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# Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, *Salvelinus fontinalis*

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The relationship between the effective number of breeders ( $N_b$ ) and the generational effective size ( $N_e$ ) has rarely been examined empirically in species with overlapping generations and iteroparity. Based on a suite of 11 microsatellite markers, we examine the relationship between  $N_b$ ,  $N_e$  and census population size ( $N_c$ ) in 14 brook trout (*Salvelinus fontinalis*) populations inhabiting 12 small streams in Nova Scotia and sampled at least twice between 2009 and 2015. Unbiased estimates of  $N_b$  obtained with individuals of a single cohort, adjusted on the basis of age at first maturation ( $\alpha$ ) and adult lifespan (AL), were from 1.66 to 0.24 times the average estimates of  $N_e$  obtained with random samples of individuals of mixed ages (i.e.  $\hat{N}_{b(\text{adj})}/\text{mean}(\hat{N}_{e(\text{mixed ages})})$ ). In turn, these differences led to adjusted  $N_e$  estimates that were from nearly five to 0.7 times the estimates derived from mixed-aged individuals. These differences translate into the same range of variation in the ratio of effective to census population size ( $\hat{N}_{e(\text{adj})}/\hat{N}_c$ ) within populations. Adopting  $\hat{N}_{e(\text{adj})}$  as the more precise and unbiased estimates, we found that these brook trout populations differ markedly in their effective to census population sizes (range approx. 0.3 to approx. 0.01). Using AGENE, we then showed that the variance in reproductive success or reproductive skew varied among populations by a factor of 40, from  $V_k/k \approx 5$  to 200. These results suggest wide differences in population dynamics, probably resulting from differences in productivity affecting the intensity of competition for access to mates or redds, and thus reproductive skew. Understanding the relationship between  $N_e$ ,  $N_b$  and  $N_c$ , and how these relate to population dynamics and fluctuations in population size, are important for the design of robust conservation strategies in small populations with overlapping generations and iteroparity.

## 1. Introduction

The genetically effective size of a population ( $N_e$ ) is the size of an ideal population that loses genetic diversity at the same rate as the actual population under study [1,2]. Effective size influences both the rate of random genetic drift and the effectiveness of natural selection acting on the population, and is thus a fundamental concept in evolutionary genetics. Because wild populations usually depart from ideal conditions, effective population sizes are generally smaller than the corresponding census population sizes [3,4], but how much smaller remains uncertain [5]. The uncertainty is in part due to the fact that effective population size is notoriously difficult to estimate precisely and without bias. One important source of bias arises from the use of estimation methods that assume discrete generations for species and populations that actually have overlapping generations and iteroparous reproduction [6]. Analysis of individuals belonging to a single cohort (using the extent of linkage disequilibrium (LD))

can provide estimates of the annual effective size or the number of effective breeders ( $\hat{N}_b$ ) in the parental generation (i.e. the generation that gave rise to that particular cohort). Generational  $\hat{N}_e$  has traditionally been approximated as the product  $\hat{N}_b \times G$  ( $G$ : generation length) [7,8]. However, this estimation has recently been shown to be problematic if applied to iteroparous species, with estimates of  $\hat{N}_b$  sometimes exceeding  $N_e$  [9]. Fortunately, the actual ratio of  $\hat{N}_e$  to  $\hat{N}_b$  can be predicted if several simple life-history traits are known: age at maturation, adult lifespan (AL) or number of reproductive cycles and variance among breeders in reproductive success [9,10] (and see [6]). Using the empirical quantitative relationships described by Waples *et al.* [10], it is now possible to obtain unbiased estimates of  $N_b$  or  $N_e$  for iteroparous species from single cohort estimates of  $N_b$  based on LD. Our goal in this study is to examine the relationship between census population size and these two measures of effective size,  $N_e$  and  $N_b$  for 14 small populations of brook trout (*Salvelinus fontinalis*). We compare biased and unbiased estimates for each of the populations and explore reasons for the variation in the relationship among populations.

Stream fish populations are often used for landscape genetics studies as the linear nature of stream habitats can lead to the simplification of genetic models used to understand their evolutionary dynamics [11–14]. Resident salmonid populations inhabiting small streams generally exhibit relatively short generation times, facilitating the study of the relationship between effective and census population sizes. Recent studies have produced variable estimates of  $N_b/N_e$  for stream brook trout populations, with large differences among studies as well as among populations within studies in these ratios [15–17]. The differences have been linked to differences in habitat variability and habitat quality, which in turn result in differences in census population size and potentially also in life-history traits such as age and size at maturation, and age-specific survival or reproductive lifespan [15,17]. None of these studies, however, considered the extent to which biases in the estimates of  $N_e$  stemming from the use of models developed for semelparous species might have influenced their estimates, nor whether variation in such biases among populations might account for some of the among population variation observed in their systems.

In this study, we first describe the structure of 14 brook trout (*S. fontinalis*) populations inhabiting 12 independent streams in eastern Canada and estimate their level of connectivity. The streams are small (maximum 5 km in length), and are located in independent and parallel watersheds that empty into the Bay of Fundy along the northwest shore of Nova Scotia. Some of the populations are landlocked owing to coastal or upstream waterfalls, and the others have intermittent connectivity to the sea during high water events. Although anadromy and interdrainage migration is in principle possible for these populations, we find that all 14 populations are essentially isolated.

