


RESEARCH ARTICLE

Using genetic data to advance stream fish reintroduction science: a case study in brook trout

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Widespread extirpation of native fish populations has led to a rise in species reintroduction efforts worldwide. Most efforts have relied on demographic data alone to guide project design and evaluate success. However, the genetic characteristics of many imperiled fish populations including low diversity, local adaptation, and hatchery introgression emphasize the importance of genetic data in the design and monitoring of reintroduction efforts. Focusing on a case study of brook trout (*Salvelinus fontinalis*) in North Carolina, we show how the combined use of genetic and demographic data can support reintroduction efforts by improving source population selection and providing opportunities to evaluate genetic viability and adaptive potential in restored populations. Using this combined approach, we reintroduced brook trout into a restored stream from two source populations and monitored changes in genetic diversity and population size in source and recipient populations. Three years after the initial translocation, the reintroduced population had comparable density, but higher genetic diversity, than either source population. This study demonstrates the utility of genetic and demographic data for reintroduction efforts, particularly when extant populations are genetically depauperate and maintaining adaptive potential is a primary restoration goal. However, we emphasize the value of continued monitoring at longer temporal and spatial scales to determine the effects of stochastic process on the long-term adaptive capacity and persistence of reintroduced populations. Overall, inclusion of genetic data in reintroduction efforts offers increased ability to meet project goals while simultaneously conserving critical sources of adaptive variation that exist across the landscape.

Key words: imperiled, microsatellite, monitoring, restoration, translocation

Implications for practice

- Integration of genetic and demographic data provides a more robust dataset for use in the design and monitoring of fisheries reintroduction efforts.
- Genetic data can assist in source population selection by providing information about candidate population genetic diversity and introgression with hatchery lineages.
- Post-translocation genetic data provide more insights into long-term population viability by providing estimates of genetic diversity and individual reproductive success.
- Using genetic data to plan future reintroduction efforts may be a valuable tool for preserving adaptive genetic variation and increasing species persistence across the landscape.

Introduction

Although freshwater ecosystems occupy less than 1% of the Earth's surface, they are a critical nexus between humans and the environment (Strayer & Dudgeon 2010). Centuries of increasing anthropogenic demands on freshwater resources have caused these key biodiversity hotspots to experience rapid declines in species diversity and ecosystem functioning, with some estimating that up to 20,000 freshwater species are extinct

or imperiled due to human activity (Strayer & Dudgeon 2010; Brauer & Beheregaray 2020; Tickner et al. 2020).

With continued threats to freshwater ecosystem health, river restoration is now an essential component of natural resource management and conservation (Wohl et al. 2005). Many restorations seek to recover endemic diversity by returning physical, chemical, and hydrological habitat to more historical states (Wohl et al. 2015). Implicitly, it is often assumed that habitat restoration will lead to recolonization and population growth of endemic fish species (Palmer et al. 1997). However, given the linear, and often limited, dispersal patterns of most freshwater-obligate taxa (Rodríguez 2002), it is often not possible for individuals to recolonize restored habitats through immigration (Larson & Moore 1985; Stranko et al. 2012; Shelley

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et al. 2021). Even when habitats are connected, immigrants from nearby populations may lack the genetic and/or phenotypic diversity needed for securing long-term population viability and evolutionary potential. Given these limitations, human-assisted translocation is often needed to reestablish native fish communities (Lake et al. 2007; Kanno et al. 2016a).

The science guiding translocations has been largely developed through studies in plant and wildlife populations (Stadtman & Seddon 2018). These efforts have been instrumental for developing theoretical frameworks that emphasize the importance of demographic and genetic processes when designing translocation projects (Weeks et al. 2011; Batson et al. 2015). However, it can be challenging to apply many established guidelines to fish reintroduction efforts (Table 1). Similar to many imperiled wildlife and plant species, stream fish populations often exhibit patterns of low genetic diversity and strong local adaptation owing to a long history of isolation and downstream bias in dispersal (Lamphere & Blum 2012). This can make it difficult to identify donor populations that have the potential to produce a self-sustaining population with sufficient adaptive capacity for contemporary and future environmental conditions (George et al. 2009; Lutz et al. 2021).

Donor site selection is further complicated in fishes because many species of conservation concern occur in such low abundance that removal of relatively few individuals can jeopardize the demographic and genetic outlook of existing populations (George et al. 2009; Furlan et al. 2020). Additionally, for some species there is the risk of introgression with hatchery lineages (Araki et al. 2007), and translocation of these individuals risks the unintended and undesired spread of domesticated, non-endemic genes throughout the landscape (Anderson et al. 2014; Kazyak et al. 2021).

The increased prevalence of translocation efforts provides an opportunity for future studies to advance the science that drives fish reintroduction ecology. Although demographic data will undoubtedly be a critical component to future reintroductions (Chauvenet et al. 2013), abundance data can overlook the deleterious effects of inbreeding/outbreeding depression, reproductive bias, and genetic drift that ultimately threaten long-term population persistence. Accordingly, a growing body of literature has started to highlight the benefits of including genetic data throughout all phases of a reintroduction effort (Table 1; Cochran-Biederman et al. 2015; Attard et al. 2016). In particular, compared to demographic data alone, genetic data can be used to better evaluate the condition of candidate source populations, help quantify long-term evolutionary potential, and explain changes in demography that may be associated with inbreeding/outbreeding depression, genetic drift, or reproductive bias. Ultimately, understanding these processes can improve the success of translocations and be used to predict the best strategies in future reintroduction efforts.

The purpose of this article is to highlight how a genetic and demographic approach can be used in the design and monitoring of a stream fish reintroduction. We focus on brook trout (*Salvelinus fontinalis*), a species that has experienced widespread population declines and extirpations throughout its native range on the east coast of the United States (Hudy et al. 2008). Brook

trout are threatened by many factors including climate change, habitat loss, competition with nonnative species, and historical overharvest. As a species with high cultural and ecological value, many agencies and organizations are actively engaged in brook trout restoration projects, and considerable resources have been invested in species recovery (EBTJV 2019).

Re-establishment of self-sustaining brook trout populations can be challenging due to the species' limited dispersal ability and the presence of natural and anthropogenic movement barriers in many streams. Because many brook trout populations are isolated in small headwater streams, they are often characterized by low genetic diversity and strong local adaptation, particularly within the southern extent of the species' range (Kazyak et al. 2022). Therefore, it is highly improbable that natural recolonization will lead to the establishment of a demographically and genetically robust brook trout population. This makes translocation an appealing tool for brook trout restoration, but few have incorporated genetic data into the design and monitoring of translocation efforts (but see Richards et al. 2008; Wood et al. 2018 for two examples).

