Spatiotemporal relationship between adult census size and genetic population size across a wide population size gradient

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Abstract

Adult census population size (N) and effective number of breeders (N_b) are highly relevant for designing effective conservation strategies. Both parameters are often challenging to quantify, however, making it of interest to determine whether one parameter can be generalized from the other. Yet, the spatiotemporal relationship between N and N_b has not been well characterized empirically in many taxa. We analysed this relationship for 5–7 consecutive years in twelve brook trout populations varying greatly in N (49-10032) and N_b (3-567) and identified major environmental variables affecting the two parameters. N or habitat size alone explained 47–57% of the variance in N_b, and N_b was strongly correlated with effective population size. The ratio N_b/N ranged from 0.01 to 0.45 and increased at small N or following an annual decrease in N, suggesting density-dependent constraints on N_b. We found no evidence for a consistent, directional difference between variability in N_b and/or N_b/N among small and large populations; however, small populations had more varying temporal variability in N_b/N ratios than large populations. Finally, N_b and N_b/N were 2.5- and 2.3-fold more variable among populations than temporally within populations. Our results demonstrate a clear linkage between demographic and evolutionary parameters, suggesting that N_b could be used to approximate N (or vice versa) in natural populations. Nevertheless, using one variable to infer the other to monitor trends within populations is less recommended, perhaps even less so in small populations given their less predictable N_b vs. N dynamics.

Keywords: census population size, effective number of breeders, effective population size, genetic monitoring, mark–recapture, stream fish

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Introduction

The estimation of adult census population size (N), effective number of breeders (N_b) and effective population size (N_e) are important for designing effective conservation strategies (Katzner *et al.* 2011; Spitzer *et al.* 2016), identifying threatened or endangered populations (Vié *et al.* 2008) and forecasting changes in population dynamics (Grueber *et al.* 2012; Fordham *et al.* 2014). While demographic events contribute to changes in N (recruitment failure, increased mortality, etc.), N_b and

N_e influence genetic processes (drift, inbreeding) and provide information about a population's reproductive biology or evolutionary history (Duong *et al.* 2013; Waples *et al.* 2014). Despite their importance, however, N, N_b and N_e can be challenging or prohibitively costly to estimate depending on the situation (Fraser *et al.* 2013; Spitzer *et al.* 2016). As a result, for practical reasons, there is substantial interest in determining whether especially the demographic (N) and genetic (N_b, N_e) parameters can be used to infer the other (Antao *et al.* 2008; Palstra & Fraser 2012).

Monitoring N_b is of particular interest for assessing population size trends in natural populations. In routinely being based on the sampling of a single cohort

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produced by a group of reproductive adults, N_b can often be more easily quantified than N_e in iteroparous species using genetic data (Waples 2002; Palstra & Fraser 2012) yet be strongly linked to N_e (Waples *et al.* 2013). N_b can also potentially be used to infer N, if N and N_b are positively correlated and if N_b/N ratios remain stable across time or within specific taxa (Schwartz *et al.* 2007; Tallmon *et al.* 2010; Palstra & Fraser 2012; Perrier *et al.* 2016). Simulation studies have indeed suggested that N_b could be used to reflect trends in N (Antao *et al.* 2011), and N_b has recently been adopted as a possible lower-cost alternative (or complement) to demographic monitoring in order to facilitate conservation management decisions in mammals (Jansson *et al.* 2012), birds (Johnson *et al.* 2010; Grueber *et al.* 2012), fishes (Osborne *et al.* 2010) and reptiles (Hoehn *et al.* 2012). Nevertheless, while some empirical studies found positive correlations between N and N_b (Osborne *et al.* 2010; Charlier *et al.* 2012), others found no relationship at all (Ardren & Kapuscinski 2003; Araki *et al.* 2007; Palstra *et al.* 2009; Berry & Kirkwood 2010; Belmar-Lucero *et al.* 2012; Johnstone *et al.* 2012; Osborne *et al.* 2012; Serbezov *et al.* 2012; Duong *et al.* 2013; Dowling *et al.* 2014; Whiteley *et al.* 2015). These disparities could be partly attributed to several factors that were not fully accounted for in many studies. Studies either included only one or few populations, had no temporal replication, incorrectly linked N_b and N, did not corroborate the degree to which N_b could be interpreted in the context of N_e (in accounting for population differences in life history), or could not estimate N with precision for logistical reasons. Hence, there is still very much a need for a better understanding of the relationship between N_b and N and how it relates to population size fluctuations, environmental conditions and population persistence.

It is important to consider a broad range of populations and population sizes when investigating spatial variation in N_b and N_b/N ratios. Intraspecific populations can vary widely in N_b/N ratios (Palstra & Fraser 2012), and population density is an important determinant of reproductive rates in many taxa, including fishes (Ardren & Kapuscinski 2003; Dowling *et al.* 2014; Perrier *et al.* 2016), birds (Grueber *et al.* 2012) and mammals (Rödel *et al.* 2004; Stewart *et al.* 2009). This has led most researchers to test for a negative correlation between the N_b/N ratio and N to infer density-dependent influences on reproductive rates, but relating a ratio to its denominator (N_b/N to N) results in spurious statistical relationships (Jackson & Somers 1991).

It is also important to assess N_b, N and N_b/N across sequential years among and within populations to better understand the factors driving N_b below N in natural populations, and to confirm the consistency of N_b/N ratios among and within populations for conservation initiatives. Differences in N_b and N_b/N could be attributed to temporal fluctuations in N or variance in reproductive success (Frankham 1995; Nomura 2002; Lee *et al.* 2011), and how these interplay with specific environmental variables (Kanno *et al.* 2015) and fluctuating environmental conditions. For example, habitat conditions can become more variable as populations become smaller and fragmented, thus increasing environmental variability (Fraser *et al.* 2014; Wood *et al.* 2014). In some years, certain small populations might therefore experience ecological conditions driving N_b below N by reducing recruitment, while other small populations might be found in higher quality, stable habitats that are simply too small to accommodate larger populations. Conversely, large populations might be found in larger, more spatially heterogeneous habitats that are temporally stable in environmental conditions, and hence may be more stable in N_b vs. N dynamics.

Iteroparous salmonid fishes are ideal models for characterizing the spatiotemporal relationship between N and N_b. Their reproductive biology, high fecundities and features of their environments likely result in highly different N_b/N ratios among populations, and their populations often vary in N. In particular, density-dependent processes could be the result of spawning habitat limitations on the proportion of breeding individuals (Chebanov 1991; Ardren & Kapuscinski 2003). For instance, in brook trout (*Salvelinus fontinalis*), spawning habitat requirements can limit the availability of suitable spawning sites, resulting in an increased variance in individual reproductive success at high population density, a potential mechanism for driving N_b below N (Chebanov 1991; Curry & Neakes 1995; Blanchfield & Ridgway 1997, 2005; Guillemette *et al.* 2011). Recently, Wood *et al.* (2014) and Whiteley *et al.* (2013, 2015) found striking correlations between N_b and either habitat size or spawning habitat quantity or quality among different brook trout populations. Collectively, these points raise the possibility that with only a minimum amount of information about a population or its environment, one might be able to derive accurate estimates of adult census sizes or genetic population sizes from the other.

