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Effective number of breeders provides a link between interannual variation in stream flow and individual reproductive contribution in a stream salmonid

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Abstract

The effective number of breeders that give rise to a cohort (N_b) is a promising metric for genetic monitoring of species with overlapping generations; however, more work is needed to understand factors that contribute to variation in this measure in natural populations. We tested hypotheses related to interannual variation in N_b in two long-term studies of brook trout populations. We found no supporting evidence for our initial hypothesis that $\hat{N}_b$ reflects $\hat{N}_c$ (defined as the number of adults in a population at the time of reproduction). $\hat{N}_b$ was stable relative to $\hat{N}_C$ and did not follow trends in abundance (one stream negative, the other positive). We used stream flow estimates to test the alternative hypothesis that environmental factors constrain N_b. We observed an intermediate optimum autumn stream flow for both $\hat{N}_b$ ($R^2 = 0.73$, $P = 0.02$) and full-sibling family evenness ($R^2 = 0.77$, $P = 0.01$) in one population and a negative correlation between autumn stream flow and full-sib family evenness in the other population ($r = -0.95$, $P = 0.02$). Evidence for greater reproductive skew at the lowest and highest autumn flow was consistent with suboptimal conditions at flow extremes. A series of additional tests provided no supporting evidence for a related hypothesis that density-dependent reproductive success was responsible for the lack of relationship between N_b and N_C (so-called genetic compensation). This work provides evidence that N_b is a useful metric of population-specific individual reproductive contribution for genetic monitoring across populations and the link we provide between stream flow and N_b could be used to help predict population resilience to environmental change.

Keywords: effective number of breeders, genetic monitoring, linkage disequilibrium, stream fishes, stream flow

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Introduction

Effective population size (N_e) is one of the most fundamental variables in evolutionary biology and conservation genetics (Waples 2005; Luikart *et al.* 2010; Hare *et al.* 2011). N_e is defined as the size of a theoretical and ideal population affected by genetic drift at the same rate per generation as the population under consideration (Wright 1931). N_e allows prediction of a population's adaptive potential in response to environmental change because it is closely related to a population's vulnerability to genetic drift (Hare *et al.* 2011). For iteroparous organisms, it is challenging to reliably estimate the effective size of an entire generation, or generational N_e (Waples & Yokota 2007; Waples & Do

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2010; Waples *et al.* 2014). It is more straightforward to apply N_e estimators to single cohorts (defined as a group of individuals born in a given year and thus with the same age; Caswell 2001) to obtain an estimate of the effective number of breeders (N_b) that gave rise to that cohort (Waples 2005; Palstra & Fraser 2012; Whiteley *et al.* 2012; Waples *et al.* 2014). Variation in cohort-specific estimates of N_b appears to be largely influenced by the number and size of families that occur within a cohort, hereafter referred to as that cohort's family structure. Thus, major influences on N_b include number of families an individual contributes to, the size of those families, and whether survival from fertilization to the time of sampling is family dependent (Waples & Do 2010; Christie *et al.* 2012). For age-structured species, N_b is more relevant than N_e for understanding eco-evolutionary processes that unfold during breeding cycles, such as sexual selection (Waples & Antao 2014; Waples *et al.* 2014).

Estimates of N_b would be useful for tracking population trends if $\hat{N}_b$ reflects the number of adults in a population at the time of reproduction ($\hat{N}_C$, Hypothesis 1, Fig. S1, Supporting information). In this case, $\hat{N}_b$ would provide a complementary metric to more intensive abundance monitoring or possibly could be used in the place of abundance monitoring (Tallmon *et al.* 2010). A correlation between $\hat{N}_b$ and $\hat{N}_C$ would require that successful reproductive contribution is proportional to adult abundanc and that variance in reproductive success does not increase with abundance. A significant positive correlation between $\hat{N}_b$ and $\hat{N}_C$ has been detected in some long-term studies (Osborne *et al.* 2010; Charlier *et al.* 2012) but not in others (Ardren & Kapuscinski 2003; Palstra *et al.* 2009; Berry & Kirkwood 2010; Serbezov *et al.* 2012; Duong *et al.* 2013; Johnstone *et al.* 2013; Dowling *et al.* 2014).

A lack of relationship between $\hat{N}_b$ and $\hat{N}_C$ could be due to environmental factors that constrain N_b (Hypothesis 2, Fig. S1, Supporting information). Environmental factors that influence the number or variation in size of families produced or that affect family-dependent survival could constrain N_b for a given population (Christie *et al.* 2012). This could include environmental factors that limit quantity of reproductive habitat, and therefore adult density, or reproductive habitat quality (Belmar-Lucero *et al.* 2012). Adult density could influence reproductive success and N_b through competition for mates or mating locations or possibly through environmental sensitivity of age at maturity (Belmar-Lucero *et al.* 2012). Higher habitat quality could also allow greater family production (contribution to more families in one season or breeding cycle) in a manner independent of adult densities. Support for this hypothesis would suggest that cohort-specific $\hat{N}_b$ is a useful population-specific indicator of the number of adults that successfully contribute to a cohort and the variance in that contribution. In other words, $\hat{N}_b$ would summarize successful individual reproductive contribution per breeding cycle for a population. This broad formulation of an environmental constraint on N_b hypothesis has received little attention to date (but see Belmar-Lucero *et al.* 2012; Wood *et al.* 2014). However, a more restrictive hypothesis related to density-dependent reproductive success has received more attention. The hypothesis that positive density-dependent reproductive success causes the relationship between $\hat{N}_b$ and $\hat{N}_C$ to be flat or negative (Hypothesis 2a, Fig. S1, Supporting information) has been termed 'genetic compensation' (Ardren & Kapuscinski 2003). Evidence for genetic compensation comes from several studies of rainbow trout (*Oncorhynchus mykiss*) (Ardren & Kapuscinski 2003; Araki *et al.* 2007) and may generally hold across salmonid fishes (but see concerns raised by Palstra & Ruzzante 2008).

We focus on habitat-dependent constraints on N_b in stream-dwelling salmonids (trout, char, salmon). Work with salmonids has revealed that stream flow is an important environmental factor that can be used to help predict species distributions under climate change (Wenger *et al.* 2011; Isaak *et al.* 2015). Other work has demonstrated strong relationships between stream flow and demographic rates (Letcher *et al.* 2014). As variance in reproductive success, the number of families produced and family-dependent survival have a large influence on N_b (Waples & Do 2010; Christie *et al.* 2012), the number of successful redd (nest) sites should be the environmental factor most likely to influence N_b for these species. We hypothesize that interannual variation in stream flow is the environmental factor most likely to influence redd site quantity and quality, and therefore redd success. Low flow during reproduction might limit spawning habitat and influence N_b via density-dependent effects on reproductive success. That is, at high density, there might be more competition for spawning territories (Blanchfield *et al.* 2003), more competition for mates, higher redd superimposition (Chebanov 1991; Blanchfield & Ridgway 2005), elevated embryo mortality caused by delayed spawning (Springate *et al.* 1984) or a combination of these factors (Ardren & Kapuscinski 2003). Both low and high flow during reproduction might create elevated habitat heterogeneity and suboptimal spawning conditions that, in the absence of uniform influence on all reproducing adults, could lead to elevated skew in reproductive success. Flow conditions during egg incubation or the early juvenile stage might also cause reproductive skew by influencing family-dependent survival. Extreme flows at these life stages might limit habitat or food availability

(low flow) or destroy entire families (high flow). To our knowledge, this study will provide the first attempt to link interannual variation in stream flow with a key variable (N_b) that influences contemporary eco-evolutionary dynamics (Waples & Antao 2014; Waples *et al.* 2014).

Here, we use genetic and demographic data collected from two long-term brook trout (*Salvelinus fontinalis*) studies to test hypotheses related to variation in $\hat{N}_b$ over time. First, we tested whether $\hat{N}_b$ reflects $\hat{N}_C$ (Hypothesis 1 in Fig. S1, Supporting information) by examining the correlation between these two measures in each long-term study population. Finding no correlation, we tested the second hypothesis that environmental factors constrain N_b through effects on variance in reproductive success (Hypothesis 2 in Fig. S1, Supporting information) and more narrowly that environmental constraints on N_b act through density-dependent variance in reproductive success (Hypothesis 2a in Fig. S1, Supporting information). Specifically, our tests provide some of the most comprehensive support to date that cohort-specific $\hat{N}_b$ summarizes successful individual reproductive contribution per breeding cycle for a population because of underlying relationships to the quantity or quality of available reproductive and juvenile rearing habitat.

Methods

Study species and sites

The brook trout is a cold-water indicator species in eastern North America (Hudy *et al.* 2008). Competition with non-native species and habitat fragmentation and degradation threaten its persistence (Hudy *et al.* 2008). Whiteley *et al.* (2012) demonstrated that N_b can be reliably and precisely estimated for brook trout, based on a single cohort [young-of-the-year (YOY)] and a sampling design that avoids family over-representation. Long-term studies within the native eastern distribution of brook trout provide detailed demographic data (Letcher *et al.* 2007, 2014) and an opportunity to examine N_b over time in multiple populations.

