Conserv Genet (2015) 16:31–42
DOI 10.1007/s10592-014-0637-5

RESEARCH ARTICLE

Fine-scale genetic structure of brook trout in a dendritic stream network

Suzanne J. Kelson · Anne R. Kapuscinski · Dianne Timmins · William R. Ardren

Received: 6 January 2014 / Accepted: 8 July 2014 / Published online: 24 July 2014

Abstract Conservation management of threatened species requires identifying the landscape features that shape population structure. Within river ecosystems, the dendritic nature of river networks and physical barriers, such as waterfalls, can strongly shape population structure. We examined population structure of native brook trout in a river network in northern New Hampshire, USA, including above and below waterfalls. We genotyped fish at 12 microsatellite loci including samples from six tributaries, mobile adults from three mainstem rivers, and fish from the hatchery broodstock that had been earlier stocked in the study region. We found that two subpopulations in tributaries above waterfalls were distinguished as unique genetic clusters with high levels of among population genetic diversity (average pairwise $F_{ST} = 0.20$) and low levels of within population genetic diversity (average allelic richness $A_R = 3.55$), including one sub-population above a waterfall. With only one exception, subpopulations below waterfalls exhibited patterns of genetic diversity within and among populations consistent with contemporary gene flow among these subpopulations (average $F_{ST} = 0.03$; $A_R = 5.83$). Most mobile adult fish caught in the mainstem rivers were genetically similar to those found in tributaries without waterfalls, suggesting that mobile individuals are likely connecting below-barrier subpopulations. Despite recent hatchery stocking in this system, we did not observe evidence of hatchery introgression with wild-caught fish. The complex metapopulation of naturally isolated and connected subpopulations of brook trout described in this study highlights the importance of considering fine scale genetic structure in conservation management.

Keywords Metapopulation · Isolated population · *Salvelinus fontinalis* · Hatchery · One-way gene flow · Landscape genetics

Electronic supplementary material The online version of this article (doi:10.1007/s10592-014-0637-5) contains supplementary material, which is available to authorized users.

S. J. Kelson (✉)
Dartmouth College, University of California, Berkeley,
130 Mulford Hall #3114, Berkeley, CA 94720, USA
e-mail: skelson@berkeley.edu

A. R. Kapuscinski
Environmental Studies Program, Dartmouth College, HB 6182,
Hanover, NH 03755, USA
e-mail: Anne.Kapuscinski@dartmouth.edu

D. Timmins
New Hampshire Fish and Game, Region One Office,
629B Main Street, Lancaster, NH 03584, USA
e-mail: Dianne.Timmins@wildlife.nh.gov

W. R. Ardren
U.S. Fish and Wildlife Service, Western New England Complex,
11 Lincoln St., Essex Junction, VT 05452, USA
e-mail: William_Ardren@fws.gov

Introduction

River ecosystems are structured in dendritic networks that are heterogeneous on fine spatial scales due to variation in elevation, stream order, stream temperature, and physical barriers. Such fine-scale heterogeneity affects dispersal, migration, and connectivity of riverine taxa (Fausch et al. 2002). In river networks, the interactions between obligatory aquatic populations necessarily occur in one dimension as individuals move longitudinally within the stream channel. Consequently, a single barrier can greatly reduce gene flow. The disruption of dispersal and gene flow via

barriers and habitat fragmentation can transform a metapopulation into a series of isolated, peripheral populations (Lesica and Allendorf 1995; Dias et al. 2013), which can increase the chance of local and system-wide extinction (Rieman and Dunham 2000; Miyazono and Taylor 2013). These barriers to gene flow can be manmade or natural. Large dams in the Kansas River basin have restricted gene flow between populations of the Canadian greenside darter (*Semotilus atromaculatus*), as the darters cannot disperse across lentic reservoir habitats (Hudman and Gido 2013). Similarly, steelhead trout were unable to swim upstream over large waterfalls in the Russian River, California, resulting in genetically distinct, isolated populations above waterfalls (Deiner et al. 2007).

Trout species (Salmonidae) are generally cold-water, riverine fish whose populations are predicted to decline with the warmer water temperatures and lower flow conditions predicted by climate change models in many regions of the world (Wenger et al. 2011). One of the major theoretical principles guiding trout conservation is the need to maintain genetic diversity within and among populations that reside in interconnected river networks across the landscape (Haak and Williams 2012). However, most local and regional management of trout populations occurs at the subwatershed scale, rather than the landscape scale, so numerous efforts are underway to downscale landscape scale conservation models (Peterson et al. 2013). This downscaling requires detailed information on the geographical features that shape evolutionary dynamics of metapopulations in local river networks, including natural disperal barriers like waterfalls (Rieman and Dunham 2000; Austin et al. 2011). The extent to which a physical barrier of a given size will impede gene flow varies within and among trout species (Whiteley et al. 2004; Reilly et al. 2014).

Brook trout (*Salvelinus fontinalis*) are native to northeastern North America and threatened throughout their native range due to a suite of factors including land-use change (Nislow and Lowe 2003; Baldigo et al. 2007), habitat fragmentation (Poplar-Jeffers et al. 2009; Nislow et al. 2011; Pépino et al. 2012), and the introduction of nonnative species (Marschall and Crowder 1996). Within river networks, brook trout can persist in diverse habitats from small headwater streams (Whiteley et al. 2013) to larger mainstem rivers (Kanno et al. 2014), and some populations are anadromous. Previous research has suggested that some brook trout may be quite mobile, moving between tributary and mainstem habitats at various stages of their life history (Gowan et al. 1994; Curry et al. 2002; Petty et al. 2012; Mollenhauer et al. 2013).

Given their affinity for headwater streams and their ability to move among streams, brook trout have the potential to be structured as interconnected populations or as small isolated headwater populations, depending on the amount of gene flow. Recent studies have analyzed genetic structure of brook trout populations within streams of a single watershed in the U.S.A. in Connecticut (Kanno et al. 2011a) and Virginia (Whiteley et al. 2013) and found that brook trout structuring is influenced by natural landscape features, including stream slope and water temperature. Here we extend this body of work further north to New Hampshire, one of the few geographic regions within the native range of brook trout that has not experienced significant human impact (Esselman et al. 2011).

The overall goal of this study is to analyze brook trout population structure in low-impacted, interconnected streams to help identify factors that shaped metapopulation structure and genetic diversity of this species before widespread habitat disturbance in their native range. Specifically, we used genetic structure and assignment methods to: (1) determine the fine-scale genetic structure of brook trout including the influence of past hatchery stocking, (2) determine the origin of migratory adult fish captured in the mainstem rivers, and (3) understand how waterfalls and geographic distance influence structure among tributaries and connecting mainstem rivers. Our data provide critical information on the natural genetic structure of brook trout metapopulations in one of the few remaining interconnected river networks in their native range.