In this study, we report on results from 5 to 7 consecutive years of N and N_b monitoring on 12 landlocked populations of brook trout. We first investigated the relationship between N_b and N and assessed the correspondence between N_b and N_e. Our large empirical survey provides one of the clearest, positive, nonlinear relationships between N_b and N reported in the literature; this result was corroborated between two commonly used genetic approaches for estimating N_b. We also confirmed that the genetic estimation of N_b based

on annual cohort sampling might be readily interpreted in the context of N_e per generation, after accounting for different life histories of our study populations following Waples *et al.* (2013). We further investigated N_b vs. N dynamics to demonstrate a significant effect of environmental and adult census size changes on N_b and N_b/N variance, both among populations and temporally within populations. Our results suggest that under certain biological and sampling conditions, conservation resources could be saved on interpreting N_b in terms of N and vice versa.

Materials and methods

Study site

We studied twelve, pristine, naturally fragmented populations of brook trout located on Cape Race (hereafter CR), Newfoundland, Canada (Fig. 1). The diversity of CR trout populations offers an ideal model to study environmental and demographic influences shaping N_b/N ratios. CR populations originate from a common ancestor (Danzmann *et al.* 1998), inhabit a fine geographic scale (20 × 20 km) and vary widely in abundance (Wood *et al.* 2014), phenotypic characteristics (Wood *et al.* 2015) and life history (Hutchings 1993; Belmar-Lucero *et al.* 2012). Varying environmental characteristics of CR streams are described in Wood *et al.* (2014) and are known to affect brook trout reproductive biology, including the number, area and quality of spawning sites (Belmar-Lucero *et al.* 2012). Additionally, N and the habitat of CR populations can be comprehensively sampled as they inhabit small streams (0.27–8.10 km in length). CR trout do not experience

significant interspecific competition or predation: only three of the streams (UO, LO and WN) contain small, natural populations of Atlantic salmon (*Salmo salar*) and two additional streams (CC, WN) harbour occasional American eels (*Anguilla rostrata*). Finally, all CR trout populations are genetically distinct from one another (Fraser *et al.* 2014; Wood *et al.* 2014). Five of the 12 study populations occur in isolated stream drainages. The remaining seven populations within another three drainages are physically isolated from each other by obvious barriers within drainages (see details below), but might exchange intermittent gene flow.

Adult census population size (N)

We estimated annual N each summer (June–July) for 4–6 years per population from 2010 to 2015. N was defined as the total number of adults; this corresponds to trout of age 1+ and older at CR (Hutchings 1993; Belmar-Lucero *et al.* 2012). The size distribution of age-0 and age-1+ trout does not overlap. The sampling protocol in 2012–2015 was standardized accordingly to the years 2010 and 2011 described in Wood *et al.* (2014). N was estimated from mark–recapture methods with a single recapture event (Petersen 1985). Individuals were marked by adipose fin clips or numerical Floy Tags (Floy Tag & Mfg. Inc., Seattle, WA, USA) (FD-68B Fine Fabric Anchor Tags; Floy Tag and Manufacturing, LOC), depending on the population. As CR populations inhabit very small to small streams (median length = 1.2 km), 1 week between marking and recapture events left the trout enough time to redisperse, thus minimizing any potential downward biases associated with increased recapture probabilities.
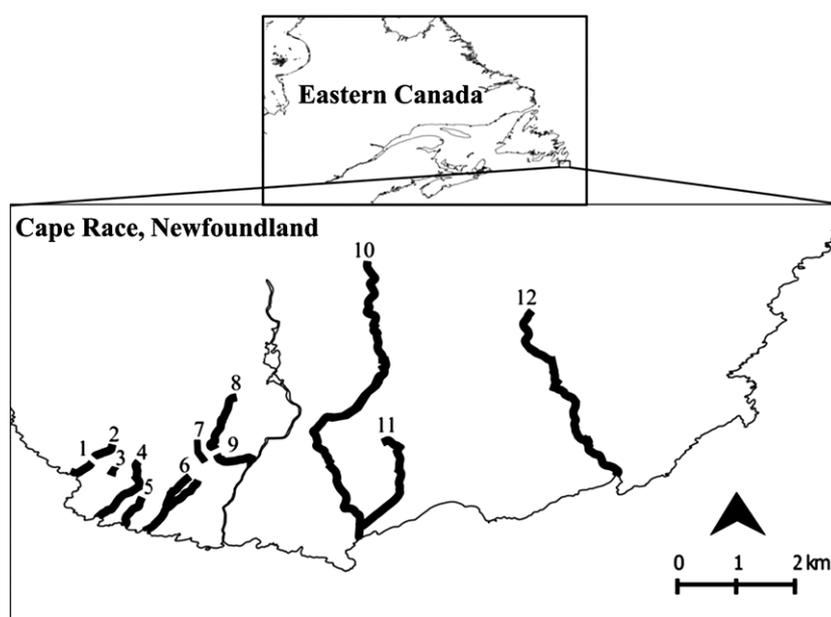

Fig. 1 CR brook trout populations. From West to East; (1) Lower Coquita (LC), (2) Upper Coquita (UC), (3) Hermitage (HM), (4) Bob's Cove (BC), (5) Still There By Chance (STBC), (6) Whale Cove (WC), (7) Ditchy (DY), (8) Upper Ouananiche Beck (UO), (9) Lower Ouananiche Beck (LO), (10) Watern (WN), (11) Blackfly (LB) and (12) Cripple Cove (CC).

Genetic data

We sampled five to seven annual young-of-the-year (YOY or age 0) cohorts per population from 2010 and 2015 to derive cohort N_b estimates within populations corresponding to the years 2009–2014. The total number of cohorts sampled across years and populations was 66 (mean sample size per cohort = 68, range 5–237; see Tables 1 and S1 for detailed sample sizes, Supporting information). We also sampled age-1+ individuals in 2010 and derived five additional N_b estimates for 2008 cohorts (adult length frequency histograms were preliminary screened for bimodality). Caudal fin clips were preserved in 95% ethanol until DNA was extracted using a phenol–chloroform protocol in sampling years 2010–2012 (described in Belmar-Lucero *et al.* 2012) and a Chelex protocol in 2013–2015 (Hua & Orban 2005). YOY were sampled evenly along each stream to maximize both the potential number of families sampled and the sample size for precise N_b estimates. In total, our study comprised 4796 YOY genotypes, 1306 of which originated from Wood *et al.* (2014; years 2010/2011). All trout were genotyped at 13 microsatellite loci; details of PCR conditions, electrophoresis using a Genetic Analyzer and allele scoring are found in Wood *et al.* (2014); and alleles were manually scored using GENEMAPPER 3.2 and PEAK SCANNER (Applied Biosystems Inc.). As two study loci (*Sco204*, *Sco218*) were found to be linked in a considerable number of cohorts, *Sco218* was removed from subsequent analyses.

