NRC Research Press

ARTICLE

Simulation and empirical analysis of novel sibship-based genetic determination of fish passage[1]

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Abstract: We develop and test a new analytic approach, termed "sib-split", to detect fish passage through road crossings. This new approach is based on the genetic analysis of full-siblings on opposite sides of potential barriers. We used simulations and data from two empirical case studies involving brook trout (*Salvelinus fontinalis*) movement with respect to barriers that varied in strength of effect on fish passage. Simulations revealed that both sib-split and the population assignment-based method (STRUCTURE) were highly accurate (mean accuracy > 99%) under easy-to-detect conditions (moderate to strong genetic differentiation and no movement). However, under difficult-to-detect simulated conditions (no genetic differentiation, 10% movement each generation), sib-split had higher accuracy (mean accuracy = 98%) than STRUCTURE (mean accuracy = 84%). Sib-split also outperformed STRUCTURE (mean accuracy 98% versus 89%) under a more difficult-to-detect simulated management scenario (simulated construction of a new complete barrier to movement). Sib-split provided more reliable and easily interpretable movement detection in both easy- and difficult-to-detect empirical case studies. With the empirical case studies, sensitivity to the prior on migration rate precluded use of STRUCTURE by itself, but a two-step approach where sib-split results were used to provide an informed migration prior for STRUCTURE provided additional information for both case studies.

Résumé : Nous avons mis au point et validé une nouvelle approche analytique désignée « sib-split » pour déceler le passage de poissons par des franchissements de route. Cette approche repose sur l'analyse génétique de groupes de fratrie de part et d'autre de barrières potentielles. Nous avons utilisé des simulations et les données de deux études empiriques de cas portant sur les déplacements des ombles de fontaine (*Salvelinus fontinalis*) par rapport à des barrières présentant différentes intensités d'effet sur le passage des poissons. Les simulations ont démontré que tant l'approche sib-split que la méthode d'affectation à des populations (STRUCTURE) étaient très exactes (exactitude moyenne > 99 %) pour des conditions de détection facile (différentiation génétique modérée à forte et aucun déplacement). Cependant, dans des conditions simulées de détection difficile (pas de différentiation génétique, 10 % de déplacements pour chaque génération), l'approche sib-split était plus exacte (exactitude moyenne = 98 %) que STRUCTURE (exactitude moyenne = 84 %). La méthode sib-split a également donné de meilleurs résultats que STRUCTURE (exactitude moyenne de 98 % contre 89 %) pour un scénario de gestion simulé avec détection plus difficile (construction simulée d'une nouvelle barrière complètement étanche aux déplacements). L'approche sib-split permettait une détection des déplacements plus fiable et facile à interpréter pour les études de cas empiriques de détection facile et difficile. En ce qui concerne les études de cas empiriques, la sensibilité aux informations a priori sur le taux de migration n'a pas permis la seule utilisation de STRUCTURE, mais une approche en deux étapes dans laquelle les résultats de l'approche sib-split étaient utilisés pour fournir de l'information a priori sur la migration pour la méthode STRUCTURE a fourni des renseignements supplémentaires pour les deux études de cas. [Traduit par la Rédaction]

Introduction

Complete or partial barriers to aquatic organism passage fragment populations and increase risk of local extirpation (Dunham et al. 1997; Letcher et al. 2007; Olden et al. 2010). There is a strong need to assess potential barriers to passage, remediate confirmed barriers, and to monitor passage if a barrier has been removed or replaced (Januchowski-Hartley et al. 2013; McKay et al. 2013; Mahlum et al. 2014; Neville and Peterson 2014, this issue). Observation of individual upstream passage provides evidence that the removal or replacement of a barrier has been effective. This evidence can be obtained in a number of different ways. Direct evidence for individual passage can be accomplished by mark-and-detection studies (Blank et al. 2006; Bourne et al. 2011). However, because movement is highly episodic and only a small fraction of the population may move during any given time interval (Kanno et al. 2014), traditional tagging studies frequently have high probabilities of not detecting movement when it actually occurs (type II error) while still incurring substantial time and personnel costs. For example, tagging studies require at least two visits to each monitored site and possibly four visits for a before–after replacement design (mark and recapture both before and after). Evidence gained from genetic techniques may provide a more efficient way to evaluate individual passage.

Standard, indirect, individual-based but population-level genetic approaches, such as assignment tests (Cornuet et al. 1999; Pritchard et al. 2000), lack resolution to detect individual movement at an ecological time scale in many situations (Waples and Gaggiotti 2006). These methods rely on substantial genetic (allele frequency)

Received 21 March 2014. Accepted 23 June 2014.

Paper handled by Associate Editor Eric Taylor.

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[1]This is a companion paper to Neville and Peterson, this issue [doi:10.1139/cjfas-2014-0138].

divergence, which depends on the age of the barrier, generation time of the organisms affected, and effective population size (N_e). Sufficient genetic divergence is unlikely to be met in many cases where assessment of recent individual movement is needed for management purposes (Waples and Gaggiotti 2006).

An alternative genetic approach proposed here takes advantage of the fact that most stream-dwelling species have point distributions of reproduction and exhibit family-level genetic structure. Examples of point distributions of reproduction include salmonid (Einum et al. 2008) and lamprey (Nika and Virbickas 2010) redds or sculpin (Fiumera et al. 2002) and stream salamander (Bruce 2005) nests. Dispersal of siblings from these point sources can be quantified from a genetic survey and subsequent assignment of individuals (parents and offspring) to family groups (Hudy et al. 2010; Kanno et al. 2011). When coupled with a sampling design that includes barrier assessment, family members captured on opposite sides of the potential barrier demonstrate individual passage. A promising aspect of this approach is that it might work for individuals collected from a single cohort. We call this new combination of appropriate field collections and existing sibship reconstruction software the "sibship-splitting" approach, or "sib-split" for short (Fig. S1[2]). This approach builds on the growing number of recent attempts to use sibship and parentage reconstruction in conjunction with more traditional population genetic approaches to assess fine-scale genetic structure and connectivity (Clark et al. 2010; Vollestad et al. 2012; Kanno et al. 2014). Sib-split has the potential to provide managers with a cost-effective method to monitor aquatic organism passage. However, simulation-based validation and field testing are required to fully explore and evaluate this new approach.

In this paper, we used simulations and data from two empirical case studies to test the accuracy of our new sib-split approach and compare it with a population-level assignment method as implemented by the program STRUCTURE (Pritchard et al. 2000). First, we used sib-split and STRUCTURE to estimate simulated movement in relation to barriers with varying degrees of passability and varying above-barrier versus below-barrier genetic divergence. Additionally, we used simulation to test the ability of both methods to detect movement for two management scenarios (barrier remediation and barrier installation). Second, we used data from two empirical eastern brook trout (*Salvelinus fontinalis*) case studies to compare movement estimates produced by sib-split and STRUCTURE. The first case study (easy-to-detect) involved sampling brook trout before and after the removal of a small dam; the second (difficult-to-detect) involved sampling on both sides of a perched culvert acting as a partial barrier within a well-studied brook trout metapopulation.

Methods

Simulations

We conducted two sets of simulations to test the accuracy of sibship-based and assignment test-based genetic methods to estimate fish movement in relation to barriers and to test realistic management scenarios (Table 1). All simulations were run for three generations. Individual per-generation movement probabilities were set to either 0 or 0.1. In the first set of simulations, our goal was to test the accuracy of sib-split and assignment test approaches by varying movement probability and genetic divergence of two populations on either side of a barrier. We simulated a two-way complete barrier with no movement in any of the three generations, and we simulated a moderate barrier with movement (movement probability = 0.1) during all three generations (Table 1). The three moderate barrier simulations differed in the amount of genetic divergence between the populations separated by the barrier (Table 1). These simulations were meant to represent barriers to movement that would be progressively more difficult to detect by the two genetic approaches.

Table 1. Scenarios simulated to assess performance of sib-split and STRUCTURE.

Scenario	Movement probability			Initial *D*	Generations moving
Simulation set 1					
Two-way barrier	0	0	0	0.25	None
Two-way barrier	0	0	0	0.10	None
Moderate barrier	0.1	0.1	0.1	0.25	All three
Moderate barrier	0.1	0.1	0.1	0.10	All three
Moderate barrier	0.1	0.1	0.1	0	All three
Simulation set 2					
New barrier	0.1	0.1	0	0	First two
Remediated barrier	0	0	0.1	0.25	Last one

Note: Movement probabilities are shown for each the three simulated generations sequentially. Jost's *D* is the measure of genetic differentiation used. The first set of simulations was designed to test detectability of a strong (two-way) or moderate (some movement in both directions) barrier from easier- to more difficult-to-detect. The second set of simulations was designed to test two management scenarios. A barrier was created between generations two and three for "New barrier". A barrier was removed between generations two and three for "Remediated barrier".