Materials and methods

Study area

The Diamond River system in the Androscoggin watershed of northern New Hampshire includes the Dead Diamond, Swift Diamond, and Magalloway rivers (Fig. 1) and is one of the few remaining systems of heterogeneous, interconnected streams containing brook trout in the United States (Hudy et al. 2008). This river network provides high quality brook trout habitats ranging from small headwater streams to the large, mainstem reaches of the Magalloway River. We sampled brook trout from tributaries and mainstem rivers to characterize genetic diversity among distinct habitats within this system. Specifically, we sampled three tributaries of the Swift Diamond River [Fourmile Brook (FB), Alder Brook (AB), and Larry's Brook (LB)], three tributaries of the Dead Diamond River [South Branch (SB), Loomis Valley (LOV), and Lamb Valley (LAV)], as well as the Swift Diamond, Dead Diamond, and Magalloway rivers (Fig. 1; Table 1). The upper sampling site on the tributary Fourmile Brook is separated from the lower site and the downstream Swift Diamond River by a 3.2 m-high waterfall (Fig. 1). Similarly, the South Branch site is separated from the Dead Diamond River by a series of three waterfalls, 2.7, 3.0 and 4.3 m high (Fig. 1). In both

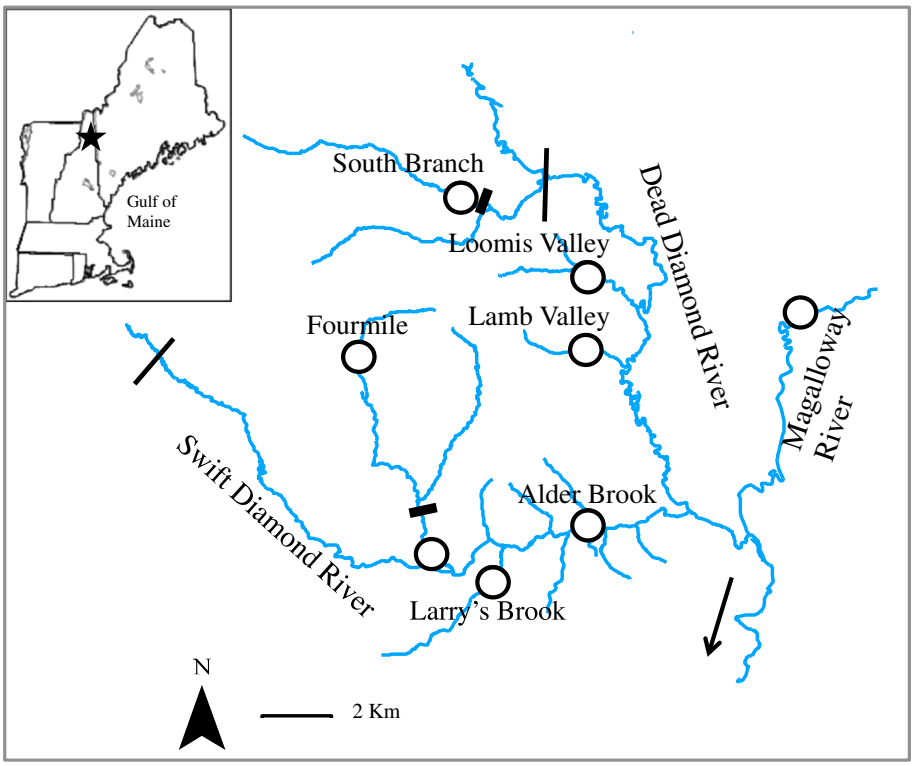

Fig. 1 Map of the Diamond River system. *Inset* shows the location of the Diamond River system in New Hampshire, U.S.A. On the large map, *open circles* show sample sites on six tributaries (Fourmile Brook has two sample locations) and on the Magalloway River. Samples not shown on map include migratory adults captured in the Swift and Dead Diamond rivers. *Short black lines* represent natural waterfalls (3.2 m vertical waterfall on Fourmile Brook, series of three waterfalls of average 3.3 m on SB). *Arrow* represents the direction of river flow

Table 1 Summary of number of brook trout sampled (N), and year sampled by stream location

Sub-basin	Sample stream	Site divisions	Historic date (N)	Recent date (N)	Total (N)
Dead Diamond R.	Loomis Valley (LOV)	Lower	2003 (4)	2011 (25)	47
–	–	Upper	2003 (11)	2011 (7)	–
Dead Diamond R.	South Branch (SB)	–	2003 (16)	2009 (24)	40
Dead Diamond R.	Lamb Valley (LAV)	–	2005 (11)	2011 (32)	43
Swift Diamond R.	Larry's Brook (LB)	–	2005 (16)	2011 (30)	46
Swift Diamond R.	Alder Brook (AB)	–	2003 (16)	2010 (32)	48
Swift Diamond R.	Fourmile Brook (FB)	Lower	2004 (16)		48
–	–	Upper	–	2011 (32)	–
Swift and Dead Diamond R.	Migratory (MG)	–	–	2011 (20)	20
Magalloway R.	Magalloway (MA)	–	2002 (13)	2007 (19)	32
Indian Stream	Outgroup (OG)	East Indian	–	2011 (24)	48
–	–	Tabor Brook	–	2011 (24)	–
–	Hatchery (HA)	–	–	–	29

cases, it is likely that the waterfalls are only passable in high flows. As a genetic outgroup for our analyses, we also sampled individuals from Tabor Brook and Indian Stream in the Connecticut River watershed of New Hampshire (hereafter referred to as 'outgroup').

Field sampling

We captured individuals from the tributaries and outgroup sites by electrofishing in August from 2002 to 2011 (not all tributaries were sampled every year, details in Table 1). We used a Smith-Root model 12B backpack electrofishing unit (DC) to conduct three-pass depletion surveys on a 100 m reach at each site, with a block net on the upstream and downstream boundaries. In the larger Dead Diamond, Swift Diamond and Magalloway rivers, where the water level was too high for electrofishing, we instead sampled fish via hook and line. At capture, we measured each individual's mass (g) and standard length (mm), and took an adipose fin clip for genetic analyses (immediately preserved in 95 %

ethanol) and scale samples for aging. The dried scales were later wet-mounted on glass slides, viewed under a transmitted light, and photographed. Two authors (SK and DT) aged the fish using annuli on scales following the methods of Stolarski and Hartman (2008). Overall, we captured a total of 372 individuals across sample sites and sample sizes ranged from 20 to 48 fish (representing a range of ages, from 0 to 3 years) per site.

Hatchery stocking

Historically, the New Hampshire Department of Fish and Game (NHFG) stocked Rome brook trout, an out-of-basin domesticated strain, into the Diamond River system in an effort to improve recreational fishing. The Swift Diamond River was stocked from 1938 to 1989 with an average of 7,700 Rome hatchery individuals per year, and from 1990 to 2006 with an average of 600 individuals per year. The Dead Diamond River was stocked from 1938 to 1978 with an average of 6,300 individuals per year, but has not been stocked since that time. Stocking events included combinations of fingerlings, yearlings, and adult brook trout. The effect of interbreeding between hatchery and native brook trout populations has been argued to be negligible (Marie et al. 2010), but in other cases, stocking has also been shown to reduce the fitness of wild brook trout populations (Hayes et al. 1996; Humston et al. 2012; Harbicht et al. 2014). To explore the potential for introgression between hatchery and wild fish in our study area, we took adipose fin clips in 2011 from age-3 Rome broodstock fish at the Berlin Hatchery, NH (source hatchery for Diamond River stockings) and incorporated these tissues into our analyses.