For each population, we then estimate effective size using a variety of methods and census size. We then assess the extent of estimate bias when using methodology that assumes semelparity by examining the relationship between  $\hat{N}_b$ ,  $\hat{N}_e$  and census population size  $\hat{N}_c$  for adjusted and non-adjusted estimates of  $N_e$ . We find that the ratio of the effective population size estimated with a sample of mixed ages ( $\hat{N}_{e(\text{mixed ages})}$ ) to that derived from an adjusted estimate of the number of breeders ( $\hat{N}_{e(\text{adj2})}$ ) varied widely across populations, ranging from

$\hat{N}_{e(\text{adj2})} \approx 5\hat{N}_{e(\text{mixed ages})}$  to  $\hat{N}_{e(\text{adj2})} \approx 0.7\hat{N}_{e(\text{mixed ages})}$ , highlighting the importance of precision and bias correction for the proper estimation of the  $N_e/N_c$  ratios in systems with overlapping generations and iteroparous reproduction.

## 2. Material and methods

### (a) Study sites and sample collection

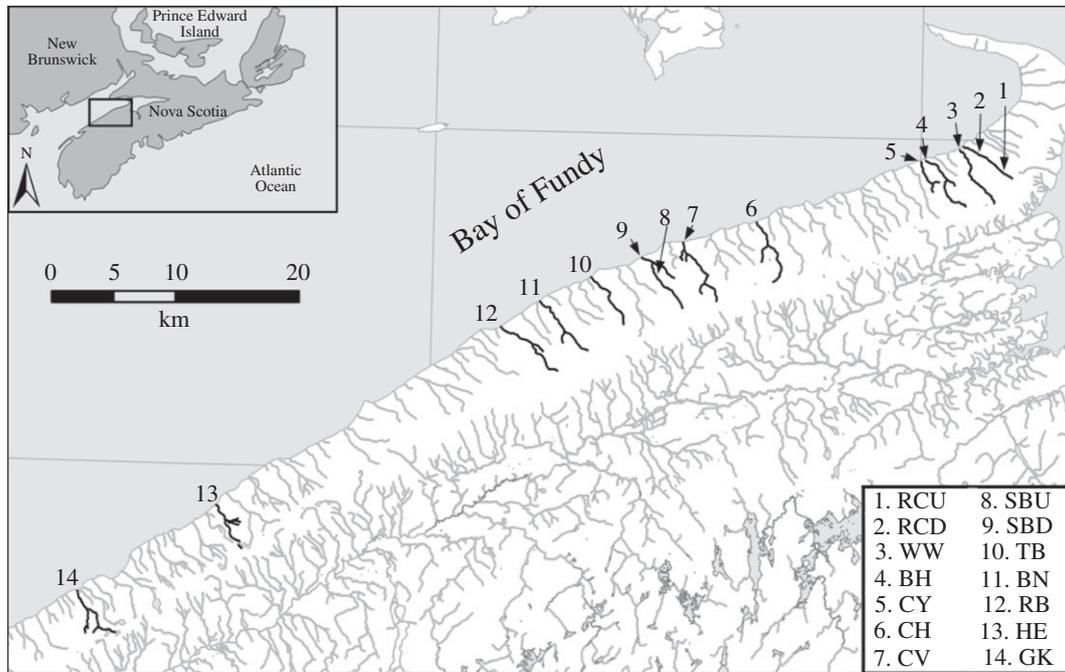
Brook trout (*S. fontinalis*) were collected from 12 coastal streams along the northwest shore of Nova Scotia in the summers of 2009–2015. DNA analysis was conducted on the samples collected between 2009 and 2013 ( $n = 1870$ ; electronic supplementary material, table S1), whereas samples collected in 2014 and 2015 were used for the estimation of census population size through mark recapture ( $n \approx 1300$  in each year), with subsets used for age ( $n = 426$ ) and sexual maturity ( $n = 66$ ) determination (see below). The 12 streams are located in independent watersheds that drain into the Bay of Fundy from near the top of the North Mountain (maximum relief 265 m; figure 1). Most streams have one or more waterfalls that are impassable for trout, some at the coast, resulting in completely landlocked populations, and others along the stream length, creating upstream landlocked populations and downstream populations that may receive immigrants from upstream (figure 1). Sampling was non-lethal. In 2009/2010 and 2012, brook trout were caught with baited minnow traps set for approximately 24 h at intervals along the streams. In 2013–2015, fish were collected by electrofishing. Fish were measured (fork length, FL) and fin clipped (adipose fin) before release. Fin clips were stored in 95% ethanol for subsequent DNA analysis.

### (b) Population size ( $\hat{N}_c$ ) estimation

Population (census) size  $N_c$  was estimated by mark–recapture using the Lincoln–Petersen method with each population sampled twice, two weeks apart in 2014, and either the same day or the next day in 2015. Fish sampled for the purposes of estimating  $\hat{N}_c$  (2014 and 2015) were not used for the genetic analysis in this study. In early July 2014 (8–11 July), 81 fish were captured, measured, marked and released in each stream population. Capture began in the section where fish had been sampled for genetic analysis in previous years. Electrofishing proceeded until more than 80 fish had been captured, fin clipped and released. The length of the sampled section was determined from GPS coordinates. Two weeks later (22–25 July in 2014) or the same or next day (July 2015), the same section of each stream was re-sampled, and the number of recaptures determined. One section of streambed was electrofished in 2014, whereas in 2015, we electrofished and resampled either the entire stream (RCD) or between two and four sections of streambed separated from each other by at least 500–750 m (two sections: SBU; three sections: RCU, WW, SBD; four sections: CV). We used the recapture rate and the number of fish (1+ or older) caught in the sampled length of stream to estimate density of adult fish (no of fish per m stream length). Population census size was then estimated by extrapolating to the length of stream over which there are no physical barriers to fish movement, this is a conservative estimate of  $N_c$ . Stream segment lengths were determined using ARCGIS (1 : 50 000). Streams were walked to locate waterfalls or other significant barriers (e.g. impassable culverts).

