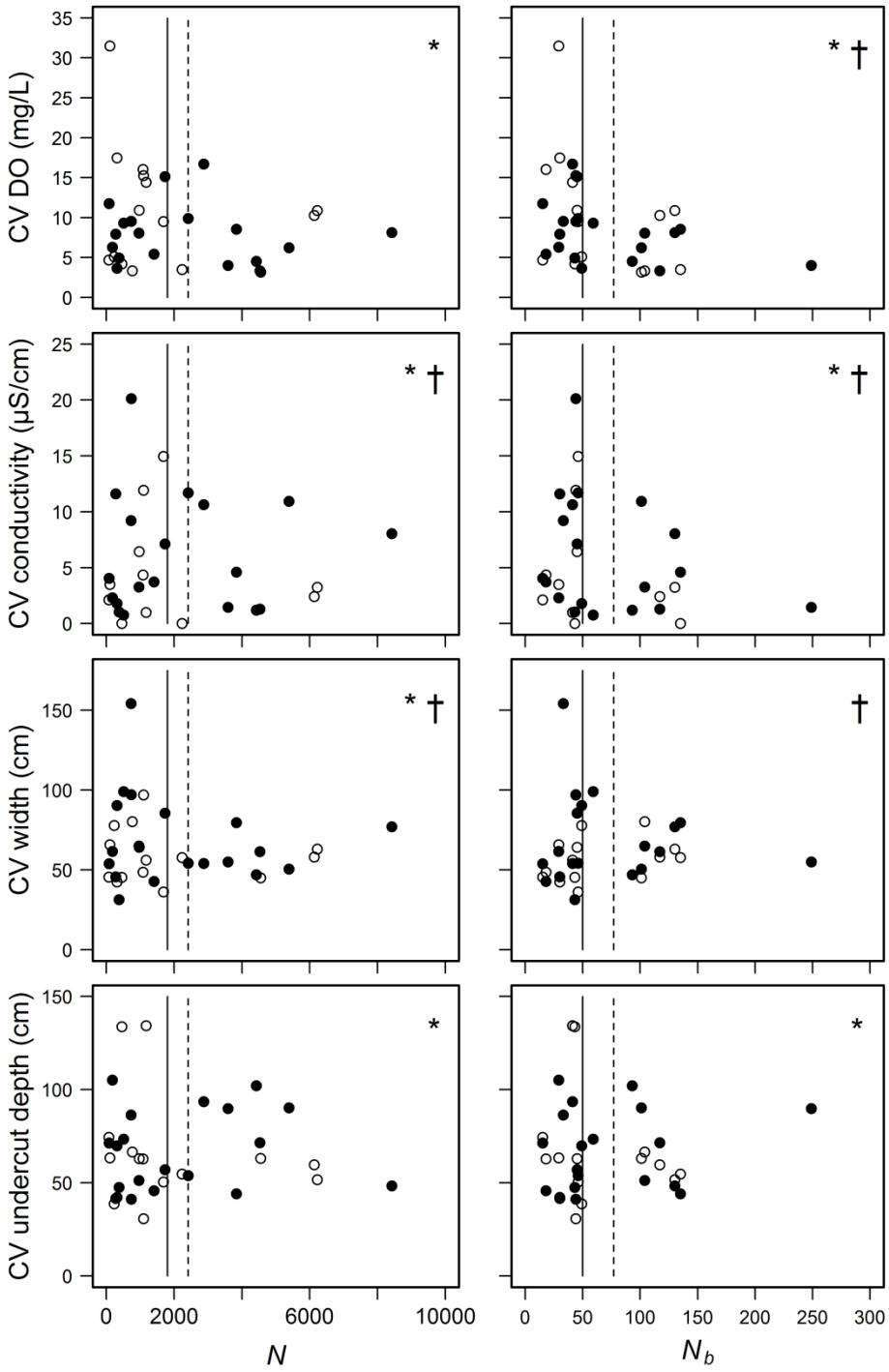


Appendix G (Fig. G1): Variable Hypothesis: habitat parameter means vs. N and N_b in 2010 (○) and 2011 (●). Cut-offs for population size bins are represented by solid lines for 2010 and dashed lines for 2011. Trends for increased variability at small populations size in 2010 and 2011 are indicated by (*) and (†), respectively.

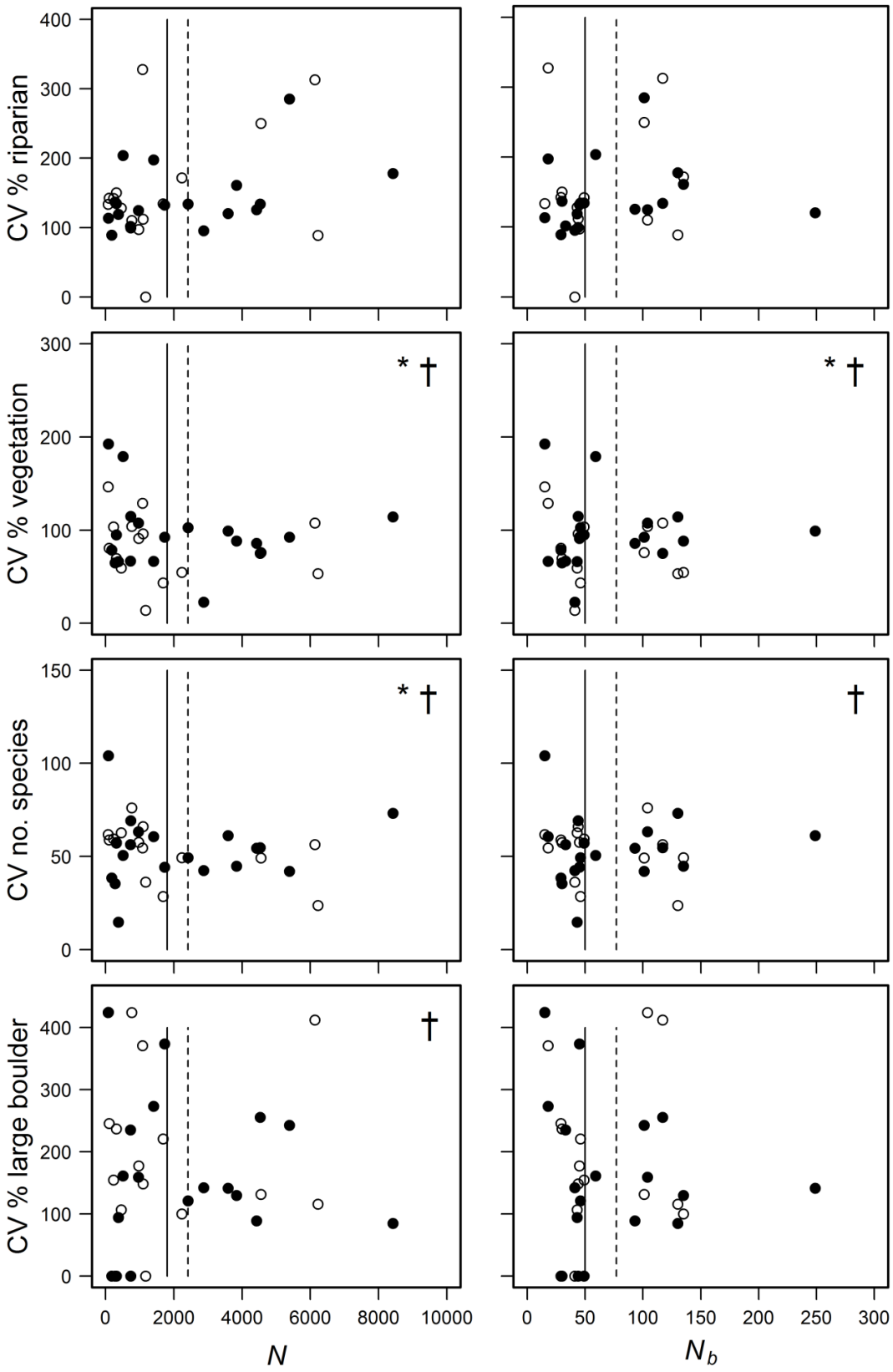

Mean % riparian
Mean % vegetation
Mean no. of species
Mean % large boulder
*
* †
* †
N
N_b

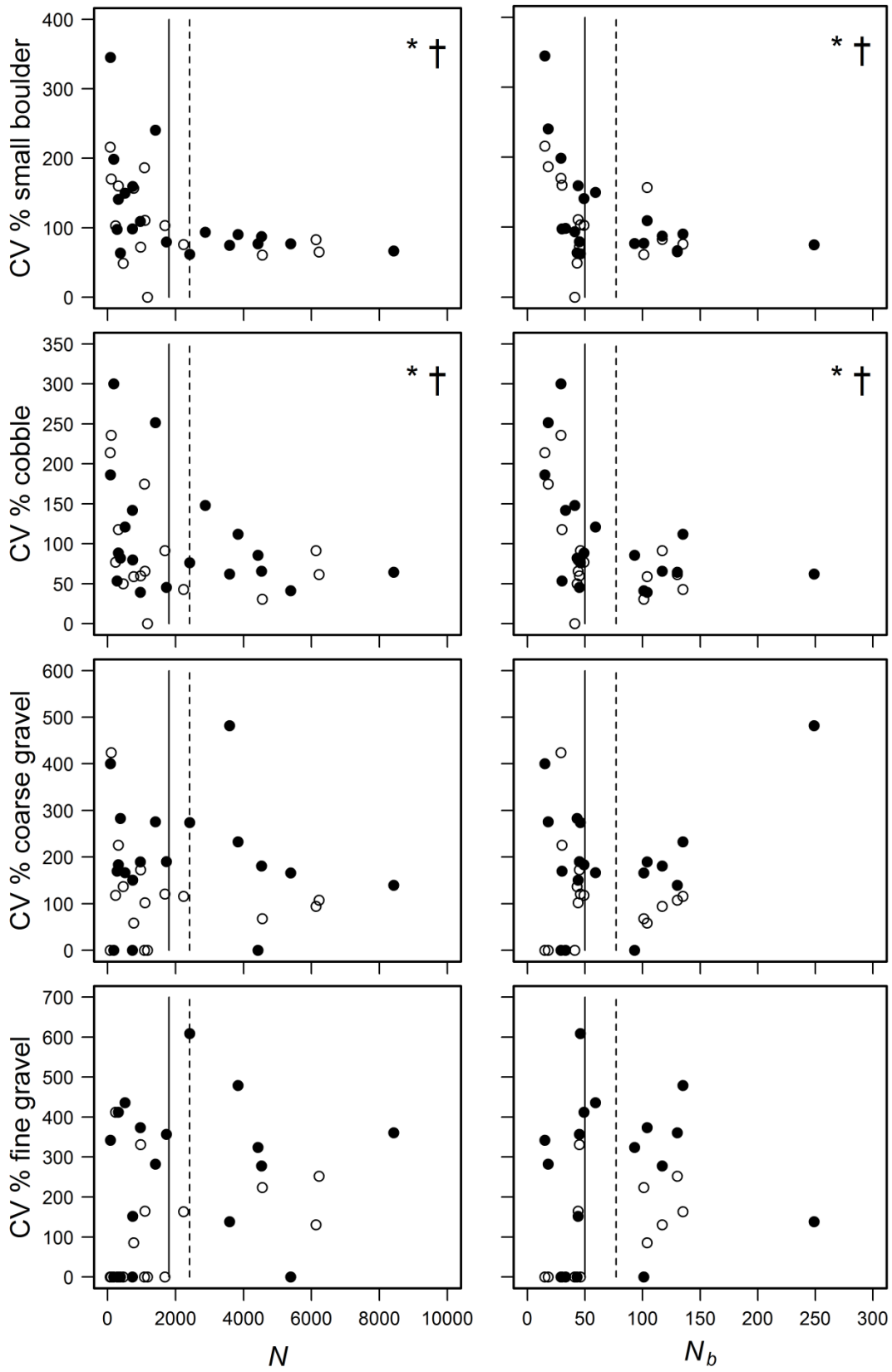

Mean % small boulder
Mean % cobble
Mean % coarse gravel
Mean % fine gravel
* †
* †
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
1.0
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0
2000
4000
6000
8000
10000
50
100
150
200
250
300
N
N_b

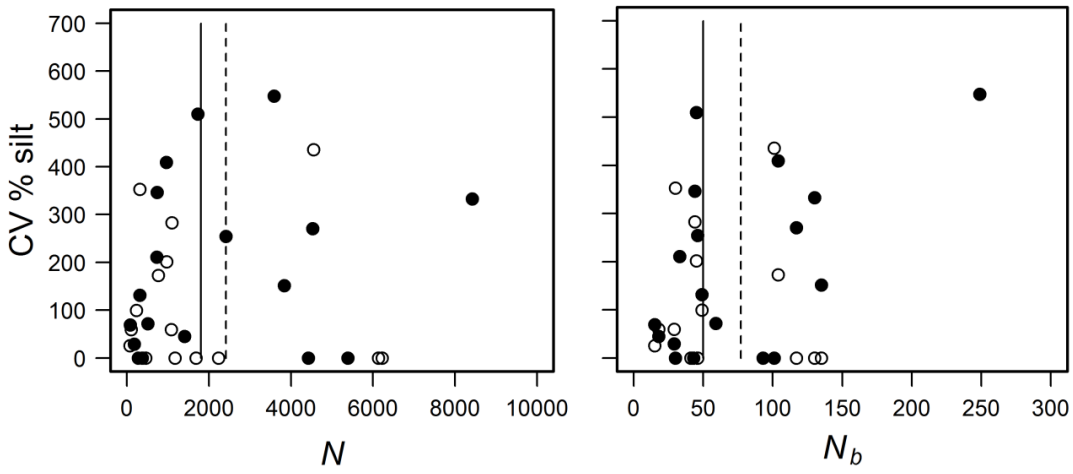

Mean % silt
1.0
0.8
0.6
0.4
0.2
0.0
0
2000
4000
6000
8000
10000
N
* †
0
50
100
150
200
250
300
N_b
* †

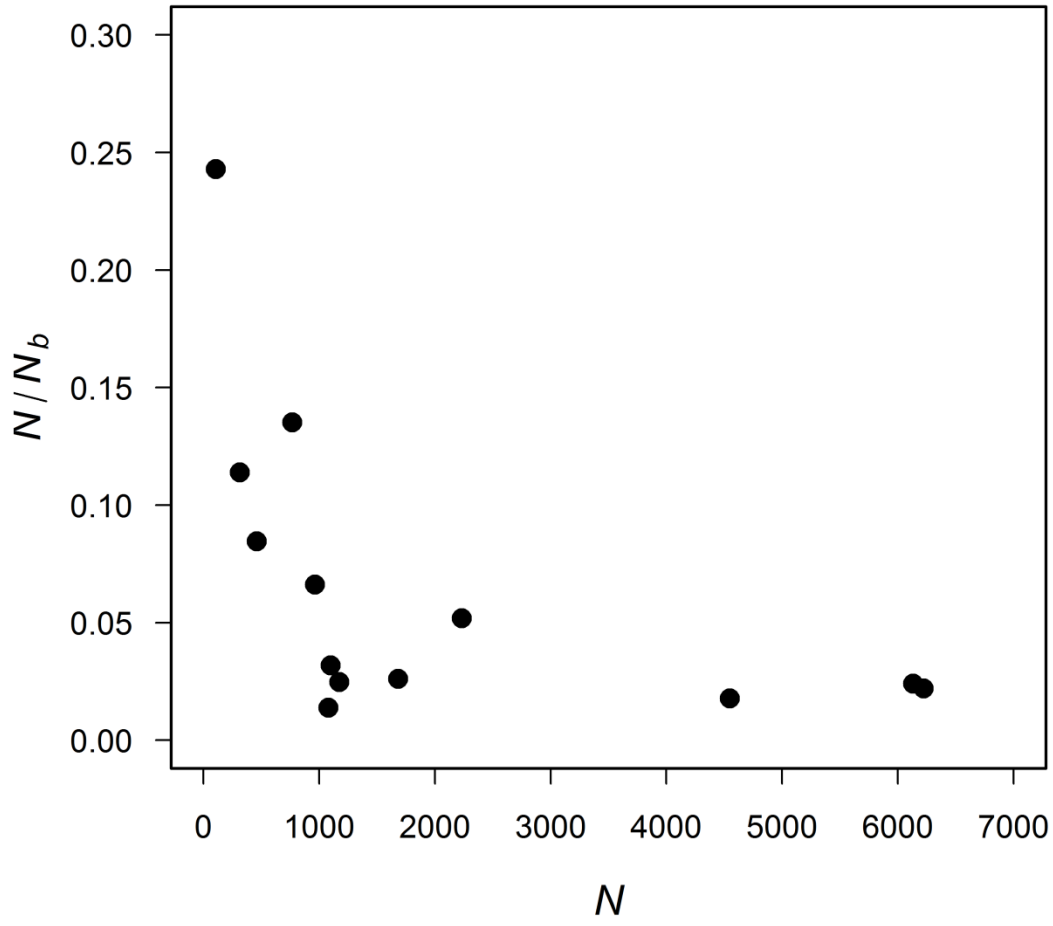


Appendix H (Fig. H1): Variable Hypothesis: habitat parameter CVs vs. N and N_b in 2010 (○) and 2011(●). Cut-offs for population size bins are represented by solid lines for 2010 and dashed lines for 2011. Trends for increased variability at small populations size in 2010 and 2011 are indicated by (*) and (†), respectively.


CV % riparian
CV % vegetation
CV no. species
CV % large boulder
* †
* †
* †
†
0
100
200
300
400
0
100
200
300
0
50
100
150
0
100
200
300
400
0
2000
4000
6000
8000
10000
N
0
50
100
150
200
250
300
N_b

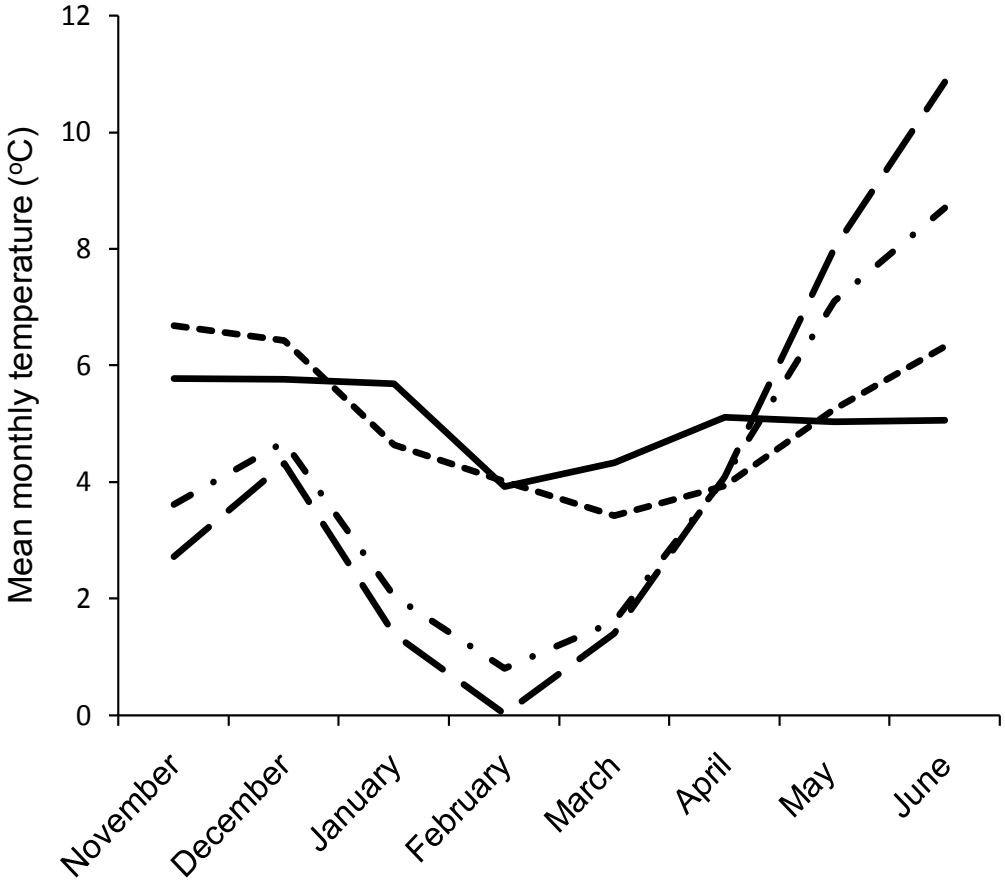

CV % small boulder
CV % cobble
CV % coarse gravel
CV % fine gravel
* †
N
N_b


CV % silt
700
600
500
400
300
200
100
0
0
2000
4000
6000
8000
10000
N
0
50
100
150
200
250
300
N_b



Appendix I (Fig. I1): Correctly matched N_b/N ratios (N_b from 2010, N from 2011) for 13 populations of brook trout at Cape Race, Newfoundland.

Chapter 2 Appendices



Appendix A (Fig. A1): Mean monthly temperatures in spawning areas for four Cape Race brook trout populations in 2010. Spawning area temperature data was unavailable for all populations due to the malfunction of temperature loggers that were placed in the streams.

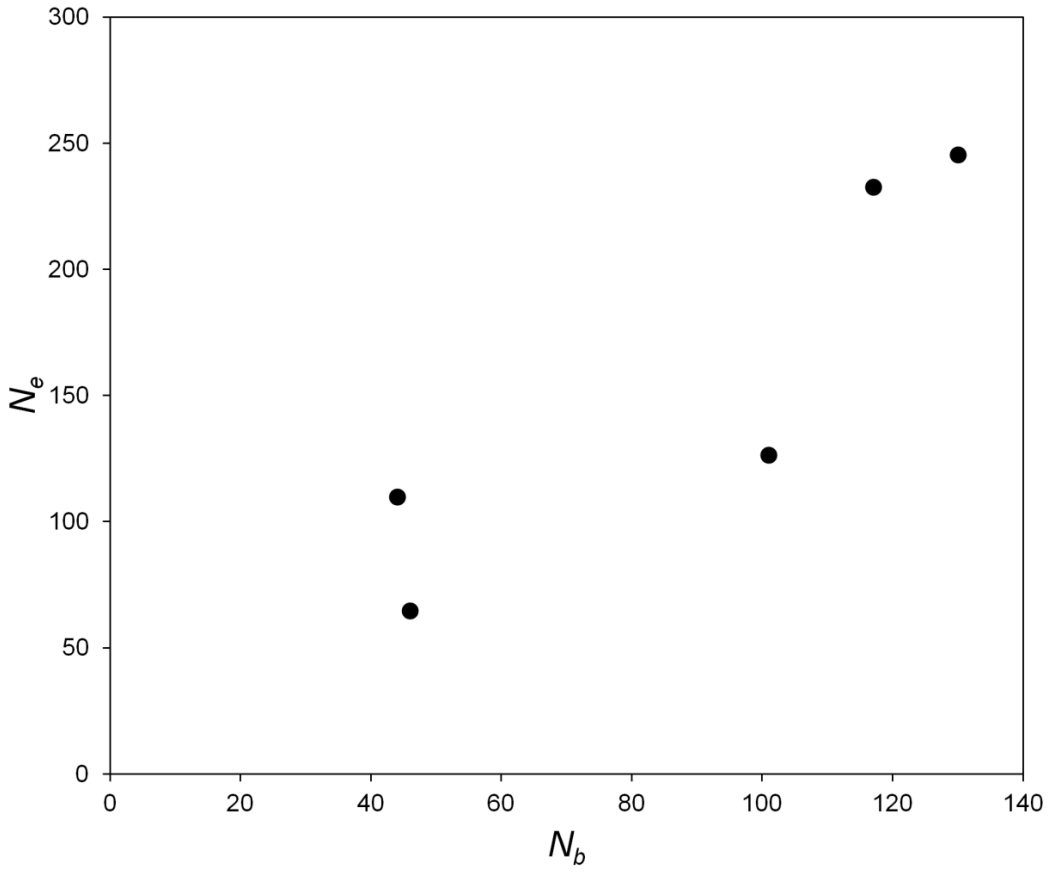
Appendix B (Table B1): Cape Race trout population census size for 2011 as well as the effective number of breeders (N_b). N_b reported is the weighted harmonic mean of point estimates across cohorts within a population. The range of point estimates are in parentheses. See Wood et al. 2013 for the 95% CI for each individual cohort.

Population	2011 *N* (95% CI)	N_b	C	Sample size
Freshwater	5385 (5076-5743)	101 (81-452)	3	45, 95, 114
Still There By Chance	1405 (1211-1696)	18 (7-72)	3	93, 42, 40
Whale Cove	735 (626-936)	44 (35-87)	3	66, 48, 108
Ditchy	179 (132-265)[a]	29 (26-34)	2	26[d], 35[d]
Upper OuananicheBeck	3835 (3355-6269)	135 (93-231)	3	67, 36, 93
Watern Cove	8416 (7225-10255)	130 (119-137)	3	59, 96, 133
Lower Blackfly	1731 (1148-2238)	45 (30-64)	3	46, 54, 52
Cripple Cove	2412 (2231-2632)	46 (28-101)	3	80, 76, 71

C = number of cohorts sampled. Unless otherwise stated; cohort sample sizes screened at microsatellite loci are listed in this order (3 = 2009, 2010, 2011; 2 = 2010, 2011; 1 = 2011).

[a]Schnabel method used for *N* estimation.

[b]2010 and 2011 cohorts, respectively.



Appendix C (Fig. C1): The relationship between the weighted harmonic mean N_b and N_e for five CR populations (Whale Cove, Watern Cove, Freshwater, Cripple Cove, and Bob's Cove; Wood et al. 2013) for which detailed life history data was available (Pearson's $r = 0.90$, $p = 0.039$). N_e was calculated using methods outlined in Waples et al. (2013).

Appendix D (Table D1): Regression coefficients estimated using GLMMs to evaluate the effect of shipment on mean trait values for six early life-history traits for eight Cape Race brook trout populations.

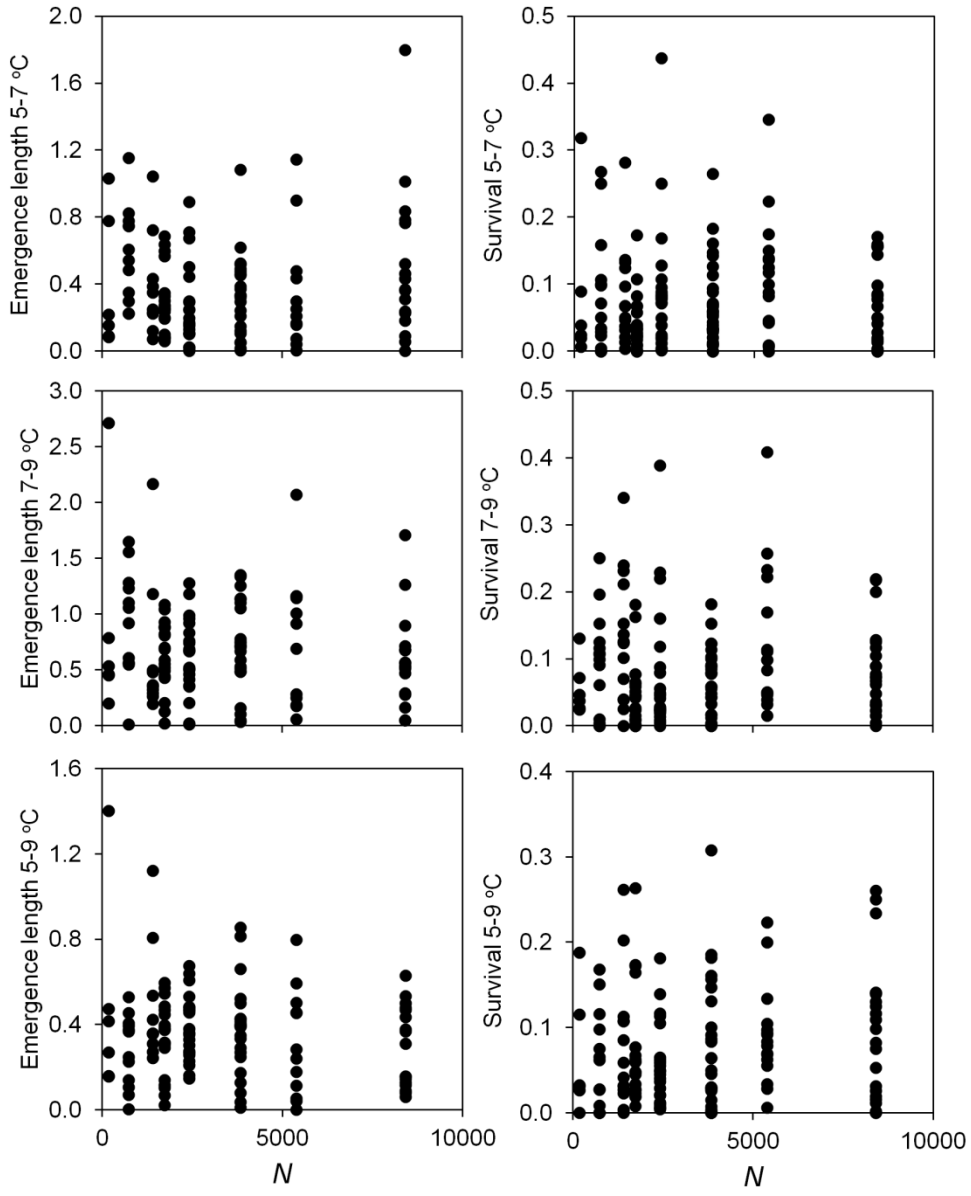
Fixed effects	Hatch date	Hatch length	Yolk-sac volume	Emergence length	Yolk-sac conversion efficiency	Survival
Cripple Cove						
Intercept	506.23*** (15.84)	13.14*** (0.70)	15.14*** (4.16)	20.67*** (0.70)	0.54*** (0.087)	0.40 (0.41)
Cold treatment	42.55*** (1.08)	1.28*** (0.068)	-5.07*** (0.45)	1.59*** (0.056)	0.14*** (0.018)	0.013 (0.048)
Medium treatment	43.29*** (1.04)	0.35*** (0.070)	0.25 (0.46)	1.19*** (0.056)	0.021 (0.017)	-0.019 (0.047)
Shipment	-5.19 (5.21)	-0.41 (0.24)	-0.83 (1.49)	0.045 (0.16)	0.017 (0.028)	0.15 (0.13)
Egg size	-0.94 (0.74)	0.12 *** (0.031)	0.62*** (0.18)	0.15*** (0.034)	-0.011** (3.5×10^{-3})	5.9×10^{-4} (0.019)
Family size	-5.38** (1.82)	-0.51*** (0.12)	-2.69*** (0.79)	-0.098 (0.096)	0.036 (0.028)	0.10 (0.082)
Random effect						
Family	9.83 (0.44)	0.44 (0.38)	2.65 (0.34)	0.52 (0.43)	0.039 (0.44)	0.28 (0.65)
Watern Cove						
Intercept	480.26*** (15.00)	11.24*** (1.37)	-2.80 (12.07)	18.73*** (1.38)	0.66*** (0.18)	0.37 (0.31)
Cold treatment	38.66*** (1.06)	1.14*** (0.077)	-5.33*** (0.48)	0.85*** (0.081)	0.12*** (0.023)	0.021 (0.046)
Medium treatment	45.41*** (1.40)	0.59*** (0.078)	0.24 (0.49)	1.32*** (0.080)	0.047* (0.023)	0.043 (0.046)
Shipment	3.05 (5.46)	0.13 (0.33)	-3.27 (2.86)	-0.056 (0.56)	0.040 (0.040)	0.034 (0.14)