As we highlight in our brook trout case study, a combined demographic and genetics approach may be particularly informative in cases where large numbers of individuals cannot be translocated. Other fish translocation efforts have benefited from the availability of source stocks that have thousands of spawning adults (Dunham et al. 2011) or were able to move hundreds of individuals to the reintroduced population (Kanno et al. 2016a). While movement of many individuals from large source populations is ideal, it may not be an option when working with many species of conservation concern due to the low abundance of extant populations. In these instances, increased understanding of genetic diversity in source populations can help maximize the transfer of endemic genetic diversity to the reintroduced population while minimizing negative effects to source populations.

Methods

Study Area

We introduced brook trout to Purllear Creek, a second-order stream located in northwest North Carolina (Fig. 1). The stream is divided into upper and lower sections by a >25 m cascade waterfall which prevents upstream migration. There is approximately 650 and 700 m of suitable stream habitat above and below the waterfall, respectively. Habitat is similar in both sections, with a mean wetted width of approximately 3 m and watershed area of 2 km².

Purllear Creek was severely degraded by historical logging and recent cattle grazing that led to excessive sedimentation, high nutrient inputs, and elevated water temperatures not conducive to brook trout persistence (Spangler 2007). Stream restoration was initiated in 2006 and included stream channel and bank stabilization, riparian buffer planting, and instream habitat installation. By the late 2010s Purllear Creek had more pools, increased pool depth, reduced substrate embeddedness, and increased canopy cover making the habitat comparable to that of other streams that support self-sustaining brook trout in surrounding watersheds (Gerow 2012; T.C. Johnson 2021, North Carolina Wildlife Resources

Table 1. Genetic and demographic objectives that are frequently used to guide reintroduction activities, and the challenges to implementing those objectives when working with imperiled fish populations (see Weeks et al. [2021] for more in-depth discussion of reintroduction objectives). Practical solutions for overcoming these challenges and appropriate evaluation strategies are noted, along with the resources that were available for the example case study of brook trout reintroduction in Purlear Creek, North Carolina.

<i>Fundamental Objective</i>	<i>Challenges to Implementation</i>	<i>Operational Approach</i>	<i>Resources Available for Purlear Creek Reintroduction</i>	<i>Evaluation Strategy</i>
Establish a self-sustaining population	Most donor populations are small and vulnerable	Move as many donor fish as practicable, given logistics and other constraints		Electrofishing surveys
Reintroduce a population representing an endemic lineage	Genetic data not available for all streams; hatchery stocking records are often incomplete	Determine spatial scale that captures the most genetic relatedness among populations; use microsatellite data to assess hatchery introgression and pick donor streams without introgression signals	Microsatellite data available for hundreds of wild brook trout populations in North Carolina (Kazyak et al. 2021)	Genetic surveys of subsequent cohorts to document genetic relatedness
Establish a population that is well-adapted to the new environment	Not always clear what metrics are most important for assessing adaption	Use donor populations with adaptations appropriate to novel environment; use multiple source populations to provide broader basis for adaptation to new habitat	Use fish from source populations in the same watershed	Not specifically evaluated—future opportunity for genomics and/or detailed studies of fitness
Provide sufficient genetic diversity for adaptation to future environmental change	Source populations are often genetically depauperate; uncertainty as to what kinds of genetic variability is important for future adaptive potential	Capture as much genetic diversity of donor population(s) possible; consider combining multiple source populations	Microsatellite data available for hundreds of wild brook trout populations in North Carolina (Kazyak et al. 2021)	Genetic surveys of subsequent cohorts
Establish a large effective population size	Effective population size limited by habitat availability and often low in source populations; aquatic habitats are stochastic environments with potential for frequent bottlenecks	Recognize limitations of suitable habitat and improve when possible; increase resources for adaptive genetic monitoring and rescue; prioritize reintroduction into larger, more suitable habitats		Long-term genetic monitoring
Establish a single reproducing population	Some previous work has suggested reintroductions with multiple sources may maintain separate populations in sympatry (Richards et al. 2008)	Avoid source populations with high levels of differentiation or clear different life history traits; attempt reintroduction with an adaptive management framework that recognizes the potential for assortative mating		Long-term genetic monitoring
Minimize inbreeding depression	Source populations are often dominated by small numbers of families; spawning habitat in donor and recipient populations is often limited; reproductive skew is common	Use multiple source populations; translocate fish across multiple years and/or seasons; increase spawning habitat availability prior to translocation		Recognize limitations of suitable habitat
Minimize outbreeding depression	Mixing multiple, highly differentiated source populations is often unavoidable	Adopted an adaptive management framework that recognizes the potential for failure if outbreeding depression does occur; estimate probability of translocation failure to determine if risk is acceptable	Review of topic in Frankham et al. (2011)	Evaluate ancestry of recruits to assess for potential outbreeding depression

Table 1. Continued

<i>Fundamental Objective</i>	<i>Challenges to Implementation</i>	<i>Operational Approach</i>	<i>Resources Available for Purllear Creek Reintroduction</i>	<i>Evaluation Strategy</i>
Avoid disease transmission	Many infectious diseases go undetected in wild populations; translocation stress or holding in captivity may lead to disease outbreak	Fish health screening prior to transfer; potentially quarantine donor individuals	Fish health screening via Southeastern Cooperative Fish Parasite and Disease Laboratory, Auburn University	Continued screening in restoration reach and surrounding waters
Avoid mixing deeper evolutionary lineages	The spatial distribution of evolutionary lineages is often unknown	Use genetic or genomic data to identify major lineages and where they occur	Microsatellite data available for hundreds of wild brook trout populations in North Carolina (Kazyak et al. 2021)	
Avoid negative impacts (demographic or genetic) to donor populations	Many source populations exist in low abundance; we do not know how many fish can be safely removed from source populations without unacceptable levels of risk	Use multiple source stocks; wait to perform translocation in years with high abundance and/or recruitment	Relative abundance (CPUE) estimates; estimates of effective population size and family structure	Genetic and demographic monitoring following translocation

Commission, written communication). Additionally, water temperature monitoring by the NCWRC showed a $>1.5^{\circ}\text{C}$ decrease in median summertime temperatures after restoration.