### (c) Life-history characteristics

The key life-history characteristics needed for Waples *et al.*'s [10] corrections are age at maturity and AL or number of reproductive cycles. We used samples captured in July 2014 as our baseline population. The young of the year (YOY) class was almost certainly



**Figure 1.** Streams sampled along the North Mountain in Nova Scotia. Streams run into the Bay of Fundy. The streams with their associated codes are from east to west: 1: Ross Creek upstream (RCU) and 2: Ross Creek downstream (RCD) of a major waterfall; 3: Woodworth (WW); 4: Black Hole (BH); 5: Cobby Irving (CY); 6: Chipman (CH); 7: Church Vault (CV); 8: Saunders Brook upstream (SBU) and 9: Saunders Brook downstream (SBD) of a major waterfall; 10: Turner (TB), 11: Brown (BN); 12: Robinson (RB); 13: Healy (HE); 14: Gaskill (GK). Inset shows study area in Nova Scotia. Populations at seven sites had intermittent access to the sea: RCD, WW, CH, SBD, RB, BN and GK. The remaining seven sites contained landlocked brook trout populations located upstream of major waterfalls (RCU, BH, CY, CV, SBU, HE) or a culvert impeding upstream brook trout passage (TB).

underrepresented in our sampling owing to their lower capture efficiency and possible differences in habitat use. Capture efficiency should have been similar for year classes older than YOY, and these individuals could thus be used for the determination of age structure as well as age at maturity.

A subsample of fish older than YOY ( $n \approx 30$  per site for a total of 426 individuals) were weighed (live weight) and aged using scales to determine the relationship between size and age for these populations. The size range of each age class cohort and the weight–length relationship were determined. This information was then used to determine the age structure of the populations for the July 2013 sample (1151 fish in all). Annual mortality rate was also estimated for fish aged 1+ and 2+ years using  $n = 1 - (\text{number in } T + 1 \text{ year class} \div \text{number in } T \text{ year class})$  [18].

Brook trout in Nova Scotia spawn from September to November, and alevin hatch the following spring. Fish ( $n = 66$ ) from three sites (Ross Creek, Woodworth Creek and Cobby Irving Brook) were sacrificed to determine size (FL) and age at maturity. The abdominal cavity was opened to determine sex and maturity status (presence of ripe gonads). The female–male ratio for immature trout was assumed to be 1 : 1. This ratio was then used to approximate the proportion of mature females and mature males in each 1 cm length interval for the YOY, 1+ year and 2+ year age cohorts. Generation time,  $G$ , was then estimated by using a standard life table approach.

#### (d) Molecular protocol

Samples were digested overnight using proteinase K. DNA was extracted following a glassmilk protocol modified from Elphinstone *et al.* [19] using a Perkin Elmer Multiprobe II Plus Liquid Handling System (Perkin Elmer, Waltham, MA). Polymorphism was examined at 11 PCR amplified microsatellite loci (details in electronic supplementary material) labelled with either 700 or 800 nm M13 for downstream visualization. The resulting

PCR products were imaged on Li-COR 4200/4300 DNA Analyzers (Li-Cor Biosciences, Lincoln, NE). Alleles were scored automatically using SAGA Automated Microsatellite Software 3.3 (Li-Cor Biosciences) and checked for repeatability of scoring (electronic supplementary material).

#### (e) Within-sample analysis

MICROCHECKER (v. 2.2.3) [20] was used for detection of potential null alleles and large allele dropout. Expected and observed heterozygosities as well as deviations from Hardy–Weinberg equilibrium (HWE) and pairs of loci in LD were identified in ARLEQUIN v. 3.5 [21]. The number of private alleles per stream was calculated using GENALEX v. 6.4 [22]. Allelic richness was estimated using FSTAT v. 2.9.3.2 [23].

#### (f) Population structure

Pairwise  $F_{ST}$ s were estimated using GENALEX v. 6.4 [22]. A principal coordinates analysis based on the matrix of pairwise  $F_{ST}$ s was conducted in R [24] using the package ‘ape’ [25]. Genetic structure was analysed using STRUCTURE v. 2.3.4 [26] with an admixture model and no location priors. Simulations were run for 1 000 000 iterations with an initial burnin of 500 000. Three replicates were run for each  $K$ -value from 1 to 15 using the entire dataset, and again for each subsequently identified cluster. The most probable  $K$ -values were chosen using the Evanno method [27] implemented in STRUCTURE HARVESTER v. 0.6.92 [28]. Once the most probable number of genetic clusters had been identified, seven more replications were produced, at that appropriate  $K$ -value, using STRUCTURE resulting in 10 total replicates. These 10 replicates were combined using CLUMPP v. 1.1.2 [29]. The resulting output from CLUMPP was visualized using DISTRICT v. 1.1 [30]. Recent migration rates between the 14 sites were estimated using BAYESASS v. 3.0.3 [31] with 30 million iterations (3 million burnin and parameters  $a = 0.2$ ,  $f = 0.2$  and  $m = 0.05$ ).