Egg size	-0.35 (0.87)	0.16 (0.086)	1.65* (0.76)	0.26** (0.080)	-0.018 (0.011)	1.7×10^{-4} (0.015)
Family size	-9.01*** (2.17)	0.095 (0.17)	-0.54 (1.05)	0.026 (0.16)	0.030 (0.050)	0.12 (0.093)
Random effects						
Family	10.44 (0.50)	0.45 (0.44)	4.11 (0.51)	1.14 (0.59)	0.048 (0.46)	0.29 (0.66)
Still There by Chance						
Intercept	510.81*** (15.59)	11.16*** (1.37)	3.74 (4.91)	17.35*** (1.59)	0.77*** (0.16)	-0.15 (0.48)
Cold treatment	44.80*** (1.89)	0.81*** (0.14)	-4.02*** (0.64)	1.82*** (0.17)	0.19** (0.051)	0.089 (0.054)
Medium treatment	45.80*** (1.94)	0.64*** (0.15)	-1.16 (0.66)	1.37*** (0.17)	0.029 (0.050)	0.23*** (0.053)
Shipment	-12.21 (6.57)	-0.29 (0.53)	-0.86 (2.10)	-0.96 (0.92)	0.19* (0.090)	0.051 (0.21)
Egg size	-1.93 (1.26)	0.11 (0.11)	0.98* (0.39)	0.21 (0.13)	-0.024 (0.011)	0.044 (0.039)
Family size	-3.84 (5.33)	0.76 (0.44)	1.49 (2.00)	0.81 (0.51)	0.0028 (0.11)	0.057 (0.17)
Random effects						
Family	7.43 (0.41)	0.52 (0.47)	2.20 (0.42)	0.69 (0.44)	3.0×10^{-7} (3.5×10^{-6})	0.24 (0.65)

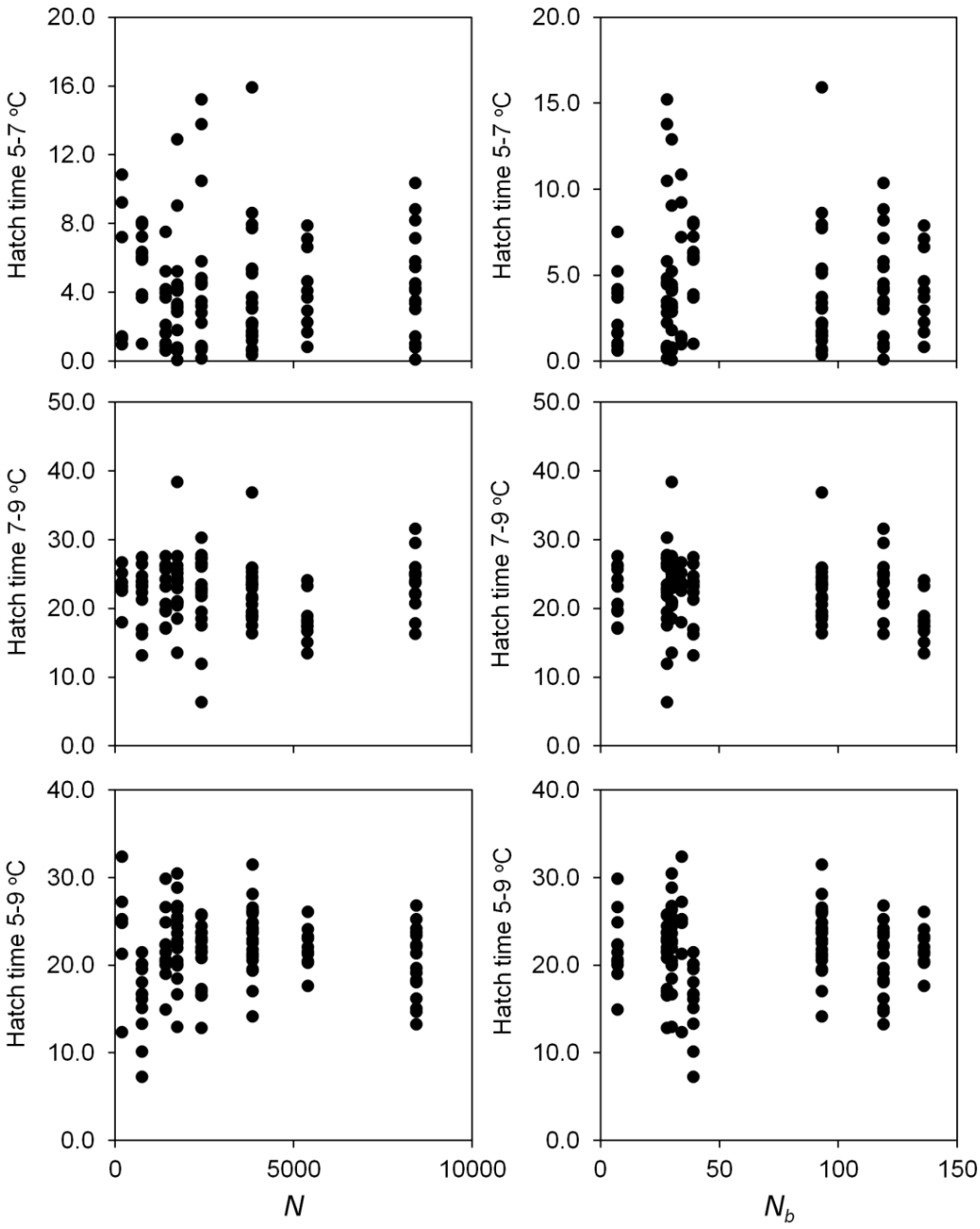
Appendix E (Table E1): Regression coefficients estimated using GLMMs to evaluate the effect of temperature regime, *N*, egg size, family size, and interactions on mean trait values for six early life-history traits for eight Cape Race brook trout populations.

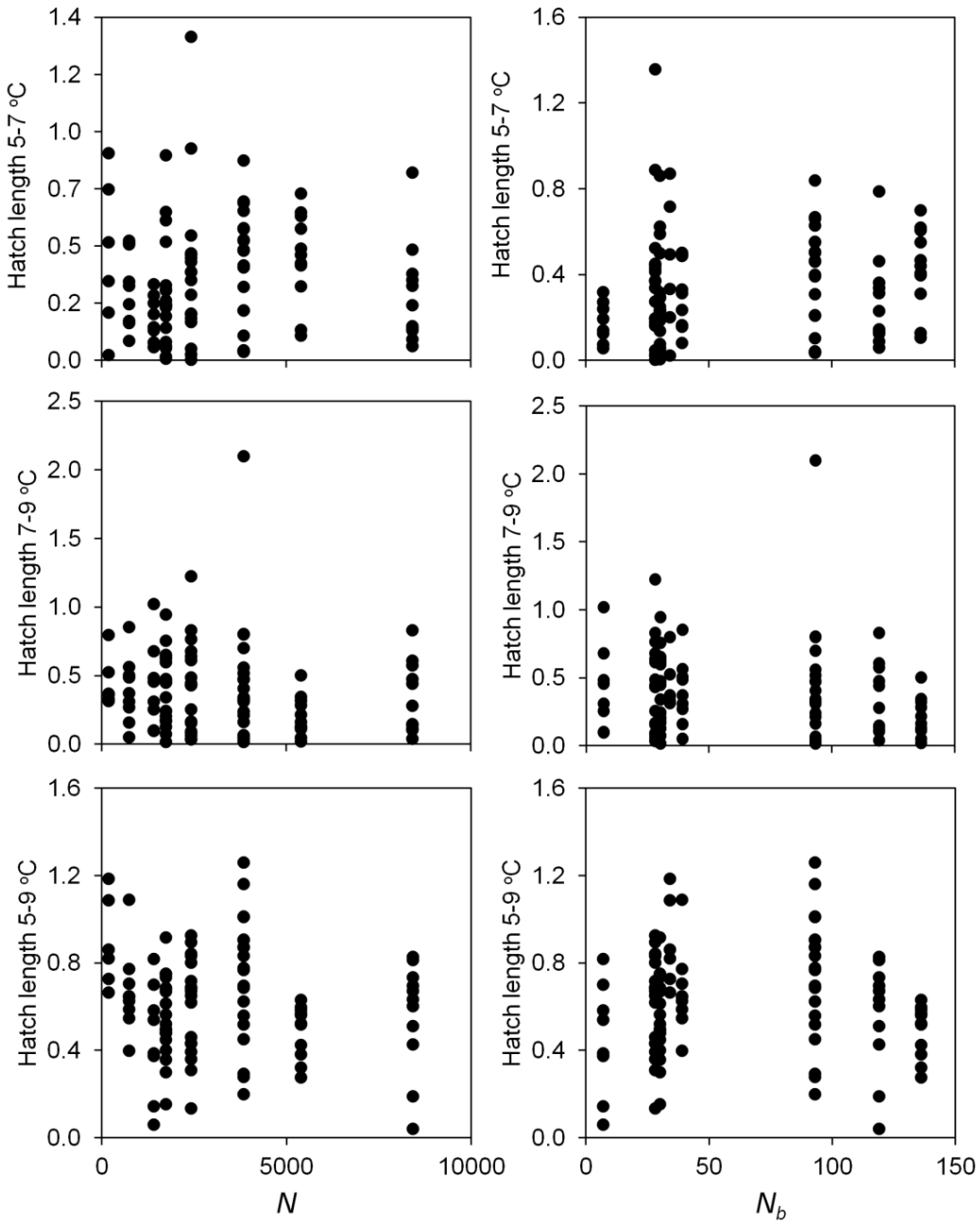
Fixed effects	Hatch date	Hatch length	Yolk-sac volume	Emergence length	Yolk-sac conversion efficiency	Survival
Intercept	4.6×10^{2}***	11.41***	8.17***	19.63***	0.91	-0.18
	(10.71)	(0.47)	(2.38)	(0.72)	(1.67)	(1.22)
Cold treatment	43.47***	1.01***	-4.72***	0.53**	1.00	0.30
	(2.48)	(0.15)	(0.99)	(0.16)	(1.59)	(1.19)
Medium treatment	49.50***	1.17***	-1.31	0.69***	0.23	1.20
	(2.43)	(0.16)	(1.01)	(0.16)	(1.51)	(1.19)
N	4.4×10^{-3}	-6.5×10^{-5}	-2.5×10^{-4}	-1.2×10^{-4}	-5.2×10^{-5}	-1.7×10^{-5}
	(2.7×10^{-3})	(1.3×10^{-4})	(7.3×10^{-4})	(1.7×10^{-4})	(4.7×10^{-4})	(2.4×10^{-4})
Egg size	0.75	0.14***	0.84***	0.14***	-0.082	0.026
	(0.54)	(0.026)	(0.14)	(0.039)	(0.091)	(0.066)
Family size	-2.02	-0.26*	-0.38	0.10	0.15	0.020
	(1.65)	(0.12)	(0.75)	(0.11)	(0.89)	(0.70)
Cold treatment × *N*	-4.0×10^{-4}*	-2.9×10^{-5}*	1.3×10^{-4}	-6.4×10^{-5}***	-1.9×10^{-5}	-3.4×10^{-5}
	(1.9×10^{-4})	(1.3×10^{-5})	(8.1×10^{-5})	(1.2×10^{-5})	(1.2×10^{-4})	(9.9×10^{-5})
Medium treatment × *N*	-1.7×10^{-4}	-3.0×10^{-5}*	5.3×10^{-7}	6.3×10^{-6}	1.2×10^{-5}	-3.2×10^{-5}
	(1.8×10^{-4})	(1.3×10^{-5})	(8.3×10^{-5})	(1.2×10^{-5})	(1.2×10^{-4})	(9.9×10^{-5})
Cold treatment × egg size	-0.27 *	1.0×10^{-2}	-0.16**	0.055***	-0.019	-0.021
	(0.12)	(7.6×10^{-3})	(0.049)	(7.6×10^{-3})	(0.081)	(0.060)
Medium treatment × egg size	-0.12	-3.2×10^{-3}	0.087	0.047***	4.5×10^{-4}	-0.069
	(0.13)	(7.8×10^{-3})	(0.050)	(7.7×10^{-3})	(0.079)	(0.060)
N × egg size	-1.4×10^{-4}	4.9×10^{-6}	2.7×10^{-5}	1.2×10^{-5}	3.0×10^{-6}	-5.9×10^{-9}
	(1.3×10^{-4})	(7.1×10^{-6})	(4.2×10^{-5})	(8.6×10^{-6})	(2.4×10^{-5})	(1.2×10^{-5})
Cold treatment × family size	5.20***	0.15	1.46*	0.091	-0.013	0.34
	(1.33)	(0.092)	(0.59)	(0.092)	(0.92)	(0.71)

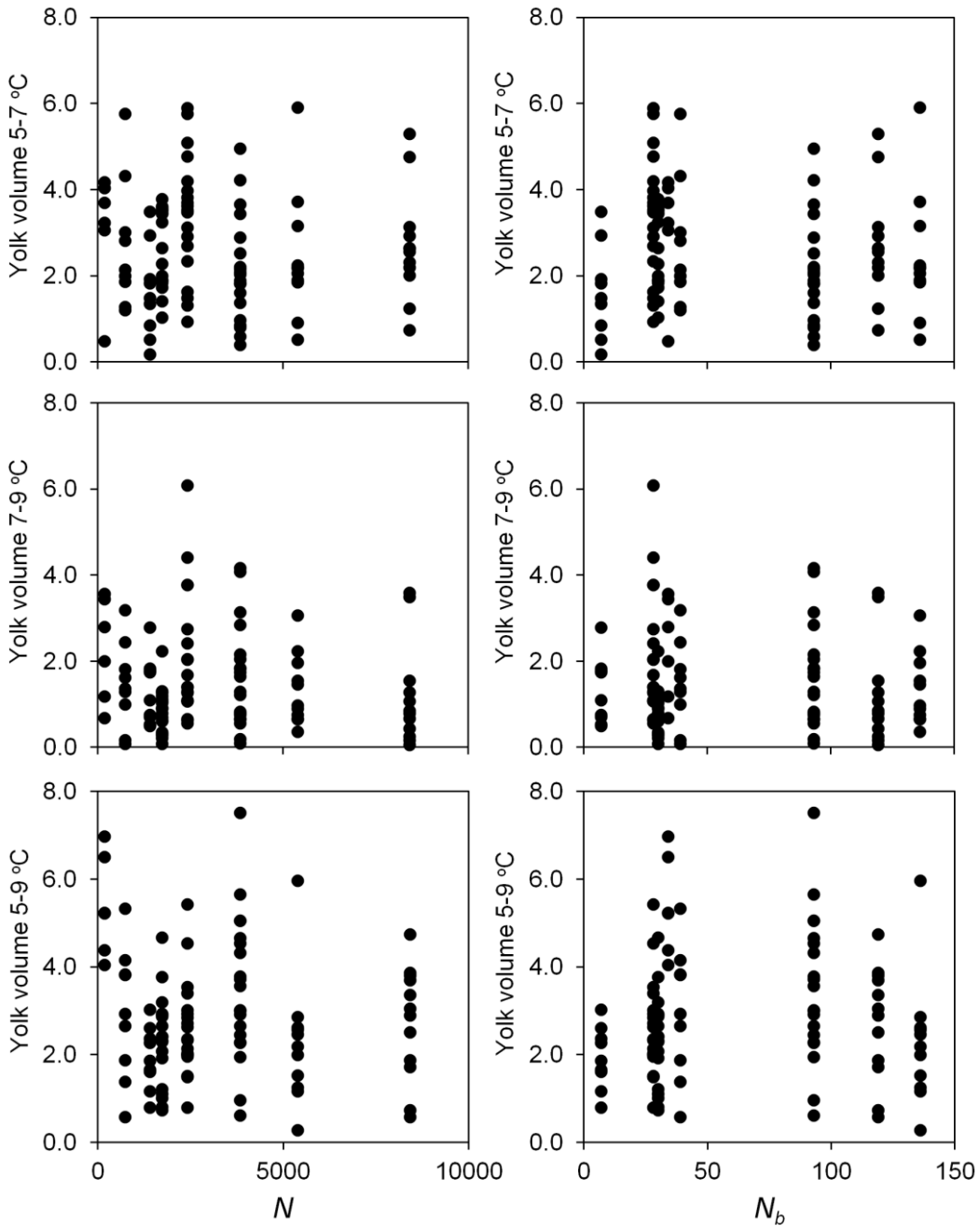
Medium treatment × family size	-2.70* (1.22)	-0.48*** (0.089)	-0.57 (0.57)	-0.14 (0.084)	-0.089 (0.88)	0.11 (0.70)
N × family size	-9.7×10^{-4}* (3.9×10^{-4})	4.1×10^{-5} (2.8×10^{-5})	-2.4×10^{-4} (1.8×10^{-4})	-3.3×10^{-5} (2.6×10^{-5})	-1.7×10^{-5} (2.2×10^{-4})	-2.7×10^{-5} (1.4×10^{-4})
Random effects						
Family	10.09 (0.31)	0.42 (0.31)	2.75 (0.37)	0.78 (0.35)	0.95 (0.78)	0.94 (0.81)
Stream	11.15 (0.34)	0.29 (0.22)	0.20 (0.027)	0.64 (0.29)	0.27 (0.22)	0.23 (0.19)



Appendix F (Fig. F1): Directional Hypothesis: Absolute values of slopes between three temperature regimes in relation to N and N_b for six early life-history traits to assess the magnitude of plasticity in relation to population size for eight brook trout populations at Cape Race, NL.


Hatch time 5-7 °C
Hatch time 7-9 °C
Hatch time 5-9 °C
N
N_b


Hatch length 5-7 °C
Hatch length 7-9 °C
Hatch length 5-9 °C
N
N_b


Yolk volume 5-7 °C
Yolk volume 7-9 °C
Yolk volume 5-9 °C
0.0
2.0
4.0
6.0
8.0
0
5000
10000
N
0
50
100
150
N_b

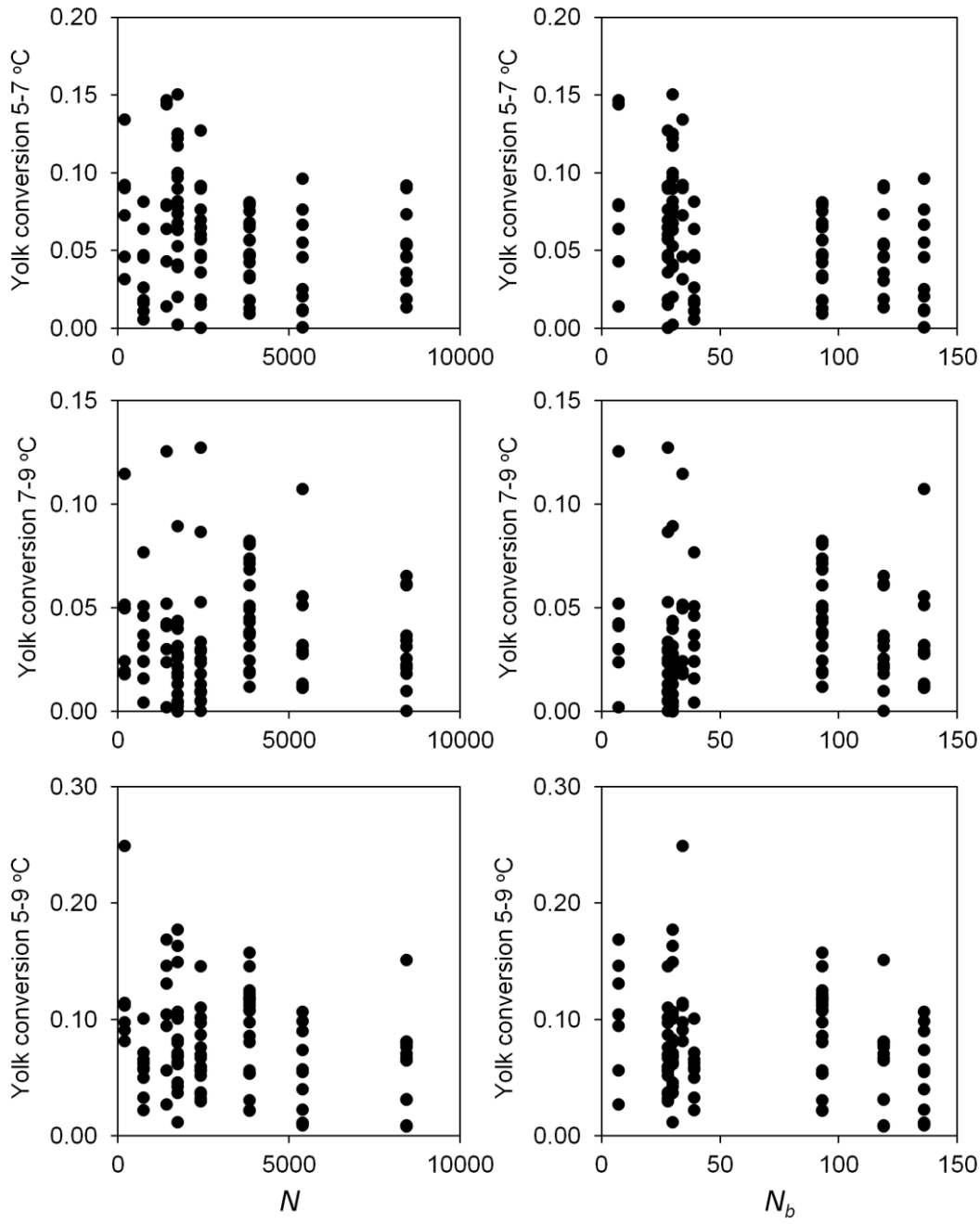

Yolk conversion 5-7 °C
Yolk conversion 7-9 °C
Yolk conversion 5-9 °C
0.00
0.05
0.10
0.15
0.20
0.30
0
5000
10000
50
100
150
N
N_b

Table F1: Spearman's correlations for the relationship between the absolute value of slopes (magnitude of plasticity) between three temperature regimes and population size (N and N_b) for eight Cape Race brook trout populations.

	N_b		N	
	ρ	p-value	ρ	p-value
Hatch date				
5-7	-0.055	0.56	-0.049	0.61
7-9	-0.080	0.40	-0.089	0.35
5-9	0.14	0.15	0.070	0.46
Hatch length				
5-7	0.16	0.12	0.11	0.27
7-9	-0.12	0.24	-0.18	0.063
5-9	0.088	0.38	-0.084	0.40
Yolk-sac volume				
5-7	-0.052	0.60	-0.041	0.68
7-9	0.0089	0.93	-0.063	0.53
5-9	0.10	0.30	-0.050	0.61
Emergence length				
5-7	-0.049	0.59	-0.045	0.63
7-9	0.025	0.79	-0.052	0.58
5-9	-0.028	0.76	-0.053	0.56
Yolk-sac conversion				
5-7	-0.20	0.052	-0.20	0.048
7-9	0.17	0.10	0.072	0.48
5-9	-0.049	0.63	-0.17	0.087
Survival				
5-7	0.049	0.56	0.091	0.28
7-9	-0.049	0.56	0.046	0.59
5-9	0.043	0.61	0.13	0.11

Table F2: GLMM regression coefficients (±SE) for the effect of N_b, egg size, family size, and interactions on magnitude of plasticity for three of six early life-history traits for eight Cape Race trout populations. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets.

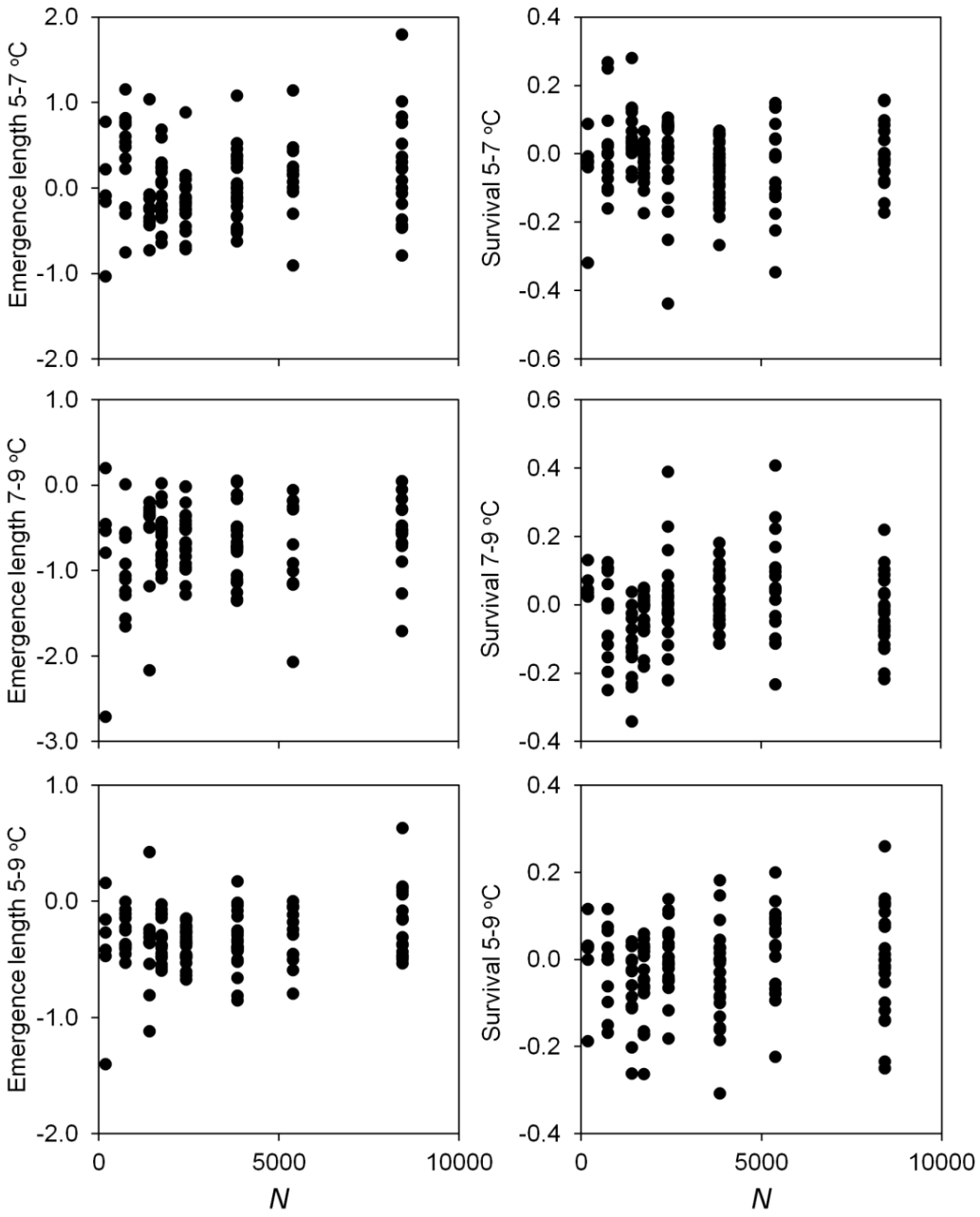
	Hatch date			Yolk-sac volume			Emergence length		
Fixed effects	5-7°C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	4.37 (3.38)	28.43*** (4.58)	21.86*** (4.93)	-1.15 (1.21)	1.24 (1.23)	2.18 (1.72)	2.5×10^{-3} (0.31)	0.59 (0.40)	0.52* (0.21)
N_b	4.7×10^{-3} (0.043)	-0.10 (0.059)	-0.010 (0.060)	0.026 (0.016)	2.3×10^{-3} (0.017)	-5.6×10^{-3} (0.023)	4.5×10^{-3} (3.8×10^{-3})	-3.3×10^{-3} (5.1×10^{-3})	-3.9×10^{-3} (2.6×10^{-3})
Egg size	-0.24 (0.17)	-0.31 (0.24)	-0.10 (0.25)	0.19** (0.058)	0.031 (0.064)	0.070 (0.085)	0.018 (0.016)	0.020 (0.020)	-6.9×10^{-5} (0.010)
Family size	4.30* (1.99)	-1.18 (2.76)	0.94 (2.61)	1.28 (0.74)	-0.16 (0.71)	-0.32 (0.87)	0.092 (0.19)	-0.22 (0.27)	-7.2×10^{-4} (0.13)
N_b × egg size	2.1×10^{-3} 2.2×10^{-3}	5.5×10^{-3} (3.0×10^{-3})	1.5×10^{-3} (2.9×10^{-3})	-1.1×10^{-3} (8.3×10^{-4})	-1.8×10^{-5} (8.4×10^{-4})	2.3×10^{-4} (1.1×10^{-3})	-2.1×10^{-4} (1.9×10^{-4})	-8.7×10^{-6} (2.5×10^{-4})	1.9×10^{-4} (1.3×10^{-4})
N_b × family size	-0.043* (0.021)	0.014 (0.029)	-0.011 (0.027)	-0.016 (8.6×10^{-3})	-4.7×10^{-3} (8.2×10^{-3})	8.3×10^{-6} (0.010)	-1.2×10^{-3} (2.0×10^{-3})	3.1×10^{-3} (2.8×10^{-3})	-8.2×10^{-5} 1.4×10^{-3}
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	1.08 (0.24)	1.19 (0.21)	2.41 (0.37)	0.00 (0.00)	0.40 (0.27)	1.07 (0.45)	0.093 (0.23)	0.00 (0.00)	0.041 (0.16)

Table F3: GLMM regression coefficients (±SE) for the effect of *N*, egg size, family size, and interactions on magnitude of plasticity for six early life-history traits for eight Cape Race trout populations. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets.