We performed a second set of simulations to compare detection accuracies for two specific scenarios relevant to management. The first scenario was meant to test accuracy after the installation of a new barrier. Movement could occur during the first two generations but not the third (new barrier installed prior to third generation). The second scenario was meant to test accuracy following barrier remediation (removal). Movement could occur during the third generation but not the first two (remediated barrier prior to third generation; Table 1).

We simulated data using the program PEDAGOG version 1.24 (Coombs et al. 2010). We used this software because of its ability to track individual pedigree and genotype information; allow manipulation of genetic, demographic, and error parameters; and automatically format the simulated output into input files for pedigree reconstruction programs. Two baseline populations were parameterized to have one age-class with a 1-year lifespan and one bout of reproduction. There was a 0.5 probability of each individual being a female. Survival, maturation, and capture probability were all given a value of one. Reproduction followed a polyandrous mating system. Males mated with one female, females mated with the number of males drawn from a Poisson distribution with a mean of two. Female fecundity followed an exponential distribution (λ = 8), which was parameterized to create realistic family-size distributions for natural populations of brook trout (Hudy et al. 2010; Whiteley et al. 2012). Simulated family-size distributions were similar to empirical observations for this species (Hudy et al. 2010; Whiteley et al. 2012). For example, 88% of individuals were contained by full-sib families of size three or greater in a natural Virginia population (Hudy et al. 2010) compared with 83% with the simulated data. Offspring contribution from multiple males was drawn from a Uniform distribution. Cohort size was maintained at a constant value of 450. We performed five replicates for each set of conditions.

Genetic data within the simulations were parameterized from empirical microsatellite data from a wild brook trout population in Virginia (Hudy et al. 2010). This data set consisted of variation at eight microsatellite loci. Mean expected heterozygosity (H_S) across loci for this population was 0.792, and the mean number of alleles (A_O) across loci was 10.8. PI_{ave} (the average probability of

[2]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2014-0137.

identity, that is, the probability of observing two copies of any multilocus genotype in a population) was 6.4×10^{-6} and PI_{ave}-sibs (a maximum value for PI_{ave} calculated for sibs) (Taberlet and Luikart 1999) was 3.6×10^{-4}. Probability of parent exclusion (PE-first parent; where no parent is known) was 99.9%. It is worth noting that PI_{ave}-sibs and PE-first parent are the most conservative versions of these statistics. For initial simulation parameterization with more than eight loci, we increased the genetic panel from 8 to 12 or 16 loci by randomly selecting without replacement and copying all alleles and their empirical allele frequencies for four loci (for the 12-locus panel) or copying all alleles and their frequencies for all loci (16-locus panel). Founding cohorts for each of the three populations were drawn from a population pool of 10 000 animals, whose genotypes were assigned randomly from population-specific allele frequencies for the set of 8, 12, or 16 loci.

Population genetic differentiation was estimated with Jost's D_{est}, hereafter D (Jost 2008), or with θ (Weir and Cockerham 1984) as a measure of F_{ST}. We used values of D = 0, 0.1, and 0.25 to simulate no, moderate, and strong genetic differentiation, respectively. We initially set Population 2 allele frequencies to those of Population 1. We then specified a D value within PEDAGOG, and the program randomly altered allele frequencies at each locus in Population 2. These became the D-specific allele frequencies used for all simulations for a given value of D.

We reconstructed full-sib relationships with PEDIGREE (Smith et al. 2001). We chose this program because of its speed for the large number of simulation-generated files. While it is possible to use parentage assignment in addition to sibship reconstruction to help assign the birth location of an individual (Fig. S1[2]), here we only used sibship reconstruction within a single cohort to evaluate the robustness of the method using the simplest and most conservative option. We used reconstructed full-sib families of size three or greater to assess movement across the simulated barrier. We used a majority-rule approach to determine movement direction. A family was assumed to have originated on the side of the barrier where the majority of family members were sampled. For example, for a full-sib family of size N = 3, if two individuals were sampled upstream and the last downstream, we assumed that family originated on the upstream location.

For the purpose of comparison with the new sib-split approach, we tested the accuracy of the Bayesian genetic clustering program STRUCTURE version 2.3.4 (Pritchard et al. 2000; Falush et al. 2003). We used 10 000 replicates and 10 000 burn-in cycles for all STRUCTURE analyses. We used sampling location as a prior under the "Use population information to test for migrants" option. We set GENSBACK = 0 ("generations back" infers only whether an individual itself is a migrant) and MIGRPRIOR = 0.1 (prior on migration rate). We tested sensitivity to the value of the MIGRPRIOR for a subset of STRUCTURE analyses. We varied MIGRPRIOR (values = 0.001, 0.05, 0.10, 0.15, and 0.25) for the analysis of simulations with initial migration of 0 (true realized migration = 0 with simulations) or initial migration of 0.1 (true realized migration = 0.12) and with initial D of 0 or 0.25. We used eight-locus data sets for this analysis.

We estimated accuracy of both sib-split and STRUCTURE by two methods. First, we define "overall accuracy" as the proportion of all individuals that were correctly designated as either having remained in their population of birth (non-migrant) or moved to the other population (migrant). This calculation consisted of the mean of the total number of correctly assigned individuals for Population 1 and Population 2 divided by the total number of individuals in each population. Individuals from each of the two populations could be incorrectly assigned to the other population (incorrectly inferred migrant) or incorrectly assigned to the birth population (incorrectly inferred non-migrant). Our accuracy measurement accounted for all four types of erroneous assignments (each population could have the two types of error) and therefore provides a comprehensive estimate of accuracy that is most relevant to management decisions.

We also estimated accuracy as the proportion of correctly inferred migrants, which we define as "inferred migrant accuracy". This was calculated as the mean of the number of correctly inferred migrants from Population 1 and Population 2 divided by the total number of inferred migrants for each population. This second measure of accuracy was only presented for simulations with migration = 0.1, because it could only be calculated when simulated migration was >0. When simulated migration was 0, inferred migration was either 100% incorrect (all inferred migrants were incorrect) or 100% correct (no migrants were inferred). We developed software (available from the authors upon request) to summarize the accuracy of sibship and population assignment for all simulations.

Empirical case studies

We compared sib-split and STRUCTURE for two empirical case studies of natural populations of eastern brook trout. The first case study consisted of a before–after analysis associated with the removal of a strong barrier to migration (hereafter the "easy-to-detect" case study). The second case study consisted of analysis of fish movement in relation to a typical road-crossing culvert (hereafter the "difficult-to-detect" case study). In both case studies, we targeted a single brook trout age-class (age-0).

For the easy-to-detect case study, we sampled before and after the removal of a small dam on Browns Creek in the Huron–Manistee National Forests, Michigan. Browns Creek (Fig. S2[2]) had a small earthen dam with a concrete–culvert water control structure that was removed on 26 July 2011. Prior to removal, a 1- to 2-acre (1 acre = 0.404 ha) pond occurred upstream of the dam. This barrier served as a highly likely but unconfirmed barrier to brook trout movement owing to physical and thermal reasons. We sampled young-of-the-year (YOY, age-0) brook trout upstream (above) from the pond on 8 July 2011, hereafter referred to as the "above–before" sample. Brook trout YOY do not occur in the pond during summer, but seasonal use is possible. There were three contiguous upstream sample sections for a total distance of 933 m. We sampled YOY downstream (below) the dam on 18 July 2011, hereafter the "below–before" sample. There were three contiguous downstream sample sections for a total distance of 800 m. A third sample was collected at the same contiguous downstream sample sections after dam removal, on 4 November 2011, hereafter the "below–after" sample.

For the difficult-to-detect case study, we sampled on both sides of a perched culvert within the West Brook watershed, in Whately, Massachusetts (Fig. S3[2]). The culvert is located at the mouth of small tributary stream (Mitchell Brook, hereafter "tributary", mean wetted width is 3 m) to the main stem of West Brook (hereafter "main stem", mean wetted width is 4.5 m). The outlet to this culvert is perched 0.75 m above the stream bed (Kanno et al. 2014). Reproduction occurs in the tributary and main stem (Kanno et al. 2014), and YOY were sampled in autumn 2004 from both sites. Previous results based on PIT tag recaptures, PIT tag antenna detections, and sibship reconstruction (full-sib families > 3) revealed that adult brook trout occasionally move upstream through the perched culvert into the tributary to reproduce. This study did not detect upstream movement into the tributary by YOY (Kanno et al. 2014). Emigration out of the tributary to the main stem by YOY was detected, likely to maximize individual survival and growth (Kanno et al. 2014). There were three approximately 100 m contiguous sample sections upstream from the culvert in the tributary for a total distance of 280 m. There were 10 approximately 100 m contiguous sample sections in the main stem for a total distance of 940 m. The tributary joins the main stem such that we sampled the main stem 380 m downstream from the confluence and 560 m upstream of the confluence. We used single-pass electrofishing to sample the tributary. We used two-pass electrofishing with block

nets deployed each 20 m to sample the main stem. Detection probability in the autumn is high in this system (mean = 0.64; B.H. Letcher, unpublished data).

For both case studies, individual length and location were recorded upon capture with electrofishing, and a tissue sample (anal fin clip) was taken as a source of genetic material.