We use data from two long-term brook trout study sites in Massachusetts (West Brook) and Virginia (Fridley Gap). West Brook (hereafter WB) is a headwater stream in western Massachusetts, USA, described in detail by Letcher *et al.* (2007) and Kanno *et al.* (2014). The study area consists of a 1-km-long third-order mainstem (mainstem West Brook, WB; mean wetted width = 4.5 m), and two 0.3-km-long second-order tributaries (Open Large, OL; mean wetted width = 3 m and Open Small, OS; mean wetted width = 2 m). Impassable waterfalls are located at the upstream boundaries of the two tributary study reaches. Fish in the mainstem move regularly between the two tributaries (Kanno *et al.* 2014). Passive integrated transponder (PIT) tag antennas are located at the upstream and downstream boundaries of the mainstem and at the mouths of the two tributaries (Kanno *et al.* 2014). Fish were sampled in a spatially continuous manner on four occasions per year (spring = late March, summer = June, autumn = late September and early October, winter = early December) using double-pass electrofishing and block nets at the ends of 20-m sections (Kanno *et al.* 2014). The mainstem has been sampled since 2000 and sampling of the tributaries began in 2002. For genetic analyses within WB, we used entire cohort samples (Table 1). This included fish of any age at initial capture that could be assigned to a given cohort. Cohort assignments are based on length frequency histograms and a growth model (Letcher *et al.* 2014). For genetic analyses, we pooled fish from the mainstem and two tributaries based on evidence that this population acts as a functional metapopulation with high connectivity (Kanno *et al.* 2014). Upon capture, fish were measured, weighed and PIT-tagged, and an anal fin clip was taken as a tissue sample.

Fridley Gap (hereafter FG) is a 1.8-km-long headwater stream in northcentral Virginia, USA, described in detail in Hudy *et al.* (2010). This population was re-established from a single source in 1993 after limestone remediation to counter low pH (Hudy *et al.* 2000). The source population (DV-a; Whiteley *et al.* 2013) was located in a nearby stream (37 km away) and possessed above average genetic diversity for this species (approximately 11 alleles per locus; Whiteley *et al.* 2013). A small dam occurs at the downstream extent of fish habitat. A small tributary (250 m long, mean wetted width = 1.8 m) enters the mainstem 1.5 km upstream of the dam. Mean wetted width of the mainstem is 3.8 m. The upstream extent of the brook trout distribution is determined by intermittent flows in the headwaters. Fish were sampled throughout the available habitat with single-pass electrofishing in summer (July) beginning in 2004. Upon capture, fish were measured and an anal fin clip was taken for a source of genetic material. For genetic analyses within FG, we used all YOY captured each summer to represent a given cohort.

Genetic analyses

We examined variation at either 12 (WB) or eight (FG) microsatellite loci in 6177 fish from WB and 1374 fish from FG (Table 1). DNA was extracted from fin clip tissue samples following a standard salt precipitation procedure. We used the following eight loci for all populations: *Sfo-C113*, *Sfo-C88*, *Sfo-D75*, *Sfo-D100*, *Sfo-C24*, *Sfo-C115*, *Sfo-C129* (King *et al.* 2012) and *Ssa-D237*

Table 1 Genetic summary statistics and $\hat{N}_b$ for brook trout cohorts from two long-term study sites

Cohort	N_G	HW	LD	A_R	H_S	F_{IS}	$\hat{N}_{fam}$	$\hat{FE}$	NB mean	σ^2	$\hat{N}_{b-LDNe}$	$\hat{N}_{b-fam}$	$\hat{N}_C$
West Brook (WB)													
2001	1094	10	61	9.0	0.614	0.058	340	0.935	3.2	7.5	124.4 (110.4–139.3)	368.7	–
2002	789	11	65	9.2	0.635	0.067	219	0.898	3.6	11.8	94.6 (84.7–105.3)	131.0	–
2003	909	9	61	8.7	0.614	0.039	269	0.914	3.4	10.1	152.7 (131.2–176.8)	234.7	1075.3 (618.1–3218.2)
2004	816	10	64	8.9	0.632	0.019	269	0.919	3.0	7.7	108.6 (95.3–123.2)	241.0	704.3 (625.1–818.7)
2005	590	5	60	9.5	0.620	0.040	242	0.936	2.4	4.3	157.3 (139.6–177.0)	275.6	470.6 (432.0–522.2)
2006	432	11	58	9.6	0.631	0.018	116	0.855	3.7	15.8	52.8 (45.5–60.9)	67.1	579.7 (526.7–666.6)
2007	288	4	30	9.4	0.614	0.033	139	0.953	2.1	2.4	141.2 (121.6–164.5)	243.8	352.8 (307.4–413.3)
2008	513	10	60	8.3	0.607	0.019	143	0.879	3.6	12.6	65.1 (55.8–75.5)	79.1	220.0 (193.6–265.2)
2009	746	9	62	9.0	0.610	0.032	180	0.916	4.1	14.3	109.3 (95.5–124.6)	182.5	208.0 (182.3–257.1)
Fridley Gap (FG)													
2004	899	7	28	9.5	0.787	0.055	177	0.896	5.1	26.2	116.6 (98.6–136.9)	–	439.9
2006	96	1	10	9.3	0.778	0.077	49	0.938	2.0	2.4	118.7 (87.8–168.8)	–	416
2009	69	2	7	9.3	0.773	0.070	37	0.955	1.9	1.9	82.5 (63.7–110.6)	–	612 (489.5–734.6)
2010	99	1	6	9.5	0.788	0.053	52	0.953	1.9	1.9	131.2 (95.0–192.7)	–	1028 (901.4–1154.3)
2011	211	4	25	9.3	0.783	0.056	96	0.940	2.2	3.6	101.6 (88.3–117.1)	–	1004 (882–1145)

Cohorts are defined by the year of emergence. N_G is the number of individuals genotyped per cohort. HW is the number of significant tests for departures from Hardy–Weinberg proportions following B-Y FDR correction for 108 (WB) or 40 (FG) tests ($\alpha = 0.05$). LD is the number of significant tests for linkage disequilibrium following B-Y FDR correction for 594 (WB) or 140 (FG) tests ($\alpha = 0.05$). A_R is mean allelic richness, based on the minimum sample size of 288 (WB) or 69 (FG) individuals. H_S is mean expected heterozygosity. F_{IS} is a measure of departure from HW proportions. $\hat{N}_{fam}$ is the number of estimated full-sib families. $\hat{FE}$ is family evenness, a measure inversely related to variance in the full-sib family distribution of each cohort. NB mean and variance (σ^2) are from a negative binomial distribution fitted to full-sib family distributions of each cohort. $\hat{N}_{b-LDNe}$ is the effective number of breeders estimated with LDNe, shown with 95% CI. $\hat{N}_{b-fam}$ is based on equation 6 from Waples & Waples (2011). $\hat{N}_C$ (shown with 95% CI) is the number of adults (age-1 and older) estimated from the fall previous to the listed spring-defined cohort. For years lacking probability of detection (p) estimates, we used average p across years and calculated a point estimate for abundance only. For WB, only the mainstem was sampled in 2001, and therefore, the connected tributaries (OS and OL) are not included in these estimates.

(King *et al.* 2005). Four additional loci were added for the WB: *Sfo-C38*, *Sfo-C86*, *Sfo-B52* and *Sfo-D91a* (King *et al.* 2012). DNA extraction and amplification followed Whiteley *et al.* (2013). Loci were electrophoresed on an ABI Prism 3130xl genetic analyser (Applied Biosystems Inc., Foster City, California), and alleles were hand-scored using GENEMAPPER version 3.2 and PEAK SCANNER version 1.0 (Applied Biosystems Inc.). Positive controls of brook trout with known genotypes were used for each set of PCR and electrophoresis.

We calculated summary statistics and tested for violations of Hardy–Weinberg (HW) proportions and linkage (gametic) disequilibrium (LD) for each cohort within each population. We used CREATE version 1.33 (Coombs *et al.* 2008) to make input files for FSTAT version 2.9.3.2 (Goudet 2001) and GENEPOP version 4.0.10 (Rousset 2008). We calculated mean number of alleles per cohort (A_O); mean allelic richness, standardized to the cohort with the lowest number of individuals (A_R); mean expected heterozygosity (H_S); and F_{IS} for each cohort (Table 1). In FG, two very large alleles (> 795 bp) were discovered and scored accurately in one cohort (2011) but were scored as a null allele in the earlier four cohorts. We chose to retain this locus in the analysis because comparisons within the cohort where we scored the two large alleles indicated little difference in point estimates or confidence intervals of $\hat{N}_b$ with or without their inclusion (see Appendix S1).

Tests for departures from HW proportions within each cohort across loci and for LD were performed with GENEPOP. The loci used here have generally conformed to HW proportions and show negligible signal of LD (Kanno *et al.* 2011; Annett *et al.* 2012; Kazyak *et al.* 2015), except when large single-cohort samples have been examined (Whiteley *et al.* 2013). The later study found that elevated variance in family size was an important predictor of significant HW departures and LD. Because we had large sample sizes per cohort (range 288–1094 for WB, 69–899 for FG) and many large full-sib families within cohorts (details about estimation below), we suspected that we would observe substantial signal of HW deviations and LD due to both statistical (large sample size) and biological (family- and population-level structure) reasons (Meirmans 2015). To aid in interpretations, we chose to use both a less conservative (B-Y FDR; Benjamini & Yekutieli 2001; Narum 2006) and highly conservative (Bonferroni) correction for multiple tests. We also randomly sampled one full-sib per

family (hereafter $RS_{1perfam}$) following Whiteley *et al.* (2013) to minimize full-sib family effects on tests for deviations from HW proportions and LD. It is possible that the number of significant tests would decline with this subsampling procedure due to reduced sample size and the associated reduction in power. To account for possible reductions in power associated with subsampling, we also randomly selected the number of individuals equal to the estimated number of full-sib families for each cohort without consideration of each individual's full-sib family membership (hereafter RS).

We assessed genotyping error by randomly selecting 100 individuals and performing a second DNA extraction and amplification of all 12 loci in WB. Ninety-one of the 100 individuals yielded complete genotypes. Seven of 2184 allele scores differed, resulting in a per allele error rate of 0.32%. A single individual accounted for four of the seven differing alleles and was likely due to a process error for that individual.