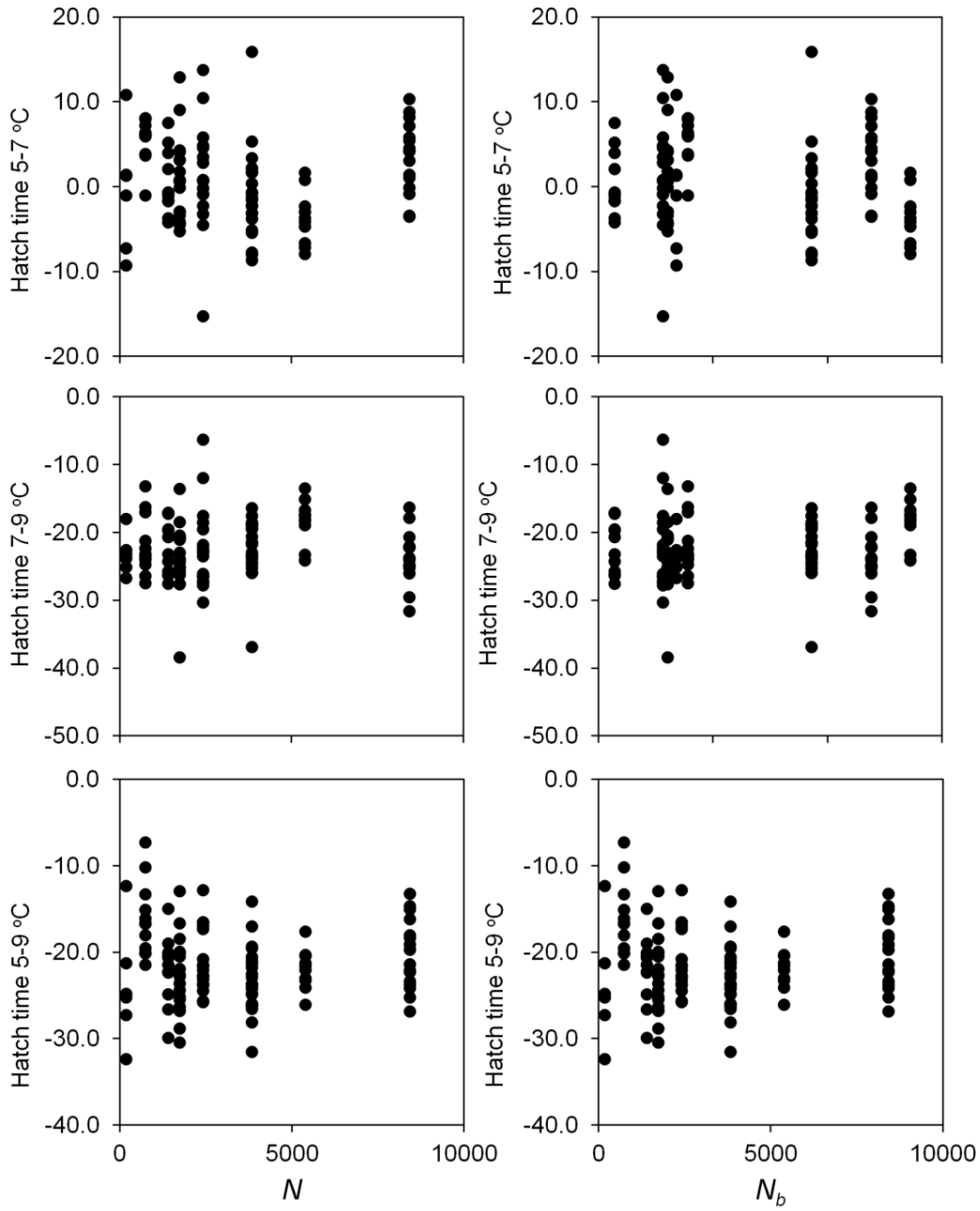
	Hatch length			Yolk-sac conversion efficiency			Survival		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	0.25 (0.25)	0.46 (0.30)	0.52* (0.26)	0.12*** (0.031)	0.055* (0.028)	0.16*** (0.041)	0.061 (0.052)	0.16* (0.059)	0.11* (0.045)
N	2.4×10^{-5} (7.6×10^{-5})	-7.6×10^{-5} (9.3×10^{-5})	1.6×10^{-5} (7.8×10^{-5})	-2.8×10^{-6} (9.3×10^{-6})	-8.1×10^{-7} (8.4×10^{-6})	-1.0×10^{-5} (1.2×10^{-5})	-1.2×10^{-5} (1.1×10^{-5})	-2.0×10^{-5} (1.2×10^{-5})	-6.6×10^{-6} (9.6×10^{-6})
Egg size	5.9×10^{-3} (0.013)	-4.0×10^{-3} (0.015)	6.9×10^{-3} (0.013)	-5.0×10^{-3}** (1.6×10^{-3})	-1.1×10^{-4} (1.4×10^{-3})	-4.2×10^{-3} (2.1×10^{-3})	3.8×10^{-3} (2.6×10^{-3})	-2.0×10^{-3} (3.0×10^{-3})	-9.5×10^{-4} 2.3×10^{-3}
Family size	-0.022 (0.13)	0.055 (0.17)	0.044 (0.13)	0.036* (0.016)	-0.021 (0.015)	-4.7×10^{-3} (0.022)	-0.054 (0.034)	-0.061 (0.036)	-0.042 (0.030)
N × egg size	-1.7×10^{-6} (3.8×10^{-5})	3.3×10^{-7} (4.7×10^{-6})	-1.3×10^{-6} (3.8×10^{-6})	4.3×10^{-7} (4.7×10^{-7})	-2.1×10^{-7} (4.2×10^{-7})	2.3×10^{-7} (6.3×10^{-7})	1.4×10^{-7} (5.8×10^{-7})	6.1×10^{-7} (6.2×10^{-7})	3.7×10^{-7} (5.1×10^{-7})
N × family size	8.3×10^{-6} (3.3×10^{-5})	-1.6×10^{-5} (4.2×10^{-5})	-1.5×10^{-5} (3.2×10^{-5})	-8.2×10^{-6}* 3.9×10^{-6}	5.3×10^{-6} 3.6×10^{-6}	3.3×10^{-6} 5.2×10^{-6}	1.1×10^{-5} 7.6×10^{-6}	1.3×10^{-5} 7.8×10^{-6}	5.8×10^{-6} 6.7×10^{-6}
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	0.066 (0.27)	0.00 (0.00)	0.11 (0.48)	7.1×10^{-3} (0.24)	6.5×10^{-3} (0.24)	0.011 (0.26)	0.00 (0.00)	0.022 (0.28)	0.00 (0.00)

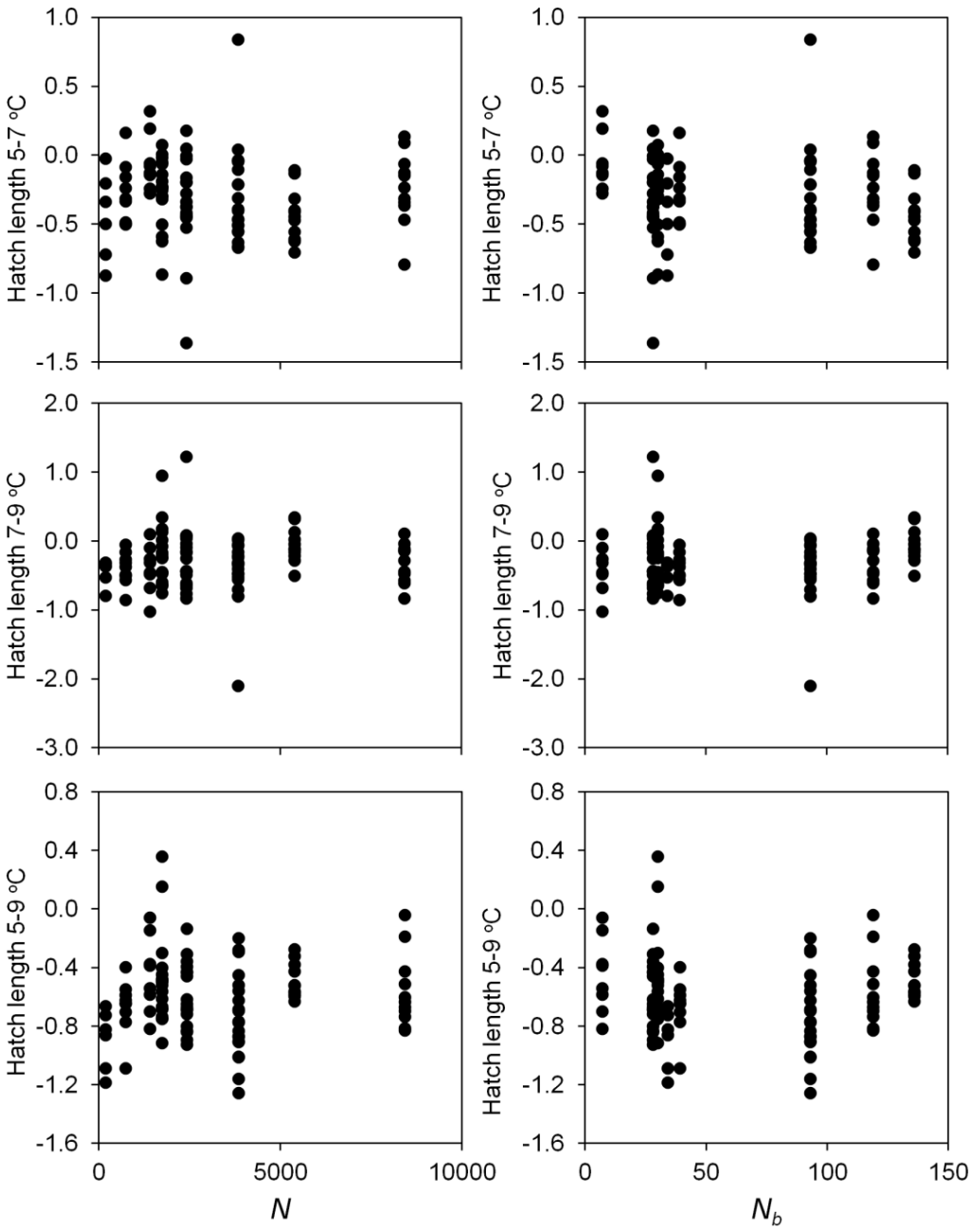
	Hatch date			Yolk-sac volume			Emergence length		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	6.29*	27.50*	21.75*	-0.52	1.09	2.19	0.11	0.36	0.40

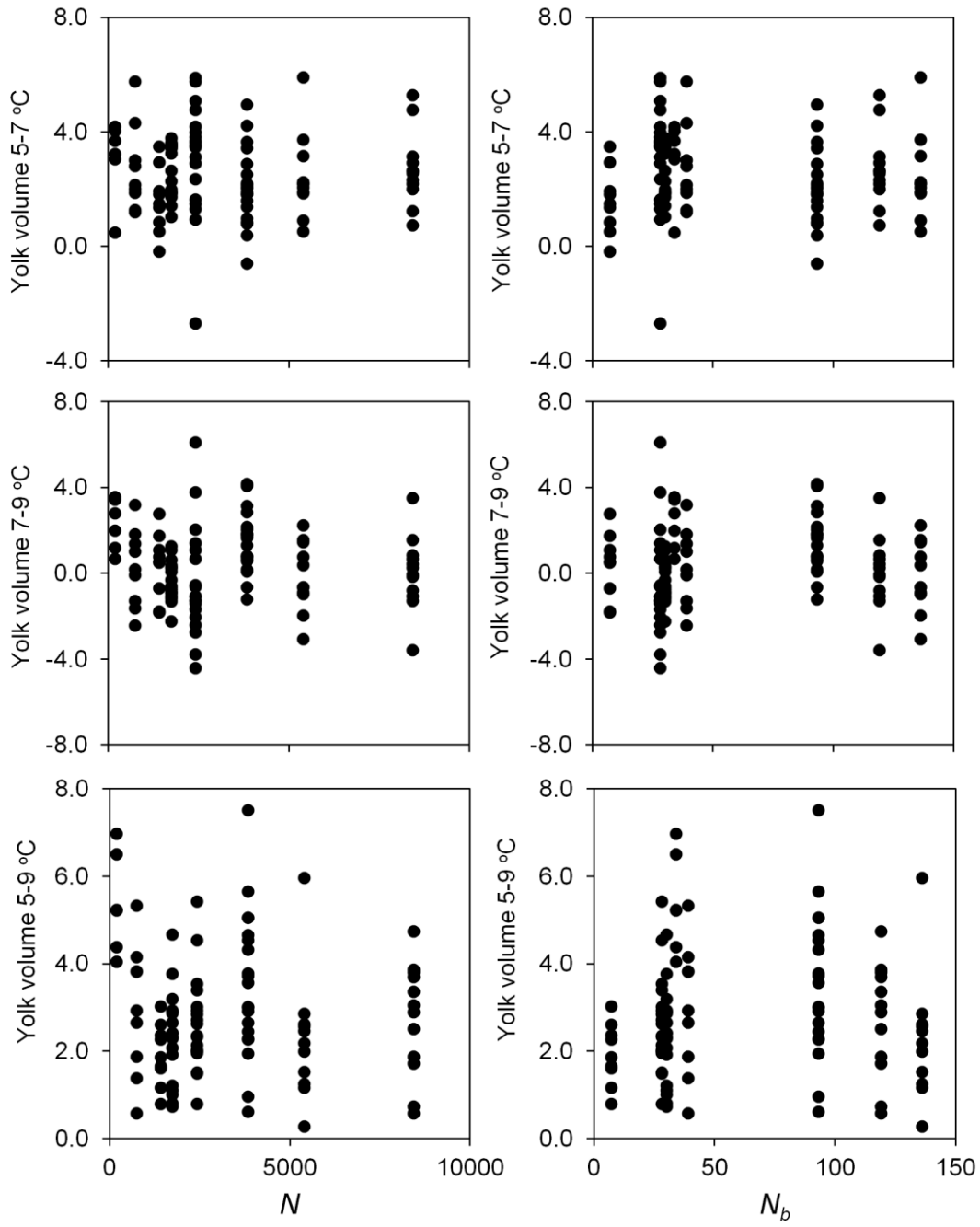
	(2.91)	(3.91)	(4.16)	(1.16)	(1.11)	(1.51)	(0.26)	(0.34)	(0.17)
N	-5.4×10^{-4}	-1.9×10^{-3}	-2.4×10^{-4}	3.5×10^{-4}	8.9×10^{-5}	-1.3×10^{-4}	6.8×10^{-5}	9.9×10^{-7}	-3.8×10^{-5}
	(7.7×10^{-4})	(1.0×10^{-3})	(1.0×10^{-3})	(3.6×10^{-4})	(3.4×10^{-4})	(4.5×10^{-4})	(6.3×10^{-5})	(8.5×10^{-5})	(4.2×10^{-5})
Egg size	-0.16	-0.26	-0.078	0.17**	0.048	0.11	0.015	0.037*	-2.1×10^{-3}
	(0.15)	(0.20)	(0.20)	(0.060)	(0.057)	(0.075)	(0.013)	(0.017)	(8.3×10^{-3})
Family size	0.85	-0.94	1.18	0.67	-0.22	-0.80	-0.013	-0.26	0.43
	(1.69)	(2.30)	(2.16)	(0.63)	(0.58)	(0.71)	(0.16)	(0.22)	(0.11)
N × egg size	3.4×10^{-5}	1.0×10^{-4}	3.1×10^{-5}	-1.6×10^{-5}	-4.4×10^{-6}	-7.0×10^{-6}	-3.7×10^{-6}	-6.0×10^{-6}	2.3×10^{-6}
	(3.9×10^{-5})	(5.3×10^{-5})	(5.1×10^{-5})	(1.8×10^{-5})	(1.7×10^{-5})	(2.2×10^{-5})	(3.1×10^{-6})	(4.3×10^{-6})	(2.1×10^{-6})
N × family size	-8.2×10^{-5}	2.0×10^{-4}	-3.4×10^{-4}	-1.7×10^{-4}	-8.8×10^{-5}	1.4×10^{-4}	1.3×10^{-6}	8.8×10^{-5}	-1.5×10^{-5}
	(4.0×10^{-4})	5.5×10^{-4}	(5.1×10^{-4})	(1.5×10^{-4})	(1.4×10^{-4})	(1.7×10^{-4})	(3.7×10^{-5})	(5.2×10^{-5}	(2.6×10^{-5})
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	0.84	1.02	2.38	0.31	0.36	0.98	0.077	0.00	6.4×10^{-7}
	(0.25)	(0.22)	(0.57)	(0.26)	(0.34)	(0.75)	(0.25)	(0.00)	(0.22)



Appendix G (Fig. G1): Variable Hypothesis: Values of slopes between three temperature regimes in relation to N and N_b for six early life-history traits to assess the extent of plasticity in relation to population size for eight book trout populations at Cape Race, NL.


Hatch time 5-7 °C
Hatch time 7-9 °C
Hatch time 5-9 °C
20.0
10.0
0.0
-10.0
-20.0
-30.0
-40.0
-50.0
0
5000
10000
N
N_b


Hatch length 5-7 °C
Hatch length 7-9 °C
Hatch length 5-9 °C
1.0
0.5
0.0
-0.5
-1.0
-1.5
2.0
-2.0
-3.0
0.8
0.4
-0.4
-0.8
-1.2
-1.6
0
5000
10000
50
100
150
N
N_b


Yolk volume 5-7 °C
Yolk volume 7-9 °C
Yolk volume 5-9 °C
8.0
4.0
0.0
-4.0
-8.0
6.0
2.0
0
5000
10000
50
100
150
N
N_b

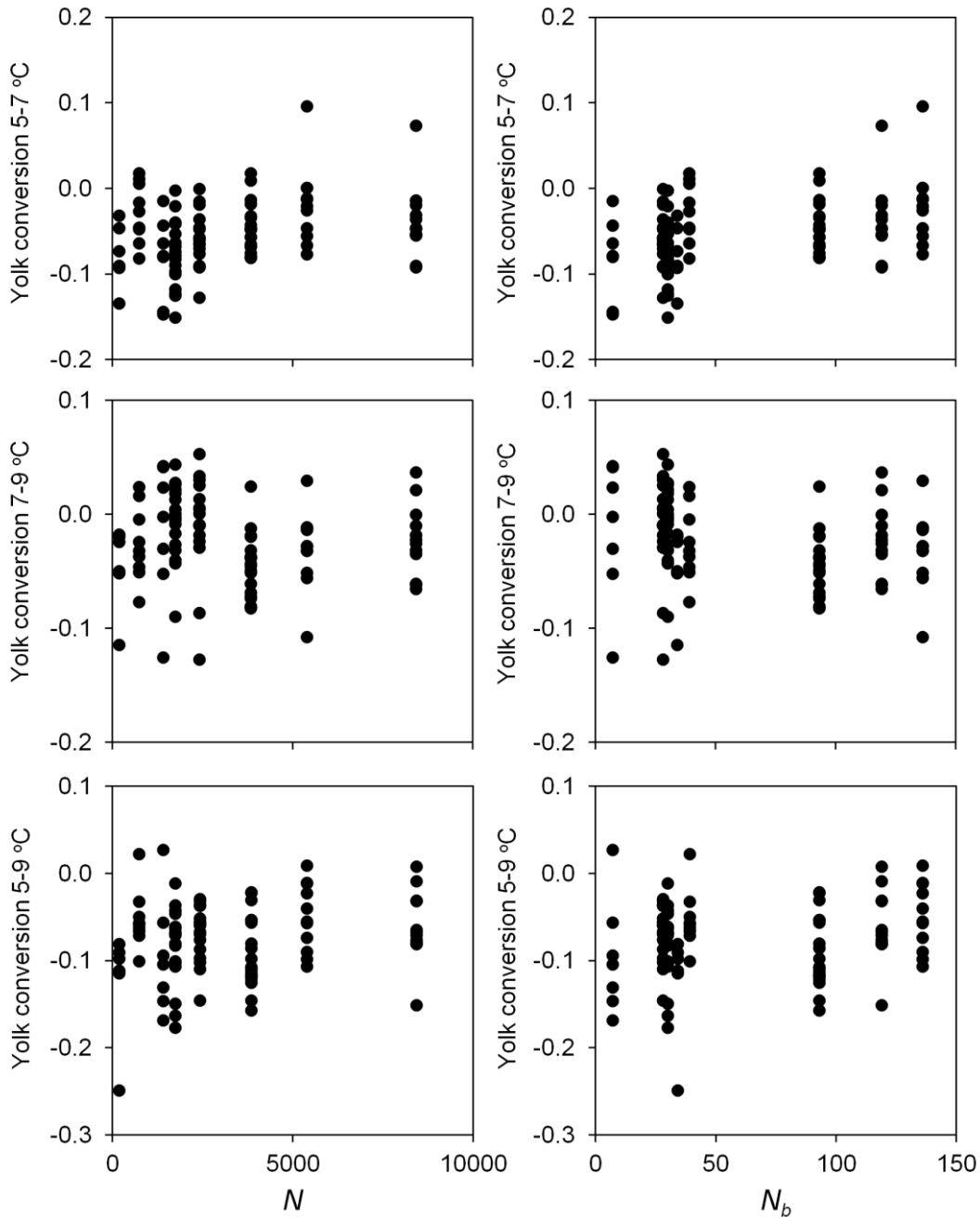

Yolk conversion 5-7 °C
Yolk conversion 7-9 °C
Yolk conversion 5-9 °C
0.2
0.1
0.0
-0.1
-0.2
-0.3
0
5000
10000
N
50
100
150
N_b

Table G1: GLMM regression coefficients (±SE) for the effect of N_b, egg size, family size, and interactions on extent of plasticity for three of six early life-history traits for eight Cape Race trout populations. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets.

	Hatch date			Yolk-sac volume			Emergence length		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	5.07 (5.82)	-28.43*** (4.58)	-21.86*** (4.93)	-1.14 (1.27)	3.39 (1.97)	2.18 (1.72)	-0.45 (0.46)	-0.51 (0.40)	-0.45 (0.23)
N_b	-0.11 (0.071)	0.10 (0.059)	0.010 (0.060)	0.025 (0.018)	-0.023 (0.027)	-5.6×10^{-3} (0.027)	7.0×10^{-3} (5.7×10^{-3})	2.5×10^{-3} (5.2×10^{-3})	4.5×10^{-3} (2.9×10^{-3})
Egg size	-0.24 (0.29)	0.31 (0.24)	0.10 (0.25)	0.20*** (0.066)	-0.13 (0.10)	0.070 (0.085)	0.035 (0.024)	-0.022 (0.020)	-4.3×10^{-3} (0.011)
Family size	-2.23 (3.07)	1.18 (2.76)	-0.94 (2.61)	0.96 (0.84)	-1.57 (1.12)	-0.32 (0.87)	-0.21 (0.29)	0.18 (0.28)	-0.023 (0.16)
N_b × egg size	4.4×10^{-3} (3.5×10^{-3})	-5.5×10^{-3} (3.0×10^{-3})	-1.5×10^{-3} (2.9×10^{-3})	-1.2×10^{-3} (9.3×10^{-4})	1.0×10^{-3} (1.3×10^{-3})	2.3×10^{-4} (1.1×10^{-4})	-3.8×10^{-4} (2.8×10^{-4})	3.5×10^{-5} (2.6×10^{-4})	-1.5×10^{-4} (1.5×10^{-4})
N_b × family size	0.029 (0.032)	-0.014 (0.029)	0.011 (0.027)	-0.013 (9.7×10^{-3})	0.013 (0.013)	8.3×10^{-6} (0.010)	8.9×10^{-4} (3.0×10^{-3})	-2.7×10^{-3} (2.8×10^{-3})	-9.8×10^{-4} (1.6×10^{-3})
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	2.87 (0.37)	1.19 (0.21)	2.41 (0.37)	0.00 (0.00)	0.70 (0.29)	1.07 (0.45)	0.12 (0.18)	0.00 (0.00)	1.3×10^{-5} (1.3×10^{-6})

Table G2: GLMM regression coefficients (±SE) for the effect of *N*, egg size, family size, and interactions on extent of plasticity for six early life-history traits for eight Cape Race trout populations. Values for the random effect are standard deviations with the proportion of total variation attributed to the random effect in brackets.

	Hatch length			Yolk-sac conversion efficiency			Survival		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	-0.048 (0.31)	-0.46 (0.40)	-0.55 (0.28)	-0.13*** (0.038)	-0.022 (0.039)	-0.10*** (0.066)	0.083 (0.082)	-0.15 (0.087)	-0.10 (0.066)
N	-7.7×10^{-5} (9.3×10^{-5})	4.5×10^{-5} (1.2×10^{-4})	-1.0×10^{-5} (8.6×10^{-5})	1.1×10^{-5} (1.1×10^{-5})	-1.3×10^{-6} (1.2×10^{-5})	1.6×10^{-5} (1.4×10^{-5})	2.7×10^{-6} (1.7×10^{-5})	1.2×10^{-5} (1.7×10^{-5})	1.6×10^{-5} (1.4×10^{-5})
Egg size	-0.013 (0.016)	6.2×10^{-3} (0.021)	-4.0×10^{-3} (0.015)	5.4×10^{-3}** (2.0×10^{-3})	-1.3×10^{-3} (2.0×10^{-3})	4.4×10^{-3} (3.4×10^{-3})	-7.7×10^{-3} (4.2×10^{-3})	0.010* (4.4×10^{-3})	4.4×10^{-3} (3.4×10^{-3})
Family size	-0.039 (0.16)	9.0×10^{-4} (0.21)	-0.048 (0.14)	-0.029 (0.020)	0.027 (0.020)	1.1×10^{-3} (0.044)	0.043 (0.049)	-0.040 (0.048)	1.1×10^{-3} (0.044)
N × egg size	4.3×10^{-6} (4.6×10^{-6})	-2.4×10^{-6} (6.1×10^{-6})	7.9×10^{-7} (4.2×10^{-6})	-7.4×10^{-7} (5.7×10^{-7})	5.1×10^{-7} (5.8×10^{-7})	-4.8×10^{-7} (7.5×10^{-7})	5.8×10^{-7} (8.5×10^{-7})	-9.6×10^{-7} (8.6×10^{-7})	-4.8×10^{-7} (7.5×10^{-7})
N × family size	2.4×10^{-6} (3.9×10^{-5})	1.3×10^{-5} (5.3×10^{-5})	1.5×10^{-5} (3.5×10^{-5})	6.6×10^{-6} (4.8×10^{-6})	-9.3×10^{-6} (4.8×10^{-6})	-4.8×10^{-6} (9.9×10^{-6})	-1.53×10^{-5} (1.1×10^{-5})	9.9×10^{-6} (1.0×10^{-5})	-4.8×10^{-6} (9.9×10^{-6})
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	0.097 (0.33)	0.11 (0.27)	0.12 (0.47)	0.011 (0.31)	0.012 (0.33)	0.00 (0.00)	0.031 (0.30)	0.048 (0.47)	0.00 (0.00)

	Hatch date			Yolk-sac volume			Emergence length		
Fixed effects	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Intercept	5.67	-27.50*	-21.80*	-0.68	3.03	2.19	-0.37	-0.27	-0.30

	(4.92)	(39.10)	(4.16)	(1.27)	(1.83)	(1.51)	(0.39)	(0.35)	(0.20)
N	-1.9×10^{-3}	1.9×10^{-3}	2.4×10^{-4}	3.7×10^{-4}	-4.4×10^{-4}	-1.3×10^{-4}	1.2×10^{-4}	-2.3×10^{-5}	4.7×10^{-5}
	1.2×10^{-3}	(1.0×10^{-3})	(1.0×10^{-3})	(4.0×10^{-4})	(5.6×10^{-4})	(4.5×10^{-4})	(9.4×10^{-5})	(8.6×10^{-5})	(5.0×10^{-5})
Egg size	-0.17	0.26	0.078	0.18**	-0.079	0.11	0.033	-0.041*	-4.0×10^{-3}
	(0.24)	(0.20)	(0.21)	(0.066)	(0.094)	(0.075)	(0.019)	(0.017)	(9.8×10^{-3})
Family size	-2.22	0.94	-1.18	0.51	-1.49	-0.80	-0.27	0.23	-0.038
	(2.54)	(2.30)	(2.16)	(0.71)	(0.93)	(0.71)	(0.24)	(0.22)	(0.13)
N × egg size	7.7×10^{-5}	-1.0×10^{-4}	-3.1×10^{-5}	-1.8×10^{-5}	9.2×10^{-6}	-7.0×10^{-6}	-8.0×10^{-6}	6.9×10^{-6}	-5.8×10^{-7}
	(6.0×10^{-5})	(5.3×10^{-5})	(5.1×10^{-5})	(2.0×10^{-5})	(2.8×10^{-5})	(2.2×10^{-5})	(4.7×10^{-6})	(4.4×10^{-6})	(2.5×10^{-6})
N × family size	6.4×10^{-4}	-2.0×10^{-4}	3.4×10^{-4}	-1.5×10^{-4}	2.8×10^{-4}	1.4×10^{-4}	4.8×10^{-5}	-8.1×10^{-5}	-1.4×10^{-5}
	6.0×10^{-4}	(5.5×10^{-4})	(5.1×10^{-4})	(1.7×10^{-4})	(2.3×10^{-4})	(1.7×10^{-4})	(5.6×10^{-5})	(5.3×10^{-5})	(3.0×10^{-5})
Random effect	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C	5-7 °C	7-9 °C	5-9 °C
Stream	2.90	1.02	2.38	0.24	0.78	0.98	0.091	0.00	0.00
	(0.59)	(0.22)	(0.57)	(0.18)	(0.46)	(0.75)	(0.19)	(0.00)	(0.00)

Table G3: Results of White's tests for residual heteroscedasticity in slope values between three temperature regimes in relation to *N* for six early life-history traits for eight brook trout populations at Cape Race, NL.

	N		
	White's	df	*p*-value
Hatch date			
5-7	0.12	2	0.94
7-9	0.95	2	0.62
5-9	10.14	2	0.0063
Hatch length			
5-7	0.40	2	0.82
7-9	1.54	2	0.46
5-9	0.50	2	0.78
Yolk-sac volume			
5-7	0.66	2	0.72
7-9	0.49	2	0.78
5-9	1.26	2	0.53
Emergence length			
5-7	6.95	2	0.031
7-9	3.15	2	0.21
5-9	4.12	2	0.13
Yolk-sac conversion			
5-7	0.78	2	0.68
7-9	1.51	2	0.47
5-9	2.70	2	0.26
Survival			
5-7	0.047	2	0.98
7-9	0.30	2	0.86
5-9	4.90	2	0.086

Chapter 3 Appendices

Appendix A (Table A1)**:** Cape Race trout population census size and N_b for 2011. N_b reported is the weighted harmonic mean of point estimates across cohorts within a population. The range of point estimates are in parentheses. See Wood et al. 2013 for the 95% CI for each individual cohort.

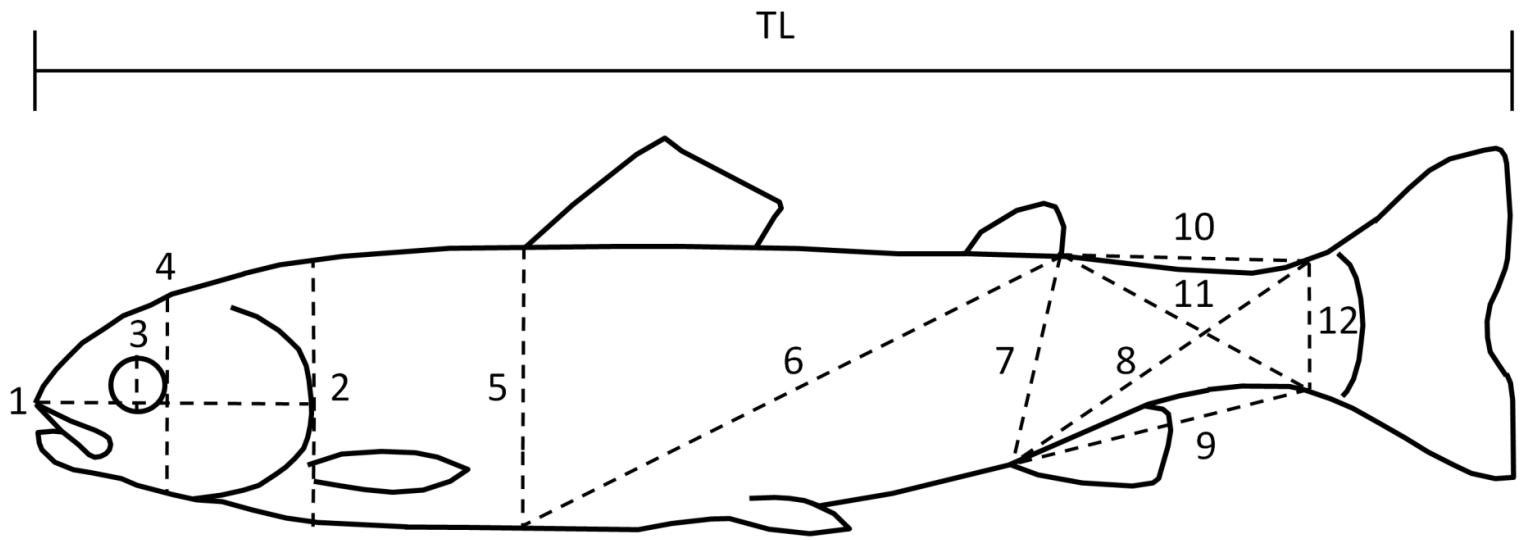
Population Code	2011 *N* (95% CI)	N_b	C	Sample size
FW	5385 (5076-5743)	101 (81-452)	3	45, 95, 114
BC	4527 (4052-5167)	117 (69-423)	3	62, 95, 105
STBC	1405 (1211-1696)	18 (7-72)	3	93, 42, 40
WC	735 (626-936)	44 (35-87)	3	66, 48, 108
DY	179 (132-265)[a]	29 (26-34)	2	26[d], 35[d]
UO	3835 (3355-6269)	135 (93-231)	3	67, 36, 93
WN	8416 (7225-10255)	130 (119-137)	3	59, 96, 133
LB	1731 (1148-2238)	45 (30-64)	3	46, 54, 52
CC	2412 (2231-2632)	46 (28-101)	3	80, 76, 71

Population Codes: FW=Freshwater River, BC=Bob's Cove, STBC=Still There By Chance River, WC=Whale Cove, DY=Ditchy Brook, UO=Upper Ouananiche Beck, WN=Watern Cove, LB=Lower Blackfly River, CC=Cripple Cove.