Genetic methods

We used either eight (easy-to-detect case study) or 12 (difficult-to-detect case study) microsatellite loci to genotype individual age-0 brook trout. The eight-locus set consisted of *SfoC113*, *SfoD75*, *SfoC88*, *SfoD100*, *SfoC115*, *SfoC129*, *SfoC24* (King et al. 2012), and *SsaD237* (King et al. 2005). Four additional loci were added for the more difficult-to-detect case study. These four loci were *SfoC38*, *SfoC86*, *SfoB52*, and *SfoD91a* (King et al. 2012). We followed protocols for DNA extraction and amplification detailed in Whiteley et al. (2013). Loci were electrophoresed on either an ABI Prism 3100-Avant or an ABI Prism 3130xl genetic analyzer (Applied Biosystems, Inc., Foster City, California), and alleles were hand-scored using GENEMAPPER version 3.2 and PEAK SCANNER version 1.0 software (Applied Biosystems, Inc.).

We used GENODIVE version 2.0b22 (Meirmans and Van Tienderen 2004) to estimate allele frequencies, observed (H_O) and expected (H_E) heterozygosity per locus and population, mean number of alleles (A), F_{IS}, F_{ST}, and D. We calculated PI_{ave} and PI_{ave}-sibs (Taberlet and Luikart 1999) and probability of parental exclusion (PE) for the case where no parent is known with GenAlEx version 6.5 (Peakall and Smouse 2012). We used GENEPOP version 4.0.10 (Rousset 2008) to test for deviations from Hardy–Weinberg (HW) expectations and linkage disequilibrium (LD). Because of the large number of assumptions associated with these tests, we used the conservative Bonferroni correction (Rice 1989) at an alpha of 0.05 to correct for inflated type I error rates due to multiple testing (Narum 2006). We reconstructed full-sib families for all individuals sampled for each empirical case study with COLONY version 1.2 (Wang 2004). Long computational time prohibited use of COLONY for the analysis of simulations, but we used it for analysis of the empirical data because of improved accuracy (Wang and Santure 2009). We allowed one parent to be polygamous and the other monogamous. We estimated the mean number of individuals per full-sib family by fitting a Poisson distribution to a frequency distribution of full-sib family size. We used full-sib families of size two or greater to assess movement across the perched culvert. Inference of movement direction was based on families of size three or greater using the same majority-rule approach described above. We have previously conducted empirically parameterized simulations to assess sibship reconstruction accuracies for the same 12 microsatellite loci in the difficult-to-detect study system (Letcher et al. 2011). These simulations revealed that for reconstructed full-sib families composed of at least two individuals, the accuracy of correct family inference was 91.2% (0.7% SE). For full-sib families composed of at least five individuals, the correct family inference was 97.7% (0.4% SE).

We also tested for movement in relation to the barriers with STRUCTURE. For the easy-to-detect case study, we incorporated a prior on sampling location for the two before-removal samples with the "Use population information to test for migrants" option. We set GENSBACK = 0 and we varied MIGRPRIOR (0, 0.001, 0.01, 0.05, and 0.1) to test for model sensitivity. Under these settings, the location prior did not apply to the below–after sample. Instead, those individuals were assigned to the above or below group with the admixture model. We inferred a separate α (the Dirichlet parameter for degree of admixture; Falush et al. 2003) for each population. We used the correlated allele frequencies model with an initial λ of 1, where λ parameterizes the allele frequency prior and is based on the Dirichlet distribution of allele frequencies. We allowed F to assume a different value for each population (above versus below barrier), which allows for different rates of drift among populations. We also implemented the same admixture model with no location prior for any of the samples. For the difficult-to-detect case study, we incorporated a prior on sampling location in the same manner as the easy-to-detect case study. We used the same settings for GENSBACK and MIGRPRIOR, including sensitivity testing across the same range of values. For all runs, we used 100 000 replicates and 20 000 burn-in cycles. We performed five replicates for all sets of conditions.

Results

Simulations

Family structure and sibship assignment

Our simulated conditions produced a large number of full-sib families useful to detect movement and assign movement direction. Median full-sib family size was eight, and 83% of individuals were contained within families of size three or larger. Sibship assignment accuracy was dependent on the number of loci. Overall accuracy of sibship reconstruction across all eight-locus simulations was 0.804 (SD = 0.095), across all 12-locus simulations was 0.935 (SD = 0.030), and across all 16-locus simulations was 0.967 (SD = 0.016; Table 2; Fig. S4[2]). Parentage exclusion (PE) across all eight-locus simulations was 97.96% (SD = 0.020), across all 12-locus simulations was 99.82% (SD = 0.014), and across all 16-locus simulations was 99.98% (SD = 0.0002; Table 2).

Detecting movement

For assessment of movement, all patterns elaborated on below were the same for inferred migrant accuracy and overall accuracy (Table 2; Fig. S5[2]). Inferred migrant accuracy was always lower than overall accuracy because the former does not include individuals correctly inferred to be non-migrants (Table 2; Fig. S5[2]). We used overall accuracy for the remainder of analyses.

Sib-split and STRUCTURE were both highly accurate under easy-to-detect conditions (no migration, moderate (initial D = 0.10) or strong (initial D = 0.25) differentiation; Table 2). For these simulations, overall accuracy was >0.99 across all locus sets for sib-split and 1.0 across locus sets for STRUCTURE (Table 2). In contrast, sib-split consistently outperformed STRUCTURE under difficult-to-detect conditions — 10% migration and either no (initial D = 0) or low to moderate (initial D = 0.1 or 0.25) genetic differentiation (Table 2). Overall accuracy of sib-split increased in a similar manner across the locus sets for each level of genetic differentiation (Table 2; Fig. 1). Mean overall accuracy of sib-split for eight-locus simulations was 0.961 (SD = 0.018), for 12-locus simulations was 0.989 (SD = 0.013), and for 16-locus simulations was 0.992 (SD = 0.007; Table 2). STRUCTURE accuracy was lower than sib-split for all locus sets and surprisingly decreased slightly with increased number of loci (mean overall accuracy of STRUCTURE for eight-locus simulations was 0.901 (SD = 0.034), for 12-locus simulations was 0.911 (SD = 0.028), and for 16-locus simulations was 0.867 (SD = 0.091); Table 2). The tendency for STRUCTURE to incorrectly assign entire full-sib families, particularly large families, to the wrong natal population appeared to be responsible for declining accuracy with increasing number of loci. Close inspection of a subset of misassigned families revealed that those particular families were more genetically similar (based on F_{ST}) to the population on the opposite (and incorrect) side of the barrier. STRUCTURE was also highly sensitive to the prior used for migration rate (Table 3). When there was no genetic differentiation between the two populations (D = 0), estimated migration rates from STRUCTURE were similar to the migration prior (Table 3). With strong genetic differentiation (D = 0.25), estimated migration rates closely corresponded to true migration rates (Table 3).

In simulations of barrier removal and barrier establishment, the relative performance again depended strongly on initial genetic divergence. Both methods were highly accurate at detecting

Table 2. Summary of simulations to test the accuracy of sib-split and STRUCTURE for detecting migrants across barriers.

