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Title: Which species, how many, and from where: Integrating habitat suitability, population genomics, and abundance estimates into species reintroduction planning

Running head: An integrated approach to reintroductions

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Extirpated organisms are reintroduced into their former ranges worldwide to combat species declines and biodiversity losses. The growing field of reintroduction biology provides guiding principles for reestablishing populations, though criticisms remain regarding limited integration of initial planning, modeling frameworks, interdisciplinary collaborations, and multi-species approaches. We used an interdisciplinary, multi-species, quantitative framework to plan reintroductions of three fish species into Abrams Creek, Great Smoky Mountains National Park, USA. We first assessed the appropriateness of habitat at reintroduction sites for banded sculpin (*Cottus carolinae*), greenside darter (*Etheostoma blennioides*), and mottled sculpin (*Cottus bairdii*) using species distribution modeling. Next, we evaluated the relative suitability of nine potential source stock sites using population genomics, abundance estimates, and multiple-criteria decision analysis (MCDA) based on known correlates of reintroduction success. Species distribution modeling identified mottled sculpin as a poor candidate, but banded sculpin and greenside darter as suitable candidates for reintroduction based on species-habitat relationships and habitats available in Abrams Creek. Genotyping by sequencing revealed acceptable levels of genetic diversity at all candidate source stock sites, identified population clusters, and allowed for estimating the number of fish that should be included in translocations. Finally, MCDA highlighted priorities among candidate source stock sites that were most likely to yield successful reintroductions based on differential weightings of habitat assessment, population genomics, and the number of fish available for translocation. Our integrative approach represents a unification of multiple

recent advancements in the field of reintroduction biology and highlights the benefit of shifting away from simply choosing nearby populations for translocation to an information-based science with strong *a priori* planning coupled with several suggested *posteriori* monitoring objectives. Our framework can be applied to optimize reintroduction successes for a multitude of organisms and advances the science of reintroduction biology by simultaneously addressing a variety of past criticisms of the field.

Introduction

Human domination and transformation of global ecosystems has caused widespread species losses and range truncations (Vitousek *et al.*, 1997). This is because anthropogenic alterations to natural landscape structures and functions negatively affected organism abundance, distribution, and diversity within ecosystems ranging from terrestrial (Butchart *et al.*, 2010) to marine (Worm *et al.*, 2006) and freshwater (Dudgeon *et al.*, 2006). These biodiversity losses affect not only the recipient ecosystems and their organisms, but have wide-ranging consequences for human well-being and livelihoods (Cardinale *et al.*, 2012; Dodds *et al.*, 2013). Consequently, worldwide efforts to mitigate or reverse biodiversity losses have been developed (Strayer & Dudgeon 2010; Alkemade *et al.*, 2009) and local-scale programs targeting ecosystem restoration are becoming increasingly common (Aronson & Alexander 2013). Among restoration methods, reintroduction biology is emerging as a field of study that targets improved understanding of how ecosystems might be restored through reestablishment of organisms where they once occurred naturally (Seddon *et al.*, 2007).

Reintroduction biology is the study of reestablishing species in portions of their natural range through intentional movement and release of organisms. Though humans have moved organisms for a variety of reasons over millennia, only within the last century have

movements targeting biodiversity or ecosystem preservation existed, and only within the last 30 years have such movements become increasingly prevalent (Seddon *et al.*, 2007).

Conservation translocation as defined by the International Union for the Conservation of Nature Species Survival Commission (IUCN SSC) involves intentional movement of an organism(s) with the implicit goal of improving conservation status or restoring natural ecosystems. Guiding principles for determining if conservation translocations are necessary and feasible as well as how they might be planned are now developed (IUCN SSC 2013). If a species is absent from the focal restoration site(s) but is still abundant in other portions of its natural range, then population restoration through reintroduction, or the movement of wild-caught individuals from one or multiple locations to another, is the preferred approach in terms of cost and effort (Griffith *et al.*, 1989; Fischer & Lindenmayer 2000; Parker 2008).

When organisms are rare across their entire range, an alternative form of translocation is the propagation of individuals in captivity and eventual release at target sites (Balmford *et al.*, 1996; Rakes *et al.*, 1999; Tenhumberg *et al.*, 2004). A critical step in reintroduction planning is the careful selection of suitable source stock(s) that are large enough to sustain harvest without harmful impacts while establishing a new population(s) (Kleiman 1989; Berger 1990; Montalvo *et al.*, 1997; He *et al.*, 2016). Regardless of the source of founders (captive or wild), source stock should come from populations that are genetically fit to ensure their persistence and adaptive potential (Schwartz *et al.*, 2007; Miller *et al.*, 2009; Weeks *et al.*, 2011). Though reintroduction efforts have traditionally focused on restoring singular species, species in need of conservation, or economically-important species (e.g., Griffith *et al.*, 1989), reintroductions of multiple, non-economically-important species are increasingly viewed as essential to addressing widespread biodiversity declines because of interactions among organisms within ecosystems (Richmond *et al.*, 2005, Gaston 2010; Lindenmayer *et al.*, 2011). In fact, ecosystem-level restoration approaches involving the reintroduction of

multiple species are becoming more common because of recognition that multi-species interactions are critical for maintaining communities post-reintroduction (Soulé *et al.*, 2003; Hooper *et al.*, 2005). In light of these recent recognitions, reintroduction biology is moving in the direction of addressing ecosystem-level goals involving multiple organisms. These ecosystem restorations require integrative and interdisciplinary approaches to ensure proper implementation (Armstrong & Seddon 2008; IUCNSSC 2013).

Advancing the field of reintroduction biology requires addressing current challenges regarding study planning, quantitative assessments of potential and realized success, and integrating interdisciplinary and multispecies approaches. Reintroduction biology as a field historically suffered from a largely retrospective bias due to a lack of initial planning and relatively few studies that elaborated on reintroduction failures or complications despite many thousands of translocations occurring (Fischer & Lindenmayer 2000; Seddon *et al.*, 2007). Although some animal reintroductions are purely opportunistic and arise over timelines that limit *a priori* quantitative planning (e.g., Sarrazin & Barbault 1996; Kleiman *et al.*, 2000; Converse *et al.*, 2013), many reintroductions could be improved by critical planning built from previous attempts (Anderson *et al.*, 2014; Miller *et al.*, 2014). Developing reintroduction biology into a predictive, theory-based science requires first developing general patterns based on multiple observations of failure and success (Griffith *et al.*, 1989; Sutherland *et al.*, 2010; Chauvenet *et al.*, 2013). Unfortunately, many reintroduction failures continue to be poorly documented or unpublished (but see Soorae 2016), leaving little room for improvement or application of “lessons learned”. Addressing this challenge necessitates structured study designs, careful planning, and even inclusion of hypothetical-deductive testing based on observations, statistical models, and potentially experimental trials (Sarrazin & Barbault 1996; IUCNSSC 2013; Ewen *et al.*, 2014; Cochran-Biederman *et al.*, 2015).