Migratory fish

Size and age structure of brook trout suggested that in this system older, larger fish move out of tributaries and into mainstem rivers (Table S-1). To understand the movement of the older individuals in these rivers, NHFG conducted telemetry studies in 2005–2011. Twenty large brook trout (>200 mm, fork length) that were captured in the Swift Diamond and Dead Diamond River were implanted with radio tags. The location, and thus movement, of these fish was documented over the summer months of years 2005–2011 via mobile tracking. Tracking revealed that these fish made regular movements in the months of June, July, and August between the Swift Diamond, Dead Diamond, and Magalloway rivers (Timmins 2005, 2006, 2007, and unpublished data). However, no fish were observed moving into tributaries during these months. Hereafter, we refer to the tissues collected from mobile adults captured in mainstem rivers as "migratory" fish.

Genetic analyses

Genotyping

All individuals were genotyped at 12 microsatellite loci: *SfoC113, SfoB52, SfoD100, SfoD38, SfoD75, SfoD88, SfoC86, SfoC129, SfoC28, SfoC24, SfoC115,* and *SfoD91a* (King et al. 2012). We extracted DNA from brook trout fin or scales using Qiagen Dneasy Blood and Tissue Kit standard protocol and reagents. We completed polymerase chain reactions (PCR) using Qiagen Multiplex PCR Kit and fluorescently labeled primers. PCRs were performed in 10 μL volumes in three multiplexes (Table S-2) using an Eppendorf Mastercylcer® ep generation Thermal Cycler. PCR profile included a 15 min activation period at 95 °C, 40 cycles of 30 s of denaturing at 94 °C, 90 s of annealing at 57 °C, and 90 s of extension at 72, and a 60 °C final extension. To prepare samples for fragment analysis, we completed a 1:45 dilution of PCR product, and added 0.5 μL of each sample to 9.30 μL of Applied Biosystems Hi-Di Formamide and 0.20 μL of Applied Biosystems GeneScan 500 Rox size standard. We completed a fragment analysis of samples using an ABI 3730 DNA Analyzer. Multilocus genotypes were scored using the Applied Biosystems Peak Scanner Software v1.0. To minimize scoring errors, two authors (SK and WA) scored all genotypes prior to compilation, and any inconsistent genotypes (<2 % of all samples) were scored again by both authors until an agreement was reached.

Population genetic structure

We characterized brook trout spatial population structure using STRUCTURE version 2.3.1 (Pritchard et al. 2000). Our analysis assumed the most likely number of genetically similar clusters (K) of individuals in our complete data set. We conducted twenty replicate runs of each K, from K = 1–14, with a burn in of 200,000 iterations, followed by 200,000 iterations of data collection. The symmetric similarity coefficient (SSC) (Jakobsson and Rosenberg 2007) was used to determine the similarity of outcomes among the 20 replicate STRUCTURE runs for each value of K. Using the LargeKGreedy algorithm of CLUMPP (Jakobsson and Rosenberg 2007) with 1,000 random input sequences, we determined the number of distinct modes among the 20 runs at each K by grouping pairs of runs that had SSC > 0.9. Graphical displays of STRUCTURE plots were generated using DISTRUCT software (Rosenberg 2004) with the membership of each individual representing the mean membership over the replicate runs. We used the delta K method of Evanno et al. (2005) to infer the most likely number of genetic clusters

(K) for each STRUCTURE analysis. Estimates of delta K and posterior probability for each K were summarized using STRUCTURE HARVESTER (Earl and Von Holdt 2012).

In addition to estimating the number of genetic clusters in STRUCTURE, we included measures of genetic diversity within and among sample sites. To estimate genetic relationships among sample sites, we used POPULATIONS version 1.2.28 (Langella 2002) to generate an unrooted neighbor-joining dendrogram based on Nei's genetic distance D_A (1972, 1987). We assessed confidence in the observed topology of the unrooted neighbor-joining dendrogram via the bootstrap procedure in POPULATIONS based on 1,000 resampled replicates across loci. A consensus unrooted neighbor-joining dendrogram was generated in TREEVIEW version 1.66 (Page 1996).

Finally, we calculated pairwise F_{ST} values (Weir and Cockerham 1984) and allelic richness (A_R) for sample sites in FSTAT (Goudet 1995). We evaluated the statistical significance of F_{ST} values at $\alpha = 0.05$, adjusted sequentially by the Bonferroni method (Rice 1989). We also checked for deviations from Hardy–Weinberg equilibrium (HWE) at each locus using the Markov-chain Monte Carlo methods of Guo and Thompson (1992), with 10,000 dememorization steps, 20 batches, and 5,000 iterations per batch, via the program GENEPOP version 4.0.10 software (Rousset 1997). Again, we corrected for multiple tests using the Bonferroni method. The inbreeding coefficient [F_{IS}, Weir and Cockerman (1984)] for each sample site was also calculated in GENEPOP.

Origin of migratory fish

We used assignment tests in ONCOR (Kalinowski 2008) to determine the origin of the adult migratory fish captured in the Swift Diamond and Dead Diamond rivers. Baseline populations for the assignment test included all of the sampled tributaries, the Magalloway River, and the hatchery strain.

Influence of geography on population structure

We tested for patterns of genetic isolation by geographic distance (IBD) using the one-dimensional model developed by Rousset (1997) which compares all pairwise $F_{ST}/(1-F_{ST})$ values with pairwise fluvial distances. Fluvial distances were calculated as the shortest distance between two points following the river channel. A Mantel test (Mantel 1967) with 1,000 permutations was conducted using IBDWS version 3.23 (Jensen et al. 2005) to test for statistical significance of patterns observed between fluvial distance and genetic distance in the IBD analysis.

We conducted a STREAMTREE analysis (Kalinowski et al. 2008) to examine other relationships between the physical geography and genetic structure for samples sites within the Diamond River system, following the methods of Krosch et al. (2011) and using TREEVIEW (Page 1996) to visualize the results. The STREAMTREE analysis relates genetic distance between populations to the number of sections of stream that connects them. Long branches in a STREAMTREE plot are suggestive of physical barriers between sites. Mobile migratory fish were excluded from the STREAMTREE and IBD analyses because they were collected from several locations. Hatchery fish were also excluded because they were not sampled from within the river system.

Sampling considerations

Because many of our samples were collected from small tributaries, which often have low effective populations (Kanno et al. 2011b), we were concerned that individuals sampled in a single sampling event could be from the same sibship, which can bias genetic structure results (Rodríguez-Ramilo and Wang 2012; Garza et al. 2014). To estimate the relatedness of our sample groups, we used a multi-pronged approach, including estimating Wang's mean pairwise relatedness (r_{xy}, 2002) and comparing samples collected at different times to determine the likelihood that our genetic analyses were biased from sibship sampling. A description of these analyses can be found in Supplemental Material (Appendix S-1). In brief, mean pairwise relatedness values of sample sites (Table 3) and other analyses suggested that genetic differentiation was not an artifact of sibship sampling.

Another concern was the ability to accurately characterize genetic structure with our relatively small sample sizes. However, simulations conducted by Kalinowski (2005) suggested that for populations that are substantially different ($F_{ST} > 0.2$), reliable estimates of genetic differentiation can be gathered using as few as 16 individuals, and for populations that are less differentiated ($F_{ST} \leq 0.02$), estimates will be reliable with 32 individuals. Sample sizes in our study ranged from 32 to 48 fish, suggesting our analyses should yield reliable estimates of genetic differentiation. One exception was the hatchery sample, which included only 20 individuals, but this sample was substantially different from other sample sites. Finally, it has been shown that increasing the number of loci will be more effective in producing reliable results than increasing the sample size, especially if $F_{ST} > 0.2$ (Kalinowski 2005). Consequently we amplified all 12 loci that were available for brook trout (see also Drinan et al. 2011).

