

Relationship of habitat variability to population size in a stream fish

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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). We investigated these alternatives based on multiyear 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.

Key words: adaptation; brook trout; Cape Race, Newfoundland, Canada; effective population size; environmental variance; evolution; fragmentation; natural selection; salmonid; *Salvelinus fontinalis*.

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 Hoffmann 2012) and their subsequent responses to environmental change, which will be largely influenced by population size (Lynch and Lande 1997).

Herein, we considered 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 (Fig. 1; Willi and Hoffmann 2012). 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 et al. 1985), parasitism (Kruess and Tscharnkte 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.

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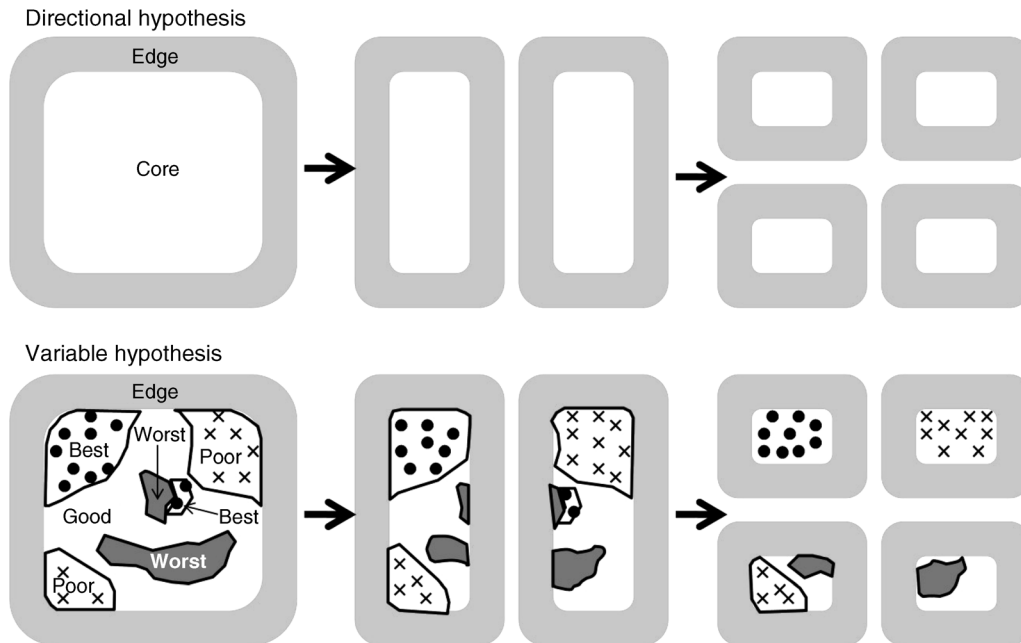


FIG. 1. Two alternatives for the effect of habitat fragmentation on selection regimes within fragments occupied by populations of varying size. Habitat fragmentation may shift selection regimes in a consistent direction within fragments inhabited by populations of similar size (directional hypothesis), or, 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). Different patterns correspond to different types of habitat (i.e., habitat patches of varying environmental conditions).

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 (Fig. 1; Willi and Hoffmann 2012). 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, Vanwalleghe 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 our 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 toward 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 Hoffmann 2012).

Our purpose was to determine whether the habitats experienced by 19 differentially abundant populations of a stream fish species (brook trout, *Salvelinus fontinalis*; see Plate 1) consistently differ in a number of physical and chemical characteristics that are related to individual fitness and population abundance. To test the directional hypothesis, we investigated whether the relationship between habitat parameters and population size differs from zero. For the variable hypothesis, we explored whether (1) small populations exhibit greater among-fragment variability in mean habitat parameters

