RESEARCH ARTICLE

Sibship reconstruction for inferring mating systems, dispersal and effective population size in headwater brook trout (*Salvelinus fontinalis*) populations

Yoichiro Kanno · Jason C. Vokoun · Benjamin H. Letcher

Abstract Brook trout *Salvelinus fontinalis* populations have declined in much of the native range in eastern North America and populations are typically relegated to small headwater streams in Connecticut, USA. We used sibship reconstruction to infer mating systems, dispersal and effective population size of resident (non-anadromous) brook trout in two headwater stream channel networks in Connecticut. Brook trout were captured via backpack electrofishing using spatially continuous sampling in the two headwaters (channel network lengths of 4.4 and 7.7 km). Eight microsatellite loci were genotyped in a total of 740 individuals (80–140 mm) subsampled in a stratified random design from all 50 m-reaches in which trout were captured. Sibship reconstruction indicated that males and females were both mostly polygamous although single pair matings were also inferred. Breeder sex ratio was inferred to be nearly 1:1. Few large-sized fullsib families (>3 individuals) were inferred and the majority of individuals were inferred to have no fullsibs among those fish genotyped (family size = 1). The median stream channel distance between pairs of individuals belonging to the same large-sized fullsib families (>3 individuals) was 100 m (range: 0–1,850 m) and 250 m (range: 0–2,350 m) in the two study sites, indicating limited dispersal at least for the size class of individuals analyzed. Using a sibship assignment method, the effective population size for the two streams was estimated at 91 (95%CI: 67–123) and 210 (95%CI: 172–259), corresponding to the ratio of effective-to-census population size of 0.06 and 0.12, respectively. Both-sex polygamy, low variation in reproductive success, and a balanced sex ratio may help maintain genetic diversity of brook trout populations with small breeder sizes persisting in headwater channel networks.

Keywords Sibship reconstruction · Polygamy · Dispersal · Effective population size (N_e) · *Salvelinus fontinalis* · Headwater streams

Introduction

Understanding how small populations persist is important in today's increasingly fragmented landscape. Conservationists working with small populations are interested in reproductive biology of the species of interest, including the breeding population size, mating systems, and breeding locations. Such ecological information is difficult to obtain from direct field observations, particularly for cryptic species such as fish. However, highly polymorphic genetic markers are an indirect but efficient alternative.

Sibship reconstruction based on neutral genetic markers is one method used to understand reproduction in the wild populations. It infers the relationship among individuals by identifying pairs of individuals that share both parents (i.e., fullsibs) and one parent (i.e., halfsibs). Sibship information has been used to determine individual variability in reproductive success (Hudy et al. 2010; Liu and Ely 2009) and dispersal (Hudy et al. 2010) for fishes. It can provide an insight into the mating systems by inferring genotypes of unknown parents (Wang 2004). Sibship assignment also

Y. Kanno (✉) · J. C. Vokoun
Department of Natural Resources and the Environment,
University of Connecticut, 1376 Storrs Road, Storrs,
CT 06269, USA
e-mail: yoichiro.kanno@uconn.edu

B. H. Letcher
Silvio O. Conte Anadromous Fish Research Center,
United States Geological Survey, P.O. Box 796,
One Migratory Way, Turners Falls, MA 01376, USA

helps infer effective population size, N_e (Wang 2009), which is defined as the size of an ideal population experiencing the same rate of random genetic change over time as the real population under consideration (Palstra and Ruzzante 2008). Genetic stochasticity and diversity depend on N_e, so estimation of N_e has received much attention in evolutionary and conservation biology (Luikart et al. 2010).

Brook trout (*Salvelinus fontinalis*) represents an aquatic species for which information gained by sibship reconstruction is potentially useful from the conservation perspective. Brook trout are native to streams and lakes in eastern North America (Power 1980), but populations have declined in much of the native range (Hudy et al. 2008). Brook trout have been lost from over 50% of the historical habitat in Connecticut, USA, (Hudy et al. 2008), and lotic populations are primarily located in small, cold headwater streams (Kanno and Vokoun 2008). Land development and resulting habitat alteration has often been associated with population declines (Kanno et al. 2010; Stranko et al. 2008; Waco and Taylor 2010), and climate change raises concern for the persistence of this steno-thermal species (Chu et al. 2008; Flebbe et al. 2006).

Despite the current conservation need and many previous scientific studies on brook trout, reproduction of brook trout in headwater streams is not well known. Brook trout females spawn during fall by digging a nest (called a "redd") in the gravel substrate, preferentially in areas of groundwater upwelling within streams (Essington et al. 1998) and lakes (Blanchfield and Ridgway 1997; Curry and Noakes 1995). Using parentage and sibship assignment, Hudy et al. (2010) reported limited dispersal and low variation in reproductive success among individuals from a headwater brook trout population in Virginia. However, it is unknown if the results represent the typical patterns across the species' native range, or specific patterns at the southern distributional range.