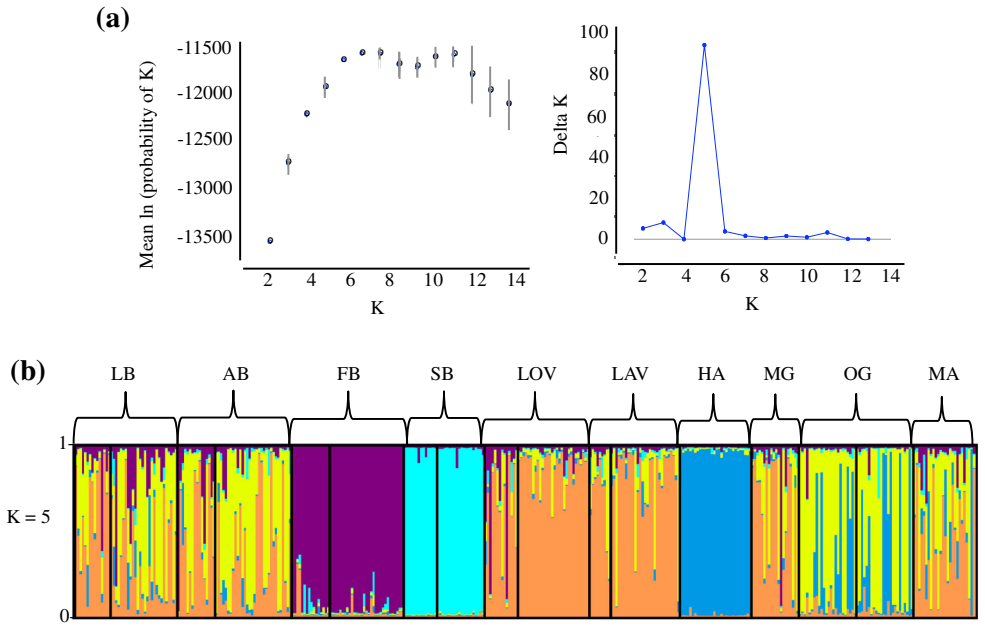

Fig. 2 Results from STRUCTURE used to infer the most likely number of distinct brook trout genetic clusters (K) in the Diamond River system. Brook trout were sampled from migratory fish and n = 7 sites in the Diamond River system. Samples from the Rome Hatchery Strain of brook trout and two outgroup locations in the Connecticut River Basin of New Hampshire are also included. **a** Likelihood values for each K = 1–14; Delta K values for K = 1–13. **b** Proportional membership of brook trout individuals to K = 5 genetic clusters. Each vertical bar represents an individual and each genetic cluster is represented by a different color. Fish are grouped by sample site. Full names of sites are listed in Table 1

Results

Population structure and hatchery influence

The STRUCTURE analysis revealed the presence of five genetic clusters: three distinct genetic clusters that were associated with three sample sites (South Branch, Fourmile Brook, and Hatchery) and two clusters that were mixed among the other sites. The estimated natural log probability of the data peaked at ~K = 6, but K = 5 had a much greater delta K value (Fig. 2a) and was consequently used for the number of genetic clusters.

Our analyses revealed that two sample sites, South Branch and Fourmile Brook, both of which contain waterfalls, were genetically distinct from the other sampled streams. The STRUCTURE analysis assigned both of these tributaries a unique genetic cluster (Fig. 2b). Genetic diversity measures also supported the isolation of these tributaries: expected and observed heterozygosity and allelic richness were lower in South Branch and Fourmile Brook than in other sample sites (Table 3). Additionally, pairwise comparisons between South Branch or Fourmile Brook and any other sample site generally resulted in F_{ST} values higher than comparisons between the other sample sites (Table 2). We found that the F_{ST} value between the upper and lower site of Fourmile Brook was zero, despite being separated by a waterfall, so we continued to treat Fourmile Brook as a single genetic cluster. The dendrogram also separated South Branch and Fourmile Brook from all other sample sites with a bootstrap value of 90, providing further support that these tributaries are isolated populations (Fig. 3).

Next, we observed mixed ancestry from two genetic clusters within the migratory individuals, the Magalloway River, and four tributaries: LB, LOV, LAV, and AB in the STRUCTURE analysis (Fig. 2b). The majority of the mixed ancestry of these sample sites was from two clusters, but some individuals, especially in samples from LOV and the migratory fish, demonstrated influence from the FB cluster (Fig. 2b). We observed small but statistically significant pairwise F_{ST} values between these sample sites, with an average of 0.03, and none of the values >0.05 (Table 2), suggesting some gene flow between these sites. These sites were also grouped together in the neighbor joining dendrogram (Fig. 3), which supported the relative genetic similarities of these streams.

Finally, the hatchery samples were assigned a unique genetic cluster in the STRUCTURE analysis (Fig. 2b). The pairwise F_{ST} values between the hatchery samples and other samples sites were also high (>0.1, Table 2). In addition, the neighbor joining dendrogram separated the hatchery and outgroup fish from all others with a bootstrap value of 94 (Fig. 3). There was little evidence for introgression with the hatchery strain in the Swift Diamond, Dead Diamond, and Magalloway rivers in the

Table 2 Pairwise comparisons of population connectivity and distance between sampled streams

	FB	SB	LB	AB	LOV	LAV	MG	MA	HA	OG
FB		0.286	0.153	0.175	0.183	0.201	0.17	0.208	0.456	0.134
SB	0.258		0.377	0.344	0.368	0.361	0.325	0.348	0.503	0.107
LB	0.112	0.296		0.072	0.101	0.081	0.096	0.105	0.262	0.29
AB	0.108	0.259	0.028		0.092	0.069	0.080	0.095	0.264	0.418
LOV	0.094	0.279	0.048	0.035		0.060	0.080	0.111	0.325	0.154
LAV	0.116	0.28	0.032	0.012	0.017		0.259	0.076	0.273	0.14
MG	0.095	0.27	0.039	**0.012**	0.028	**0.010**		0.094	0.259	0.139
MA	0.129	0.296	0.037	0.023	0.049	0.018	0.026		0.238	0.135
HA	0.370	0.453	0.197	0.117	0.247	0.196	0.215	0.173		0.238

Lower values are pairwise F_{ST} values, upper values upper values are Nei's genetic distance D_A (Fig. 3). Bolded F_{ST} values represent values that are not significant ($\alpha = 0.05$ adjusted for multiple comparisons) after 4,500 comparisons. See Table 1 for full sample site names