### (g) Effective population size and effective number of breeders

Estimates of effective population size ( $\hat{N}_e$ ) were obtained with the LD method implemented in LDNe [32]. Estimates were obtained for each of the 31 location per year combinations (2009/2010: 12 streams; 2012: five locations from four streams; 2013: 14 locations from 12 streams). Second, we estimated effective population size using the temporal method as implemented in MLNE [33] with samples collected in 2009–2010 (2012 in one case) representing  $t_0$  and those collected in 2013 representing  $t_1$ . Third, we also estimated effective number of breeders ( $\hat{N}_b$ ) using exclusively individuals of age class 1+. This was done only with the relatively large samples collected in 2013, for which sufficient individuals of age 1+ were available. Raw estimates of the effective number of breeders ( $\hat{N}_b$ ) were adjusted using the method that involves two life-history traits, namely age at first reproduction ( $\alpha$ ) and adult lifespan (AL), as follows [10]:

$$\hat{N}_{b(\text{adj}2)} = \frac{\text{raw } \hat{N}_b}{1.103 - 0.245 \times \log(\text{AL}/\alpha)}. \quad (2.1)$$

Adjusted estimates of effective number of breeders ( $\hat{N}_{b(\text{adj}2)}$ ) were then used to estimate  $\hat{N}_{e(\text{adj}2)}$  as follows:

$$\hat{N}_{e(\text{adj}2)} = \frac{\hat{N}_{b(\text{adj}2)}}{0.485 + 0.758 \times \log(\text{AL}/\alpha)}. \quad (2.2)$$

We thus obtained three estimates of effective population size per stream: two based on LD, one of which used mixed-ages data ( $\hat{N}_{e(\text{LD, mixed ages})}$ ), averaged over up to three estimates: mean ( $\hat{N}_{e(\text{LD, mixed ages})}$ ) and the other single cohort data ( $\hat{N}_{e(\text{adj}2)}$ ), and one based on drift between temporally spaced samples ( $\hat{N}_{e(\text{MLNE})}$ ). These estimates were then compared with the estimates of census population size, ( $\hat{N}_c$ , individuals aged 1+ or older; see above). Lastly, we examined the role of variance in family size on these empirical  $\hat{N}_e/(\hat{N}_c)$  by comparing them with ratios obtained with AGE<sub>NE</sub> [34] under varying degrees of variance in reproductive success.

## 3. Results

### (a) Size at age

Cohorts were quite easily distinguishable. Mean lengths at age, averaged across sites, were 5.8, 11.4, 15.0 and 18.3 cm for YOY, 1+, 2+ and 3+, respectively (electronic supplementary material, table S2), using upper boundaries of 8 (YOY), 13–16 (1+) and 16–19 cm (2+). Annual mortality rates (estimated from age structure) were 0.43 and 0.64 for age classes 1+ and 2+, respectively (electronic supplementary material, table S2). The absence of individuals aged 4+ years suggests that fish rarely survive past their third year in these streams. Annual mortality for brook trout aged 3+ was therefore 1.0.

### (b) Age at maturity ( $\alpha$ ) and adult lifespan

Length and age at maturity were estimated with data collected from three streams (RC, WW, CY) in the autumn (16 September) of 2014. Brook trout with FL less than 9.5 cm were immature and the proportion of mature trout increased with size from 0.09 at 10 cm FL to 1.0 at 13 cm FL, with an average across electrofished sites of 59% of trout aged 1+ being mature (electronic supplementary material, table S3a,b). The proportion of mature brook trout was similar for males and females. For females, the proportion of mature individuals was 0.0, 0.62 and 1.0 for YOY, 1+ and 2+, respectively. For males, the proportion of

mature individuals was 0.0, 0.44 and 1.0 zero for YOY, age 1+ and age 2+, respectively (electronic supplementary material, table S3c). Only two individuals aged 3+ were sampled and were not included in the evaluation. Overall, therefore, weighted age at maturity was  $\alpha = 2.41$ . Given that oldest age at reproduction is 3, adult lifespan is  $\text{AL} = 3 + 1 - 2.41 = 1.59$ . All subsequent estimates of  $\hat{N}_{b(\text{adj}2)}$  and  $\hat{N}_{e(\text{adj}2)}$  were thus obtained using  $\alpha = 2.41$  and  $\text{AL} = 1.59$ . Generation length, estimated using the proportion of eggs contributed to the next generation by females aged 1+, 2+ and 3+, was  $G = 1.88$  years (electronic supplementary material, table S4).

### (c) Census population sizes

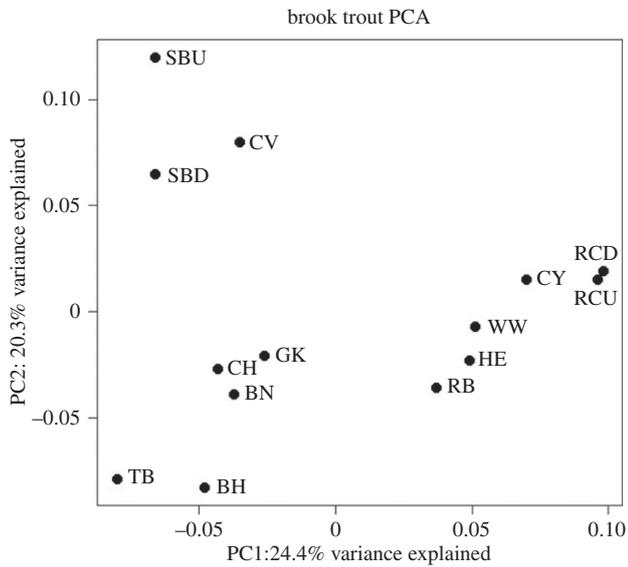
Census population size estimates,  $\hat{N}_c$ , ranged from as low as 854 individuals for BH to 12 000 individuals for CV in 2014, with an average (median) estimate across populations of between 3600 and 4000 (2100–2500), depending on whether a mixture of 2014 and 2015 or only 2014 estimates were used (electronic supplementary material, table S5).

