

ARTICLE

# Evaluating the Effects of Barriers on Slimy Sculpin Movement and Population Connectivity Using Novel Sibship-based and Traditional Genetic Metrics

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## Abstract

Population genetics-based approaches can provide robust and cost-effective ways to assess the effects of potential barriers, including dams and road-stream crossings, on the passage and population connectivity of aquatic organisms. Determining the best way to apply and modify genetic tools for different species and situations is essential for making these genetics-based approaches broadly applicable to fisheries and aquatic habitat management. Here, we used multiple genetic approaches to assess the movement and population structure of Slimy Sculpin *Cottus cognatus* at two road-stream crossings in Michigan and one dam in Massachusetts, USA. We captured and genotyped individual sculpin and assessed movement and population connectivity by using (1) a sibship-based approach, where the presence and proportional distribution of siblings on either side of a barrier indicates population connectivity and the possible direction of movement (i.e., presumed movement from higher to lower proportions), and (2) two Bayesian genetic assignment approaches (STRUCTURE and BayesAss) to identify migrants across potential barriers based on individual population assignment probabilities. We also used traditional genetic metrics to assess within-population genetic variation and among-population genetic divergence. At all three locations, we found evidence for sculpin movement across the potential barrier based on sibship reconstruction, but small family sizes limited the ability of this approach to provide robust estimates of the rate and direction of movement. At two sites, a lack of genetic differentiation between

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above- and below-barrier populations limited the effectiveness of the genetic assignment methods for identifying possible migrants. At the third site, reduced upstream allelic diversity and effective number of breeders resulted in high genetic differentiation ( $F_{ST}$ ) between above- and below-barrier populations, and both sibship and genetic assignment methods provided strong evidence of limited connectivity and bias against upstream movement. Overall, combining approaches and metrics may help overcome the limitations of any one method and maximize the value of datasets for genetics-based monitoring and assessment.

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Millions of dams and culverts that are intended mainly for human use and benefit (Graf 1999) span waterways globally (Smith et al. 2002; O'Connor et al. 2008). In addition to altering the physical ecosystem (Poplar-Jeffers et al. 2009), these barriers fragment populations of fish and other species that depend on the aquatic environment (Norman et al. 2009; Bozek 2015), potentially leading to decreased species diversity in the affected systems (Bednarek 2001; Nislow et al. 2011; Briggs and Galarowicz 2013). Habitat fragmentation affects both diadromous fish species that make long-distance migrations for spawning (Tillinger and Stein 1996; Diebel et al. 2015) and resident freshwater fishes that move within river corridors for feeding and spawning throughout their ontogeny (Tillinger and Stein 1996), preventing them from reaching habitats that are critical for different life stages (Taylor and Cooke 2012). While individual organism passage assessment has provided evidence of the degree to which a given stream crossing fragments a population and acts as a barrier, quantifying the effects of barriers on population connectivity and viability remains challenging.

Capture–mark–recapture and passive integrated transponder (PIT) tagging (e.g., installing PIT tag readers at barriers to track movement) have long been used to determine the extent to which stream crossings act as barriers to aquatic organism movement and to provide evidence for the benefits of barrier remediation (Natsumeda 2007; Goerig et al. 2015). However, these methods are cost- and labor-intensive. These traditional tagging studies may also potentially fail to detect movement because fish movement is highly episodic and not every individual will move during the designated time period (Natsumeda 2007; Whiteley et al. 2014). Traditional population genetic approaches—for example, genetic assignment tests (Manel and Holderegger 2013) and population metrics like within-population genetic diversity (heterozygosity and allelic diversity) and among-population divergence (often measured by  $F_{ST}$  or related statistics; Jost 2008; Meirmans and Hedrick 2011)—have limitations as well. Although genetic assignment approaches have the potential to capture movement directly, they require a high degree of genetic divergence to accurately detect dispersers (Whiteley et al. 2014). These methods are often not sensitive enough to detect ecologically relevant movement of individuals, especially when disruptions to gene flow have occurred only within the last several generations (Waples and Gaggiotti 2006; Landguth

et al. 2010; Whiteley et al. 2014). Among-population divergence metrics also capture gene flow on a longer temporal or evolutionary scale rather than detecting movement at an ecological time scale.

An alternative approach to assessing fish movement across barriers, called sibship-splitting or sib-split, was developed by Whiteley et al. (2014) using genetically reconstructed family-level relationships (either siblings or parent-offspring). This approach relies on the fact that most stream-dwelling species have point distributions of reproduction (Hudy et al. 2010) and that full siblings disperse from these point sources. To capture a snapshot of movement, this approach requires only a single sampling event, which makes it less time- and labor-intensive in terms of field work than traditional capture–mark–recapture and PIT-tagging designs. Moreover, compared to traditional genetic approaches that capture gene flow on evolutionary time scales, sib-split can detect individual movement at an ecological time scale, a period over which changes within a population can be observed directly. Oftentimes, to verify that aquatic organism passage improvement projects are needed or to measure their success, a snapshot of movement is all that is necessary, as opposed to a longer-term integrated approach. Sib-split can also be used to capture the movement of young-of-the-year individuals, an otherwise difficult age-class to assess. To date, the sib-split approach for detecting movement through barriers has outperformed traditional population genetic approaches for Brook Trout *Salvelinus fontinalis* (Whiteley et al. 2014) and Westslope Cutthroat Trout *Oncorhynchus clarkii lewisi* (Neville and Peterson 2014). Despite its advantages, sib-split is constrained by the accuracy of sibship estimates, the family structure (the number and variation in family sizes) of the organism that is being considered, and the need for relatively large samples of same-age individuals (Whiteley et al. 2014). Because traditional population genetic analyses are not as constrained by sampling effects (sample size or the need to sample same-age individuals), it is possible that using sib-split in combination with more traditional approaches (that rely on the same data) may be the most effective way to assess movement across barriers.

The general movement patterns and life history characteristics of individual species result in varied effects of a barrier on population connectivity (Nislow et al. 2011). Relatively sedentary fish species like Slimy Sculpin—a

benthic freshwater fish that is found in clear, cold, rocky streams (Rashleigh and Grossman 2005)—may display impeded movement even in the presence of relatively small barriers (Petty and Grossman 2004; Nislow et al. 2011). Sculpins are considered to be poor swimmers; they lack swim bladders and use fin adaptations and a flattened body shape to maintain their position in a stream (Facey and Grossman 1990; Petty and Grossman 2004). Consequently, sculpins tend to display limited movement, especially as adults. Petty and Grossman (2004) found that across 3 years, the mean movement distance across life stages ranged from 1.0 to 8.4 m, with adults moving less than juveniles; however, some individuals have been shown to move upwards of 150 m (Petty and Grossman 2004; Natsumeda 2007; Hudy and Shiflet 2009) and as far as 1,711 m (Hudy and Shiflet 2009). Nonetheless, most adult sculpins move small distances (<10 m) over several years, as they typically find foraging, spring reproduction, and winter refuge habitat all within close proximity (Petty and Grossman 2004). This characteristic limited movement combined with an annual mean female fecundity of approximately 70 eggs (Grossman et al. 2002) is believed to make sculpins vulnerable to the effects of in-stream barriers (Natsumeda 2007). For example, barriers have the potential to create small, isolated populations with decreased genetic diversity that may be at risk of inbreeding and that lack resilience to environmental changes (Coleman et al. 2018). Despite these speculations, few studies have documented the effects of barriers on sculpin movement.

In this study, we used multiple genetic approaches to evaluate our ability to detect Slimy Sculpin passage at three sites: two culverts (one with upstream passage and one without) and one dam (without upstream passage). Specifically, we compared a sibship reconstruction method (sib-split), two Bayesian genetic assignment approaches (STRUCTURE and BayesAss), and traditional population genetic metrics to assess the applicability of these methods, separately and in combination, for detecting reduced movement and associated population impairments under different barrier conditions. This study aims to enhance our understanding of the values and limitations of these approaches for this small, resident fish species, with potential applications to fish species with similar movement habits.