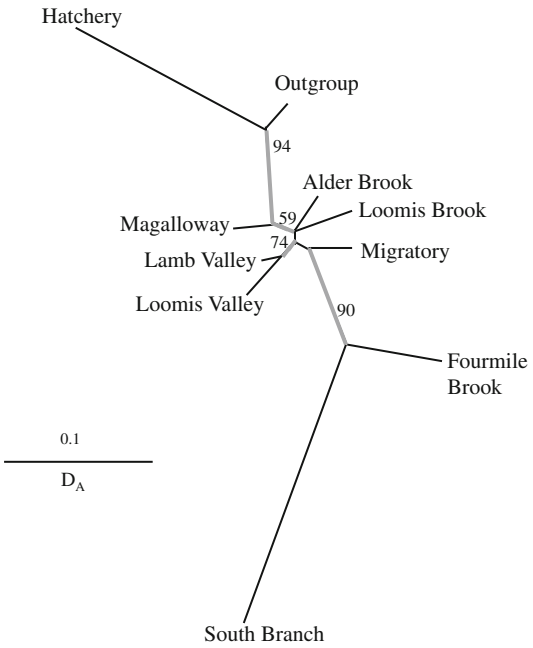

Fig. 3 Unrooted neighbor joining dendrogram of genetic clusters in Diamond River system demonstrates the genetic distance between South Branch and Fourmile Brook and the other tributaries. The absence of influence of the hatchery strain is apparent. Distance was calculated according to the Nei's genetic distance D_A (1972, 1987). Bootstrap values are based on 1,000 permutations on 12 microsatellite loci. Values over 50 are indicated by *grey branches*. Full names of sites are listed in Table 1

STRUCTURE plot, however several individuals from the outgroup sites were assigned a high probability of belonging to the hatchery cluster (Fig 2b), which likely explains why the outgroup sites were branched with the hatchery samples in the dendrogram (Fig. 3).

Overall, there were no deviations from HWE (Table S-3), and genotype frequencies conformed to expectations for randomly mating populations.

Origin of migratory fish

ONCOR assigned one migratory fish to Fourmile Brook with a probability of 1.00. This same fish was also given a high probability of belonging to the Fourmile Brook genetic cluster in the STRUCTURE analysis (Fig. 2b). ONCOR assigned the other migratory fish to tributaries of mixed ancestry. Seven fish were assigned to AB, a tributary to the Swift Diamond River. Twelve fish were assigned to tributaries to the Dead Diamond (two fish to LOV, ten fish to LAV). Mean assignment probability for all assignments was 0.87 ± 0.14 (Table S-4). The assignment probabilities may be low due to relatively little genetic differentiation between some of the tributaries (discussed above).

Influence of geography

Geographical distance was not significantly related to genetic differentiation between sample sites [pairwise $F_{ST}/(1-F_{ST})$ values, $F_{1,8} = 3.52$, $p = 0.10$]. A Mantel test between genetic differentiation and geographic distance matrices also supported the conclusion of no relationship between the two distance matrices ($Z = 9.35$, $r = -0.57$, $p = 0.88$). The STREAMTREE analysis indicated the presence of physical barriers between both South Branch and Fourmile Brook and the mainstem rivers (Fig. 4). The STREAMTREE model between observed and modeled genetic distance fit the Diamond River system well ($R^2 = 0.96$), indicating that barriers and corridors were likely influential in shaping genetic diversity.

Discussion

While many subpopulations of brook trout in the Diamond River system were genetically interconnected, two distinct genetic subpopulations were found in tributaries with

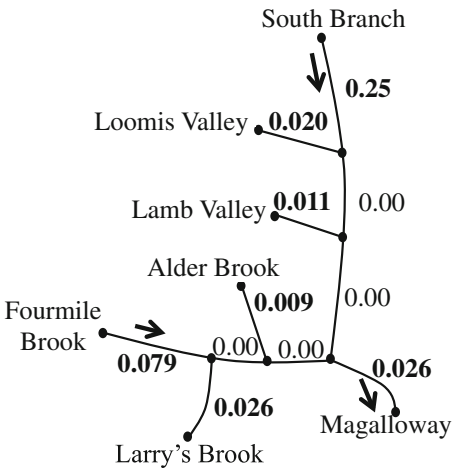

Fig. 4 STREAMTREE analysis shows the genetic distance between stream segments, displayed here as sections between an upstream and downstream dot (Kalinowski et al. 2008). Non-zero distances are shown in *bold* font. *Arrows* represent direction of flow. The high genetic distance between South Branch and Fourmile Brook and the respective adjacent stream segments suggests a physical barrier. The lack of distance between other tributaries suggests that the location below barriers in the Swift Diamond, Dead Diamond, or Magalloway River has not shaped genetic structure

physical waterfall barriers. Despite recent stocking in the study region, including in the early years that genetic sampling occurred, we found very little evidence of hatchery ancestry in wild fish. Mobile adults captured in the mainstem rivers were assigned to tributaries within the river network, suggesting that these rivers may provide important corridors for genetic exchange between tributary populations. Our results provide insight on the characteristics of brook trout metapopulation structure in a low-impacted river network within the species' native range. Below we discuss the implications of these results, including for brook trout management and conservation.

Population structure and gene flow is influenced by barriers

We found relatively high genetic similarity among four of the tributaries, the Magalloway River, and migratory fish (Figs. 2, 3; Table 2). Although the observed genetic differentiation between tributaries was low, it should not be disregarded as it could be related to the homing behavior of brook trout (O'Connor and Power 1973; D'Amelio et al. 2008). However, the mixed ancestry of these subpopulations indicate that there was some exchange of individuals among sites, providing further empirical support for maintaining river corridor habitats that connect headwater tributaries (Perkin and Gido 2012).

Our analyses identified two isolated populations above waterfalls, suggesting the importance of physical landscape features in structuring these populations. Both of these tributary populations were separated into unique clusters in STRUCTURE, were characterized by high F_{ST} values, and exhibited low heterozygosity, evidence that supports that both were small isolated peripheral populations subject to fast rates of genetic drift. The STREAMTREE analysis (Fig. 4) also suggested the existence of barriers as a mechanism for isolation. Patterns of genetic diversity in these isolated tributaries are similar to other highly distinct salmonid populations located above waterfalls (D'Amelio and Wilson 2008; Gomez-Uchida et al. 2009; Ardren et al. 2011; Scribner et al. 2012).

Interestingly, the upper and lower sample sites on one of the isolated streams, Fourmile Brook, were divided by a waterfall, but were grouped together genetically, indicating high migration rates *within* the tributary. Moreover, there was no obvious physical barrier between the lower sample site on Fourmile Brook and the mainstem, leaving open the mechanism for the separation of the lower site from the mainstem river. One possibility is that the lower population was comprised mostly of migrants from the upper site (i.e., downstream gene flow, see below), which may swamp out the genetic signal of the few migratory fish that swim up this tributary to the barrier from downstream mainstem reaches. Alternatively, behavioral and temporal differences may have prevented interbreeding with adults from other tributaries in this reach. For example, salmonid species breeding within connected streams can fail to interbreed due to differences in spawn timing (e.g., pink salmon: Gharrett et al. 2013) and microhabitat site selection (e.g., brook trout: Franssen et al. 2014), both of which could explain reduced gene flow between subpopulations. The processes that maintain genetic differentiation when physical barriers are lacking merit further attention.