### (d) Within-sample analysis

Average (median) scoring repeatability across 11 microsatellite loci was 98.5% (98.8%) (electronic supplementary material, table S6). Observed and expected heterozygosities ranged from 0.360 and 0.366 to 0.577 and 0.628, respectively (electronic supplementary material, table S7). Loci were moderately polymorphic with allelic richness, ranging from 3.37 to 6.60 (electronic supplementary material, table S7). The number of private alleles per population ranged from 0 to 4. The number and identity of loci presenting potential null alleles varied across samples and included 0 or 1 locus with potential null alleles (5 and 17 samples, respectively), 2, 3 and 4 loci with potential null alleles (five, two and two samples, respectively; electronic supplementary material, table S7). Similarly, the number (and identity) of loci out of HWE varied across samples, with eight samples exhibiting no locus out of HWE and one sample exhibiting four loci out of HWE, with other samples exhibiting an intermediate number. The lack of consistency among populations in the identity of the loci exhibiting departures from HWE suggest these departures are not owing to null alleles. Rather, they probably result from the interaction of small effective sizes with age structure (i.e. cohorts differing in allele frequencies leading to an intercohort Wahlund effect) along with the potential existence of siblings and spatial or deme structure within streams. Similarly, although eight populations exhibited at least one pair of loci in gametic phase disequilibrium (electronic supplementary material, table S7), the pair identity varied across populations, suggesting their detection results from random sampling effects rather than actual physical linkage between loci.  $F_{IS}$  overall loci ranged from 0.023 (RB) to 0.162 (HE) (median across populations,  $F_{IS} = 0.066$ ), indicating close similarity between observed and expected heterozygosities within populations (electronic supplementary material, table S8).

### (e) Population structure and gene flow

A hierarchical structure analysis revealed the existence of population structure at the level of individual streams (electronic supplementary material, figure S1). A plot of the first two coordinates of a principal coordinate analysis (PCA)



**Figure 2.** Principal coordinate analysis (PCA) based on 11 loci for brook trout collected from 12 streams (14 sites).

based on the matrix of pairwise  $F_{ST}$ s (figure 2; electronic supplementary material, table S9) provided results consistent with those obtained with STRUCTURE: RCU and RCD appear genetically similar to each other, and to a lesser degree also to the neighbouring WW and CY and to the more distant HE and RB, while they appear more different from the remaining populations. Similarly, SBU and SBD appear more similar to each other and to the neighbouring CV than to the remaining populations, with the remaining populations exhibiting varying degrees of differentiation (figure 2). Migration rates estimated with BAYESASS were low to nil (not shown), indicating little to no gene flow between populations, with one exception: RCU and RCD exhibited a sufficiently low  $\hat{F}_{ST}$  (0.008) to prevent the reliable estimation of  $m$ .

#### (f) Effective population size ( $\hat{N}_e$ ), effective number of breeders ( $\hat{N}_b$ ) and census population size ( $\hat{N}_c$ ), and their ratios using raw and adjusted estimates

Effective population sizes were estimated for each of the 31 stream/sampling year combinations (2009/2010, 2012, 2013) using the LD method and all sampled individuals regardless of age ( $\hat{N}_{e(LD, mixed\ ages)}$ ; electronic supplementary material, table S10). For each stream, we then calculated the weighted harmonic mean across years ( $\text{mean}(\hat{N}_{e(LD, mixed\ ages)})$ ) using sample sizes as weights (electronic supplementary material, table S10; table 1). Effective sizes were also estimated using the temporal method ( $\hat{N}_{e(MLNe)}$ ; electronic supplementary material, table S10; table 1). Lastly, we also estimated the raw effective number of breeders ( $\hat{N}_b$ ) (electronic supplementary material, table S11) from which we calculated  $\hat{N}_{b(adj2)}$  (electronic supplementary material, table S11) and  $\hat{N}_{e(adj2)}$  following equations (2.1) and (2.2), and assuming  $AL = 1.59$  and  $\alpha = 2.41$  (electronic supplementary material, table S11). We thus obtained three estimates of effective size for each population (table 1). Across all populations,  $\text{mean}(\hat{N}_{e(LD, mixed\ ages)}) \approx 206$  (median  $\approx 142$ ). Among the six populations for which we obtained all three estimates, the means (medians) were

$\hat{N}_{e(LD, mixed\ ages)} \approx 113$  (126),  $\hat{N}_{e(MLNe)} \approx 40$  (44) and  $\hat{N}_{e(adj2)} \approx 227$  (125). Individually,  $\hat{N}_{e(adj2)} > \hat{N}_{e(LD, mixed\ ages)}$  in five of the six cases with median ratio  $[\hat{N}_{e(adj2)}/\hat{N}_{e(LD, mixed\ ages)}] \approx 1.66$  while median ratio  $[\hat{N}_{e(adj2)}/\hat{N}_{e(MLNe)}] \approx 4.7$  and median ratio  $[\text{mean}(\hat{N}_{e(LD, mixed\ ages)})/\hat{N}_{e(MLNe)}] \approx 2.88$  (range 1.8–4.6). Individually though,  $\hat{N}_{e(adj2)}$  could be up to nearly five times larger than estimates obtained from mixed ages (CY) and 13 times larger than estimates obtained with the temporal method (CY, WW; table 1). Similarly, estimates obtained with the LD method using individuals of mixed ages could be up to nearly five times larger than estimates obtained with the temporal method.