Headwater brook trout populations are also an ideal system in which to apply sibship reconstruction since their small population size and limited spatial distribution enable the researcher to collect a spatially representative sample from the entire local population. Such a sampling scheme is important because stream-dwelling salmoninds are known to exhibit fine-scale genetic structure even over a few kilometers (Carlsson et al. 1999; Hudy et al. 2010; Spruell et al. 1999) and spatially limited sampling might result in inaccurate understanding of reproduction and spatial structure. For example, sampling from selected stream reaches might contain a high proportion of related individuals (Hansen et al. 1997), and it would bias the watershed-scale estimate of N_e. As another example, the estimation of dispersal distance for stream fish is inherently constrained by the spatial extent of sampling (Albanese et al. 2003; Gowan et al. 1994).

The goal of this study was to use sibship analysis to infer mating systems, dispersal and effective population size of resident (non-anadromous) brook trout populations in two headwater stream channel networks. We had the following specific objectives. First, reproductive strategies (i.e., polygamy versus monogamy) were inferred for both sexes without any information on parental genotypes. In light of recent genetic studies that report the prevalence of polygamy in both sexes across many fish species including salmonids (Avise et al. 2002; Garant et al. 2001; Seamons et al. 2004), we predicted that at least some proportion of brook trout of both sexes would exhibit polygamy. Second, the presence and prevalence of fullsib relationships was examined to understand the variability of reproductive success. If a small number of parent pairs makes a disproportionate reproductive contribution, large-sized fullsib families should be common in the sample. Third, spatial distributions of inferred fullsibs were recorded along the stream channel network. We were interested in examining the extent to which fullsib individuals were spatially clustered. Finally, a sibship assignment method was used to infer N_e and the ratio of effective-to-census population size (N_e/N_c) in these headwater brook trout populations.

Materials and methods

Study area

This study was conducted in two headwater watersheds located in northwestern Connecticut, USA (Fig. 1). Both study streams contained self-reproducing brook trout populations in a branching stream channel network characterized by high channel gradient (mean of 4.0% and range of 1.1–13.4% in Kent Falls Brook; mean of 3.4% and range of 0.6–11.6% in Jefferson Hill-Spruce Brook). Streams were predominately first and second order; mean summer wetted width was 4.8 m (range: 1.4–12.7 m) in Kent Falls Brook and 4.3 m (range: 1.2–8.1 m) in Jefferson Hill-Spruce Book.

The Kent Falls Brook watershed (drainage area: 14.06 km²) included approximately 4.4 km of stream channel network. The headwater channel network was isolated due to large natural waterfalls located downstream of the study area. There was no known record of brook trout stocking above the waterfalls including the study area, although the presence of naturalized non-native brown trout (*Salmo trutta*) in the downstream portion of our study area suggested that trout stocking had occurred in the past. Blacknose dace (*Rhinichthys atratulus*) was common in Kent Falls Brook. A second barrier (a series of natural waterfalls >5 m in height) existed in a first-order tributary

Fig. 1 Locations of study sites in the State of Connecticut, Northeastern USA. Brook trout were sampled throughout the entire stream channel networks in a spatially continuous manner
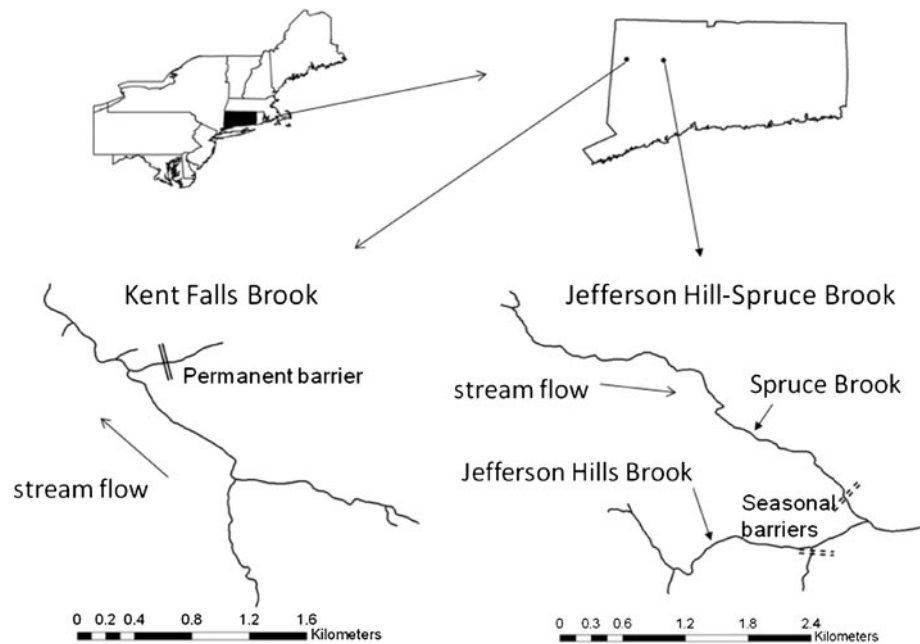
to Kent Falls Brook (Fig. 1). No brook trout were found above the natural waterfalls in this tributary.