Ultimately, transformative approaches to reintroduction biology will arise from interdisciplinary studies that integrate perspectives across multiple fields of study, including perspectives that come from understanding organism natural history, ecology, evolutionary history, and molecular architecture (Armstrong & Seddon 2008). Finally, heretofore the field as a whole has been largely limited to single-species approaches when, in fact, multiple beneficial taxa might be reintroduced simultaneously to promote restoration of a community at the ecosystem level (Ewen & Armstrong 2007, Lipsey *et al.*, 2007). Further research integrating *a priori* planning, sophisticated analytical tools, interdisciplinary perspectives, and multiple species is necessary to advance reintroduction biology (Taylor *et al.* 2017).

We used a combination of abundance, molecular, and ecological data to assess the suitability of three fish species and their potential source stocks for translocation into Lower Abrams Creek in the Great Smoky Mountains National Park, USA. The goal of this study was to develop a multi-tiered, theoretical planning framework to prioritize the reintroduction of three native fishes by first assessing species suitability, and then scoring candidate source stock suitability from a suite of metrics related to past reintroduction successes. We built collaborations among government agencies, ecologists, and molecular biologists to address multiple facets required to quantitatively plan reintroduction of multiple candidate species as highlighted by the IUCNSSC (2013). Our first objective was to determine which species were most suitable for reintroduction into the targeted portion of their former range based on habitat preferences and habitat availability at reintroduction sites. Next, we sought to determine the abundance of species at candidate source stock sites based on counts. Finally, we asked how genetically diverse populations were at each candidate site using genotyping by sequencing and population genomic statistics. We integrated all of these data into a single prioritization scheme to weight multiple priorities for source stock selection to highlight the

best location(s) to collect fishes and how many fishes should be collected from each location. Our work advances the science of reintroduction biology by simultaneously addressing past criticisms regarding *a priori* planning, application of sophisticated quantitative tools, and an interdisciplinary approach with an ecosystem perspective.

Materials and Methods

Study area, candidate species, and site selection

We chose fish reintroduction sites and candidate source stock sites distributed among tributaries to the Tennessee River Basin (Figure 1). The reintroduction site, Lower Abrams Creek, is a 5th order stream originating in the Great Smoky Mountains National Park (GSMNP) and flowing southwest into Chilhowee Reservoir, an impoundment on the Little Tennessee River. Native fishes were intentionally removed from lower sections of Abrams Creek between Abrams Falls and the embayment of Chilhowee Reservoir (19.4 km long reach) during a reclamation project targeting improvement of a non-native rainbow trout (*Oncorhynchus mykiss*) fishery in 1957 (Lennon & Parker 1959; George *et al.*, 2009). Reclamation resulted in extirpation of 53% (31 of 58) of native fish species with limited opportunity to recolonize naturally (Simbeck 1990), but through captive propagation combined with reintroduction, populations of Smoky madtom (*Noturus baileyi*), yellowfin madtom (*Noturus flavipinnis*), and Citico darter (*Etheostoma sitikuense*) are now restored in Lower Abrams Creek (Shute *et al.*, 2005). Recent establishment of Native Fish Conservation Areas (NFCA, Williams *et al.*, 2011), including the Little Tennessee River NFCA, has strengthened interest in reintroducing additional native species into Lower Abrams Creek. The goal of fish reintroductions into Lower Abrams Creek is to reestablish populations of native species with abundances comparable to nearby streams (i.e., a population goal) and to reestablish host fish communities for native mussel species (i.e., an ecosystem goal).

We selected three candidate fish species for reintroduction into Lower Abrams Creek using a three-tiered approach. First, we considered which species were historically present (Simbeck 1990) and still missing to eliminate species that naturally recolonized or were already reintroduced (Shute *et al.*, 2005). Second, we considered which missing fish species were widely distributed in nearby basins to ensure suitable source stocks would be available for translocation (George *et al.*, 2009) using unpublished fish collection data from the Tennessee Wildlife Resources Agency (TWRA) and the National Park Service (NPS). Lastly, we selected fish species that hosted a broad suite of mussel species because the National Park Service is interested in future restoration of imperiled mussel species and these fishes are critical for mussel recovery given glochidia-host associations between mussels and fishes (Barnhart *et al.*, 2008). We used existing literature (Yeager & Saylor 1995; Parmalee & Bogan 1998; Jones & Neves 2002; Jones *et al.*, 2004; Jones *et al.*, 2010) and unpublished data (J. Layzer, Tennessee Technological University; K. Moles, Arkansas Game and Fish Commission) to estimate total numbers of mussel species these fishes host. Three species were selected using these criteria, including banded sculpin (*Cottus carolinae*), greenside darter (*Etheostoma blennioides*), and mottled sculpin (*Cottus bairdii*).

We selected sites for candidate source stocks using a multi-tiered process. We first used a list of nearby sites sampled by the TWRA (unpublished data), NPS (unpublished data), and historical fish collectors (Frimpong *et al.*, 2016) to identify basins inhabited by each target species and in close proximity to Lower Abrams Creek. We used proximity as an initial criterion because nearby fish assemblages are most likely genetically similar to what previously occurred in Lower Abrams Creek (George *et al.*, 2009). A total of three watersheds per candidate species was selected, and within each watershed we selected three sites spread 1-20 km apart across a longitudinal gradient to yield an upstream, middle, and

downstream site (Figure 1). This site selection process resulted in a total of nine sample sites per species to be considered as potential source stock localities. Through this process, we selected sites within Citico Creek (CTCK), Lower Little River (LRVL), and the Middle and West Prongs of the Little Pigeon River (MPLP and WPLP) watersheds to sample for banded sculpin and greenside darter. Mottled sculpin prefer colder, steeper gradient streams (Etnier & Starnes 1993), so separate watersheds were chosen, including Upper Little River (LRVU), Deep Creek (DPCK), and the Oconaluftee River (OCOR).

Abundance estimates

We used snorkel surveys to collect abundance data for each site to verify populations were robust and capable of sustaining harvest for translocation. Snorkel surveying is a standardized fisheries data collection method commonly used to estimate abundances with minimal impact on individuals and their habitats (Bonar *et al.*, 2009) and is typically used to validate modeled estimates of population sizes (Mollenhauer & Brewer 2017). Our protocol included surveying multiple (3-5) reaches within each site using three snorkelers spaced equidistant from each other and moving upstream along a transect in unison (Davis *et al.*, 2011). A minimum total of 50 linear meters was surveyed at each site to improve detection and because this distance allowed for multiple reach replicates to be surveyed (Hagler *et al.*, 2011). Snorkelers recorded counts of only target species observed by carefully lifting rocks within a meter of each side of their longitudinal transect as they moved upstream. Count data from all snorkelers and all reaches within a site were summed, divided by the total area surveyed [i.e., (2 m width observation area) x (3 snorkelers) x (total length (m) of transect)], and multiplied by 100 to yield an estimate of target fish density (fish/100 m²) at a site (Ensign *et al.*, 1995, Gibbs *et al.*, 2014).