Population genetic analyses

Departures from linkage and Hardy–Weinberg equilibrium were verified using GENEPOP version 4.2 across the 71 sampled cohorts (66 YOY, five age 1+; Raymond & Rousset 1995). Significance levels were adjusted to control for type I error via Bonferroni correction. We used GENALEX version 6.5 (Peakall & Smouse 2012) to quantify alleles per locus, observed and expected heterozygosity for each locus in each cohort sample. Fst values were assessed in Fraser *et al.* (2014) and Wood *et al.* (2015). Although most stream drainages at CR constitute single trout populations (Wood *et al.* 2014), we used STRUCTURE version 2.3.4 (Pritchard *et al.* 2000) to evaluate the temporal stability of within drainage population structuring found in 2010/2011 in three drainages by Wood *et al.* (2014; see Table S2 for details, Supporting information). In the three drainages, multiple populations were consistently demarcated across sampling years (Table S2, Supporting information). These were associated with clear geographic divisions such as waterfalls (UC vs. LC) or fragmentation and isolation of stream beds (DY vs. UO vs. LO; BF vs. WN).

Table 1 Population summary statistics from twelve CR brook trout populations sampled between 2008 and 2015 (see details per year/cohort in Table S1, Supporting information)

	N	LDNe N_b	Mean N	Mean N_b	Mean S	Total S	A	Ae	Ho	He
UC	6	5	65 (49–79)	20 (13–48)	19 (5–35)	115	3.09 (2.42–4.33)	1.90 (1.57–2.42)	0.39 (0.28–0.54)	0.41 (0.31–0.53)
HM	4	4	66 (52–80)	5 (3–5)	25 (4–61)	126	2.08 (1.42–3.17)	1.39 (1.09–1.66)	0.15 (0.06–0.20)	0.22 (0.06–0.52)
DY	6	5	116 (84–179)	10 (3–34)	26 (9–35)	153	3.85 (3.00–4.33)	2.47 (2.06–2.72)	0.54 (0.49–0.61)	0.52 (0.45–0.57)
LC	6	6	338 (250–798)	31 (11–117)	43 (11–60)	299	3.82 (2.25–5.58)	2.23 (1.70–2.96)	0.44 (0.40–0.50)	0.48 (0.37–0.59)
LO	5	5	470 (372–625)	44 (23–188)	31 (25–39)	155	4.35 (3.75–4.67)	2.62 (2.34–2.81)	0.56 (0.47–0.62)	0.56 (0.51–0.60)
WC	6	6	783 (530–1148)	31 (21–52)	98 (48–153)	586	5.04 (3.83–6.17)	2.71 (2.21–3.12)	0.52 (0.46–0.56)	0.55 (0.48–0.58)
STBC	6	5	917 (587–1405)	28 (14–54)	63 (40–97)	444	2.48 (1.83–3.50)	1.54 (1.48–1.61)	0.27 (0.24–0.30)	0.28 (0.25–0.31)
LB	4	5	1184 (877–1383)	52 (34–83)	55 (52–58)	274	5.30 (4.75–5.75)	2.94 (2.74–3.08)	0.54 (0.50–0.60)	0.56 (0.53–0.57)
CC	4	5	1862 (1471–5246)	74 (65–99)	104 (71–163)	520	3.81 (3.33–4.58)	2.09 (2.03–2.24)	0.43 (0.40–0.47)	0.42 (0.40–0.46)
UO	6	6	2569 (1949–3835)	62 (41–87)	113 (36–237)	679	5.92 (5.25–6.50)	3.25 (3.04–3.32)	0.60 (0.56–0.64)	0.62 (0.61–0.63)
BC	6	4	4693 (4044–6132)	355 (267–567)	123 (62–223)	738	4.60 (4.17–5.25)	2.23 (2.17–2.29)	0.46 (0.44–0.49)	0.45 (0.44–0.46)
WN	4	7	7801 (6713–10 032)	178 (110–267)	101 (56–160)	707	7.18 (6.50–8.00)	3.29 (3.05–3.53)	0.57 (0.56–0.60)	0.59 (0.56–0.60)

Number of N and LDNe N_b estimates from different years, harmonic means of N and N_b, mean annual sample size (Mean S), total number of genotyped YOY (Total S), mean annual number of alleles per locus (A), mean annual effective number of alleles per locus (Ae), mean annual observed heterozygosity (Ho), mean annual expected heterozygosity (He). Ranges across years are between parentheses.

N_b, associations between N_b and N_e

We first employed the one-sample linkage disequilibrium method implemented in LDNe (Waples & Do 2010) to derive contemporary N_b for each sampled cohort. To avoid upward bias due to rare alleles, we used an allele exclusion criterion Pcrit = 0.05 for sample sizes <25, Pcrit = 0.02 for sample sizes between 25 and 100 and Pcrit = 0.01 for sample sizes exceeding 100 (Waples & Do 2010). The effect of sample size and P_{crit} choice on N_b estimation was also evaluated (see Appendix S1 for details), an issue we treat more in the Discussion. To complement this analysis, we compared LDNe N_b estimates to those derived from the sibship assignment method implemented in COLONY (Wang 2004; Appendix S2). As preliminary investigations showed that LDNe N_b estimates with and without sibling groups were tightly correlated ($P < 0.001$ d.f. = 59, $r^2 = 0.88$; Fig. S1, Supporting information), all fish (including sibling groups) were used to derive N_b.

To determine how well N_b estimates may reflect contemporary N_e within CR trout populations, we investigated the relationship between mean N_b estimates across years from LDNe and COLONY and the N_e estimates generated using the modelling of Waples *et al.* (2013). This modelling accounts for the potential influence of different life histories on the variance in the relationship between N_b/N_e in iteroparous species, chiefly through three traits: age-at-maturity, adult lifespan and coefficient of variation in fecundity. CR population-specific age-at-maturity (approximated as mean spawning age) and maximum adult lifespan were derived from Hutchings (1993) (CC, WN) and Belmar-Lucero *et al.* (2012) (WC, BC), and similarly from scale ageing of spawning fish collected in streams in 2014 for all other populations. Female fecundity data were obtained from egg stripping of a random sample of mature females collected from all populations in the fall of 2010, 2011 (Wood *et al.* 2015) and/or 2014. We did not estimate N_e using the temporal method because the number of generations spanning our cohort samples within CR populations was too low (1.5–2; mean spawning age is commonly 2+ to 3+ years), which is well known to generate significant biases (Waples & Yokota 2007); LDNe also outperforms the temporal method for detecting population declines (Waples & Antao 2014).

Stream environmental data

To obtain fine-scale habitat coverage across the entire length of each stream, we collected summer habitat data annually from a total of 1850 stream cross-sections (Table S3, Supporting information), with 18–32 cross-sections per population in 2010 and 18–64 cross-sections per population in subsequent years. GPS coordinates of each cross-section were recorded in 2010 or 2011; the same cross-sections were sampled each year (± 0–5 m due to fine-scale GPS uncertainties). Here, we focus on environmental conditions occurring several months prior to the breeding season; however, it is noteworthy that environmental conditions occurring during or after spawning are also known to affect brook trout population dynamics (Kanno *et al.* 2015, 2016). We focused on stream flow, temperature and stream length as previous work supported that these are important environmental variables shaping brook trout population dynamics (Whiteley *et al.* 2013, 2015; Wood *et al.* 2014; Xu *et al.* 2010b). For instance, low stream flow during the summer can affect adult survival and body condition by reducing habitat availability and quality (Hakala & Hartman 2004; Xu *et al.* 2010b), which could delay or reduce spawning and negatively affect N_b. Additionally, high summer temperature can also affect survival and body condition (Xu *et al.* 2010b); temperature-induced stress and metabolism changes can also affect gonad development (Pankhurst & Munday 2011) and lead to delayed spawning and reduced red construction (Warren *et al.* 2012), thus potentially reducing N_b. Stream flow and length were measured as in Wood *et al.* (2014). Stream temperature was recorded every 75 min between June and September using two loggers per stream affixed to the stream bottom (Table S3, Supporting information). The environmental variables were measured the same years as N, and they were therefore likely to affect the breeding adult generation described by N_b.