Beyond revealing specific patterns of fine scale structuring in our system, our study also highlights that simple geographical distance cannot be used to infer genetic structuring of brook trout populations at fine spatial scales. Instead, genetic structure appeared to be driven by barriers between tributaries, which occur independently of geographic distance and location (Fig. 4). The genetic divergence of subpopulations in tributaries above waterfalls supports the idea that physical features (e.g., waterfalls, stream temperature, stream slope) can play a strong role in structuring riverine fish populations. Fine-scale structuring in streams by landscape features has been demonstrated for brook trout (Kanno et al. 2011a; Whiteley et al. 2013) and other freshwater fishes (e.g., *Salmo salar*, Dillane et al. 2008; *Etheostoma raneyi*, Sterling et al. 2012; *Salvelinus namaycush*, McCracken et al. 2013). These results suggest that local conservation management requires information about fine-scale genetic structuring to supplement larger-scale landscape genetics studies of brook trout (Castric et al. 2001;

Castric and Bernatchez 2003; Poissant et al. 2005) and other organisms (see Manel et al. 2003 for a review).

Large migratory fish connect subpopulations

To explore a mechanism for gene flow between tributaries, we genetically assigned large mobile fish to their tributary of origin. We found that the majority of radio-tracked adult trout in the Swift Diamond and Dead Diamond rivers originated from the four tributaries that were of mixed ancestry (Table S-4). Although we could not track the individuals to their spawning sites due to the limited battery life of the tags, the relative genetic similarity of these tributaries indicates that there must be gene flow between them. Similarly, a study by Kanno et al. (2014) demonstrated that some brook trout may move between and breed in both tributaries and mainstem rivers. In their system, adult brook trout movements resulted in substantial gene flow, with adult mainstem trout producing nearly half of their surviving offspring in tributaries (Kanno et al. 2014). Similarly, the earlier radio-tracking study in the Diamond River system suggests that brook trout move between tributaries and mainstem sites (Timmins 2005, 2006, 2007), and our genetic data provide evidence of gene flow between these same sites.

We also observed a pattern of one-way, downstream gene flow via migratory individuals from an isolated population in Fourmile Brook. One of the migratory individuals captured in the Swift Diamond River was assigned to Fourmile Brook, as the most likely source population. Evidence of fish dispersing from Fourmile Brook into other tributaries was also observed in the STRUCTURE plots, with two fish sampled in the LOV displaying high assignment probability to Fourmile Brook (Fig. 2). Thus one-way gene flow appears to be contributing to the higher allelic richness and observed heterozygosity found in the other sample sites (Table 3). Patterns of one-way downstream gene flow have been reported in other brook trout populations in the Connecticut (Kanno et al. 2011a) and Salmon Trout river basins (Scribner et al. 2012). We concur with Allendorf and Luikart (2007) that important sources of genetic variability, such as isolated populations that provide one-way gene flow to larger populations, should be given consideration in conservation efforts.

Table 3 Summary of sample sites shows expected heterozygosity (H_E), observed heterozygosity (H_O), rarefied allelic richness (A_R), the inbreeding coefficient (F_{IS}), and mean pairwise Wang (2002) relatedness (r_{xy})

Sample Site	H_E	H_O	A_R	F_{IS}	r_{xy}
FB	0.48 (0.28)	0.43 (0.25)	3.8 (2.4)	0.088	−0.096
SB	0.35 (0.31)	0.30 (0.29)	3.3 (1.9)	0.136	−0.090
LB	0.62 (0.15)	0.65 (0.17)	5.5 (2.3)	−0.053	0.12
AB	0.70 (0.12)	0.64 (0.09)	6.8 (2.7)	0.085	−0.095
LOV	0.66 (0.17)	0.57 (0.17)	5.3 (2.1)	0.142	−0.015
LAV	0.70 (0.12)	0.67 (0.14)	5.7 (2.9)	0.04	−0.0080
MG	0.62 (0.18)	0.55 (0.17)	6 (2.4)	0.114	0.0011
MA	0.60 (0.11)	0.56 (0.09)	5.6 (1.6)	0.052	0.030
HA	0.54 (0.16)	0.50 (0.16)	4.6 (1.6)	0.072	−0.079
OG	0.34 (0.15)	0.32 (0.15)	6.6 (2.8)	0.057	−0.050

For H_E, H_O, and A_R, standard deviations are in parenthesis. Calculations were averaged over 12 microsatellite loci. See Table 1 for full sample site names

Absence of hatchery influence despite historic stocking

Both the STRUCTURE analysis (Fig. 2) and the neighbor-joining dendrogram (Fig. 3) grouped the hatchery individuals into a unique cluster, suggesting that any introgression between hatchery and native fish did not have a lasting impact on native fish genetic diversity in the Diamond River system. Annett et al. (2012) found a similar lack of introgression with hatchery strains in Cape Cod brook trout due to the low survival and reproductive success of hatchery fish in natural habitats. Indeed, hatchery strains are known to have decreased reproductive abilities relative to wild strains (Araki et al. 2007; Leonard et al. 2013), as well as reduced survival and growth (Vincent 1960; Fraser 1981). It is important to note, however, that this is not the case in all systems. Humston et al. (2012) found the colonization and natural reproduction of hatchery-hybridized brook trout to be common in headwater streams in Virginia. Previous studies have noted that usable habitat size and stocking density influences the extent of hatchery hybridization (Marie et al. 2010, 2012), suggesting reasons for different outcomes in different regions. In the Diamond River system recent stocking has been at low levels and only in headwater regions, both of which likely contributed to the lack of hatchery ancestry in wild fish. In contrast, the outgroup sample from Indian Stream (Connecticut, USA), which is currently stocked, appears to have some individuals with hatchery ancestry, in addition to full hatchery individuals. This result highlights the fact that it is not safe to assume a lack of hatchery introgression in all river systems, especially in currently stocked streams.

Conservation implications and conclusions

Our characterization of a complex brook trout metapopulation in a dendritic stream network demonstrates the importance of understanding structure at a fine spatial scale in river networks. We found higher levels of genetic differentiation between populations within a watershed than has been previously documented for brook trout (Kanno et al. 2011a; Whiteley

et al. 2013), possibly because of fast rates of genetic drift in small, isolated populations. In addition, our evidence of one-way gene flow from an isolated, genetically differentiated population provides empirical support for the importance of maintaining isolated populations, despite their isolation and small size, as such populations may serve as source populations (Allendorf and Luikart 2007; Letcher et al. 2007).

Our results also suggest that fragmentation at a fine spatial scale (i.e., implementation of a single barrier in a headwater stream) may significantly alter genetic structure, producing an isolated, above-barrier population. For brook trout, headwater streams often maintain small effective population sizes (Kanno et al. 2011b), and thus isolated populations may be even more susceptible to demographic stochasticity. A series of isolated peripheral populations could pose a greater challenge for conservation management than similarly sized populations connected via dispersal and gene flow (Letcher et al. 2007). On a landscape scale, fragmentation of naturally interconnected tributaries via human land use (e.g., construction of dams and culverts, channelization of streams) is widespread throughout the native range of brook trout (Hudy et al. 2008), and many other river networks (Fullerton et al. 2010).

Our study highlights the importance of interconnected, heterogeneous stream networks for maintaining diverse brook trout populations and resilient metapopulations. Given the importance of small tributaries as breeding habitat and potential source populations, management actions should preserve headwater tributaries located above presumed barriers and the downstream mainstem habitats that provide dispersal corridors among subpopulations. Conservation efforts that include both headwater and mainstem populations of trout should greatly increase the resilience of trout stock complexes.