Habitat suitability

We used species distribution modeling as a measure of habitat suitability to determine how well habitats used by candidate source stocks matched available habitats in Lower Abrams Creek. We developed boosted regression tree (BRT) models using historical fish data from the entire Tennessee River Basin. This process included using data from IchthyMaps (Frimpong *et al.*, 2016) to denote occurrence of each target species along National Hydrography Dataset (NHD) version 2 plus polylines (EPA 2012). BRT modeling is a machine learning process that relies on an ensemble approach to develop multiple simple trees iteratively to “boost” performance of final models (De’Ath 2007). Because BRT models require presence and absence data, we relied on the protocol described by Huang & Frimpong (2015) to assign absences by assuming that any sampling that detected one target species was equally likely to detect the other two. Thus, when only one target species was reported on a polyline, the other two were considered absent (Huang & Frimpong 2015). We then parameterized BRT models with 13 environmental predictor variables used by previous studies (Elith *et al.*, 2008; Huang & Frimpong 2015) for predicting the occurrence of fish species (Table 1). These variables describe stream ecosystem size, elevation, and precipitation and temperature regimes, all of which are known to influence aquatic organism distributions (Vannote *et al.* 1980), especially fishes (Huang & Frimpong 2015). Species-specific outputs from BRT models were used to predict the probability of occurrence of each species at each candidate source stock site and at all polylines in Lower Abrams Creek (n = 13). We then used the difference in probability of occurrence between candidate source sites and the average probability in Lower Abrams Creek to quantify similarity between habitats as habitat match (H_M) using the equation:

$$H_M = 1 + (SITEprob - ABRAMSprob)$$

Where *ABRAMSprob* is the average predicted probability of occurrence across the 13 segments in Lower Abrams Creek and *SITEprob* is the predicted probability of occurrence at a given candidate source stock site. Using this equation, an H_M value of 1 represents an identical match in habitat, values greater than 1 represent movement from superior habitat, and values less than 1 represent movement from inferior habitat. We developed BRT models using the ‘dismo’ package in the R Statistical Environment (Hijmans *et al.*, 2017) and optimized fits with code from Elith *et al.*, (2008) using tree complexity = 5, bagging fraction = 0.5, and species-specific tree numbers and learning rates. The banded sculpin model included 2,400 trees with learning rate 0.001, the greenside darter model included 1,200 trees with learning rate 0.0005, and the mottled sculpin model included 3,200 trees with learning rate 0.001. We assessed model predictive performance using the area under the curve (AUC) statistic and classified models with AUC = 0.5-0.7 as only slightly greater than random predictions, and models with AUC = 0.7-0.8, 0.8-0.9, and > 0.9 as having “acceptable”, “excellent”, and “outstanding” predictive power, respectively (Hosmer & Lemeshow 2000; Pittman & Brown 2011). BRT models were fit with R version 3.4.0 (R Core Team 2016).

Population genomics

We used genotyping by sequencing (GBS) to identify and characterize single nucleotide polymorphisms (SNPs) across nine candidate source stock sites for all three target species from each site. We elected to use GBS because this approach is economical, allows for joint marker discovery and genotyping, and is an emerging technology in ecological and conservation studies (Narum *et al.*, 2013), particularly in fishes (Li and Wang 2017). Conservation-oriented studies with objectives similar to this study have implemented GBS to assess fish population structuring and genomic diversity (Li *et al.*, 2014; Nunez *et al.*, 2015; Underwood *et al.*, 2016). We sampled all candidate source stock sites using backpack

electrofishing and collected up to 15 individuals of each target species across several hundred meters of stream from multiple habitat types. Length (mm), weight (g), and a clip from the lower caudal fin (Osborne *et al.*, 2014) were taken prior to releasing individuals back to the site. We flame sterilized all fin clipping equipment with alcohol between samplings and fin clips were kept in DNA/RNA Shield (Zymo Research, Tustin, CA, USA) in the field, transported back to the lab, and stored in a -20° C freezer until processing.

We extracted genomic DNA from fin clips using a Qiagen DNeasy Blood and Tissue kit (Qiagen Inc., Hilden, Germany) according to manufacturer's protocol, but eluted in water instead of the supplied buffer because Ethylenediaminetetraacetic acid (EDTA) within the buffer may adversely impact downstream reactions during library preparation and sequencing. Genomic DNA was quantified using Quant-iT™ PicoGreen™ dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA) and all samples were standardized to 5 ng/ul (50 ng total genomic DNA). Extracted DNA was pooled, digested with the restriction enzyme ApeKI, adapters were ligated, and PCR amplified as described by Elshire *et al.*, (2011). Target fragment size was quantified using an Agilent 2100 BioAnalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). The resulting library was sequenced using Illumina NexSeq 500 (Illumina Inc., San Diego, CA, USA) with a 75 bp single-end read chemistry. We identified and filtered SNPs using the TASSEL 3.0 UNEAK pipeline (Glaubitz *et al.*, 2014) and determined the number of reads per individual using the *process_radtags* function in STACKS 2.0 (Catchen *et al.*, 2013). We ligated uniquely identifying barcode adapters to DNA fragments for each individual fish sequenced so that locality and biological metadata could be matched to sequence data, and we analyzed each species separately. We first removed individuals with low SNP coverage using a threshold of 0.01 proportion of sites present for each species (i.e., individuals with <1% of possible reads

were removed; Labate & Robertson 2015). We then filtered SNP occurrence for each species and retained only those genotyped in at least 75% of individuals (we also assessed SNP occurrences using 50% of individuals but only analyzed the more conservative 75% threshold). We then removed SNPs with a minimum allele frequency of < 0.05 in all candidate source stock sites per species (Larson *et al.*, 2014; Li *et al.*, 2014; Nunez *et al.*, 2015).

We used filtered SNPs to estimate the structure of genetic variation between sampling sites and to assess levels of genetic variation within putative populations by calculating multiple population genomic attributes including analysis of molecular variance (AMOVA), observed heterozygosity (H_o), allelic richness (A_r), mean pair-wise fixation index between a site and all other sites (F_{ST}), and genetic effective population size (N_e). These metrics are widely used in reintroduction biology (Weeks *et al.*, 2011), conservation genetics (Schwartz *et al.*, 2007; Allendorf *et al.*, 2010), and fisheries fields (Drauch *et al.*, 2008; Huff *et al.*, 2010, Coleman *et al.*, 2013; Ozer & Ashley 2013; Larson *et al.*, 2014). We first used the R package ‘adegenet’ (Jombart & Ahmed 2011) to produce discriminant analysis of principal components (DAPC) plots (Jombart & Collins 2015) to assess structuring of genetic variation among watersheds and sites. DAPC makes no assumptions about Hardy Weinberg equilibrium or linkage disequilibrium and therefore did not require SNP filtering prior to analysis (Jombart *et al.*, 2010). Second, we produced assignment plots to show membership probabilities to putative population clusters identified using two programs. First, we used the *find.clusters* function from ‘adegenet’ to identify the optimal k (i.e., number of population clusters) for each species using a Bayesian Information Criterion (BIC) process (Jombart & Ahmed 2011). Second, we used STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) with parameter settings described by Porras-Hurtado *et al.* (2013) and STRUCTURE HARVESTER (Earl

2012) to estimate assignment probabilities to putative populations for each individual. All species analyses were conducted independently.

