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Evaluation of Genetic Structuring within GIS-Derived Brook Trout Management Units

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Abstract

Delineation of management units across broad spatial scales can help to visualize population structuring and identify conservation opportunities. Geographical information system (GIS) approaches can be useful for developing broad-scale management units, especially when paired with field data that can validate the GIS-based delineations. Genetic data can be useful for evaluating whether management units accurately represent population structuring. The Eastern Brook Trout Joint Venture, a regionwide collaborative group, delineated patch-based management units for Brook Trout *Salvelinus fontinalis* by using GIS approaches to inform conservation strategies across the eastern United States. The objectives of this research were to (1) evaluate how well the patches predicted Brook Trout genetic structuring in Connecticut, USA; (2) modify the patches as needed to represent contemporary genetic structuring; and (3) identify catchment- and patch-scale riverscape characteristics that predict genetic diversity. Patches with dams and

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high levels of upstream impervious surfaces (>3%) had increased intrapatch genetic structuring, which we incorporated into our revised patch delineation algorithm. Patch area and catchment area were the best predictors of genetic diversity, suggesting the importance of maintaining connectivity and incorporating patch-scale processes into conservation actions. The modified patch layer could be used as the basis for Brook Trout management units to help predict population structuring in the absence of watershed-scale genetic data, allowing opportunities for Brook Trout conservation to be identified.

Development and prioritization of conservation strategies require accurate delineations of spatially explicit management units. Here, we define management units as populations of individuals whose demographics are driven by local processes (i.e., birth and death rates) rather than immigration (Palsbøll et al. 2007). For stream fishes, such population dynamics often occur at spatial scales beyond the stream reach and are influenced by biological and geophysical processes across the “riverscape” (Schlosser 1991; Fausch et al. 2002). Delineating management units at the riverscape scale can be limited by biological data, but advancements in GIS approaches and availability of regional data have greatly enhanced the development of management units for stream fishes and their habitat (Wang et al. 2011, 2012). However, GIS-based approaches typically require some aspect of validation using biological field data before they can be reliably used to make conservation decisions.

Genetic techniques can be useful for validating GIS-based riverine management units at broad spatial scales. The larger spatial scale also enables genetic approaches to identify landscape-scale environmental factors influencing gene flow and subsequently the structuring of populations (i.e., “landscape genetics”; Manel et al. 2003). Applications of landscape genetic approaches to river systems (i.e., “riverscape genetics”) have become increasingly popular as a method to indirectly measure dispersal of stream fishes (Kanno et al. 2011; Davis et al. 2018). Additionally, genetic metrics can also be used to monitor populations and provide managers with important information about population dynamics that can be used to inform conservation strategies (Schwartz et al. 2007; Scribner et al. 2016).

Contemporary populations of Brook Trout *Salvelinus fontinalis* are commonly fragmented and limited to headwater streams due to a combination of physical barriers, warming main stems, and nonnative species (Hudy et al. 2008). The Eastern Brook Trout Joint Venture (EBTJV) was established as a collaborative effort among agencies, universities, tribes, and nonprofit organizations to promote and coordinate Brook Trout conservation efforts across the native range on the U.S. East Coast (<http://easternbrooktrout.org>). As part of the efforts to achieve regional conservation goals, a GIS-based patch layer representing collections of nearby occupied stream habitats that were likely to experience shared management actions was

developed using historical catch data and the presence of dams (Whiteley et al. 2013, 2014; Coombs and Nislow 2015). Patches were assumed to represent interconnected populations of Brook Trout and thus be useful for making decisions about conservation strategies and prioritizing management. Therefore, in this study we consider the EBTJV patches to be management units.

Genetic data were not used to delineate the EBTJV patches, but if successfully validated with genetic data the patches could become an even more useful management tool. The patches could be used by managers to identify opportunities for reconnecting genetically fragmented populations via barrier removal or to prioritize restoration of Brook Trout habitat. If patches do not align with extant genetic structuring, they may need to be refined to provide more accurate predictions of Brook Trout population structure. To date, genetic evaluations of the patch layer have been limited to Virginia (Whiteley et al. 2013, 2014); more northerly patches in states like Maine or Vermont may present challenges due to increased patch size and more gene flow among populations. Additional evaluation of patches covering a wider range of environmental conditions would strengthen their utility as a conservation tool.