Each of these estimates was then related to census population size  $\hat{N}_c$ . The ratio of the mean ( $\hat{N}_{e(LD, mixed\ ages)})/\hat{N}_c < 0.10$  in all but three cases, with the lowest ratio being approximately 0.01 (CV) and the largest being approximately 0.18 (median = 0.078 [0.046] for the six populations with available  $\hat{N}_{e(adj2)}$ ; table 1). Such wide range in the ratio of effective to census size is also observed among the  $[\hat{N}_{e(adj2)}/\hat{N}_c]$  ratios (table 1), where they lie in the 20–30% range in two populations (WW, CY), in the 10% in one (GK), 5% in two (RCU, HE) and 1% in the remaining two (CV, TB) of the seven populations for which  $\hat{N}_{e(adj2)}$  could be estimated (median = 0.048; table 1). Such vast differences in effective to census size, regardless of how effective size is measured implicate vast differences in reproductive dynamics among these brook trout populations.

Predictably, the median ( $\hat{N}_{e(MLNe)}/\hat{N}_c$ ) was lower than with the other methods (0.011), but still, important differences were observed among populations (table 1) with one population exhibiting a ratio = 0.133, five exhibiting ratios in the 0.02–0.05 range, and seven exhibiting ratios less than or equal to 0.01 (table 1), once again implicating difference in reproductive dynamics among populations.

Next, we examined the range of reproductive skew required to attain these ratios using AGE<sub>NE</sub> based on each population's age-specific survival rates (electronic supplementary material, table S2) and relative fecundity [35]. We assumed relative fecundities for 2+ and 3+ males were, respectively, three and four times that of a 1+ male, a reasonable assumption given that a 3+-year-old is approximately twice as long as a 1+-year-old. This resulted in a generation length for males similar to that for females. Using the above values for these life-history traits, the Poisson factor (variance in reproductive success over its mean,  $V_k/\bar{k}$ ) ranged from a low of between 5 and 10 for four populations (RCD, WW, BH, CY) to a high of 120 and 200 for two populations (TB and CV, respectively) and was intermediate (20–50) in the other four (RCU, CH, SBD, HE; table 1). In general, and using estimates of age-specific survival rates averaged over all populations, observing a ratio  $\hat{N}_e/\hat{N}_c \approx 0.180$  requires a skew in reproductive success (Poisson factor)  $\approx 10$ , a ratio  $\hat{N}_e/\hat{N}_c$  in the range of 0.040–0.020 requires a skew in the range of 50–100, and a ratio  $\hat{N}_e/\hat{N}_c \approx 0.010$  requires a skew  $\approx 200$ .

## 4. Discussion

Waples *et al.* [10] recently presented the methods for adjusting estimates of effective population size ( $N_e$ ) and effective number of breeders ( $N_b$ ) in iteroparous populations by accounting for the impact of AL and age at first maturation. We have shown the importance of those adjustments for small brook trout populations; adjusted estimates of  $N_e$  derived from adjusted

**Table 1.** Mean ( $\hat{N}_{e(\text{mixed ages})}$ ) and  $\hat{N}_{e(\text{MLNE})}$ : effective population size estimates obtained, respectively, from linkage disequilibrium in a random sample of individuals of mixed ages, and from genetic drift between temporal samples (both from electronic supplementary material, table S11).  $\hat{N}_{e(\text{adj})}$ : effective population size, adjusted on the basis of two life-history traits, adult lifespan (AL = 1.59) and age at maturation ( $\alpha = 2.41$ ) (from electronic supplementary material, table S11). Ratios of  $\hat{N}_{e(\text{adj})}/\text{Mean}(\hat{N}_{e(\text{LD, mixed ages})}) \cdot \hat{N}_C$  and  $\hat{N}_{e(\text{MLNE})}/\hat{N}_C$ : census population size over stem length over which there is no barrier to fish movement (from electronic supplementary material, table S5). Mean ( $\hat{N}_{e(\text{mixed ages})}/\hat{N}_C$ ),  $\hat{N}_{e(\text{MLNE})}/\hat{N}_C$  and  $\hat{N}_{e(\text{adj})}/\hat{N}_C$ : ratios of population effective to census size, where effective sizes were derived, respectively, from mixed ages, from the temporal method, and  $\hat{N}_{e(\text{adj})}$ . Variance in reproductive success over its mean or Poisson factor ( $V_{\mu}/k$ ) as entered in AgeNE as entered in AgeNE to obtain the observed  $\hat{N}_{e(\text{adj})}/\hat{N}_C$  (when available) or the observed mean ( $\hat{N}_{e(\text{mixed ages})}/\hat{N}_C$ ) given each population age-specific survival rates as shown in electronic supplementary material, table S2.

stream	mean ( $\hat{N}_{e(\text{LD, mixed ages})}$ )	$\hat{N}_{e(\text{MLNE})}$ (95% CI)	$\hat{N}_{e(\text{adj})}$ (95% CI)	$\hat{N}_{e(\text{adj})}/\text{mean}(\hat{N}_{e(\text{LD, mixed ages})})$	$\hat{N}_{e(\text{adj})}/\hat{N}_{e(\text{MLNE})}$	$\hat{N}_C$	mean ( $\hat{N}_{e(\text{LD, mixed ages})}/\hat{N}_C$ )	$\hat{N}_{e(\text{MLNE})}/\hat{N}_C$	$\hat{N}_{e(\text{adj})}/\hat{N}_C$	Poisson factor ( $V_{\mu}/k$ )
RCU	127.3 (41–∞)	40.8 (28–68)	189 (55–∞)	1.48	4.63	3953–4680	0.032–0.027	0.009–0.010	0.040–0.048	50
RCD	235.0 (72–∞)	32.8 (25–44)	— (158–∞)	—	—	1369–690	0.172–0.341	0.024–0.048	—	5–10
WW	124.6 (58–∞)	27.2 (21–36)	363 (109–∞)	2.91	13.35	1370–1800	0.091–0.070	0.015–0.020	0.202–0.265	7.5
BH	151.3 (36–∞)	114.0 (62–341)	— (65–∞)	—	—	854	0.177	0.133	—	10
CY	135.4 (55–∞)	50.1 (35–79)	646 (52–∞)	4.77	12.89	2235	0.061	0.022	0.289	5
GH	446.5 (80–∞)	24.4 (18–34)	— (90–∞)	—	—	6139	0.073	0.004	—	30
CV	141.6 (40–∞)	46.4 (33–70)	98 (75–∞)	0.69	2.11	12166	0.012	0.004–0.006	0.008–0.013	200
SBU	595 (32–∞)	—	— (48–∞)	—	—	5066–3850	0.117–0.155	—	—	10–15
SBD	155.5 (63–∞)	72.4 (47–133)	— (356–∞)	—	—	2780–2000	0.056–0.078	0.026–0.036	—	30
TB	— (108–∞)	47.4 (31–81)	74 (23–∞)	—	1.56	5296	—	0.009	0.014	120
BN	— (168–∞)	15.0 (12–17)	— (195–∞)	—	—	1417	—	0.011	—	—
RB	— (123–∞)	37.3 (25–63)	—	—	—	10833	—	0.003	—	—
HE	48.6 (27–∞)	18.8 (15–25)	89 (49–701)	1.83	4.73	1878	0.026	0.010	0.047	50
GK	102.5 (49–∞)	57.4 (36–111)	125 (75–949)	1.22	2.18	1299	0.079	0.044	0.096	20