We then used the R package ‘SNPRelate’ (Zheng *et al.*, 2012) to filter SNPs that were not in Hardy Weinberg equilibrium ($P < 0.05$) or were in linkage disequilibrium (threshold = $R^2 > 0.8$, Larson *et al.*, 2014) for remaining population genomic analyses. We used GenAlEx 6.5 (Peakall & Smouse 2012) to convert files for additional population genomic parameter estimates. We conducted hierarchical AMOVAs for each species using Arlequin 3.5 (Excoffier & Lischer 2010) to determine how genomic variance was partitioned among sites and watersheds. We calculated average H_o and F_{ST} for each site using the R package ‘adegenet’ (Jombart & Ahmed 2011) and A_r using the R package “PopGenReport” (Adamack & Gruber 2014).

Estimates of multiple cohort effective population size (N_e) were obtained using the linkage disequilibrium method (Hill 1981), which is based on linkage disequilibrium (LD) of physically unlinked loci that arises in finite populations due to drift. Estimating N_e using SNP data and the linkage disequilibrium method requires removing SNPs under putative selection (Waples & Do 2010). We removed SNPs with outlier F_{ST} values using Arlequin 3.5 (Excoffier & Lischer 2010) to exclude those under putative selection based on a hierarchical island model with 20,000 simulations, 100 simulated demes, 10 simulated groups, minimum expected heterozygosity of 0 and maximum expected heterozygosity of 1 to identify and remove loci with $P < 0.05$ (Larson *et al.*, 2014). Filtered datasets used for N_e were then converted into Genepop files and imported into NeEstimator (Do *et al.*, 2014) to calculate effective population size using the linkage disequilibrium method. We used a random mating

model and retained values from the 95% parametric confidence interval with a minimum allele frequency cutoff of 0.02 (Larson *et al.*, 2014).

Finally, we used published meta-analyses of ratios between N_e and adult census size (N) to approximate the minimum number of fish that should be collected over time for translocation to ensure preservation of genetic diversity comparable to source populations in the reintroduced population(s). We used this approach rather than our own census size estimates because our estimates did not represent total population size for adults and were not appropriately linked to estimated N_e values (*sensu* Palstra & Fraser 2012). Published estimates of N_e/N included in our analyses were 0.11 (Frankham 1995), 0.14 (Palstra & Ruzzante 2008; Waples 2005), and 0.23 (Palstra & Fraser 2012). These adult census size range estimates can be used by fisheries biologists to ensure sufficient numbers of fishes are translocated over time to Lower Abrams Creek. We acknowledge uncertainty exists regarding exact numbers of organisms that must be moved and suggest this meta-analysis approach provides a starting point for making decisions regarding how many individuals should be translocated. An additional benefit of these estimates is their use in providing long-term restoration goals for newly repatriated populations (Hansen *et al.*, 2006; Diefenbach *et al.*, 2015).

Prioritizing source stock sites

Selecting the most appropriate source stock location is a challenge in reintroduction biology. This is particularly true of reintroduction programs such as Lower Abrams Creek in which genetic comparisons with archived tissues from missing populations cannot be used to guide source stock selection (George *et al.*, 2009). We used a published meta-analysis of existing freshwater fish reintroduction literature (Cochran-Beiderman *et al.*, 2015) and multiple-

criteria decision analysis (MCDA; reviewed by Hajkowitz *et al.*, 2000) to include uncertainty in the relative importance of factors determining reintroduction success in our source stock prioritization. Reintroduction success can be measured in four ways, including (1) author-defined measures of success, (2) evidence of survival, (3) spawning, or (4) recruitment (Cochran-Beiderman *et al.*, 2015). Because water quality in Lower Abrams Creek is relatively pristine (Denton *et al.*, 2004) and non-native introductions were consistent across all segments, we focused on habitat, population genomics, and abundance estimates as parameters for source stock prioritization (Griffith *et al.*, 1989; Martinez-Meyer *et al.*, 2006; Chauvenet *et al.*, 2013; Allendorf *et al.*, 2010; Weeks *et al.*, 2011). Cochran-Beiderman *et al.*, (2015) showed that the relative importance of habitat assessment ranged 10-100% among studies in which the original cause of extirpation was already addressed (as is the case for Lower Abrams Creek), population genetics ranged 10-20%, and the number of fish stocked ranged 0-10%. We quantified habitat using the H_M metric developed from BRT model output, population genetics using A_r , H_o , and F_{ST} metrics developed from GBS output, and the number of fish potentially stocked using density data developed from abundance estimates. Higher values of any of these attributes represent higher priorities for inclusion in conservation translocations, thus we developed a composite site suitability score using the equation:

$$S_s = (H_M + A_r + H_o + (1-F_{ST}) + S_D)$$

Where S_s is a site-specific suitability score, H_M is habitat match, A_r is allelic richness, H_o is observed heterozygosity, $1-F_{ST}$ is fixation index rescaled so that larger values represent a site with less isolation, and S_D is the density of fish at a site. We then ranked sites based on their S_s across 40 different combinations of weightings, including H_M multiplied by 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0, A_r , H_o and $1-F_{ST}$ each multiplied by 0.1 or 0.2, and S_D multiplied by 0.0 or 0.1. For each weighting scenario, we ranked S_s values to assess the order

of sites for source stock selection priority (1 = first priority) given the associated weightings as a means of illustrating the importance of weighting selection criteria. Finally, we used the combination of highest weightings for habitat (1.0), genetics (0.2 applied to all metrics), and abundance (0.10) to illustrate the most robust (with regard to past predictors of fish reintroduction success; Cochran-Beiderman *et al.*, 2015) ranking of sites and illustrated the proportional S_s scores for all sites relative to the highest priority.

Results

Abundance estimates

Abundances varied among species, watersheds, and sites. Densities were intermediate for banded sculpin and included 1-6 fish/100m² among sites in Little River, 3-9 fish/100m² in Little Pigeon, and 2-3 fish/100m² in Citico Creek (Table 2). Densities were lowest for greenside darter and included 1-2 fish/100m² in Little River, 2-3 fish/100m² in Little Pigeon, and 1-2 fish/100m² in Citico Creek. Densities were highest for mottled sculpin and included 1-13 fish/100m² in Little River, 6-18 fish/100m² in Deep Creek, and 9-17 fish/100m² in the Oconaluftee River.

Habitat suitability

BRT model performance varied across the three target species. The banded sculpin model had acceptable predictive power (AUC = 0.74) and environmental predictors contributing to >10% of explained variance were minimum elevation (24.3%), precipitation (17.6%), air temperature (15.9%), and maximum elevation (10.7%; Table 1). Banded sculpin probability of occurrence increased where elevations were low, precipitation was less, temperature was warmer, and urban land in the watershed was minimal, and minimum and maximum elevation, precipitation, and temperature variables interacted most (Figure 2). Banded sculpin

probability of occurrence ranged 0.40-0.61 among candidate source stock sites (Table 2), averaged 0.45 in Lower Abrams Creek, H_M values ranged 0.84-1.05, and there was some evidence for longitudinal improvement in habitat from upstream to downstream in Lower Abrams Creek (Figure 3). The greenside darter model had predictive power that was only slightly greater than random (AUC = 0.56) and environmental predictors contributing to >10% of explained variance were minimum elevation (17.6%) and temperature (15.2%). Greenside darter probability of occurrence increased where minimal elevation was intermediate, precipitation was less, temperature was cooler, velocities were intermediate, and discharge was least, and precipitation interacted most with other variables. Greenside darter probability of occurrence ranged 0.39-0.44 among candidate source stock sites, averaged 0.40 in Lower Abrams Creek, H_M values ranged 0.99-1.04, and there was some evidence for longitudinal improvement in habitat from downstream to upstream in Lower Abrams Creek (Figure 3). The mottled sculpin model had excellent predictive power (AUC = 0.88) and environmental predictors contributing to >10% of explained variance were minimum elevation (31.9%), precipitation (21.4%), and maximum elevation (12.5%). Mottled sculpin probability of occurrence increased where elevations were highest, precipitation was most, temperature was highest, and stream slope was steepest, and precipitation interacted most with other variables. Mottled sculpin probability of occurrence ranged 0.40-0.71 among candidate source stock sites, averaged 0.04 in Lower Abrams Creek, H_M values ranged 1.22-1.64, and there was limited evidence for longitudinal improvement in habitat from downstream to upstream in Lower Abrams Creek (Figure 3).