## METHODS

*Study sites.*—Three streams that had populations of Slimy Sculpin upstream and downstream of a barrier were selected as study sites: Peterson Creek, with a culvert that was impassable to upstream sculpin movement prior to 2012 and passable afterwards; Arquilla Creek, with a culvert that was impassable to upstream movement; and Fall

River, with a dam that was impassable to upstream movement. Peterson Creek, a third-order stream, and Arquilla Creek, a second-order stream, are both tributaries to the Manistee River in the Huron–Manistee National Forest in lower Michigan (Figure 1A). Peterson Creek had a  $2.13 \times 26.52$  m corrugated metal pipe culvert that was installed around 1960 (Table 1; Figures 1A, 2A–C). The culvert was undersized relative to the bankfull width of the channel, which resulted in an increase in flow velocity in the culvert relative to upstream and subsequent scouring of the downstream channel bed. This caused the culvert outlet to be perched above the average water level, making it a suspected barrier to small and weak-swimming fish, including Slimy Sculpin. In 2006, a channel-spanning berm of small boulders, cobbles, and gravel was installed immediately below the culvert outlet scour pool, raising the water level above the culvert bottom and increasing the probability of upstream migration of Slimy Sculpin and other aquatic organisms through the culvert (C. Riley, unpublished data). However, given the steep downstream face of the berm and how stream flow passed through and over its component rock material, the berm potentially functioned as a physical barrier to the passage of fish under all conditions but the highest flows. The culvert and berm were eventually removed and replaced in 2012 with a timber bridge in an effort to restore proper channel function and aquatic organism passage. Arquilla Creek had a  $0.91 \times 34.14$  m corrugated metal pipe culvert that was installed around 1960 (Table 1; Figure 2D). At the time of sampling, the downstream end of the culvert was perched at approximately 0.3 m, so the barrier was presumed to be impassable to the upstream movement of Slimy Sculpin. The third study site was Fall River, a tributary of the Connecticut River in western Massachusetts (Table 1; Figures 1B, 2E). A stone and timber dam measuring roughly  $9.14 \times 2.44 \times 1.52$  m (Naley 2014) was constructed along the tributary in the mid-1800s to provide fire protection and to pump water to a local paper mill, but the dam has not been operational since the late 1930s. The dam was presumed to be impassable to the upstream movement of fish (Naley 2014). All three barriers allowed downstream, passive organism passage during high flows.

*Sample collection.*—Young-of-the-year sculpins (i.e., those spawned in late April; Grossman et al. 2002) were sampled both upstream and downstream of each barrier. In Peterson Creek, sculpin were collected in November 2011 before the culvert replacement and in October and November 2013 after the culvert had been replaced by a bridge (Figure 2A–C). In both years, the sampling was conducted in a 305-m reach downstream of the culvert and a 220-m reach upstream of the culvert. In Arquilla Creek, sculpin were sampled in September 2014 in a 130-m reach downstream of the culvert and in a 160-m reach upstream of the culvert. Sculpin were sampled in Fall

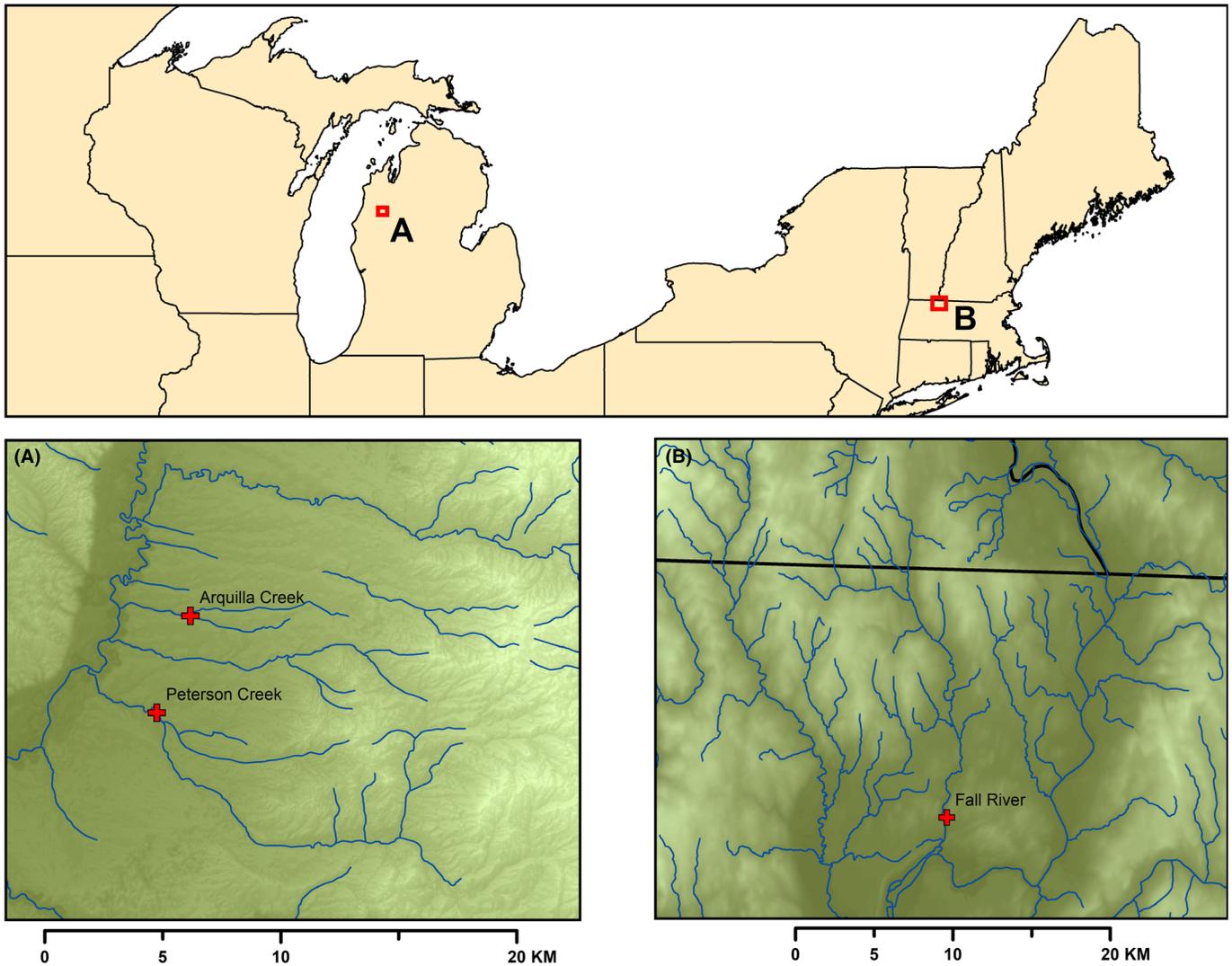


FIGURE 1. Location of sampling sites (A) Peterson Creek and Arquilla Creek in the Huron–Manistee National Forest, Michigan, and (B) Fall River in western Massachusetts.

River in December 2014 in a 200-m reach downstream of the dam and a 150-m reach upstream of the dam and an associated small impoundment. At all of the sampling sites, the fish were collected using a backpack electroshocker and dip net and/or a 1.5- $\times$ 3.6-m seine net. Each sampled individual was assigned a unique identifier, measured for total length, and had a piece of its caudal fin clipped as a source for genetic material before being returned to the stream. At all of the study sites, we used length-frequency histograms to determine length cutoffs for the young-of-the-year age-class, using a cutoff of 50 mm for Arquilla and Peterson creeks (Whiteley et al. 2012) and a cutoff of 59 mm for Fall River.

*Genetic methods.*— We genotyped the young-of-the-year sculpins following the protocols for DNA extraction and

amplification that are detailed in King et al. (2005). We extracted all DNA following a standard salt-precipitation genomic DNA extraction protocol. All of the individuals were genotyped at nine microsatellite loci (Fujishin et al. 2009): *Cco02*, *Cco08*, *Cco09*, *Cco10*, *Cco13*, *Cco14*, *Cco15*, *Cco16*, and *Cco17*. The loci were PCR-amplified following the Qiagen Master Mix protocol (Qiagen, Valencia, California) on an Eppendorf Mastercycler PRO PCR System (Fisher Scientific, Pittsburgh, Pennsylvania) and electrophoresed on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Inc., Foster City, California). Alleles for individuals sampled from Arquilla Creek and individuals sampled from Peterson Creek in 2011 were hand-scored using GENEMAPPER version 4.0 and PEAK SCANNER version 1.0 (Applied Biosystems, Inc.).

TABLE 1. Stream and barrier characteristics for each study location.