Acknowledgments This project was made possible with funds from the Kaminsky Family Fund Award, Trout Unlimited, the Dartmouth Outing Club Northern Studies Internship, the James O. Freedman Presidential Scholar Assistantship, and the Sherman Fairchild Professorship in Sustainability Science of A.R. Kapuscinski. We thank R. Piampiano and K. Evans for supporting research at the Second College Grant, F.J. Kull for the use of his lab at Dartmouth College, NHFG staff for help with electrofishing, S. Julian with U.S. Fish and Wildlife Service (USFWS) for donating PCR primers, and D. Seiders and D. Boucher with Maine Department of Inland Fisheries and Wildlife and J. Martell from Antioch College for sharing scale samples. We also thank M. Ayres, M. Bogan, S. Carlson, J. C. Garza, A. Sturrock, B. Taylor, and two anonymous reviewers for valuable comments that improved this paper. The findings and conclusions in the article are those of the authors and do not necessarily represent the views of the USFWS.

References

Allendorf FW, Luikart G (2007) Conservation and the genetics of populations. Blackwell Publishing, Malden

Annett B, Gerlach G, King TL, Whiteley AR (2012) Conservation genetics of remnant coastal brook trout populations at the southern limit of their distribution: population structure and effects of stocking. Trans Am Fish Soc 141:1399–1410

Araki H, Cooper B, Blouin MS (2007) Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318:100–103

Ardren WR, DeHaan PW, Smith CT (2011) Genetic structure, evolutionary history, and conservation units of bull trout in the coterminous United States. Trans Am Fish Soc 140:506–525

Austin JD, Jelks HL, Tate B, Johnson AR, Jordan F (2011) Population genetic structure and conservation genetics of threatened Okaloosa darters (*Etheostoma okaloosae*). Conserv Genet 12:981–989

Baldigo BP, Lawrence G, Simonin H (2007) Persistent mortality of brook trout in episodically acidified streams of the southwestern Adirondack mountains, New York. Trans Am Fish Soc 136: 121–134

Castric V, Bernatchez L (2003) The rise and fall of isolation by distance in the anadromous brook charr (*Salvelinus fontinalis* Mitchill). Genetics 163:983–996

Castric V, Bonney F, Bernatchez L (2001) Landscape structure and heirarchical genetic diversity in the brook charr, *Salvelinus fontinalis*. Evoultion 55:1016–1028

Curry RA, Sparks D, van De Sande J (2002) Spatial and temporal movements of a riverine brook trout population. Trans Am Fish Soc 131:551–560

D'Amelio S, Mucha J, Mackereth R, Wilson CC (2008) Tracking coaster brook trout to their sources: combining telemetry and genetic profiles to determine source populations. North Am J Fish Manag 28:1343–1349

Deiner K, Garza JC, Coey R, Girman DJ (2007) Population structure and genetic diversity of trout (*Oncorhynchus mykiss*) above and below natural and man-made barriers in the Russian River, California. Conserv Genet 8:437–454

Dias MS, Cornu J, Oberdorff T, Lasso CA, Tedesco PA (2013) Natural fragmentation in river networks as a driver of speciation for freshwater fishes. Ecography 36:683–689

Dillane E, McGinnity P, Coughlan JP, Cross C, de Eyto E, Kenchington E, Prodohl P, Cross TF (2008) Demographics and landscape features determine intrariver population structure in Atlantic salmon (*Salmo salar* L.): the case of the River Moy in Ireland. Mol Ecol 17:4786–4800

Drinan DP, Kalinowski ST, Vu NV, Shephard BB, Muhlfied C, Campbell MR (2011) Genetic variation in westslope cutthroat trout *Oncorhynchus clarkii lewisi*: implications for conservation. Conserv Genet 12:1513–1523

Earl D, Von Holdt B (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

Esselman PC, Infante DM, Wang L, Wu D, Cooper AR, Taylor WW (2011) An index of cumulative disturbance to river fish habitats of the conterminous United States from landscape anthropogenic activities. Ecol Restor 19:1–2

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Fausch KD, Torgersen CE, Baxter CV, Li HW (2002) Landscapes to riverscapes: bridging the gap between research and conservation of stream fishes. Bioscience 52:483–498

Franssen J, Lapointe M, Magnan P (2014) Geomorphic controls on fine sediment reinfiltration into salmonid spawning gravels and the implications for spawning habitat rehabilitation. Geomorphology 211:11–21

Fraser J (1981) Comparative survival and growth of planted wild, hybrid, and domestic strains of brook trout (*Salvelinus fontinalis*) in Ontario. Can J Fish Aquat Sci 38:1672–1684

Fullerton AH, Burnett KM, Steel EA, Flitcroft RL, Pess GR, Feist BE, Torgersen CE, Miller DJ, Sanderson BL (2010) Hydrological connectivity for riverine fish: measurement challenges and research opportunities. Freshw Biol 55:2215–2237

Garza JC, Gilbert-Horvath EA, Spence BC, Williams TH, Fish H, Gough SA, Anderson JH, Hamm D, Anderson EC (2014) Population structure of steelhead in coastal California. Trans Am Fish Soc 143:134–152

Gharrett AJ, Joyce J, Smoker WW (2013) Fine-scale temporal adaptation within a salmonid population: mechanism and consequences. Mol Ecol 22:4457–4469

Gomez-Uchida D, Knight TW, Ruzzante DE (2009) Interaction of landscape and life history attributes on genetic diversity, neutral divergence and gene flow in a pristine community of salmonids. Mol Ecol 18:4854–4869

Goudet J (1995) FSTAT (Version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486

Guo S, Thompson E (1992) Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48:361–372

Haak AL, Williams JE (2012) Spreading the risk: native trout management in a warmer and less-certain future. North Am J Fish Manag 32:387–401

Hudman SP, Gido KB (2013) Multi-scale effects of impoundments on genetic structure of creek chub (*Semotilus atromaculatus*) in the Kansas River basin. Freshw Biol 58:441–453

Hudy M, Thieling TM, Gillespie N, Smith EP (2008) Distribution, status, and land use characteristics of subwatersheds within the native range of brook trout in the eastern United States. North Am J Fish Manag 28:1069–1085

Humston R, Bezold KA, Adkins ND, Bisey RJ, Huss J, Meekins BA, Cabe PR, King TL (2012) Consequences of stocking headwater impoundments on native populations of brook trout in tributaries. North Am J Fish Manag 32:100–108

Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806

Jensen J, Bohonak A, Kelley S (2005) Isolation by distance, Web service. http://ibdws.sdsu.edu/

Kalinowski ST (2005) Do polymorphic loci require large sample sizes to estimate genetic distances? Heredity 94:33–36

Kalinowski ST (2008) Oncor: software for genetic stock identification. Department of Ecology, Montana State University. http://www.montana.edu/kalinowski/Software/ONCOR.htm/

Kalinowski ST, Meeuwig MH, Narum SR, Taper ML (2008) Stream trees: a statistical method for mapping genetic differences between populations of freshwater organisms to the sections of streams that connect them. Can J Fish Aquat Sci 65:2752–2760

Kanno Y, Vokoun JC, Letcher BH (2011a) Fine-scale population structure and riverscape genetics of brook trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks. Mol Ecol 20:3711–3729

Kanno Y, Vokoun JC, Letcher BH (2011b) Sibship reconstruction for inferring mating systems, dispersal and effective population size in headwater brook trout (*Salvelinus fontinalis*) populations. Conserv Genet 12:619–628