C = number of cohorts sampled. Unless otherwise stated; cohort sample sizes screened at microsatellite loci are listed in this order (3 = 2009, 2010, 2011; 2 = 2010, 2011; 1 = 2011).

[a]Schnabel method used for *N* estimation.

[b]2010 and 2011 cohorts, respectively.



Appendix B (Fig. B1): Landmarks used for morphometric analyses on Cape brook trout: (1) head length; (2) head depth at the posterior edge of the operculum; (3) eye diameter; (4) head depth at the most posterior point of the eye; (5) body depth; (6) posterior adipose insertion to point of maximum body depth, ventral (BD: ADP); (7) anterior insertion of the anal fin to posterior insertion of adipose fin (ANA: ADP); (8) anterior insertion of anal fin to caudal peduncle, dorsal (ANA: CPD); (9) anterior insertion of anal fin to caudal peduncle, ventral (ANA: CPV); (10) posterior insertion of adipose fin to caudal peduncle, dorsal (ADP: CPD); (11) posterior insertion of adipose fin to caudal peduncle, ventral (ADP: CPV); (12) caudal peduncle depth (CPD: CPV); (TL) total length (used as a covariate in analyses).

Appendix C (Table C1): Variance component estimates (95% CI) for additive genetic variance (V_A), maternal variance (V_M), and residual environmental variance (V_R) for 21 traits measured from 9 brook trout populations at Cape Race, Newfoundland.

Population	Trait	V_A	V_M	V_E
FW	Hatch time	125.07(40.63-256.24)	57.25(13.40-152.62)	77.84(16.63-130.16)
	Hatch length	0.46(0.30-0.59)	0.61(0.31-1.29)	0.071(0.029-0.16)
	Yolk volume	9.96(4.25-23.08)	28.83(12.42-49.45)	10.60(4.07-14.80)
	Emergence length	0.72(0.43-0.92)	2.35(1.26-4.19)	0.16(0.074-0.31)
	Yolk conversion	0.0025(0.00060-0.019)	0.002(0.0005-0.0096)	0.010(0.001-0.019)
	Survival	0.019(0.0033-0.067)	0.020(0.0032-0.058)	0.010(0.0028-0.053)
	Head length	0.0075(0.0036-0.018)	0.004(0.0023-0.0099)	0.016(0.01-0.020)
	Head width	0.022(0.011-0.037)	0.008(0.0034-0.018)	0.013(0.0059-0.021)
	Eye diameter	0.00090(0.00030-0.0029)	0.0007(0.0003-0.0018)	0.0034(0.0022-0.0043)
	Head depth behind eye	0.011(0.0036-0.024)	0.0032(0.0015-0.0086)	0.017(0.0092-0.023)
	Body depth	0.011(0.0053-0.024)	0.0059(0.0029-0.013)	0.014(0.0087-0.021)
	BD: ADP	0.019(0.0080-0.037)	0.0153(0.0077-0.033)	0.036(0.024-0.046)
	ANA: ADP	0.018(0.0090-0.032)	0.0072(0.0035-0.017)	0.015(0.0071-0.021)
	ANA: CPD	0.017(0.0078-0.031)	0.012(0.0058-0.026)	0.019(0.011-0.026)
	ANA: CPV	0.027(0.0096-0.058)	0.018(0.0074-0.040)	0.033(0.015-0.046)
	ADP: CPD	0.0076(0.0036-0.017)	0.0027(0.0012-0.0074)	0.008(0.0034-0.012)
	ADP: CPV	0.0087(0.0038-0.015)	0.0049(0.0023-0.0115)	0.0065(0.0032-0.0098)
	CPD: CPV	0.0016(0.00070-0.0040)	0.0018(0.0007-0.0035)	0.005(0.0035-0.0064)
	Pre-stimulus foraging	0.97(0.32-1.42)	0.128(0.0255-0.766)	0.11(0.0311-0.543)
	Latency	65.36(47.97-92.12)	299.85(139.27-863.7)	48.91(37.54-67.20)
	Post-stimulus foraging	3.46(0.54-5.58)	0.357(0.0865-2.194)	0.95(0.164-2.97)
BC	Hatch time	76.20(29.80-125.80)	11.8 (3.1- 86.4)	36.1(5.6-55.0)
	Hatch length	0.13 (0.060-0.51)	0.095(0.019-0.56)	0.21(0.041-0.30)
	Yolk volume	2.81(0.86-16.03)	11.45(2.79-49.94)	13.14(6.84-18.22)
	Emergence length	1.01 (0.38-1.46)	0.48(0.075-2.54)	0.25(0.058-0.58)
	Yolk conversion	0.0015(0.00040-0.0089)	0.0066(0.0011-0.031)	0.0031(0.0007-0.0089)
	Survival	0.0092(0.0022-0.042)	0.047(0.007-0.20)	0.012(0.0031-0.034)
	Head length	0.057(0.027-0.085)	0.040(0.0053-0.21)	0.0081(0.0028-0.029)

	Head width	0.086(0.027-0.13)	0.038(0.0044-0.20)	0.014(0.0043-0.057)
	Eye diameter	0.0018(0.00030-0.0097)	0.0024(0.0005-0.014)	0.011(0.007-0.016)
	Head depth behind eye	0.035(0.016-0.052)	0.022(0.0034-0.13)	0.006(0.0018-0.018)
	Body depth	0.030(0.0072-0.068)	0.048(0.0083-0.221)	0.022(0.0044-0.038)
	BD: ADP	0.062(0.018-0.28)	0.054(0.0079-0.34)	0.093(0.015-0.17)
	ANA: ADP	0.014(0.0033-0.092)	0.035(0.0057-0.161)	0.055(0.016-0.082)
	ANA: CPD	0.15(0.052-0.23)	0.0323(0.0055-0.245)	0.027(0.0077-0.095)
	ANA: CPV	0.046(0.0085-0.17)	0.032(0.0053-0.20)	0.072(0.010-0.11)
	ADP: CPD	0.048(0.0097-0.080)	0.0056(0.0012-0.041)	0.017(0.0024-0.041)
	ADP: CPV	0.062(0.014-0.094)	0.014(0.0021-0.097)	0.012(0.0028-0.043)
	CPD: CPV	0.014(0.0031-0.024)	0.0079(0.0012-0.043)	0.0061(0.0011-0.012)
	Pre-stimulus foraging	0.18(0.046-2.19)	0.14(0.0451-1.119)	1.41(0.36-2.36)
	Latency	101.73(63.59-172.29)	332.42(116.59-1300.3)	72.95(49.33-124.32)
	Post-stimulus foraging	1.01(0.24-8.51)	0.632(0.165-4.917)	4.42(0.43-6.67)
STBC	Hatch time	144.98(101.88-179.10)	86.89(15.89-252.90)	11.51(4.65-33.46)
	Hatch length	0.33(0.15-0.43)	0.048(0.011-0.27)	0.044(0.013-0.14)
	Yolk volume	1.50(0.44-7.70)	6.91(2.51-18.83)	6.12(2.79-7.95)
	Emergence length	0.20(0.071-0.53)	0.65(0.26-1.74)	0.23(0.059-0.32)
	Yolk conversion	0.0031(0.00040-0.012)	0.0027(0.0003-0.012)	0.0022(0.0004-0.0094)
	Survival	0.046(0.0061-0.091)	0.013(0.0028-0.062)	0.0115(0.0028-0.0564)
	Head length	0.016(0.0037-0.044)	0.015(0.0026-0.052)	0.016(0.0033-0.026)
	Head width	0.017(0.0030-0.044)	0.018(0.0031-0.065)	0.018(0.0034-0.027)
	Eye diameter	0.00070(0.00010-0.0036)	0.0006(0.0001-0.0027)	0.0022(0.0007-0.0032)
	Head depth behind eye	0.49(0.11-4.26)	0.32(0.077-1.36)	2.96(0.52-3.82)
	Body depth	0.011(0.0024-0.037)	0.022(0.005-0.067)	0.015(0.0034-0.024)
	BD: ADP	0.084(0.023-0.13)	0.031(0.0067-0.14)	0.021(0.0068-0.062)
	ANA: ADP	0.054(0.018-0.083)	0.012(0.0025-0.058)	0.012(0.0033-0.036)
	ANA: CPD	0.046(0.016-0.073)	0.031(0.0076-0.12)	0.012(0.0041-0.0321)
	ANA: CPV	0.038(0.0081-0.067)	0.031(0.007-0.11)	0.019(0.0041-0.036)
	ADP: CPD	0.013(0.0025-0.039)	0.0079(0.0016-0.032)	0.017-0.0034)
	ADP: CPV	0.0067(0.0014-0.027)	0.0093(0.0024-0.035)	0.015(0.0034-0.0205)
	CPD: CPV	0.0016(0.00050-0.0097)	0.0048(0.0008-0.016)	0.0051(0.0014-0.0076)

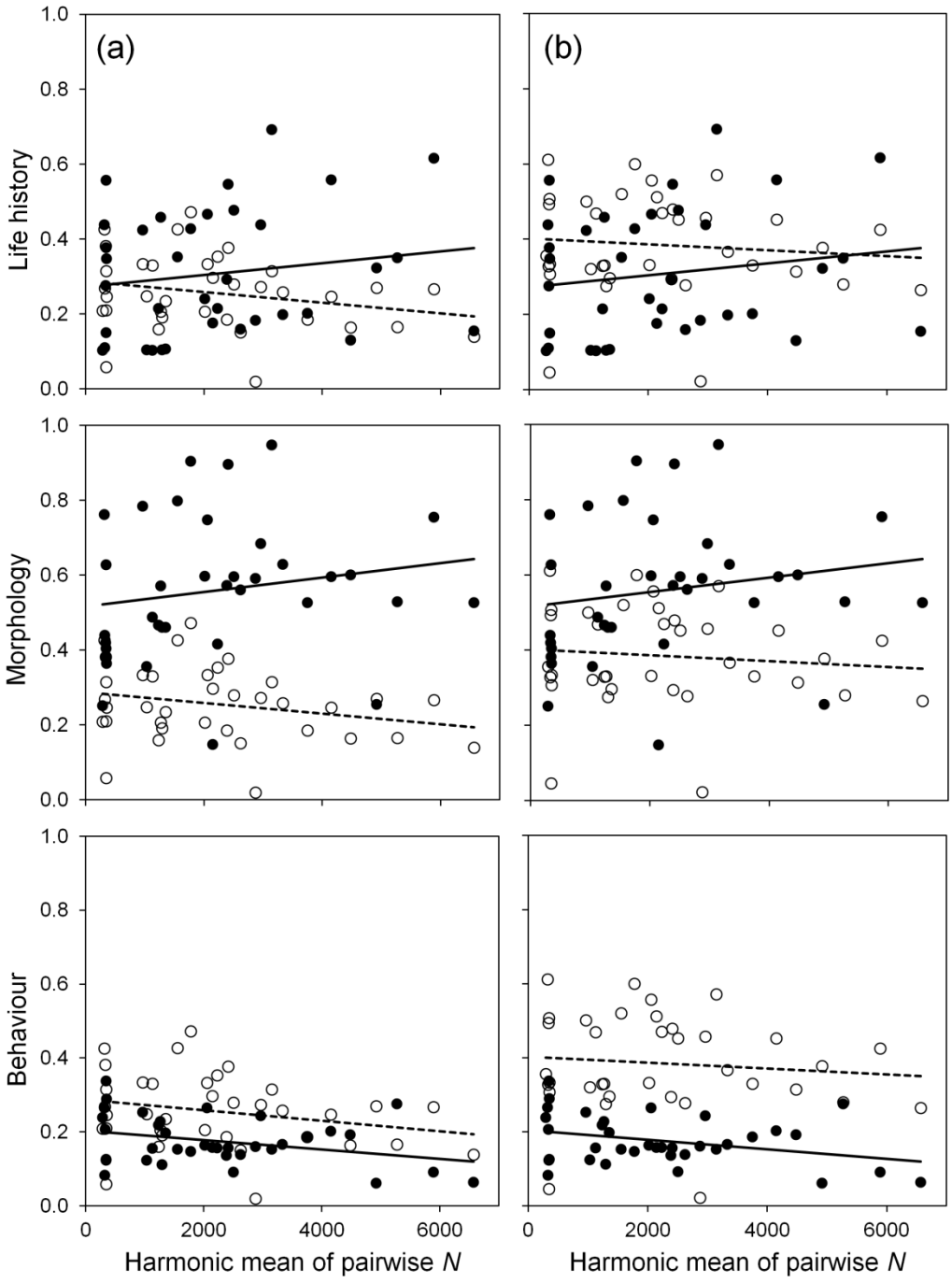
	Pre-stimulus foraging	0.18(0.045-1.50)	0.172(0.037-1.20)	0.76(0.12-1.20)
	Latency	59.15(40.22-96.29)	218.06(85.32-940.72)	43.74(31.66-70.96)
	Post-stimulus foraging	1.14(0.25-9.73)	1.02(0.23-6.09)	8.98(3.86-13.79)
WC	Hatch time	247.62(210.27-291.64)	231.02(76.11-708.75)	18.19(10.01-33.32)
	Hatch length	0.48(0.40-0.57)	0.16(0.039-0.63)	0.030(0.017-0.059)
	Yolk volume	9.19(3.47-23.12)	5.22(1.16-21.08)	9.00(1.69-12.39)
	Emergence length	0.98(0.69-1.25)	0.58(0.12-1.95)	0.10(0.044-0.27)
	Yolk conversion	0.00090(0.00010-0.0053)	0.0005(0.0001-0.0033)	0.0014(0.0002-0.0047)
	Survival	0.078(0.0086-0.16)	0.040(0.0051-0.15)	0.026(0.0055-0.11)
	Head length	0.0059(0.0023-0.020)	0.0059(0.0021-0.019)	0.016(0.0096-0.024)
	Head width	0.015(0.0048-0.029)	0.0049(0.0018-0.021)	0.012(0.0043-0.020
	Eye diameter	0.0014(0.00040-0.0038)	0.0011(0.0003-0.0039)	0.0018(0.0004-0.0026)
	Head depth behind eye	0.011(0.0034-0.021)	0.0046(0.0014-0.018)	0.0083(0.0027-0.014)
	Body depth	0.013(0.0042-0.027)	0.0052(0.0020-0.019)	0.014(0.0056-0.021)
	BD: ADP	0.037(0.011-0.089)	0.021(0.008-0.097)	0.045(0.020-0.071)
	ANA: ADP	0.010(0.0036-0.027)	0.01(0.0025-0.033)	0.013(0.0049-0.019)
	ANA: CPD	0.017(0.0079-0.037)	0.011(0.0039-0.038)	0.016(0.0071-0.025)
	ANA: CPV	0.018(0.0061-0.047)	0.011(0.003-0.036)	0.021(0.0076-0.033)
	ADP: CPD	0.016(0.0083-0.025)	0.003(0.0011-0.014)	0.0044(0.0018-0.010)
	ADP: CPV	0.0077(0.0030-0.0192)	0.0031(0.0013-0.013)	0.0086(0.0033-0.014)
	CPD: CPV	0.0027(0.00080-0.0074)	0.0019(0.0006-0.0075)	0.0042(0.0018-0.0063)
	Pre-stimulus foraging	0.17(0.039-1.63)	0.15(0.028-1.02)	0.88(0.081-1.30)
	Latency	70.16(44.58-104.66)	281.61(96.14-1109.60)	48.72(36.03-79.56)
	Post-stimulus foraging	0.43(0.12-3.54)	0.38(0.11-3.04)	3.91(1.91-5.81)
DY	Hatch time	269.08(204.92-354.45)	37.81(7.91-268.40)	21.45(9.61-55.05)
	Hatch length	0.25(0.0997-0.43)	0.072(0.011-0.58)	0.062(0.016-0.18)
	Yolk volume	2.10(0.48-18.94)	3.37(0.50-28.23)	12.87(3.94-18.29)
	Emergence length	0.21(0.038-0.67)	0.41(0.044-2.57)	0.25(0.040-0.40)
	Yolk conversion	0.0011(0.00020-0.0080)	0.0011(0.0002-0.015)	0.0008(0.0002-0.0068)
	Survival	0.0065(0.0014-0.048)	0.0084(0.001-0.088)	0.0077(0.0013-0.042)
	Head length	0.032(0.011-0.050)	0.0054(0.0012-0.042)	0.0066(0.0019-0.021)
	Head width	0.036(0.011-0.050)	0.0092(0.0022-0.064)	0.013(0.004-0.033)

	Eye diameter	0.0038(0.00040-0.0078)	0.0009(0.0001-0.0069)	0.0009(0.0002-0.0045)
	Head depth behind eye	0.031(0.015-0.046)	0.0081(0.0014-0.052)	0.0048(0.0019-0.016)
	Body depth	0.0066(0.0028-0.025)	0.0073(0.0021-0.055)	0.014(0.0053-0.021)
	BD: ADP	0.027(0.0096-0.080)	0.023(0.0057-0.14)	0.040(0.015-0.063)
	ANA: ADP	0.0080(0.0028-0.027)	0.012(0.0022-0.085)	0.016(0.0065-0.024)
	ANA: CPD	0.023(0.0082-0.051)	0.030(0.0039-0.10)	0.019(0.0063-0.032)
	ANA: CPV	0.021(0.0049-0.053)	0.013(0.0032-0.088)	0.025(0.0082-0.041)
	ADP: CPD	0.0156(0.0035-0.083)	0.0074(0.0017-0.058)	0.045(0.0065-0.063)
	ADP: CPV	0.0097(0.0031-0.020)	0.0038(0.0008-0.023)	0.0064(0.0019-0.012)
	CPD: CPV	0.0039(0.0012-0.0090)	0.0019(0.0005-0.014)	0.0035(0.0011-0.0059)
	Pre-stimulus foraging	4.35(1.00-9.18)	0.87(0.15-7.59)	0.79(0.22-4.16)
	Latency	67.50(40.08-116.55)	225.11(69.50-1408.70)	53.03(31.16-85.57)
	Post-stimulus foraging	0.75(0.18-8.26)	0.70(0.13-6.85)	5.00(0.90-8.37)
UO	Hatch time	176.41(93.06-236.24)	62.92(16.76-171.68)	32.48(7.57-77.04)
	Hatch length	0.59(0.35-0.76)	0.74(0.36-1.55)	0.096(0.035-0.22)
	Yolk volume	4.35(1.04-14.22)	17.59(8.19-35.13)	14.09(8.40-17.27)
	Emergence length	0.26(0.093-0.54)	1.05(0.54-1.91)	0.24(0.091-0.34)
	Yolk conversion	0.0083(0.0034-0.027)	0.20(0.10-0.37)	0.0087(0.0034-0.022)
	Survival	0.019(0.0023-0.046)	0.022(0.0033-0.051)	0.0094(0.0020-0.033)
	Head length	0.013(0.0059-0.021)	0.0063(0.003-0.015)	0.0099(0.0048-0.014)
	Head width	0.0082(0.0035-0.021)	0.0071(0.0029-0.016)	0.016(0.0089-0.021)
	Eye diameter	0.0010(0.00030-0.003)	0.0008(0.0004-0.0022)	0.0032(0.0020-0.0043)
	Head depth behind eye	0.0063(0.0019-0.016)	0.0028(0.0012-0.0064)	0.014(0.0076-0.018)
	Body depth	0.0044(0.0022-0.011)	0.0042(0.0021-0.0093)	0.012(0.0085-0.016)
	BD: ADP	0.026(0.011-0.070)	0.020(0.0086-0.045)	0.088(0.062-0.12)
	ANA: ADP	0.0093(0.0047-0.021)	0.0068(0.0029-0.015)	0.014(0.0083-0.020)
	ANA: CPD	0.013(0.0061-0.023)	0.014(0.0064-0.031)	0.012(0.0075-0.018)
	ANA: CPV	0.014(0.0060-0.030)	0.011(0.0045-0.024)	0.019(0.010-0.026)
	ADP: CPD	0.011(0.0042-0.020)	0.0039(0.0013-0.011)	0.0065(0.0026-0.012)
	ADP: CPV	0.0071(0.0028-0.016)	0.003(0.0015-0.0083)	0.010(0.0051-0.014)
	CPD: CPV	0.003(0.0010-0.0074)	0.0019(0.0007-0.0049)	0.0049(0.0029-0.0073)
	Pre-stimulus foraging	0.28(0.066-3.06)	0.33(0.054-2.31)	1.82(0.30-3.21)

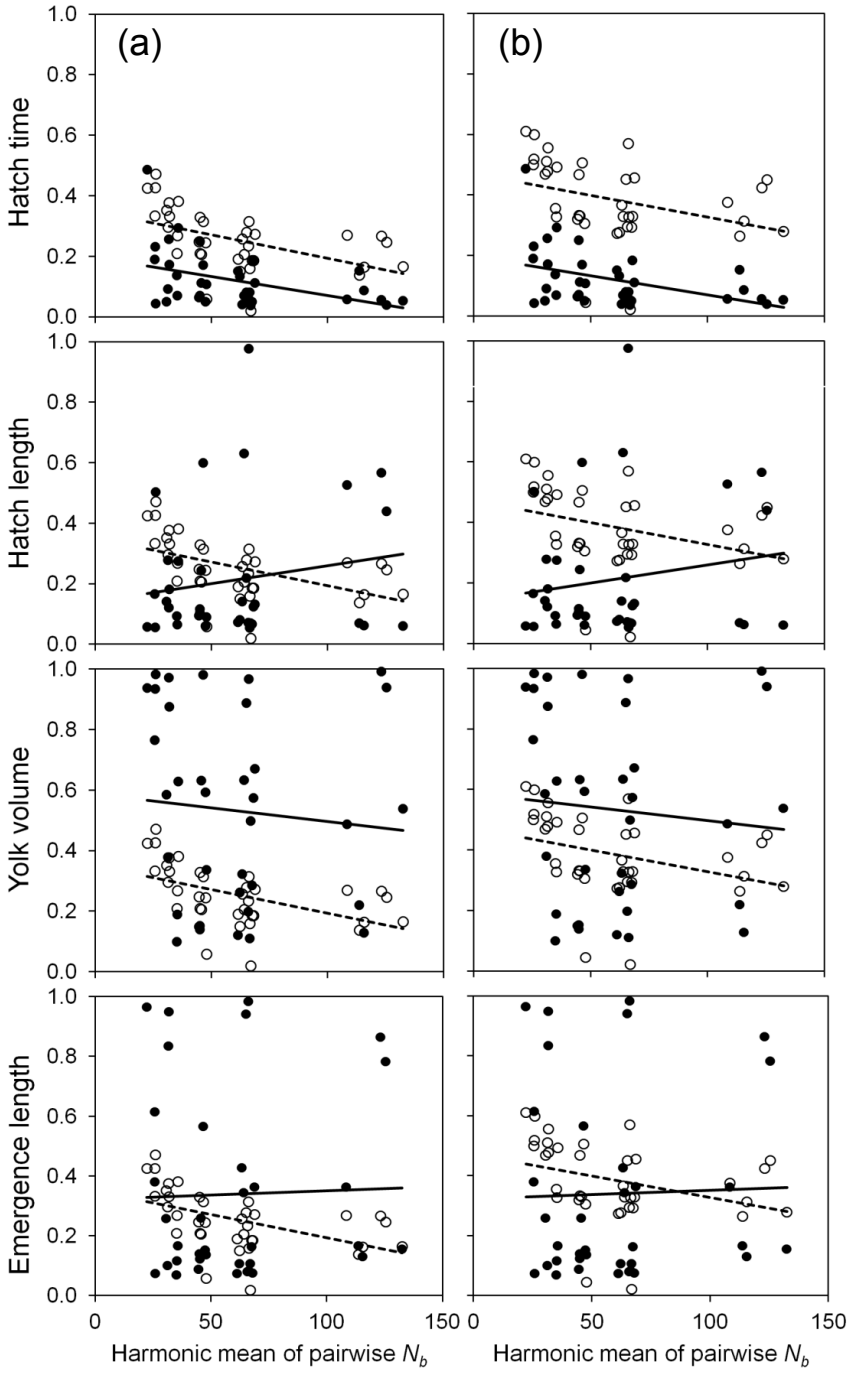
	Latency	83.66(50.54-153.71)	236.59(88.67-1011.50)	66.33(41.80-113.86)
	Post-stimulus foraging	0.67(0.17-6.95)	0.52(0.14-4.32)	3.95(0.72-6.99)
WN	Hatch time	234.8(124.05-284.41)	51.28(14.70-219.00)	32.28(9.84-83.71)
	Hatch length	0.47(0.34-0.61)	0.28(0.076-0.77)	0.059(0.022-0.13)
	Yolk volume	3.40(1.29-14.38)	30.63(15.04-67.64)	15.48(9.48-18.76)
	Emergence length	0.59(0.37-0.86)	1.26(0.59-2.92)	0.14(0.058-0.29)
	Yolk conversion	0.00040(0.00010-0.0025)	0.0027(0.00070-0.0071)	0.0010(0.00020-0.0024)
	Survival	0.014(0.0032-0.036)	0.051(0.024-0.13)	0.051(0.024-0.13)
	Head length	0.0048(0.0017-0.012)	0.0031(0.0015-0.0082)	0.0031(0.0015-0.0082)
	Head width	0.012(0.0036-0.031)	0.0084(0.0027-0.022)	0.0084(0.0027-0.022)
	Eye diameter	0.0094(0.0035-0.034)	0.0091(0.0032-0.024)	0.0091(0.0032-0.024)
	Head depth behind eye	0.0058(0.0016-0.020)	0.049(0.0016-0.014)	0.049(0.0016-0.014)
	Body depth	0.0063(0.0025-0.021)	0.0065(0.0027-0.017)	0.0065(0.0027-0.017)
	BD: ADP	0.019(0.0059-0.058)	0.026(0.01-0.063)	0.026(0.010-0.063)
	ANA: ADP	0.0077(0.0028-0.019)	0.0047(0.0022-0.012)	0.0047(0.0022-0.012)
	ANA: CPD	0.012(0.0052-0.030)	0.0088(0.0038-0.021)	0.0088(0.0038-0.021)
	ANA: CPV	0.018(0.0064-0.0576)	0.014(0.0055-0.034)	0.014(0.0055-0.034)
	ADP: CPD	0.0064(0.0016-0.018)	0.0051(0.0014-0.014)	0.0051(0.0014-0.014)
	ADP: CPV	0.0089(0.0032-0.019)	0.0037(0.0013-0.0093)	0.0037(0.0013-0.0093)
	CPD: CPV	0.0036(0.0010-0.010)	0.0016(0.0007-0.0054)	0.0016(0.0007-0.0054)
	Pre-stimulus foraging	1.93(0.71-2.90)	0.26(0.057-1.48)	0.26(0.057-1.48)
	Latency	71.67(52.82-100.24)	328.72(151.59-937.55)	328.72(151.59-937.55)
	Post-stimulus foraging	0.92(0.18-5.40)	0.59(0.12-2.48)	0.59(0.12-2.48)
LB	Hatch time	298.71(206.53-357.48)	48.16(11.46-286.16)	22.90(9.64-70.78)
	Hatch length	0.62(0.43-0.79)	0.21(0.046-0.97)	0.073(0.030-0.18)
	Yolk volume	6.03(2.003-19.086)	19.12(6.71-61.03)	10.50(3.52-13.82)
	Emergence length	0.50(0.27-0.72)	1.62(0.65-4.79)	0.15(0.069-0.29)
	Yolk conversion	0.0020(0.00040-0.012)	0.0027(0.0004-0.017)	0.0023(0.0004-0.010)
	Survival	0.0084(0.0018-0.049)	0.036(0.0029-0.14)	0.017(0.029-0.049)
	Head length	0.0054(0.0023-0.015)	0.0029(0.0013-0.0081)	0.010(0.0054-0.014)
	Head width	0.0059(0.0024-0.026)	0.010(0.0031-0.028)	0.020(0.0086-0.025)
	Eye diameter	0.0015(0.00040-0.0037)	0.0004(0.0002-0.0014)	0.0020(0.0007-0.003)

	Head depth behind eye	0.0058(0.0021-0.019)	0.009(0.0032-0.024)	0.0095(0.0042-0.015)
	Body depth	0.0059(0.0021-0.017)	0.0059(0.0025-0.017)	0.016(0.0090-0.021)
	BD: ADP	0.031(0.011-0.081)	0.016(0.0062-0.047)	0.052(0.025-0.074)
	ANA: ADP	0.0089(0.0035-0.023)	0.005(0.0019-0.013)	0.015(0.0071-0.021)
	ANA: CPD	0.010(0.0048-0.026)	0.0068(0.0032-0.020)	0.018(0.0096-0.025)
	ANA: CPV	0.0086(0.0035-0.029)	0.0082(0.0030-0.022)	0.024(0.013-0.033)
	ADP: CPD	0.0057(0.0015-0.015)	0.0024(0.0009-0.0073)	0.012(0.0058-0.016)
	ADP: CPV	0.0068(0.0023-0.016)	0.0024(0.0009-0.0085)	0.0078(0.0031-0.012)
	CPD: CPV	0.0046(0.0016-0.020)	0.0045(0.0015-0.017)	0.048(0.036-0.061)
	Pre-stimulus foraging	0.40(0.12-5.67)	0.36(0.086-1.97)	2.97(0.27-4.43)
	Latency	79.21(48.60-116.38)	213.87(98.77-779.47)	55.28(38.97-84.27)
	Post-stimulus foraging	0.771(0.21-8.30)	0.67(0.17-4.64)	4.50(0.79-7.33)
CC	Hatch time	204.50(167.29-243.60)	40.50(9.34-195.64)	16.21(6.37-35.17)
	Hatch length	0.42(0.19-0.64)	0.16(0.046-0.70)	0.14(0.036-0.27)
	Yolk volume	4.28(1.04-14.86)	10.50(3.42-36.20)	14.51(8.52-17.72)
	Emergence length	0.48(0.18-0.79)	0.29(0.065-0.94)	0.22(0.057-0.37)
	Yolk conversion	0.0022(0.00030-0.0056)	0.0011(0.0002-0.0063)	0.0012(0.00020-0.0037)
	Survival	0.012(0.0023-0.075)	0.0099(0.0025-0.059)	0.042(0.0090-0.083)
	Head length	0.0074(0.0033-0.014)	0.0037(0.0015-0.012)	0.0076(0.0035-0.010)
	Head width	0.0085(0.0030-0.026)	0.0058(0.0021-0.020)	0.022(0.011-0.027)
	Eye diameter	0.001(0.00030-0.0029)	0.0006(0.0002-0.0022)	0.0036(0.0024-0.0046)
	Head depth behind eye	0.0058(0.0018-0.021)	0.0053(0.0018-0.020)	0.018(0.0099-0.024)
	Body depth	0.0088(0.0037-0.021)	0.0060(0.0024-0.021)	0.014(0.0080-0.019)
	BD: ADP	0.020(0.0079-0.062)	0.016(0.0067-0.057)	0.072(0.050-0.094)
	ANA: ADP	0.0097(0.0034-0.035)	0.0068(0.0024-0.021)	0.032(0.018-0.041)
	ANA: CPD	0.016(0.0062-0.046)	0.012(0.0049-0.040)	0.054(0.037-0.068)
	ANA: CPV	0.017(0.006-0.051)	0.014(0.0042-0.040)	0.042(0.024-0.055)
	ADP: CPD	0.0060(0.0017-0.021)	0.0042(0.0015-0.016)	0.015(0.0056-0.019)
	ADP: CPV	0.0047(0.0019-0.013)	0.0029(0.0012-0.0098)	0.0097(0.0055-0.013)
	CPD: CPV	0.0014(0.00060-0.0048)	0.0014(0.0005-0.0044)	0.0085(0.0065-0.011)
	Pre-stimulus foraging	1.67(0.42-5.08)	0.51(0.098-2.30)	1.79(0.25-3.03)
	Latency	46.37(34.66-67.46)	239.62(97.29-792.34)	36.50(27.33-49.45)