We used the program LDNE version 1.31 (Waples & Do 2008) to obtain estimates of N_b ($\hat{N}_b$) for each cohort (Table 1). This is the most extensively tested single-sample effective population size estimator (Luikart *et al.* 2010). A monogamous mating model was assumed because brook trout appear to conform much more closely to monogamy than random mating (Coombs 2010). N_b estimates were derived using a minimum allele frequency cut-off (P_{crit}) of 0.02. P_{crit} = 0.02 has been shown to provide an adequate balance between precision and bias across sample sizes (Waples & Do 2008). 95% confidence intervals were generated using the jackknife approach. We also used equation 6 from Waples & Waples (2011) to estimate N_b (hereafter $\hat{N}_{b-fam}$) as:

$$\hat{N}_b = \frac{2S - 2}{\left(\left(\sum(k_i^2)/2s\right) - 1\right)}$$

where S is the total number of offspring in a cohort and k_i equals the number of offspring contributed by parent i. For data without a known pedigree, this approach requires estimation of family groups. We implemented this approach with estimates of the number and size of full-sibling families for each cohort with the assumption of a monogamous mating system (no half-sib families). This method is expected to be highly sensitive to sample size because it relies on an accurate estimate of the distribution of full-sib families. Therefore, we only estimated $\hat{N}_{b-fam}$ for WB because we used entire cohort samples for this site.

We used COLONY version 1.2 (Wang 2004) for full-sib family reconstruction. A previous study based on empirically parameterized simulations confirmed high accuracies of sibship reconstruction in WB based on the same 12 microsatellite loci using COLONY version 1.2 (Letcher *et al.* 2011). Reconstructed full-sib families composed of at least two individuals had a rate of correct family inference of 91.2% (0.7% SE). For full-sib families composed of at least five individuals, the rate of correct family inference was 97.7% (0.4% SE) (Letcher *et al.* 2011). Other single-sample estimators of $\hat{N}_b$ are available (Tallmon *et al.* 2008; Wang 2009) but appear to be biased when applied to simulated data from our focal populations (A. R. Whiteley, unpublished data).

Full-sib family reconstruction allowed us to examine the influence of family structure (cohort-specific number and size of families) on estimates of N_b. We obtained estimates of the number of full-sibling families produced ($\hat{N}_{fam}$) from COLONY. We estimated family evenness ($\hat{FE}$) as a measure of variance in family size (Whiteley *et al.* 2013). $\hat{FE}$ was calculated as $FE = H'/H'_{Max}$, where $H' = -\sum_1^S p_i \ln(p_i)$ and $H'_{Max} = \ln(S)$ (Mulder *et al.* 2004). S, which usually represents the number of species in an evenness calculation, here represented the number of families and p_i represented the proportion comprised of the i-th family. We also calculated the mean (μ) and overdispersion parameter (k) for a negative binomial distribution fit to the full-sib family distribution for each cohort with the MASS package in program R version 3.1.1 (R Development Core Team 2006). We calculated the variance of these negative binomial distributions as $\mu + \mu^2/k$ (Bolker 2008).

The relationship between N_e and N_b might lead to bias in $\hat{N}_b$ for species with overlapping generations (Waples *et al.* 2014), such as the brook trout. To accommodate this, Waples *et al.* (2014) introduced a bias correction based on life history information. We constructed a life table from demographic data in WB (Letcher *et al.* 2014) (Table S1, Supporting information). We then used program AGENE (Waples *et al.* 2011) to calculate the N_b-to-N_e ratio. From AGENE, $\hat{N}_b$ was 70 and $\hat{N}_e$ was 100 (N_b/N_e ratio = 0.70). Applying equation 8 from Waples *et al.* (2011) reveals that $\hat{N}_b$ from our study could be biased high by approximately 3.4%. We chose not to apply this bias correction because we were interested in comparing relative $\hat{N}_b$ over time and the bias correction would not influence these comparisons.

$\hat{N}_b$ can also be biased by population substructure. Exclusion of habitat that is important for reproduction or inclusion of cryptically genetically differentiated sites can both lead to bias (Waples & England 2011; Neel *et al.* 2013). For WB, we used a metapopulation estimate of N_b based on the mainstem and two connected tributaries. These three sites exhibit low genetic differentiation (mean pairwise F_{ST} of overyearlings = 0.03; Kanno *et al.* 2014) and include important spawning and early rearing habitat as well as habitat used for adult growth (Kanno *et al.* 2014; Letcher *et al.* 2014). This spatial scale of analysis allowed us to avoid possible biases (likely

downwards) that might have occurred had we estimated N_b for only part of the metapopulation. Population substructure was not an important factor in FG (Hudy *et al.* 2010).

Demographic measures

We define N_C as the number of adults in a population at a specific point in time (Luikart *et al.* 2010). For brook trout, this includes all age-1 fish and older because both sexes tend to start reproducing at age-1, although age at maturity is variable in this species (Hutchings 1994). Further, age-0 brook trout (YOY) are readily field identifiable based on body size (Hudy *et al.* 2010). In each study system, we estimated $\hat{N}_C$ and $\hat{N}_{YOY}$ based on autumn (WB) or summer (FG) samples, because YOY can be distinguished from overyearlings in summer or autumn and we wanted to compare estimates of annual census sizes (either $\hat{N}_C$ or $\hat{N}_{YOY}$) to annual $\hat{N}_b$. A given $\hat{N}_b$ corresponded to each spring-defined cohort for year t (Waples 2005; Charlier *et al.* 2012). The appropriate $\hat{N}_C$ for comparison to the $\hat{N}_b$ from year t was from the previous year ($t - 1$) because that was when reproduction that gave rise to a spring-born cohort occurred (Waples 2005; Charlier *et al.* 2012). The appropriate $\hat{N}_{YOY}$ for comparison to the $\hat{N}_b$ from year t was from summer or autumn of the same year a cohort was born (year t).

For WB, demographic measures were obtained from a long-term individual-based field study (Letcher *et al.* 2014). $\hat{N}_C$ was estimated as the count of age-1 and older fish divided by the probability of capture (p). We obtained estimates of $\hat{p}$ from a CJS model (Letcher *et al.* 2014). Fish age or body size was not included in this model. To incorporate uncertainty, we subsampled $\hat{p}$ from each of 200 chains of the model described in Letcher *et al.* (2014). We divided season-specific (autumn) and river-specific (WB, OL or OS) fish counts (YOY or adults) by river-specific autumn subsampled $\hat{p}$ for each of the 200 chains to obtain a distribution of abundance estimates per year and per river. We summarized these distributions with the median, 5% and 95% quantiles. We summed each of these estimates from WB, OL and OS to obtain a point estimate and 95% confidence interval for metapopulation $\hat{N}_C$ and $\hat{N}_{YOY}$ for each year. We used the same $\hat{p}$ for YOY and adults; however, $\hat{p}$ for YOY is generally lower than it is for adults (Letcher *et al.* 2014). Therefore, the $\hat{N}_{YOY}$ reported here were likely to be biased low. Demographic estimates were available from 2002 to 2009 for WB. Therefore, $\hat{N}_C$ were available for comparison to the 2003–2009 cohorts because of the 1-year time lag. $\hat{N}_{YOY}$ were available for comparison to the 2002–2009 cohorts.

For FG, $\hat{N}_C$ and $\hat{N}_{YOY}$ were based on single-pass electrofishing capture–mark–recapture (CMR) estimates. We used the Lincoln–Petersen model (Seber 1982) based on two sampling events separated by at most 2 weeks. We calculated the standard error and used this to calculate 95% confidence intervals (Smith & Smith 2001). CMR was performed in 2006–2011. To obtain point estimates for summer 2003 adults (for comparison to 2004 cohort $\hat{N}_b$), summer 2004 YOY (for comparison to 2004 cohort $\hat{N}_b$) and summer 2005 adults (for comparison to 2006 cohort $\hat{N}_b$), we divided counts by 2006–2011 CMR-based mean $\hat{p}$ for adults (0.511) and YOY (0.209) separately. CMR-based abundance estimates were available for all other cohorts for which we obtained $\hat{N}_b$.

Habitat constraints on $\hat{N}_b$

We initially tested for constraints on $\hat{N}_b$ by examining the stability of $\hat{N}_b$ relative to $\hat{N}_C$. More stable measures over time should have lower coefficients of variation (CV). We calculated unbiased CV (Sokal & Rohlf 1995). We used the harmonic mean as the measure of central tendency for $\hat{N}_b$, $\hat{N}_C$ and $\hat{N}_{YOY}$.

Constraints on $\hat{N}_b$ might occur through environmental effects on habitat quantity or quality. We tested the influence of stream flow during reproduction (autumn), egg incubation (winter) and the early juvenile life stage (spring) on variation in $\hat{N}_b$. Autumn stream flow represented a surrogate for spawning habitat quality and availability. We used estimates of autumn discharge from a 3-month window from 1 October to 31 December. This window includes time preceding, during and immediately following reproduction, which occurs from late October to mid-November. As a surrogate for egg incubation and overwintering habitat quality, we used estimates of winter discharge (Kovach *et al.* 2015). The 2-month window from 1 January to 28 February was used for winter flow. As a surrogate for early rearing habitat quality, we used spring discharge for a 3-month window from 1 March to 31 May. The spring measure was correlated with 4-month (1 February to 31 May, $r = 0.93$, $P = 0.0003$) and 5-month (1 February to 30 June, $r = 0.90$, $P = 0.0009$) windows in WB. We retained the 3-month spring window for subsequent analyses. If we found significant results for these larger windows of time in each season, we subsequently used monthly discharge estimates to help pinpoint the period with greatest environmental influence on $\hat{N}_b$.