In this study, we had three objectives that centered around using Brook Trout genetic data from Connecticut to evaluate the EBTJV patch layer. Objective 1 was to evaluate the amount of genetic structuring within EBTJV patches. We modeled genetic structuring as a function of physical barriers and environmental factors against a null hypothesis of isolation by distance. We expected to observe moderate levels of genetic structuring following an isolation-by-distance pattern if the patches represented observed genetic structuring. Alternatively, if physical barriers or environmental factors limiting gene flow were present within the patches, we expected to see a stronger relationship between genetic structuring and barriers compared to distance alone. Objective 2 was to modify the patch delineation algorithm as necessary to produce a revised patch layer that accurately represented observed genetic structuring and would represent improved Brook Trout management unit delineations. Objective 3 was to identify stream- and watershed-scale (i.e., patch) riverscape characteristics that influence stream-scale genetic diversity. Identification of riverscape features that influence genetic

diversity can help managers to develop management strategies by identifying opportunities to conserve genetic diversity among Brook Trout patches. We predicted that habitat area would be positively associated with genetic diversity due to increased carrying capacity in larger patches and gene flow among streams within patches. We also expected genetic diversity to be positively associated with “high-quality” habitat, such as areas with low anthropogenic development and cooler temperatures, due to positive effects on Brook Trout population dynamics.

METHODS

Existing geospatial data.—We used a combination of two preexisting geospatial databases in our data collection and analytical approaches. First, we used the EBTJV patches to guide sampling efforts, as these were hypothesized to represent Brook Trout structuring (Figure 1C). Coombs and Nislow (2015) developed the patches by merging National Hydrography Dataset Plus version 2 (hereafter, “NHD”) catchments (1:100,000 scale; https://nhdplus.com/NHDPlus/NHDPlusV2_home.php) based on Brook Trout occupancy data and barrier locations (Figure 1B). The barrier layer was based on the National Anthropogenic Barrier Dataset (Ostroff et al. 2013) and was supplemented by other regional and state databases (Coombs and Nislow 2015). Contiguous NHD catchments with records of Brook Trout occupancy (based on available data from the states) and no barriers were considered a single patch. Based on the scale of the underlying NHD catchments, patches could span main-stem habitat that does not support Brook Trout occupancy throughout the year (Figure 1A). Although such habitat is often considered unsuitable, discrete populations of Brook Trout in headwater streams can be genetically connected due to gene flow at the watershed scale (Aunins et al. 2015; Kelson et al. 2015; Nathan et al. 2019). Mean patch size across the region (Maine to Georgia, latitude range: 34.4–47.6) was 19 km² (range = 0–4,114 km²) and followed a general pattern of increasing size at the northern extent of the range. Patches in Connecticut were slightly below the rangewide average, with an average size of 8 km² (range = 0–110 km²).

For our second geospatial database, we used Spatial Hydro-Ecological Decision System (SHEDS) “catchments” from the NHD High-Resolution Delineation version 2 (<https://ecosheds.org>; Figure 1D) to delineate local stream watersheds. The layer consisted of a total of 453,824 catchments ranging from Maine to Virginia (latitude range: 36.5–47.6), with a mean catchment area of 1.63 km² (range = 0.00–213.01 km²). Regional trends in catchment area were less distinct than the EBTJV patches; however, the northernmost region had a slightly larger mean catchment area (1.94 km²). Brook Trout populations can be structured by confluences (Kanno et al. 2011), so we used the catchment

delineations as a proxy for local populations, which then comprised larger, interconnected populations represented by EBTJV patches (Figure 1). The SHEDS catchments provided a slightly finer resolution (mean area in Connecticut = 1.49 km²; range = 0.00–12.65 km²) compared to the NHD catchments that were used in the original EBTJV patch delineations (mean area in Connecticut = 1.64 km²; range = 0.00–61.90 km²). We therefore hypothesized that we could use the SHEDS catchments to delineate patches at a finer resolution. The SHEDS database also includes covariate data from a variety of sources that are summarized at multiple spatial scales. We provide a more detailed description of the covariates in the *Genetic diversity* Methods section below, and additional information can be found on the SHEDS data page (<http://conte-ecology.github.io/shedsGisData/>).