Simulation conditions	Initial *D*	Final *D*	No. of loci	Sibship accuracy	PE	Overall accuracy sib-split	Overall accuracy STRUCTURE	Inferred migrant accuracy sib-split	Inferred migrant accuracy STRUCTURE
Two-way barrier (0, 0, 0) — easy-to-detect									
Moderate genetic	0.10	0.174 (0.090–0.257)	8	0.866 (0.844–0.889)	0.989 (0.988–0.991)	0.994 (0.990–0.997)	0.998 (0.993–1.003)	—	—
differentiation	0.10	0.163 (0.107–0.22)	12	0.920 (0.905–0.935)	0.997 (0.997–0.998)	1.000 (1.000–1.000)	0.999 (0.998–1.000)	—	—
	0.10	0.172 (0.143–0.201)	16	0.968 (0.948–0.988)	1.000 (1.000–1.00)	1.000 (1.000–1.00)	1.000 (1.000–1.00)	—	—
Strong genetic	0.25	0.462 (0.402–0.522)	8	0.635 (0.563–0.708)	0.937 (0.927–0.947)	1.000 (1.000–1.000)	1.000 (1.000–1.000)	—	—
differentiation	0.25	0.437 (0.405–0.470)	12	0.881 (0.835–0.927)	0.995 (0.994–0.997)	1.000 (1.000–1.000)	1.000 (1.000–1.000)	—	—
	0.25	0.364 (0.334–0.394)	16	0.935 (0.914–0.957)	0.999 (0.999–0.999)	1.000 (1.000–1.000)	1.000 (1.000–1.000)	—	—
Moderate barrier (0.1, 0.1, 0.1) — difficult-to-detect									
No genetic	0	0.023 (0.004–0.041)	8	0.856 (0.822–0.89)	0.989 (0.987–0.991)	0.948 (0.924–0.972)	0.873 (0.841–0.905)	0.692 (0.600–0.784)	0.403 (0.353–0.454)
differentiation	0	0.011 (0.005–0.017)	12	0.969 (0.964–0.974)	0.999 (0.999–0.999)	0.988 (0.982–0.994)	0.890 (0.860–0.921)	0.919 (0.888–0.95)	0.462 (0.343–0.582)
	0	0.012 (0.007–0.017)	16	0.980 (0.976–0.983)	1.000 (1.000–1.000)	0.994 (0.985–1.003)	0.754 (0.695–0.813)	0.955 (0.909–1.00)	0.187 (0.119–0.255)
Moderate genetic	0.10	0.063 (0.029–0.098)	8	0.867 (0.841–0.894)	0.993 (0.992–0.994)	0.965 (0.944–0.986)	0.927 (0.896–0.958)	0.776 (0.671–0.881)	0.576 (0.462–0.691)
differentiation	0.10	0.055 (0.044–0.067)	12	0.939 (0.934–0.943)	0.999 (0.998–0.999)	0.989 (0.980–0.999)	0.927 (0.907–0.947)	0.933 (0.870–0.997)	0.619 (0.535–0.702)
	0.10	0.064 (0.045–0.084)	16	0.981 (0.977–0.986)	1.00 (1.00–1.00)	0.993 (0.987–1.00)	0.936 (0.902–0.969)	0.967 (0.945–0.989)	0.608 (0.496–0.721)
Strong genetic	0.25	0.139 (0.053–0.225)	8	0.807 (0.794–0.82)	0.988 (0.985–0.991)	0.97 (0.952–0.987)	0.905 (0.865–0.944)	0.821 (0.752–0.89)	0.544 (0.426–0.663)
differentiation	0.25	0.136 (0.103–0.169)	12	0.943 (0.935–0.951)	0.999 (0.999–0.999)	0.989 (0.981–0.997)	0.914 (0.87–0.958)	0.944 (0.894–0.994)	0.599 (0.420–0.778)
	0.25	0.144 (0.099–0.189)	16	0.964 (0.95–0.978)	1.00 (1.00–1.00)	0.987 (0.978–0.996)	0.912 (0.862–0.962)	0.946 (0.907–0.985)	0.554 (0.361–0.746)
New barrier (0.1, 0.1, 0)									
	0	0.019 (−0.001–0.038)	8	0.882 (0.862–0.902)	0.988 (0.987–0.99)	0.965 (0.958–0.972)	0.930 (0.893–0.968)	—	—
	0	0.020 (0.006–0.035)	12	0.966 (0.952–0.979)	0.999 (0.999–0.999)	0.994 (0.99–0.997)	0.918 (0.890–0.947)	—	—
	0	0.015 (0.008–0.022)	16	0.980 (0.973–0.987)	1.000 (1.000–1.000)	0.999 (0.997–1.000)	0.826 (0.786–0.866)	—	—
Remediated barrier (0, 0, 0.1)									
	0.25	0.225 (0.190–0.26)	8	0.712 (0.641–0.783)	0.973 (0.968–0.979)	0.984 (0.969–0.999)	0.978 (0.965–0.992)	0.901 (0.806–0.996)	0.839 (0.735–0.942)
	0.25	0.230 (0.183–0.277)	12	0.927 (0.903–0.952)	0.999 (0.998–0.999)	0.988 (0.976–1.00)	0.989 (0.973–1.000)	0.956 (0.916–0.996)	0.937 (0.867–1.000)
	0.25	0.17 (0.137–0.203)	16	0.963 (0.941–0.984)	1.000 (1.000–1.000)	0.992 (0.986–0.998)	0.978 (0.966–0.989)	0.968 (0.943–0.993)	0.875 (0.819–0.931)

Note: Simulation conditions follow Table 1, with movement probability for each of three generations in parentheses. We consider the two-way barrier with no movement "easy-to-detect" and a moderate barrier with movement each generation "difficult-to-detect". Initial *D* is the amount of genetic divergence at the beginning of each three-generation simulation. Summary statistics are reported as the mean and 95% CI for the five replicates for each set of conditions. Final *D* was used as the estimate of genetic differentiation after three generations. Number of loci indicates the number of markers used for a simulation set. Sibship accuracy is the proportion of correctly assigned full-sibs. PE is the probability of exclusion for one parent when the genotype for the other parent is not known. Overall accuracy of sib-split is the proportion of all individuals that were correctly assigned to their population of birth, assessed for both sib-split and STRUCTURE. Inferred migrant ancestry is the proportion of correctly inferred migrants, assessed for both sib-split and STRUCTURE.

Fig. 1. Overall accuracy of sib-split and STRUCTURE for simulations with 10% migration. Overall accuracy is defined as the proportion of all individuals that were correctly assigned to their population of birth. Mean and 95% confidence intervals for the five replicates of each combination of number of loci and degree of genetic differentiation are shown. No differentiation (*a*) corresponds to $D = 0$, moderate differentiation (*b*) corresponds to $D = 0.1$, and strong differentiation (*c*) corresponds to $D = 0.25$.

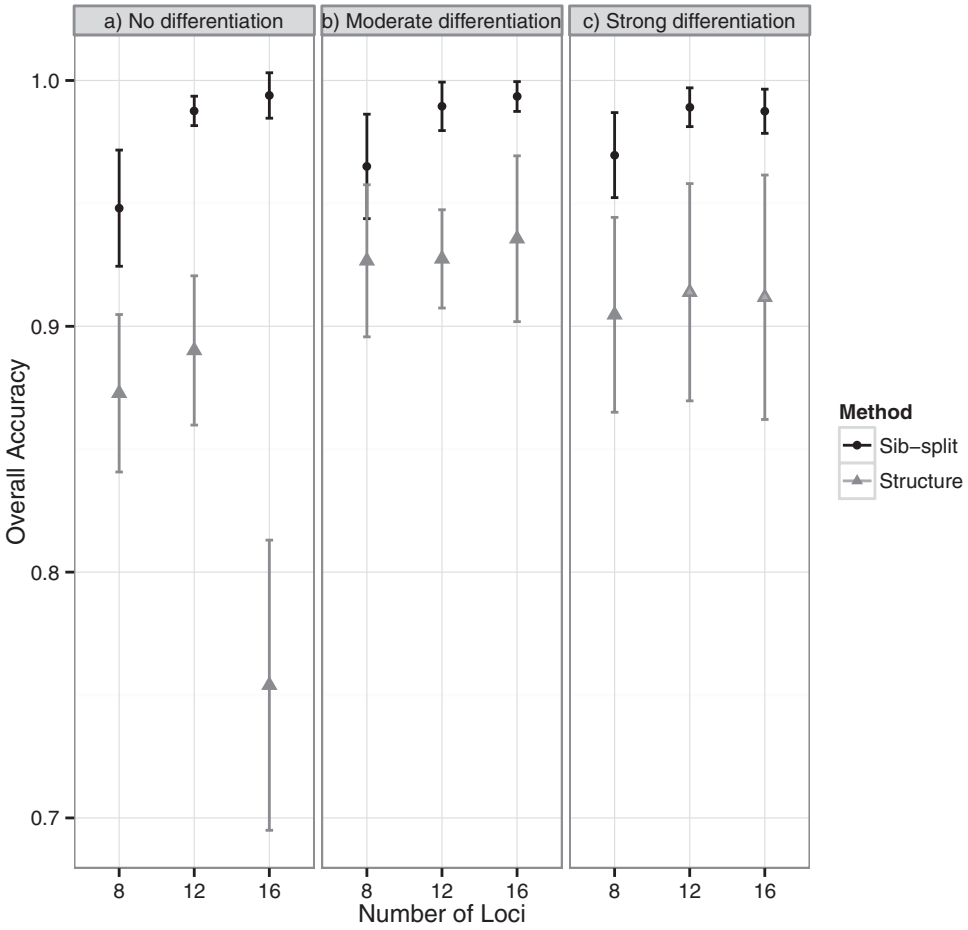

Table 3. Test of sensitivity to migration prior (MIGRPRIOR) in STRUCTURE for simulations.

	True migration = 0.00		True migration = 0.12	
Migration prior	$D = 0$	$D = 0.25$	$D = 0$	$D = 0.25$
0.001	0.00	0.00	0.00	0.12[b]
0.05	0.03	0.00	0.05	0.12[b]
0.10	0.06	0.00	0.12[a]	0.12[b]
0.15	0.07	0.00	0.17	0.12[b]
0.20	0.09	0.00	0.20	0.12[b]

Note: Values are estimated migration rates. True migration between populations 1 and 2 is shown. *D* is the measure of genetic differentiation used.

[a]Accuracy = 0.5.