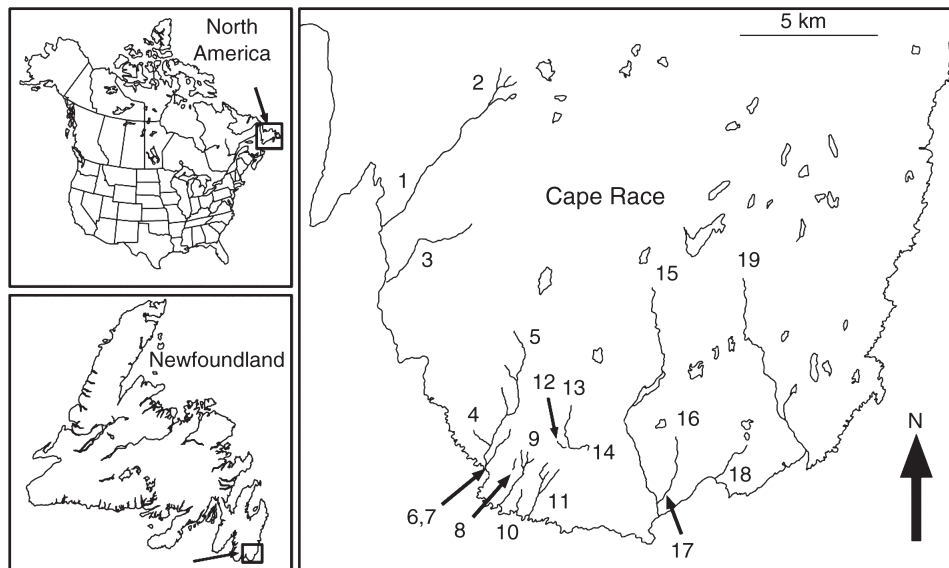


FIG. 2. The geographic locations of study streams on Cape Race, Newfoundland, Canada. Numbers on the inset correspond to streams situated from east to west: (1) Lower Whelan's, (2) Upper Whelan's, (3) Cotton River, (4) Perdition Brook, (5) Freshwater River, (6) Lower Coquita, (7) Upper Coquita, (8) Bella's 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 Appendix A: Table A1.

than large populations, and (2) 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, we also quantified the relationship between population size and fragment size (drainage area) for our study populations.

We tested our hypotheses 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-Lucero et al. 2012, Palstra and Fraser 2012).

MATERIALS AND METHODS

Study site

Cape Race, Newfoundland, Canada (Fig. 2; see Plate 1), 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 (0.27–8.10 km), low-order streams, with most harboring resident populations of brook trout (Appendix A: Table A1). Several attributes make these populations ideal for investigating the relationship of habitat variability to population size in vertebrates: (1) the small size of Cape Race streams is amenable to thorough sampling and measuring of population and habitat characteristics; (2) populations are pristine and largely unexploited due to the small average size of individuals (typically <15 cm); (3) most streams terminate in a 30–50 m waterfall emptying directly into the sea, which we found effectively eliminated gene flow among populations; and (4) 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, (5) phylogeographic work suggests that isolation of Cape Race populations occurred from a common ancestor since the late-Wisconsinan deglaciation (10 000–12 000 years before present; Danzmann et al. 1998).

Habitat data

To quantify spatial and temporal habitat variability across trout streams, we initially collected data on 31 habitat parameters from 875 transects across 13 Cape Race stream drainages in 2010 and 2011. Highly correlated variables were dropped, yielding a reduced set of 17 transect-level habitat parameters per stream (Appendix A: Table A2; see Appendix A for a complete list of habitat parameters and data collection method-

TABLE 1. Brook trout populations (*Salvelinus fontinalis*) census sizes (N) for 2010 and 2011, as well as the effective number of breeders (N_b), at Cape Race, Newfoundland, Canada.

Population	2010 N (95% CI)	2011 N (95% CI)	N_b	C	Sample size
Lower Whelan's	NS	4421 (3883–5190)	93 (45–1363) [†]	1	48
Upper Whelan's	NS	3588 (3206–4107)	249 (114– ∞) [†]	1	74
Cotton River	1174 (692–2042)	2871 (2016–4240)	41 (29–59)	2	56, 47
Perdition Brook	NS	726 (636–853)	33 (17–115) [†]	1	24
Freshwater River	4550 (4171–5028)	5385 (5076–5743)	101 (81–452)	3	45, 95, 114
Lower Coquita	316 (229–452) [‡]	278 (173–483)	30 (26–36)	3	48, 59, 42
Upper Coquita	76 (50–99) [‡]	79 (43–196)	15 (15–16)	2	19, § 25§
Bella's Brook	NS	510 (309–1169)	59 (39–105) [†]	1	48
Bob's Cove	6132 (4500–9739) [‡]	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) [‡]	735 (626–936)	44 (35–87)	3	66, 48, 108
Ditchy	107 (76–161)	179 (132–265) [‡]	29 (26–34)	2	26, ¶ 35¶
Upper Ouananiche Beck	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, ¶ 25¶
Watern Cove	6223 (5049–8434)	8416 (7225–10 255)	130 (119–137)	3	59, 96, 133
Upper Blackfly	235 (164–418)	317 (95–1055)	49 (14–138)	2	41, § 28§
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) [#]	3	90, 53, 44
Cripple Cove	1683 (992–2927)	2412 (2231–2632)	46 (28–101)	3	80, 76, 71