Population genomics

We sequenced fin clips from 135 banded sculpin, 134 greenside darter, and 94 mottled sculpin, but retained 129, 115, and 88, respectively after removing individuals with low SNP coverage (Table 2). Distributions of sequence reads per individual varied across the three species, with a median of 1.6 million for banded sculpin, 1.6 million for greenside darter, and 2.4 million for mottled sculpin (Appendix S1). Alignments produced by TASSEL identified 337,872 putative SNPs across all three candidate species before low coverage SNPs were removed. TASSEL protocols filtered the number of SNPs to 171,519 for banded sculpin, 111,994 for greenside darter, and 130,776 for mottled sculpin. Filtering SNPs based on >75% coverage reduced SNP counts to 230 for banded sculpin (although not analyzed, 6,031 SNPs occurred at 50% coverage), 268 for greenside darter (7,430 at 50% coverage), and 479 for mottled sculpin (12,222 at 50% coverage), and these SNPs were used for DAPC. Average read depth (minimum-maximum) across SNPs was 14 (2-160) for banded sculpin, 8 (2-79) for greenside darter, and 12 (2-210) for mottled sculpin. Removal of SNPs that were not in Hardy Weinberg equilibrium and linkage disequilibrium left 188 SNPs for banded sculpin, 254 for greenside darter, and 400 for mottled sculpin, and these SNPs were used for calculation of A_r , H_o , and F_{ST} statistics. Removal of SNPs with large outlier F_{ST} values and therefore considered under putative selection left 172 SNPs for banded sculpin, 225 for greenside darter, and 348 for mottled sculpin. These filtered SNPs were used for AMOVAs and calculation of N_e estimations.

Both discriminant analyses of principal components and assignment tests revealed population structure across some watersheds, apparent gene flow across some watershed boundaries, and high levels of gene flow among sites within watersheds (Figure 4). For all three candidate species, the first three principal components (PC) and two discriminant

analyses (DA) were retained and explained the most genetic variation. For banded sculpin, PC 1-3 explained 41.9% of variation in genomic structure and optimal k was 4 for both 'adegenet' and STRUCTURE (Figure 4). Banded sculpin population clusters included Citico Creek (CTCK 1-3), the West Prong Pigeon River (WPPR 1-2), and the Lower Little River plus the Middle Prong of the Little Pigeon River (LRVL 1-3, MPLP 3). The fourth cluster consisted of a mixture of low probability of assignment for individuals in Citico Creek (CTCK) and relatively greater assignment of individuals in Little River (LRVL 1). For greenside darter, PC 1-3 explained 12.5% of variation in genomic structure and optimal k was 2 in 'adegenet' and 3 in STRUCTURE. Greenside darter population clusters included Citico Creek (CTCK 1-3) and all sites in the Lower Little River and West and Middle prongs of the Little Pigeon River (LRVL 1-3, WPLP 1-2, MPLP 3). The third cluster identified by STRUCTURE showed low probability of assignment for individuals in the Little River and Little Pigeon River cluster. For mottled sculpin, PC 1-3 explained 34.2% of variation in genomic structure and optimal k was 3 in 'adegenet' and 5 in STRUCTURE. Mottled sculpin population clusters included Little River (LRVU 1-3), Deep Creek (DPCK 1-3), and the Oconaluftee River (OCOR 1-3). The other two clusters identified in STRUCTURE showed low probability of assignment for individuals in Deep Creek.

Population genomic statistics revealed moderate levels of diversity and minimal isolation within candidate source stock sites (Table 2). Putative populations were relatively diverse with little isolation across all sites for banded sculpin (mean $H_o = 0.34$, mean $A_r = 1.55$, and mean $F_{ST} = 0.12$), greenside darter (mean $H_o = 0.38$, mean $A_r = 1.79$, and mean $F_{ST} = 0.04$), and mottled sculpin (mean $H_o = 0.37$, mean $A_r = 1.30$, mean $F_{ST} = 0.08$). Results from AMOVA revealed a relatively large amount of structure partitioned by watershed for

banded sculpin (15.29%), an intermediate amount for mottled sculpin (7.82%), and a small amount for greenside darter (2.27%; Table 3).

Multiple cohort effective population size (N_e) calculated for each site varied across species. Banded sculpin N_e estimates ranged 2-615 among sites and included infinity at four sites, but lower 95% confidence envelope values allowed for estimating the minimum number of fish to target for translocation at eight sites (Table 4). N_e estimates ranged 34-142 among sites for greenside darter and included infinity at three sites, but lower 95% confidence envelope values allowed for estimating the minimum number of fish to target for translocation at eight sites. Mottled sculpin N_e estimates ranged 38-200 among sites and included infinity at 6 sites, but lower 95% confidence envelope values allowed for estimating the minimum number of fish to target for translocation at four sites. Depending on site and N_e/N values, the minimum number of banded sculpin ranged 9-1,200, greenside darter ranged 109-2,945, and mottled sculpin ranged 130-927.

Source stock priorities

Multiple criteria decision analysis highlighted the interactions of habitat, genetic, and abundance criteria in choosing source stock sites. Altering weighting of habitat, abundance, and genetics affected source stock selection for all species. For banded sculpin, 40 weighting scenarios revealed top ranked sites as LRVL3 ($n = 23$), LRVL2 ($n = 16$), and WPLP1 ($n = 1$), and LRVL3 was the top ranked site in the scenario of highest weightings for all criteria (Figure 5). For greenside darter, top ranked sites were WPLP1 ($n = 19$), LRVL1 ($n = 15$), MPLP3 ($n = 5$), and LRVL3 ($n = 1$), and WPLP1 was the top ranked site in the scenario of highest weightings for all criteria. For mottled sculpin, top ranked sites were LRVU2 ($n = 18$), OCOR1 ($n = 11$), LRVU1 ($n = 5$), DPCK3 ($n = 5$), and LRVU3 ($n = 1$), and LRVU2 was

the top-ranked site in the scenario of highest weightings for all criteria. The two top-ranked sites were members of the same population clusters for banded sculpin and greenside darter, but belonged to two different clusters for mottled sculpin.