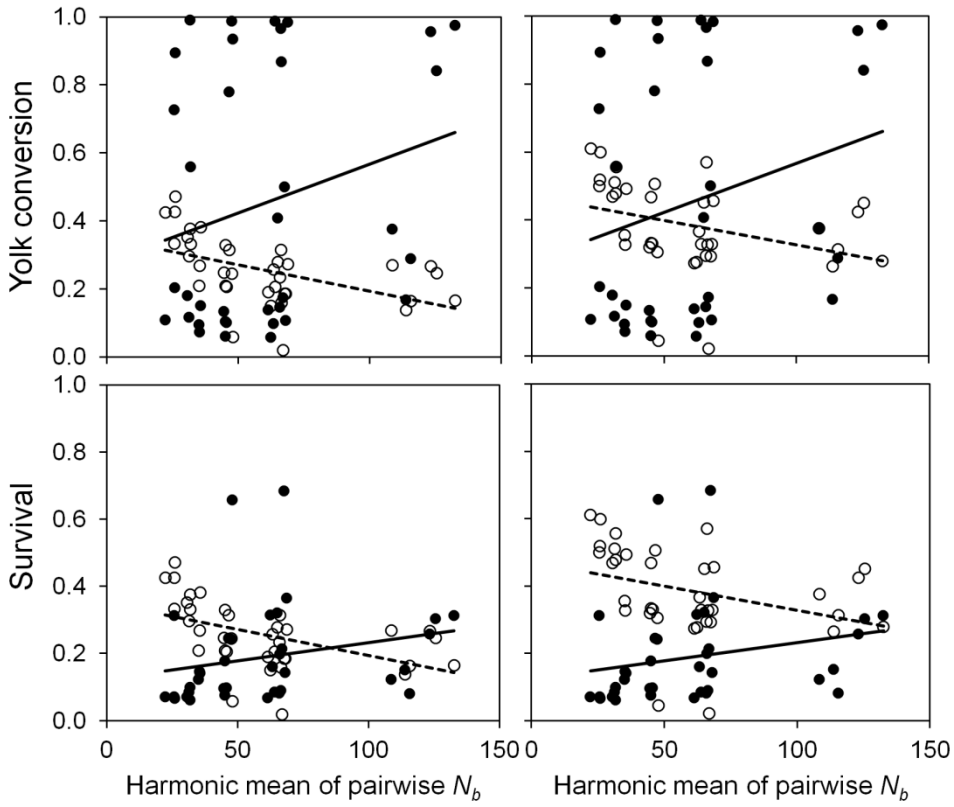
Post-stimulus foraging	1.40(0.41-8.70)	1.29(0.36-6.38)	14.56(9.97-20.50)

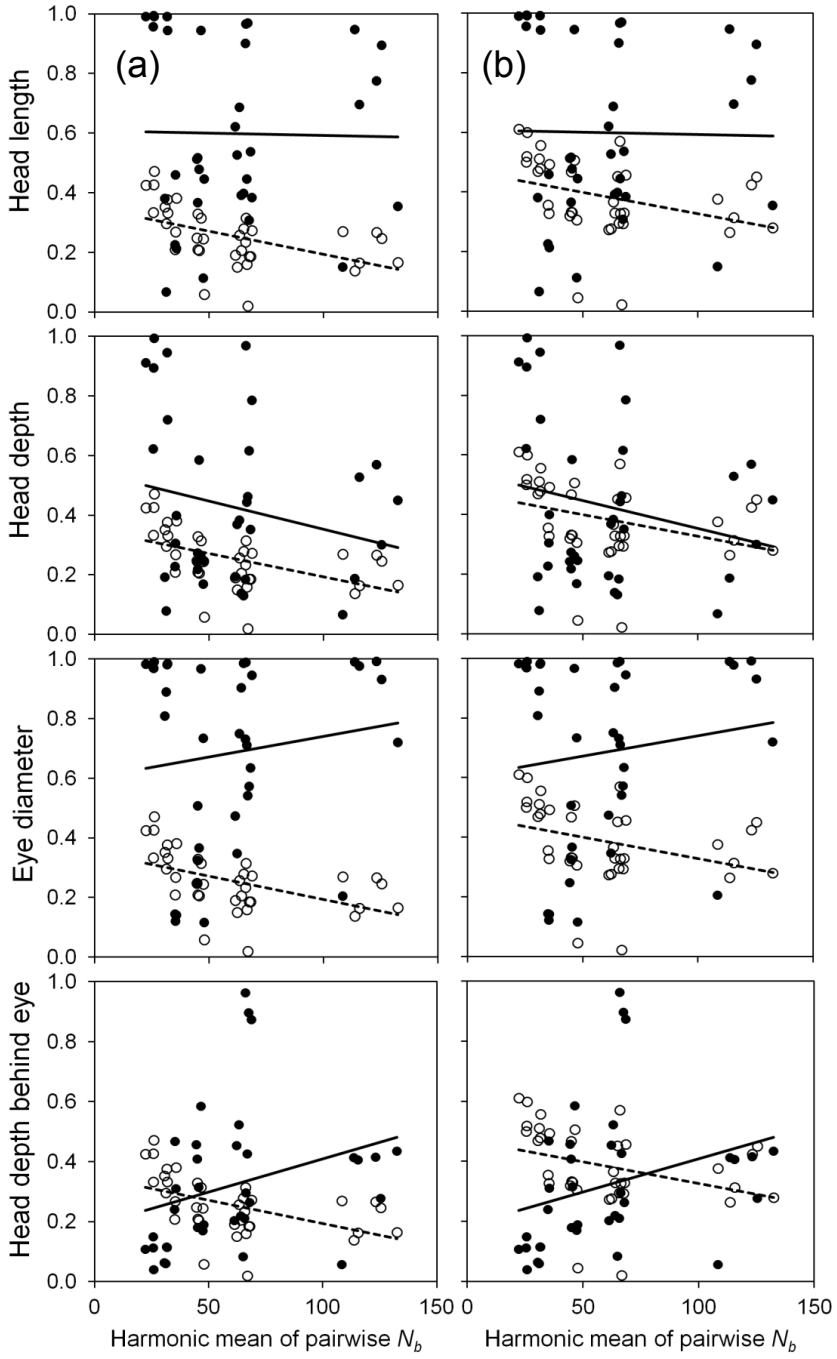


Appendix D (Fig. D1): Mean Q_{ST} (●) and F_{ST} (○) vs. N across traits in each of three trait categories. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$

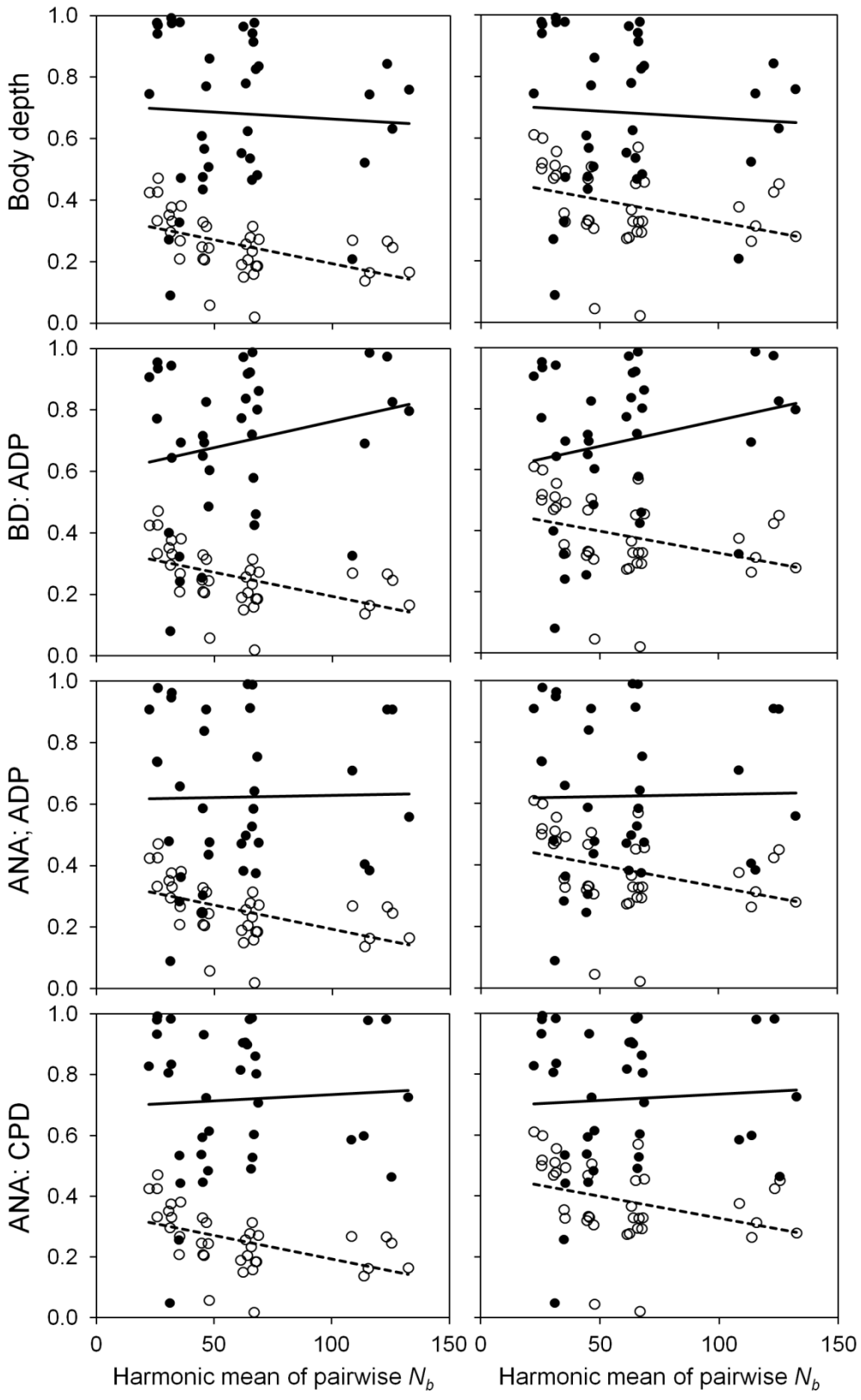


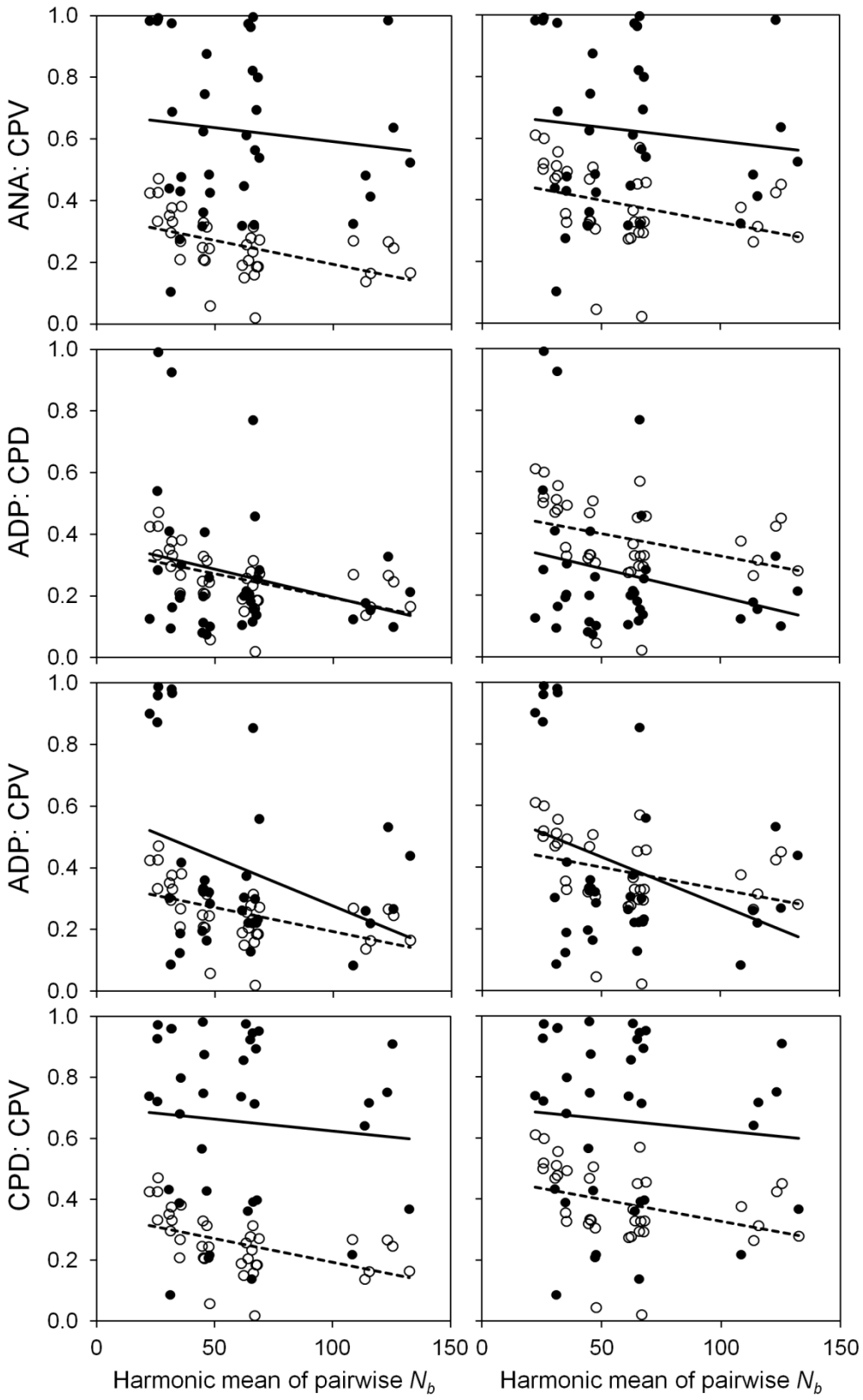
Appendix E (Fig. E1): Q_{ST} (●) and F_{ST} (○) vs. N_b for six early life traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$

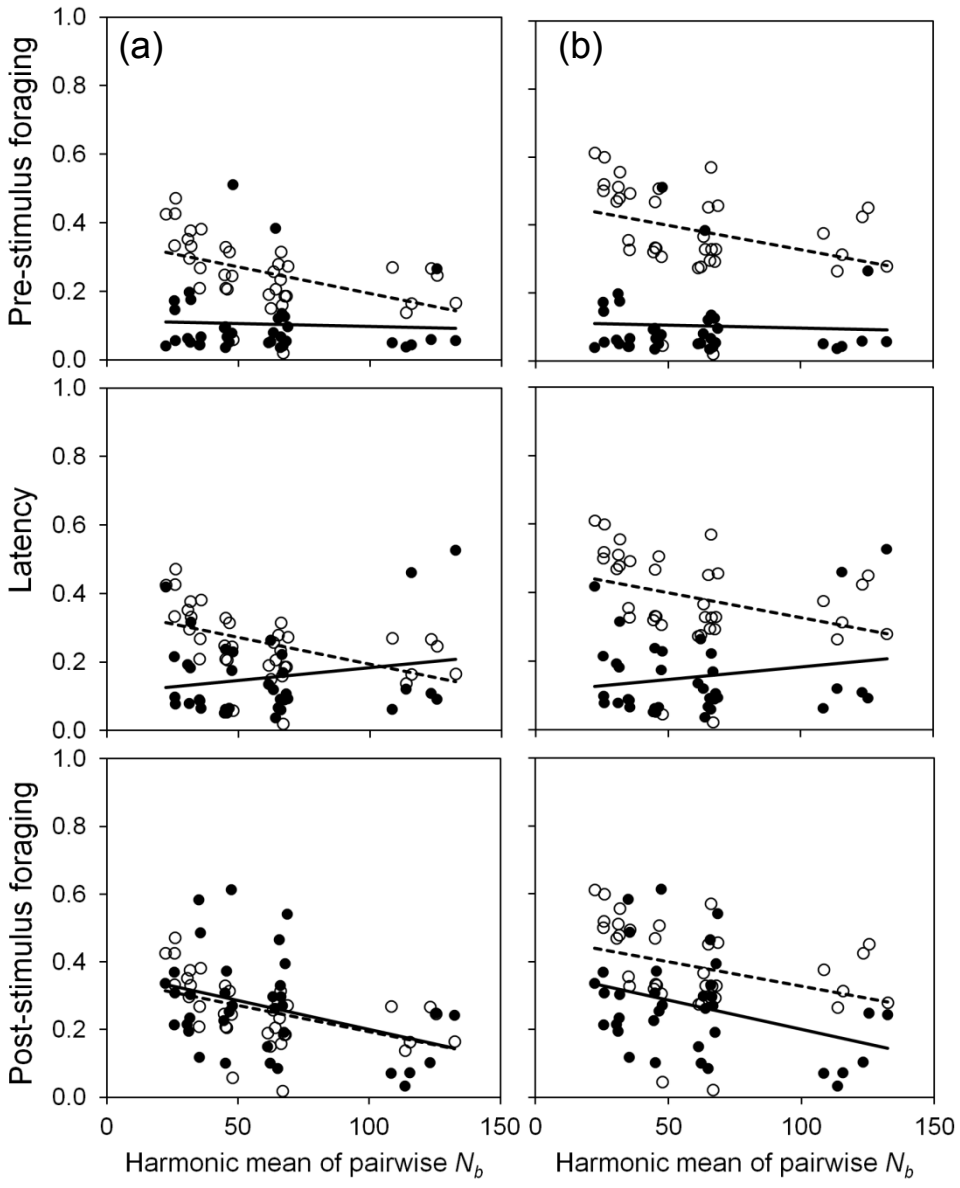

Yolk conversion
Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b



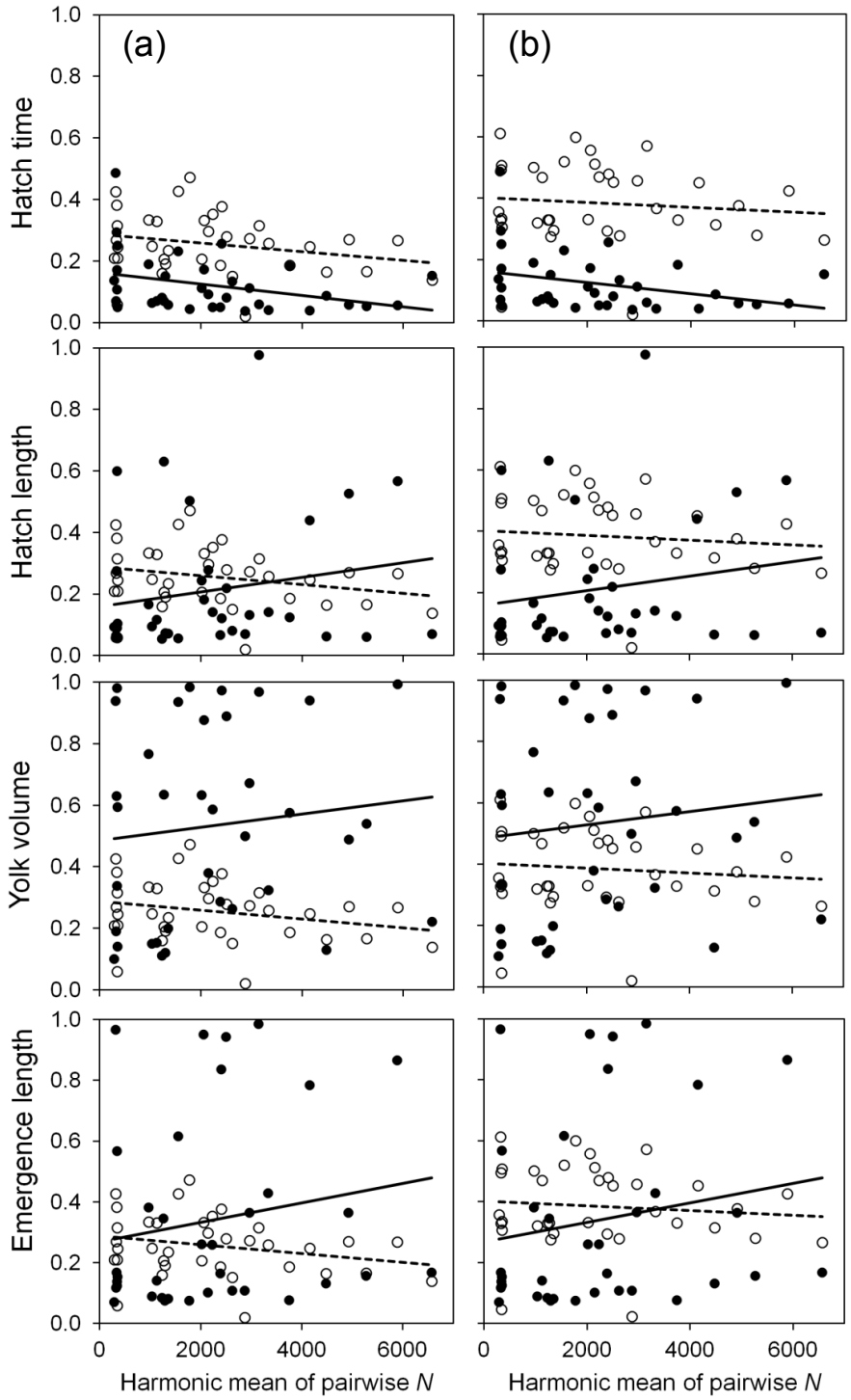
Appendix E (Fig. E2): Q_{ST} (●) and F_{ST} (○) vs. N_b for twelve morphological traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$


Body depth
BD: ADP
ANA; ADP
ANA: CPD
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b

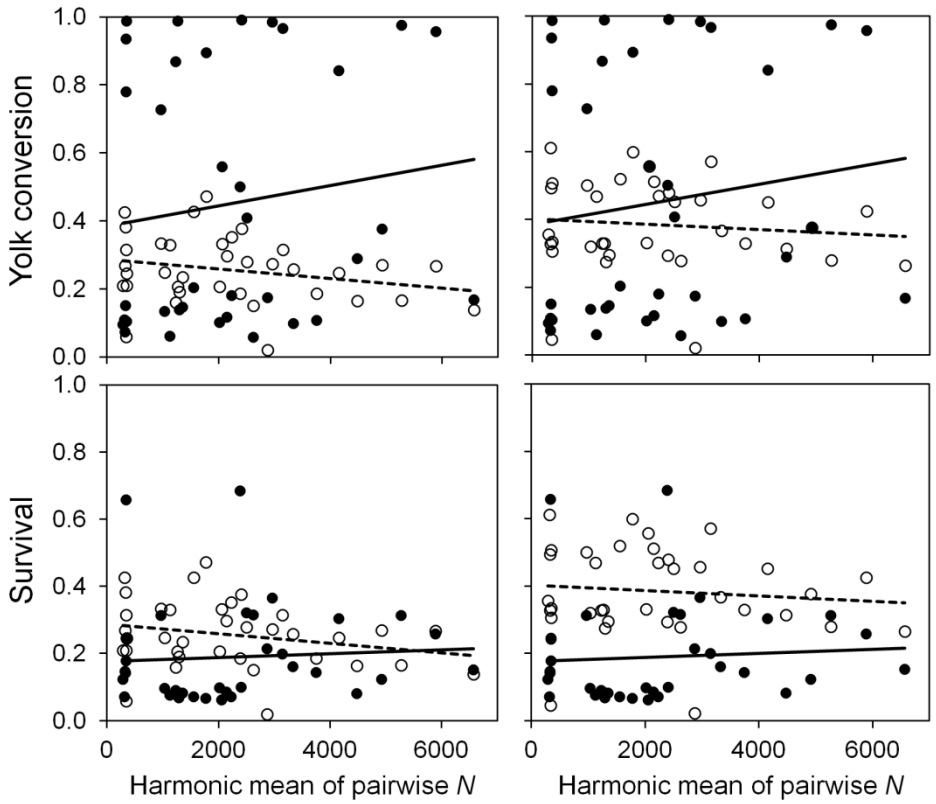

ANA: CPV
ADP: CPD
ADP: CPV
CPD: CPV
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b

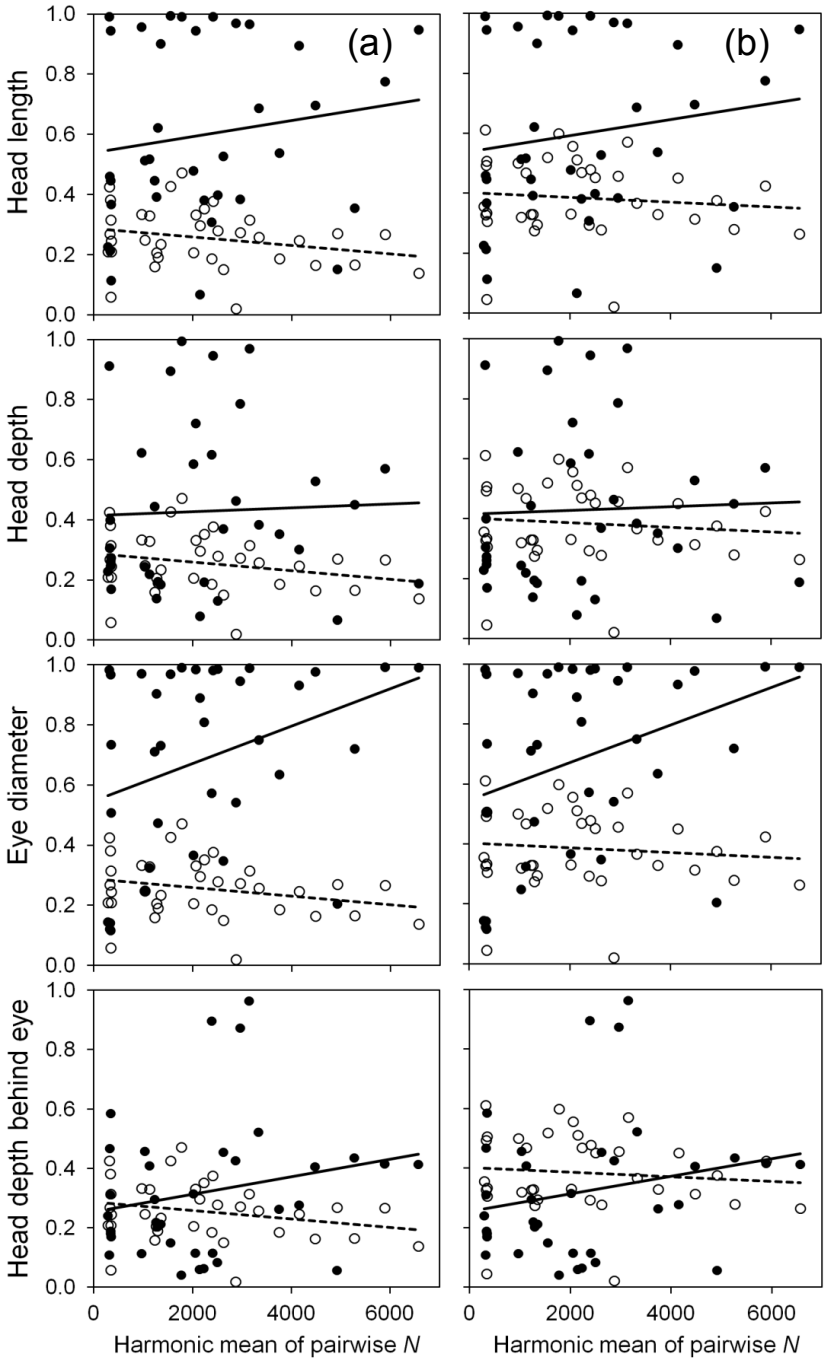


Appendix E (Fig. E3): Q_{ST} (●) and F_{ST} (○) vs. N_b for three behavioural traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$

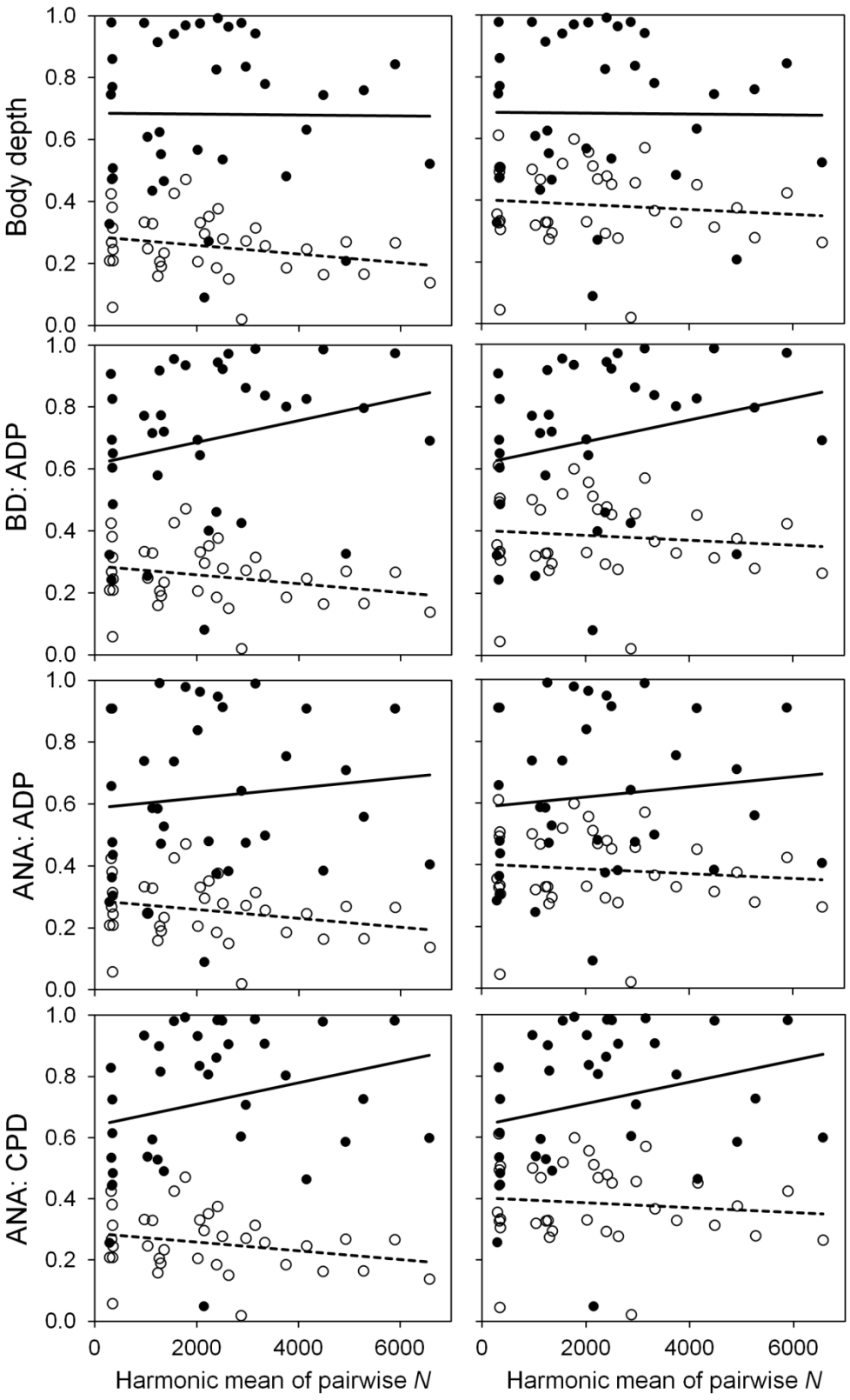


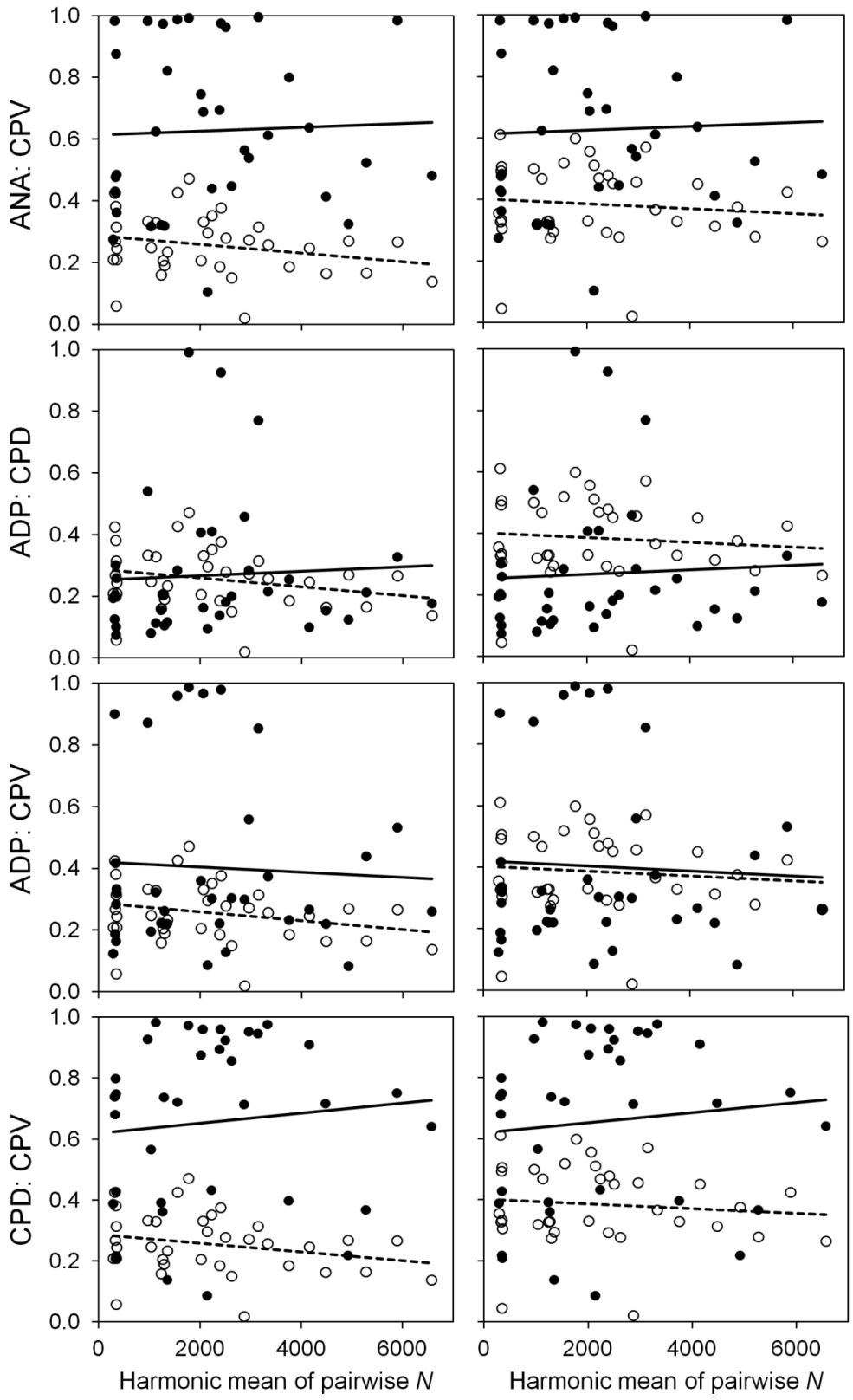
Appendix E (Fig. E4): Q_{ST} (●) and F_{ST} (○) vs. N for six early life traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for F_{ST}.