We lagged autumn discharge estimates by 1 year (time $t - 1$) relative to spring-defined cohorts (time t). Winter and spring discharge estimates were from the same year as spring-defined cohorts (time t). Estimates of discharge were in cubic metres per second (cms). For WB, we used a flow extension model based on discharge from a USGS gauging station located on the Mill River (Xu *et al.* 2010). We used the mean of daily mean

flow over each window of time. For the period 6 February 2007 to 1 March 2007, ice prevented the gauge from taking a daily measure. We used mean daily flow of available days for that period. For FG, manual discharge measurements were measured monthly.

We used linear models separately with autumn discharge, winter discharge and spring discharge as explanatory factors and $\hat{N}_b$, $\hat{F}\hat{E}$ or $\hat{N}_{fam}$ as the response variables. For each of these three seasons, we fit a model with and without a quadratic term to test for intermediate optima. For FG, we tested for correlations with flow in each season and $\hat{N}_b$ because we lacked sufficient data to perform regressions with a quadratic term.

Genetic compensation

The genetic compensation hypothesis postulates that variance in reproductive success will be greater at high adult densities at the time of reproduction. Under genetic compensation, a series of predictions can be tested. (i) A negative relationship between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_C$ is predicted (Ardren & Kapuscinski 2003). Low $\hat{N}_C$ should be associated with relatively high $\hat{N}_b$ under genetic compensation; however, the need to standardize $\hat{N}_b$ by $\hat{N}_C$ has led to the common approach of testing the relationship between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_b$. However, this approach suffers from relating a ratio to its denominator (Palstra & Ruzzante 2008) and does not directly test for a relationship between variance in reproductive success and adult abundance or density (Araki *et al.* 2007). We used $\hat{F}\hat{E}$, as an inversely related surrogate for variance in reproductive success to more directly test for genetic compensation. (ii) $\hat{F}\hat{E}$ is predicted to be negatively correlated with adult abundance (high evenness at low adult abundance). (iii) The number of full-sib families produced relative to the number of breeding adults (ratio of $\hat{N}_{fam}$ to $\hat{N}_C$) is predicted to be positively correlated with $\hat{F}\hat{E}$. That is, reproductive cycles with few adults that produce a relatively large number of full-sib families (high value of this ratio) should be the reproductive bouts with the greatest evenness under genetic compensation.

For each study site, we tested correlations among the variables $\hat{N}_b$, $\hat{N}_C$, $\hat{N}_{YOY}$, $\hat{F}\hat{E}$, $\hat{N}_{fam}$ or specified ratios. The genetic compensation hypothesis specifically predicts that variance in reproductive success should vary with adult density at the time of reproduction. We calculated autumn surface areas (m^2) as mean width of each section (m) × section length (m) × total number of sections of each of the metapopulation components (mainstem, OS, OL). We calculated adult densities (number of fish/m^2) in WB by dividing metapopulation $\hat{N}_C$ by the summed surface areas of mainstem, OS and OL (Table S2, Supporting information). Mean metapopulation surface areas ranged from 6263 to 8300 m^2 (mean = 7204 m^2, SD = 811). $\hat{N}_C$ and adult densities were highly correlated ($r = 0.97$, $P = 0.0003$). We chose to use $\hat{N}_C$ throughout the manuscript because density estimates were not available for FG.

Results

Genetic variation

West Brook. Mean A_O per cohort ranged from 8.7 to 10.4, mean A_R (standardized to lowest sample size per cohort; $N_{2007} = 288$) ranged from 8.7 to 9.6, mean H_S ranged from 0.607 to 0.635, and mean F_{IS} ranged from 0.018 to 0.067 (Table 1). For the nine WB cohorts, significant departures from HW proportions occurred in 89 of 108 (82%) tests performed ($P < 0.05$), where five were expected by chance ($\alpha = 0.05$; Table 1). Following B-Y FDR correction for 108 tablewide tests ($\alpha = 0.05$), 79 (73%) tests remained significant (Table 1). Following Bonferroni correction for 108 tablewide tests ($\alpha = 0.05$), 69 (64%) tests remained significant. Randomly subsampling one individual per full-sib family ($RS_{1perfam}$) reduced but did not eliminate the signal of departures from HW proportions. Significant departures from HW proportions occurred in 23 of 108 (21%) tests following B-Y FDR correction. Following Bonferroni correction, seven of 108 (6%) tests remained significant. $RS_{1perfam}$ reduced the signal of deviations from HW proportions more than the RS subsampling procedure, which was meant to mimic the power reduction that occurred when we conducted the $RS_{1perfam}$ subsampling procedure. There remained 37 (34%; B-Y FDR) and 22 (20%; Bonferroni) significant tests after applying the RS subsampling procedure.

Significant LD was detected in 558 of 594 (94%) tests performed ($P < 0.05$), where 30 were expected by chance ($\alpha = 0.05$). Following B-Y FDR correction for 594 tablewide tests ($\alpha = 0.05$), 521 (88%) LD tests remained significant. Following tablewide Bonferroni correction, 445 (75%) LD tests remained significant. $RS_{1perfam}$ reduced the signal of LD. Significant LD occurred in 50 of 594 (8%) tests following B-Y FDR adjustment and 25 tests (4%) following Bonferroni correction. Substantially more signal of LD occurred with RS. Significant LD occurred in 229 of 594 (39%) tests following B-Y FDR adjustment and 135 tests (23%) following Bonferroni correction.

Fridley Gap. Mean A_O per cohort ranged from 9.3 to 10.9, mean A_R (standardized to $N_{2009} = 69$) ranged from 9.1 to 9.5, mean H_S ranged from 0.773 to 0.788, and F_{IS} ranged from 0.053 to 0.077. For the five FG cohorts,

significant departures from HW proportions occurred in 20 of 40 (50%) tests performed ($P < 0.05$), where two were expected by chance ($\alpha = 0.05$). Following B-Y FDR correction for 40 tablewide tests ($\alpha = 0.05$), 15 (38%) tests remained significant (Table 1). Following a tablewide Bonferroni correction, 13 (33%) tests remained significant. We observed fewer departures from HW proportions the $RS_{1perfam}$ subsampling procedure. Significant departures from HW proportions occurred in five of 40 (13%) tests following a tablewide B-Y FDR correction and four of 40 (10%) tests following Bonferroni correction. These four significant tests occurred with *Ssa*-D237, the locus known to have null alleles. For the 2011 cohort, where we removed the null allele problem, *Ssa-D237* conformed to HW proportions once one random full-sib per family was selected ($P = 0.672$). RS provided an intermediate number of significant tests for deviations from HW proportions (data not shown).

Significant LD was detected in 96 of 140 (69%) tests performed ($P < 0.05$), where seven were expected by chance ($\alpha = 0.05$). Following B-Y FDR correction for 140 tablewide tests ($\alpha = 0.05$), 76 (54%) tests remained significant (Table 1). Following Bonferroni correction for 140 tablewide tests ($\alpha = 0.05$), 59 (42%) tests remained significant. $RS_{1perfam}$ strongly reduced the LD signal. Significant LD occurred in three of 140 (2%) tests following B-Y FDR adjustment and one of 140 (0.71%) tests following Bonferroni correction. RS provided an intermediate number of significant tests for LD (data not shown).

Patterns of variation in $\hat{N}_b$ *and abundance*

West Brook. $\hat{N}_{b-LDNe}$ was highly correlated with $\hat{N}_{b-fam}$ ($r = 0.80$, $P = 0.01$; Fig. S2, Supporting information). Point estimates of $\hat{N}_{b-LDNe}$ based on entire cohorts and for the entire WB metapopulation ranged from 52.8 to 157.3 (Table 1, Fig. 1a). Point estimates of $\hat{N}_{b-fam}$ based on entire cohorts and for the entire WB metapopulation ranged from 67.1 to 368.7 (Table 1). The harmonic mean of $\hat{N}_{b-LDNe}$ across cohorts was 98.6 (SD = 36.6), and the harmonic mean of $\hat{N}_{b-fam}$ was 151.2 (SD = 97.6). $\hat{N}_{b-LDNe}$ was positively related to number of families ($\hat{N}_{fam}$; $r = 0.54$, $P = 0.13$) and family evenness ($\hat{FE}$; $r = 0.87$, $P = 0.002$) and negatively related to variance of the negative binomial distribution fitted to the full-sib family distribution of each cohort ($r = -0.76$, $P = 0.02$)(Fig. S3a–c, Supporting information). $\hat{N}_{b-fam}$ showed similar relationships (Fig. S3d–f, Supporting information). We focus on $\hat{N}_{b-LDNe}$ for the remainder of the study. $\hat{FE}$ and variance of the negative binomial distributions were highly correlated ($r = -0.86$, $P = 0.003$). We focus on $\hat{FE}$ as a surrogate for variance in reproductive success for the remainder of the study due to its stronger relationship with $\hat{N}_b$. Cohorts with relatively low $\hat{N}_b$ had more uneven full-sib family distributions (Figs S3b,e and S4, Supporting information). For example, the two lowest $\hat{N}_b$ occurred in the 2006 and 2008 cohorts when the family size distribution was skewed ($\hat{FE}$ was low; Fig. S4f,h, Supporting information). These cohorts with lower $\hat{N}_b$ also had fewer full-sib families (Fig. S4f,h, Supporting information). Large $\hat{N}_b$ (e.g. 2005 and 2007 cohorts) were associated with even family distributions, large numbers