Relationship between N_b, N and the environment among populations

We evaluated the relationship between N, N_b and the environment to test the predictions that there would exist (i) a positive correlation between N and N_b given that as the number of adults increases, more individuals may contribute to the next generation; (ii) a positive correlation between stream length and N and/or N_b as suggested by recent publications (Whiteley *et al.* 2013; Wood *et al.* 2014); and (iii) a positive correlation between flow and N_b and a negative correlation between summer temperature and N_b as high temperatures and low flow during the summer could reduce or delay spawning and negatively affect N_b (Xu *et al.* 2010b; Letcher *et al.* 2014; Whiteley *et al.* 2015).

We used generalized linear mixed models (GLMMs) with a Poisson lognormal distribution (Elston *et al.* 2001) and a log link function to evaluate the effect of N and environmental variables on N_b. Environmental variables from all rivers in each sampled year were

summarized as means; N_b was natural-log-transformed as preliminary investigation revealed a nonlinear relationship with N_b. In all models, explanatory variables were centred and scaled, and we included a random intercept-by-population term, regardless of its significance, to account for basal variations in mean N_b. We also included an observation-level random effect to model the extra-Poisson variation in N_b (Elston et al. 2001). We did not include interaction terms in any of the models as it led to convergence failure.

GLMMs were performed using the lme4 package (Pinheiro et al. 2013) in R Studio 0.99.484 (Team RC 2013). Model comparison was conducted by stepwise removal of nonsignificant fixed effects ($P > 0.05$) using likelihood ratio tests. We also used a corrected version of Akaike's information criterion, AICc (Burnham & Anderson 2004), to compare the relative fit between alternative models, as well as marginal and conditional R^2 (Nakagawa & Schielzeth 2013; R_m^2 = variability explained by fixed effects; R_c^2 = variability explained by fixed and random effects) to compare the variance explained by alternative models.

Variance inflation factor analyses were conducted in all models to check for multicollinearity between environmental variables and N. High VIF scores indicated that stream size and N were strongly collinear. To determine whether N or stream size best explained N_b, mixed models were fitted separately with N and stream size and the models' explained variance were compared. N, stream length, summer flow and temperature were treated as fixed effects. To determine whether N or stream length alone explained a substantial amount of variability in N_b, reduced mixed models including only N or stream length as a fixed effect were fitted and the explained variances were compared with those of the fuller models.

We also used a GLMM with a Poisson lognormal distribution and a log link function to determine whether a significant correlation existed between N and environmental variables. The GLMM included N as a response variable, stream length, summer flow and temperature as explanatory variable and a random intercept-by-population term. Stream length was natural-log-transformed to increase normality and reduce heteroscedasticity.

Relative trends in N, N_b and N_b/N within populations

A relationship between annual changes in N and N_b or N_b/N within populations would suggest that conservation resources could potentially be saved by estimating only one variable to monitor genetic *and* demographic trends within natural populations. Two alternative predictions were investigated: (i) a positive correlation

would exist between a change in N_b and a change in N, as more adults in the population could allow more parents to contribute; and (ii) a negative correlation between a change in N and in N_b/N might be predicted. For the latter, a substantial increase in N could heighten the competition for access to spawning areas, which would increase the variance in reproductive success and reduce N_b/N (Ardren & Kapuscinski 2003).

We used per cent change (PC) to express relative annual changes in N, N_b and N_b/N where $PC = \frac{(V_x - V_{x-1})}{V_{x-1}} \times 100$ (V_x and V_{x-1} = value in year x and in the preceding year). As preliminary analyses suggested that our data fulfilled linear regression assumptions, we used PC in N_b or N_b/N (separately) as response variables and PC in N as the explanatory variable. Because this analysis required two consecutive N_b estimates correctly matched to two consecutive N estimates within populations, there were only a total of 24 PC data points for the analysis from our time series. We could not include by-population random intercepts to estimate within-population variability, as three of 12 populations only had one data point.

Spatial and temporal variability in N_b, N and N_b/N

We tested a series of alternative predictions regarding the relative extant of spatial and temporal variability in N_b, N and N_b/N. This analysis is relevant towards a better understanding of the sampling conditions under which one variable can reliably be used to infer the other. First, we tested whether there was a significant difference in temporal variability between N_b and N. Indeed, Whiteley et al. (2015) suggested that the use of N_b to monitor trends was limited as it may be more temporally constrained than that of N. Second, we tested two predictions related to variability and N_b or N_b/N and population size: (i) small populations might be more temporally variable in N_b and/or N_b/N than larger populations; (ii) there might be more variability among small populations in temporal variance N_b and/or N_b/N among small populations (*sensu* Wood et al. 2014, 2016).

We used coefficients of variation (CVs; Tables S4 and S5 for sample calculations, Supporting information) as a standardized measure of relative variability (spatial and temporal) in N_b, N and N_b/N. To compare spatial and temporal dispersion in N_b, we examined boxplots of spatial and temporal CVs. To investigate whether CV in N_b and N_b/N was related to N, we plotted the temporal CV against harmonic mean of N for each stream. We then used simple linear regression to test whether there was a significant correlation between the average N and the temporal CV in N_b and N_b/N. To investigate whether there was more variability in temporal CVs

among small populations, we used White's test (White 1980) implemented in the lmtest package (Zeileis & Hothorn 2002) to check for significant heteroscedasticity of the residuals in the fitted linear models.

Results

Intrapopulation genetic variation

CR trout populations were moderately genetically diverse with an average number of alleles per locus ranging from 2.08 to 7.18 alleles and an average heterozygosity from 0.15 to 0.60 (Tables 1 and S1, Supporting information). For the 71 cohorts sampled, significant LD was detected in only 3.1% of 4686 LD tests (71 cohorts*66 pairwise locus comparisons) after Bonferroni correction and removing locus Sco218. Significant departures from HWE occurred in 4.6% of the 852 (71 cohorts*12 locus) tests after Bonferroni correction and were spread across loci and populations.

N, N_b, associations between N_b and N_e, and N_b/N ratios

Harmonic mean N per population ranged from 65 to 7801 with annual estimates ranging from 49 to 10 032. Harmonic mean N_b per population using LDNe and COLONY ranged from 5 to 355 with annual estimates ranging from 3 to 567 (Tables 1 and S1, Supporting information; Fig. 2). We used harmonic means to compute averages that would not be biased by possible outliers.