With no effective connectivity to other populations, natural recolonization of brook trout in Purllear Creek was not possible. Therefore, NCWRC decided to reintroduce brook trout to Purllear Creek, and specifically sought to establish a self-sustaining population that was genetically similar to other wild brook trout populations in the region. However, it was not clear which source population(s) would be best for achieving the reintroduction goal. Despite historical stocking in the region, most brook trout populations in the watershed have minimal hatchery introgression (Kazyak et al. 2018). Still, many populations are characterized by low allelic richness and heterozygosity owing to a history of isolation and genetic drift (Kazyak et al. 2021). Translocating fish from such genetically depauperate sources risked establishment of a population with low genetic viability and adaptive potential (Houde et al. 2015), thereby jeopardizing long-term success of the reintroduced population. Moreover, brook trout populations at many candidate source sites were so small that removal of relatively few individuals risked deleterious demographic and genetic effects to the existing population. Therefore, we used a multistep filtering process to identify source populations that had the greatest potential to transfer endemic genetic diversity to Purllear Creek and were large enough to support removal of individuals without considerable risk of demographic and/or genetic bottlenecks.

Source Population Selection

Our filtering process aimed to identify potential source populations that met the following criteria: (1) limited evidence of hatchery introgression, (2) relatively large and stable population size, (3) at least moderate levels of endemic genetic diversity,

and (4) found in streams with comparable habitat to that in Purllear Creek. Therefore, we first identified extant brook trout populations in North Carolina with existing demographic and genetic data. Demographic data were collected by NCWRC and primarily consisted of indices of relative abundance (e.g. catch per unit effort [CPUE]) collected during single-pass backpack electrofishing surveys over multiple years. The genetic data we used are described in detail by Kazyak et al. (2021), from which we primarily focused on estimates of effective population size and allelic richness generated from microsatellite loci. There were over 450 populations that met our data availability criteria, from which we eliminated all populations that occurred outside of the same drainage as Purllear Creek (as identified by the eight-digit hydrologic unit code). This removed most candidate populations, but previous findings of hierarchical genetic structuring suggested that populations within this spatial extent would have similar genetic lineage to the extirpated population in Purllear Creek (Kazyak et al. 2021), thereby reducing the probability that maladapted alleles would be introduced to the restored population.

We then sought to balance the goals of maximizing the amount of endemic genetic diversity that would be translocated to Purllear Creek with the need to minimize risk to source populations. Accordingly, we removed from consideration all populations that occurred at relatively low abundance (CPUE < 20 mature adults/hour) or had indications of hatchery introgression (Kazyak et al. 2018). A comparison of observed heterozygosity and allelic richness across remaining candidate source populations suggested no single source could support the establishment of a population with high genetic diversity. Therefore, we chose to translocate fish from two sources, which is a strategy that has been effective for increasing genetic diversity in reintroduced populations while preserving local adaptation in other taxa (St. Clair et al. 2020). Final source population selection only

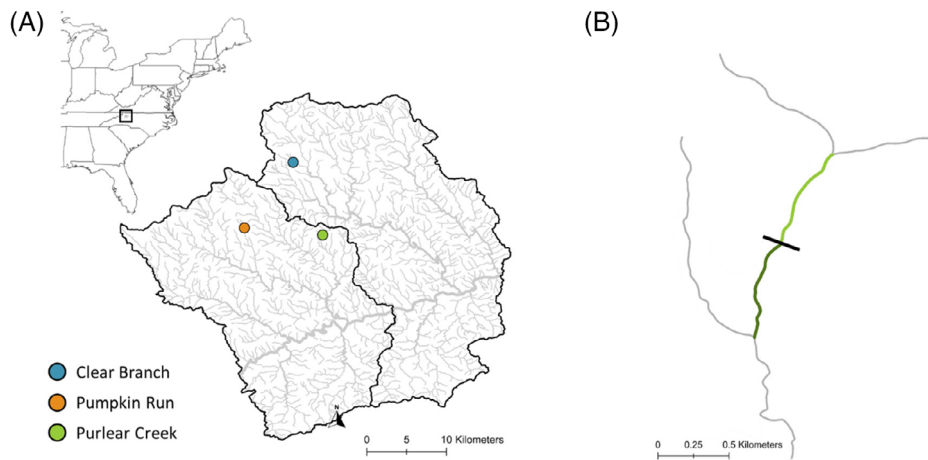


Figure 1. (A) Location of two source populations Clear Branch (blue) and Pumpkin Run (orange) relative to the Purlear Creek restoration site (green). Black outline indicates watershed boundaries (10-digit hydrologic unit code). (B) Fine-scale map of Purlear Creek, which was divided into an approximate 400-m upper (light green) and 400-m lower (dark green) reach by a waterfall (black line). Demographic and genetic monitoring was conducted at two randomly selected 50-m sites within both the upstream and downstream reaches from 2019 to 2021. We also monitored for range expansions in habitats outside of the study reach (gray lines).

considered streams that occurred at the same elevation as Purlear Creek (i.e. ± 250 m), as elevation is strongly correlated to spawning phenology and differences in phenology could reduce post-translocation admixture (Kulp et al. 2017).

Our filtering processes identified Pumpkin Run and Clear Branch as the best source populations for translocation (Fig. 1). Both populations occurred at similar abundance and had minimal indications of hatchery introgression; however, estimates of effective population size and heterozygosity were higher in Clear Branch (Kazyak et al. 2021). The two populations were also highly genetically differentiated ($F'_{ST} = 0.82$), which could be prognostic of reduced admixture following translocation. However, we did not weigh this metric in our selection criteria because most brook trout populations in the region are highly differentiated (Kazyak et al. 2021). Moreover, population differentiation is a common challenge for stream fish reintroductions and post-translocation admixture presents an opportunity for increased genetic diversity with minimal risk to donor populations (George et al. 2009). Prior to translocation we screened both populations for the myxosporean *Myxobolus cerebralis*, as whirling disease (a parasitic infection that can cause mortality of infected individuals) has been recently detected in trout in the southern Appalachians (Ksepka et al. 2020).