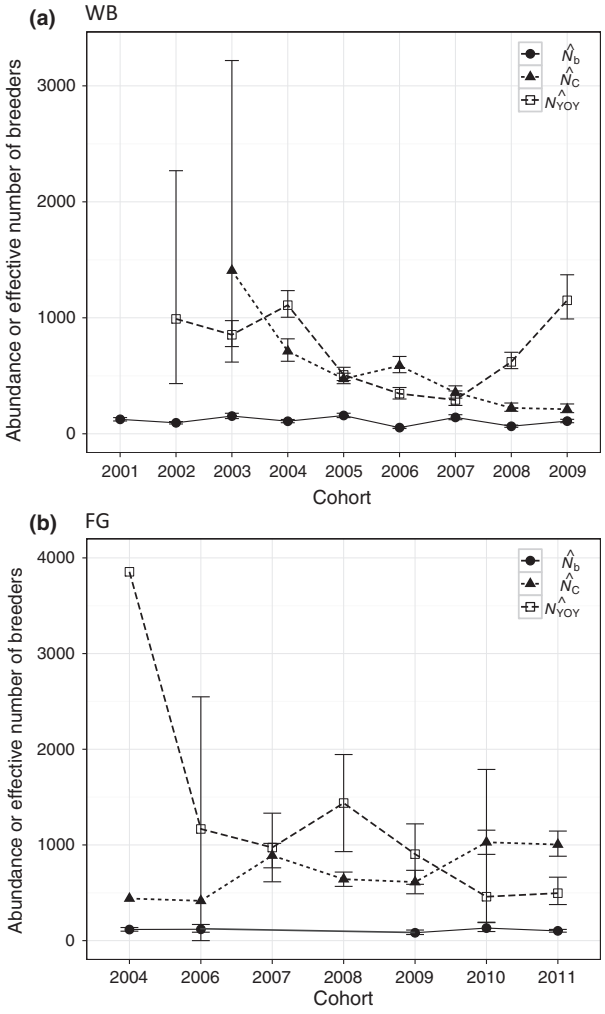

Fig. 1 Estimates of $\hat{N}_b$ and adult ($\hat{N}_C$) or juvenile ($\hat{N}_{YOY}$) abundance over time within the (a) Massachusetts (WB) and (b) Virginia (FG) brook trout study sites. $\hat{N}_b$ is shown with solid lines and filled circles. $\hat{N}_C$ is shown with dashed lines and closed triangles. $\hat{N}_C$ is lagged by 1 year relative to the spring-defined birth year of cohorts. That is, $\hat{N}_C$ represents the fall from the year prior to the year shown on the *x*-axis. $\hat{N}_{YOY}$ is shown with the dashed lines and open squares. $\hat{N}_{YOY}$ and $\hat{N}_b$ are from the same years as shown on the *x*-axis. $\hat{N}_b$ were estimated from the program LDNe ($\hat{N}_{b-LDNe}$).

of singleton full-sib families and fewer large families (Fig. S4e,g, Supporting information).

$\hat{N}_b$ was more stable than adult or juvenile abundance and did not vary in relation to either of these demographic measures in WB (Table S2, Supporting information, Fig. 1a). Variation in $\hat{N}_b$ occurred in a regular pattern (relatively high values followed by relatively low values) with no positive or negative trend (Table 1; Fig. 1a). The CV of $\hat{N}_b$ across cohorts was 0.34. $\hat{N}_C$ (lagged 1 year relative to $\hat{N}_b$) declined over the study period (Table S2, Supporting information; Fig. 1a). The correlation between $\hat{N}_C$ and $\hat{N}_b$ was positive but nonsignificant ($r = 0.34$, $P = 0.45$; Table S2, Supporting information, Fig. 1a). $\hat{N}_C$ had a higher coefficient of variation than $\hat{N}_b$ (harmonic mean = 380.7, SD = 306.5, CV = 0.80; Table S2, Supporting information; Fig. 1a). $\hat{N}_{YOY}$ was not correlated with $\hat{N}_b$ ($r = 0.03$, $P = 0.94$) and was less stable than $\hat{N}_b$ (harmonic mean = 556.6, SD = 345.6, CV = 0.62; Table S2, Supporting information; Fig. 1a). $\hat{N}_C$ and $\hat{N}_{YOY}$ were also not correlated ($r = 0.16$, $P = 0.73$), providing no evidence that the number of adults available to spawn determines the strength of the following cohort. This correlation was weak because production of a large number of YOY was produced by relatively few parents in some of the years (Fig. 1a). For example, 2009 had the lowest $\hat{N}_C$ during the autumn reproductive bout (autumn 2008) but the highest $\hat{N}_{YOY}$ point estimate at recruitment the following year (autumn 2009).

Fridley Gap. $\hat{N}_{b-LDNe}$ ranged from 82.5 to 131.2 and was positively but not significantly correlated with $\hat{N}_{fam}$ ($r = 0.19$, $P = 0.76$) and negatively but not significantly correlated with $\hat{FE}$ ($r = -0.25$, $P = 0.69$) (Fig. S5, Supporting information). Full-sib family distributions were highly similar across years (Fig. S6, Supporting information). The largest cohort (2004 cohort) had a similar $\hat{N}_b$ as other years because low $\hat{FE}$ was offset by a substantially larger $\hat{N}_{fam}$ (Fig. S6, Supporting information).

$\hat{N}_b$ did not vary in relation to either adult or juvenile abundance in FG (Table S2, Supporting information, Fig. 1b). $\hat{N}_b$ was highly stable during the 7-year period ($\hat{N}_b$ was available for five of these years; Fig. 1b). The harmonic mean of $\hat{N}_b$ was 107.3 (SD = 18.7, CV = 0.18). $\hat{N}_C$ (lagged 1 year relative to $\hat{N}_b$) increased steadily from 2003 to 2010 (Fig. 1b) and was weakly positively correlated with $\hat{N}_b$ ($r = 0.12$, $P = 0.85$). Harmonic mean $\hat{N}_C$ was 638.5 (SD = 255.8, CV = 0.40; Table S2, Supporting information; Fig. 1b). $\hat{N}_{YOY}$ tended to be greater in the first half of the study period with a peak in 2004 (Fig. 1b) and was weakly positively correlated with $\hat{N}_b$ ($r = 0.15$, $P = 0.81$). Harmonic mean $\hat{N}_{YOY}$ was 859.9 (SD = 1167.3, CV = 1.36; Table S2, Supporting information; Fig 1b). $\hat{N}_C$ and $\hat{N}_{YOY}$ were also not correlated ($r = 0.12$, $P = 0.85$).

Habitat constraints and genetic compensation

West Brook. There was a significant quadratic (intermediate optimum) relationship between stream discharge and $\hat{N}_b$ during autumn (Table 2; Fig. 2). Stream discharge during other periods of time was weakly and nonsignificantly associated with $\hat{N}_b$ (Table 2). Autumn

Table 2 Tests for habitat constraints on $\hat{N}_b$ in WB

	$\hat{N}_b$ (dependent variable)			
Predictor(s)	*F*	*P*	d.f.	R^2
Autumn	0.5	0.50	1,7	0.07
Autumn quadratic	8.2	0.02	2,6	0.73
Winter	2.1	0.19	1,7	0.23
Winter quadratic	2.2	0.19	2,6	0.43
Spring	1.6	0.24	1,7	0.19
Spring quadratic	1.5	0.29	2,6	0.34

$\hat{N}_b$ (from the program LDNE) was the dependent variable, and seasonal flow (autumn, winter, spring) with or without a quadratic term in the linear model was used as a predictor. Autumn flow was the mean of mean daily stream discharge from 1 October to 31 December in the year prior to the emergence of a spring-defined cohort. Winter flow was mean stream discharge from 1 January to 28 February in the year of emergence of a spring-defined cohort. Spring flow was mean stream discharge from 1 March to 31 May in the year of emergence of a spring-defined cohort. Quadratic terms were added to test for intermediate optima. Shown are *F*-values (*F*), *P*-values (*P*), degrees of freedom (d.f.) and proportion of variance explained (R^2).

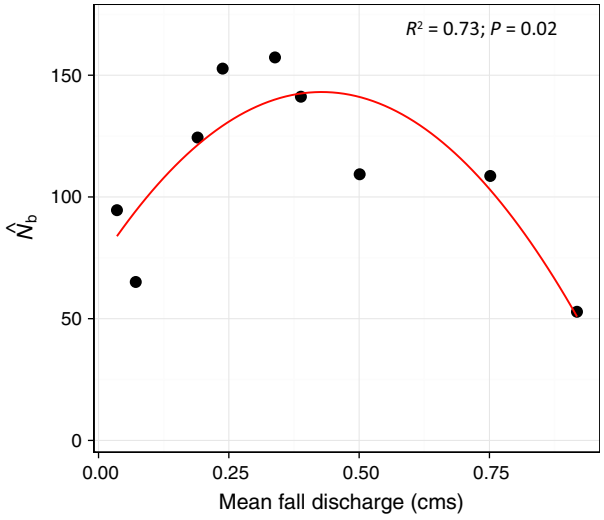

Fig. 2 Relationship between autumn discharge and $\hat{N}_{b-LDNe}$ in the Massachusetts population (WB). Mean fall discharge is the average of mean daily discharge taken from 1 October to 31 December in the year preceding a spring-born cohort, that is, when reproduction occurred. Results from a quadratic regression are shown.