The Jefferson Hill-Spruce Brook watershed (drainage area: 14.56 km^2) spanned approximately 7.7 km in stream channel length. It was not isolated and drained into a large stream (5th order stream with mean width of 20 m) at the downstream end of the study area (Fig. 1). Brook trout were stocked every year by the state fisheries agency in this large stream and were observed in the downstream end of our study area. Common fish species observed in Jefferson Hill-Spruce Brook included blacknose dace, longnose dace (*Rhinichthys cataractae*), and white sucker (*Catostomus commersoni*). These species were more common in the downstream portion of the study area.

Field sampling

Brook trout were collected in a spatially continuous manner across the entire stream channel networks in both study sites. Prior to fish collection, the study streams were travelled by foot, and riparian trees were marked at an interval of ≈50 m (each 50 m-zone is called a "reach" hereafter). Single-pass backpack electrofishing surveys (Smith-Root model LR-24, Vancouver, WA, USA) were conducted without blocknets in June and August of 2008. Trout count was recorded in each reach, and each fish was measured for total length (±1 mm) and weight (±0.25–1.00 g depending on fish size). An anal fin clip was removed from all trout captured, except age 0+ individuals that had hatched in early spring of 2008. Age 0+ trout were easily distinguished from older fish due to their small body size.

Adipose fins were also removed and used as a permanent mark, so that tissue samples were not collected twice from the same individuals. During the summer of 2009, three-pass electrofishing samples were conducted to estimate electrofishing capture probabilities in selected reaches (eight reaches in Kent Falls Brook and 15 reaches in Jefferson Hill-Spruce Brook, which was approximately 10% of the total study areas).

Laboratory methods

A sub-sample was genotyped for eight microsatellite loci: *Sfo*C-113 (trinucleotide), *Sfo*D-75 (tetranucleotide), *Sfo*C-88 (trinucleotide), *Sfo*D-100 (tetranucleotide), *Sfo*C-115 (dinucleotide), *Sfo*C-129 (trinucleotide), *Sfo*C-24 (trinucleotide) and *Ssa*D-237 (tetranucleotide). The same set of loci had been used in a previous brook trout study (Hudy et al. 2010). We used genotype data on trout ranging 80–140 mm in both study sites, in order to target a single cohort as best as possible for statistical analysis. Body size is a reasonable surrogate for age, however it is not possible to determine age clearly for brook trout older than 0+ years, based on length-frequency distributions (Hudy et al. 2010; Kennedy et al. 2003). Presumably, fish in the size range genotyped were mostly age 1+ individuals. An attempt was made to genotype a similar number of trout (3–4 individuals) randomly selected from each 50 m reach, without considering differences in trout density among reaches. In addition, fin clips from stocked trout collected at the downstream area of Jefferson Hill-Spruce Brook were genotyped to assess the level of introgression in this

study area. Stocked trout were reliably identified in the field from a combination of body size and external characteristics.

Genomic DNA was extracted by fin tissue digestion in cell lysis buffer with proteinase K and RNase A, and treatment with a protein precipitation solution of isopropanol and ethanol before being rehydrated with TE buffer solution. Microsatellite markers were amplified with polymerase chain reaction (PCR) in a thermal cycler (MJ DNA Engine Dyan, model PTC-220; MJ Research, Inc., Waltham, MA, USA). The PCR program included 94°C for 2 min; 35 cycles at 94°C for ¾ min, 56°C for ¾ min, and 72°C for 2 min; followed by 72°C for 10 min, and finally 6°C until samples were removed. Each locus was amplified with a fluorescent-dye-labeled forward primer with a corresponding no-dye-labeled reverse primer. A 10-µl PCR cocktail was composed of 1.9–2.3 µl of ddH$_2$O, 2 µl of PCR buffer, 0.8 µl of MgCl$_2$, 1 µl of dNPTs, 0.1 µl of *Taq* DNA, and 0.7–0.9 µl of each of forward and reverse primer. After amplification, PCR products were treated with a solution composed of formamide and ROX 350 size standard (MCLAB, South San Francisco, CA, USA). Alleles were sized on an ABI PRISM 3100-Avant Genetic Analyzer using the PEAK SCANNER version 1.0 software (Applied Biosystems, Carlsbad, CA, USA).