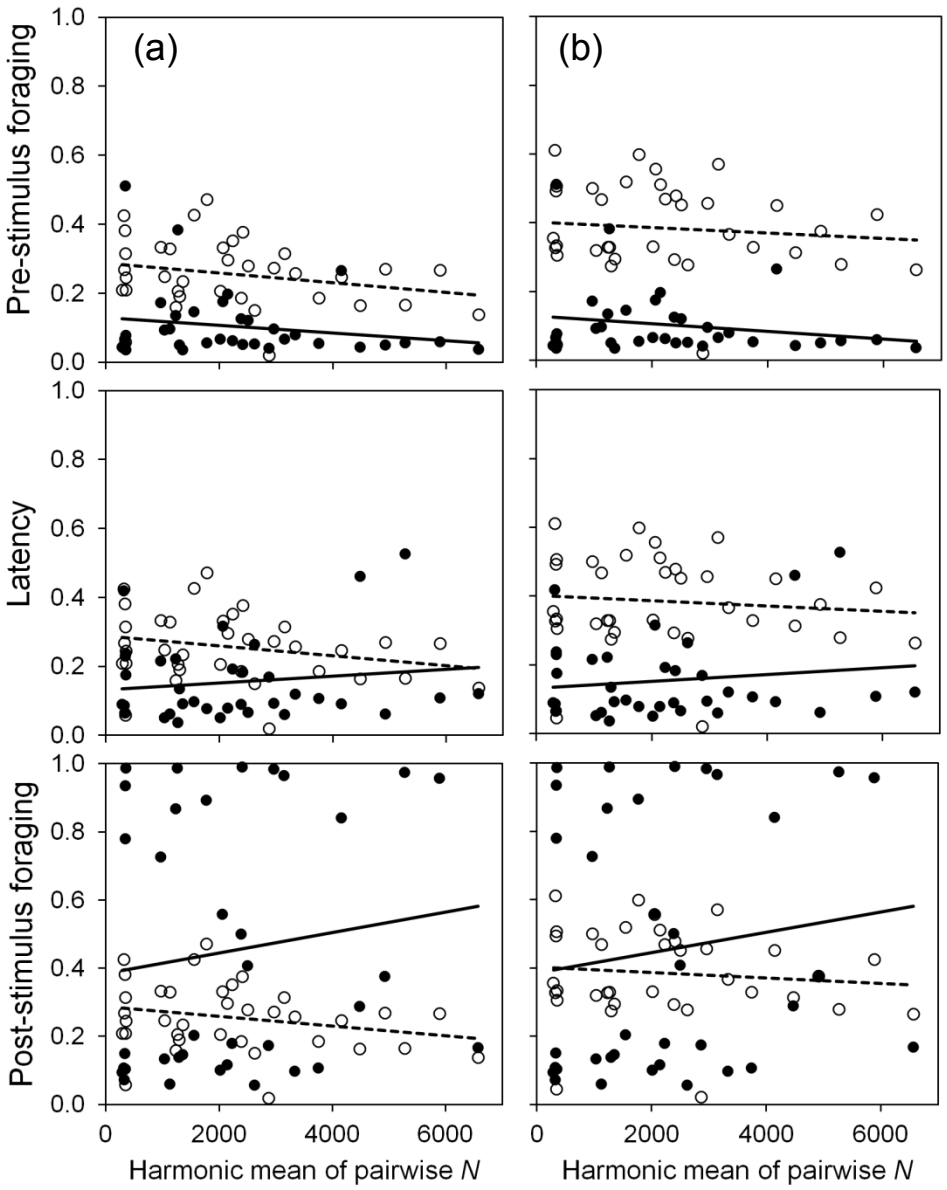

Yolk conversion
Survival
1.0
0.8
0.6
0.4
0.2
0.0
0
2000
4000
6000
Harmonic mean of pairwise N
Harmonic mean of pairwise N



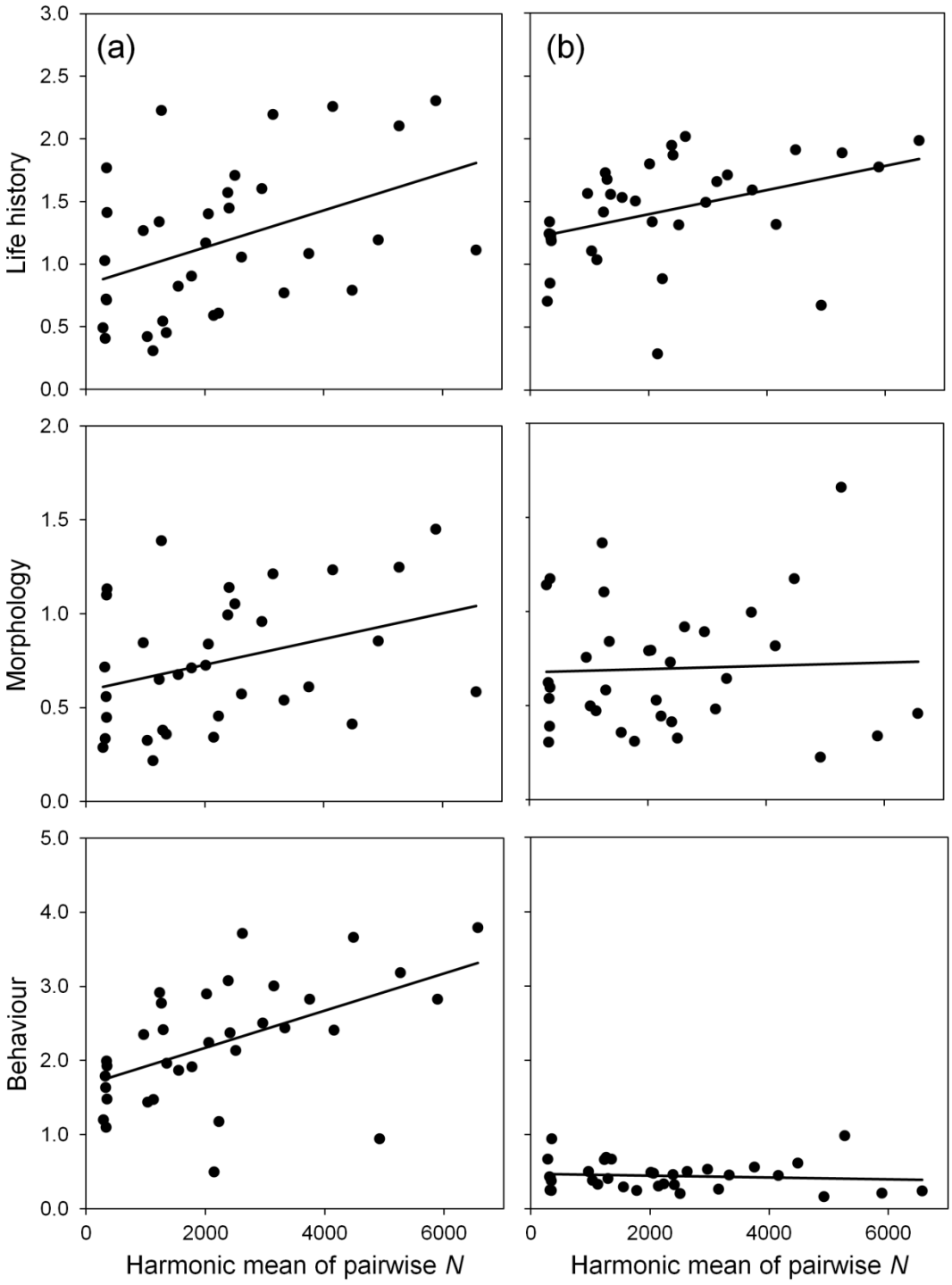
Appendix E (Fig. E5): Q_{ST} (●) and F_{ST} (○) vs. N for twelve morphological traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$


Body depth
BD: ADP
ANA: ADP
ANA: CPD
1.0
0.8
0.6
0.4
0.2
0.0
0
2000
4000
6000
Harmonic mean of pairwise N
Harmonic mean of pairwise N

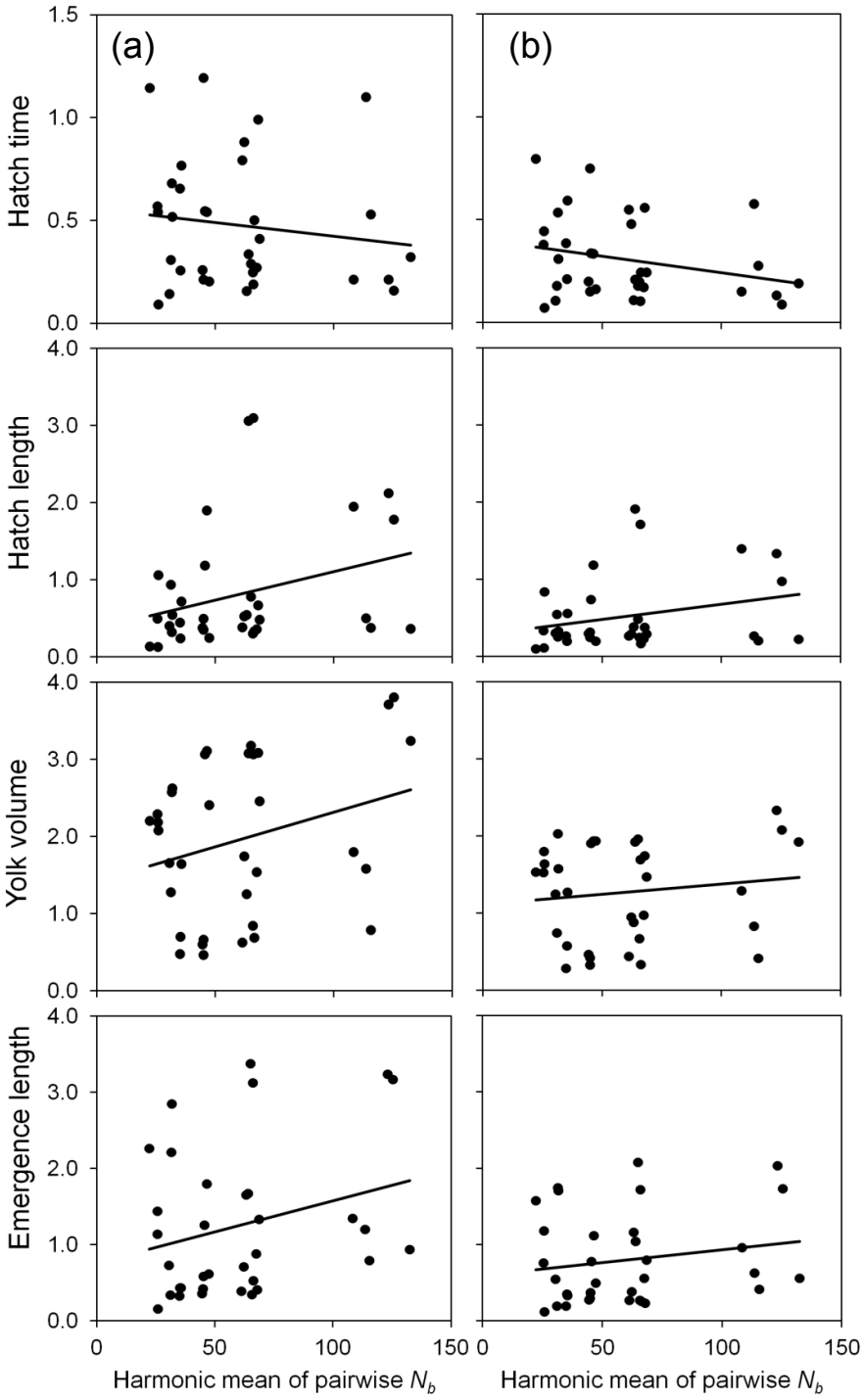

ANA: CPV
ADP: CPD
ADP: CPV
CPD: CPV
1.0
0.8
0.6
0.4
0.2
0.0
0
2000
4000
6000
Harmonic mean of pairwise N
Harmonic mean of pairwise N



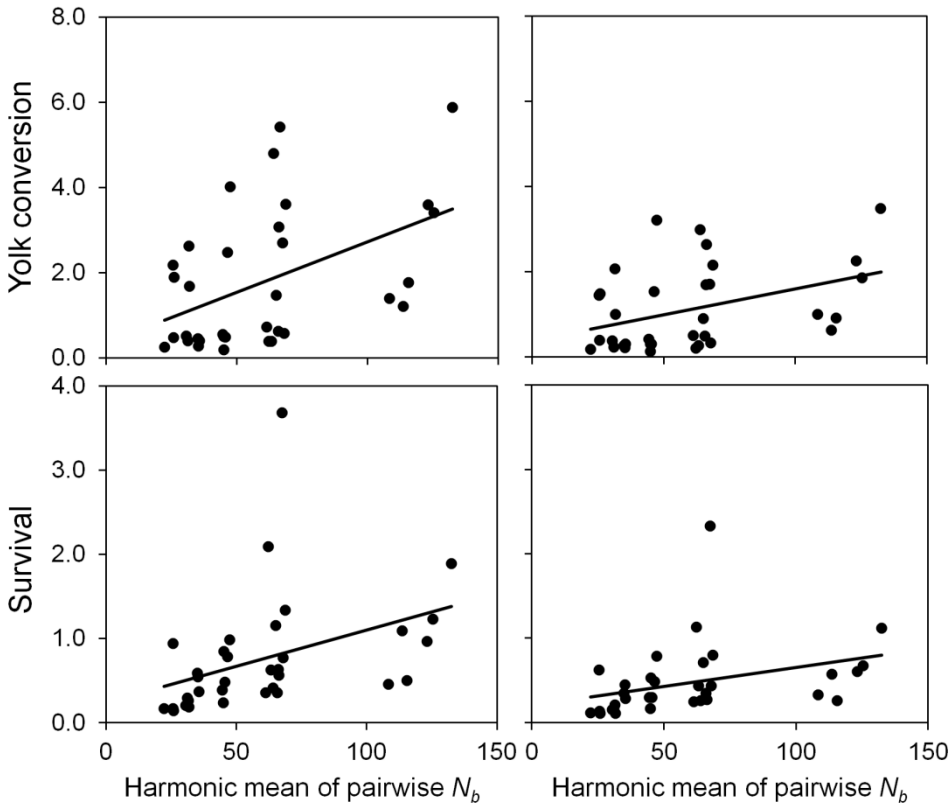
Appendix E (Fig. E6): Q_{ST} (●) and F_{ST} (○) vs. N for three behavioural traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Best-fit linear regressions are represented by solid lines for Q_{ST} and dashed lines for $F_{ST.}$

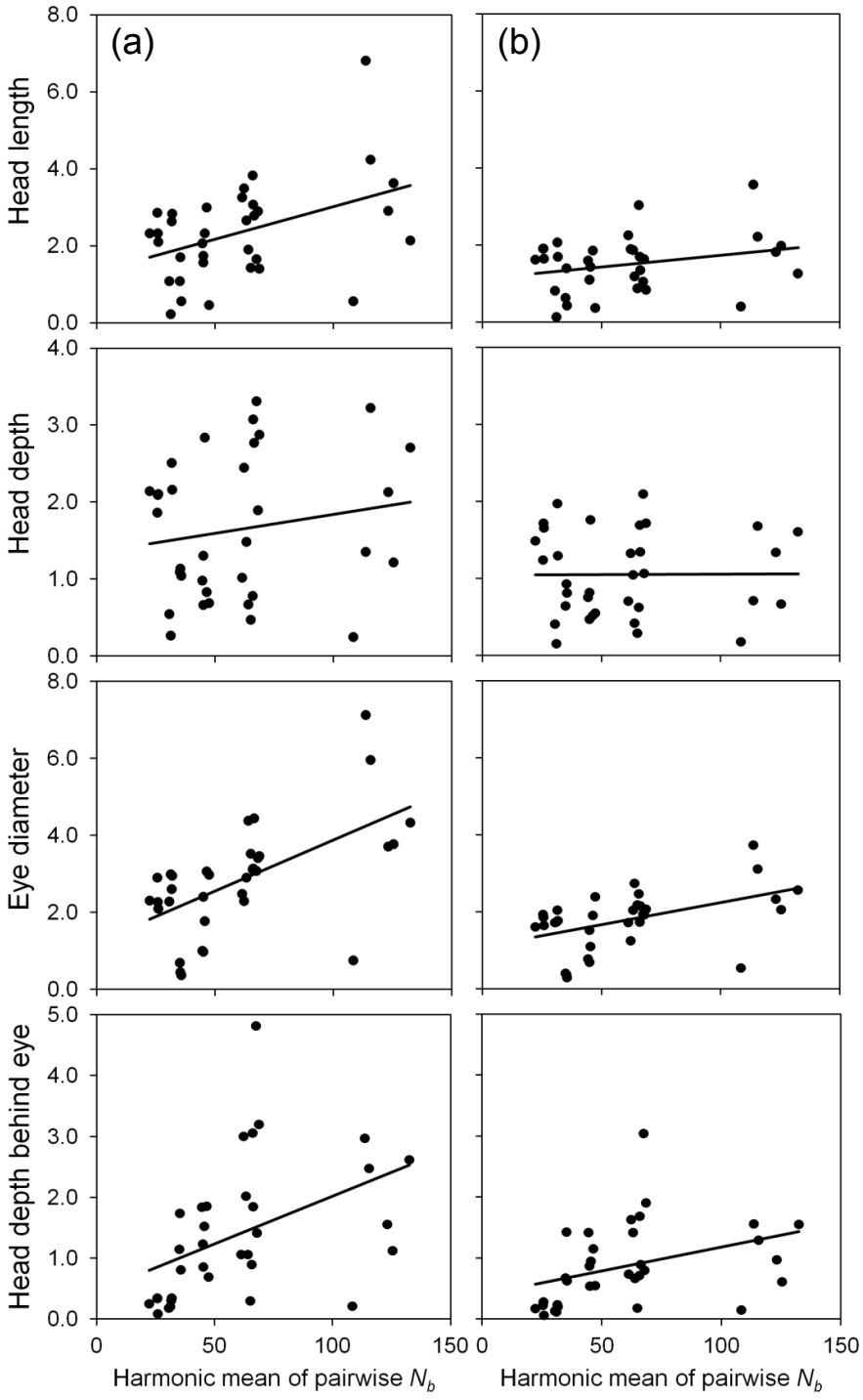


Appendix F (Fig. F1): Mean Q_{ST} /F_{ST} vs. N across traits in each of three trait categories. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.

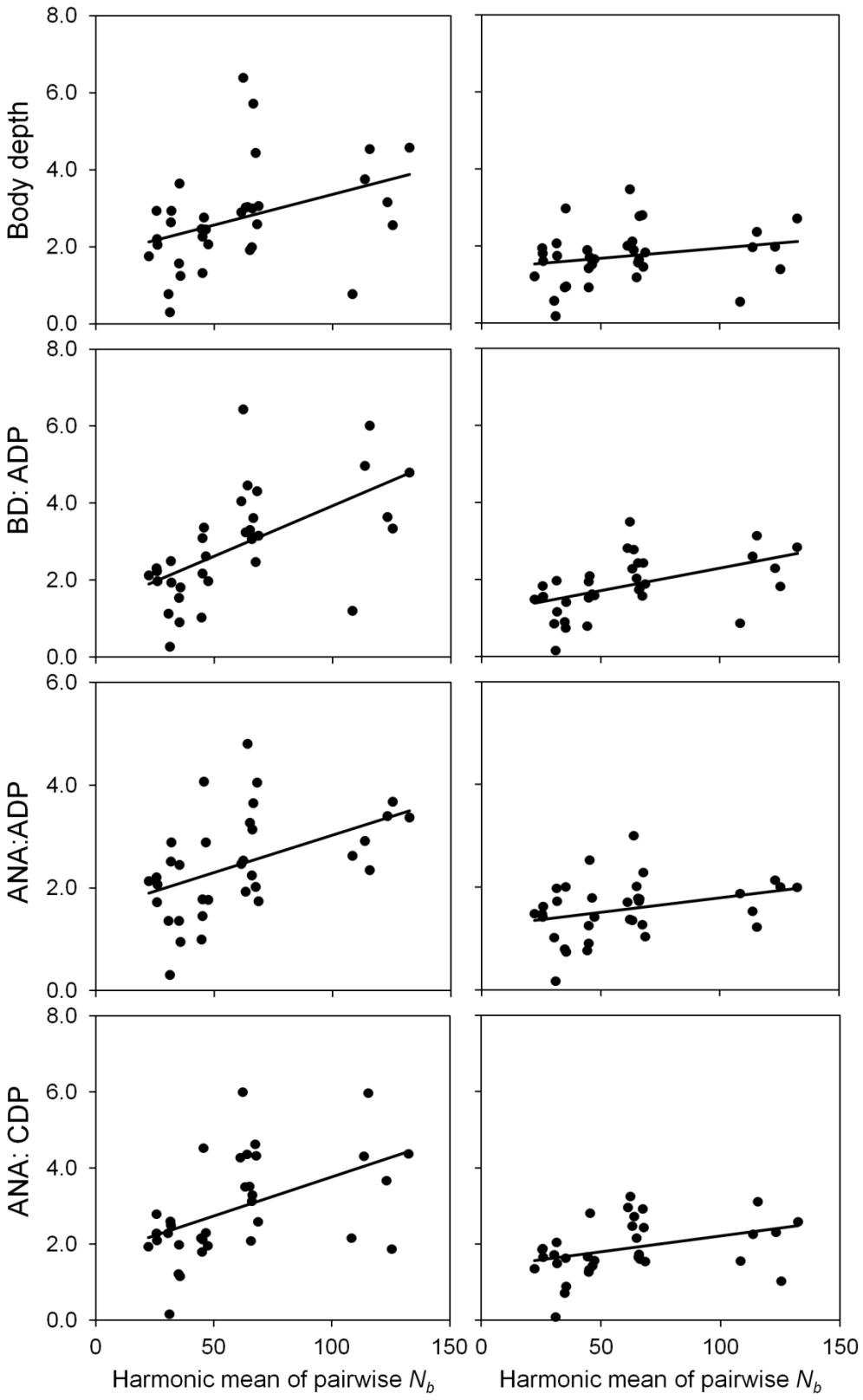


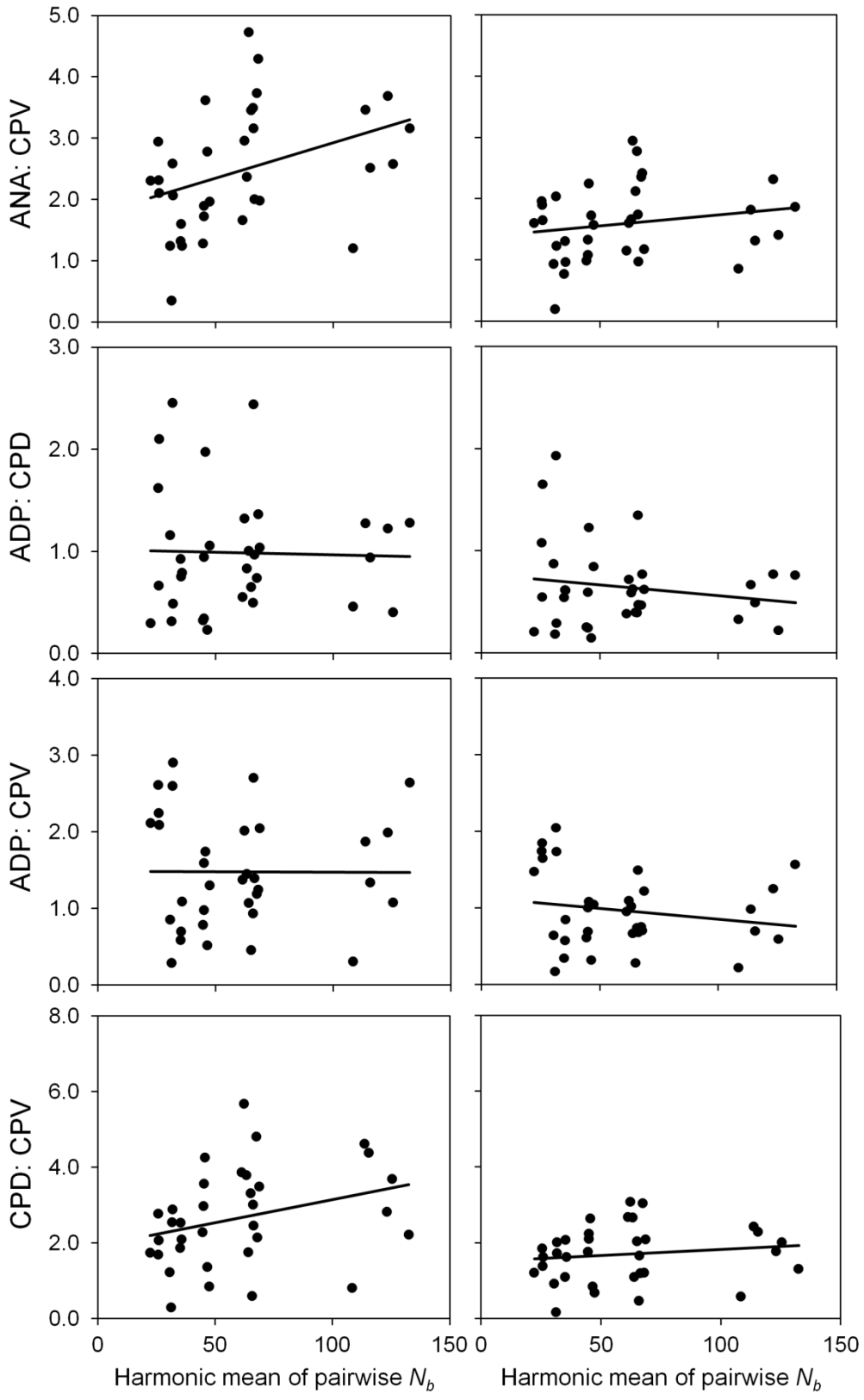
Appendix G (Fig. G1): Q_{ST}/F_{ST} vs. N_b for six early life traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines represent best-fit linear regressions.