Field collection.—To evaluate the genetic structuring within patches, we collected 2,220 Brook Trout from 102 SHEDS catchments (i.e., streams; <https://ecosheds.org>) nested within 78 EBTJV patches delineated in the regional patch layer (Figure 2; Coombs and Nislow 2015). Average sampled EBTJV patch size was 17.31 km², and patch size ranged from 1.83 to 110.09 km². Of the 78 patches, 19 (894 individuals) had multiple catchments and allowed for the assessment of within-patch variation (Figure 2; Table 1). We captured a mean of 22 Brook Trout (range = 10–58) at each catchment via backpack electrofishing, distributed across an average of 274 m (range = 83–539 m) to minimize the oversampling of a family group (Hudy et al. 2010). All samples consisted of mixed-age Brook Trout ranging in total length (TL) from 40 to 325 mm (mean = 108 mm). Caudal fin clips were collected from each individual and stored in molecular-grade 95% ethanol until processing. The field methods used in this study were approved by the Institutional Animal Care and Use Committee at the University of Connecticut (Protocol A14-024) and by the state of Connecticut (Scientific Collection Permit SC-14029).

Genetic methods.—We conducted DNA extractions using a 96-well Promega extraction kit or a DNALand Scientific individual extraction kit and normalized samples to a concentration of 20 ng/μL. To genotype individuals, we used a suite of eight microsatellites: *SfoC-24*, *SfoD-75*, *SfoC-88*, *SfoD-100*, *SfoC-113*, *SfoC-115*, *SfoC-129* (King et al. 2012), and *SsaD-237* (King et al. 2005). We followed the amplification protocols described by Nathan et al. (2019). We manually scored genotypes using a Beckman Coulter GenomeLab GeXP Genetic Analysis System and a Beckman Coulter Genetic Analysis System (Beckman Coulter, Indianapolis, Indiana). After manual scoring, we identified errors associated with repeat motifs or topographical errors by using a custom R script (available at <https://github.com/lrnathan/RiverscapeGenetics>). All analyses in this study were conducted in R version 3.4.1 (R Core Team 2017). We used results of previous analyses with these data to identify and remove putative hatchery