Statistical analysis

Genotypes were checked for scoring errors arising from stutter products, large allele dropout, or the presence of null alleles using the program MICRO-CHECKER version 2.2 (Van Oosterhout et al. 2004). Observed (H_O) and expected (H_E) heterozygosity was calculated using the program GENEPOP version 4.0.10 (Raymond and Rousset 1995). Exact tests for gametic disequilibrium between loci, and calculation of the inbreeding coefficient, F_{IS}, for each locus were performed in the program FSTAT version 2.9.3.2 (Goudet 1995). The presence of stocked trout in the downstream area of Jefferson Hill-Spruce Brook prompted us to quantify the degree of introgression in this stream. The program GENECLASS2 (Piry et al. 2004) was used to calculate the probability that each individual fish of 80–140 mm was assigned to the stocked trout population. The computation was done using the Bayesian method of Rannala and Mountain (1997) and 10,000 simulations with the Monte Carlo resampling method of Paetkau et al. (2004).

Sibship analysis was conducted using program COLONY version 2.0 (Jones and Wang 2010). The program's approach is computationally robust because it considers the likelihood of the entire pedigree structure, as opposed to relatedness of individuals on a pair-wise basis (Herbinger et al. 2006). Three different runs were executed specifying different mating strategies in each study stream, and the likelihood of observed genotype data was compared among the different hypotheses of mating strategies. The mating strategies tested were: both-sexes monogamy, both-sexes polygamy, or one-sex monogamy and the other-sex polygamy. Because sex was not known for our fish, the third model did not specify which sex was monogamous or polygamous. Using the most plausible mating strategy identified, the frequency distribution of inferred fullsib family sizes was constructed to examine the individual variation of reproductive success. The breeding population size (N_b) was determined from sibship analysis by counting the number of inferred parents that produced the genotype data.

Fish dispersal was estimated to the nearest 50 m by measuring stream channel pair-wise distance between individuals inferred to belong to the large-sized fullsib families (>3 individuals). Fullsib families containing two or three individuals were not included because the identification of small-sized fullsib families is less accurate in program COLONY (Hudy et al. 2010) and other sibship reconstruction programs (e.g., Herbinger et al. 2006). Applying the same set of eight microsatellite loci to a different headwater brook trout population, Hudy et al. (2010) reported a high accuracy rate of 95.2% when inferred families contained at least three individuals.

The contemporary effective population size (N_e) was inferred using the sibship assignment method (Wang 2009) available in program COLONY. Among the many genetic methods available for estimating N_e (Luikart et al. 2010), the sibship assignment method was chosen for our data because it does not assume random mating; weak isolation-by-distance patterns were observed in both study sites, and additionally, genetically distinguishable subpopulations were identified in Jefferson Hill-Spruce Brook (Kanno *unpublished data*). The method assumes that a random sample of offspring was taken from a single cohort (as our subsampling scheme was designed for), and that there was little or no immigration (as supported by our spatially continuous and extensive sampling design at the headwater channel network/watershed scale).

The census population size (N_c) was estimated by calculating the capture probabilities of trout via single-pass electrofishing, using a removal method (Zippin 1958) based on the three-pass electrofishing data collected in 2009, and applying those probabilities to fish count recorded in the 2008 August survey. Capture probabilities were estimated separately for trout 80–140 mm and those larger than 140 mm because electrofishing is size selective (Reynolds 1996). We followed Luikart et al.'s (2010) definition of N_c as the number of individuals that have reached sexual maturity. Because both males and females of approximately 100 mm total length were reproductively

mature (i.e., expressing milt and eggs) during fall in the study sites (Kanno *personal observation*), the N_c was based on the estimated abundance of trout ≥ 100 mm during the 2008 August survey. This was immediately prior to the spawning season and electrofishing efficiency was high due to late-summer low flows.

Results

A total of 1,732 adipose fin clips was collected from brook trout of 80–140 mm total length in the two study sites (779 in Kent Falls Brook and 953 in Jefferson Hill-Spruce Brook). The stratified random design resulted in a sub-sample of 740 individuals for genotyping in the two sites combined; 267 in Kent Falls Brook and 473 in Jefferson Hill-Spruce Brook. A mean of 3.3 individuals per reach (standard deviation (SD): 1.1) and 3.1 individuals per reach (SD: 1.1) were genotyped in Kent Falls Brook and Jefferson Hill-Spruce Brook, respectively. Additionally, 26 stocked trout individuals (mean 258 mm; range 167–348 mm) were genotyped in Jefferson Hill-Spruce Brook. The assignment test indicated no evidence of introgression by stocked fish in this stream. None of the 473 individuals (80–140 mm) belonged to the stocked trout population (assignment probability <0.0001).