Characteristics	Peterson Creek	Arquilla Creek	Fall River
Type of barrier	Corrugated metal pipe culvert	Corrugated metal pipe culvert	Dam
Year of barrier installation	1960	1960	mid-1880s
Barrier location	Huron–Manistee National Forest, Michigan, USA	Huron–Manistee National Forest, Michigan, USA	Gill/Greenfield, Massachusetts, USA
Latitude, longitude (°)	44.26281, –85.84408	44.2891, –85.8313	42.62537, –72.54905
Passability rating	Enhanced <sup>a</sup>	Impassable	Impassable
Drainage area (km <sup>2</sup> )	73.3	8.0	82.90
Channel width (m)	5.5	3.1	9.10
Outflow drop height (m)	0.0 <sup>b</sup>	0.3	2.44 <sup>c</sup>
Basin slope (%)	0.6	1.7	15.50

<sup>a</sup>Following the replacement of the culvert with the timber bridge. Prior to replacement, the culvert was rated as impassable.

<sup>b</sup>This outflow drop height was created by the 2006 installation of a rock berm below the culvert outlet. Previously, the drop height was approximately 0.08 m.

<sup>c</sup>Height of Fall River dam.

For samples that originated in Fall River and in Peterson Creek in 2013, the alleles were sized for each locus using Geneious version R7 (Kearse et al. 2012).

*Population genetic summary statistics.*—We used GDA version 1.1 (Lewis and Zaykin 2001) to estimate allele frequencies, observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosity, and mean number of alleles ( $A$ ) for each sampling location. Population differentiation ( $F_{ST}$ ) and allelic richness ( $AR$ ) were calculated with FSTAT version 2.9.3.2 (Goudet 1995). To quantify the power of the genetic marker panel for pedigree reconstruction, we calculated the probability of identity in both its unbiased ( $PI$ ) and sibling ( $PI_{SIBS}$ ) forms by using GIMLET version 1.3.3 (Valière 2002). We used GENEPOP (Raymond and Rousset 1995; Rousset 2008) to test for locus deviations from Hardy–Weinberg (HW) expectations and for linkage disequilibrium (LD). To correct for inflated type I error rates due to multiple testing (Narum 2006), we used the conservative sequential Bonferroni correction (Rice 1989) at an alpha of 0.05. We note that single cohort samples can deviate from HW expectations and show nonrandom associations among alleles at unlinked loci (linkage disequilibrium) due to family structure (Whiteley et al. 2013). Given that we only genotyped young-of-the-year individuals, we considered the need to purge siblings to account for family structure, as the inclusion of many closely related individuals could upwardly bias the population genetic metrics (Waples and Anderson 2017). However, based on the limited family structure that was observed, sib-purging did not appear to be necessary for our data set.

We estimated the effective number of breeders ( $N_b$ )—reflecting the quantity or quality of spawning and early rearing habitat (Whiteley et al. 2017)—in each population

to provide further context for inferences based on genetic diversity and genetic differentiation. The estimates of  $N_b$  obtained from same-age (single cohort) samples are largely determined by the number of reproducing adults (from overlapping generations) and variance in family size (including family-correlated survival until the time of sampling) of the cohort in question (Waples and Do 2008; Whiteley et al. 2012). At each site,  $N_b$  was estimated for young-of-the-year sculpin using the single-sample linkage disequilibrium method within the program NeEstimator version 2.1 (Do et al. 2014). Estimates of  $N_b$  were derived assuming a random mating model and a minimum allele frequency cutoff ( $P_{crit}$ ) of 0.02, which has been shown to provide a balance between precision and bias across sample sizes (Waples and Do 2008). Confidence intervals (95%) were generated using the jackknife approach. We used GENEPOP to perform genic exact tests to evaluate whether we could pool upstream and downstream samples when calculating  $N_b$ . In evaluating the significance of each locus in each population, we again used a Bonferroni correction at an alpha of 0.05. Given the results of these tests, we estimated  $N_b$  separately for the upstream and downstream reaches, even if there was no evidence of allele frequency heterogeneity at a given locus.

*Sibship-based migrant detection (sib-split).*—We reconstructed full-sibling families using COLONY version 2.0 (Jones and Wang 2010) and estimated the mean number of individuals per full-sibling family by fitting a Poisson distribution to a frequency distribution of full-sibling family size. In our COLONY analyses, we assumed a genotyping error rate of 0.005 and used a full-likelihood model with medium-likelihood precision and a medium run time. Species was set as dioecious and diploid. We assigned

## Peterson Creek



## Arquilla Creek



## Fall River



FIGURE 2. The barriers at Peterson Creek (A) before and (B) after the 2006 rock berm installation and (C) following the culvert removal; (D) Arquilla Creek; and (E) Fall River.

both males and females a polygamous mating system with no inbreeding and no clone inference. We used sibship scaling, but we did not use an informative sibship prior or update allele frequencies. One run was performed with a random number seed of 1234.

For full-sib families of three or more that had individuals on both sides of a barrier, we attempted to use a majority rule approach to infer the directionality of movement (Whiteley et al. 2014) and the number of directional migrants. The majority rule approach uses the assumption that the family originated on the side of the barrier with the most siblings present. In calculating both upstream ( $R_U$ ) and downstream ( $R_D$ ) migration rates, we used the parameters  $M_U$  and  $M_D$  to represent the numbers of putative upstream and downstream migrants respectively. The symbols  $N_U$  and  $N_D$  represent the numbers of individuals that were sampled upstream and downstream, respectively, from families of size three or greater:

$$R_U = M_U / (N_D + M_U - M_D)$$

$$R_D = M_D / (N_U + M_D - M_U).$$

Given the purported impassability of the Arquilla Creek culvert and Fall River dam to young-of-the-year sculpin, we additionally calculated migration rates for

those two sites assuming that all movement occurred in the downstream direction. Thus, instead of using the majority rule to determine migration rates, we assumed that the upstream migration rate ( $M_U$ ) was zero and allowed for the occurrence of multiple full-sibs to move downstream over the barrier. Downstream migration rates were then recalculated according to the same formula as above, with  $M_U$  set to zero.

*Sibship reconstruction simulations.*—The power of the locus panel to reconstruct full-sibling families accurately was assessed through the use of simulated data that was generated by the program PEDAGOG version 1.2 (Coombs et al. 2010a). For the simulated populations, the genetic parameters were derived from the sampled individuals for each study population, while the demographic parameters were derived from the primary literature (Supplemental File 1 available in the online version of this article; Owens and Noguchi 1998; Meyer et al. 2008; Gray et al. 2018). The population sizes and capture probabilities were modified for each simulated population to produce simulated sample sizes and mean family sizes that resembled those of their empirical counterpart. The sibship reconstructions were conducted on the simulated data by using COLONY version 2, and accuracy assessments were performed by using PEDAGREE version 1.06 (Coombs et al. 2010b). A total of 20 replicate simulations were performed for each study

population (see Supplemental File 2 available in the online version of this article).

*Migrant detection using Bayesian models.*—In addition to the sibship reconstructions, we used two Bayesian admixture models—STRUCTURE version 2.3.3 (Pritchard et al. 2000) and BayesAss edition 3.0 (Rannala 2007)—to test for migration between the upstream and downstream sample sites of each river system. For STRUCTURE, we set the GENSBACK (G) parameter to 0 to evaluate the migration of currently sampled individuals and we tested the sensitivity of the model to three values of  $\nu$ , the probability that an individual was an immigrant. Following Pritchard et al.'s (2000) recommendations, we used values for MIGRPRIOR ( $\nu$ ) of 0.03, 0.05, and 0.10 (assuming, therefore, that each individual had a 3, 5, and 10% probability of being a migrant). We chose values closer to the 0.10 upper limit because we suspected relatively high rates of downstream migration in our study systems. All of the runs had a burn-in period of 30,000 steps followed by 50,000 Markov chain–Monte Carlo sample repetitions. We calculated the migration rates as described above for the sib-split approach for each value of  $\nu$ , and we present the STRUCTURE migration rate estimates as a range of values. A limitation of STRUCTURE is that it assumes that migration is symmetrical and constant only for the last generation (when  $G=0$ ). Unlike STRUCTURE, BayesAss allows for asymmetrical migration, but it also assumes constant migration rates over the last two generations. We ran BayesAss using the default parameters, including 5,000,000 iterations and a random number seed of 10, except for the mixing parameter for inbreeding coefficients, for which we adjusted the proposal step length to 0.30 based on the acceptance rates from the initial runs.