The N_b estimates derived from LDNe and COLONY were positively correlated (Appendix S2; $P < 0.001$, d.f. = 62, $R^2 = 0.44$). Furthermore, within populations, harmonic mean N_b with LDNe or COLONY were strongly, linearly correlated with population-specific N_e using the model of Waples et al. (2013) (Appendix S3; both $P < 0.001$, d.f. = 11; $R^2 = 0.995$, $R^2 = 0.977$, respectively); N_b/N_e ratios varied only 1.2- to 1.6-fold among populations, ranged from 0.55 to 0.68 and 0.34–0.57 depending on the approach taken to approximating age-at-maturity (Appendix S3, Table S3.1, Supporting information). Given these results, and that LDNe N_b estimates were more strongly linked to N and stream length than COLONY N_b estimates (Appendix S2, Table S2.1, Supporting information), LDNe N_b estimates were kept for all further analyses, while N_e and COLONY N_b estimates were no longer considered.

We obtained a total of 63 LDNe N_b estimates as those for eight of 71 sampled cohorts had no lower confidence intervals (CI) and/or point estimates (Table S1, Supporting information). The genetic data from those eight cohorts were nonetheless used to quantify genetic diversity and to assess potential population substructure. Of the 63 N_b estimates, 14 had upper CI including infinity (Table S1, Supporting information); this lack of precision can be chiefly explained by small sample sizes despite intensive sampling efforts (LC, UC, LO, DY, HM) and/or a lack of genetic diversity (HM, STBC) in small or very small populations. For one additional population (BC), unreliable N_b estimates despite large sample sizes in 2009 and 2011 were probably due to an interaction between sample size and a large N_b (see Waples & Do 2010).

We produced 43 N_b/N ratios by matching N_b estimates derived from YOY samples to N of the previous year; N_b estimates were derived from YOY shortly after hatching and therefore primarily reflect the parental generation (Waples 2005). Harmonic mean N_b/N ranged from 0.02 to 0.31 within populations, with annual estimates ranging from 0.01 to 0.45. On average, we had 3.6 N_b/N (2–5) ratios per population for further analyses (Table S1, Supporting information).

Effect of N, population, breeding year and environmental variables on N_b

The best-fit GLMM describing the relationship between N_b and N included N and stream flow as explanatory variables according to AICc and likelihood ratio tests (Table 2, Fig. 3). As predicted, N_b increased with N and when summer flow was high. This model was within 2 AICc units of the restricted N model (including N alone) suggesting that the two models were equally plausible. Most of the variability could be accounted for by N alone as shown by the relatively small difference in the explanatory power of the two models (8%; Table 2).

The best-fit GLMM describing the relationship between N_b and stream size included stream size and flow as explanatory variables (Table 2; Fig. 3): N_b was positively associated with stream size. However, the variance explained by the reduced model was nearly 42%, suggesting that stream size alone could account for nearly 2/3 of the variability of the full model. Although the model including stream size, flow and temperature was within 2 AICc units of the best-fit model, stream temperature did not significantly affect N_b according to likelihood ratio tests. Overall, N models (both full and reduced) had a greater explanatory power for N_b relative to stream size model, according to both AICc and variances explained (Table 2).

The population-specific variance term did not explain a large amount of variability in N_b in the models including N as an explanatory variable or in the full stream size model (6–13%). Conversely, in the reduced stream size model, there was twice as much variability in the random effects (28%; Table 2).
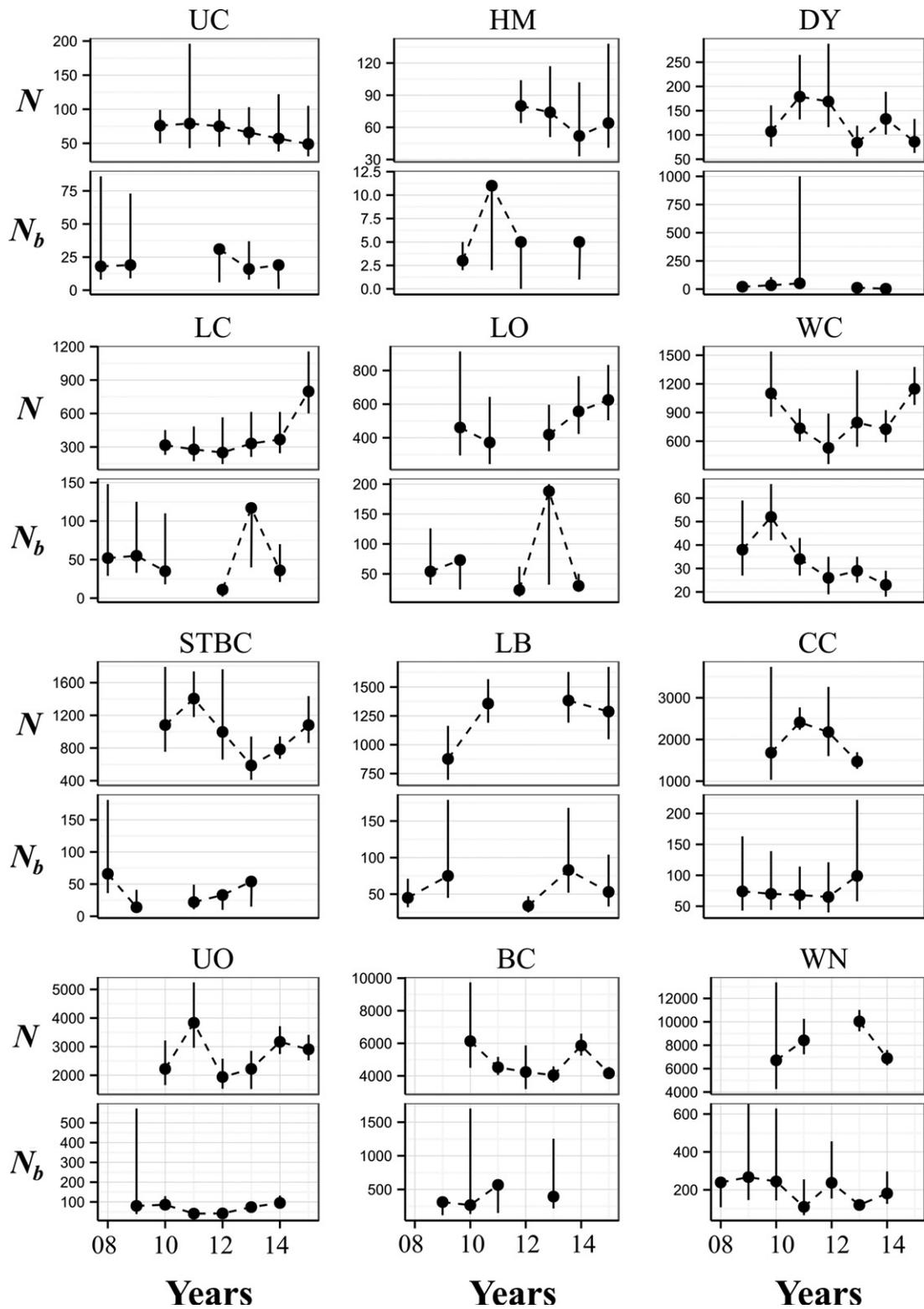
Fig. 2 Temporal trends in N and N_b estimates between 2010 and 2015 for 12 CR brook trout populations. The 95% confidence intervals around each N_b estimates are represented unless they include infinity.