Translocation to Purlear Creek

We translocated sexually mature brook trout from Pumpkin Run and Clear Branch in mid-September. This timeframe was expected to precede the onset of spawning (Johnson 2016; Kulp et al. 2017), so we anticipated that most fish would reproduce soon after translocation. To minimize risk to source populations while establishing a total density of approximately 50 fish/km (Kanno et al. 2016a), we translocated 11 and 10 fish from each source population in 2018 and 2019, respectively (Table 2). Others have reported success when stocking more individuals

and multiple age classes of brook trout (Kanno et al. 2016a). However, because translocating large numbers of individuals was not possible, we focused our effort on sexually mature adults to maximize reproduction of translocated individuals. Additionally, brook trout populations often have a weak stock-recruitment relationship (Kanno et al. 2016b), and so translocating relatively few individuals provided an opportunity for a rapid increase in young-of-year abundance while still maintaining minimum risk to source populations. Translocating fish in two consecutive years was intended to maximize transfer of genetic diversity from each source while minimizing the probability that stochastic processes and competition would limit reproduction and admixture. Additionally, sequential transfer spread the risk to source populations over multiple years.

Brook trout were collected from donor streams on the same day using backpack electrofishing. Each fish was anesthetized, measured for total length (TL) and weight, and an adipose fin clip was removed for subsequent genetic analyses (see below). Fish were transported to Purlear Creek in an oxygenated hauling tank. Prior to release, we created random male–female pairs using guidelines by Kazyak et al. (2013) to sex fish from external morphology. To minimize competition for spawning habitat, we randomly stocked pairs of brook trout into calm, deep pools that were evenly spaced throughout 400-m study reaches in both the upper and lower sections of Purlear Creek (Fig. 1).

Demographic Monitoring

We completed post-translocation demographic monitoring in Purlear Creek in the summer from 2019 to 2021. Notably, the average lifespan of brook trout in the region is approximately 3 years (Habera & Moore 2005). Therefore, in 2019, the only brook trout expected to be present were the adults we translocated in fall 2018 and young-of-year that were produced from mating of translocated individuals. By 2020 and 2021, we

expected multiple year classes including some surviving adults that were translocated, year-1 individuals that were reproduced by translocated fish, and young-of-year that have uncertain parentage due to potential backcrossing and mating among year-1 individuals (Fig. 2).

For each year of monitoring, we used a stratified random selection process to identify two 50-m sites in both the upstream and downstream reaches to sample fish. The stratified process ensured that we sampled one site from the upper and lower sections of each reach (but see below for limitations of this study design). At each site, we completed a three-pass depletion survey, with block nets placed at the upstream and downstream extents (except where a natural movement barrier was present). All brook trout were measured for length and weight, and an adipose fin clip was taken from young-of-year for genetic

monitoring efforts. We only collected fin clips from young-of-year as previous sampling efforts would have characterized the genetics of older age classes and because this was the only size class we could confidently assign to a cohort.

We used the R package FSA (Ogle et al. 2018) to estimate the number of fish (with 95% confidence intervals) in each 50-m site using the method of Carle and Strub (1978). The two estimates within the upper and lower reach were added to get a single estimate of the number of fish captured in upper and lower Purlear Creek for each year. We then converted estimated population size to density (fish/100 m²).

To evaluate whether brook trout were colonizing habitat outside of the 800-m study reach (i.e. 400 m below and above the waterfall) we also completed single-pass electrofishing surveys upstream of the highest stocking point, downstream of the

Table 2. Genetic diversity metrics for brook trout from two source populations (Pumpkin Run and Clear Branch) that were used to reintroduce brook trout to Purlear Creek. Adults from the source populations were randomly sampled in 2017 to assess baseline hatchery introgression and genetic diversity. Values for 2018 and 2019 represent diversity metrics for fish that were transplanted to Purlear Creek. We then quantified diversity metrics for young-of-year sampled in upper and lower Purlear Creek in 2019 and 2020. Metrics quantified included rarefied allelic richness (rA_R , rarefied to 40 alleles), observed heterozygosity (H_O), and unbiased expected heterozygosity (uH_E), and the fixation index (F).

Population Type	Sample Year	Location	<i>N</i>	rA_R	H_O	uH_E	<i>F</i>
Source populations	Pre-translocation 2017	Pumpkin Run	22	1.61	0.16	0.19	0.08
		Clear Branch	18	4.46	0.37	0.37	-0.03
	Translocated in 2018	Pumpkin Run	11	1.69	0.23	0.21	-0.13
		Clear Branch	11	2.31	0.34	0.40	0.11
	Translocated in 2019	Pumpkin Run	10	1.62	0.25	0.21	-0.25
		Clear Branch	10	2.23	0.38	0.43	0.07
Reintroduced population	2019	Upper Purlear Creek	47	2.86	0.63	0.52	-0.25
		Lower Purlear Creek	10	1.62	0.22	0.18	-0.18
	2020	Upper Purlear Creek	43	2.33	0.33	0.28	-0.15
		Lower Purlear Creek	30	2.26	0.64	0.42	-0.53

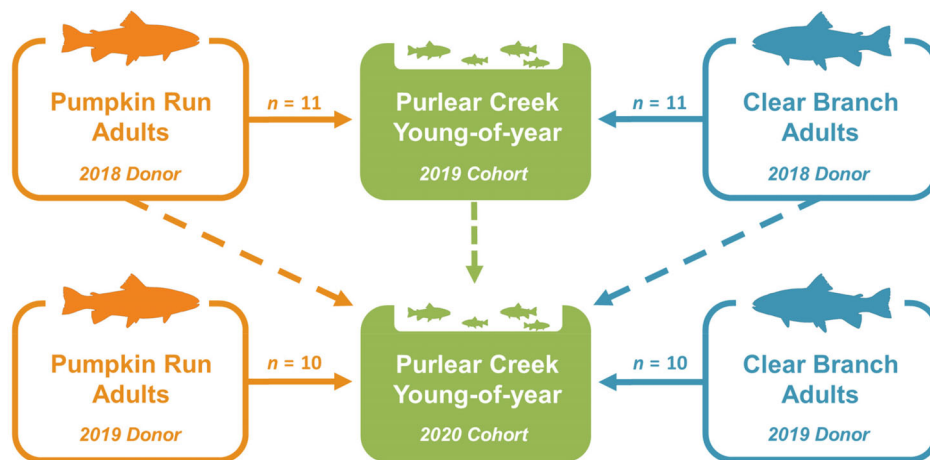


Figure 2. Design of two-step reintroduction effort to translocate brook trout from two source populations, Pumpkin Run and Clear Branch, to one recipient population, Purlear Creek. In 2018, we translocated 11 fish from each source population, and these adults produced the 2019 young-of-year cohort. In 2019, we translocated 10 fish from each donor population and these individuals, along with 2018 donors and individuals from the 2019 cohort, produced the 2020 young-of-year 2020. We did not translocate fish in 2020, and so possible parents for the 2021 cohort include all previous donors and individuals from the 2019 and 2020 cohort that had reached sexual maturity. Solid lines indicate fish that were recently translocated and therefore likely contributed the most towards reproduction of a cohort. Dashed lines indicate fish that were either translocated the previous year or were from the previous year's cohort, and so the number individuals that may have contributed to reproduction is unknown.