## RESULTS

### Peterson Creek, Michigan

In 2011, two loci (*Cco14* and *Cco16*), both in the downstream population, deviated significantly from HW expectations and one test for LD was significant. In 2013, two loci (*Cco02* and *Cco13*, both in the upstream population) deviated significantly from HW expectations and five tests for LD were significant. In 2011, five loci (*Cco02*, *Cco09*, *Cco10*, *Cco14*, and *Cco16*) exhibited significant allele frequency heterogeneity with genic exact tests, while two loci (*Cco10* and *Cco15*) exhibited significant allele frequency heterogeneity with genic exact tests in 2013.

We sampled 394 young-of-the-year sculpins in 2011 (268 individuals downstream of the culvert and 126 individuals upstream) and 182 young-of-the-year sculpins in 2013 (90 from downstream of the culvert and 92 from upstream). In both years, all of the individuals were genotyped at all loci. In 2011, the young-of-the-year sculpins ranged in length from 27 to 50 mm, with a mean length of

39.9 mm. In 2013, the sampled fish ranged in length from 24 to 50 mm with a mean length of 40.5 mm. In both 2011 before the culvert replacement and 2013 after the culvert replacement,  $A$  and  $AR$  (standardized to a sample size of 126 for 2011 and 90 for 2013) were greater in the downstream population than in the upstream population (Table 2). Expected heterozygosity was greater in the downstream population in 2011 and in the upstream population in 2013 (Table 2). In both years, only very slight genetic differentiation was observed between the upstream and downstream samples (2011  $F_{ST}=0.003$ , 95% CI: 0–0.006; 2013  $F_{ST}=0.004$ , 95% CI: 0.001–0.01). In 2011, the estimate of effective number of breeders was greater downstream of the culvert ( $N_b = 239.7$ , 95% CI: 161.1–403.2) than upstream ( $N_b = 192.0$ , 95% CI: 102.9–632.0) of the culvert, although the 95% CIs overlapped (Table 2). In 2013,  $N_b$  was again greater downstream ( $N_b = 153.8$ , 95% CI: 81.5–550.5) than upstream ( $N_b = 73.1$ , 95% CI: 48.0–125.9).

The 394 young-of-the-year sculpin that were genotyped in 2011 were reconstructed into 279 families, ranging in size from one to five individuals (mean family size = 1.41), while the 183 individuals that were genotyped for the 2013 cohort were reconstructed into 127 families, ranging in size from one to four individuals (mean family size = 1.43). Twenty-seven of the full-sibling families that were reconstructed using the 2011 data (9.7%) contained three or more members. Of those, 12 (44%) provided evidence of movement through the culvert, with seven having a majority of members downstream of the culvert with one member upstream (seven putative upstream migrants, Table 3; Figure 3A), while five had a majority of members upstream of the culvert with one member downstream (five putative downstream migrants, Table 3; Figure 3A). For families of size three or greater, the Prob(Inc.) values from the COLONY output, which are the probability estimates that the individuals in each reconstructed family are full siblings, ranged from 0.19 to 0.98, with a mean Prob(Inc.) of 0.75. The Prob(Exc.) values, representing the probability that each family had been optimally reconstructed (not split), ranged from 0.19 to 0.96, with a mean value of 0.69. For the 2013 families, five of ten families (50%) with three or more members provided evidence of movement. Two families had a majority of members downstream of the bridge (two putative upstream migrants, Table 3), and three families had a majority of members upstream of the bridge (three putative downstream migrants, Table 3; Figure 3B). The Prob(Inc.) values ranged from 0.30 to 0.99, with an average Prob(Inc.) of 0.71, and the Prob(Exc.) values ranged from 0.30 to 0.99, with an average value of 0.66. In 2011, we estimated an upstream migration rate of 0.11 and a downstream migration rate of 0.21 (Table 3). For the 2013 individuals, we estimated an upstream migration rate of 0.22 and a

TABLE 2. Genetic summary statistics for a 9-loci microsatellite panel for the downstream (DS) and upstream (US) populations at the three study sites;  $n$  = sample size;  $A$  = number of alleles;  $AR$  = allelic richness;  $H_O$  = mean observed heterozygosity;  $H_E$  = mean expected heterozygosity;  $N_b$  = effective number of breeders (95% CI); Inf. = infinity.

Location	Sample	$n$	$A$	$AR$	$H_O$	$H_E$	$N_b$ (95% CI)
Peterson Creek 2011	DS	268	10.8	10.1	0.612	0.635	239.7 (161.1–403.2)
	US	126	9.0	9.0	0.612	0.615	192.0 (102.9–632.0)
Peterson Creek 2013	DS	90	9.4	9.4	0.607	0.610	153.8 (81.5–550.5)
	US	92	8.6	8.5	0.568	0.611	73.1 (48.0–125.9)
Arquilla Creek	DS	154	8.7	7.4	0.632	0.646	91.8 (59.0–161.4)
	US	45	6.7	6.6	0.580	0.598	27.5 (16.1–54.5)
Fall River	DS	83	12.4	12.3	0.613	0.612	1,006.6 (137.1–Inf.)
	US	162	14.3	12.7	0.623	0.631	1,959.1 (308.0–Inf.)

downstream migration rate of 0.12 (Table 3). Across both years, simulated sibship reconstruction accuracies were 79% overall and 84% for families with three or more full siblings.

In both 2011 and 2013, STRUCTURE estimated zero to one migrant within the upstream samples across probabilities that an individual was an immigrant ( $v$ ) of 0.03 to 0.10 (Table 4; Supplemental File 3 available in the online version of this article). The mean assignment probability to the assigned population was 0.94 across values of  $v$  in both 2011 and 2013. The estimated migration rates ranged from 0.0 to 0.004 (2011) and from 0.0 to 0.01 (2013). For the downstream samples, zero to three migrants were estimated in 2011 over values of  $v$  ranging from 0.03 to 0.10, and zero migrants were estimated consistently in 2013 over the same prior probability range (Table 4). The estimated migration rates ranged from 0.0 to 0.02 in 2011, and were zero across the range of  $v$  in 2013.

In 2011, BayesAss estimated that just over 31% of sculpin sampled in the downstream population ( $n = 84$ ; 95% CI: 0.29–0.34) were migrants from upstream and 1% of

sculpin in the upstream population ( $n = 1$ ; 95% CI: –0.01–0.02) originated in the downstream population. This corresponded to an estimated upstream migration rate of 0.01 and an estimated downstream migration rate of 0.40. In 2013, 28% of the downstream individuals ( $n = 25$ ; 95% CI: 0.21–0.35) were estimated as migrants from upstream and 11% of sculpin in the upstream population ( $n = 10$ ; 95% CI: 0.05–0.18) were estimated to be migrants that originated downstream. There was an estimated upstream migration rate of 0.13 and an estimated downstream migration rate of 0.23 (Table 3).

#### Arquilla Creek, Michigan

In Arquilla Creek, we identified three loci that deviated significantly from HW proportions. One deviation (*Cco14*) occurred in the upstream population, and the remaining two deviations occurred in the downstream samples (*Cco13* and *Cco17*). Five tests for linkage disequilibrium were significant, and seven loci (*Cco09*, *Cco10*, *Cco13*, *Cco14*, *Cco15*, *Cco16*, and *Cco17*) exhibited significant allele frequency heterogeneity with genic exact tests.

TABLE 3. Estimated migration rates within each study site as estimated through sib-split when assuming the majority rule, sib-split when assuming exclusively downstream movement, BayesAss, and STRUCTURE.

Study site	Location	Number of putative migrants (majority rule)	Sib-split (majority rule)	Sib-split	BayesAss	STRUCTURE
				(downstream only)		
Peterson Creek 2011	US	7	0.11	N/A	0.01	0.00–0.004
	DS	5	0.21	N/A	0.40	0.00–0.02
Peterson Creek 2013	US	2	0.22	N/A	0.13	0.00–0.01
	DS	3	0.12	N/A	0.23	0.00
Arquilla Creek	US	2	0.06	0.00	0.02	0.02–0.03
	DS	2	0.10	0.26	0.11	0.05–0.09
Fall River	US	1	0.17	0.00	0.37	0.00
	DS	5	0.29	0.43 <sup>a</sup>	0.12	0.00

<sup>a</sup>This estimate includes the family that had two members on either side of the barrier as potential migrants.