Notes: Total census population size (N) is reported with the 95% CIs in parentheses. N_b reported is the weighted harmonic mean of point estimates across cohorts within a population, with the range of point estimates in parentheses. Appendix C: Table C1 includes the 95% CI for each individual cohort. NS represents a stream not sampled; and C is the 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).

[†] 95% CI reported if only one cohort was sampled.

[‡] Schnabel method used for N estimation.

§ 2009 and 2010 cohorts.

¶ 2010 and 2011 cohorts.

Two cohort estimates of ∞ not included.

ology). Several stream-level characteristics were measured in the laboratory, using Google Earth version 6.0 (see Appendix A: Table A1; Google, *available online*),⁶ and drainage area was measured using Google Earth in conjunction with MapWindow GIS open source GIS software version 4.7 (MapWindow Open Source, *available online*).⁷ Habitat data in most stream drainages (11 of 13) were collected in July for both years; remaining drainages were assessed in October 2011 (Appendix A: 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 25–100 m apart, depending on stream length (Appendix A: Table A1). We 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 –3 m 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 50-m or 100-m interval from the stream mouth, depending on stream length. Details of microsatellite PCR and polymorphism screening are found in Appendix B.

We used STRUCTURE 2.1 (Pritchard et al. 2000) 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). We

⁶ <http://www.google.com/earth/index.html>

⁷ <http://www.mapwindow.org/>



PLATE 1. A typical male brook trout from the streams of Cape Race, Newfoundland, and surrounding scenery. Photo credits: J. L. A. Wood.

determined K 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, 4–7; see Fig. 2) or fragmentation and isolation of the stream bed (populations 12–14, 16, 17). Therefore, for all subsequent analyses, these samples were treated as separate populations (Table 1). A suggestion of subpopulation 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 *Results*, first section). Nevertheless, Freshwater River was treated as one population because individual assignments within clusters were ambiguous and no evident spatiotemporal 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), we then used GENEPOP 4.0 (Raymond and Rousset 1995) 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)

We used either the Schnabel (1938) or Petersen (1896) method to estimate annual adult census population sizes (N) in Cape Race streams in 2010 and 2011 (see Table 1 for individual population details). Population size was estimated for the number of age 1+ yr and older individuals, which, we found, for most streams, roughly corresponds to adult population size since these constitute reproductive ages at Cape Race (Hutchings 1993). 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, LOC) 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; we also simultaneously tagged more individuals (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)

We also overlapped habitat data with population size data relating to genetics. We specifically estimated the effective number of breeders (N_b) of each cohort for each population, using the linkage disequilibrium method implemented in LDNe (Appendix C; Waples and Do 2008). 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_b 's across multiple cohorts (comprising the equivalent of one generation) could be biased. For simplicity, here we used the harmonic mean of N_b where multiple cohorts were available rather than N_e , weighting each cohort 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), we excluded alleles 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 our 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 nonzero 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. We used penalized cubic regression splines and assumed that errors were 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, we divided all populations 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 cutoff in 2010 and above the cutoff in 2011; we included Cripple Cove in the “large” size bin in both years for consistency, and to increase sample size in 2010. We used generalized linear mixed models (GLMM) 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, we estimated and compared relative proportions of variance in habitat parameters among years for “small” and “large” N bins, 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, we plotted the coefficients of variation (CV; a normalized measure of dispersion where $CV = \sigma/\mu$ [where σ is standard deviation and μ is the mean]) of habitat parameters for each stream against N and N_b in each year. We then used White's test (White 1980) 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

TABLE 2. GAM results for habitat parameters vs. N and N_b for Cape Race trout populations.