Table 2 Generalized mixed-effect modelling of the effect of N and environmental variables on N_b among 12 CR brook trout populations

	Full N model	Restricted N model	Full stream length model	Restricted stream length model
Fixed-effects estimates (SE)				
Intercept	3.88 (0.12)	3.85 (0.14)	3.93 (0.15)	3.86 (0.19)
N or stream length	0.63 (0.15)*	0.83 (0.14)***	0.49 (0.17)*	0.77 (0.19)***
Flow	0.41 (0.15)***	—	0.53 (0.16)**	—
Random-effects variance components (SD)				
Population	0.07 (0.26)	0.13 (0.36)	0.19 (0.43)	0.39 (0.62)
Individual level	0.32 (0.57)	0.34 (0.58)	0.29 (0.54)	0.25 (0.50)
AICc	317.2	318.8	323.8	329.0
R_m^2	0.65	0.57	0.58	0.47
R_c^2	0.71	0.69	0.74	0.79
n	41	43	42	63

SE = standard error; SD = standard deviation; AICc = corrected Akaike's information criteria; R_m^2 = marginal R square (fixed effects only); R_c^2 = conditional R square (fixed and random effects); n = number of observations, significance codes according to likelihood ratio test: <0.001***, <0.01 **, <0.05 *.
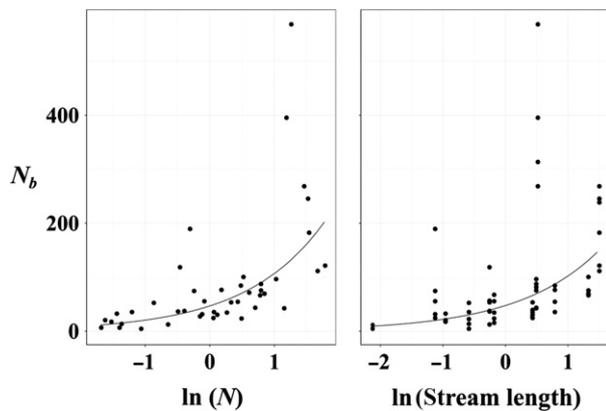
Fig. 3 Relationship of N_b with N and stream length in 12 CR brook trout populations.

Our Poisson model with a log link supported that log N_b is a linear function of log N (Fig. 3), suggesting that untransformed N_b and N or stream length follow a fractional power trend: as N and stream length increased, N_b increased at a decreasing rate.

The best-fit GLMM describing N only included stream length as an explanatory variable (Table S6; Fig S2, Supporting information). Longer streams harboured larger trout populations, and up to 69% of the variability in N could be explained by stream length alone.

Relative trends in N, N_b and N_b/N within populations

There was no relationship between a change in N and a change in N_b, or between a change in N and a change in N_b/N ($P = 0.76$ and 0.32, respectively, d.f. = 21). However, this lack of relationship was mainly driven by a single, extreme outlier (a nearly 10-fold increase in N_b in a very small population, LC, in 2013 observable

in Fig. 2 as an increase in adult recruitment in 2015). Without the outlier, per cent changes in N and N_b remained uncorrelated (Fig. 4a; $P = 0.12$, d.f. = 21), yet per cent changes in N and N_b/N became significantly negatively correlated (Fig. 4b; $P < 0.001$, d.f. = 21, $r^2 = 0.47$). The relationship between changes in N and changes in N_b/N was not proportional: on average, N_b/N was expected to decrease by -1.55% per 1% increase in N.

Spatial and temporal variability in N_b, N and N_b/N

Across populations, spatial variability was significantly greater than temporal variability as shown by the nonoverlapping notches of the boxplot. Spatial CVs in N, N_b and N_b/N were respectively 5.1-, 2.5- and 2.3-fold greater than temporal variation within populations (Fig. 5a).

The linear model showed no relationship between adult census size and temporal variability in N_b or N_b/N (Fig. 5b, c: $P = 0.15$ and 0.77, d.f. = 10). However, we found some evidence for increased variability among small populations in temporal variances; although the White's test was only significant for the temporal variability in N_b/N (Fig. 5c; $P = 0.04$, d.f. = 1), a similar trend was observed for temporal variability in N_b (Fig. 5b; $P = 0.22$, d.f. = 1).

Discussion

Spatiotemporal variation in N_b and N

Our study provides evidence for a strong, positive, nonlinear relationship between N and N_b over a broad range of population sizes in a vertebrate species
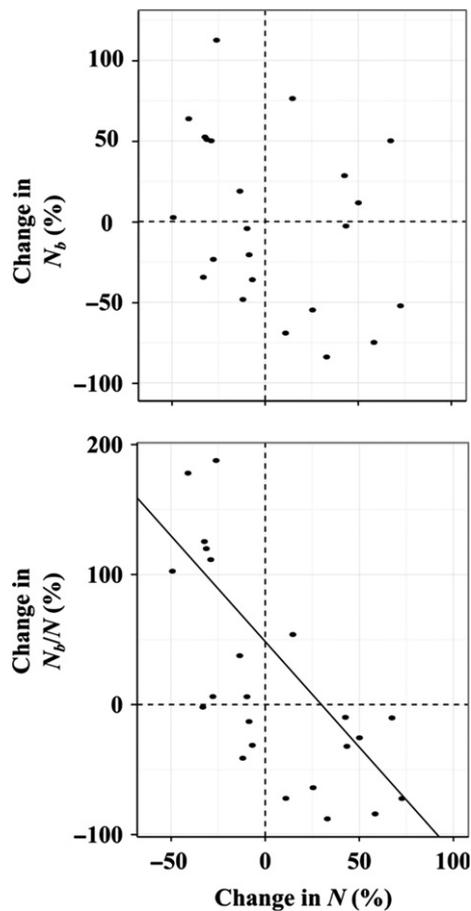
Fig. 4 Relationships between per cent changes in annual N and the per cent change in annual N_b or the per cent change in annual N_b/N after removal of a far outlier.

(N = 49-10032 and N_b = 3-567). Much of the variability in N_b could be accounted for by N or stream size alone: larger habitats tended to harbour larger populations with a larger N_b. This result is consistent with island biogeography models at the species level (MacArthur & Wilson 1967) and other studies documenting correlations between habitat patch size, adult census size and genetic population sizes within fishes (Whiteley *et al.* 2013; Wood *et al.* 2014) and small mammals (Pardini *et al.* 2005). The strength and consistency of the relationships between N, N_b and habitat size across populations and years in our study were especially intriguing given the variety of habitat conditions, trout population sizes and trout diversity (e.g. adult body size, mean spawning age) at CR (Hutchings 1993; Belmar-Lucero *et al.* 2012; Wood *et al.* 2014).