lowest stocking point, and in an unnamed tributary to Purlear Creek (Fig. 1). To monitor for potential demographic effects of translocation in source streams, we completed three-pass demographic assessments in a 66-m site in Clear Branch and a 95-m site in Pumpkin Run in summer 2021. Estimates in source streams also serve as a baseline for evaluating demographic success of the reintroduced population in Purlear Creek.

Genetic Monitoring

Fins clips collected from adults translocated from Pumpkin Run and Clear Branch and young-of-year sampled in Purlear Creek in 2019 and 2020 were genotyped using methods described in Kazyak et al. (2018). Briefly, all individuals were screened at 13 microsatellite loci (King et al. 2012) on an ABI 3130XL or 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.) and were scored using GeneMapper software (Applied Biosystems).

To evaluate whether adults from Pumpkin Run and Clear Branch were interbreeding or maintaining separate lineages in sympatry (Richards et al. 2008), we performed a Bayesian clustering analysis in the program STRUCTURE (Pritchard et al. 2000). We used ancestry coefficients (Q -scores) from STRUCTURE to identify young-of-year whose parents both came from the same source population (Q -scores of approximately 0 or 1) or were admixed between populations (Q -scores of approximately 0.5). Importantly, all parents are known for young-of-year sampled in 2019, so Q -scores can be used to definitively determine each parent's source location. However, parent source location is less certain for young-of-year sampled in 2020 due to potential reproduction of year-1 individuals. While backcrossing would be evidenced by Q -scores of approximately 0.75, other mating scenarios that involve at least one year-1 individual produce a Q -score that is commensurate with breeding between translocated adults. Overall, while complex patterns of ancestry confusate interpretation of Q -scores for the 2020 cohort, we find the analysis informative as we expect that year-1 individuals had a proportionally small contribution to total reproductive effort and results are still indicative of the extent of genetic admixture between source stocks.

For all STRUCTURE runs, we assigned Pumpkin Run and Clear Branch as reference populations and allowed for admixture between these sources. Preliminary analyses showed no indication of temporal changes in diversity and differentiation in either source population, so we combined fish translocated in 2018 and 2019 such that each population was represented by a single, pooled collection. Consistent with the number of source populations, we only evaluated $K = 2$ and retained 200,000 repetitions after a burn-in of 200,000 steps for each of 10 replicate runs. Results from STRUCTURE were visualized using STRUCTURESelector (Li & Liu 2018).

We also evaluated offspring ancestry using a principal component analysis (PCoA) performed in the program GenAlEx (Peakall & Smouse 2006, 2012). In addition to relaxing assumptions about Hardy–Weinberg equilibrium and linkage disequilibrium (which could be problematic in small populations with reproductive skew; Waples 2015), PCoA can help visualize

patterns of admixture, particularly when there is suspected backcrossing.

Although STRUCTURE and PCoA identify the extent of genetic admixture between source populations, we also wanted to quantify bias in reproductive success among translocated individuals. Therefore, we performed a pedigree analysis in the program COLONY 2.0.6.2 (Jones & Wang 2010) to identify the most likely mother and father for each young-of-year sampled in 2019. We did not consider young-of-year sampled in 2020 because there was the potential for individuals from the 2019 cohort to contribute to reproduction and so all candidate parents were not known. Collections made in upstream and downstream reaches were analyzed separately, but we included all individuals translocated in 2018 as possible parents for both reaches because we did not monitor individual stocking location, and fish stocked upstream of the waterfall could have moved to the downstream section. Parentage was determined using the full-likelihood method with a long run length, high precision, updated allele frequency, and no sibship scaling. We assumed a model of polygamous mating and no inbreeding, and family size estimates were obtained with maximum likelihood estimation. Sex data for translocated adults were included in the analysis, and the probability of the father and mother being in the collection was set to 1.0.

Due to low genetic diversity in translocated individuals, some parents could not be identified with high probability. Therefore, we only assigned a mother and a father to each individual if the combination had >0.80 likelihood of being the parents. Combinations with lower likelihood were generally the result of only one parent's identity being uncertain. In these instances, we assigned one parent if the two most likely combinations included the same mother or father. Occasionally, one parent could not be identified with high probability, but all candidate parents came from the same source stream. These parents were identified as unknown but from the identified source location.

For each reach and year, we estimated observed (H_O), unbiased expected heterozygosity (uH_E), and the fixation index (F) in the program GenAlEx (Peakall & Smouse 2006, 2012) and rarefied allelic richness (rA_R) standardized to 40 alleles (equivalent to a sample of 20 individuals) in the program HP-Rare (Kalinowski 2005). We also used GenAlEx to estimate pairwise F'_{ST} between streams and collection years. It is important to note that genetic diversity estimates are based on collections from young-of-year individuals collected from 50-m sites. Because spatial aggregation of families has been reported in young-of-year from other streams (Hudy et al. 2010), admixture and genetic diversity may be underestimated, and sampling more individuals from a larger spatial extent could increase detection of more family groups. As such, our estimates of genetic diversity and admixture should be viewed as conservative estimates.

Results

Demographic

Demographic monitoring suggested that translocated fish successfully survived and reproduced, eventually leading to a

Table 3. Demographic estimates for post-translocation monitoring in two source populations (Pumpkin Run and Clear Branch) in 2021 and for upper and lower sections of the reintroduced population, Purlear Creek, from 2019 to 2021. Source populations were surveyed at a single site. Upper and lower reaches of Purlear Creek were each surveyed at two randomly selected 50-m sites, and then site-specific estimates were combined to get one estimate per reach. The 95% confidence intervals (CIs) and standard errors (SE) appear in parentheses.