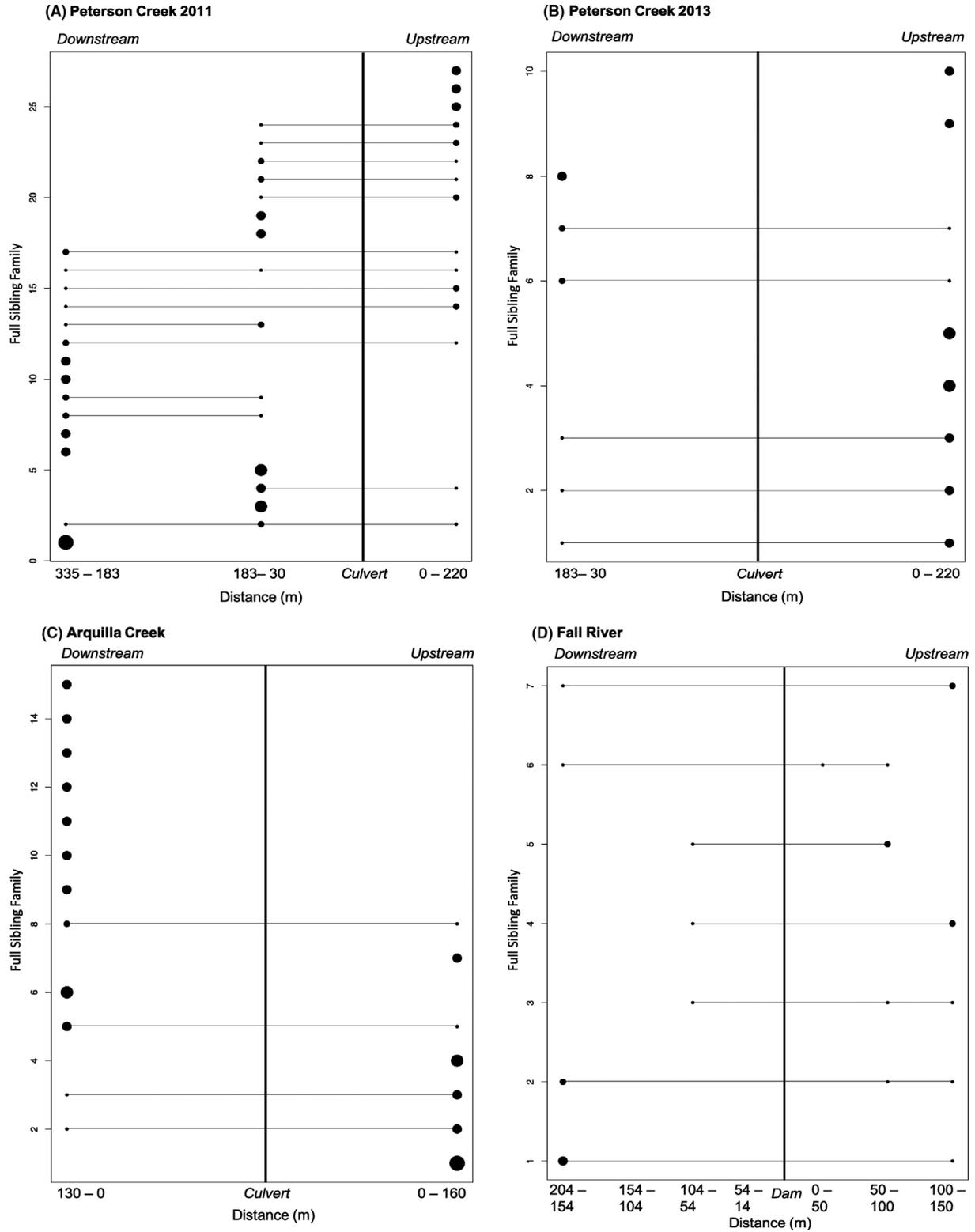


FIGURE 3. Movement of full-sibling families of size three or greater in (A) Peterson Creek in 2011 and (B) 2013; (C) Arquilla Creek, and (D) Fall River. Each row represents individuals (points, with larger points representing more individuals), and a horizontal line connecting individuals shows family members on either side of the barrier. The vertical dashed lines represent the locations of the barriers relative to the sampling sites. Upstream and downstream reaches are indicated in meters from the barrier.

TABLE 4. The number of migrant individuals per sampling site for varying migration rate prior probabilities estimated by STRUCTURE.

Location	Sample	Migration prior probability		
		0.03	0.05	0.10
Peterson Creek 2011	DS	0	1	3
	US	0	0	1
Peterson Creek 2013	DS	0	0	0
	US	0	0	1
Arquilla Creek	DS	2	2	4
	US	3	3	4
Fall River	DS	0	0	0
	US	0	0	0

We sampled 199 young-of-the-year sculpins in this system: 45 individuals were collected upstream of the culvert and 154 were collected downstream. Overall across all of the sampled individuals, 95% of the loci were successfully genotyped—3% of the loci in the upstream population and 6% of the loci in the downstream population could not be genotyped. Individual fish ranged in length from 26–42 mm, with a mean length of 33.6 mm. The mean number of alleles,  $AR$  (standardized to a sample size of 40), and  $H_E$  were all greater in the downstream population than in the upstream population (Table 2). The upstream and downstream samples exhibited significant genetic differentiation ( $F_{ST}=0.041$ , 95% CI: 0.018–0.070). The estimated  $N_b$  was 91.8 (95% CI: 59.0–161.4) for the downstream population and 27.5 (95% CI: 16.1–54.5) for the upstream population.

The 199 genotyped individuals were reconstructed into 135 full-sibling families (mean family size = 1.47). Fifteen of the 135 full-sib families (11%) were composed of three to five individuals, accounting for 52 of the 199 fish (26%). Of these families, four provided evidence of migration through the culvert (Figure 3C). Two of these families had a majority of members downstream of the culvert with one member upstream (two putative upstream migrants, Table 3; Figure 3C), while two families had a majority of members upstream of the culvert with one member downstream (two putative downstream migrants, Table 3, Figure 3C). There were, in total, four putative migrants, with an estimated upstream migration rate of 0.06 and an estimated downstream migration rate of 0.10 (Table 3). When we assumed that all migration occurred in the downstream direction, the estimated downstream migration rate was 0.26. For families with three or more members, the Prob(Inc.) values ranged from 0.05 to 1.0, with an average Prob(Inc.) of 0.75 and Prob(Exc.) values ranged from 0.05 to 0.98, with an average value of 0.57. The simulated sibship reconstruction accuracies were 81%

overall and 90% for families with three or more full siblings.

The number of upstream migrants estimated with STRUCTURE analyses was insensitive to the assumed values of the probability that an individual was an immigrant ( $v$ ), while the estimated downstream migration rate demonstrated some sensitivity to  $v$  (Supplemental File 3). For the upstream samples, 3 or 4 migrants were consistently estimated over values of  $v$  ranging from 0.03 to 0.10 (Table 4). The mean assignment probability to the assigned population was 0.95 across values of  $v$ . Of the four estimated migrants, one belonged to a full-sibling family that contained three or more members and was a putative upstream migrant in the sibship reconstruction analysis as well. Using the four estimated migrants, the estimated upstream migration rate equaled 0.03 (Table 3). For the downstream samples, 2 to 4 migrants were estimated over the assumed values of  $v$  (Table 4). Of the four estimated migrants, one belonged to a full-sibling family that contained three or more members. This migrant, assigned to a four-member family, was a putative downstream migrant in the sibship reconstruction analysis. Based on four estimated migrants, the estimated downstream migration rate was 0.09 (Table 4), which was similar to the sib-split estimate. BayesAss estimated that 6% of the individuals in the upstream population ( $n=3$ ; 95% CI: 0.01–0.11) had originated downstream, and 3% of the individuals in the downstream population ( $n=5$ ; 95% CI: 0 – 0.06) were migrants from upstream. The upstream migration rate was estimated as 0.02, and the estimated downstream migration rate was 0.11 (Table 3).

#### Fall River, Massachusetts

In Fall River, none of the tests for LD were significant following a Bonferroni correction, but one locus (*Cco08*) in the downstream population deviated significantly from HW proportions. None of the loci exhibited significant allele frequency heterogeneity based on genic exact tests. We genotyped 245 young-of-the-year sculpins, including 83 individuals that were sampled downstream of the dam and 162 individuals sampled upstream. Across all of the individuals, 97% of loci were successfully genotyped; 4% of loci in the upstream population and 2% of loci in the downstream population could not be genotyped. Individual fish ranged in length from 37 to 59 mm, with a mean length of 48.0 mm. Unlike in Peterson Creek and Arquilla Creek,  $A$ ,  $AR$  (standardized to a sample size of 77),  $H_O$ , and  $H_E$  were all greater in the upstream population than in the downstream population in Fall River (Table 2). Minimal genetic differentiation was observed between the upstream and downstream samples ( $F_{ST}=0.001$ , 95% CI: 0–0.007). The estimate of  $N_b$  was much larger in Fall River than in the other two study systems, with a

downstream estimate of 1,006.6 (CI: 137.1–infinity) and an upstream estimate of 1,959.1 (CI: 308.0–infinity; Table 2).