All eight microsatellite loci were polymorphic (Table 1). There was no clear evidence of stutter products, large allelic dropout, or null alleles based on the MICRO-CHECKER results. Genetic diversity was generally lower in Kent Falls Brook than in Jefferson Hill-Spruce Brook, perhaps due to isolation of Kent Falls Brook by the natural waterfalls. Observed (H_O) and expected (H_E) heterozygosity was 0.195–0.835 and 0.209–0.847, respectively, in Kent Falls Brook. In Jefferson Hill-Spruce Brook, H_O was

0.514–0.871, and H_E was 0.537–0.922. Gametic disequilibrium was not significant after Bonferroni corrections for any pair-wise comparison of loci in the two sites.

Observed genotype data had the best support under a both-sexes polygamy model in Kent Falls Brook and Jefferson Hill-Spruce Brook (Table 2). The inferred number of partners ranged from 1 to 9 for males (mean = 4.2) and from 1 to 8 for females (mean = 3.9) in Kent Falls Brook, and it ranged from 1 to 8 for males (mean = 3.2) and from 1 to 7 for females (mean = 3.4) in Jefferson Hill-Spruce Brook. The inferred number of partners did not differ between sexes in either study site (*t*-test: $t = -0.75$, $p = 0.458$ in Kent Falls Brook; $t = 0.64$, $p = 0.521$ in Jefferson Hill-Spruce Brook). Both males and females were typically inferred to have mated with more than one partner, although some single pair matings were also inferred. An example of the mating distribution for a first-order stream is shown in Fig. 2. This tributary was chosen as an example because it was a semi-isolated habitat due to the presence of a series of natural chutes (all <2 m) that appeared to act as a seasonal barrier to fish movement (Fig. 1). Thus, the tributary represented a mating system of brook trout in a small, semi-isolated habitat. In this tributary, 30 genotyped trout were inferred to have derived from 22 unknown breeders with a 1:1 sex ratio (11 males and 11 females). A 1:1 sex ratio was similarly observed at the watershed scale; 51 versus 49 in Kent Falls Brook ($N_b = 100$), and 115 versus 111 in Jefferson Hill-Spruce Brook ($N_b = 226$). These values translated to about two and three successful breeders per 100 m in Kent Falls Brook and Jefferson Hill-Spruce Brook, respectively.

Few large-sized fullsib families were identified in either study stream; five fullsib families containing >3 individuals were identified in Kent Falls Brook and nine were identified in Jefferson Hill-Spruce Brook (Fig. 3).

Table 1 Summary statistics and allelic diversity for eight microsatellite loci from a subsample of brook trout (80–140 mm) collected in Jefferson Hill-Spruce Brook and Kent Falls Brook

	SfoC-113	SfoD-75	SfoC-88	SfoD-100	SfoC-115	SfoC-129	SfoC-24	SsaD-237
Kent Falls Brook ($n = 267$)								
H_E	0.596	0.579	0.209	0.743	0.836	0.537	0.547	0.847
H_O	0.581	0.547	0.195	0.715	0.813	0.494	0.562	0.835
A	5	6	4	10	16	5	5	18
F_{IS}	0.026	0.056	0.069	0.037	0.028	0.079	−0.028	0.014
Jefferson Hill-Spruce Brook ($n = 473$)								
H_E	0.792	0.821	0.732	0.691	0.874	0.710	0.537	0.922
H_O	0.755	0.765	0.660	0.660	0.869	0.679	0.514	0.871
A	11	15	6	8	17	7	4	24
F_{IS}	0.047	0.067	0.099	0.045	0.005	0.044	0.043	0.055

Number of individuals genotyped (n), number of alleles (A), observed (H_O) and expected (H_E) heterozygosity, and inbreeding coefficient (F_{IS}) are shown

Table 2 Log likelihood values for different mating strategies calculated in the COLONY version 2.0 software for brook trout (80–140 mm) collected in Kent Falls Brook and Jefferson Hill-Spruce Brook

Mating strategies	Kent Falls Brook	Jefferson Hill-Spruce Brook
Both sex monogamy	−3778.092	−9623.301
One sex monogamy, the other polygamy	−3583.797	−9056.056
Both sex polygamy	−3178.417	−8324.094
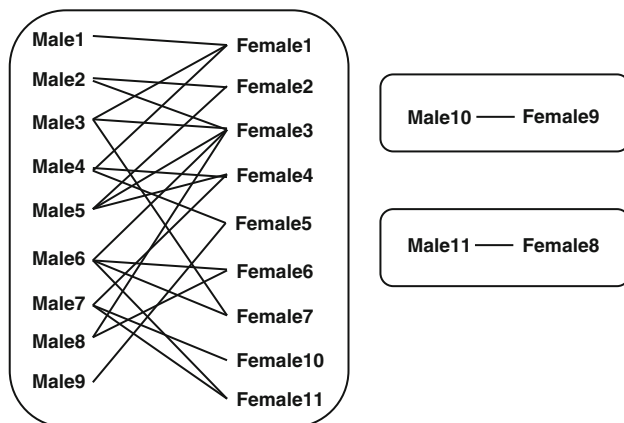
Fig. 2 An example inferred mating system in the semi-isolated tributary in Jefferson Hill Brook

The majority of individuals were inferred to have no full-sibs among those fish genotyped. A total of 200 fullsib families were inferred among the 267 genotyped individuals in Kent Falls Brook, and 155 fullsib families were composed of a single individual. Fullsib family sizes ranged from 1 to 7 individuals (mean = 1.34). Similarly, 308 of the 374 fullsib families inferred in Jefferson Hill-Spruce Brook were composed of a single individual. Fullsib family sizes ranged from 1 to 8 individuals (mean = 1.26).