[b]Accuracy = 1.0.

a remediated barrier with strong genetic divergence prior to barrier removal (migration was zero for the first two generations and 10% for the third generation; Table 2; Fig. 2*b*). When the simulated barrier was removed at generation three, both methods accurately detected this removal and subsequent true individual movement (Table 2; Fig. 2*b*). Both methods therefore avoided type II errors (probability of falsely rejecting movement) under these simulated conditions. In contrast, sib-split outperformed STRUCTURE for the more difficult of the two simulated management scenarios (new barrier, for which migration was simulated to be 10% for the first two generations and zero for the third generation; Table 2; Fig. 2*a*). Overall accuracy of sib-split was always greater than 95% across locus sets and increased with screening of more loci (Table 2; Fig. 2*a*). STRUCTURE results were more variable and less accurate (Table 2; Fig. 2*a*). Further, accuracy again decreased with more loci for STRUCTURE (Fig. 2*a*). The errors committed by STRUCTURE in this case should be classified as false detection of migrants (type I error) because migration was zero in generation three.

Empirical case studies

Easy-to-detect conditions

We examined 116 brook trout at eight microsatellite loci for three samples (above–before, below–before, and below–after). The mean observed number of alleles per locus (A_O) for the three samples ranged from 5.9 to 6.1. Mean expected heterozygosity (H_S) ranged from 0.629 to 0.699 (Table 4). PE ranged from 88.0% to 95.6%. PI_{ave} and PI_{ave}-sibs were highest in below–before and lowest in below–after (Table 4). Four loci deviated significantly from HW proportions following Bonferroni correction for eight locus tests per population. Two of these tests occurred in the below–before sample, and two tests were in the below–after sample. Eleven tests for LD remained significant following Bonferroni correction for a total of 84 tests. Deviations from HW proportions and LD were likely caused by family structure (Whiteley et al. 2013). These loci do not exhibit deviations from HW proportions or LD for mixed-aged samples or when family structure is removed

Fig. 2. Overall accuracy of sib-split and STRUCTURE for simulations for two management scenarios. In panel (*a*), a new barrier was simulated with generation 1 migration = 0.1, generation 2 migration = 0.1, and generation 3 migration = 0. In panel (*b*), a remediated barrier was simulated with generation 1 migration = 0, generation 2 migration = 0, and generation 3 migration = 0.1. Overall accuracy is defined as the proportion of all individuals that were correctly assigned to their population of birth. Mean and 95% confidence intervals for the five replicates of each combination of number of loci and management scenario are shown. For panel (*a*), *D* was initially set to 0; in panel (*b*), *D* was initially set to 0.25.

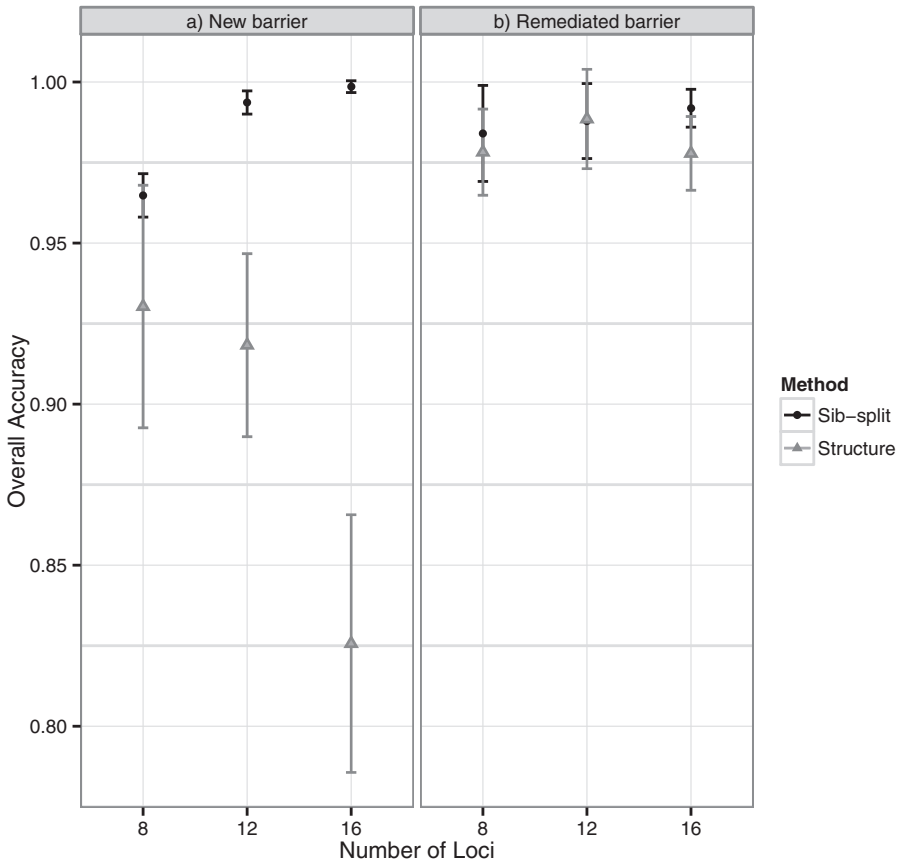

Table 4. Genetic summary statistics for young-of-the-year (YOY) brook trout captured in Browns Creek, Michigan.

Sample	Date collected	*N*	A_O	H_S	PI_{ave}	PI_{ave}-sibs	PE	F_{IS}
Easy-to-detect								
Above–before	8 July 2011	64	5.9	0.660	3.2e-7	2.0e-3	0.921	−0.119
Below–before	18 July 2011	23	5.1	0.629	1.6e-6	3.0e-3	0.880	0.023
Below–after	4 November 2011	29[a]	6.1	0.699	5.8e-8	1.0e-3	0.956	−0.040
Difficult-to-detect								
Tributary	7–8 October 2004	66	5.1	0.600	2.6e-9	2.4e-4	0.960	−0.071
Main stem	13 September–7 October 2004	60	7.1	0.630	2.1e-10	1.4e-4	0.984	0.057

Note: Data include sample names, collection dates, number of individuals genotyped (*N*), mean number of observed alleles (A_O), mean expected heterozygosity (H_S), probability of identity (PI_{ave}), probability of identity among siblings (PI_{ave}-sibs), parentage exclusion (PE), and inbreeding coefficient values (F_{IS}).

[a]Two individuals were removed prior to calculation of summary statistics because they were recaptures identified genetically.

(Whiteley et al. 2013). The two predam removal samples exhibited moderate to strong genetic differentiation (F_{ST} = 0.09, *D* = 0.17). The inclusion of the postdam removal sample lowered genetic differentiation (F_{ST} = 0.05, *D* = 0.10) and provides initial evidence for brook trout movement after the dam was removed.

We analyzed the three samples jointly to estimate sibship. Two genetic recaptures (individuals sampled in below–after collection with genotypes identical to individuals sampled in below– or above–before collections) were removed prior to sibship reconstruction. The remaining 114 individuals were assigned to a total of 37 estimated full-sib families ($\hat{N}_{fam}$) (Fig. 3*a*). Mean family size ($\hat{\lambda}$) was 2.95. There were 12 full-sib families (71% of YOY) with three or more members and 18 full-sib families (81% of YOY) with two or more members (Table 5). The largest full-sib family had 17 members (Table 5; Fig. 3*a*).

The sib-split approach provided no evidence of migration before barrier removal but substantial evidence for movement after the removal of the barrier (Table 5). None of the prebarrier removal individuals (before–above or before–below) was assigned to full-sib families on the opposite side of the barrier. Following dam removal, a presumed genetic recapture from family 13 was sampled upstream in above–before and downstream in below–after

Fig. 3. Full-sibling family size distribution in (*a*) easy-to-detect case study (Browns Creek, Michigan) and (*b*) difficult-to-detect case study (West Brook, Massachusetts). *N* is the number of individuals sampled in each site, $\hat{N}_{fam}$ is the total number of estimated full-sib families, and $\hat{\lambda}$ is mean family size.