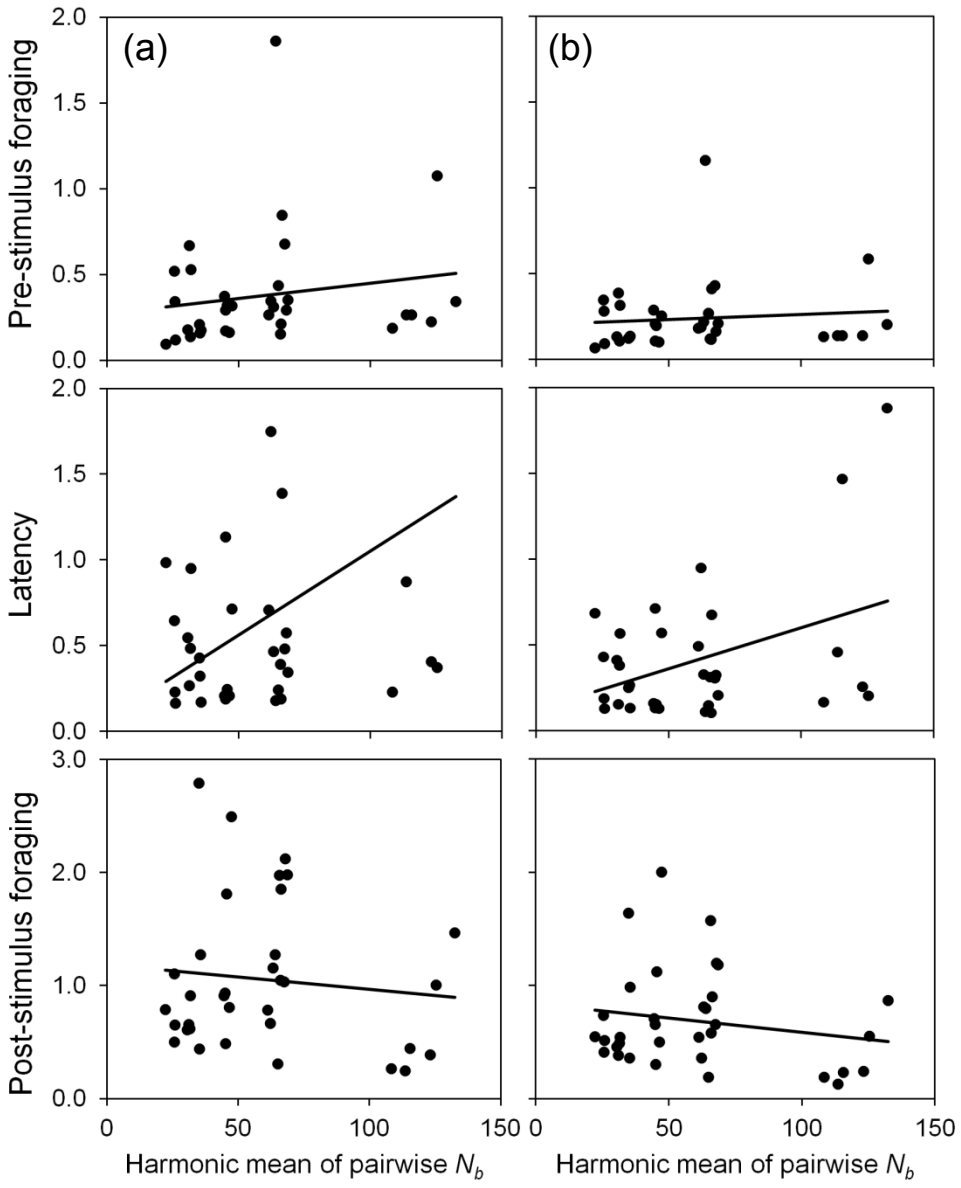

Yolk conversion
Survival
8.0
6.0
4.0
2.0
0.0
4.0
3.0
2.0
1.0
0.0
0
50
100
150
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b



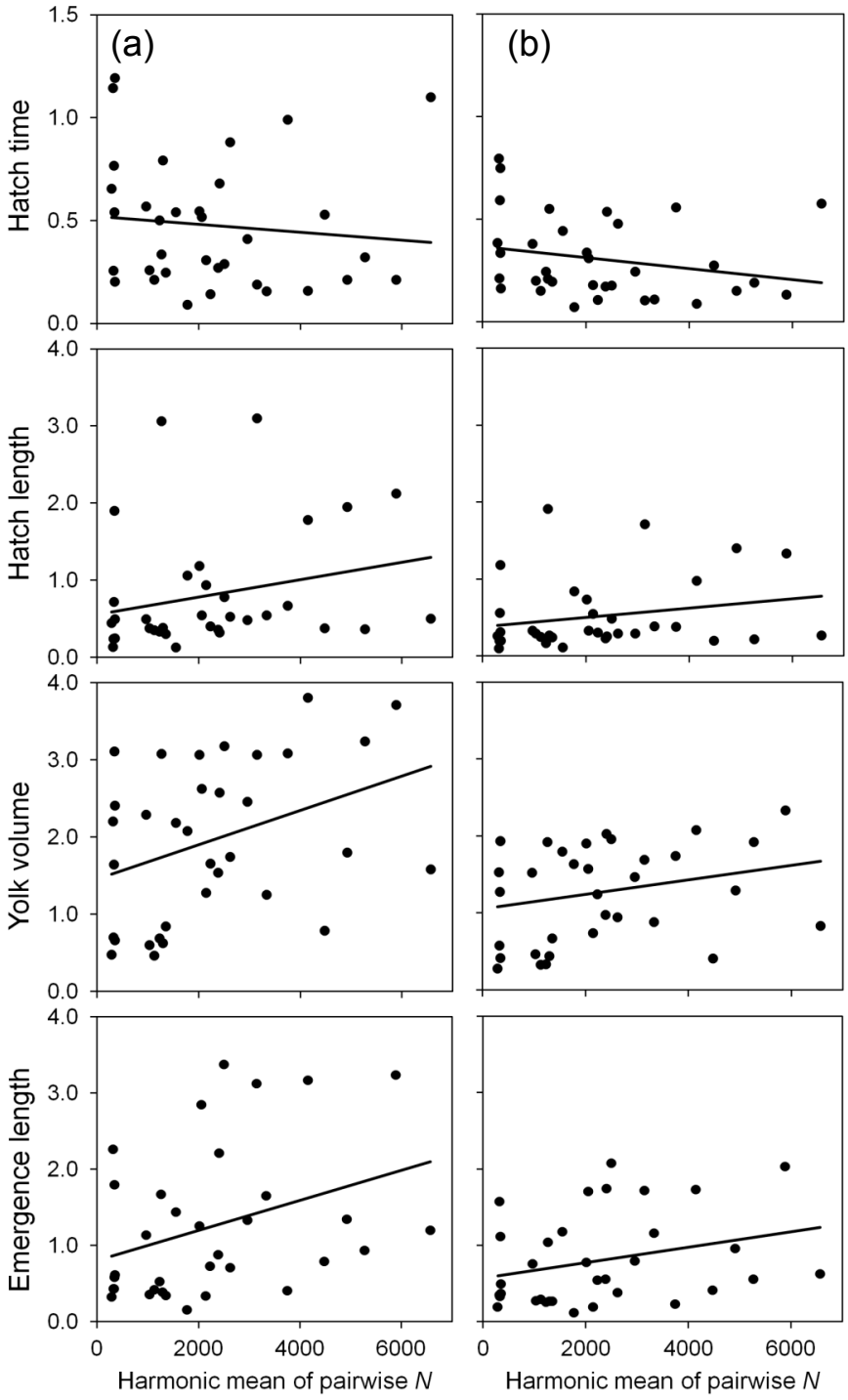
Appendix G (Fig. G2): Q_{ST}/F_{ST} vs. N_b for twelve morphological traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.


Body depth
BD: ADP
ANA:ADP
ANA: CDP
8.0
6.0
4.0
2.0
0.0
0
50
100
150
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b

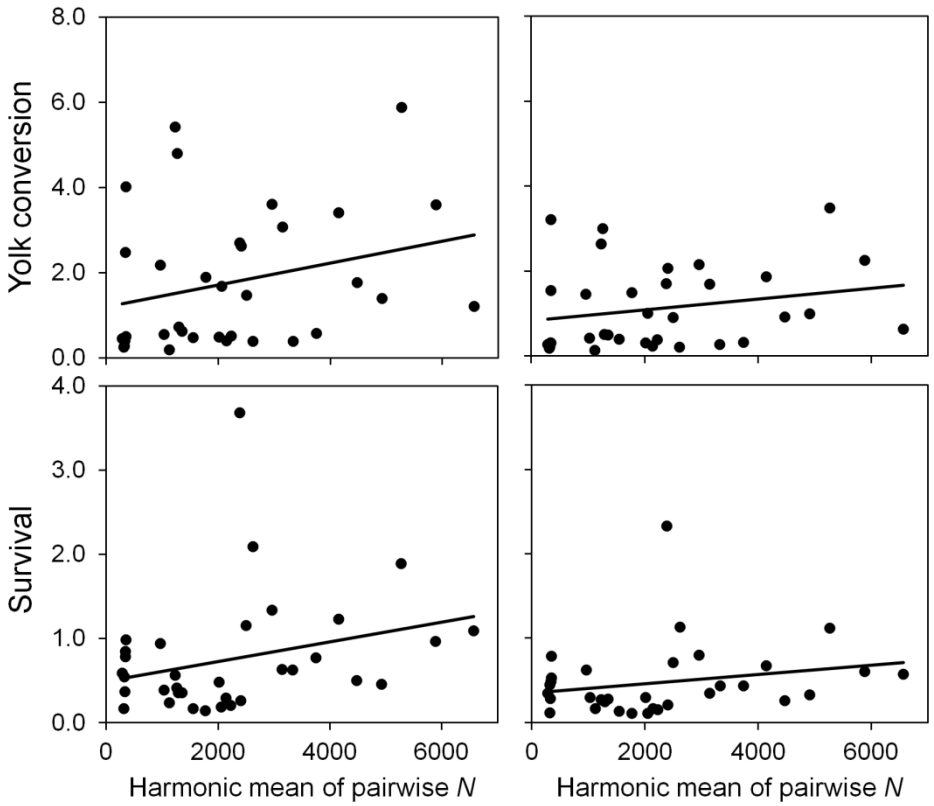

ANA: CPV
ADP: CPD
ADP: CPV
CPD: CPV
Harmonic mean of pairwise N_b
Harmonic mean of pairwise N_b

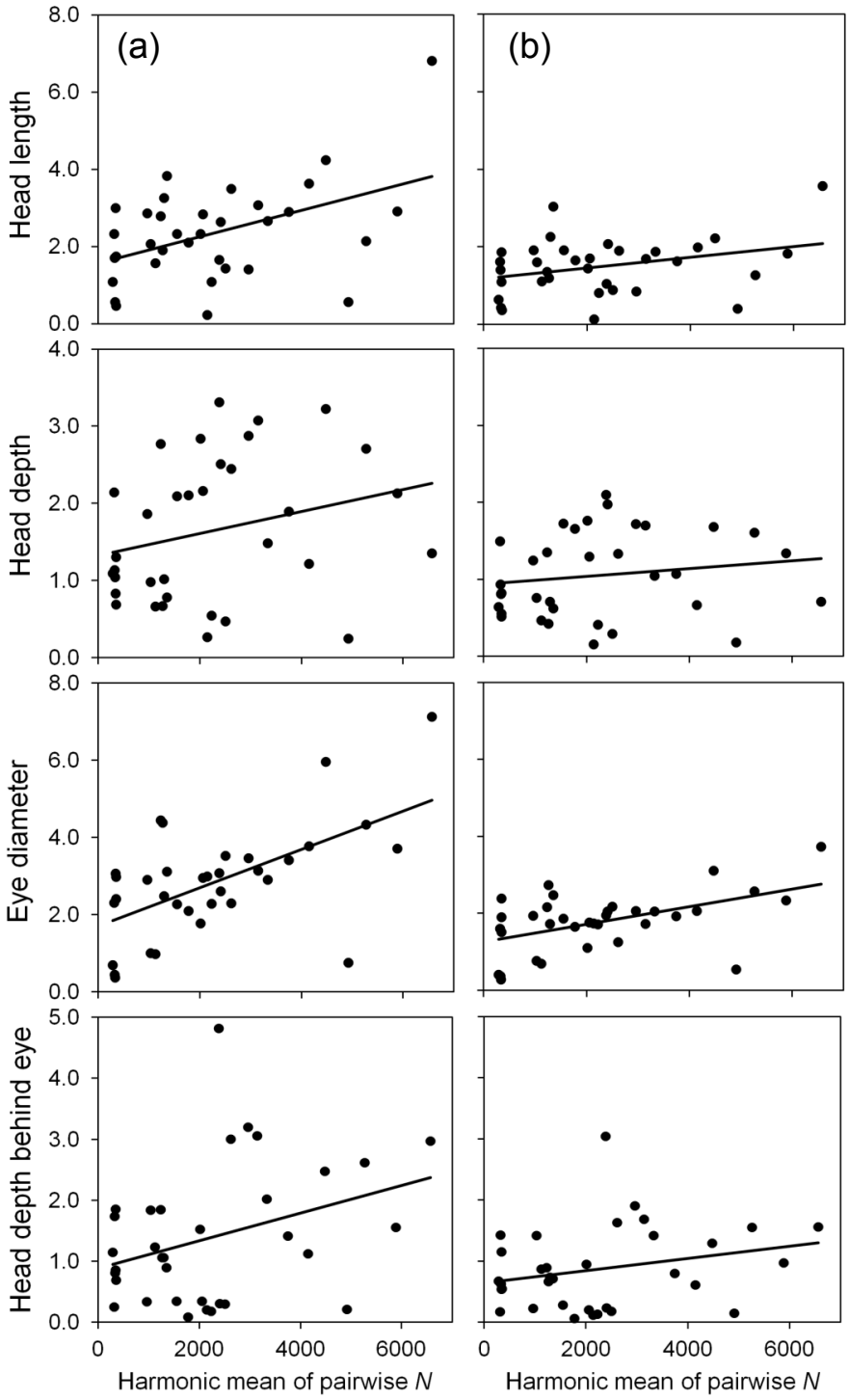


Appendix G (Fig. G3): Q_{ST}/F_{ST} vs. N_b for three behavioural traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.

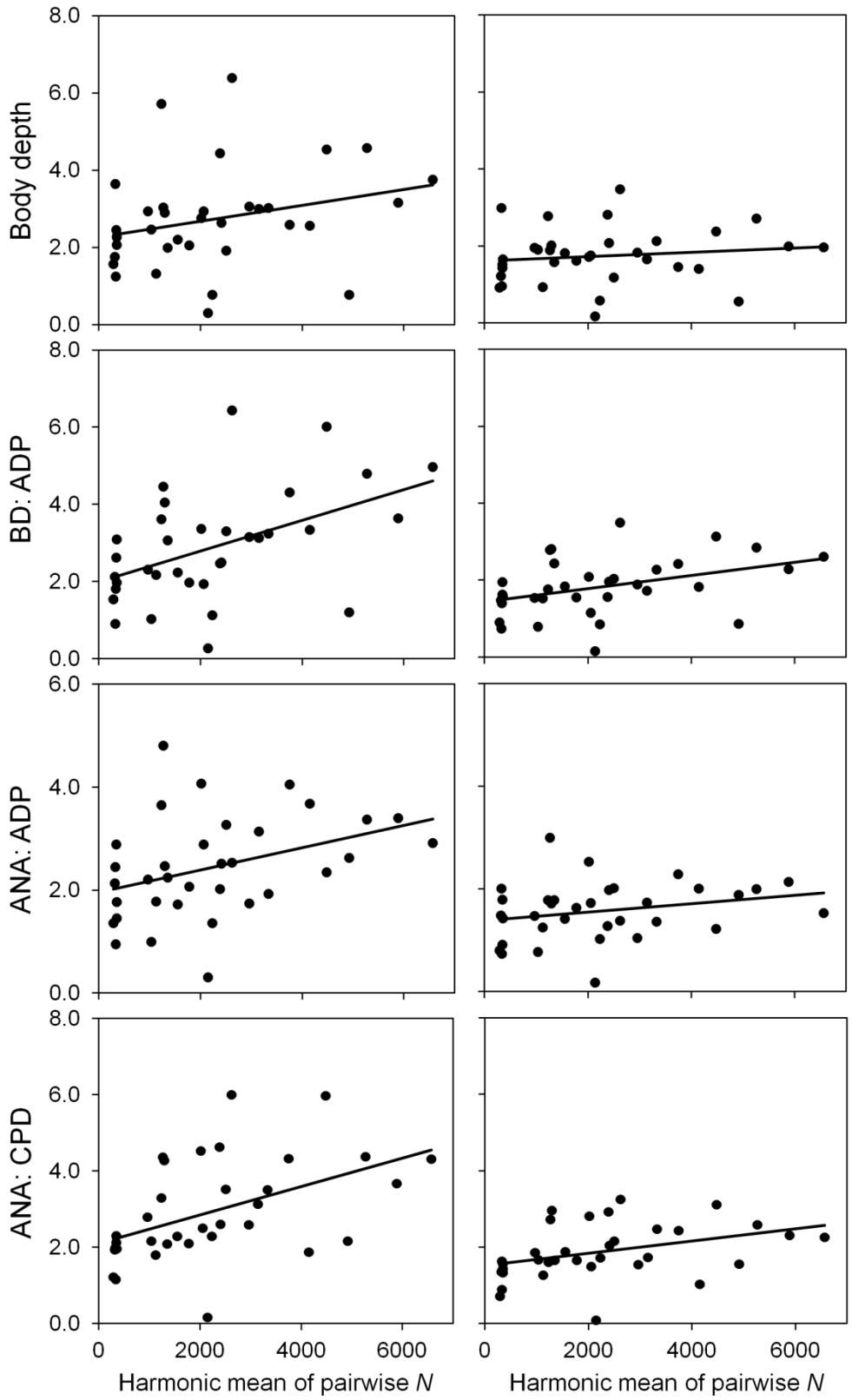


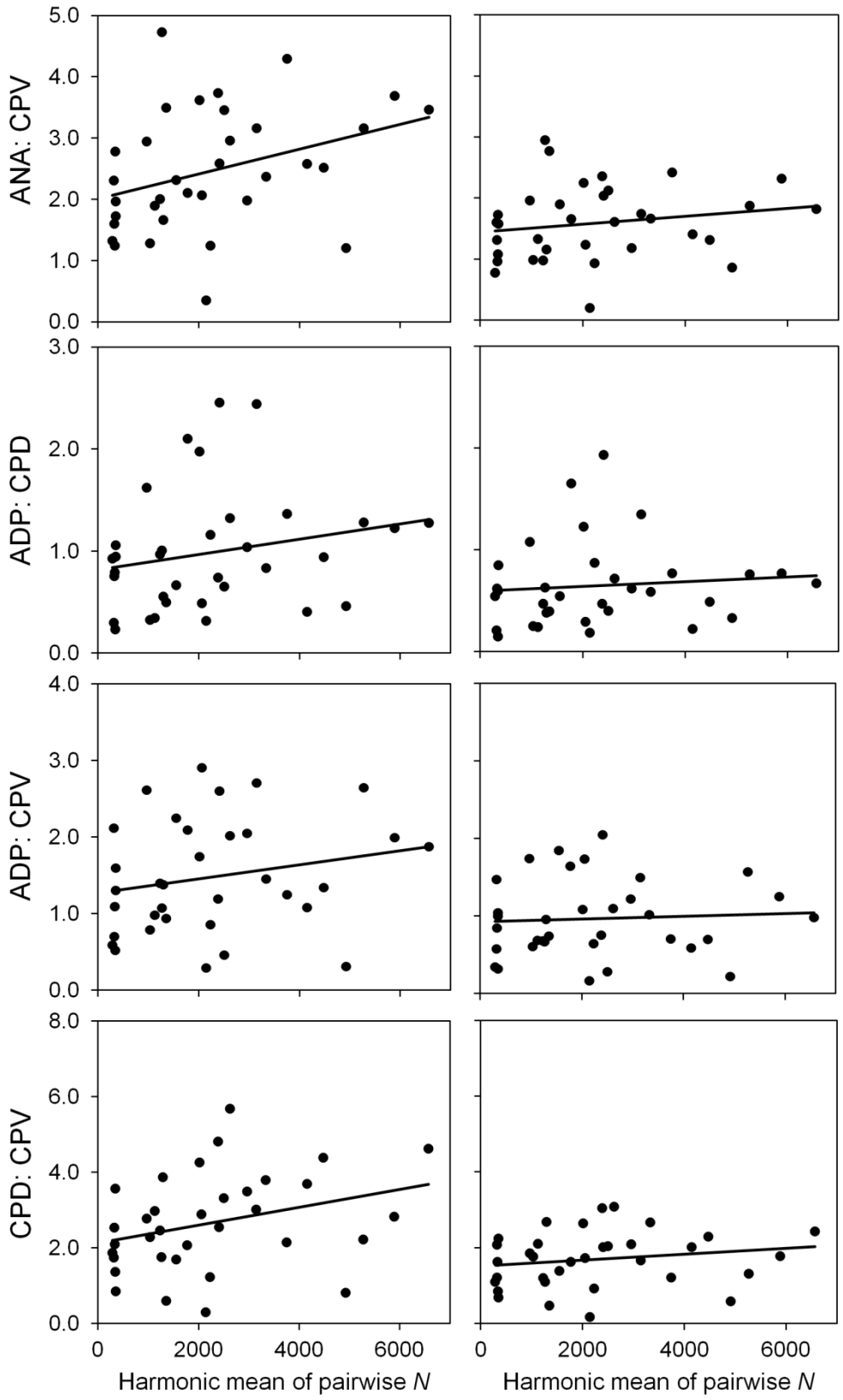
Appendix G (Fig. G4): Q_{ST}/F_{ST} vs. N for six early life traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.

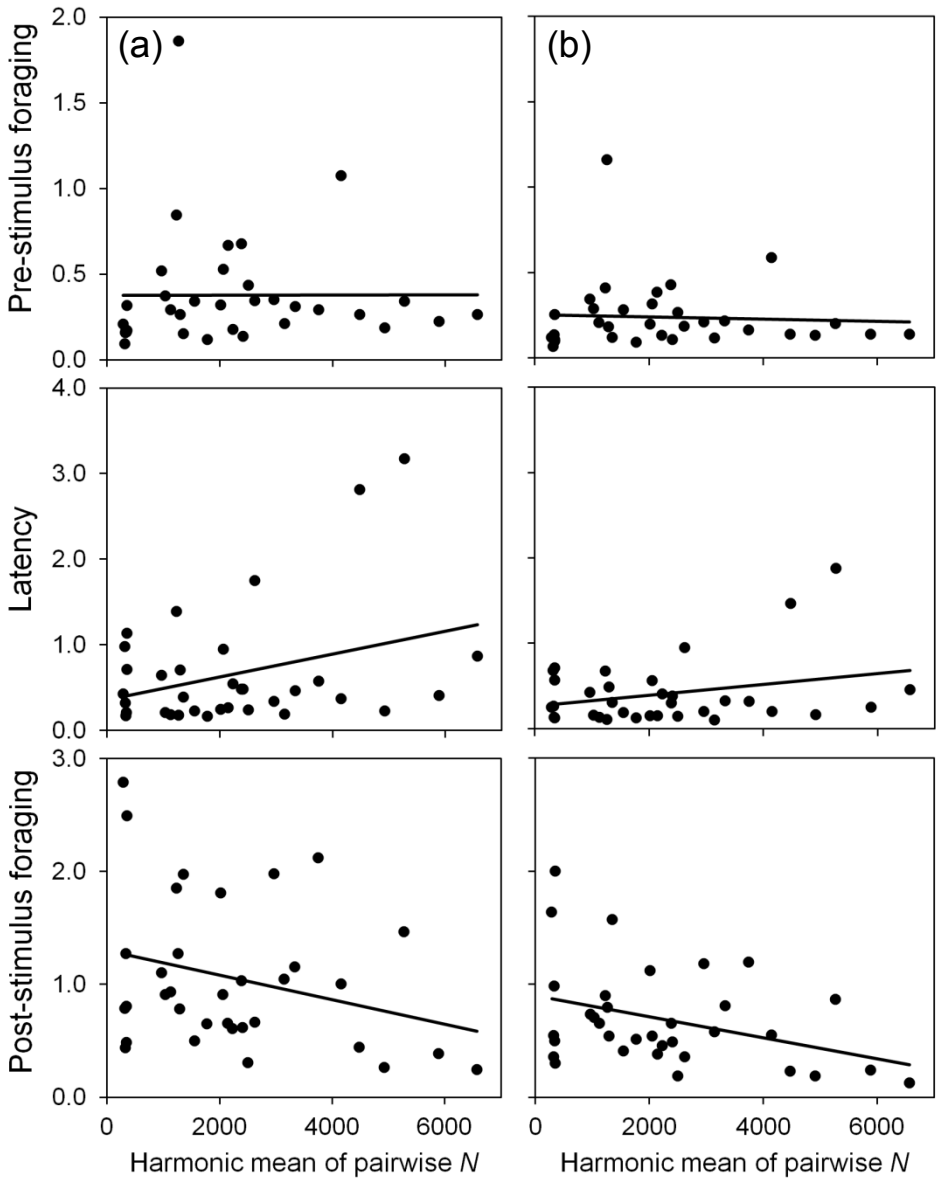

Yolk conversion
Survival
8.0
6.0
4.0
2.0
0.0
4.0
3.0
2.0
1.0
0.0
0
2000
4000
6000
Harmonic mean of pairwise N
Harmonic mean of pairwise N



Appendix G (Fig. G5): Q_{ST}/F_{ST} vs. N for twelve morphological traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.

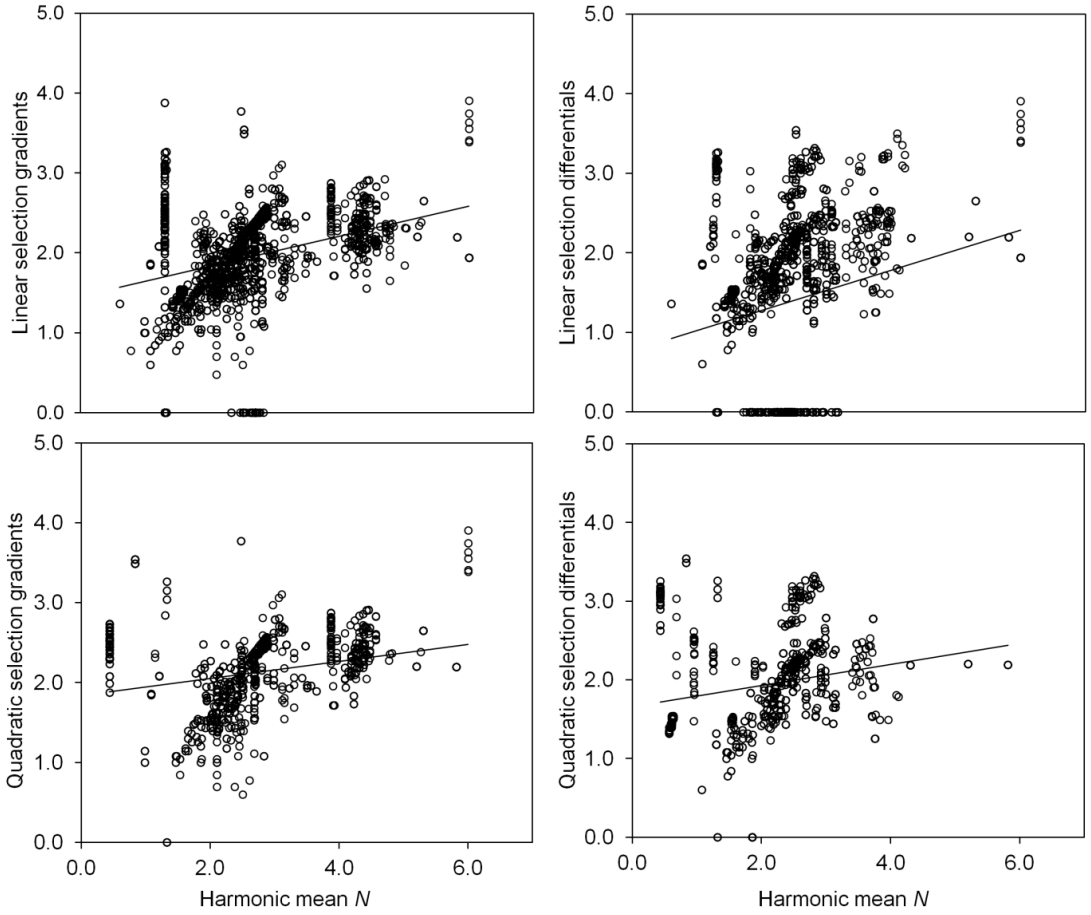

Body depth
BD: ADP
ANA: ADP
ANA: CPD
0.0
2.0
4.0
6.0
8.0
0
2000
4000
6000
Harmonic mean of pairwise N
Harmonic mean of pairwise N


ANA: CPV
ADP: CPD
ADP: CPV
CPD: CPV
Harmonic mean of pairwise N
Harmonic mean of pairwise N

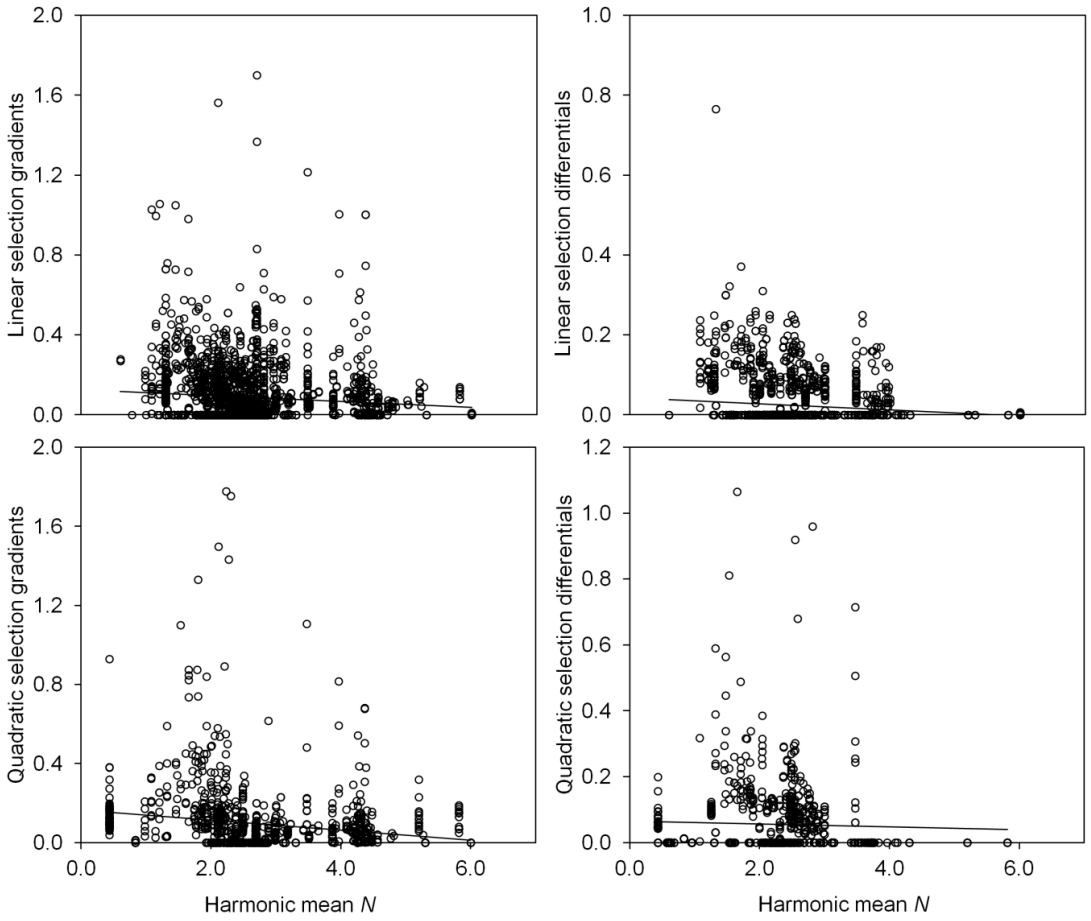


Appendix G (Fig. G6): Q_{ST}/F_{ST} vs. N for three behavioural traits. F_{ST} values among populations pairs was estimated using (a) microsatellite loci, and (b) SNPs for each trait. Solid lines are best-fit linear regressions.