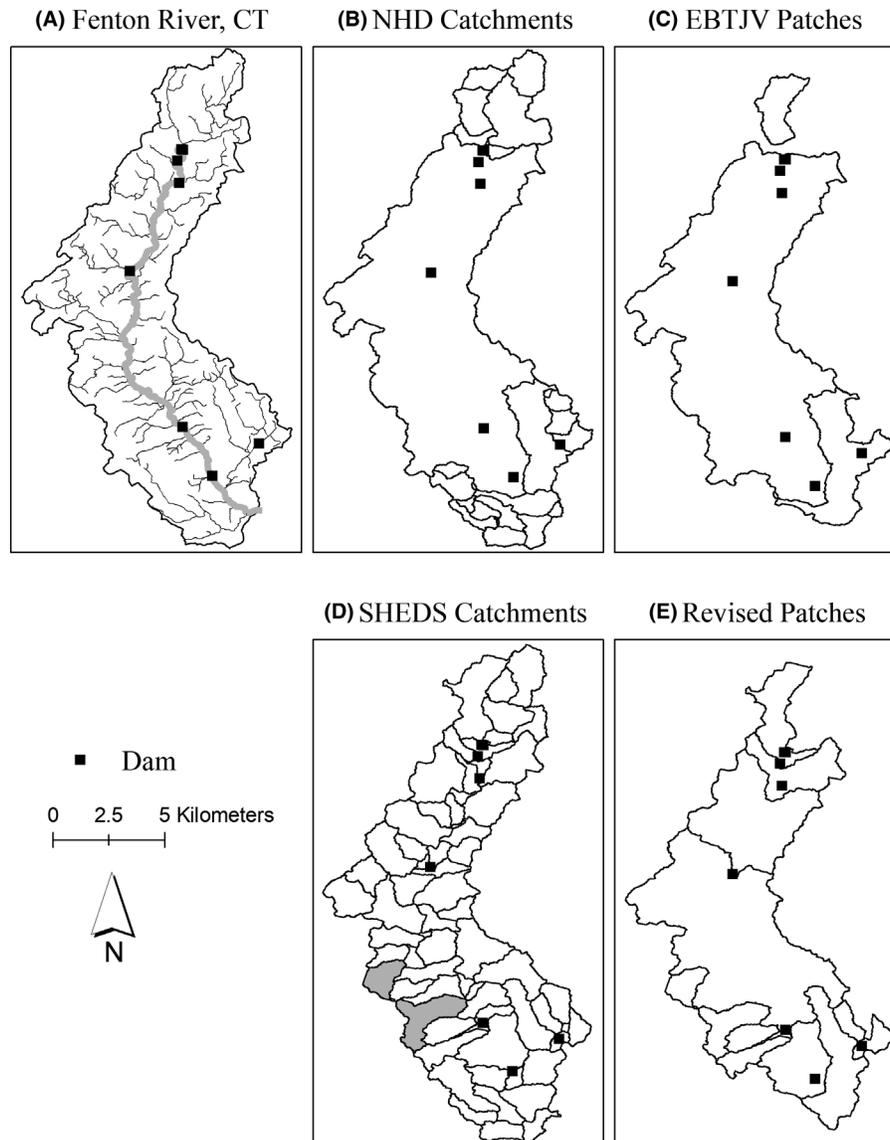


FIGURE 1. Examples of spatial databases used in this study based on a single watershed. (A) The Fenton River watershed was used as a representation of Brook Trout distribution in Connecticut. Main-stem habitat (gray line in panel A) is typically not occupied, although Brook Trout can be found in headwater streams (black lines in panel A). (B) Catchments from the National Hydrography Dataset Plus version 2 (NHD) were merged based on contiguous Brook Trout occupancy and lack of barriers to produce (C) the original Eastern Brook Trout Joint Venture (EBTJV) patch delineations. (D) Catchments from the Spatial Hydro-Ecological Decision System (SHEDS) were used in this study to delineate (E) the revised patches, which provided finer resolution compared to the original patches. Shaded SHEDS catchments (in panel D) are those that were above the 3% upstream impervious surface threshold used in the patch delineation algorithm.

hybrids or admixed individuals (Nathan et al. 2019; D. Greene, L. Nathan, and J. Vokoun, University of Connecticut, unpublished data).

Intrapatch genetic structure.—We estimated four genetic metrics to assess the genetic structuring within the 19 EBTJV patches with multiple catchment samples (Figure 2; Table 1). First, we calculated genic differentiation (GD) among catchments within patches based on genic exact tests using the Genepop package (Rousset 2008) to

test whether the alleles varied significantly among catchments within patches. We then pooled together genotypes across catchments within patches to assess deviations from Hardy–Weinberg equilibrium (HWE) expectations, linkage disequilibrium (LD), and genetic differentiation (Nei's F_{ST} ; Nei 1973) at the patch scale. We identified significant HWE deviations based on 1,000 permutations and calculated significant LD using the chi-square approximation test in the pegas package (Paradis 2010), which can be

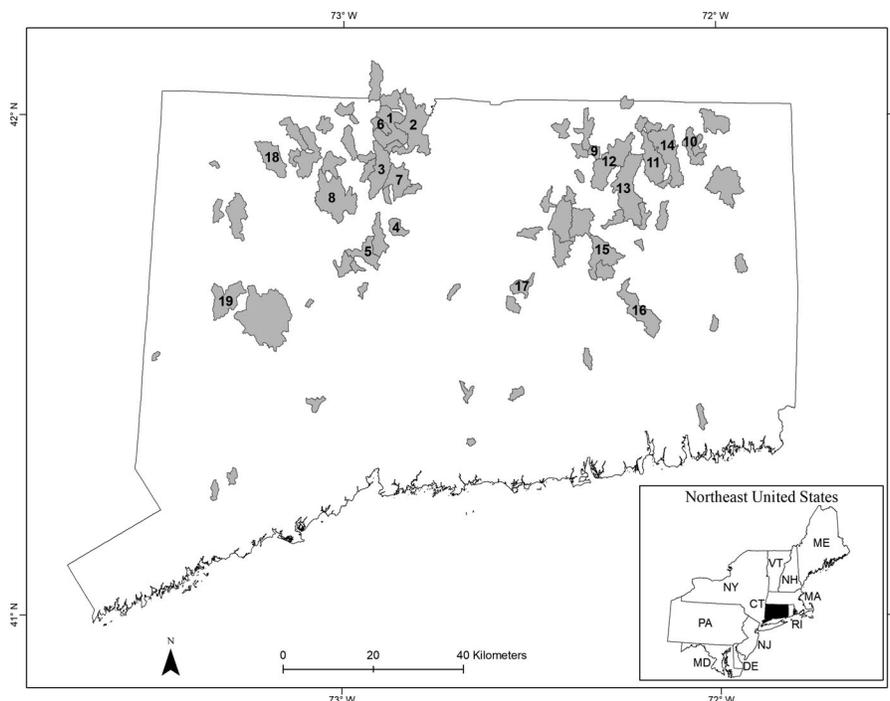


FIGURE 2. Map of original Eastern Brook Trout Joint Venture patches sampled for Brook Trout genetics. Patches with numbers contained two or more Spatial Hydro-Ecological Decision System catchments and were included in the intrapatch genetic analyses. Numbers correspond to the Patch ID column in Table 1.