The 245 genotyped individuals were reconstructed into 194 full-sibling families, ranging in size from one to four individuals (mean family size = 1.26). Seven of these families (3.6%) consisted of three or four members, and all seven showed evidence of movement across the dam (Figure 3D). Of these, five had a majority of members upstream of the dam with one member downstream (five putative downstream migrants, Table 3), one had a majority of members downstream of the dam with one member upstream (one putative upstream migrant, Table 3), and one had two members upstream and two members downstream of the dam (Figure 3D). We estimated an upstream migration rate of 0.17 and a downstream migration rate of 0.29 (Table 3). When recalculating migration rates assuming entirely downstream migration, we estimated a downstream migration rate of 0.43 (Table 3). For families with three or more members, the Prob(Inc.) values ranged from 0.16 to 0.86, with an average Prob(Inc.) of 0.57, and the Prob(Exc.) values ranged from 0.12 to 0.86, with an average value of 0.56. The simulated sibship reconstruction accuracies were 82% overall and 86% for families with three or more full siblings.

The number of estimated upstream and downstream migrants with STRUCTURE was zero (Table 4; Supplemental File 3). The mean assignment probability to the assigned population was 0.94 across values of  $v$ . Based on the results from BayesAss, just over 21% of the individuals (95% CI: 0.07–0.36) in the downstream population ( $n = 18$ ) were estimated as being migrants from the upstream population and 24% of the individuals in the upstream population ( $n = 39$ ; 95% CI: 0.20–0.27) were estimated as being migrants from downstream. We estimated the upstream migration rate as 0.37 and the downstream migration rate as 0.12.

## DISCUSSION

At all three study sites examined herein, juvenile sculpins appeared to be able to migrate across the barriers, although the magnitude of the barrier effect on population connectivity and other population-level consequences varied among the sites and genetic approaches.

While sib-split appears to have accurately detected movement at the spatial scales ( $\approx 400$  m) used at all three of the study sites (i.e., we assumed that downstream movement during high flows was possible at all of the sites), inference regarding directionality of movement based on sibship reconstruction alone was not possible (i.e., upstream movement was suggested at the presumed impassable barriers). In previous studies that used sib-split, the majority rule was used to infer the directionality of movement under the assumption that every full-sibling

family originated on the side of the barrier with the most full-siblings present (Whiteley et al. 2014). In this study, the largest sculpin family that was reconstructed consisted of five individuals and the overall mean family size was 1.39 individuals, making majority-rule inferences extremely sensitive to sample reach size, capture efficiency, and survival. If full siblings had moved outside of our sampling reaches then their presence would not have been reflected in our analyses, thus underestimating migration and potentially affecting our ability to make inferences about directionality; this would be particularly problematic for species with high dispersal rates (not sculpins) and should be accounted for in the sampling design.

Our ability to capture siblings (and thus use the majority rule) is also dependent on their survival both above and below the barriers. At our study sites, we have no evidence of differential survival among the reaches, although differences in habitat and habitat availability and abundances of predators may affect the survival of juvenile sculpins as well as their movement and capture probability. While it appears that the majority rule can be appropriately applied to species such as Brook Trout that have relatively large family sizes (Whiteley et al. 2014 reported a mean family size of 2.95 and the largest family out of 114 assigned individuals was 17 members), application to species with small family sizes should be undertaken with caution.

Information on genetic variation and population differentiation, which reflect gene flow over many generations, helped with our interpretations of migration rates and the detection of movement based on sib-split. Though pairs of loci were in linkage disequilibrium across the populations examined herein, there was no consistent pattern of association. In Peterson Creek, we expected downstream but not upstream movement by young-of-the-year Slimy Sculpin prior to the culvert replacement, as well as an increase in movement in 2013 following the replacement of the culvert with a timber bridge, yet the sib-split results were consistent with movement occurring during both sampling periods. If gene flow was unidirectional (downstream) prior to the culvert replacement, we might expect reduced genetic variation in the upstream reach and significant genetic differentiation between above and below barrier populations. However,  $H_E$  and  $AR$  were similar upstream and downstream of the culvert in 2011 and  $F_{ST}$  values for both sample years were consistent with high average rates of gene flow over past generations. It is possible that these genetic metrics are not sensitive to recent changes; while  $F_{ST}$  can change quickly if migration across a barrier weakens genetic differentiation, it may take more than 200 generations for  $F_{ST}$  to detect a new barrier and more than 100 generations to remove historical discontinuities if dispersal movements are small (Landguth et al. 2010), as with sculpins. The retention of genetic variation upstream of the culvert could also be

explained by a large effective population size in the reach above the culvert. The relatively large estimate of the effective number of breeders upstream of the culvert in 2011 suggests that generational effective population size ( $N_e$ ) is also likely to be large, although further work to understand the relationship between  $N_b$  and generational  $N_e$  in Slimy Sculpin is needed.

Estimates for  $N_b$  in Fall River had wide confidence intervals (Table 2), suggesting that  $N_b$  was large both upstream and downstream of the dam. We suspect that, similar to Peterson Creek, the upstream population contained enough habitat to support a large population that was not threatened by loss of genetic diversity or the demographic effect of juvenile emigration. Unlike in Peterson and Arquilla creeks,  $A$ ,  $AR$ ,  $H_O$ , and  $H_E$  in Fall River were all greater in the upstream than in the downstream reaches (Table 2). The number of individuals sampled in this system was greater upstream ( $n=162$ ) than downstream ( $n=83$ ; Table 2), which may affect  $A$  and  $H_O$  but should not influence  $AR$  or  $H_E$ . The greater genetic variation in the upstream reach suggests that there was more suitable habitat upstream than downstream of the dam—in fact, there is limited downstream habitat (~1.2 km) before fish reach the much larger Connecticut River, which does not support sculpins.

Of the three populations evaluated here, Arquilla Creek showed the greatest differentiation between upstream and downstream populations ( $F_{ST}=0.041$ ), suggesting that population subdivision was present and that the culvert had a negative effect on population connectivity. This high rate of genetic differentiation combined with small  $N_b$  and higher genetic drift than in the other two systems made it comparatively difficult to group differentiated families. Low upstream  $N_b$  was consistent with limited spawning and early rearing habitat in the upstream portion of this stream; we estimated that a very small amount of habitat (924 m) was available upstream before reaching additional perched culverts in the two upstream branches of Arquilla Creek. Reduced  $A$ ,  $AR$ ,  $H_O$ , and  $H_E$  values in the upstream population (Table 2) were consistent with limited-to-absent upstream gene flow, despite the fact that the sib-split method inferred upstream migration. Due to its smaller effective size and presumed net loss of migrating individuals, the upstream population in Arquilla Creek was subjected to the greatest impact by the presence of the barrier, and this sculpin population might be susceptible to inbreeding depression and reduced adaptive potential.

In systems with high genetic differentiation between upstream and downstream populations, using both sib-split and genetic assignment methods—here, STRUCTURE and BayesAss—can help assess the reliability of the results. Arquilla Creek was the site for which the STRUCTURE results appeared to be the most reliable, as

it had the most four and five individual families and the number of estimated migrants was less dependent upon the assumed values of  $\nu$  (the probability that an individual was an immigrant; Table 4); consequently, this is the site for which a direct comparison of migration rates using all three metrics is the most informative. In this system, the migration rates that were estimated by using BayesAss and STRUCTURE were similar to those that were estimated using sib-split when assuming the majority rule (Table 3). Collectively, sib-split, STRUCTURE, and BayesAss point to a strong bias against upstream movement in Arquilla Creek, which is expected given the purported impassability of the culvert. This alignment suggests that using a combination of genetic approaches that rely on the same data allows for the best interpretation of movement across barriers, particularly in species with small families. It is also likely, though, that in systems such as Arquilla Creek and Fall River, migration rates estimated using sib-split when assuming strictly downstream migration may most accurately represent true migration rates in the system.