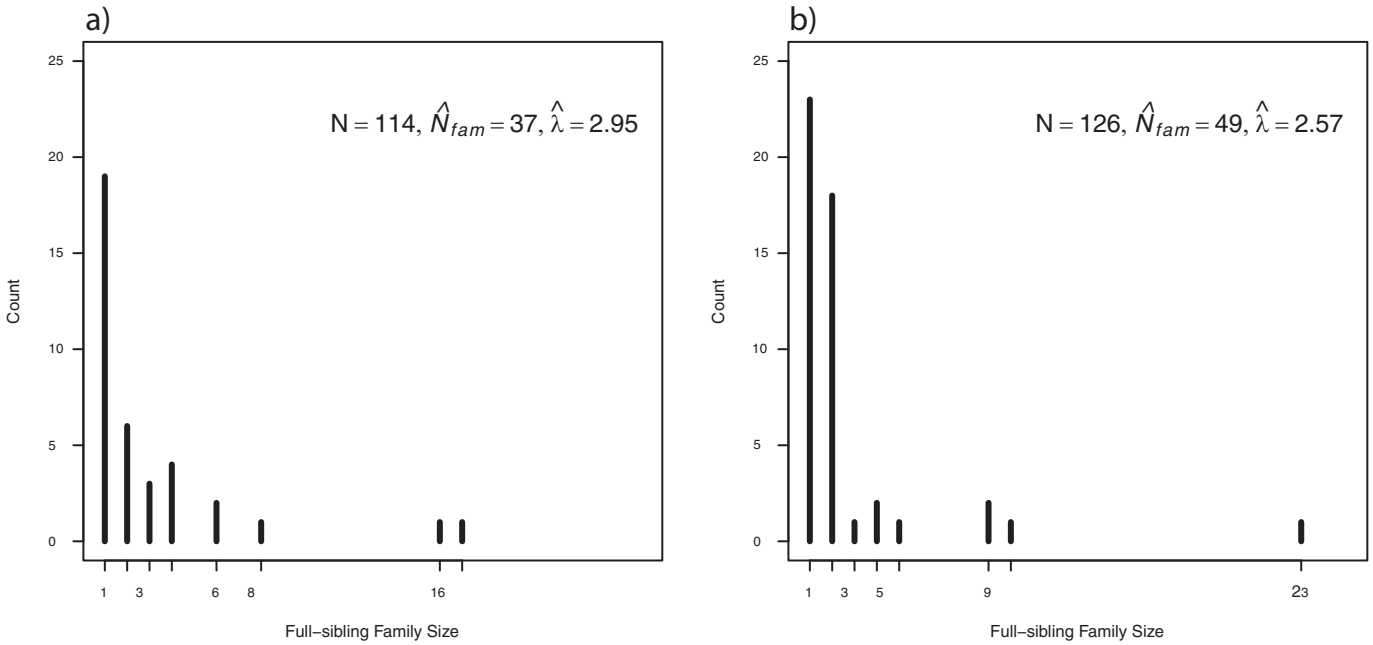

Table 5. Full-sibling family locations for age-0 brook trout in Browns Creek, Michigan.

	Downstream of dam			Upstream of dam		
Family (size)	800 to 350 m	358 to 246 m	245 to 0 m	220 to 507 m	508 to 707 m	708 to 853 m
1 (17)	3, 1GR		14			
2 (16)	1[a]			8	5	2
3 (8)	3[a]			1	1	3
4 (6)	1[a]			4	1	
5 (6)	1[a]			3	4	
6 (5)	2[a]				3	
7 (4)	1	1	2			
8 (4)	4[b]					
9 (4)					2	2
10 (3)	3[b]					
11 (3)	2		1			
12 (3)	1[a]			1	1	
13 (2)	1GR[a]				1	1
14 (2)				1	1	
15 (2)	1		1			
16 (2)					2	
17 (2)				2		
18 (2)				1	1	

Note: Full-sib families are rank-ordered by family size. Family size (number of full-sibs assigned to a family) is shown in parentheses in the family column. Sampling location of each full-sib is shown for six stream sections, three downstream of the dam and three upstream of the dam. Section numbers represent distance (m) from the dam. The dam was removed prior to the last downstream sample. Upstream sections start 220 m upstream from the dam because of unsuitable former pond habitat. Genetic recaptures (i.e., individuals with identical genotypes sampled at separate times) are labeled GR. All putative downstream migrants were from the "below–after" sample.

[a]Putative downstream migrant(s).

[b]Entire family sampled only in the "below–after" sample.

and therefore provides direct evidence for downstream movement. Based on sib-split, nine individuals from below–after assigned to six otherwise above-barrier families and therefore were putative downstream migrants (total migration rate = 10/112 = 0.09; Table 5). We conservatively assume that two below-barrier families (families 8 and 10) that were composed of individuals sampled only in the below–after sample were not downstream migrants, although it is possible that these entire families moved.

STRUCTURE analyses were inaccurate at initial barrier assessment but accurate at migrant detection for the easy-to-detect case study. Prior to barrier removal, the number of inferred migrants (all upstream) increased with the prior on migration (Table 6). Therefore, conclusions about barrier status based on these results alone would be tenuous. However, in this situation, we were highly confident that migration was zero prior to dam removal. With a very low migration prior (MIGRPRIOR = 0.001), there were no inferred migrants prior to dam removal. Following dam removal, 10 individuals from below–after were assigned as downstream migrants, regardless of the value of the migration prior (Table 6). These included the nine individuals identified by sib-split analysis and an additional individual that assigned to a singleton family (full-sib family of size N = 1). Results from the admixture model with no prior demonstrated the sensitivity of STRUCTURE to family-level structure. Compared with MIGRPRIOR = 0.1, there was one additional upstream migrant and an entire large downstream family (family 1, N = 17 individuals; N = 14 from before–below, N = 3 from below–after) was assigned upstream, leading to highly erroneous before and after results.

Difficult-to-detect conditions

For the difficult-to-detect case study, we examined 126 brook trout at 12 microsatellite loci for two samples (above culvert = tributary, below culvert = main stem). The tributary had lower genetic diversity (A_O and H_S), lower PE, and higher PI_{ave} and PI_{ave}-sibs than the main stem (Table 4). Five loci deviated significantly from HW proportions following Bonferroni correction for 12 locus tests per population. All of these tests occurred in the tributary sample, where family structure was more pronounced. Forty-nine tests for linkage disequilibrium remained significant following Bonferroni correction for a total of 132 tests. Forty-eight of these tests occurred in the tributary sample and again were likely caused by the stronger family structure in the tributary. The two

Table 6. Summary of STRUCTURE results for easy-to-detect brook trout case study.

	No. of migrants			Migration rate			
Migration prior	Upstream before	Downstream before	Downstream after	Upstream before	Downstream before	Total before	Downstream after
0.001	0	0	10	0	0	0	0.14 (10/74)
0.01	1	0	10	0.04 (1/24)	0	0.01 (1/87)	0.14 (10/73)
0.05	4	0	10	0.15 (4/27)	0	0.05 (4/87)	0.14 (10/70)
0.1	7	0	10	0.23 (7/30)	0	0.08 (7/87)	0.15 (10/67)
None	8	14	13	0.47 (8/17)	0.20 (14/70)	0.25 (22/87)	0.16 (13/83)

Note: Models were run with sampling location used as a prior for the two before-removal samples and the admixture model for the after-removal sample. The prior on migration is shown. "None" represents no location prior and the admixture model. "Upstream before" migrants were individuals collected in the above–before sample that assigned to the below–before sample. "Downstream before" migrants were individuals collected in the below–before sampled that assigned to the above–before sample. "Downstream after" migrants were individuals collected in the below–after sample that assigned to the above–before sample. Upstream migration following dam removal was not assessed. Migration rates were determined four ways. "Upstream before" was the proportion of fish sampled upstream that assigned downstream out of the total number of fish assigned downstream. "Downstream before" was the proportion of fish sampled downstream that assigned upstream out of the total number of fish assigned upstream. "Total before" was the number of migrants divided by the total number of before individuals (N = 87). "Downstream after" was the proportion of fish sampled downstream that assigned upstream out of the total number of fish that assigned upstream. Numbers used to calculate nonzero migration rates are shown in parentheses.

samples exhibited weak genetic differentiation (F_{ST} = 0.02, D = 0.03).

We analyzed the tributary and mainstem samples jointly to estimate sibship. The 126 individuals were assigned to a total of 49 estimated full-sib families ($\hat{N}_{fam}$; Fig. 3*b*). Mean family size ($\hat{\lambda}$) was 2.57. There were eight full-sib families (53% of YOY) with three or more members and 26 full-sib families (82% of YOY) with two or more members (Table 7). The largest full-sib family had 23 members (Table 7). The tributary contained the five largest families, and the main stem tended to have a greater number of small families (Table 7).

Sib-split identified movement across the culvert, with a total of five migrants (Table 7). Four of the five migrants were assigned to full-sib families composed of three or more YOY, and thus directionality could be assigned. The majority-rule approach suggested that all four of these migrants moved downstream from the tributary through the culvert to the main stem (Table 7). Family 19 ($N_{full\text{-}sibs}$ = 2) had one full-sib on either side of the culvert (Table 7). We can identify movement but not directionality from this family. For migrants inferred from families of size three or greater, the total migration rate was 0.06 (four migrants out of a total of 67 YOY), all of which occurred in the downstream direction. The total migration rate was 0.05 (5/103) when we considered families of size two or greater.

Results from STRUCTURE analyses for the difficult-to-detect case study were highly dependent on the migration prior (Table 8). Upstream migration rates ranged from 0 to 0.44, and downstream migration rates ranged from 0 to 0.20 (Table 8). The results for a MIGRPRIOR = 0.05 (one upstream migrant, three downstream migrants) were most similar to the sib-split results. Here, the three downstream migrants identified by STRUCTURE were identical to those from sib-split for family 1 (N = 2 migrants) and family 4 (Table 7). The individual from family 2 identified by sibship splitting as a migrant from the tributary (Table 8) was not identified by STRUCTURE, but the probability of assignment to the main stem was low (0.543). The upstream migrant with MIGRPRIOR = 0.05 (assignment probability = 0.872) was from family 19 (N = 2 full-sibs), the family for which directionality could not be determined with sib-split.