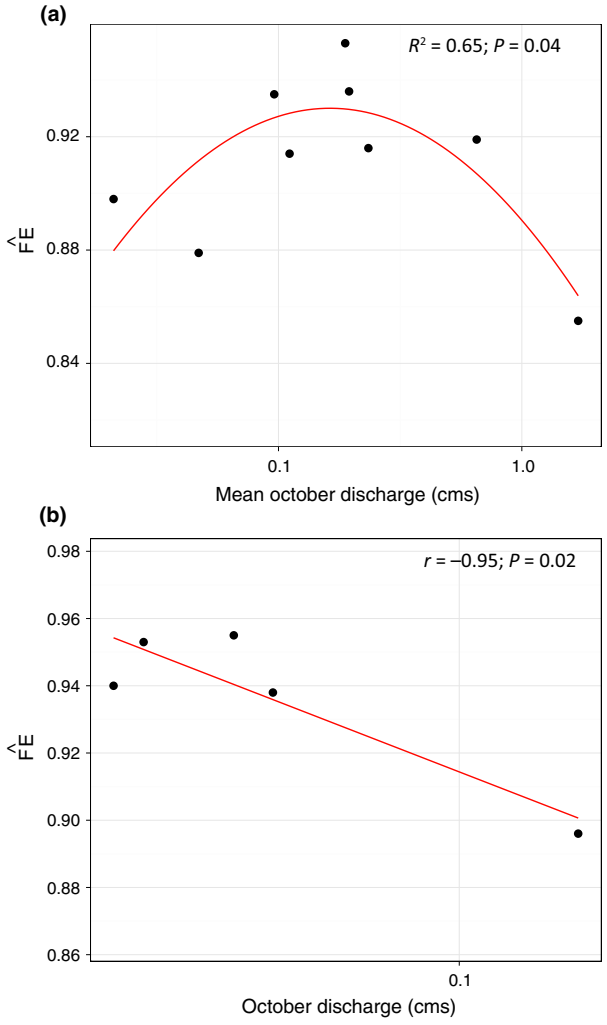

Fig. 3 Relationship between mean October discharge and family evenness ($\hat{FE}$) for (a) WB and (b) FG. Family evenness is a metric of variance in the full-sib family size distribution. Mean October discharge is the average of mean daily discharge taken from 1 October to 31 October of the autumn preceding a spring-born cohort, that is when the reproductive bout occurred. Results from quadratic regressions (a) or correlation coefficients and associated *P*-values (b) are shown.

stream discharge had a stronger quadratic relationship with $\hat{FE}$ ($F_{2,6}$ = 10.0, P = 0.01, R^2 = 0.77) than $\hat{N}_{fam}$ ($F_{2,6}$ = 0.50, P = 0.62, R^2 = 0.15). To investigate the autumn relationship further, we examined discharge monthly. The quadratic regression with mean flow during the month of October as the predictor explained more than 60% of the variation in $\hat{N}_b$ and $\hat{FE}$. This regression was significant with $\hat{FE}$ as the dependent variable ($F_{2,6}$ = 5.5, P = 0.04, R^2 = 0.65; Fig. 3a), was marginally significant with $\hat{N}_b$ as the dependent variable ($F_{2,6}$ = 4.6, P = 0.06, R^2 = 0.61) and was nonsignificant with $\hat{N}_{fam}$ as the dependent variable ($F_{2,6}$ = 0.64, P = 0.56, R^2 = 0.18). Mean November and December discharge was not significantly related to $\hat{N}_b$ (P > 0.70 for each month). Discharge during autumn 2005 (highest mean October discharge) relative to the 2006 cohort (lowest $\hat{N}_b$) drove the quadratic shape of the flow–$\hat{N}_b$ relationship in WB. The mean of mean daily discharge in October 2005 was 1.7 cms (mean October discharge from 2000 to 2009 = 0.34 cms). Nine days had mean daily discharge >1 cms (range 0.01–18.3 cms) between 8 October and 27 October. Average discharge in November 2005 was 0.49 cms (3 days with discharge > 1 cms). This provides limited evidence that the critical window of time during which extremely high stream discharge influences $\hat{N}_b$ and $\hat{FE}$ is immediately preceding and during the beginning of the seasonal reproductive cycle.

A significant negative correlation between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_C$ was consistent with genetic compensation in WB (Table 3; Fig. 4a). However, $\hat{FE}$ and $\hat{N}_C$ were not correlated (prediction = negative; Table 3). The correlation between $\hat{FE}$ and the $\hat{N}_{fam}/\hat{N}_C$ ratio was in the predicted direction but was not significant (Table 3; Fig. 4b).

Fridley Gap. $\hat{N}_b$ was also stable relative to $\hat{N}_C$ in FG. There was no evidence of an effect of flow on $\hat{N}_b$, but there was evidence for a relationship between high flow and variance in family sizes (reduced family evenness). $\hat{N}_b$ was not significantly correlated with discharge during autumn, winter or spring (P > 0.05). However, $\hat{FE}$ and autumn discharge were significantly negatively correlated (Fig. 3b). This was driven by the highest flow in 2003 (0.17 cms, average from 2004 to 2011 = 0.08 cms) and the correspondingly lowest family evenness in 2004. $\hat{FE}$ was significantly negatively correlated with flow in October (r = −0.95, P = 0.02) and November (r = −0.96, P = 0.01), but not in December (r = −0.57, P = 0.43).

A significant negative correlation between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_C$ was consistent with genetic compensation (Table 3; Fig. 4c). However, there was no additional evidence for genetic compensation in FG. The correlation between $\hat{FE}$ and $\hat{N}_C$ was opposite from the direction predicted (Table 3). Finally, the correlation between $\hat{FE}$ and the $\hat{N}_{fam}/\hat{N}_C$ ratio was in the opposite direction than predicted and was statistically significant (Table 3; Fig. 4d). This relationship was significant with or without the 2004 cohort, which appeared to be highly influential (Fig. S6, Supporting Information) because it had approximately twice as many families as the next largest cohort and had the lowest $\hat{FE}$.

Discussion

Our study provides one of the most comprehensive analyses of the mechanistic underpinnings of $\hat{N}_b$ within populations conducted to date. We obtained highly

Table 3 Tests for genetic compensation in two long-term brook trout study sites

		WB			FG		
Variables	Prediction	Dir.	r	P	Dir.	r	P
$\hat{N}_b/\hat{N}_C, \hat{N}_C$	−	−	**−0.74**	**0.05**	−	**−0.87**	**0.05**
FE, $\hat{N}_C$	−	−	−0.01	0.99	+	0.53	0.36
FE, $\hat{N}_{fam}/\hat{N}_C$	+	+	0.17	0.72	− (−)	**−0.99 (−0.92)**	**0.002 (0.03)**

Direction and significance of relationships indicated in the variables column were determined with Pearson's correlation tests. Significant values ($P \leq 0.05$) are in bold. A test with a potential outlier value removed is shown in parentheses. $\hat{N}_b$ were estimated from the program LDNE ($\hat{N}_{b-LDNe}$)

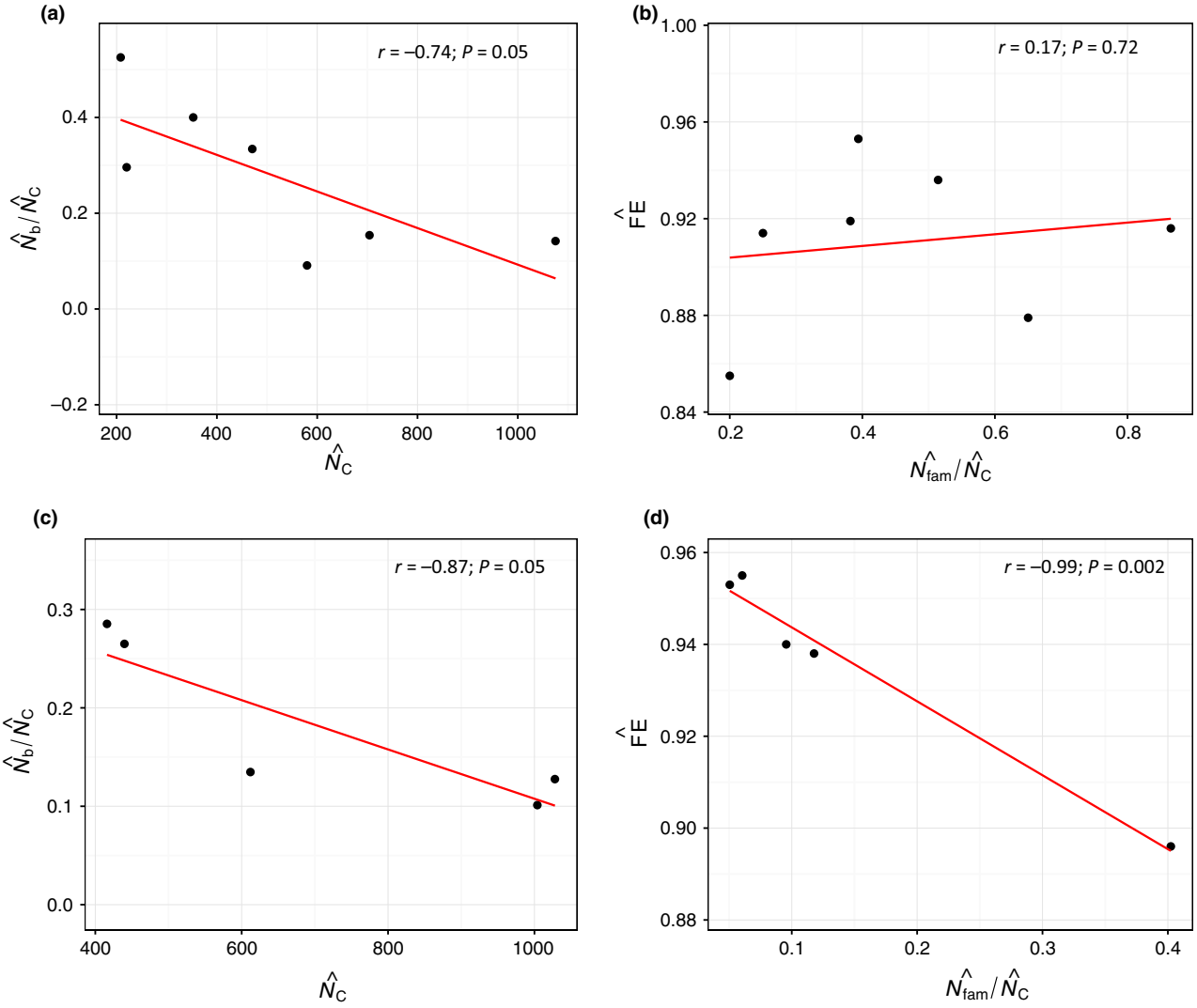

Fig. 4 Tests of genetic compensation in two brook trout populations. Results for WB appear in panels (a) and (b). Results for FG appear in panels (c) and (d). For the $\hat{N}_b/\hat{N}_C$ ratio (panels a and c), $\hat{N}_b$ from a given spring-defined cohort is compared to $\hat{N}_C$ from the previous year. For the $\hat{N}_{fam}/\hat{N}_C$ ratio, the number of families estimated from a spring-defined cohort is compared to $\hat{N}_C$ from the previous year. Pearson's correlation coefficients and associated P-values are shown in each panel. $\hat{N}_b$ were estimated from the program LDNE ($\hat{N}_{b-LDNe}$).