Discussion

Our interdisciplinary study provides a framework for empirical planning in order to determine the most suitable species and source stocks prior to moving organisms to maximize success of reintroductions. We found that although mottled sculpin was historically reported in Lower Abrams Creek, the habitat is likely insufficient to support the species (it was perhaps misidentified during the 1957 reclamation; MAK, *personal observation*). Identifying habitat limitations prior to reintroductions is necessary to avoid failures, even in pristine ecosystems such as Lower Abrams Creek. For example, the apparent failed reintroduction of spotfin chub (*Erimonax monachus*) in our study area was likely a consequence of Lower Abrams Creek habitats representing only marginal (rather than core) habitat for the species (Shute *et al.*, 2005; Gibbs 2009). Once appropriate species are determined, choosing the appropriate location(s) from which to draw source stock requires consideration from multiple perspectives. We used multiple-criteria decision analysis and found that among multiple measures of fish reintroduction success, clear priorities were obvious. Interestingly, our data-driven approach combining habitat suitability, population genomics, and census size metrics suggested potential source stock sites nearest to Lower Abrams Creek were low priorities. This conclusion is contrary to parsimonious decisions made in the absence of robust data in which nearby sites would otherwise be prioritized (George *et al.*, 2009). Given that mixing of populations is no longer possible in highly fragmented contemporary landscapes, and genetic dilution is therefore unlikely, choosing the most suitable population over the closest population for source stock represents a more sophisticated approach to reintroduction

biology (Palstra & Ruzzante 2008). Collectively, our findings emphasize the benefits of integrating quantitative, interdisciplinary, and ecosystem perspectives into reintroduction biology (Armstrong & Seddon 2008).

Ensemble approaches and integrative planning represent clear mechanisms for advancing the science of reintroduction biology (Seddon *et al.*, 2007). A wide variety of studies have used large population sizes (Raesly 2001; Shute *et al.*, 2005; Hayward *et al.*, 2007), habitat modeling (Harig *et al.*, 2000; Hirzel *et al.*, 2004), and population genetics (Drauch *et al.*, 2008; Vonholdt *et al.*, 2008; de Barba *et al.*, 2010) independently in project planning and as methods to measure overall reintroduction success. Our ensemble approach used multiple, robustly weighted measurements of known contributors to reintroduction success (Cochran-Beiderman *et al.*, 2015) that, when combined, provided a more complete ranking of population suitability for translocation. We found that sites with the highest densities of individuals (banded sculpin, WPLP2 = 9.38 fish/100m²; greenside darter, MPLP3 = 3.13 fish/100m²) did not always have corresponding high levels of genetic diversity for banded sculpin ($A_r = 1.47$, max = 1.71; $H_o = 0.28$, max = 0.41) or greenside darter ($A_r = 1.76$, max = 1.82; $H_o = 0.31$, max = 0.45). This apparent trade-off is counterintuitive and suggests prioritizations based solely on abundance estimates could have jeopardized the long-term viability and adaptive potential of populations reintroduced into Lower Abrams Creek (Minckley 1995; Meffe 1995; Weeks *et al.*, 2011). Sites with the most similar habitat template ($H_M = 1.0$, identical habitat) to Lower Abrams Creek did not have the most individuals present for banded sculpin (CTCK2 $H_M = 0.99$ with 1.93 fish/100m², max = 9.38 fish/100m²) or greenside darter (CTCK2 $H_M = 0.99$ with 0.55 fish/100m², max = 3.13 fish/100m²) despite having source stock that would otherwise be well-suited for translocation based on habitat and proximity to Lower Abrams Creek (Letty *et al.*, 2007; George *et al.*,

2009). These examples of conflicting results based on independent metrics demonstrate that singular criteria approaches to population prioritization may not always accurately reflect population suitability to be used as source stock for reintroductions.

Prioritizing species to reintroduce by considering unique habitat requirements benefits reintroduction biology from both applied and basic research perspectives. Despite using historical species occurrences to select a list of suitable candidates for reintroduction into Lower Abrams Creek (Simbeck 1990), we found that mottled sculpin may not be suitable for translocation. This finding is similar to conclusions associated with reintroduction programs for a variety of organisms other than fishes (Thatcher *et al.*, 2006; Osborne & Seddon 2012). Broad-scale habitat modeling identified mottled sculpin as a poorly-suited species with a mean probability of occurrence less than 0.10 throughout the entirety of Lower Abrams Creek. If mottled sculpin was reintroduced into Lower Abrams Creek, our findings suggest the attempt might fail and, in the process, consume valuable conservation resources. Furthermore, our species distribution modeling identified specific inter-confluence segments where habitats for banded sculpin and greenside darter were most suitable, including banded sculpin in downstream, lower elevation segments and greenside darter in upstream, higher elevation segments. This information can be used to identify ideal sites for releases during reintroduction so that each species might be more likely to establish initially and persist in the long-term (Martinez-Meyer *et al.*, 2006, Leathwick *et al.*, 2009, Wilson *et al.*, 2011). Moreover, these *a priori* predictions of species success might be used as a basis for integrating structured hypothesis testing into reintroduction biology to address a current short-coming of the field (Armstrong & Sedden 2008; Taylor *et al.* 2017). Although habitat and species distribution modeling remains an ever-changing field with complicated approaches (Araujo & Guisan 2006; Elith & Leatherwick 2009), model development prior to

reintroductions and model validation following reintroductions is a robust method for improving model performance. We suggest it is better to investigate questions of species-habitat relationships during planning phases than to make incorrect assumptions leading to irreversible decisions after reintroductions are attempted. These key points further strengthen the need to incorporate habitat modeling analyses into reintroduction planning and practice (Osborne & Seddon 2012).

Molecular ecology tools provide fine-scale resolution to prioritize populations based on diversity metrics and population interactions occurring over broad spatiotemporal scales. We did not observe consistent correlations between abundance estimates and estimates of genetic variation as might be expected from theory and observation (Frankham 1996). This highlights the importance of assessing genetic diversity of a source population before individuals are harvested, rather than assume that abundance is reflective of genetic diversity (Amos & Harwood 1998; Romiguier *et al.*, 2014; He *et al.*, 2016). Although abundance does track genetic effective population size in some short-lived fishes (e.g., Osborne *et al.*, 2010), demographic mechanisms affecting instantaneous population abundance operate on shorter time-scales than do molecular mechanisms, resulting in the decoupling of abundance and genetic effective populations sizes (Palstra & Fraser 2012; Osborne *et al.* 2014). For this reason, we used published values of N_e/N to estimate the number of individuals managers should attempt to translocate from source stocks and to create measureable long term population size goals that could help to track the success of the reintroduced population's establishment over time. We also emphasize that these estimates suffer from known and unknown biases and future work refining the application of N_e/N ratios will provide greater insight into exact numbers (Waples 2005; Palstra & Fraser 2012). For example, our estimate of N_e for banded sculpin at LRVL1 was exceedingly low (i.e., 2), and this finding could be

attributed to population structuring, admixture, or life history attributes resulting in large fluctuations in population size (Etnier & Starnes 1993; Frankham 1995; Kalinowski & Waples 2002). The location of LRVL1 is potentially a mixing zone between banded and mottled sculpin, and any hybridization (e.g., Kinziger and Raesly 2001) might have affected our estimates. Alternatively, banded sculpin is an opportunistic life history strategist with a short life span (Gebhard & Perkin 2017; Gebhard *et al.*, 2017) and tends to colonize in an upstream direction (Wells *et al.*, 2017), and the estimated small N_e at LRVL1 might reflect a truly small population. For mottled sculpin, our N_e estimates match those reported for populations in North Carolina (Fiumera *et al.*, 2002).