What we and several other studies did not find was a correlation between N and N_b when they fluctuate temporally within a population (Araki *et al.* 2007; Palstra *et al.* 2009; Ardren & Kapuscinski 2003; Johnstone *et al.* 2012; Serbezov *et al.* 2012; Duong *et al.* 2013; Whiteley

et al. 2015). In fact, N_b appeared more temporally constrained than N. This discrepancy led to instability in the N_b/N ratio within populations; as N increased, N_b/N decreased, and vice versa.

Building on previous works, we found some support for the hypothesis that N_b and N_b/N would vary more in small compared to large populations: small populations had more varying temporal variability in N_b/N ratios than large populations, and a trend for more temporally varying N_b. Put another way, some small populations were highly variable in N_b/N or N_b while others remained relatively stable over time. This supports the contention of previous work on CR trout and diverse taxa that small populations might be exposed to more variable selective pressures than larger populations (Wood *et al.* 2014, 2016). While large populations fluctuated in population sizes as well, the confidence intervals around the estimates were largely overlapping and so might be largely attributable to sampling variance. In addition to sampling variance, we likely detected other variation in population processes at low N_b/N and N_b. The higher temporal variance in these parameters at smaller population size might simply be explained by high variation in the size (proportion) of different age groups due to the increasing influence of demographic stochasticity. In the future, we hope to collate the longer-term data required on temporal trends in adult age structure within CR trout populations to examine this possibility further. Although reduced precision in N_b estimation due to small sample sizes in some small or very small populations might influence these trends, our analyses (see Appendix S1 and discussion below) and others suggested that N_b can be accurately derived in small populations of conservation concern (Luikart *et al.* 2010; Tallmon *et al.* 2010; Hoehn *et al.* 2012). Small CR streams experienced high variability in recruitment throughout the study years, and despite intensive sampling, it was sometimes extremely challenging to acquire large sample sizes from certain populations.

Density-dependent constraints on N_b

CR trout data supported that N_b/N ratios tend to be larger in small populations. Among populations, N_b increased at a decreasing rate as N became larger; the two parameters were related linearly on a log–log scale, suggesting that the untransformed variables follow a fractional power trajectory. Further compelling evidence for the population size limitations on N_b came from the strong negative correlation between temporal changes in N and N_b/N within populations. Given the weak correlation between temporal changes in N and N_b, N_b must have remained relatively stable as N increased, thus reducing N_b/N. Most previous studies used
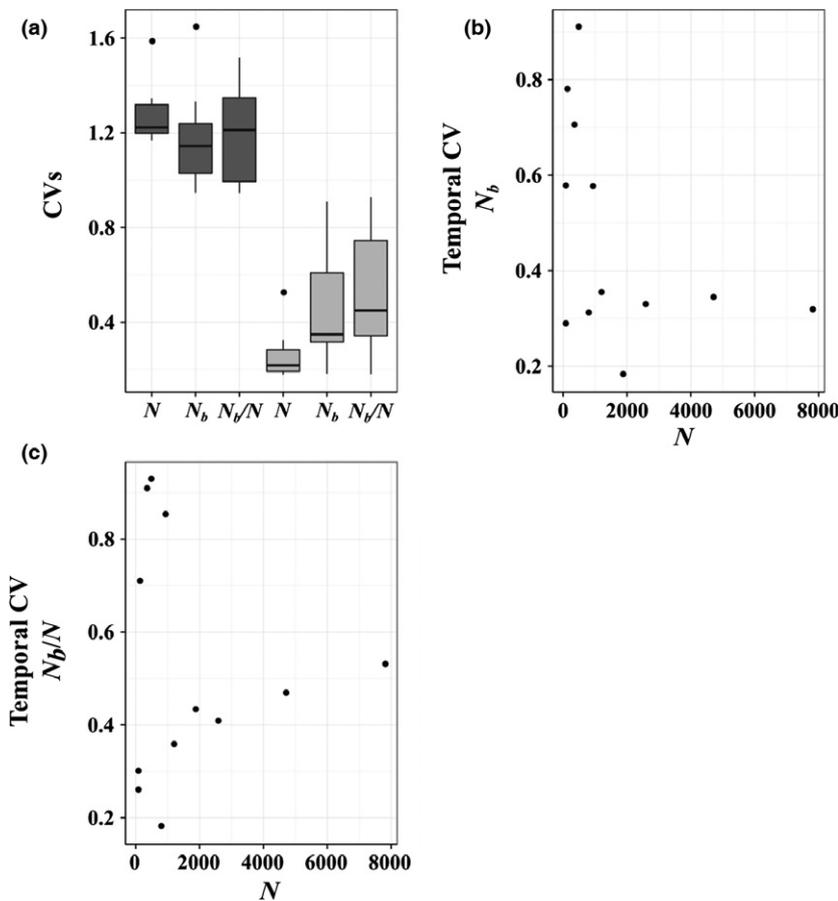
Fig. 5 (a) Among-population (spatial) variation (dark grey) and within-population (temporal) variation (light grey) in N, N_b and N_b/N; (b) relationship between harmonic mean N and variance in N_b; (c) relationship between harmonic mean N and variance in N_b/N. Coefficients of variation (CVs) were used as a standardized metric of variability.

correlations between the ratio N_b/N and N as a means to investigate density-dependent constraints on N_b [see Jackson & Somers (1991) on spurious correlations]; in avoiding this, our results strengthen the indications from previous works in providing further evidence that as the number of adults with the potential to contribute to the breeding population increases, the proportion of adults that do contribute decreases (Ardren & Kapuscinski 2003; Dowling *et al.* 2014; Perrier *et al.* 2016).

The presence of the significant correlation between stream length, N and N_b also provides a potential mechanism for density-dependent constraints on N_b. In salmonids, the initial mechanism emphasized was the breeding capacity of a stream: Chebanov (1991) suggested that as N increases, increased competition for favourable breeding places would increase the variance in reproductive success. However, Whiteley *et al.* (2013, 2015) found no indication of density-dependent changes on indicators of reproductive success in two brook trout populations.

While physical habitat availability is the likely underlying mechanism for density-dependent processes in salmonids, density-dependent adjustments to individual growth could also (and potentially synergistically)

result in temporal variation in important life history traits likely to affect N_b or N_b/N, such as fecundity or size- or age-at-maturity. At small population sizes and/ or following a sharp decline in abundance, decreased adult density and increased food availability could result in higher growth, increased maturation rates and egg production (Rose *et al.* 2001), thus allowing small populations to maintain a relatively high N_b/N. In CR streams, we observed that in years where adult abundance was high, recruits tended to be smaller in size; we hypothesize that a certain proportion may have poorer spawning success due to poor body condition, or experience other physiological stresses that impact maturation or gamete production or quality. Partial support from this hypothesis comes from Waples *et al.* (2013) who found that only a few life history variables explained most variation in N_b/N across various taxa, and a simulation study on a salmonid (Vincenzi *et al.* 2008).