Parameter	2010 N		2011 N		2010 N_b		2011 N_b	
	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 stands for the estimated degrees of freedom, and R^2 -adj is the proportion of variance explained by the model.

† $P < 0.1$; * $P < 0.05$; *** $P < 0.001$.

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.—We used Spearman's correlations 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. We furthermore calculated the CV of drainage area 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 (\pm SE) allelic richness and observed heterozygosities across the 13 loci screened within the 44 individual population cohorts of Table 1 were 5.2 ± 0.1 and 0.52 ± 0.01 , respectively (see individual population data in Appendix D, and θ_{ST} among populations in 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 pairwise 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 to 6223 and from 79 to 8416 in 2010 and 2011, respectively. Point estimates of N_b based on one to three cohorts (2009–2011) ranged from 15 to 249. N_b estimates of ∞ were not included in the weighted harmonic mean (see Table 1). Mean transect-level habitat characteristics for all Cape Race streams are found in Appendix A: 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 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. 3; 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 populations 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

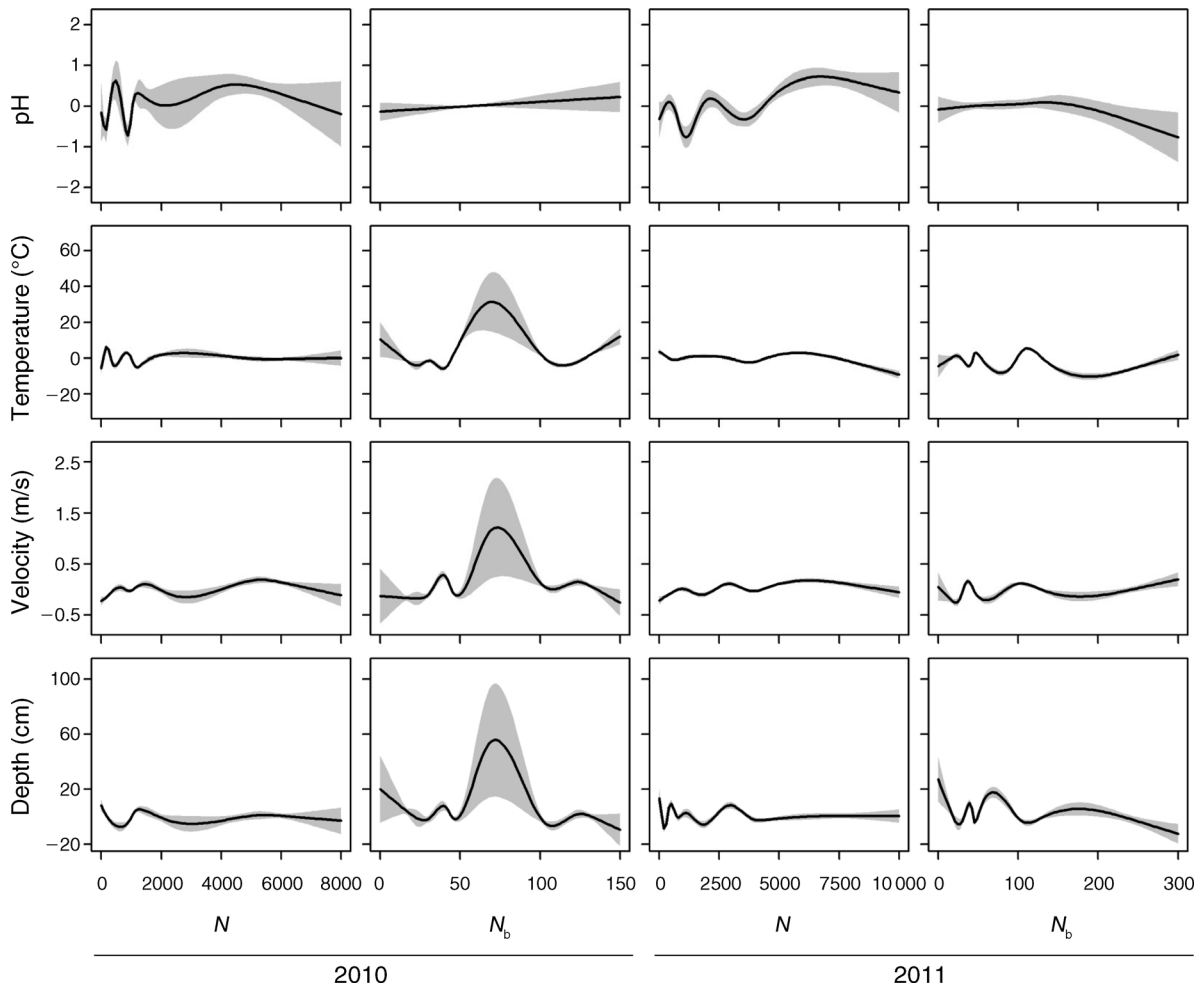


FIG. 3. Directional hypothesis generalized additive model (GAM) plots of habitat parameters vs. total population size (N) and the effective number of breeders (N_b) in 2010 and 2011 for 4 of 17 parameters that might be related to the fitness and abundance of brook trout populations (*Salvelinus fontinalis*) at Cape Race. The solid line is the cubic regression spline, and the gray areas are the 95% confidence bands. Plots for the remaining 13 habitat parameters are found in Appendix F.

population size for six habitat parameters (Table 3); for one additional case, there was only marginally more variation at the among-bin vs. 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 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 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. 4; 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. 4; Appendix G).

TABLE 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.

Variable	2010 N		2010 N_b		2011 N		2011 N_b		Across years	
	Bin	Population	Bin	Population	Bin	Population	Bin	Population	Small N	Large N
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. 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
Course 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 cutoff, with the exception of Cripple Cove in 2010, which was included in the large size bin for consistency among years.

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 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 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 4). Considering sampling years separately, 6 of 17 parameters consistently showed increased spread at small N and N_b (conductivity, temperature, mean depth, percentage vegetation, percentage fine gravel, and percentage 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 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. 5; 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 , we observed increased variability at small population sizes

for 10 of 17 parameters in 2010 and 11 of 17 parameters in 2011 (Fig. 5; 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. 5). The CV of drainage area was higher for populations in the small vs. 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. 6).

DISCUSSION

Relationship of habitat variability to population size

Our 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), our study generally revealed a wider range of variances around habitat parameter means among small vs. large populations, based on significant residual heteroscedasticity. To a lesser extent, we also observed increased variability in habitat parameter means at smaller population size. Collectively, these trends were observed in both study years and when relating habitat characteristics to both N and N_b .