The stream channel pair-wise distance between individuals belonging to large fullsib families (>3 individuals) was short relative to the spatial scale of the two study streams (Figs. 4, 5). The median stream channel pair-wise distance was 100 m (range: 0–1,850 m) in Kent Falls Brook and 250 m (range: 0–2,350 m) in Jefferson Hill-Spruce Brook. Typically, individuals of the same fullsib families were clustered spatially and fullsib families were found across the stream channel networks in both study sites (Figs. 4, 5).

The effective population size (N_e) was estimated at 91 (95%CI: 67–123) in Kent Falls Brook and 210 (95%CI: 172–259) in Jefferson Hill-Spruce Brook. Using the estimated single-pass electrofishing capture probability of 0.55 for trout 80–140 mm and 0.70 for trout >140 mm, the census population size (N_c) was estimated at 1,417 in Kent Falls Brook and 1,792 in Jefferson Hill-Spruce Brook in 2008. Accordingly, the N_e/N_c was estimated at 0.06 in Kent Falls Brook and 0.12 in Jefferson Hill-Spruce Brook.
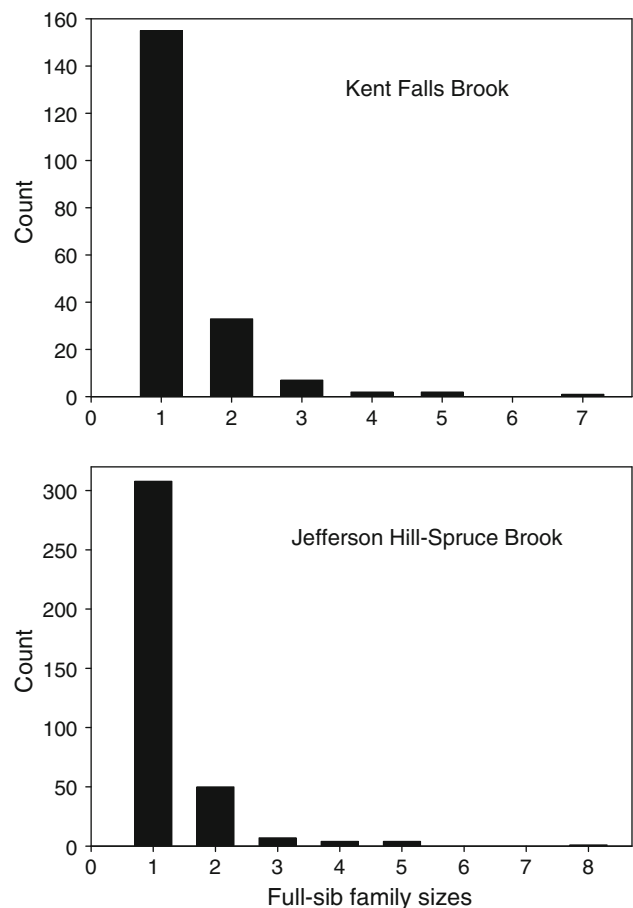
Fig. 3 Frequency distributions of inferred fullsib family sizes in Kent Falls Brook and Jefferson Hill-Spruce Brook