Effect of the environment on N_b

In agreement with other recent studies showing that environmental variables can influence N_b (e.g. Whiteley

et al. 2015), results from CR populations showed that summer flow had an effect on N_b, especially when stream size was used as a proxy for N; indeed, N_b tended to be higher when summer flow was high. Higher summer flows could improve the reproductive rate of a population by, for instance, increasing habitat availability or limiting physiological stresses on individuals. However, it is also noteworthy that larger streams tend to be characterized by faster flow on CR. Thus, the relationship between flow and N_b could be partially attributed to the relationship with stream length. On the other hand, our data were not consistent with the hypothesis that summer stream temperatures would have a negative effect on N_b. Moreover, we found no evidence for an effect of seasonal weather patterns (precipitation and air temperature) on N_b (Appendix S4). These results differed from several other studies (Xu *et al.* 2010a, b; Kanno *et al.* 2016) and could be the consequence of the specific weather pattern experienced by CR trout populations; indeed, CR is characterized by cool temperatures and constant precipitation all year round. Nevertheless, it is possible that extreme events (droughts, flood) have a greater influence on population dynamics. More importantly, our data are also congruent with the idea that conservation actions restoring or maintaining habitat quantity and/or quality could be effective at improving the likelihood of wild population persistence. For instance, preventing further habitat degradation or fragmentation could help increase and/or maintain both N_b and N, thus improving long-term persistence potential.

Correctly/incorrectly linking N_b and N

In many species, correctly linking N_b and N requires multiple sampling events, including over different years (Palstra & Fraser 2012). In many salmonids, for example, and as illustrated in our study, juveniles and adults have to be sampled in t and $t − 1$ years, respectively. In such situations, an important practical consideration is the extent to which the relationship between N_b and N might change when their estimates are incorrectly linked based on sampling conducted at the same time (e.g. in our case, summer). When we conducted an exploratory analysis on the relationship between unlinked N_b and N (sampling conducted in the same year), the relationship remained essentially the same as in the correctly linked analyses (Table S7; Fig. S3, Supporting information). These results suggest that resources (transportation and field costs, time) could be saved by reducing the number of sampling events, while obtaining reasonable information on adult census size and genetic population size.

Sampling limitations for estimating N_b

Because our study populations varied 120-fold in N and included very small populations of $N = 50$–100, sample sizes inherently differed among populations and temporally within populations (range: 5–237 sampled individuals). We investigated the effect of sample size on LDNe N_b estimates (see Appendix S1) and found no significant correlation between sample size and N_b. There was more variability in N_b estimates when sample size was very small (<25), suggesting that N_b estimates derived from small samples might be slightly less accurate. Nevertheless, this variability was clearly low compared to variability in N_b between very small, moderately large and large populations. Additionally, only five of our 43 N_b/N ratios involved N_b estimated from very small sample sizes (<25), of which only one was $n < 10$ (Table S1, Supporting information). Although the interpretation of the N_b –N relationship is complicated by differing sample sizes, these results suggest that the relationship appears to be relatively independent of sample sizes for CR populations.

Conservation implications

Empirical studies of the spatiotemporal relationship between N and N_b among populations spanning a large gradient of population sizes are rare (Van Doornik *et al.* 2013; Perrier *et al.* 2016). Our results on 12 brook trout populations demonstrate a clear linkage between demographic and evolutionary parameters, but they provide a mixed perspective on the utility of using N or N_b to infer the other.

On the one hand, there was a strong association between adult census sizes and genetic population sizes, as well as between these parameters and simple metrics of habitat size (stream length, flow) when compared among populations. Additionally, variation among populations in N_b/N and N was greater than temporal variation within populations. Collectively, these findings suggest that the relative abundance of different populations can be inferred from N_b and habitat size, from a spatial comparison standpoint. For instance, noninvasive sampling methods could be used to estimate N_b and derive the relative N of populations of species that are not easily sampled such as those that are rare, elusive or found in remote or northern areas (Kindberg *et al.* 2011), even without correctly linking N_b to N. Such knowledge could provide guidelines to set conservation targets.

Our work also adds to the growing body of evidence that inferring one variable (N_b or N) from the other might not be appropriate when monitoring population trends over time, as demonstrated by the lack of strong

temporal association between N_b and N and instability of N_b/N within populations. In particular, our results suggest that this is perhaps especially the case for very small populations (<250 adults) because their N_b –N dynamics may be less predictable than larger populations. As small populations face the greatest threat of extirpation, this finding has important management implications. For instance, it means that both genetic and the adult census population sizes should be used to monitor trends in very small populations of conservation concern.

The observed differences in mean N_b/N between small and large populations and the increased variability in N_b/N variance among small populations also have potential ramifications in a global context where wild populations tend to become increasingly small and fragmented. Our empirical analysis suggests that ecological interactions might consistently differ between small and large populations, and density-dependent processes might be an important force enhancing the resilience of very small populations. Future studies should be aimed at understanding the mechanisms underlying density-dependent growth in N_b/N, which may arise from two main population-level causes: availability and quality of physical habitat (Ardren & Kapuscinski 2003; Whiteley *et al.* 2013) and alterations in life history (Vincenzi *et al.* 2008; Waples *et al.* 2013). Additionally, small populations might be more heterogeneous than large populations with respect to their selective pressures and likelihood of persistence in a dynamic and ever-changing world. Some small populations might be more resilient to disturbances relative to others that are more likely to be funnelled into the well-documented extinction vortex. Identifying criteria to differentiate viable small populations from those that are more likely to become extirpated, such as the degree of habitat specification, the extent of plasticity or physical habitat characteristics, might help achieve genetic and demographic goals in conservation.

Acknowledgements

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Data accessibility

Brook trout microsatellite data and velocity measurements can be found at DRYAD entry. doi: 10.5061/dryad.f9f75.

Supporting information

Additional supporting information may be found in the online version of this article.

Table S1 Annual population summary statistics for 12 brook trout populations between 2008-2015, Cape Race, Newfoundland, Canada.

Table S2 STRUCTURE analysis of population subdivision in four brook trout drainages from Cape Race, Newfoundland, Canada.

Table S3 Environmental data summary statistics for 12 brook trout populations between 2010-2014, Cape Race, Newfoundland, Canada.

Table S4 Spatial variability in N, N_b and N_b/N among 12 brook trout populations from Cape Race, Newfoundland, Canada.

Table S5 Temporal variability in N, N_b and N_b/N within 12 brook trout populations from Cape Race, Newfoundland, Canada.

Table S6 Poisson lognormal mixed-effect modeling of the relationship between N and stream length among 12 brook trout populations from Cape Race, Newfoundland, Canada.

Table S7 Poisson lognormal mixed-effect modeling of the relationship between incorrectly linked N and N_b, sampled from the same season (based on adult tagging for N estimation and juvenile YOY sampling for N_b estimation).

Fig. S1 Relationship between N_b estimates inferred from the whole data sets and those inferred after removal of family structure.

Fig. S2 Relationship between N and stream size from a Poisson lognormal mixed model.

Fig. S3 Relationship between incorrectly linked N and N_b from a Poisson lognormal mixed model, sampled from the same season (based on adult tagging for N estimation and juvenile YOY sampling for N_b estimation).

Appendix S1 Effect of sample size and pcrit on N_b estimates from Cape Race.

Appendix S2 The relationship between N_b derived from a sibship method and N.

Appendix S3 Correspondence between N_b and N_e.

Appendix S4 The relationship between N_b and seasonal temperatures and precipitation in 12 brook trout populations from Cape Race, Newfoundland, Canada.