precise estimates of cohort-specific $\hat{N}_b$, and we related these estimates to demographic and environmental measures. $\hat{N}_b$ was not correlated with $\hat{N}_C$ in either population. We found evidence for environmentally driven habitat constraints (stream flow) on interannual variation in $\hat{N}_b$ but not for genetic compensation. These results have important implications for understanding cohort-specific estimates of effective size and for the use of N_b as a metric for genetic monitoring (defined as quantification of temporal changes in population genetic metrics or other population data based on molecular markers; Schwartz *et al.* 2007). The link between N_b and stream flow provides a foundation that could be used to build models of variation in N_b across space and in relation to environmental covariates. Our results also suggest that management actions aimed at habitat improvement are likely to be more effective at maintaining effective population size than efforts to directly increase abundance.

Patterns of variation in $\hat{N}_b$ and abundance

The lack of correlation between $\hat{N}_b$ and $\hat{N}_C$ observed here is consistent with several other studies of salmonids (Ardren & Kapuscinski 2003; Araki *et al.* 2007; Palstra *et al.* 2009; Serbezov *et al.* 2012; Johnstone *et al.* 2013), razorback sucker (*Xyrauchen texanus*) (Dowling *et al.* 2014), lake sturgeon (*Acipenser fulvescens*) (Duong *et al.* 2013) and an island population of the red fox (*Vulpes vulpes*) (Berry & Kirkwood 2010). Several studies of fishes have observed a positive correlation between cohort-specific $\hat{N}_b$ and $\hat{N}_C$ (Osborne *et al.* 2010; Charlier *et al.* 2012). Care must be taken in interpreting results and drawing conclusions from few studies, especially when precision of effective size estimates can be low due to sampling limitations. However, lack of correlation in ours and other studies provides growing evidence that N_b might be of limited use for detecting population trend in many cases. It remains possible that $\hat{N}_b$ more closely tracks $\hat{N}_C$ at smaller population sizes than those examined here. Indeed, in a recent comprehensive review, Palstra & Fraser (2012) provide evidence that a positive relationship between $\hat{N}_b$ and $\hat{N}_C$ might occur at very low abundance (N_C less than approximately 250).

There were several lines of evidence that N_b was constrained in our brook trout study populations. First, $\hat{N}_b$ was more stable than $\hat{N}_C$. $\hat{N}_b$ varied threefold, while $\hat{N}_C$ varied fivefold in one population (WB), and $\hat{N}_b$ varied 1.6-fold and $\hat{N}_C$ varied threefold in the other population (FG). Second, there was a lack of response in $\hat{N}_b$ despite a negative trend in abundance in WB and a positive trend in FG. In both populations, there were years with larger $\hat{N}_C$ but these did not translate into larger $\hat{N}_b$. These results are similar to other studies. For example, Ardren & Kapuscinski (2003) examined a population of rainbow trout over 18 years and found that $\hat{N}_b$ was stable during an approximately threefold decline in abundance. Duong *et al.* (2013) found that $\hat{N}_b$ did not exhibit a trend, while $\hat{N}_C$ increased over the 10-year period in a single population of lake sturgeon. $\hat{N}_b$ was stable despite a management-induced demographic decline in an island population of red fox (Berry & Kirkwood 2010).

In cases where $\hat{N}_b$ does not follow the population trend, it would remain a central among-population metric for genetic monitoring if it is an indicator of a population's cohort-specific successful individual reproductive contribution. We hypothesize that the amount of available reproductive habitat constrains the number of families produced and creates a similar distribution of family sizes from year to year. Despite the greater variability and range of $\hat{N}_b$ observed in WB relative to FG, the similar harmonic means (98.6 vs. 107.3) suggest that these two populations have approximately similar numbers of individuals contributing to reproduction. Standardization by the river lengths provides further evidence for this assertion. $\hat{N}_b$ per km was 61.6 (1.6 km sampled) and 59.6 for FG (1.8 km sampled). Further support for $\hat{N}_b$ as a metric of successful individual reproductive contribution comes from the observation of a positive relationship between $\hat{N}_b$ (range 4.9–226.5) and patch size (range 590–11 600 hectares) in brook trout patches in Virginia (Whiteley *et al.* 2014) and Newfoundland (Wood *et al.* 2014), as well as reduced $\hat{N}_b$ above dams (Whiteley *et al.* 2013). Collectively, this work suggests that N_b could be used for monitoring habitat change and successful individual reproductive contribution. We predict that habitat degradation or enhancement should lead to a sharp decline (habitat degradation) or increase (habitat improvement) in $\hat{N}_b$. Additional work before and after this type of management action as well as continued analyses across geographic space could help reveal additional aspects of the relationship between N_b and habitat quality and quantity.

Habitat constraints and genetic compensation

Constraints on N_e and N_b have been hypothesized to be due to density-dependent reproductive success, termed 'genetic compensation' (Ardren & Kapuscinski 2003). Under this hypothesis, at low adult abundance, lower variance in reproductive success leads to relatively high effective size, either per generation (N_e) or per cohort (N_b). In salmonids, genetic compensation is mechanistically hypothesized to be due to density dependence in competition for spawning territories, competition for mates, redd superimposition or embryo mortality caused by delayed spawning (Ardren & Kapuscinski

2003). We found no compelling evidence for this strict genetic compensation hypothesis in either of our study populations. We observed a significant negative relationship between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_C$ in both populations. This observation has been used as a test for genetic compensation in other studies (Ardren & Kapuscinski 2003). However, this result might be due to plotting a ratio against its denominator (Palstra & Ruzzante 2008). This problem was especially apparent in FG, for which $\hat{N}_b$ was remarkably stable. The one cohort (2009) that deviated substantially from others in $\hat{N}_b$ was likely due to bias due to smaller sample size. In FG, $\hat{N}_C$ increased over time. Given the stability of $\hat{N}_b$, the $\hat{N}_b$-to-$\hat{N}_C$ ratio must, therefore, have declined over time and the relationship between the $\hat{N}_b/\hat{N}_C$ ratio and $\hat{N}_C$ was forced to be negative. Our results caution against the use of this approach for tests of genetic compensation, especially since our additional, more comprehensive, tests that directly included a surrogate for variance in reproductive success (family evenness) were not consistent with genetic compensation in either population. We found no indication that variance in reproductive success was reduced at low abundance. Nor did we find evidence that variance in reproductive success was lower when the ratio of families produced to adult abundance was relatively large.

Both populations in our study consist of entirely resident trout populations. It is possible that relatively small differences in body size in these populations do not allow similar responses in variance in reproductive success found in anadromous salmonid populations. For example, evidence for genetic compensation by alternative life history strategies has been provided from populations of steelhead (*Oncorhynchus mykiss*) (Araki *et al.* 2007) and Atlantic salmon (*Salmo salar*) (Johnstone *et al.* 2013). In the steelhead population, reproductive contribution by relatively small resident fish was greater when adult anadromous abundance was lower (Araki *et al.* 2007). Greater contribution by smaller individuals likely leads to lower reproductive skew (and relatively greater $\hat{N}_b$) because fecundity is size dependent in salmonids. However, we observed marked reproductive skew in some cohorts in our study. The largest full-sib family size (estimated at least 7 months after reproduction) was 71, and in both populations combined, there were 37 full-sib families with 20 or more individuals. This suggests potential for high variance in reproductive success occurs with these purely resident trout populations and the potential for genetic compensation was present but did not occur.

$\hat{N}_b$ was stable relative to $\hat{N}_C$ but still varied across years within each population. We found strong support for the hypothesis that an important environmental factor (stream flow) influences this within-population interannual variation in N_b. The observed intermediate optimum autumn flow for $\hat{N}_b$ and $\hat{FE}$ occurred during a window of time corresponding to October, which occurs immediately preceding and at the beginning of the spawning period. We speculate that density-dependent reproductive success occurs at low autumn flows due to increased competition for mates or mating sites, redd superimposition or mortality due to delayed spawning. However, $\hat{FE}$ was also low during the highest autumn flows in both populations, which would also create low adult densities. For example, autumn 2005 corresponded to the highest mean autumn flow (0.92 cms) in WB and had intermediate adult abundance (579.7). Abundance (and likely density) was therefore low during reproduction but corresponded with the lowest $\hat{N}_b$ for the 2006 cohort (52.8). High autumn flows therefore appear to create suboptimal spawning conditions that are independent of adult abundance. It is possible that few families find spawning sites least affected by high flow conditions, which might include smaller tributaries that are less affected by high flows. We previously observed that the second highest autumn flow year (2003) corresponded with high reproductive success of adults from the WB that reproduced in the OS tributary (Kanno *et al.* 2014). In the 2004 cohort, the top four full-sib families were born in OS (family sizes ranged from 19 to 29 full-sibs), but this did not create enough reproductive skew to have much influence on metapopulation $\hat{N}_b$ (108.6 for 2004 cohort). During the autumn with the highest flow (2005), which corresponded with the lowest $\hat{N}_b$ (52.8) and lowest $\hat{FE}$ (0.855), the six largest families (family sizes ranging from 19 to 39) were born in the river mainstem, which suggests that refugia from high flow events also occur in the mainstem. The observation of stream flow appearing to cause interannual variation in N_b is consistent with our hypothesis that N_b is a metric of the amount of cohort-specific successful individual reproductive contribution supported by a given habitat. Habitat constraints on N_b might also have contributed to other observations of stable $\hat{N}_b$ despite trends in $\hat{N}_C$ (Ardren & Kapuscinski 2003; Araki *et al.* 2007; Duong *et al.* 2013).