Discussion

Polygamy was common in both males and females of the two headwater resident brook trout populations examined in this study. The result differs slightly from Blanchfield et al. (2003), who suggested polygyny in their lacustrine population of brook trout. Our result adds another example to an expanding list of fish species inferred to exhibit polygamous mating systems by both sexes (Garant et al. 2001; Rourke et al. 2009; Seamons et al. 2004; Thériault et al. 2007). Such mating systems potentially result from several behavioral mechanisms in brook trout. Mature salmonid males compete with other each for access to
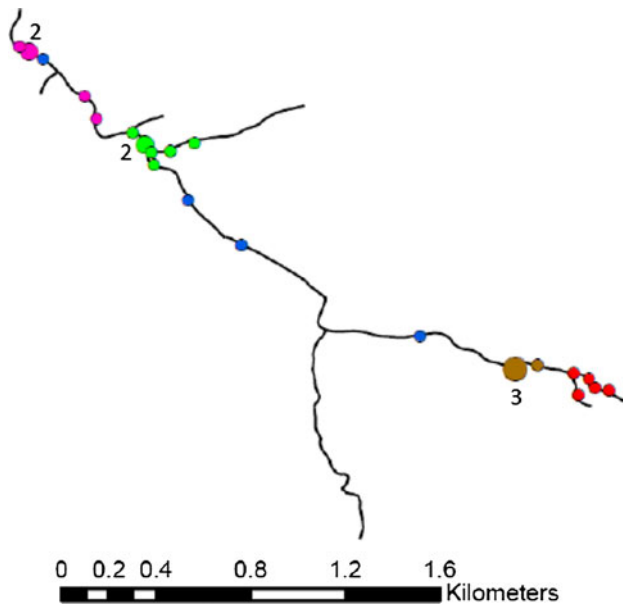
Fig. 4 Capture locations of individuals inferred to belong to large-sized fullsib families (>3 individuals) in Kent Falls Brook. Each of four fullsib families is represented by a different color. The smallest-sized *dot* indicates the presence of a single individual in a 50 m reach, and larger-sized *dots* indicate the presence of multiple individuals (fish count denoted by Arabic numerals)
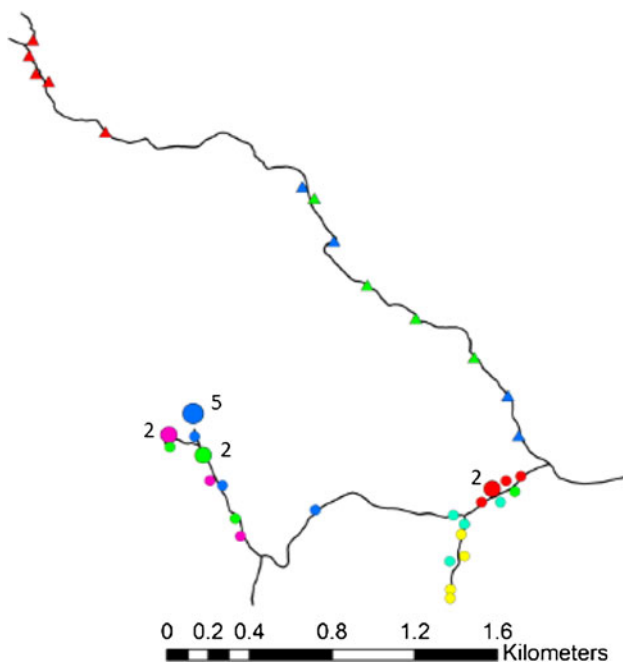

Fig. 5 Capture locations of individuals inferred to belong to large-sized fullsib families (>3 individuals) in Jefferson Hill-Spruce Brook. Each of nine fullsib families is represented by a combination of different colors and symbols. The smallest-sized *dot* indicates the presence of a single individual in a 50-m reach, and larger-sized *dots* indicate the presence of multiple individuals (fish count denoted by Arabic numerals). Some points were jittered for graphical clarity

females, and large, dominant brook trout males may be able to find multiple partners (Blanchfield et al. 2003). Male brook trout are reproductively active over a longer period of time than females (Blanchfield and Ridgway 1997; Thériault et al. 2007), which confers males a chance to mate with multiple partners. Female brook trout may construct multiple redds over a spawning season and redds may be sired by different males (Blanchfield and Ridgway 1997). Additionally, females may intentionally choose partners by delaying spawning when courted by undesirable males (Berejikian et al. 2000; de Gaudemar et al. 2000). Sneaking mating behavior is known for males among salmonids (Blanchfield et al., 2003; Thériault et al. 2007), providing an opportunity for females to become polygamous if sneaker males sire offspring successfully. Polygamy may lead to higher genetic diversity within the offspring population and reduce the risk of inbreeding (Garant et al. 2001), and such reproductive strategies are probably important for small, resident headwater brook trout populations to maintain genetic diversity and evolutionary potential.

The predominance of small fullsib family sizes indicated low variation in reproductive success among individuals in our study sites. Skewed reproductive success has been observed in other brook trout studies. Blanchfield et al. (2003) reported that larger males made a disproportionate reproductive contribution in a lacustrine population of brook trout. Also, in a stream system in which individuals of both sexes were anadromous or resident, larger (anadromous) females of brook trout were more reproductively successful than smaller (resident) females due to a fecundity advantage, but male reproductive success was not dependent on body size (Thériault et al. 2007). In salmonids, smaller males can be as reproductively successful as larger males via alternative mating tactics (i.e., sneaking behavior) (Garant et al. 2001; Saura et al. 2008; Thériault et al. 2007) or size-assortative mating (Chebanov 1990; Maekawa et al. 1994). In headwater brook trout populations dominated by young fish (most fish in our study sites are age 2+ or younger), the difference in body size is perhaps not as pronounced in determining reproductive success compared to anadromous or lacustrine populations. However, our results of low reproductive variation need to be interpreted cautiously taking into account our sampling scheme. First, our data only show that variation in reproductive success is low among successful breeders, but it is unknown what proportion of reproductively mature adults successfully spawned. The N_e/N_c estimates indicate the possibility that not all fish >100 mm total length successfully spawned in both populations. Second, our genotyping of individuals was not exhaustive. We genotyped less than half of the adipose fins collected, in an attempt to extend the spatial coverage of this study to the watershed scale.