While STRUCTURE was used to help evaluate the majority-rule inference of sib-split, it has decreased accuracy and is sensitive to the choice of migration prior and to family structure in systems with little genetic differentiation (Pritchard et al. 2000; Whiteley et al. 2014) so it cannot be solely relied on for detecting migrants. In Peterson Creek and Fall River, the lack of estimated migrants by STRUCTURE and, consequently, the low estimated migration rates (Table 3), most likely resulted from weak family structure and a lack of genetic differentiation between the upstream and downstream samples, which may be a result of individuals moving across the barriers. At both of these sites, the low genetic differentiation (i.e., low  $F_{ST}$ ) affected the calculation of migration rates, resulting in values that were dissimilar from both BayesAss and sib-split (Table 3). The migration rate estimates also differed between BayesAss and sib-split. The upstream migration rate estimates for Peterson Creek, as estimated by BayesAss, were lower for both years than those that were estimated using sib-split, while estimated downstream migration rates from BayesAss were greater than those estimated using sib-split. Conversely, in Fall River, BayesAss estimated higher upstream and lower downstream migration rates than did sib-split, both when applying the majority rule and when assuming solely downstream migration. It has been suggested that BayesAss is most accurate if the genetic differentiation ( $F_{ST}$ ) in a system is greater than 0.05 (Faubet et al. 2007), which was not the case for either Peterson Creek (2011  $F_{ST}=0.003$ ; 2013  $F_{ST}=0.004$ ) or Fall River ( $F_{ST}=0.001$ ). Given this, it is likely that in systems where there is minimal genetic divergence between the upstream and downstream populations, or in systems with ample upstream

habitat in which a population can persist, as seen here in Fall River, migration rates can be estimated most reliably using sib-split.

Our migration results can be interpreted within the context of past work on the demography, life history, and movement ecology of freshwater sculpins. Juvenile sculpins may be pushed out of high-quality habitats by territorial adults that tend to be limited in their movements (though note that adults have been shown to make occasional large-scale movements for food and resources [Petty and Grossman 2004, 2007; Natsumeda 2007; Hudy and Shiflet 2009]). Consequently, juvenile sculpins may make long-distance movements for resources and to avoid predation and competition (Freeman and Stouder 1989), potentially accounting in part for the young-of-the-year migration rates seen here. These biological and life history factors combined suggest that sculpins are sensitive to in-stream barriers at all life stages (Natsumeda 2007) which, in addition to population-level genetic effects, may lead to increased rates of intraspecific competition.

While our analysis focused on full-sibling families, half siblings are common with Slimy Sculpins and may be useful to include in future sib-split reconstructions to help better understand movement. Slimy Sculpin are known to display a polygynous mating system, where a nest-guarding male Slimy Sculpin accepts clutches of eggs from multiple females, resulting in half siblings and full siblings emerging from the same nest (Keeler and Cunjak 2007; Gray et al. 2018). However, half sibling assignments are prone to high inaccuracies (Ackerman et al. 2017) and, given our full-sibling accuracy estimates, our marker panel would not have enough power to accurately reconstruct half-sibling families. With a more powerful marker panel, analyzing half siblings would be useful to consider because it would potentially provide larger family sizes and increase our understanding of sibling movement from a point source.

Our work also helps define the situations where barriers might have the greatest adverse effects on sculpin population persistence. Systems such as Arquilla Creek, which had a perched culvert, limited upstream habitat, reduced  $N_b$  in the upstream population, and likely more genetic drift, appear to have the lowest probability of persistence. Furthermore, the net loss of migrating fish would be expected to compound the rate at which genetic variation was lost in the Arquilla Creek upstream population. Conversely, systems with limited downstream habitat should be less susceptible to genetic drift, as downstream populations are buffered by downstream migrants, which provide gene flow into the population. For streams with sufficient upstream and downstream habitat, there is lower management concern about fragmentation given the relative reduced risk to these populations. In systems such as Fall River, with large barriers but ample upstream habitat,

Slimy Sculpins appear to be the least vulnerable with regard to population persistence in the short term (i.e., tens of generations), as individuals are not prevented from reaching critical habitats at designated life stages. Although Slimy Sculpin are thought to be highly sensitive to barriers to movement due to their biology (including their poor swimming ability), the likelihood of population persistence further depends on stream characteristics like available habitat and the purported passability of the barrier.

## Conclusions

This study represents the first test of the sib-split method for assessing the population connectivity of a species with small family sizes. The applicability of the sib-split method relative to other genetic methods depends on the characteristics of the study site. When applied to sculpins and other species with small family sizes, sib-split is likely best used to infer movement but not directionality. When possible, combining results from sib-split (here, assuming solely downstream movement), genetic assignment methods, and traditional genetic metrics provides increased confidence in the accuracy of migration rate estimates, as seen here for Arquilla Creek where estimates aligned across methods. To more fully test the applicability of the sib-split method for species with small family sizes, and to better assess its ability to infer directionality, the method should be tested in other species and populations of fish with similar life histories to Slimy Sculpin, possibly coupled with a traditional capture-mark-recapture sampling design. Regardless of these limitations, sib-split is a promising technique for addressing the effects of barriers on fishes at an ecologically relevant time scale. Sib-split, in combination with genetic assignment and traditional population genetic metrics, provides a detailed understanding of connectivity within a system and of the effects that in-stream barriers have on stream fish assemblages.