Population Type	Sample Year	Location	Estimated Number of Fish Within the Sampled Site(s) (95% CI)	Fish/100 m ² (95% CI)	Mean Total Length (SE)
Source population	2021	Pumpkin Run	6 (6–6)	1.9 (1.9–1.9)	183 (6.4)
	2021	Clear Branch	19 (16.9–21.1)	16.9 (15.1–18.8)	86 (7.1)
Reintroduced population	2019	Upper Purlear Creek	55 (52.4–57.6)	17.2 (16.4–18.1)	79.5 (1.6)
		Lower Purlear Creek	10 (8.1–11.9)	3.5 (2.8–4.2)	82.5 (3.8)
	2020	Upper Purlear Creek	53 (51.6–54.4)	15.8 (15.3–16.2)	96.5 (4.9)
		Lower Purlear Creek	34 (33.3–34.7)	12.6 (12.4–12.9)	93.5 (6.0)
	2021	Upper Purlear Creek	19 (18.4–19.6)	5.0 (4.9–5.2)	123.5 (9.1)
		Lower Purlear Creek	6 (5.7–6.3)	3.0 (2.8–3.1)	158 (6.5)

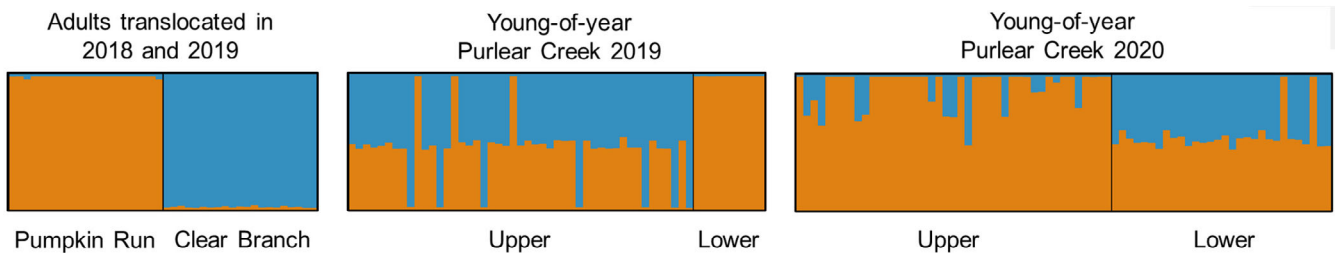


Figure 3. Proportion of individual membership to each of $K = 2$ genetic clusters inferred from STRUCTURE analysis for adult brook trout translocated from Pumpkin Run and Clear Branch and young-of-year sampled in upper and lower Purlear Creek in 2019 and 2020.

mixed-cohort population of brook trout in upper and lower reaches of Purlear Creek. Estimates of the number and density (fish/100 m²) of brook trout in upper and lower Purlear Creek increased from 2019 to 2020 but declined in 2021 (Table 3). Compared to the lower reach, the number and density of brook trout was consistently higher in upper Purlear Creek across all years. Average brook trout size increased steadily throughout the study period with mean TL being greatest in 2021 (Table 3), indicating survival and recruitment of older individuals through time. We also observed brook trout up to 400 m outside of the original area of reintroduction, suggesting rapid range expansion into unoccupied habitat.

Genetic

We genotyped 47 and 10 young-of-year brook trout from upper and lower Purlear Creek, respectively, in 2019, and 43 and 30 young-of-year in 2020. In all cases, we genotyped at least 85% of all young-of-year that were collected, suggesting our genetic analyses were representative of the individuals present at each site.

Based on results from STRUCTURE, 37 individuals (79%) in upper Purlear Creek in 2019 were of admixed ancestry, 7 (15%) had pure Clear Branch ancestry, and 3 (6%) had pure Pumpkin Run ancestry. Conversely, all parents for young-of-year captured in lower Purlear Creek in 2019 appeared to have originated from Pumpkin Run (Fig. 3). In 2020, 28 (93%) of the young-of-year sampled in lower Purlear Creek had admixed ancestry, with only two individuals inferred to have

pure Pumpkin Run ancestry. Patterns of admixture were less definitive in upper Purlear Creek in 2020. While there was no evidence of individuals with pure Clear Branch ancestry, approximately 70% of individuals appeared to have pure Pumpkin Run ancestry. The remaining 30% of individuals had Q -scores that were commensurate with either admixture between translocated adults or backcrossing of admixed year-1 individuals with translocated adults.

Results from the PCoA were congruent with results from STRUCTURE and indicated that the extent of admixture between Pumpkin Run and Clear Branch varied across space and time (Fig. 4). The PCoA also provided more substantive evidence that at least 12 young-of-year captured in upper Purlear Creek in 2020 were produced either by backcrossing of year-1 individuals with an adult translocated from Pumpkin Run or mating between two year-1 individuals.

Pedigree analyses suggested considerable reproductive bias from fish translocated in 2019 (Fig. 5). In the upper reach, we identified six fathers (two from Clear Branch and four from Pumpkin Run) and five mothers (three from Clear Branch and two from Pumpkin Run). There was evidence of polygamous mating in both sexes, and the number of offspring per male ranged from 2 to 21 and per female from 1 to 26. We identified seven full-sibling families, but nearly half (21 of 47) of the young-of-year genotyped assigned to the same mother and father. In the lower reach, we identified two fathers and four mothers, all from Pumpkin Run. Notably, in our sample of 10 young-of-year, we identified 5 full-sibling families; however, 9 young-of-year shared the same father. Interestingly, the one