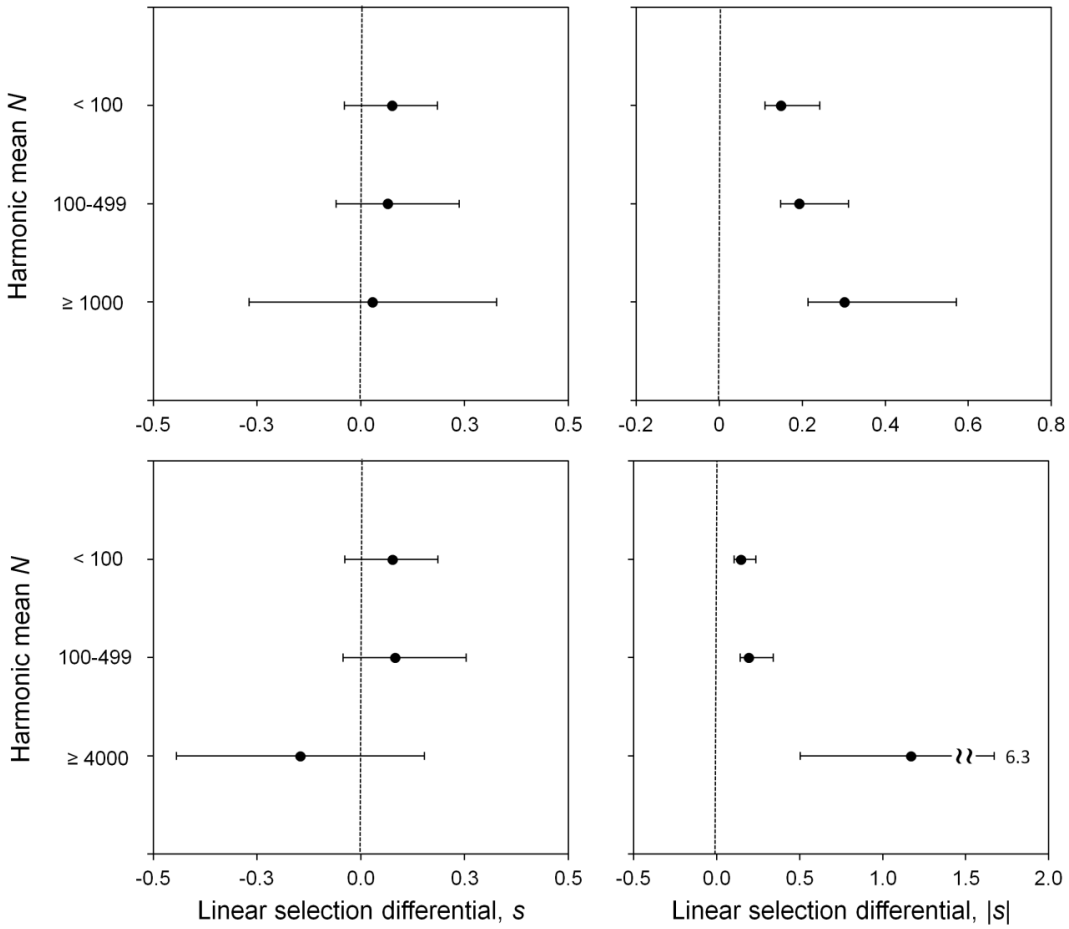
Chapter 4 Appendices



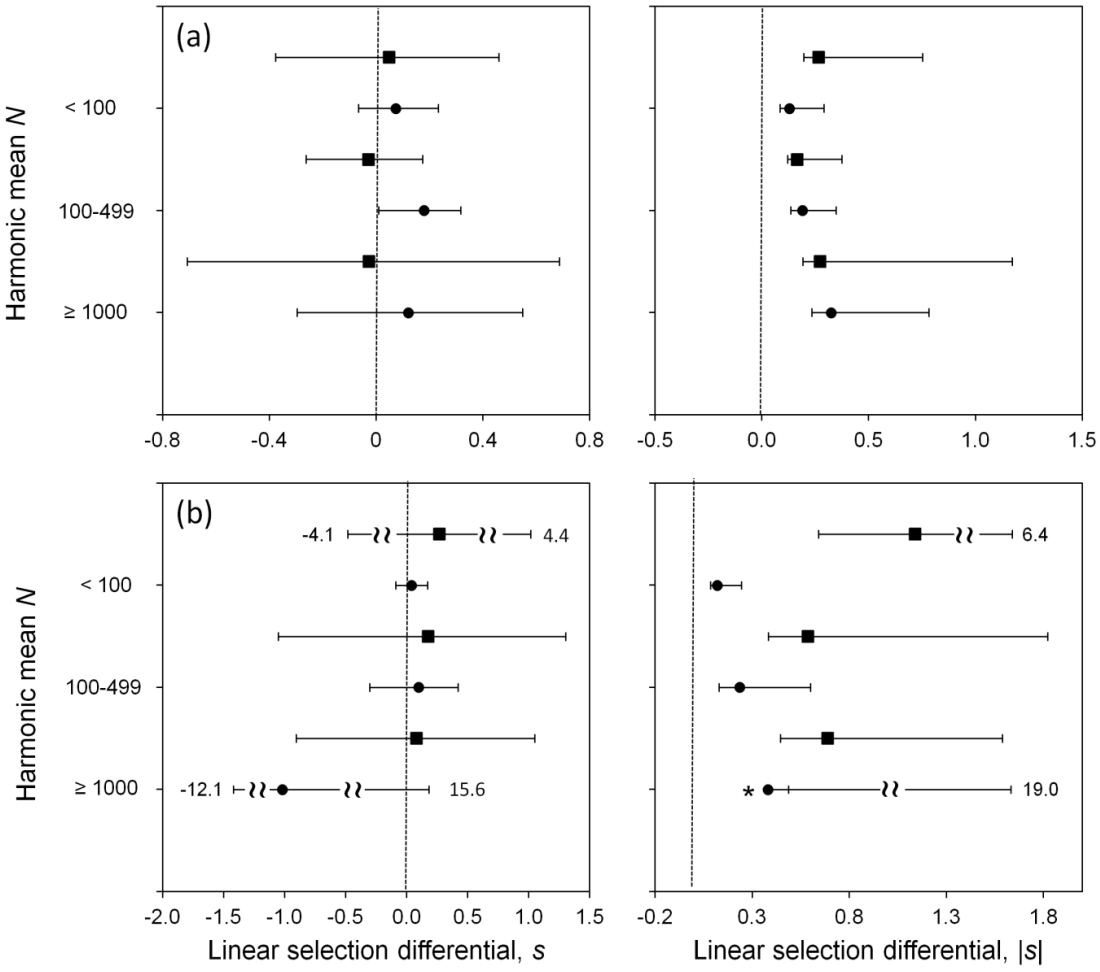
Appendix A (Fig. A1): The relationship between sample size for four types of selection coefficients and population size. The solid line represents the best fit linear regression.



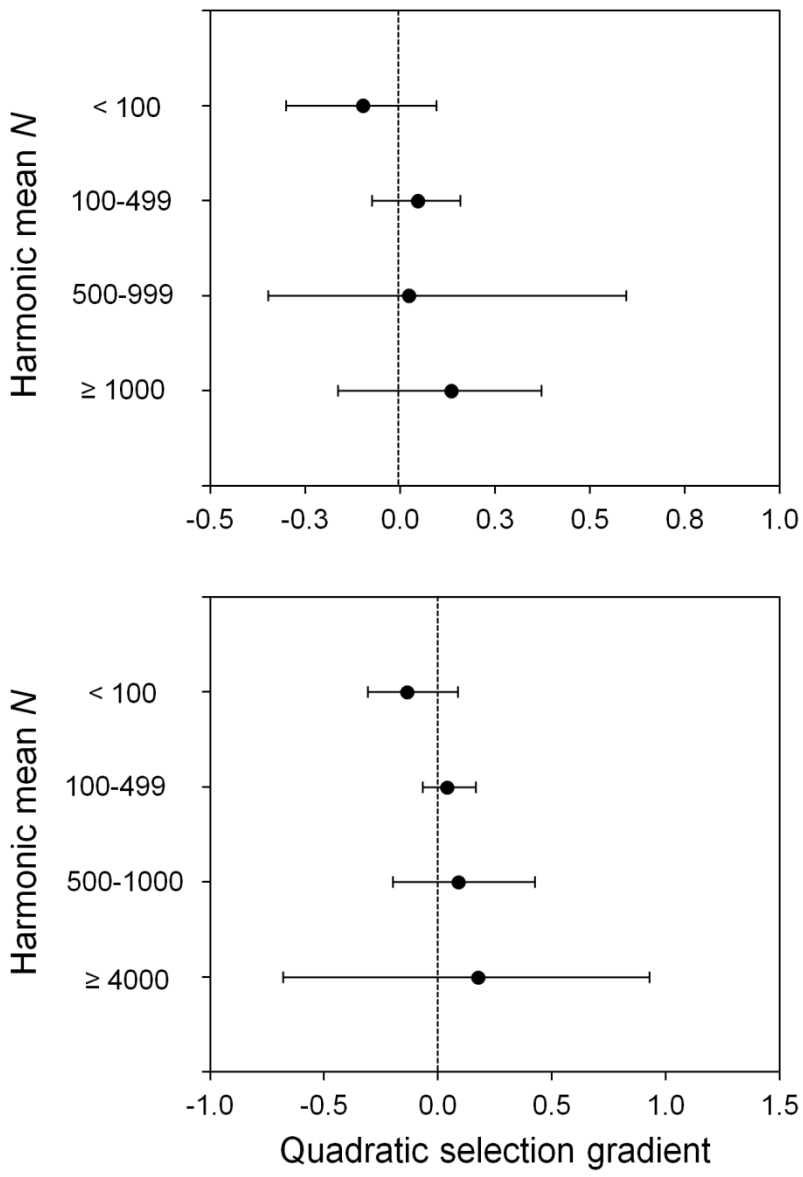
Appendix B (Fig. B1): The relationship between standard errors of selection estimates for four types of selection coefficients and population size. The solid line represents the best fit linear regression.



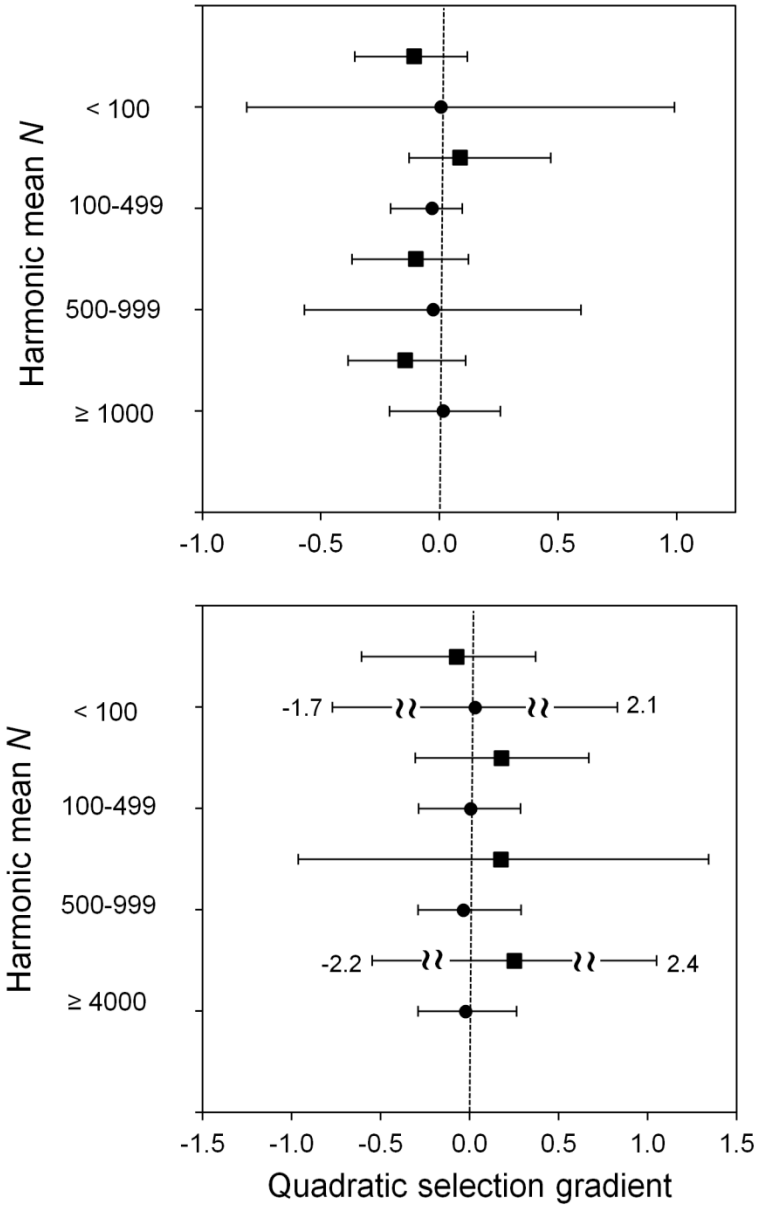
Appendix C (Fig. C1): Posterior modes of linear differential values, *s* and the magnitude of linear selection differentials, |*s*| in each of three different population size bins. The magnitude of selection was calculated using the folded binomial distribution. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large.



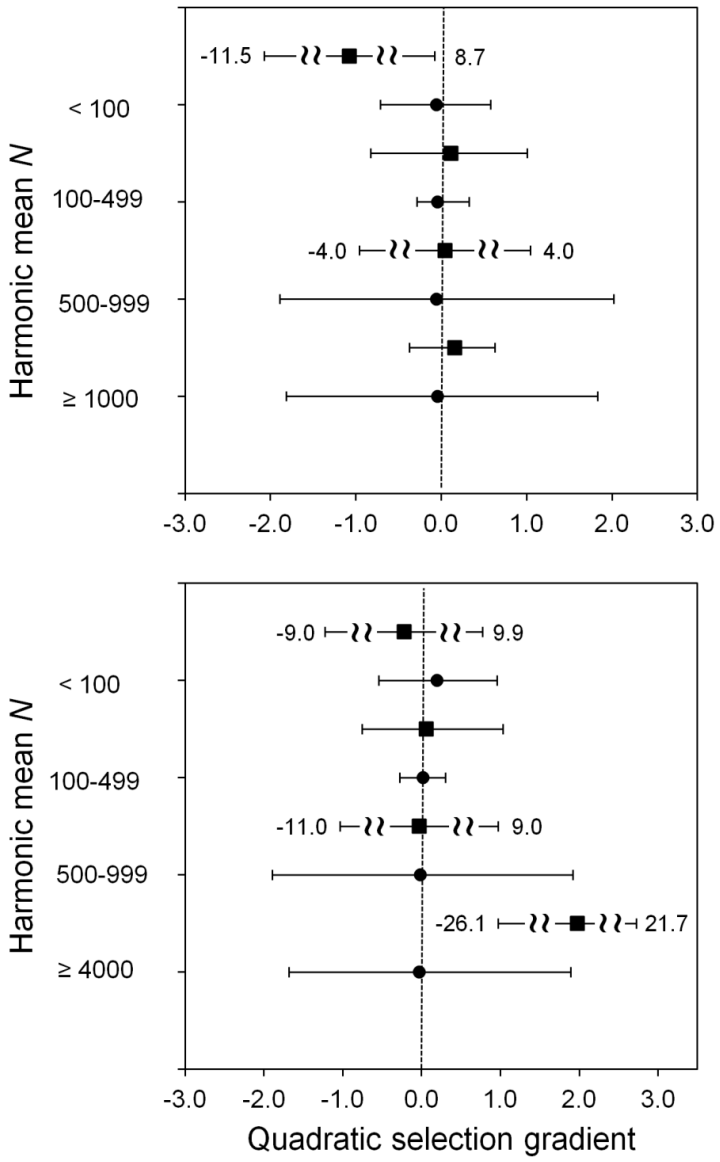
Appendix C (Fig. C2): Posterior modes of linear differential values, *s* and the magnitude of linear selection differentials, |*s*| for a) morphological (●) and life history traits (■), and b) plants (■) and vertebrates (●) in each of three population size bins. The magnitude of selection was calculated using the folded binomial distribution. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large. The asterix represents where a posterior mode was outside the calculated HPD confidence intervals, likely because of low statistical power.



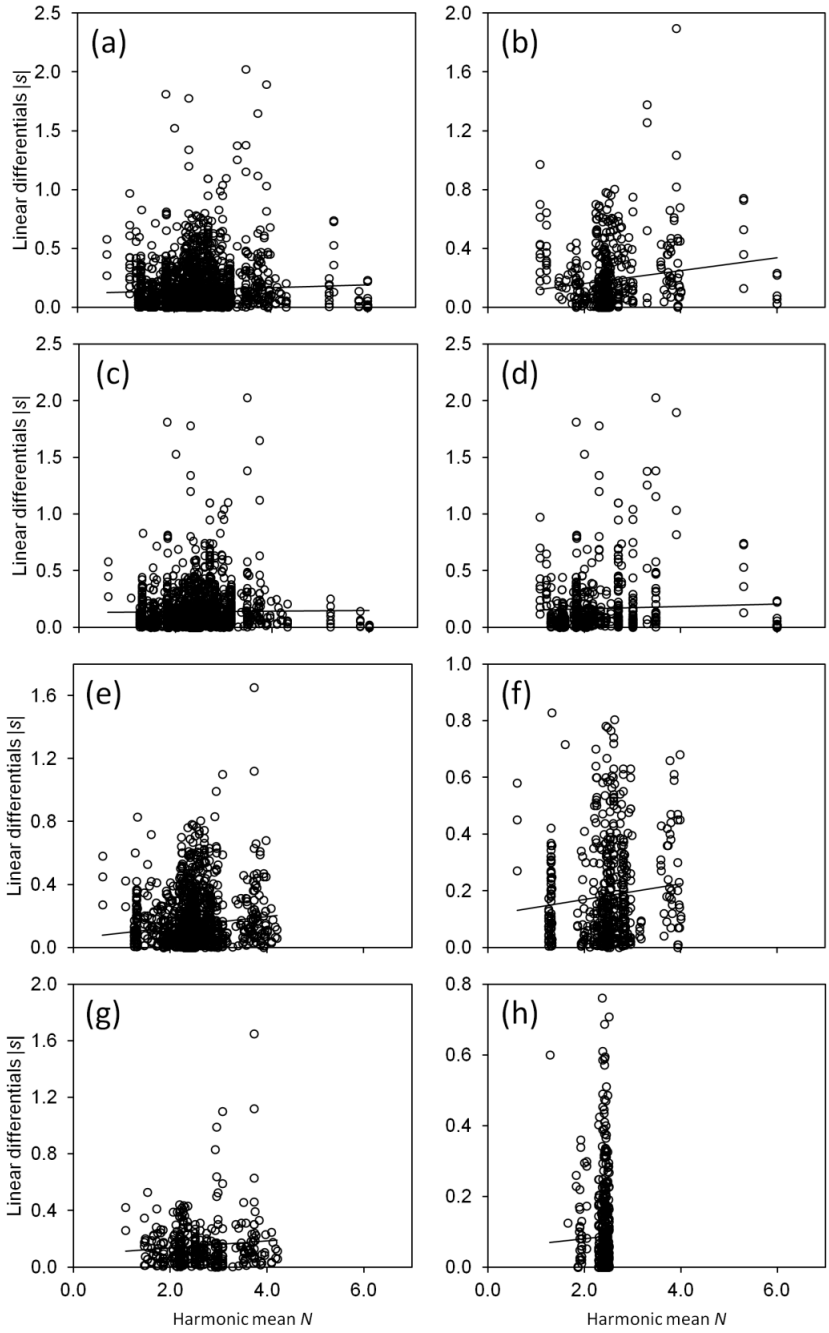
Appendix D (Fig. D1): Posterior modes of quadratic gradients in each of four different population size bins. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm.



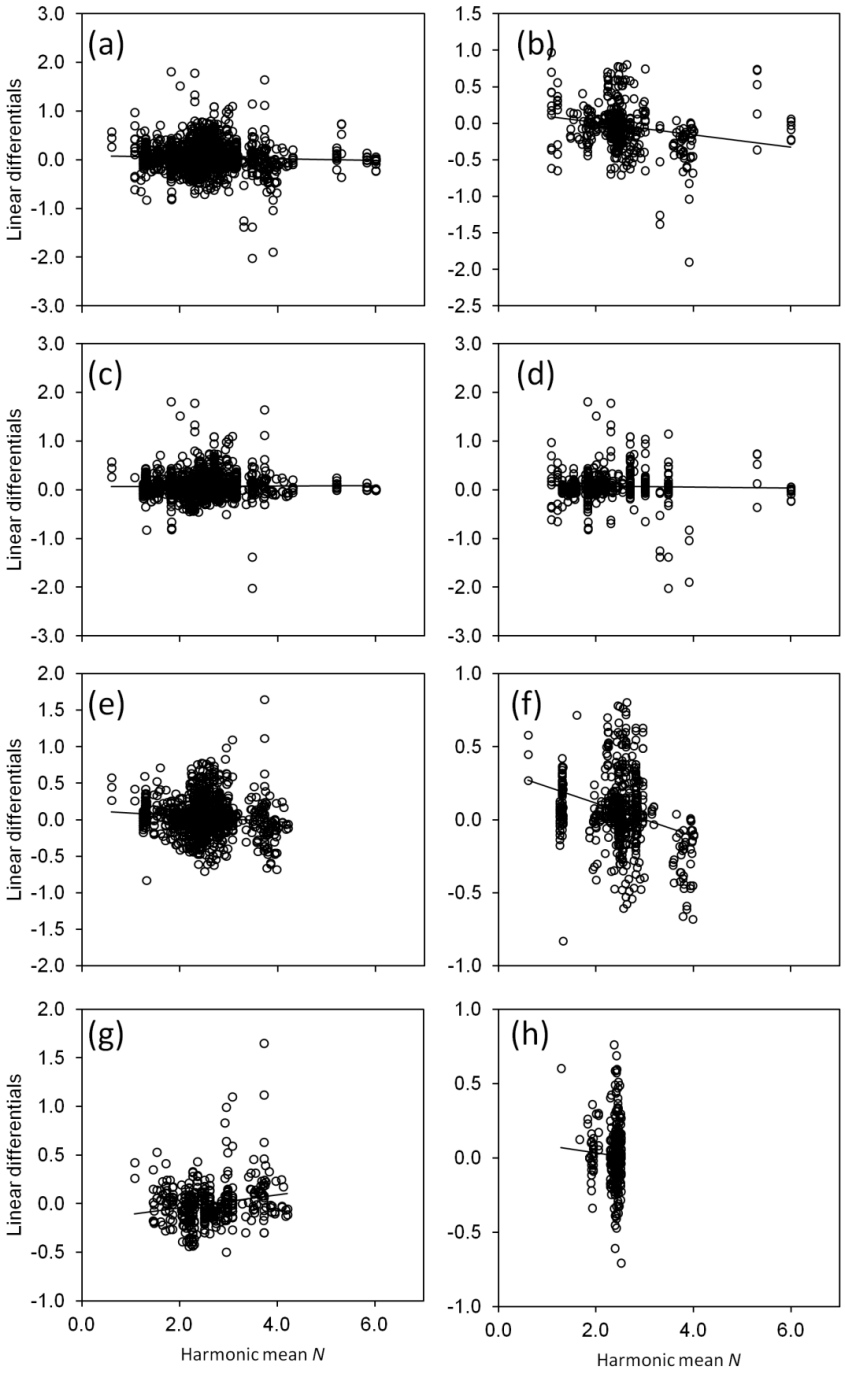
Appendix D (Fig. D2): Posterior modes for quadratic gradients for morphological traits (●) and life history traits (■) in each of four population size bins. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large.



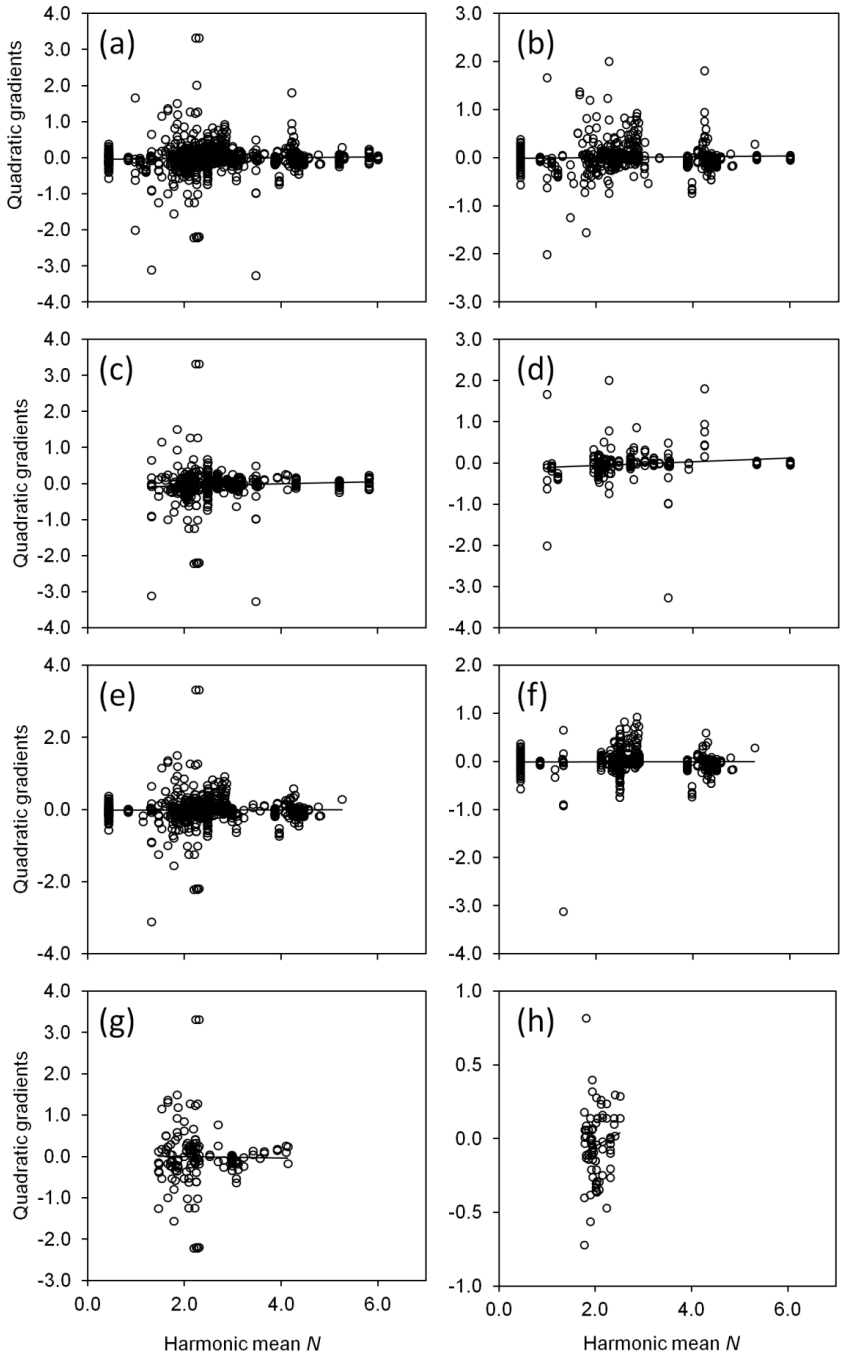
Appendix D (Fig. D3): Posterior modes for quadratic gradients for plants (■) and vertebrates (●) in each of four population size bins. Error bars represent 95% HPD confidence intervals calculated using MCMCglmm. Numbers next to error bars with breaks are the maximum 95% HPD values where confidence intervals were large.



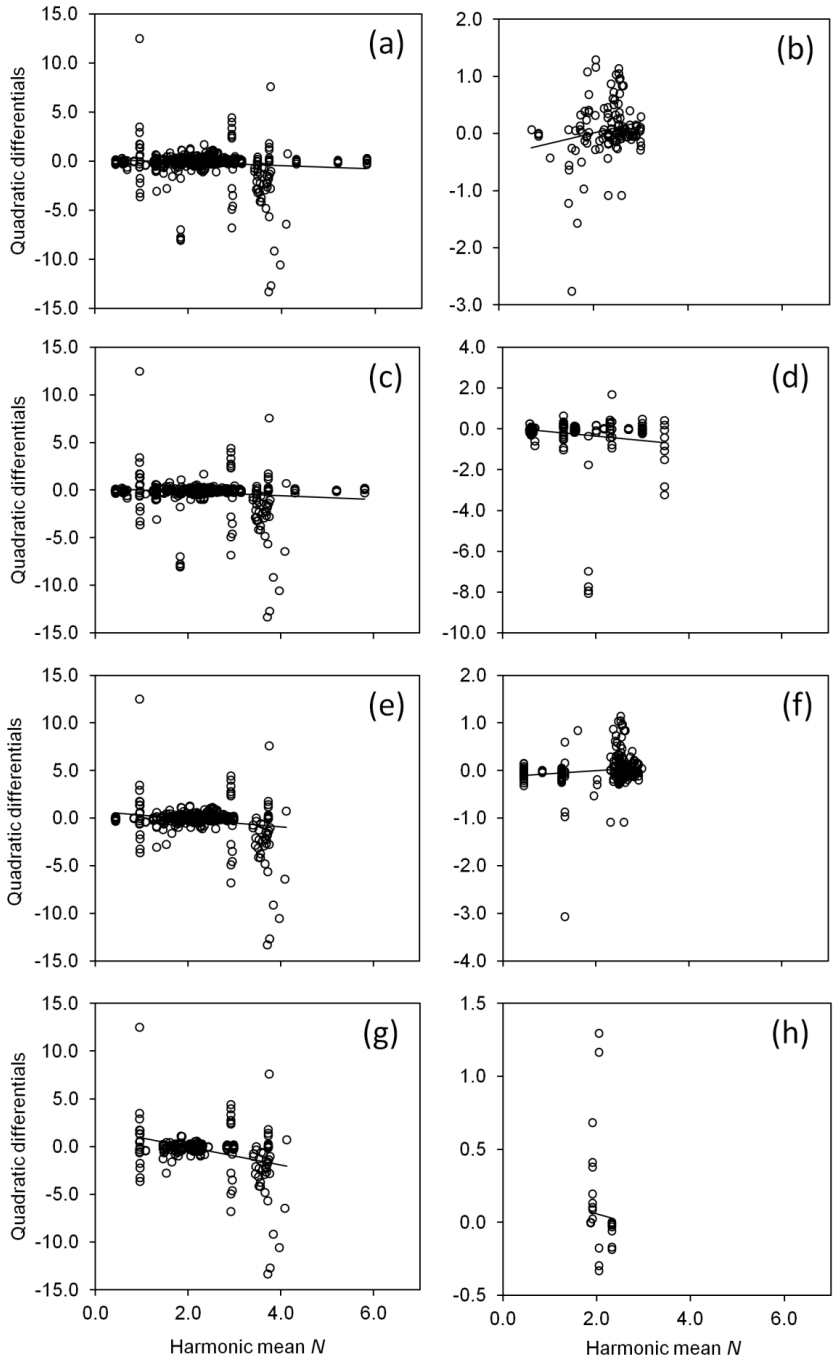
Appendix E (Fig. E1): Absolute values of linear selection differentials to assess the strength of directional selection in relation to population size across a) all taxa and trait types, b) life-history traits c) morphological traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.



Appendix F (Fig. F1): Linear selection differential estimates to assess the direction of selection in relation to population size across a) all taxa and trait types, b) life history traits, c) morphology traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.



Appendix F (Fig. F2): Quadratic selection gradient estimates to assess the direction of selection in relation to population size across a) all taxa and trait types, b) life history traits, c) morphology traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.



Appendix F (Fig. F3): Quadratic selection differential estimates to assess the direction of selection in relation to population size across a) all taxa and trait types, b) life history traits, c) morphology traits, d) plants, e) vertebrates, f) birds, g) fish, and h) mammals. The solid line represents the best fit line from a linear regression.