TABLE 1. Summary data for 19 Eastern Brook Trout Joint Venture genetic patches (with a minimum of two catchments sampled) that were used in intrapatch genetic structure analyses (Patch ID=label associated with Figure 2; tree canopy cover=minimum tree canopy cover [%] in the 15.24-m [50-ft] riparian between catchments, sourced from the Spatial Hydro-Ecological Decision System [SHEDS]; impervious surface=maximum upstream impervious surface [%] between catchments, sourced from SHEDS; N =number of individuals in the sample; N_{age0} =number of age-0 individuals [<100 mm TL] included in the sample; N_{OneSib} =number of individuals in the sample after removal of full siblings).

Patch ID	Patch area (km ²)	Catchments in patch	Dams	Distance between catchments (km)	Tree canopy cover (%)	Impervious surface (%)	N	N_{age0}	N_{OneSib}
1	39.16	3	0	4.62	49.00	1.28	55	41	37
2	61.99	2	0	5.23	44.50	2.53	28	12	27
3	36.02	2	0	7.11	32.64	0.73	52	29	37
4	10.94	2	0	2.31	73.23	10.51	50	41	29
5	21.41	3	0	7.80	56.35	5.06	72	45	50
6	9.23	2	1	3.09	77.71	2.15	60	35	39
7	27.42	2	0	4.84	58.96	3.28	60	51	37
8	62.07	2	0	8.06	28.39	1.30	55	24	48
9	5.81	2	0	1.43	65.21	0.53	26	21	17
10	17.70	2	0	0.23	65.45	0.29	29	25	16
11	35.89	2	0	3.07	76.06	1.43	57	30	39
12	50.24	3	1	10.64	25.33	4.70	75	28	64
13	62.36	2	1	6.60	50.21	0.93	46	30	32
14	47.28	2	0	7.87	63.20	0.84	27	5	27
15	34.30	2	1	7.58	54.19	3.45	52	20	41
16	39.33	3	1	7.60	48.17	0.95	53	45	31
17	13.19	2	0	2.47	68.75	1.33	33	33	19
18	29.61	2	0	6.00	48.37	0.56	32	32	19
19	35.44	2	0	5.89	44.50	1.67	32	32	12

used to identify deviations from expected genotypic proportions and nonrandom allele associations, respectively. Random mating is an underlying assumption of both tests; therefore, significant deviations could be indicative of population structuring within a patch. To control for multiple tests, we used the Benjamini–Yekutieli false discovery rate (FDR) correction (Benjamini and Yekutieli 2001; Narum 2006) for 8 tests/patch (HWE and GD) and 28 tests/patch (LD). We used the *fstat* function in the *hierfstat* package (Goudet and Jombart 2015) to calculate Nei's F_{ST} within patches, which provides a numeric estimate of population differentiation (ranging from 0 to 1) based on heterozygosity. The four tests each have unique limitations and assumptions; therefore, we used a “collective body of evidence” approach to evaluate genetic structuring. Across the four tests, a higher value (number of significant tests or differentiation estimate) was interpreted as a greater degree of structuring within a patch.

Oversampling of family groups can lead to increases in deviations from HWE and LD (Whiteley et al. 2013). Full-sibling young-of-the-year (age-0) Brook Trout are especially likely to be oversampled due to limited dispersal early in their first year (Hudy et al. 2010). Although we spatially distributed samples to reduce the oversampling of families, we anticipated some full siblings within the data set. We used COLONY version 2.0 (Jones and Wang 2010), with input files prepared using CREATE (Coombs et al. 2008), to identify putative full-sibling age-0 fish in the data set (<100 mm TL; 65% of samples). We then randomly selected one individual per family and removed all other siblings to conduct the intrapatch genetic structuring tests (Whiteley et al. 2013).