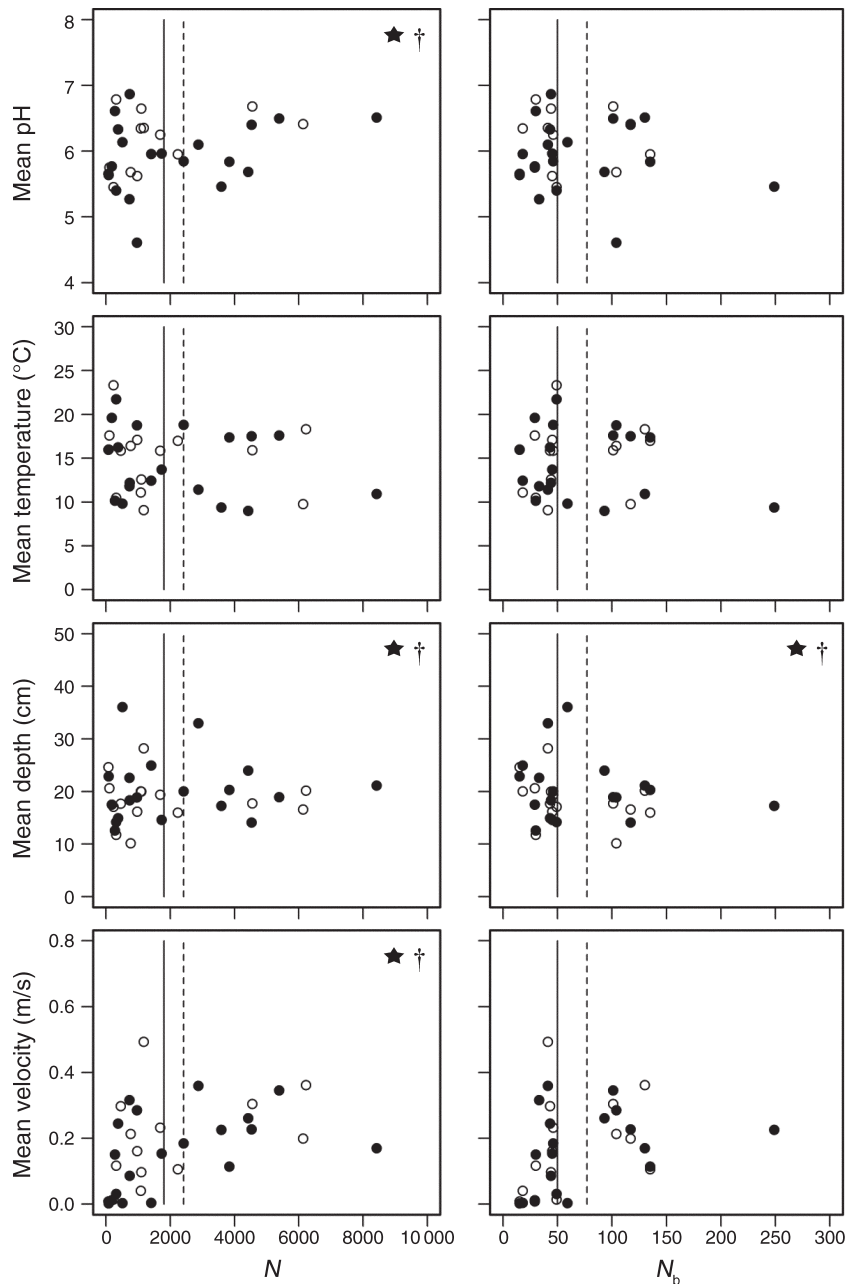


FIG. 4. Variable hypothesis plots of habitat parameter means vs. N and N_b in 2010 (open circles) and 2011 (solid circles) for 4 of 17 parameters that might be related to the fitness and abundance of trout populations at Cape Race. Trends for increased variability at small populations size in 2010 and 2011 are indicated by a star and a dagger (†), respectively. Cutoffs 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 Appendix G.

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. 3; Appendix F). Taken together, although we investigated variability in habitat parameters as a function of

TABLE 4. Results of White's test (White 1980) for residual heteroscedasticity in habitat parameter values in relation to N and N_b in two years of sampling at Cape Race.

Variable	N			N_b		
	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. 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
Course 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 (‡).

† $P < 0.1$; * $P < 0.05$; *** $P < 0.001$.

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 our choice of habitat parameters, we have not attempted to determine which ones specifically drive selection within each study stream per se, but have chosen a variety of parameters 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 (Appendix A: Table A2), and in both years our 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 (Appendix J; 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

We 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, we found a strong positive correlation 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.

We treat our study system at Cape Race 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 our Cape Race streams represent a large range of sizes relative to one another, in a broader context, all these streams are considered small; the largest Cape Race streams that we