Kanno Y, Letcher BH, Coombs JA, Nislow KH, Whiteley AR (2014) Linking movement and reproductive history of brook trout to assess habitat connectivity in a heterogeneous stream network. Freshw Biol 59:142–154

King TL, Lubinski BA, Burnham-Curtis MK, Stott W, Morgan RP II (2012) Tools for the management and conservation of genetic diversity in brook trout (*Salvelinus fontinalis*): tri- and tetranucleotide microsatellite markers for the assessment of genetic diversity, phylogeography, and historical demographics. Conserv Genet Resour 4:539–543

Krosch MN, Baker AM, Mather PB, Cranston PS (2011) Spatial population genetic structure reveals strong natal site fidelity in *Echinocladius martini* (Diptera: Chironomidae) in northeast Queensland, Australia. Freshw Biol 56:1328–1341

Langella O (2002) POPULATIONS 1.2.31. bioinformatics.org/~tryphon/populations/. bioinformatics.org/~tryphon/populations/

Leonard JBK, Stott W, Loope DM, Kusnierz PC, Sreenivasan A (2013) Biological consequences of the coaster brook trout restoration stocking program in Lake Superior tributaries within Pictured Rocks National Lakeshore. North Am J Fish Manag 33:359–372

Lesica P, Allendorf FW (1995) When are peripheral populations valuable for conservation? Conserv Biol 9:753–760

Letcher BH, Nislow KH, Coombs JA, O'Donnell MJ, Dubreuil TL (2007) Population response to habitat fragmentation in a stream-dwelling brook trout population. PLoS One 2:e1139

Manel S, Schwartz MK, Luikart G, Taberlet P (2003) Landscape genetics: combining landscape ecology and population genetics. Trends Ecol Evol 18:189–197

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Marie AD, Bernatchez L, Garant D (2010) Loss of genetic integrity correlates with stocking intensity in brook charr (*Salvelinus fontinalis*). Mol Ecol 19:2025–2037

Marie AD, Bernatchez L, Garant D (2012) Environmental factors correlate with hybridization in stocked brook charr (*Salvelinus fontinalis*). Can J Fish Aquat Sci 69:884–893

Marschall EE, Crowder LB (1996) Assessing population responses to multiple anthropogenic effects: a case study with brook trout. Ecol Appl 6:152–167

McCracken GR, Perry R, Keefe D, Ruzzante DE (2013) Hierarchical population structure and genetic diversity of lake trout (*Salvelinus namaycush*) in a dendritic system in Northern Labrador. Freshw Biol 58:1903–1917

Miyazono S, Taylor CM (2013) Effects of habitat size and isolation on species immigration–extinction dynamics and community nestedness in a desert river system. Freshw Biol 58:1303–1312

Mollenhauer R, Wagner T, Kepler MV, Sweka JA (2013) Fall and early winter movement and habitat use of wild brook trout. Trans Am Fish Soc 142:1167–1178

Nislow KH, Lowe WH (2003) Influences of logging history and stream pH on brook trout abundance in first-order streams in New Hampshire. Trans Am Fish Soc 132:166–171

Nislow KH, Hudy M, Letcher BH, Smith EP (2011) Variation in local abundance and species richness of stream fishes in relation to dispersal barriers: implications for management and conservation. Freshw Biol 56:2135–2144

O'Connor J, Power G (1973) Homing of brook trout (*Salvelinus fontinalis*) in Matamek lake, Quebec. J Fish Res Board Canada 30:1012–1014

Page RDM (1996) TreeView: an application to display phylogenetic trees on personal computers. Cabios Appl Note 12:357–358

Pépino M, Rodríguez MA, Magnan P (2012) Impacts of highway crossings on density of brook charr in streams. J Appl Ecol 49:395–403

Perkin JS, Gido KB (2012) Fragmentation alters stream fish community structure in dendritic ecological networks. Ecol Appl 22:2176–2187

Peterson DP, Wenger SJ, Rieman BE, Isaak DJ (2013) Linking climate change and fish conservation efforts using spatially explicit decision support tools. Fisheries 38:112–127

Petty JT, Hansbarger JL, Huntsman BM, Mazik PM (2012) Brook trout movement in response to temperature, flow, and thermal refugia within a complex Appalachian riverscape. Trans Am Fish Soc 141:1060–1073

Poissant J, Knight TW, Fergurson MM (2005) Nonequilibrium conditions following landscape rearrangement: the relative contribution

of past and current hydrological landscapes on the genetic structure of a stream-dwelling fish. Mol Ecol 14:1321–1331
Poplar-Jeffers IO, Petty JT, Anderson JT, Kite SJ, Strager MP, Fortney RH (2009) Culvert replacement and stream habitat restoration: implications from brook trout management in an Appalachian watershed, U.S.A. Restor Ecol 17:404–413
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
Reilly JR, Paszkowski CA, Coltman DW (2014) Population genetics of arctic grayling distributed across large, unobstructed river systems. Trans Am Fish Soc 143:802–816
Rice W (1989) Analyzing tables of statistical tests. Evolution 43:223–225
Rieman BE, Dunham JB (2000) Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. Ecol Freshw Fish 9:51–64
Rodríguez-Ramilo ST, Wang J (2012) The effect of close relatives on unsupervised Bayesian clustering algorithms in population genetic structure analysis. Mol Ecol Resour 12:873–884
Rosenberg NA (2004) Distruct: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138
Rousset F (1997) Genetic differentiation and estimation of gene flow from F-statistics under isolation by distance. Genetics 145:1219–1228
Scribner K, Huckins C, Baker E, Kanefsky J (2012) Genetic relationships and gene flow between resident and migratory brook trout in the Salmon Trout River. J Great Lakes Res 38:152–158
Sterling KA, Reed DH, Noonan BP, Warren MLJ (2012) Genetic effects of habitat fragmentation and population isolation on *Etheostoma raneyi* (Percidae). Conserv Genet 13:859–872
Stolarski JT, Hartman KJ (2008) An evaluation of the precision of fin ray, otolith, and scale age determinations for brook trout. North Am J Fish Manag 28:1790–1795
Timmins D (2005) Migration patterns of wild adult brook trout in northern New Hampshire, F50R Project Segment Report. pp 1–3
Timmins D (2006) Migration patterns of wild adult brook trout in northern New Hampshire, F50R Project Segment Report. pp 1–6
Timmins D (2007) Migration patterns of wild adult brook trout in northern New Hampshire, F50R Project Segment Report. pp 1–9
Vincent RE (1960) Some influences of domestication upon three stocks of brook trout (*Salvelinus fontinalis* Mitchill). Trans Am Fish Soc 89:35–52
Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370
Wenger SJ, Isaak DJ, Luce CH, Neville HM, Fausch KD, Dunham JB, Dauwalter DC, Young MK, Elsner MM, Rieman BE, Hamlet AF, Williams JE (2011) Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. Proc Natl Acad Sci 108:14175–14180
Whiteley AR, Spruell P, Allendorf FW (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. Mol Ecol 13:3675–3688
Whiteley AR, Coombs JA, Hudy M, Robinson Z, Colton AR, Nislow KH, Letcher BH (2013) Fragmentation and patch size shape genetic structure of brook trout populations. Can J Fish Aquat Sci 70:678–688