With MIGRPRIOR = 0.1 or the model with no location prior, STRUCTURE became highly error-prone (Table 8). Errors tended to occur owing to misassignment of entire families. For example, with MIGRPRIOR = 0.1, five entire families ranging in size from two to nine full-sibs were assigned to the population where the individuals were not sampled. With no location prior, seven entire families were misassigned. The difference in results between sib-split and STRUCTURE with no prior was most apparent with family 4. This family contained nine individuals (Table 7). With sib-split, one individual was more parsimoniously assigned as a downstream migrant. With STRUCTURE and no location prior, all eight individuals sampled in the tributary were assigned as upstream migrants.

Discussion

We used simulated and empirical data to rigorously test a novel approach (termed sib-split) to detect movement of individuals from a single age-class across incomplete barriers based on sibship assignment. Simulations revealed that both sib-split and the population-level assignment approach implemented by STRUCTURE were highly accurate under easy-to-detect conditions (moderate to strong genetic differentiation and no movement). However, under more difficult-to-detect conditions (no to weak genetic differentiation, 10% movement each generation), sib-split outperformed STRUCTURE. Sib-split also outperformed STRUCTURE under a more difficult-to-detect management scenario (simulated installation of a new barrier to movement). Empirical evaluations also suggested that the sib-split approach is likely to be more reliable than STRUCTURE in many management-relevant scenarios.

Sib-split offers promise as an approach that can fill a gap where population-based assignment approaches become unreliable. It has long been recognized that at least a moderate degree of genetic differentiation among populations is required for assignment tests like those implemented in STRUCTURE to perform well (Cornuet et al. 1999; Manel et al. 2005). Management and conservation applications often require assessment of movement where population-based assignment tests perform poorly. The application we have in mind is effectiveness monitoring for aquatic organisms through structures such as road-crossing culverts or small dams. However, there is a general need to assess organismal movement on an ecological and conservation-relevant time scale. These conditions often will occur under more difficult-to-detect situations with low divergence and low but nonzero migration.

Sib-split — simulations

Our work builds on previous studies that have used sibship and parentage assignment to test for dispersal (Clark et al. 2010; Hudy et al. 2010; Vollestad et al. 2012; Kanno et al. 2014). Our sib-split approach was designed to test for dispersal relative to specific barriers. We provide the first rigorous evaluation of sibship reconstruction used in this management context (but see Neville and Peterson 2014, this issue). Sib-split performed well under a wide

Table 7. Full-sibling family locations for age-0 brook trout in West Brook and Mitchell Brook, Massachusetts.

	Main stem										Tributary		
Family (size)	−380 to −301 m	−300 to −201 m	−200 to −101 m	−100 to 0 m	0[a] to 100 m	101 to 200 m	201 to 300 m	301 to 400 m	401 to 500 m	501 to 560 m	0 to 100 m	101 to 200 m	201 to 280 m
1 (23)					1[b]				1[b]		8	9	4
2 (10)							1[b]				7	1	1
3 (9)											5	3	1
4 (9)									1[b]		3	2	3
5 (5)											3		2
6 (4)				1				1	2				
7 (4)			1	1	1					1			
8 (3)				1				1	1				
9 (2)				1					1				
10 (2)			1	1									
11 (2)		1		1									
12 (2)			1	1									
13 (2)											1		1
14 (2)						1		1					
15 (2)				1		1							
16 (2)		1	1										
17 (2)				1			1						
18 (2)					1	1							
19 (2)									1[b,c]		1[b,c]		
20 (2)											1		1
21 (2)				1		1							
22 (2)											1	1	
23 (2)												2	
24 (2)			2										
25 (2)			1						1				
26 (2)	1			1									

Note: Full-sib families are rank-ordered by family size. Family size (number of full-sibs assigned to a family) is shown in parentheses in the family column. Sampling location of each full-sib is shown for the main stem (West Brook) and tributary (Mitchell Brook). The perched culvert is located at the mouth of the tributary. In the main stem, sections reflect downstream (negative values) or upstream (positive values) distance relative to the tributary confluence.

[a]The tributary joins the main stem at the section labeled 0; no barriers to movement occur within the main stem.

[b]Putative downstream migrant.

[c]Movement occurred but direction could not be assigned.

Table 8. Summary of STRUCTURE results for difficult-to-detect brook trout case study.

	No. of migrants		Migration rate		
Migration prior	Upstream	Downstream	Upstream	Downstream	Total
0.001	0	0	0	0	0
0.01	0	0	0	0	0
0.05	1	3	0.02 (1/58)	0.04 (3/68)	0.03 (4/126)
0.1	19	6	0.26 (19/73)	0.11 (6/53)	0.20 (25/126)
None	42	6	0.44 (42/96)	0.20 (6/30)	0.38 (48/126)

Note: Models were run with sampling location used as a prior. The prior on migration is shown. "None" represents no location prior and the admixture model. Upstream migrants were individuals collected in the tributary that assigned to the main stem. Downstream migrants were individuals collected in the main stem that assigned to the tributary. Migration rates were determined three ways. The upstream rate was the proportion of fish sampled upstream that assigned downstream out of the total number of fish assigned downstream. The downstream rate was the proportion of fish sampled downstream that assigned upstream out of the total number of fish assigned upstream. The total rate was the number of migrants divided by the total number of individuals ($N = 126$). Numbers used to calculate nonzero migration rates are shown in parentheses.

variety of simulated conditions, including conditions where genetic identification of migrants is the most difficult. For example, we accurately detected simulated movement the first generation after barrier removal. Results of our simulations revealed that the sib-split approach is unlikely to lead to erroneous inference. It is unlikely that a researcher would either mistakenly conclude that organisms have passed through or across a real barrier (type I error) or mistakenly conclude that movement of organisms has been blocked by a non-barrier (type II error).

The metric we used in our simulation analyses to assess accuracy of inferred movement by sib-split, overall accuracy, was appropriate for evaluation of this approach for management purposes. For the assessment of a barrier for management, the most important goal is to determine whether movement does or does not occur. Thus, we needed to use a comprehensive measure of accuracy that did not just take into account whether migrants were accurately assigned but rather whether all individuals were accurately assigned. "Overall accuracy" of sibship splitting was greater than 0.95 across all simulated conditions, including cases using only eight loci. The overall accuracies reported may be somewhat inflated because of the large number of individuals that did not move (initial migration was either 0 or 0.1) and were accurately assigned as such. Greater simulated migration rates would have led to fewer individuals that did not move and overall accuracies might have been lower. Our "inferred migrant accuracies" (see Methods) are more relevant for the use of sibship reconstruction for questions related to the direction of movement through a barrier of management concern. For applications where

accurate inference of movement direction is needed, we recommend the use of more loci (>12) to achieve sibship reconstruction and inferred migrant accuracy rates greater than 0.95. It also should be noted that the program we used to reconstruct sibships for the simulated data (PEDIGREE) is fast but may be less accurate than more recent programs (Wang and Santure 2009). This would lead to our reported simulation-based accuracies being lower than that attainable using other algorithms.

Sib-split — empirical case studies

We detected movement by age-0 brook trout in both empirical case studies. In the easy-to-detect case study, movement occurred following dam removal. In the difficult-to-detect case study, movement occurred through a perched culvert. In many management scenarios, detection of movement alone may be sufficient. Sib-split offers promise for inference of movement directionality as well. The observed distribution of YOY in our study allowed for cautious inference of movement direction based on larger families in both case studies. Inference of movement direction requires the assumptions that pertain to use of a majority-rule approach for families of size three or larger. Many of the putative migrants in the empirical case studies came from larger families ($N_{\text{full-sibs}} > 5$, mean size of families with putative migrants across studies = 9.2). All of these putative migrants from larger families appear to have moved in the downstream direction. Our sampling design limited us to only testing for downstream movement in the easy-to-detect case study. In the difficult-to-detect case study, previous work revealed only downstream YOY movement through the perched culvert (Kanno et al. 2014). The putative migrants from three of the four largest families were consistent with results of this previous analysis. We could infer movement but not directionality from a two-member family (family 19). In this case, the combined use of sib-split and STRUCTURE, where sib-split results provide an informed migration prior for STRUCTURE models, provided added information (see "Recommendations" below) and suggest that YOY upstream movement may be rare but possible.

Possible errors in family reconstruction need to be carefully considered with sib-split. Sibship reconstruction may suffer from type I (falsely related) or type II (falsely unrelated) errors (Wang 2013). Falsely related full-sibs that occur on either side of a barrier would lead to an incorrect movement inference (Neville and Peterson 2014, this issue). The predominant pattern of large families on one side and few full-sibs on the opposite side of the putative barrier (or former barrier) in both of our empirical case studies suggests that the inferences reported here are robust to both types of error. Two of six families that exhibited movement in the easy-to-detect case study had similar numbers of full-sibs above and below the former barrier. It is possible that these families contain falsely related individuals (type I error), but the weight of evidence from other full-sib families would still support an inference of downstream movement. Reconstruction of falsely unrelated families may be a more common problem with sibship estimation (Wang 2013). This often occurs through errantly splitting one individual from a larger full-sib family to which it truly belongs (Wang 2013; Neville and Peterson 2014, this issue). These type II errors are less likely to influence sib-split because the joint probability that the individual "shaved off" is an individual on the opposite side of a barrier (representing a missed opportunity to detect movement) should be low.