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## REFERENCES

- Ackerman, M. W., B. K. Hand, R. K. Waples, G. Luikart, R. S. Waples, C. A. Steele, B. A. Garner, J. McCane, and M. R. Campbell. 2017. Effective number of breeders from sibship reconstruction: empirical evaluations using hatchery steelhead. *Evolutionary Applications* 10:146–160.
- Bednarek, A. 2001. Undamming rivers: a review of the ecological impacts of dam removal. *Environmental Management* 27:803–814.
- Bozek, C. 2015. Removing dams: benefits for people and nature. *Solutions for a Sustainable and Desirable Future* 5:79–84.
- Briggs, A. S., and T. L. Galarowicz. 2013. Fish passage through culverts in central Michigan warm water streams. *North American Journal of Fisheries Management* 33:652–664.
- Coleman, R., B. Gauffre, A. Pavlova, L. Beheregaray, J. Kearns, J. Lyon, M. Sasaki, R. Leblois, C. Sgro, and P. Sunnucks. 2018. Artificial barriers prevent genetic recovery of small isolated populations of a low-mobility freshwater fish. *Heredity* 120:515–532.
- Coombs, J. A., B. Letcher, and K. Nislow. 2010a. PEDAGOG: software for simulating eco-evolutionary population dynamics. *Molecular Ecology Resources* 10:558–563.
- Coombs, J., B. Letcher, and K. Nislow. 2010b. PedAgree: software to quantify error and assess accuracy and congruence for genetically reconstructed pedigree relationships. *Conservation Genetics Resources* 2:147–150.
- Diebel, M. W., M. Fedora, S. Cogswell, and J. R. O'Hanley. 2015. Effects of road crossings on habitat connectivity for stream-resident fish. *River Research and Applications* 31:1251–1261.
- Do, C., R. S. Waples, D. Peel, G. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: re-implementation of software for the estimation of contemporary effective population size ( $N_e$ ) from genetic data. *Molecular Ecology Resources* 14:209–214.
- Facey, D. E., and G. D. Grossman. 1990. The metabolic cost of maintaining position for four North American stream fishes: effects of season and velocity. *Physiological Zoology* 63:757–776.
- Faubet, P., R. S. Waples, and O. E. Gaggiotti. 2007. Evaluating the performance of a multilocus Bayesian method for the estimation of migration rates. *Molecular Ecology* 16:1149–1166.
- Freeman, M., and D. Stouder. 1989. Intraspecific interactions influence size specific depth distribution in *Cottus bairdi*. *Environmental Biology of Fishes* 24:231–236.
- Fujishin, L. M., F. K. Barker, D. D. Huff, and L. M. Miller. 2009. Isolation of 13 polymorphic microsatellite loci for Slimy Sculpin (*Cottus cognatus*). *Conservation Genetics Resources* 1:429–432.
- Goerig, E., T. Castro-Santos, and N. É. Bergeron. 2015. Brook Trout passage performance through culverts. *Canadian Journal of Fisheries and Aquatic Sciences* 73:94–104.
- Goudet, J. 1995. FSTAT (version 1.2): a computer program to calculate  $F$ -statistics. *Journal of Heredity* 86:485–486.
- Graf, W. L. 1999. Dam nation: a geographic census of American dams and their large-scale hydrologic impacts. *Water Resources Research* 35:1305–1311.
- Gray, M. A., R. A. Curry, T. J. Arciszewski, K. R. Munkittrick, and S. M. Brasfield. 2018. The biology and ecology of Slimy Sculpin: a recipe for effective environmental monitoring. *Facets* 3:103–127.
- Grossman, G., K. McDaniel, and R. Ratajczak. 2002. Demographic characteristics of female Mottled Sculpin, *Cottus bairdi*, in the Coweeta Creek drainage, North Carolina. *Environmental Biology of Fishes* 63:299–308.
- Hudy, M., J. A. Coombs, K. H. Nislow, and B. H. Letcher. 2010. Dispersal and within-stream spatial population structure of Brook Trout revealed by pedigree reconstruction analysis. *Transactions of the American Fisheries Society* 139:1276–1287.
- Hudy, M., and J. Shiflet. 2009. Movement and recolonization of Potomac Sculpin in a Virginia stream. *North American Journal of Fisheries Management* 29:196–204.
- Jones, O. R., and J. Wang. 2010. COLONY: a program for parentage and sibship inference from multilocus genotype data. *Molecular Ecology Resources* 10:551–555.
- Jost, L. 2008.  $G_{ST}$  and its relatives do not measure differentiation. *Molecular Ecology* 17:4015–4026.
- Kearse, M., R. Moir, A. Wilson, S. Stones-Havas, M. Cheung, S. Sturrock, S. Buxton, A. Cooper, S. Markowitz, and C. Duran. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28:1647–1649.
- Keeler, R. A., and R. A. Cunjak. 2007. Reproductive ecology of Slimy Sculpin in small New Brunswick streams. *Transactions of the American Fisheries Society* 136:1762–1768.
- King, T. L., M. S. Eackles, and B. H. Letcher. 2005. Microsatellite DNA markers for the study of Atlantic Salmon (*Salmo salar*) kinship, population structure, and mixed-fishery analyses. *Molecular Ecology Resources* 5:130–132.
- Landguth, E. L., S. A. Cushman, M. K. Schwartz, K. S. McKelvey, M. Murphy, and G. Luikart. 2010. Quantifying the lag time to detect barriers in landscape genetics. *Molecular Ecology* 19:4179–4191.
- Lewis, P. O., and D. Zaykin. 2001. Computer program for the analysis of allelic data, version 1.0. Available: [www.phylogeny.uconn.edu/software/#](http://www.phylogeny.uconn.edu/software/#). (September 2019).
- Manel, S., and R. Holderegger. 2013. Ten years of landscape genetics. *Trends in Ecology and Evolution* 28:614–621.
- Meirmans, P. G., and P. W. Hedrick. 2011. Assessing population structure:  $F_{ST}$  and related measures. *Molecular Ecology Resources* 11:5–18.
- Meyer, K. A., J. D. Cassinelli, and F. S. Elle. 2008. Life history characteristics of the Wood River Sculpin, *Cottus leiopomus* (Cottidae), in Idaho. *Copeia* 2008:648–655.
- Naley, M. 2014. Fish passage barrier removal on Fall River (aka Fall River Dam or International Paper No. 2 Dam) on the Gill and Greenfield town line, Massachusetts. U.S. Fish and Wildlife Service Notice of Intent 168-0299. Available: <https://eeaonline.eea.state.ma.us/portal#!/search/wire>. (September 2019).
- Narum, S. R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. *Conservation Genetics* 7:783–787.

- Natsumeda, T. 2007. Movement patterns of Japanese Fluvial Sculpin *Cottus pollux* in a headwater stream. *Transactions of the American Fisheries Society* 136:1769–1777.
- Neville, H. M., and D. P. Peterson. 2014. Genetic monitoring of trout movement after culvert remediation: family matters. *Canadian Journal of Fisheries and Aquatic Sciences* 71:1680–1694.
- Nislow, K. H., M. Hudy, B. H. Letcher, and E. P. Smith. 2011. Variation in local abundance and species richness of stream fishes in relation to dispersal barriers: implications for management and conservation. *Freshwater Biology* 56:2135–2144.
- Norman, J. R., M. M. Hagler, M. C. Freeman, and B. J. Freeman. 2009. Application of a multistate model to estimate culvert effects on movement of small fishes. *Transactions of the American Fisheries Society* 138:826–838.
- O'Connor, J., J. Major, and G. Grant. 2008. Down with the dams: unchaining US rivers. *Geotimes* 53:22–27.
- Owens, R. W., and G. E. Noguchi. 1998. Intra-lake variation in maturity, fecundity, and spawning of Slimy Sculpins (*Cottus cognatus*) in southern Lake Ontario. *Journal of Great Lakes Research* 24:383–391.
- Petty, J. T., and G. D. Grossman. 2004. Restricted movement by Mottled Sculpin (*Pisces: Cottidae*) in a southern Appalachian stream. *Freshwater Biology* 49:631–645.
- Petty, J. T., and G. D. Grossman. 2007. Size-dependent territoriality of Mottled Sculpin in a southern Appalachian stream. *Transactions of the American Fisheries Society* 136:1750–1761.
- Poplar-Jeffers, I. O., J. T. Petty, J. T. Anderson, S. J. Kite, M. P. Strager, and R. H. Fortney. 2009. Culvert replacement and stream habitat restoration: implications from Brook Trout management in an Appalachian watershed, USA. *Restoration Ecology* 17:404–413.
- Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Rannala, B. 2007. BayesAss edition 3.0 user's manual. University of California, Davis.
- Rashleigh, B., and G. Grossman. 2005. An individual-based simulation model for Mottled Sculpin (*Cottus bairdi*) in a southern Appalachian stream. *Ecological Modelling* 187:247–258.
- Raymond, M., and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. *Journal of Heredity* 86: 248–249.
- Rice, W. R. 1989. Analyzing tables of statistical tests. *Evolution* 43:223–225.
- Rousset, F. 2008. Genepop'007: a complete re-implementation of the Genepop software for Windows and Linux. *Molecular Ecology Resources* 8:103–106.
- Smith, S. V., W. H. Renwick, J. D. Bartley, and R. W. Buddemeier. 2002. Distribution and significance of small, artificial water bodies across the United States landscape. *Science of the Total Environment* 299:21–36.
- Taylor, M. K., and S. J. Cooke. 2012. Meta-analyses of the effects of river flow on fish movement and activity. *Environmental Reviews* 20:211–219.
- Tillinger, T. N., and O. R. Stein. 1996. Fish passage through culverts in Montana: a preliminary investigation. Montana Department of Transportation, Research, Development, and Technology Transfer Program, Helena.
- Valière, N. 2002. GIMLET: a computer program for analyzing genetic individual identification data. *Molecular Ecology Resources* 2:377–379.
- Waples, R. S., and E. C. Anderson. 2017. Purging putative siblings from population genetic data sets: a cautionary view. *Molecular Ecology* 26:1211–1224.
- Waples, R. S., and C. Do. 2008. LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Molecular Ecology Resources* 8:753–756.
- Waples, R. S., and O. Gaggiotti. 2006. Invited review: what is a population? An empirical evaluation of some genetic methods for identifying the number of gene pools and their degree of connectivity. *Molecular Ecology* 15:1419–1439.
- Whiteley, A. R., J. A. Coombs, M. Hudy, Z. Robinson, A. R. Colton, K. H. Nislow, and B. H. Letcher. 2013. Fragmentation and patch size shape genetic structure of Brook Trout populations. *Canadian Journal of Fisheries and Aquatic Sciences* 70:678–688.
- Whiteley, A. R., J. A. Coombs, M. Hudy, Z. Robinson, K. H. Nislow, and B. H. Letcher. 2012. Sampling strategies for estimating Brook Trout effective population size. *Conservation Genetics* 13:625–637.
- Whiteley, A. R., J. A. Coombs, B. H. Letcher, and K. H. Nislow. 2014. Simulation and empirical analysis of novel sibship-based genetic determination of fish passage. *Canadian Journal of Fisheries and Aquatic Sciences* 71:1667–1679.
- Whiteley, A. R., J. A. Coombs, M. J. O'Donnell, K. H. Nislow, and B. H. Letcher. 2017. Keeping things local: subpopulation  $N_b$  and  $N_e$  in a stream network with partial barriers to fish migration. *Evolutionary Applications* 10:348–365.

## SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.