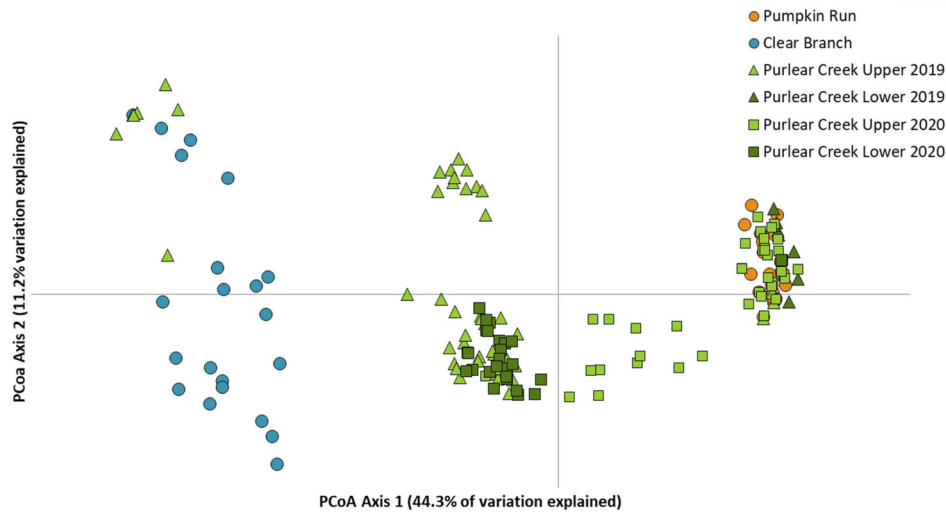


Figure 4. Results of a principal coordinates analysis (PCoA) that included adult brook trout translocated from two source locations, Pumpkin Run (orange) and Clear Branch (blue), into Purlear Creek. Young-of-year brook trout were sampled from Purlear Creek in 2019 (triangles) and 2020 (squares) in upper (light green) and lower (dark green) reaches.

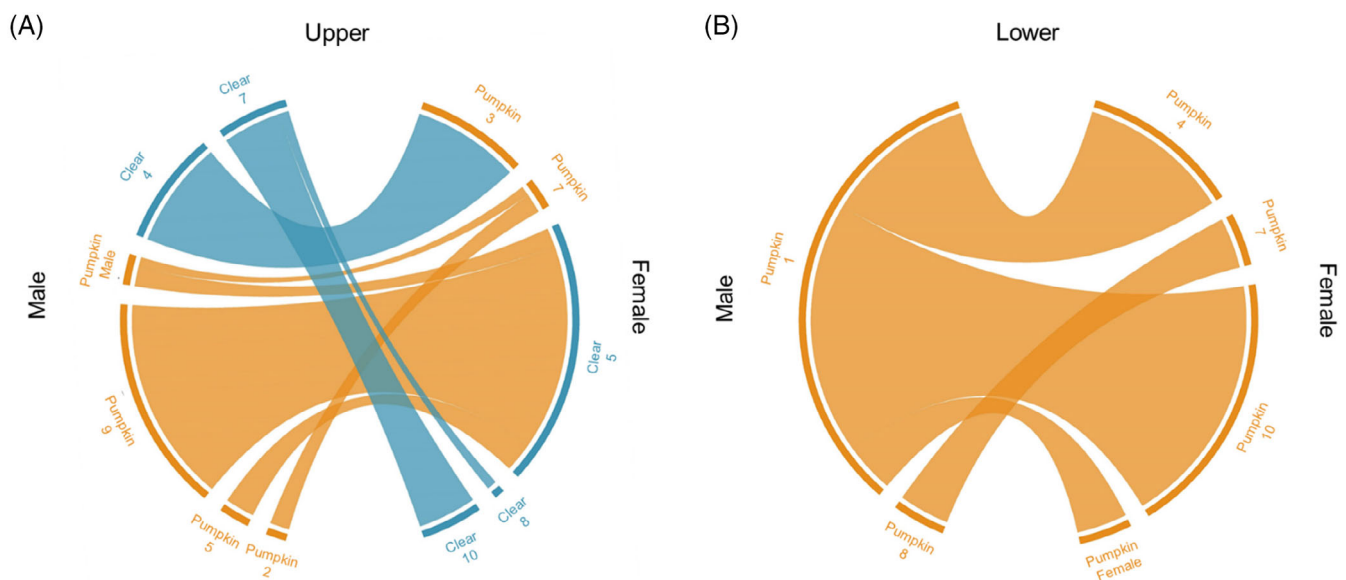


Figure 5. Chord diagrams showing parentage assignments for young-of-year genotyped from upper (A) and lower (B) Purlear Creek in 2019. In each plot, males and females are shown on the left and right, respectively, with the colored line next to each individual's numeric identifier indicating the source location (blue = Clear Branch, orange = Pumpkin Run). Lines connecting mating pairs are color-coordinated by the father's source location, with the width of the line corresponding to the number of offspring per parent pair (1–21 in upper, 1–9 in lower). Mothers and fathers that could not be identified with sufficient probability were classified as unknown.

individual with a unique father also had a unique mother, and the same mother was detected in the pedigree analysis for the upstream reach. This suggests that adults or young-of-year may have descended over the waterfall.

Although our monitoring was limited in spatial and temporal extent, we generally observed elevated genetic diversity in Purlear Creek relative to either source population. However, the magnitude of increase in genetic diversity varied across space and time and generally reflected observed patterns of admixture

(Table 2). That is, when we detected considerable admixture—as was the case in upper Purlear Creek in 2019 and lower Purlear Creek in 2020—most diversity metrics exceeded the values that were observed from fish translocated from Pumpkin Run and Clear Branch. Even when admixture was minimal, diversity was usually higher in Purlear Creek than in Pumpkin Run, and only marginally lower than values observed in Clear Branch. Consistent with the observation of more reproduction by adults from Pumpkin Run, pairwise F'_{ST} was lower between Purlear

Creek and Pumpkin Run than between Purlear Creek and Clear Branch. Additionally, F'_{ST} between upper and lower sections of Purlear Creek varied considerably, with lowest values observed between sections and years that had similar patterns of admixture (e.g. F'_{ST} was 0.10 between samples collected in upper reaches in 2020 and lower reaches in 2019 and both sections had predominant Pumpkin Run ancestry).

Discussion

This study highlights how genetic data can increase the efficacy of fisheries reintroductions by assisting in source population selection and providing more rigorous evaluation of the adaptive potential of restored populations. While demographic data remain an important consideration in planning and monitoring, genetic data provide new opportunities to minimize spread of maladapted alleles throughout the landscape, quantify individual reproductive success, and monitor the transfer and erosion of genetic diversity between source and recipient populations. Genetic data may be particularly informative in conservation of imperiled fishes, where challenges such as low genetic diversity and population sizes, local adaptation, and hatchery introgression can make it difficult to meet long-term reintroduction goals. While we present one case study in brook trout, rapid declines in freshwater fauna are likely to accelerate the need for future application and advancement of reintroduction science (Furlan et al. 2020).