Sib-split — general considerations

We have demonstrated implementation of the sib-split approach based on two samples, one upstream and one downstream from a barrier in question, taken at a single point in time and from a single age-class (age-0). Proportions of full-sibs above and below the putative barrier and the use of a majority-rule approach provide a preliminary assessment of movement direction that may not be sufficient in some cases. Additional sources of information could improve movement direction inference (Fig. S1[2]). In particular, pedigree reconstruction could be used to assign juveniles to parents from the same sites (Kanno et al. 2011, 2014). Known parental locations, ideally based on individual-based tagging information close to the time of reproduction, could be used to help identify movement direction of juveniles relative to estimated parent locations (Kanno et al. 2014). These additional sources of information would incur additional time and costs (both field and laboratory), but provide an alternative to the majority-rule approach where inference of movement direction in addition to binary identification of movement is needed (Fig. S1[2]).

There are a number of general constraints and caveats to consider for the sib-split approach. First, this approach must target a single age-class. Earliest sampling of the youngest age-class possible (e.g., early age-0) would potentially maximize full-sib family sizes, because sampling would occur prior to potentially high juvenile mortality. However, focusing on older age-0 fish has several benefits. More time between birth and capture provides more opportunity for movement to occur. It also can be difficult to efficiently capture very small age-0 fish because of positive size-dependent probability of detection. In our empirical case studies, we sampled in late summer or autumn to allow high sampling efficiency of age-0 brook trout (Hudy et al. 2010). Importantly, large full-sib families still tend to occur in late summer for this spring-emerging species (Hudy et al. 2010; Whiteley et al. 2012).

Second, age-specific differences in dispersal capability should be considered. Upstream movement detected in younger age-classes should indicate that upstream movement is possible in older age-classes, because age-0 fish probably have lower upstream dispersal capability than older fish. However, age-0 fish might be more prone to passive downstream dispersal during high flow events than older age-classes. Species specificity of age-class-specific barrier effects on dispersal should also be taken into account.

Third, sample size (combined from both sides of barrier) and genetic diversity must be sufficient to allow accurate family assignment. In our case studies, we were able to detect migrants in larger families with combined sample sizes (upstream and downstream of barrier) close to $N = 100$ in each case study. This should provide a general starting point for brook trout in small streams. Pilot data on species-specific family structure as well as a barrier's influence on abundance will likely be required. Reduced abundance on one side of the barrier may limit the sib-split approach. Reduced genetic diversity on the upstream side of a barrier might also decrease sibship reconstruction accuracies. Our simulations revealed that above-barrier versus below-barrier genetic divergence enabled accurate inference of movement with sib-split even if above-barrier sibship reconstruction accuracies decline somewhat, presumably owing to the genetically depauperate above-barrier site.

Finally, our simulation-based assessment of sib-split reflects the family structure generally observed for natural populations of brook trout, which tend to have up to three-quarters of families with more than three full-sibs (Hudy et al. 2010; Whiteley et al. 2012). Simulations modeled on an organism with many small families may yield lower accuracies with the sib-split approach. Smaller families in general present a difficulty for this approach because it is more difficult to assign individuals accurately to small families (Butler et al. 2004; Wang and Santure 2009), and directionality may not be able to be inferred.

Our assessment of sib-split was based on highly polymorphic microsatellite markers. Our simulations demonstrated that increasing the number of loci from eight to 16 increased accuracy of the sib-split approach. We demonstrated that eight highly polymorphic loci (mean number of alleles approximately 6) were sufficient to detect migration in the easy-to-detect case study. We used 12 loci to reconstruct sibships to detect movement for the

difficult-to-detect case study. We recommend that more loci be used as the difficulty to detect movement genetically increases (lower genetic differentiation and greater probability of movement). Subsequent implementation of sib-split could take advantage of single nucleotide polymorphism (SNP) markers as well. Wang and Santure (2009) demonstrated that one microsatellite has roughly the same power for sibship inference as approximately 10 SNPs. At the moment, more variable (per locus) microsatellites remain well suited for resolving family-level relationships for a wide array of organisms.

STRUCTURE — simulations

Results from using STRUCTURE were accurate under easy-to-detect simulated conditions but less accurate than sib-split under more difficult-to-detect simulated conditions. These results are consistent with expectations from population-level assignment tests (Cornuet et al. 1999; Waples and Gaggiotti 2006). Use of STRUCTURE alone under more difficult-to-detect passage assessment conditions is not recommended. STRUCTURE was also prone to type I errors with important management implications in our analysis of a simulated newly constructed barrier. This type of movement detection error following the creation of a new complete barrier would likely lead to lower prioritization of a new barrier than is warranted. In addition, we demonstrated sensitivity to the prior on migration when the genetic signal was weak (D = 0), and thus results without an informed prior under these conditions have a high degree of uncertainty.

A confounding of family-level with population-level structure might explain the observed decreasing STRUCTURE accuracy with increasing number of loci under the most difficult-to-detect simulated conditions. This problem has been generally recognized (Anderson and Dunham 2008; Rodriguez-Ramilo and Wang 2012). Reduced STRUCTURE accuracy with more loci seems counterintuitive until this confounding effect is considered. We hypothesize that increasing the number of loci, even with the use of a migration prior, increased the ability of STRUCTURE to detect underlying family-level structure. Families that were more genetically similar to the population on the opposite side of the barrier than the population in which they were sampled subsequently had all family members incorrectly assigned as migrants.

STRUCTURE — empirical case studies

Our empirical case study results also point to the possibility of an interesting interaction between choice of prior for STRUCTURE models and sensitivity of those models to underlying family-level structure. In both of our case studies, when we used an admixture model with no prior on sample location or migration, the cause of inaccuracy was the misassignment of entire large families to the location other than where the family members were sampled. This family misassignment did not occur with the use of decreasing migration priors. This outcome suggests that a low migration prior, if appropriate, overrides the sensitivity of the admixture STRUCTURE model to family-level structure.

Combining sib-split and STRUCTURE

In practice, we suggest that a two-step combined approach will yield the most insight into movement in natural populations. First, we recommend performing a sib-split analysis with the caveats mentioned above. Second, we recommend conducting a STRUCTURE analysis with a migration prior informed from the sib-split analysis. Application of this two-step approach maximized information from both of our empirical case studies. For the easy-to-detect case study, we would have used MIGRPRIOR = 0.001 and correctly inferred no migration prior to dam removal. STRUCTURE results following dam removal for the easy-to-detect case study were not sensitive to the migration prior. These results provided added information to the sib-split analysis because we gained one additional downstream migrant that arose from a single-ton family. For the difficult-to-detect case study, STRUCTURE results were highly dependent upon the migration prior. A sib-split-informed STRUCTURE migration prior of 0.05 would have led to high concordance between both methods. Further, the joint analysis caused us to examine two individuals in particular with greater scrutiny. An individual from family 2 was classified as a downstream migrant by sib-split but not by STRUCTURE. However, the assignment probability to the tributary with STRUCTURE was marginal (0.54). We would classify this individual as a putative migrant based on the joint analysis. Family 19 contained two individuals. This family was too small for us to infer movement direction for these individuals based on sib-split, but the high probability with which both individuals assigned to the main stem with STRUCTURE suggests that one of these fish is an upstream migrant. Our previous analysis based on sibship in this system did not detect upstream YOY movement into this tributary, but we only considered full-sib families of three or larger (Kanno et al. 2014). The combined analysis of sib-split and STRUCTURE suggested that upstream movement through this perched culvert is possible. However, the 0.75 m height of the culvert and difficulty for movement this would present to an age-0 brook trout suggests that even high-probability assignments from STRUCTURE models with informed migration priors should be interpreted cautiously.

In summary, our results demonstrate that the novel sib-split approach provides a valuable new tool to infer organism movement genetically on ecological time scales. This new approach, based on single genetic samples, should allow cost- and time-efficient prioritization of barriers such as stream road crossings and for application in other difficult-to-detect situations with low genetic divergence and ongoing or very recently limited gene flow. While our results are based on brook trout that reside in small streams, results from Neville and Peterson (2014, this issue) suggest that sib-split may be generally applicable to fish species that meet the sample requirements of this approach.

Acknowledgements

We thank C. Riley for sample collection from the Michigan study site. We thank M. O'Donnell and T. Dubreuil for help with sample collection from the Massachusetts study site. Genotypic data were collected by M. Page, S. Jane, and M. Burak. H. Neville, E. Taylor, and two anonymous reviewers provided helpful comments on a draft of this manuscript. This work was funded through a grant from the USDA Forest Service San Dimas Technology and Development Center (USFS 10-JV-11242307-098). A.W. received additional support from the National Institute of Food and Agriculture, US Department of Agriculture, the Massachusetts Agricultural Experiment Station, and the Environmental Conservation Department of the University of Massachusetts Amherst, under project number MAS#14.

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