We used a set of four generalized linear models to explain the variation in intrapatch genetic structuring. In each model, we used one of the four genetic structuring tests (HWE, LD, GD, and F_{ST}) as the response variable and we used the same set of four explanatory variables that we anticipated may influence genetic structuring: distance, dams, percent upstream impervious surface, and percent riparian tree canopy cover (Tables 1 and S1 [information on how to access Table S1 available in the Supplement in the online version of this article]). For each of the genetic tests described above, we anticipated moderate levels of genetic structuring given that patches extended beyond a single catchment. If no barriers were present, we expected distance to be the best predictor of genetic structuring.

We used the EBTJV barrier layer (<https://ecosheds.org>) to identify dams between catchments within patches. Although dams were a factor in the EBTJV patch delineation algorithm (Coombs and Nislow 2015; see *Existing geospatial data* section above), 5 of the 19 patches included in this study had catchments separated by a dam (Table 1). This was due to the underlying NHD catchment layer being based on hydrological data and not on the

presence of barriers. The EBTJV patch algorithm functioned by merging—but not splitting—NHD layers. Therefore, dams could be located within NHD catchments (Figure 1B) and within EBTJV patches (Figure 1C).

We included the percent upstream impervious surface and percent riparian tree canopy because they were previously associated with genetic differentiation in Connecticut Brook Trout populations (Nathan et al. 2019). Anthropogenic development and reduction of natural vegetative cover in the riparian zone can reduce shading and increase water temperatures (reviewed by Allan 2004), and upstream development can lead to degraded habitat quality through multiple mechanisms that are collectively referred to as the “urban stream syndrome” (Walsh et al. 2005). We predicted that increased development at either the riparian or upstream scale would be associated with increased structuring due to reduced gene flow through unsuitable or lower-quality Brook Trout habitat. We used the SHEDS database to extract the maximum upstream impervious surface and minimum 15.24-m (50-ft) riparian tree canopy cover among catchments to represent environmental factors that may reduce gene flow. Finally, we included the total stream distance separating all catchments within a given patch, as estimated using the NHD flowline layer and the OD Cost Distance analysis in the Network Analyst extension within ArcMap version 10.3 (ESRI, Redlands, California), to evaluate potential isolation-by-distance effects.

We used the *glm* function in R with a binomial error distribution and logit link function to fit the HWE, LD, and GD models based on the proportion of significant tests, and a normal linear regression was used for the F_{ST} model. For all four models, we used a backward stepwise regression model selection process to identify the most parsimonious model. We began with the global model for HWE, LD, GD, and F_{ST} and iteratively removed the variable with the highest *P*-value until the final model included only the intercept parameter. Finally, we performed Bayesian information criterion (BIC) model selection on the nested group of models to identify the top supported model.

Patch modification.—Based on the results of the intrapatch genetic structure analyses, we modified the patches to represent the observed genetic structuring. We extracted the SHEDS catchments that made up the EBTJV patches and used them as the basis for the modified patch delineation. Our algorithm iteratively moved from headwaters to downstream, merging catchments together until a dam (based on the EBTJV barrier layer) or a catchment with a total upstream impervious surface greater than 3% was reached. We chose this threshold based on previous occupancy (Stranko et al. 2008) and riverscape genetics (Nathan et al. 2019) studies, which found that watersheds with greater than 3% impervious surface had negative effects on Brook Trout populations. The Python script

used for the patch modification can be found at <https://github.com/lrnathan/PatchAnalyses>. To compare the modified patches to the original EBTJV patches, we ran the same set of models as described above (Table S2).

Genetic diversity.—We used a linear model to explore the influence of riverscape variables on catchment-scale genetic diversity (Table S3). We were unable to use a hierarchical model due to sample size limitations (74 of 86 patches had only one catchment). We therefore chose to randomly select a single catchment for the 12 patches with multiple catchments to prevent pseudoreplication within the data set, yielding a total of 86 estimates of catchment-scale genetic diversity from 86 unique modified patches (see Patch Modification in the Results). For each patch, we calculated allelic richness as our genetic diversity metric using a rarefaction method to correct for sample size in the PopGenReport package (El Mousadik and Petit 1996; Adamack and Gruber 2014).