The goal of the Purlear Creek reintroduction was to establish a self-sustaining brook trout population with enough endemic genetic diversity to adapt to current and future conditions. We documented successful reproduction of translocated adults followed by a rapid increase in young-of-year brook trout abundance and recruitment of older individuals into the population, suggesting maturation of age classes through time. However, given habitat limitations and the abundance and biomass of other regional brook trout populations (Kanno et al. 2016b), the establishment of a large population was never realistic, nor was it the intention of the reintroduction. Therefore, the reduction in demographic estimates observed in 2021 was not surprising, as we expected recruitment of older individuals would increase competition for limited resources and reduce juvenile abundance. Moreover, we observed substantial declines in nearby brook trout populations in 2021 (T.C. Johnson 2021, NCWRC, written communication), suggesting spatial synchrony among brook trout populations in the area and highlighting the stochastic variation that is typical of many headwater brook trout populations (Kanno et al. 2016b).

Although this demographic response to translocation is encouraging, data from short-term genetic monitoring indicate a more guarded outlook may be warranted. Young-of-year in our genetic analyses were produced by relatively few individuals from Pumpkin Run, and the extent of admixture with individuals from Clear Branch varied across space and time. This result may have been, at least in part, the product of our study design; however, reproductive bias and variable admixture have been reported in translocation studies in other taxa (Huff et al. 2011; Weise et al. 2020). While the reproduction of

multiple age classes in the second year buffered against some loss of genetic diversity in Purlear Creek, the absence of nearly half of all translocated individuals in our parentage analyses did reduce the amount of total genetic diversity that we observed in our samples of young-of-year from Purlear Creek. Therefore, while young-of-year sampled from Purlear Creek had higher genetic diversity than either Pumpkin Run or Clear Branch, there was less genetic diversity in our samples than would be expected under complete admixture between all individuals translocated from the two source populations.

It is important to note that our results are based on subsamples of individuals that were collected from 100 m in both the upstream and downstream reaches. It is possible that sampling more individuals could reveal more family groups and retention of greater genetic diversity. In particular, we detected multiple age classes approximately 300 m downstream and 450 m upstream of the study area (representing an approximate 94% range expansion from the original stocking reaches) but did not include any of these individuals in our genetic analyses. Sampling over a larger spatial extent may have allowed for detection of more family groups, which could have increased estimates of genetic diversity and admixture while decreasing estimates of reproductive bias. However, even though our analyses may have underestimated the transfer of genetic diversity, this study still demonstrates that it is possible to establish a self-sustaining brook trout population with transfer of relatively few adults from source populations.

Even though early monitoring suggests that brook trout in Purlear Creek may be vulnerable to future extirpation, the reintroduced population is demographically and genetically similar to brook trout populations in the watershed. Southern Appalachian brook trout are largely confined to small, headwater streams where isolation, genetic drift, and low carrying capacity interact to limit population size and genetic diversity (Kazyak et al. 2022). This sets an upper bound on the population density and genetic diversity that can be realistically expected for most brook trout reintroduction efforts. Although this may make each individual reintroduced population vulnerable to near-term extirpation, reintroduction of multiple populations is still likely to reduce extirpation risk of brook trout at larger, regional scales. The creation of redundant pools of genetic diversity across the landscape can allow important sources of adaptive genetic variation to persist despite loss of individual populations, which may be particularly important for species persistence in stochastic headwater streams where local extirpation events are common but natural recolonization is improbable. However, the vulnerability of reintroduced populations also underscores the importance of minimizing risk to existing populations in reintroduction planning.

Others have cautioned against the use of multiple populations in brook trout reintroductions due to the potential for pre- or postzygotic reproductive isolating mechanisms to limit admixture (Richards et al. 2008). Although it is important to consider local adaptation and genetic diversity when selecting source populations, our finding of admixture supports others (Kulp et al. 2017) and suggests that reintroductions with multiple sources can be successful, and admixture can occur between

genetically differentiated populations. Crossing of unrelated populations can produce large fitness benefits in fishes (McClelland & Naish 2007; Fitzpatrick et al. 2016; Robinson et al. 2017), and effects of outbreeding depression may be minimal (Frankham 2015). Benefits can be larger when using small source populations that may be experiencing high rates of inbreeding or drift; however, it is notable that data beyond the F2 generation is generally lacking. As such, hybrid fitness in contemporary and future environmental conditions is often unpredictable (Edmands 2002). This uncertainty, combined with studies highlighting the potential for translocation efforts to fail to meet restoration goals (Huff et al. 2011; Cochran-Biederman et al. 2015), underscores the value of an adaptive management framework for designing, monitoring, and modifying management actions.

An outstanding research need in fisheries reintroduction ecology concerns the development of more rigorous protocols for donor populations selection. Although there is unlikely to be a single most effective strategy (Moseby et al. 2014), there is currently no clear guidance in, for example, the number and age of individuals to translocate, the optimal number of donor populations to translocate from, or the number of translocations that should occur (but see Todd & Lintermans 2015, for one such discussion), particularly when working under the real constraints of at-risk species. Refinement of translocation science could be improved with the use of higher-resolution genomic data to provide more information about adaptive traits and a more comprehensive genome-wide survey of diversity than is possible through microsatellite data alone (He et al. 2016; Seaborn et al. 2021). In particular, the use of genomic data may be beneficial for preserving evolutionary trajectories that may arise due to fine-scale genetic and phenotypic divergences (Taylor et al. 2021).

Our study adds to the growing body of literature that seeks to identify best practices in restoration and translocation efforts (Attard et al. 2016). This field is rapidly evolving and would benefit from more studies conducted at larger temporal and spatial scales and that include both single- and multi-species efforts. We agree with others that the use of genetic and genomic data is poised to increase the efficacy of these future projects (Mijangos et al. 2015), while offering opportunity to define theories concerning the trajectory of inbred populations with low genetic diversity and high divergence. Similar issues have been studied in plants for several decades (McKay et al. 2005), and brook trout represent an ideal model organism to use in the development of translocation ecology for aquatic fauna. Brook trout is a particularly appealing species for such study as there are often a variety of source populations that can be used to test a range of hypotheses about the ideal timing, magnitude, and composition of translocations to meet restoration goals.

Future declines in native species distributions from climate change and habitat loss underscore the value of an adaptive, objective-driven approach to conservation (McCarthy et al. 2012; Buxton et al. 2020). Our study provides a foundation for the parallel use of genetic and demographic data for the planning, execution, and evaluation of conservation and restoration efforts. This approach presents an opportunity to better

understand the mechanisms responsible for reintroduction outcomes and improve understanding of best management practices for imperiled species.

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