The number of individuals that should be stocked during population establishment has continually been a topic of debate, and suggestions vary greatly (Griffith *et al.*, 1989; Tracy *et al.*, 2011). A successfully reintroduced population will maintain the same levels of genetic diversity as the source stock if enough individuals are relocated (Fraser *et al.*, 2007; Schwartz *et al.*, 2007; Andersen *et al.*, 2014), and this principle highlights the importance of considering effective population size. By calculating effective population size utilizing the single sample linkage disequilibrium method, we provided estimates for the minimum number of fish necessary to translocate to ensure sufficient numbers of individuals are moved to reduce negative impacts on current levels of genetic diversity (Richards *et al.*, 2008). However, the numbers of individuals collected is often a result of what can be captured at a given time and is therefore as large of a logistical problem as it is an academic problem. Consequently, individuals are sometimes collected from multiple sites as a means of lessening the impact on local abundances at any one site, though care should be taken to ensure sites are within the same population cluster to maintain unique lineages and genetic diversity (e.g., Huff *et al.*, 2010). Although genetic diversity did partition by watershed for

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banded sculpin and mottled sculpin, we did not observe signals that would suggest greenside darter gene flow was significantly altered by barriers (e.g., dams and reservoirs) that isolated sampling sites assigned to the same putative populations. This indicates either very large populations prior to riverscape fragmentation, slow evolutionary change, or that some individuals are able to migrate at a rate sufficient to reduce effects of genetic drift or isolation (Meldgaard *et al.*, 2003, Houde *et al.*, 2011). While many species of fish are known to experience genetic structuring of populations across isolated river stretches fragmented by large reservoirs (brook trout, Kanno *et al.*, 2011, Roanoke logperch, Roberts *et al.*, 2013), this was not the case for the greenside darter or banded sculpin, particularly in the Little River and Middle Prong Little Pigeon River. Given these patterns, our results provide conservation practitioners with some guidance as to how multiple sites might be combined to increase the number of individuals included in translocations without compromising unique genetic lineages. Similarly, the initial genetic metrics reported here and collected prior to reintroduction will allow conservation practitioners to establish genetic diversity goals that can measure the success of establishing viable populations of candidate species in Lower Abrams Creek (Huff *et al.*, 2010).

Prevailing directions in reintroduction biology as of ten years ago pointed towards improved planning, scaling-up thinking to the ecosystem level, quantitative research to identify and address uncertainty, and integrated perspectives built on interdisciplinary science (Armstrong & Seddon 2007). The approach we used here represents an amalgamation of these directionalities and aligns with the findings in a recent review of the field (Taylor *et al.* 2017). Reintroduction projects have begun to incorporate more robust planning to identify suitable habitats for reintroductions through broad and fine scale modeling (Hebblewhite *et al.*, 2011; D'Elia *et al.*, 2015) as well as working to critically consider benefits and determine

risks prior to releasing animals back into historical habitats (Converse *et al.*, 2013, Anderson *et al.*, 2014; Ewen *et al.*, 2014). Our approach also addresses a long-standing concern regarding post-release monitoring because infrastructure is already in place for the NPS to track reintroduced populations once translocations are conducted (Kulp *et al.*, 2015). There is a need for increased application of molecular ecology tools in the planning of reintroductions (Moyle *et al.*, 2003; DeSalle & Amato 2004; Weeks *et al.*, 2011), and our genetics results were pivotal in our source stock selection analysis. Habitat suitability alone may not directly predict reintroduction successes, but species distribution modeling is still a valuable tool to better understand mechanisms that regulate a species' distribution as well as remove uncertainty from candidate species selection while choosing future release sites (Osborne & Seddon 2012). The three fishes studied here are capable of hosting many mussel species and other reintroduction programs are incorporating similar ecosystem level approaches to restore beneficial species interactions (Gibbs *et al.*, 2008; Ritchie *et al.*, 2012; LaBar *et al.*, 2014) that have greater conservation impacts than solely focusing on restoring a singular, rare, or economically-important species. Synthesizing numerous meta-analyses and thinking beyond a specific reintroduction project, as we have done here, will help advance the science of reintroduction biology beyond existing limitations and ultimately provide greater contributions to biodiversity conservation (Taylor *et al.* 2017).

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Table 1. Thirteen environmental predictor variables used for species distribution models, data source, data description, and percent (%) contribution to variance explained by each model for three candidate fish species for reintroduction into Lower Abrams Creek, Great Smoky Mountains National Park, USA.

Habitat parameter	Source	Description	Banded sculpin	Greenside darter	Mottled sculpin
Minimum elevation	NHDplus V2	Minimum elevation (m; smoothed)	24.3	17.6	31.9
Maximum elevation	NHDplus V2	Maximum elevation (m; smoothed)	10.7	6.2	12.2
Slope	NHDplus V2	Slope of flowline (m/km) based on smoothed elevations	4.2	5.8	4.3
Stream order	NHDplus V2	Modified Strahler stream order	0.2	0.5	0.1
Drainage area	NHDplus V2	Drainage area (sq km) at bottom of flowline	2.7	6.8	2.5
Discharge	NHDplus V2	Flow from gage adjustment (cubic feet per second, cfs)	4.5	8.2	2.0
Velocity	NHDplus V2	Velocity from gage adjustment (feet per second, fps)	3.6	7.4	2.9
Precipitation	NHDplus V1	Mean annual precipitation (mm)	17.6	9.8	21.4
Air temperature	NHDplus V1	Mean annual temperature (°C)	15.9	15.2	9.3
Forest land coverage	NHDplus V1	Percent forested lands (%)	3.2	4.4	3.8
Agricultural land coverage	NHDplus V1	Percent agricultural lands (%)	5.5	3.8	3.8
Urban land coverage	NHDplus V1	Percent urban lands (%)	5.1	6.4	3.5
Water land coverage	NHDplus V1	Percent land covered by water (%)	2.5	7.7	2.2

Table 2. Density (fish/100m²), number of fish retained for molecular analysis (Fish), predicted probability of occurrence based on species distribution modeling (SDM), habitat match (H_M; see text), allelic richness (A_r), observed heterozygosity (H_o), and fixation index (F_{ST}) measured at nine candidate source stock sites for three candidate fish species for reintroduction into Lower Abrams Creek, Great Smoky Mountains National Park, USA. CTCK = Citico Creek, LRVL = Lower Little River, LRVU = Upper Little River, WPLP = West Prong Little Pigeon, MPLP = Middle Prong Little Pigeon, DPCK = Deep Creek, OCOR = Oconaluftee River.