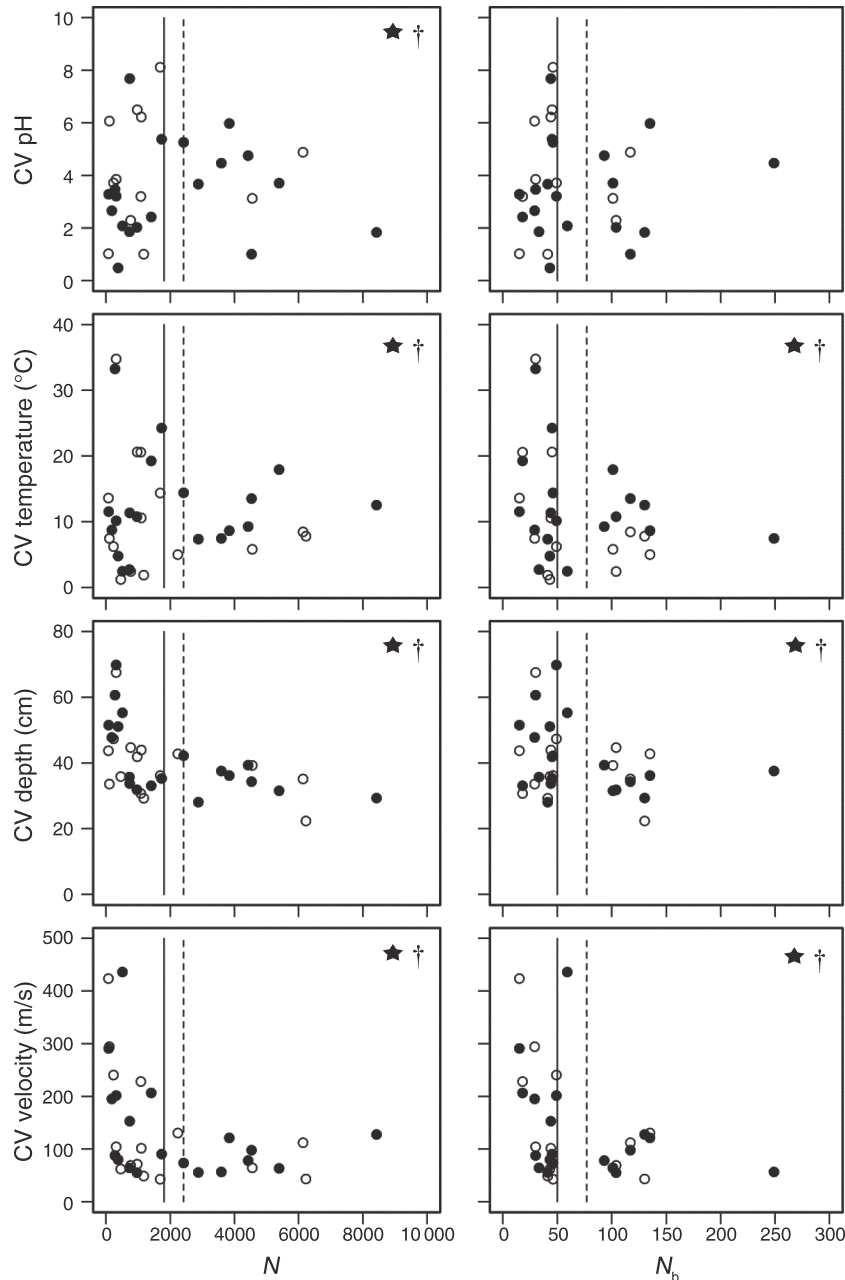


FIG. 5. Variable hypothesis plots of habitat parameter coefficients of variation (CVs) vs. N and N_b in 2010 (open circles) and 2011 (solid circles) for 4 of 17 parameters that might be related to the fitness and abundance of trout populations at Cape Race. Trends for increased variability at small populations size in 2010 and 2011 are indicated by a star and a dagger (†), respectively. Cutoffs 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 Appendix H.

examined were third-order streams, but most were of the first or second order (15 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. We compared the relationship between abundance and fragment size for Cape Race populations within the same drainage and found that the correlation between N and drainage area was similar for both upstream and downstream segments (downstream Pearson's $r = 0.89$, upstream $r = 0.81$).

We also acknowledge that in, a few cases, the trend for increased habitat variability at small N and N_b might