estimates of  $N_b$  ranged from nearly fivefold larger than to around 30% smaller than unadjusted estimates of  $N_e$  derived from random samples of individuals of mixed ages. These results suggest that published estimates of effective population size obtained with random samples of individuals of mixed ages for iteroparous species with overlapping generations and even those based on unadjusted  $\hat{N}_b$  can be biased, and should thus be considered with caution. As seen in other studies [15–17], we also found that  $\hat{N}_{e(\text{adj2})}/\hat{N}_c$  ratios were variable across populations in the same geographical area, ranging from a high of 0.29 (CY) to a low of around 0.01 (e.g. CV, TB). These differences presumably reflect differences in population dynamics potentially mediated through differences among streams in environmental characteristics and productivity, which will probably affect the intensity of competition for access to mates, or for redd sites. In fact, we found that, given the observed age-specific survival rates and the known age-specific relative fecundities, very high variances in reproductive success in the order of  $V_k/k \approx 50\text{--}200$  are needed to exhibit  $\hat{N}_e/\hat{N}_c$  ratios in the range of 0.05–0.01. Irrespective of the differences, the majority of the  $\hat{N}_{e(\text{adj2})}/\hat{N}_c$  ratios were very low, the median ratio was 0.048, considerably smaller than the 0.20 median ratio described by Palstra & Ruzzante [4] for small salmonid populations. In this study, this ratio was between 20% and 30% in two populations (WW, CY) and was otherwise less than or equal to 0.1 (table 1).

Before discussing the results in detail, we raise four caveats. First, we have used estimates of age at first maturation ( $\alpha$ ), AL and generation length ( $G$ ) that were averages across populations. It is possible, indeed likely, that these parameters varied somewhat across populations, but much more intensive sampling would be required to accurately measure this variation. Second, our three  $\hat{N}_e$  estimates refer to slightly different though overlapping time periods. Mean  $\hat{N}_{e(\text{LD, mixed ages})}$  represents averages (harmonic means) over the period 2009–2013,  $\hat{N}_{e(\text{MLNe})}$  is a temporal estimate over the same period, and  $\hat{N}_{e(\text{adj2})}$  is derived from an annual effective size estimate ( $\hat{N}_b$ ) based on samples of 1+ year old individuals collected in 2013. The  $\hat{N}_c$  estimates were instead obtained in 2014 with some estimates repeated in 2015. Thus, our  $\hat{N}_e$  and  $\hat{N}_c$  estimates do not refer to the same time period and this may have introduced an unknown bias in our ratio estimates (5) if population size varies dramatically between years. In our analysis, we have thus assumed population size remained constant between years. Third, 23 of the 31 samples exhibited between one and four loci out of HWE even after correction for multiple tests. These departures probably reflect real biological phenomena including the combined effect of small effective sizes and age structure, where cohorts differ in their allele frequencies resulting in an intercohort Wahlund effect, along with the potential existence of siblings and spatial or deme structure within streams. The extent to which these departures from HWE affect downstream analyses has unfortunately not been examined in detail [36]. Fourth, the  $N_e$  temporal estimates were generally smaller than those based on LD. This is not surprising given that the temporal method is expected to provide downwardly biased estimates when generations are not discrete and fewer than three to five generations have elapsed between samples. Regardless of this downward bias, the  $(\hat{N}_{e(\text{MLNe})}/\hat{N}_c)$  ratios differed among populations, and we inferred this is due to differences in reproductive dynamics among populations. In doing so, we assumed the

potential bias introduced by age structure does not differ among populations.