Species	Location	Density	Fish	SDM	H _M	A _r	H _o	F _{ST}
Banded sculpin								
	LRVL1	2.86	14	0.40	1.05	1.60	0.41	0.12
	LRVL2	6.44	14	0.50	0.95	1.58	0.41	0.12
	LRVL3	1.27	15	0.61	0.84	1.60	0.36	0.12
	WPLP1	5.84	15	0.43	1.02	1.71	0.38	0.09
	WPLP2	9.38	15	0.51	0.94	1.47	0.28	0.13
	MPLP3	3.40	14	0.47	0.98	1.46	0.27	0.13
	CTCK1	2.33	13	0.43	1.02	1.46	0.29	0.14
	CTCK2	1.93	15	0.46	0.99	1.53	0.32	0.11
	CTCK3	2.49	14	0.53	0.92	1.56	0.35	0.10
Greenside darter								
	LRVL1	1.14	12	0.42	1.02	1.82	0.45	0.04
	LRVL2	1.29	12	0.42	1.02	1.82	0.45	0.04
	LRVL3	1.53	12	0.40	1.00	1.81	0.44	0.03
	WPLP1	2.39	15	0.44	1.04	1.77	0.37	0.04
	WPLP2	1.88	13	0.43	1.03	1.79	0.37	0.03
	MPLP3	3.13	13	0.43	1.03	1.76	0.31	0.04
	CTCK1	0.78	14	0.41	1.01	1.79	0.37	0.04
	CTCK2	0.55	13	0.39	0.99	1.75	0.32	0.04
	CTCK3	2.21	15	0.39	0.99	1.79	0.36	0.03
Mottled sculpin								
	LRVU1	0.96	9	0.70	1.63	1.30	0.37	0.09
	LRVU2	6.39	9	0.71	1.64	1.29	0.37	0.09
	LRVU3	13.01	9	0.49	1.42	1.31	0.40	0.09
	DPCK1	5.61	15	0.43	1.36	1.33	0.36	0.06
	DPCK2	6.70	14	0.29	1.22	1.33	0.37	0.06
	DPCK3	17.63	8	0.21	1.14	1.35	0.42	0.06
	OCOR1	9.38	10	0.68	1.61	1.28	0.36	0.08
	OCOR2	8.87	6	0.61	1.54	1.28	0.34	0.08
	OCOR3	16.79	8	0.40	1.33	1.26	0.29	0.09

Table 3. Analysis of molecular variance (AMOVA) results assessing genomic variation among watersheds, among sites within watersheds, and within candidate source stock sites for three candidate fish species for reintroduction into Lower Abrams Creek, Great Smoky Mountains National Park, USA. Degrees of freedom (D.F.) and percentage of variation at each level are given for each species.

Species	Source of variation	D.F.	Percentage of variation
Banded sculpin	Among watersheds	2	15.29
	Among sites within watersheds	6	3.74
	Within sites	249	80.97
Greenside darter	Among watersheds	2	2.27
	Among sites within watersheds	6	-0.85
	Within sites	227	98.58
Mottled sculpin	Among watersheds	2	7.82
	Among sites within watersheds	6	0.96
	Within sites	167	91.22

Table 4. Estimates of genetic effective population size (N_e) with 95% confidence intervals (95% CI) and the estimated minimum number of individuals necessary to establish translocated populations without bottleneck effects based on three previously published ratios of genetic effective population size to number of individuals in a population (N).

Species and location	N_e (95% CI)	$N_e/N = 0.23^A$	$N_e/N = 0.14^B$	$N_e/N = 0.11^C$
Banded sculpin				
CTCK1	∞ (132- ∞)	574	943	1200
CTCK2	∞ (∞ - ∞)	-	-	-
CTCK3	34 (23-61)	100	164	209
LRVL1	2 (2-2)	9	14	18
LRVL2	∞ (92- ∞)	400	657	836
LRVL3	615 (55- ∞)	239	393	500
MPLP3	17 (12-26)	52	86	109
WPLP1	49 (29-130)	126	207	264
WPLP2	28 (19-47)	83	136	173
Greenside darter				
CTCK1	∞ (324- ∞)	1409	2314	2945
CTCK2	142 (71-3,768)	309	507	645
CTCK3	54 (39-83)	170	279	355
LRVL1	∞ (∞ - ∞)	-	-	-
LRVL2	34 (25-53)	109	179	227
LRVL3	77 (35- ∞)	152	250	318
MPLP3	80 (49-209)	213	350	445
WPLP1	49 (30-128)	130	214	273
WPLP2	80 (45-277)	196	321	409
Mottled sculpin				
DPCK1	200 (102-2,342)	443	729	927
DPCK2	38 (31-48)	135	221	282
DPCK3	∞ (∞ - ∞)	-	-	-
LRVU1	∞ (∞ - ∞)	-	-	-
LRVU2	149 (56- ∞)	243	400	509
LRVU3	∞ (∞ - ∞)	-	-	-
OCOR1	46 (30-92)	130	214	273
OCOR2	∞ (∞ - ∞)	-	-	-
OCOR3	∞ (∞ - ∞)	-	-	-

^APalstra & Fraser (2012)

^BPalstra & Ruzzante (2008); Waples (2005)

^CFrankham (1995)

Figure Captions

Figure 1. Map showing region of interest, Great Smoky Mountains National Park (GSMNP), 18 candidate source stock sites, and Lower Abrams Creek, TN. The candidate source stock site panel shows four letter site acronyms used for each watershed that are color coded to identify each separate basin and the corresponding site number (1 = upstream, 2 = middle, 3 = downstream) next to each point. CTCK = Citico Creek, LRVL = Lower Little River, LRVU = Upper Little River, WPLP = West Prong Little Pigeon, MPLP = Middle Prong Little Pigeon, DPCK = Deep Creek, OCOR = Oconaluftee River.

Figure 2. Partial dependence plots for boosted regression tree analyses illustrating interactions among the top five predictor variables for three candidate fish species for reintroduction into Lower Abrams Creek, Great Smoky Mountains National Park, USA. Positive fitted function values indicate increased probability of occurrence and arrow thickness indicates interaction strength (range: 0-1) between predictor variables.

Figure 3. Probability of occurrence (range: 0-1) based on species distribution modeling for three candidate reintroduction fish species at 13 inter-confluence stream segments in Lower Abrams Creek, Great Smoky Mountains National Park, USA.

Figure 4. Summary of discriminant analyses of principal components (left) and assignment plots from R Package ‘adegen’ (upper right) and Program STRUCTURE (lower right) for banded sculpin, greenside darter, and mottled sculpin collected from sites throughout the Tennessee River Basin (see Figure 1 for locations). Site abbreviations are as in Figure 1.

Figure 5. Candidate source stock site rankings based on multiple-criteria decision analysis for banded sculpin, greenside darter, and mottled sculpin. Points represent priority ranks (1 = highest rank) for candidate source stock sites across combinations of weightings for abundance (0.0, 0.1), genetic diversity (0.1, 0.2), and habitat (0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0) measurements at each site (see text for details). Final rankings are from the scenario with highest weights assigned to abundance (0.1), genetic diversity (0.2), and habitat (1.0) and are shown as continuous scores relative (proportional) to the highest rank. Colors correspond to sampling sites (see Figure 1) for weighting scenarios and population clusters (see Figure 4) for final ranking scores. Site abbreviations are as in Figure 1.