Our results do not rule out additional compensatory factors that might serve to elevate N_b when adult densities are low, beyond the narrowly defined genetic compensation hypothesis. For example, it remains possible that relatively more full-sib families are produced or more families tend to survive at low N_C. Another compensatory factor could be density-dependent age at maturity. Despite the lack of trend in $\hat{N}_b$ in WB, we observed a regular oscillation of $\hat{N}_b$ that consisted of relatively high values followed by relatively low values. We hypothesize that this oscillation was due to age-1

fish maturing more often at lower density. When a larger proportion of age-1 fish mature, there would be more individuals contributing to offspring production and possibly a larger $\hat{N}_b$. This could be caused by both production of more families and lower variance in family size because age-1 fish tend to be smaller and would have lower fecundity; thus, family evenness would be expected to be greater. The following year, densities would be expected to be higher. Density-dependent maturation rates would be lower, leading to a smaller proportion of mature age-1 fish. Fewer mature age-1 fish could lead to a lower $\hat{N}_b$. Our analysis was unable to detect this effect, if present, because age at maturity was unknown in our populations and we included all age-1 and older fish in our estimates of $\hat{N}_C$. Further, while these compensatory effects remain possible, the link between flow and N_b that we have demonstrated shows that environmental factors have the potential to overwhelm compensatory factors.

General implications for $\hat{N}_b$

Our ability to examine components of family structure across multiple cohorts reveals novel insights about N_b. Variation in $\hat{N}_b$ was shaped by both number of families and family evenness. We hypothesize that family structure mediates the cohort-specific signal of LD that shapes $\hat{N}_b$, although nonindependence of estimates of the variables involved ($\hat{N}_b$, $\hat{N}_{fam}$, $\hat{FE}$) due to their estimation from the same data source must be acknowledged. Independent estimation of these variables would help to test this hypothesis. However, our results suggest that examination of the distribution of full-sib families itself is critical for interpretation of $\hat{N}_b$ values. We found that relatively large $\hat{N}_b$ were arrived at by either production of many full-sib families, low variance in full-sib family size or both. On the other hand, relatively low values of $\hat{N}_b$ were only arrived at when the number of full-sib families was low and variance in family size was high (e.g. 2002, 2006, 2008 WB cohorts). The FG 2004 cohort was highly informative in this regard. $\hat{FE}$ was low, but we did not observe a response in $\hat{N}_b$ because $\hat{N}_{fam}$ was large. Here, we bracket aside the relatively low $\hat{N}_b$ that was likely due to relatively small sample size in the FG 2009 cohort. Otherwise, our sample sizes were large and we can rule out the influence of bias and lack of precision in these interpretations. Instead, our results suggest that low $\hat{N}_b$ based on sufficient sample size and appropriate sampling strategies (Whiteley *et al.* 2012) indicates that individual reproductive contribution has been low and variable for a given cohort.

Caveats and assumptions

Several caveats in our analysis are worth mentioning. We observed a very strong signal of deviation from HW proportions and LD, particularly in WB. This signal was likely due to the combination of family structure and population substructure. Large sample sizes also translated to strong statistical power and the possibility of statistically but not biologically meaningful results. The random data reduction step helped demonstrate the influence of family structure on our HW and LD results, as deviations were reduced following random sampling. Aside from the null alleles at one locus in FG, which has been reported previously (Hudy *et al.* 2010), we found no compelling reason to suspect data quality issues underlying the HW results. Rather, the lack of consistent HW deviations or LD in other studies that used the same set of markers (Kanno *et al.* 2011; Annett *et al.* 2012; Kazyak *et al.* 2015) and the modest F_{IS} values observed support our interpretation that deviations had a biological origin.

The lack of supporting evidence for the genetic compensation hypothesis could be influenced by bias in $\hat{N}_C$ or estimates of fish density. Based on our thorough sampling, especially in WB, our estimates of $\hat{N}_C$ should contain little bias (Letcher *et al.* 2014). However, we assumed all age-1 fish were mature and included them in $\hat{N}_C$. Density-dependent maturation appears to be common for brook trout (Hutchings 1994; Belmar-Lucero *et al.* 2012). $\hat{N}_C$ might be biased high at large N_C due to reduced rates of maturation of age-1 fish at high abundance. It follows that our $\hat{N}_b/\hat{N}_C$ ratios might be biased low at high $\hat{N}_C$. This bias, if present, would further weaken the limited evidence observed for genetic compensation that was based on relating the $\hat{N}_b/\hat{N}_C$ ratio to $\hat{N}_C$. Bias in $\hat{N}_C$ could also have influenced our ability to observe the predicted patterns involving $\hat{N}_C$, $\hat{N}_{fam}$ and $\hat{FE}$. However, the evidence for genetic compensation was minimal enough that it is unlikely that biases associated with this factor would change our interpretations. Additionally, the genetic compensation hypothesis specifically predicts that variance in reproductive success should vary with adult density at the time of reproduction. We used $\hat{N}_C$ as a surrogate for fish density but we also used density estimates from WB based on stream width measurements collected every 20 m at the time of sampling. These estimates of density did not yield different patterns related to the genetic compensation hypothesis. We cannot rule out the possibility that the use of fish density estimates could alter interpretations in FG, but this seems unlikely given the magnitude and direction of observed effects.

Finally, our assumption of a monogamous mating system could influence interpretations regarding the relationship between $\hat{N}_b$ and stream flow. Brook trout in WB appear to conform much more closely to a monogamous mating model than random mating (Coombs 2010). The mating system has not been investigated in FG. Polygamy has been observed in other populations of this species (Blanchfield *et al*. 2003; Theriault *et al*. 2007). Estimates of N_b from LDNe are approximately doubled for the monogamy model relative to the random mating model and will be biased high if we assume monogamy when the true mating system is polygamous. In terms of evidence for genetic compensation, our use of metrics that directly estimate variance in family size avoids this assumption. If the degree of polygamy is similar among years, our interpretations will not be affected. However, if the degree of polygamy is greatest at intermediate flows, the observed relationship between N_b and stream flow would be weakened. We suspect that this is unlikely. Instead, the degree of polygamy is likely to be greatest at the lowest flows, when competition for mates and mating sites would be greatest. The possibility that $\hat{N}_b$ from low flow years are biased high would strengthen the observed relationship between N_b and stream flow.

Conclusions

We have demonstrated that $\hat{N}_b$ in these populations is constrained and more strongly affected by habitat features and environmental conditions than by abundance. Our results suggest that N_b might not be appropriate for tracking abundance, at least for brook trout populations that range in adult abundance from approximately 200–1000. It remains possible that N_b might track abundance in extremely small populations of this species (<200 adults). Importantly, our work adds to a growing body of evidence that N_b can be useful as an index of habitat quality and for monitoring population response following habitat restoration or degradation. If the management goal is to enhance effective population size and minimize the influence of stochastic evolutionary processes, measures designed to enhance quality or quantity of habitat (e.g. stream restoration) might have a greater influence than efforts to directly increase population abundance (e.g. stocking). Finally, the link we established between interannual variation in stream flow and N_b could provide a foundation for future efforts to understand environmental variation on evolutionary processes.

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A.R.W. designed research, analysed data and wrote the manuscript, J.A.C. helped design research, collected samples and analysed data; M.C. analysed data and helped with manuscript preparation; M.J.O. collected samples and helped with data analysis; M.H. conceived the Fridley Gap portion of the project; K.H.N. helped conceive the West Brook portion of the project and helped with manuscript preparation; and B.H.L. helped conceive the West Brook portion of the project and helped with manuscript preparation.

Data accessibility

Data files with individual information (sample date and location, cohort, body size, full-sib family ID and microsatellite genotypes) for both study sites are available from DRYAD (doi:10.5061/dryad.v6k91). Environmental and abundance data appear in Supplemental Tables.

Supporting information

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

Appendix S1 Null allele in FG at locus Ssa-D237

Fig. S1 Hypotheses related to variation in N_b over time in two long-term brook trout study sites.

Fig. S2 Relationship between $\hat{N}_b$ from LDNe ($\hat{N}_{b-LDNe}$) and $\hat{N}_b$ calculated from full-sib family distributions ($\hat{N}_{b-fam}$), following Waples & Waples (2011) for WB.

Fig. S3 Contribution of number of families, family evenness, and variance obtained from fitted negative binomial distributions to variation in $\hat{N}_b$ for WB.

Fig. S4 Full-sibling family distributions for the 2001–2009 cohorts from WB.

Fig. S5 Contribution of number of families, family evenness, and variance obtained from fitted negative binomial distributions to variation in $\hat{N}_b$ for FG.

Fig. S6 Full-sibling family distributions for five cohorts from FG. Family size is the number of estimated full-sibs per family.

Table S1 Life table data for brook trout from West Brook.

Table S2 Demographic and environmental data for two brook trout populations (WB and FG).