### (a) Effective number of breeders to effective population size ( $(\hat{N}_{b(\text{adj2})}/\hat{N}_{e(\text{LD, mixed ages})})$ ratios

We examined the relation between the annual effective size ( $N_b$ ) and the generational effective population size ( $\hat{N}_e$ ). This relationship had until recently been examined in detail largely only for semelparous age-structured species [37,38] (but see [8,39]). Following these studies, it was assumed that in iteroparous species, effective sizes were  $\hat{N}_e \leq \hat{N}_b * G$ , where  $G$  is generation length, which in turn implies that  $\hat{N}_b \leq \hat{N}_e$  [10,40]. It has since been shown that this is not necessarily true and that in iteroparous species  $\hat{N}_b$  can indeed be  $>\hat{N}_e$ , with evidence the  $\hat{N}_b/\hat{N}_e$  ratio reported to vary across species by a factor of 6 from 0.3 to 1.6 [10]. In fact, here we show a similar range of variation in this ratio among the six populations for which we were able to estimate both  $\hat{N}_{b(\text{adj2})}$  and  $\hat{N}_{e(\text{LD, mixed ages})}$ ;  $\hat{N}_{b(\text{adj2})}/\hat{N}_{e(\text{LD, mixed ages})} = 1.66$  in one population (CY),  $\hat{N}_{b(\text{adj2})}/\hat{N}_{e(\text{LD, mixed ages})} \approx 1$  in another (WW) and  $\hat{N}_{b(\text{adj2})}/\hat{N}_{e(\text{LD, mixed ages})}$  between 0.63 and 0.24 in the remaining four populations. These wide interpopulation differences in  $\hat{N}_b/\hat{N}_e$  ratios suggest, again, the existence of important differences in population dynamics across streams within a single reproductive season (see above). An issue currently under investigation in our laboratory concerns the degree to which within-population temporal changes in  $\hat{N}_b$  match the among-population differences in  $\hat{N}_b$  and how they relate to variation in  $\hat{N}_c$  (see below and [37,38]). It should be noted though, that estimates of  $N_{e(\text{LD, mixed ages})}$  have been shown to provide estimates of  $N_e$  that are up to 93% of the true  $N_e$  in species with life histories similar to those of our brook trout populations [10]. In other words,  $N_e$  is supposed to be slightly underestimated when using a random sample of individuals of mixed ages. In fact, the majority (five out of six) of our  $\hat{N}_{e(\text{LD, mixed ages})}$  are smaller than  $\hat{N}_{e(\text{adj2})}$ . In the following section, we discuss our analyses of the ratios of effective to census population size based on both  $\hat{N}_{e(\text{LD, mixed ages})}$  and  $\hat{N}_{e(\text{adj2})}$  (table 1).

### (b) Effective population size to census population size ( $(\hat{N}_e/\hat{N}_c)$ ratios

Census population size was estimated by extrapolating fish density over the electrofished streambed section (estimated through mark recapture), to the length of the stream over which there was no obvious barrier to fish movement. This scale is well within the range of adult brook trout movement [41,42]. Our results indicate that at this spatial scale, the  $(\hat{N}_e/\hat{N}_c)$  ratios are both low relative to the ratios generally expected in small populations of salmonids ( $\hat{N}_e/\hat{N}_c \sim 0.20$ , [4]) and extremely variable across populations (table 1).

Palstra & Fraser [5] argued that uncertainty in the estimates of effective population size ( $\hat{N}_e$ ) translates into uncertainty into the ratios  $(\hat{N}_e/\hat{N}_c)$ . Although we have considerably reduced the uncertainty in the individual  $\hat{N}_e$  estimates, we still find major differences in the  $\hat{N}_e/\hat{N}_c$  ratios across populations within a single species. These results suggest that uncertainty is not the only cause, perhaps not even the major cause for intraspecific variation in these ratios. The variation probably reflects a real biological

phenomenon and is a function of variation in the effective number of breeders (see also [14,39,43]) and in reproductive success. In fact, given the estimated age-specific survival rates and age- or size-specific relative frequencies, we have shown with AGENE [34] that very high variances in reproductive success (between 50 and 100 times higher than the mean reproductive success) are necessary to generate  $\hat{N}_e/\hat{N}_c$  in the 0.05–0.02 range (200 times higher to generate ratios  $\approx 0.01$ ).

Theory predicts that  $N_e/N_c$  ratios should be low in species with early maturity and long reproductive lifespan because of the large variance in reproductive success that can ensue from such combination of life-history traits [10]. Here we have shown that these ratios can also be low even when AL and age at maturity are relatively short. Our estimates reflect the  $N_e/N_c$  ratios across a number of brook trout populations at a particular point in time. We have not quantified how these ratios vary over time within populations and how they relate to variation in  $\hat{N}_c$ ; this is the focus of our ongoing work. Long-lived sturgeon, for instance, have been described as having relatively stable interannual variation in  $\hat{N}_b/\hat{N}_c$  ratios (0.27–0.86) despite a 40-fold difference in annual larval production [44]. This result was attributed to a low variance in reproductive success probably stemming from the polygamous mating system and the large number of adults breeding in a temporally stable population [44]. Similarly, recent studies on stream brook trout populations found that  $\hat{N}_b$  was stable relative to  $\hat{N}_c$  with no evidence of a relationship between these two metrics but a link between  $\hat{N}_b$  and stream

flow [39]. Understanding relationships between  $N_e$ ,  $N_b$  and  $N_c$  and how these relate to population dynamics and fluctuations in population size are important for the design of robust strategies for species of conservation concern, and we encourage further efforts to improve our understanding of these relationships.

**Ethics.** The sampling was conducted under fishing permit no. 321158 issued to the Inland Fisheries Division of the Nova Scotia Department of Fisheries and Aquaculture.

**Data accessibility.** Genotypic data have been uploaded to Dryad and are available for download: <http://dx.doi.org/10.5061/dryad.nh448>.

**Author contributions.** D.E.R. and S.J.W. designed the study. K.H., S.P. and G.R.M. produced the molecular data and conducted initial analyses as part of two honours theses (K.H. 2010, S.P. 2014). A.C. and J.M. conducted the analysis on life-history traits. D.E.R. and G.R.M. conducted further analysis. D.E.R. wrote the first draft with input from S.J.W., G.R.M. and J.M. All authors contributed to fieldwork.

**Competing interests.** We declare we have no competing interests.

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