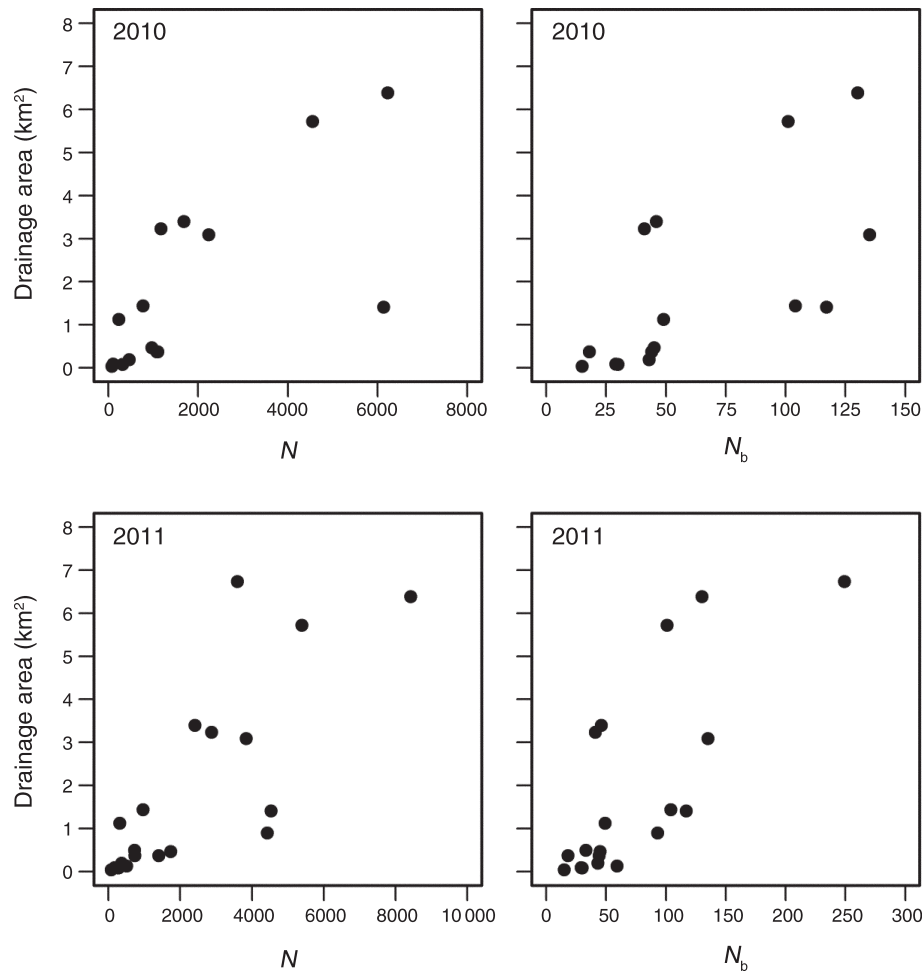


FIG. 6. The relationship between drainage area and N and N_b in 2010 and 2011 for fragmented populations of brook trout at Cape Race.

be driven by one or two outliers, such as the coefficients of variation for pH and temperature (Fig. 4). But outliers cannot explain most cases of increased variability. Furthermore, the implicit assumption of our competing alternatives that habitat fragments support near-pristine 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 Cape Race 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 effects 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 Cape Race populations may have occurred quite rapidly (T. Burdon and D. J. Fraser, *unpublished data*), and thus, may not differ so greatly from a scenario of human-caused fragmentation.

Similarly, our 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, our 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 (T. Burdon and D. J. Fraser, *unpublished data*).

Conclusions and conservation implications

In a naturally fragmented system of populations of a vertebrate, we found evidence that small populations exhibited a wider range of variances in habitat parameters than large populations. We also found 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 unfavorable conditions (Brown 1984, Hoffmann 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 harboring 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 “evolu-

tionary stochasticity” would represent an underappreciated 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.

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SUPPLEMENTAL MATERIAL

Appendix A

Habitat data collection methodology and habitat character estimates for 2010 and 2011 ([Ecological Archives A024-062-A1](#)).

Appendix B

Polymerase chain reaction (PCR) conditions for microsatellite DNA analyses for samples collected in 2010 and 2011 ([Ecological Archives A024-062-A2](#)).

Appendix C

Number of breeders (N_b) estimated for each sampled cohort of each population using LDNe ([Ecological Archives A024-062-A3](#)).

Appendix D

Within-population genetic diversity characteristics based on 13 microsatellite loci for all sampled cohorts of each population ([Ecological Archives A024-062-A4](#)).

Appendix E

Pairwise θ_{ST} estimates between each cohort from all study populations estimated using GENETIX 4.0 ([Ecological Archives A024-062-A5](#)).

Appendix F

Directional hypothesis: generalized additive model (GAM) plots of habitat parameters vs. N and N_b in 2010 and 2011 ([Ecological Archives A024-062-A6](#)).

Appendix G

Variable hypothesis: habitat parameter means vs. N and N_b in 2010 and 2011 ([Ecological Archives A024-062-A7](#)).

Appendix H

Variable hypothesis: habitat parameter coefficients of variation (CVs) vs. N and N_b in 2010 and 2011 ([Ecological Archives A024-062-A8](#)).

Appendix I

Correctly matched N_b/N ratios for 13 populations ([Ecological Archives A024-062-A9](#)).