If all fin clips collected had been genotyped, more large-sized families could have been detected in our study streams and more skewed reproductive success might have been revealed. Nonetheless, studies conducting such exhaustive genotyping at a finer spatial scale found similarly exponential distributions of family size (Coombs 2010; Hudy et al. 2010).

Spatial distributions of individuals from the same fullsib families (median pair-wise distance 100 m in Kent Falls Brook and 250 m in Jefferson Hill-Spruce Brook) were generally confined relative to the dispersal potential in the study sites characterized with very few permanent barriers (4.4 and 7.7 km, respectively, of channel networks). In a headwater brook trout population in Virginia, Hudy et al. (2010) similarly found spatial clustering of related brook trout individuals including age 2+ trout. Such restricted movement, if common, may be responsible for fine-scale population structuring often reported in stream resident salmonids (Carlsson et al. 1999; Letcher et al. 2007). However, the conclusion of restricted movement remains incomplete in our study areas because only one size class of brook trout (80–140 mm: presumably age 1+) was genotyped. Additional research is warranted by examining older trout or estimating population-level dispersal rates.

The estimated N_e values in our study (91 in Kent Falls Brook and 210 in Jefferson Hill-Spruce Brook) fell near the mean N_e (149) of wild populations of species of conservation concern (Palstra and Ruzzante 2008). It is unknown if these populations have historically maintained such low N_e values or N_e values have been reduced in recent times due to anthropogenic disturbances. It should be stressed that the low N_e in our study appears to be due primarily to the small number of successful breeders in headwater streams ($N_b = 100$ in Kent Falls Brook and $N_b = 226$ in Jefferson Hill-Spruce Brook). Variance in reproductive success and unequal sex ratio reduce N_e in wild populations (Frankham 1995), but there was no evidence that these factors were operating in our study. The N_e value is also reduced when a population size fluctuates over time (Frankham 1995); stream salmonids are known to exhibit such temporal patterns (Elliott 1994; Lobón-Cerviá 2009), although data is not temporally available in our study sites. Headwater habitat is inherently small with limited physical space. Redd locations are typically associated with areas of groundwater upwelling in streams (Essington et al. 1998) and in lakes (Blanchfield and Ridgway 1997; Curry and Noakes 1995), and such a habitat preference may further decrease spawning habitat availability. Later-spawning females of brook trout have been reported to re-use redd sites, which may reduce the number of successful breeders by removing previously deposited eggs (Essington et al.

1998). High stream discharge may scour redds, resulting in high egg mortality (Carline and McCullough 2003). All of these factors potentially limit the N_b of brook trout inhabiting headwater stream networks.

The N_e/N_c ratios obtained for brook trout in our study sites (0.06 in Kent Falls Brook and 0.12 in Jefferson Hill-Spruce Brook) were very close to the mean (0.10–0.11: Frankham 1995) or median value (0.14: Palstra and Ruzzante 2008) reported from wild populations across many taxa. The comparison of N_e/N_c ratios among studies is not straight-forward due to different analytical approaches to estimate those ratios, but still our estimated N_e/N_c ratios are comparable to those of other salmonid studies. Notably, Palstra and Ruzzante (2008) showed an inverse relationship between N_e/N_c and N_c (i.e., genetic compensation) among salmonids, and our estimates of N_e/N_c and N_c fall right on this empirical relationship (see Fig. 3 of their study). The N_e/N_c ratios for salmonids were typically below 0.2, when N_c exceeded 1,000 individuals (Palstra and Ruzzante 2008). Very low N_e/N_c estimates (0.00001–0.001) have often been reported among marine fishes with high fecundity and family-related survival (Hoarau et al. 2005; Turner et al. 2002). Clearly, such a "sweepstakes effect" (Hedrick 2005) was not observed in our resident headwater populations of brook trout.

In summary, this study examined mating systems, dispersal and effective population size of resident brook trout populations in headwater stream channel networks. Spatially continuous field sampling and polymorphic markers allowed us to investigate genetic characteristics of headwater brook trout populations at the watershed scale. The headwater brook trout populations were characterized with low N_e, but polygamy, low variation in reproductive success, and a balanced sex ratio may help maintain genetic diversity and evolutionary potential. As habitat alteration continues and climate change is projected to inflict major environmental changes, understanding mechanisms of population persistence for small populations, such as headwater brook trout, is important and necessary for their conservation and management.

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