Within the model, we included seven explanatory variables covering a range of biological and geophysical characteristics at the patch and catchment scales (Table 2). At the patch scale, we estimated patch area (km²) using the geometry calculator and calculated river complexity by dividing the number of river confluences by total river kilometers per patch using the “spatial join” tool in ArcMap version 10.3 (ESRI). An increase in habitat area is often positively associated with genetic diversity (Castric et al. 2001; Neville et al. 2006; Kovach et al. 2015; Buonaccorsi et al. 2017) due to increased availability of spawning habitat and increases in the effective number of breeders (N_b ; Whiteley et al. 2013; Wood et al. 2014). We therefore predicted positive relationships with catchment area and patch area. Confluences can be associated with genetic structuring (Kanno et al. 2011), so we predicted a positive relationship with genetic diversity. We also used the EBTJV database to determine the presence of nonnative salmonids at the patch scale (Coombs and Nislow 2015) due to competitive interactions that could reduce N_b (Whiteley et al. 2014).

We extracted catchment-scale explanatory variables from the SHEDS database, which were sourced from various regional and national sources (<https://ecosheds.org>). Use of the SHEDS covariate data had the benefit of aligning with the spatial resolution of our revised patches; furthermore, by spanning the native Brook Trout range in eastern USA, the data are readily available for use in future studies with the goal of replicating the approaches used here. We selected the covariates based on their potential influence on Brook Trout population dynamics, which may influence genetic diversity. We used average percent impervious surface due to the negative impact of anthropogenic development on Brook Trout occupancy (Stranko 2008; DeWeber and Wagner 2015; Kanno et al. 2015b) and gene flow (Nathan et al. 2019). Surficial coarse

geology was used as a proxy for groundwater influx potential, which is associated with spawning habitat (Witzel and MacCrimmon 1983; Essington et al. 1998) and can buffer stream temperatures and keep them within suitable temperature ranges for Brook Trout despite high levels of watershed development (Stranko et al. 2008). We used annual maximum air temperature (a proxy for stream temperature), August precipitation (a proxy for reduced streamflow during the dry season), and percent waterbodies on the stream network (Table 2) to determine the extent to which temperature and flow may affect genetic diversity due to the influence on Brook Trout distribution and abundance (McKenna and Johnson 2011), growth (Xu et al. 2010b; Letcher et al. 2015; Bassar et al. 2016), age-0 abundance (Kanno et al. 2016), and survival (Xu et al. 2010a; Letcher et al. 2015; Kanno et al. 2015a). For all variables, we used the upstream data set, representing the total watershed upstream of a given catchment. Impervious surface was log transformed to meet normality assumptions.

RESULTS

Intrapatch Genetic Structure

Of the 579 age-0 Brook Trout analyzed from the 19 patches, we identified a total of 306 families with an average size of two individuals (range of family size = 1–10). After we randomly selected one sibling per family and removed all others, the resulting data set consisted of 621 individuals (306 age-0 fish and 315 presumed age-1 or older fish), with an average of 33 individuals/patch (range = 12–64). Previous error assessments of 1,378 repeated allele calls from these data found an allelic scoring error rate of 2.18% (Nathan et al. 2019).

The original EBTJV patches displayed varying levels of genetic structuring. Fifteen of the 19 patches (79%) had at least one significant LD, GD, or HWE test, and three patches had 10 or more total significant tests (Figure 3; Table S1). Global F_{ST} estimates indicated highly variable genetic differentiation, ranging from –0.015 to 0.139 (Figure 3; Table S1). Patches with dams and higher levels of upstream impervious surfaces tended to have the greatest genetic structuring based on the results of the four models (Tables 3, S4). “Dams” was the last variable included in the F_{ST} , LD, and HWE backward stepwise model selection processes and was a significant variable in every top-ranked model. “Impervious” (percent upstream impervious surface) was also included in the top supported LD, GD, and HWE models, although it was only significant in the LD and GD models. “Distance” was only included in the GD model, and it also appeared to have a moderate effect on global F_{ST} despite not being included in the top supported models (Figure 3).

TABLE 2. Summary data for variables used in linear models of Brook Trout genetic diversity in modified patches.

Variable (code)	Description	Source	Predicted relationship	Mean (range)
Waterbodies on stream (Waterbodies)	Percentage of catchment area covered by wetland, lakes, and ponds that intersect the stream network	USFWS ^a	–	4.30 (0.00–17.77)
Mean annual maximum temperature (Temp)	30-year (1981–2010) average annual maximum air temperature (°C) in the catchment; proxy for stream temperature	PRISM ^b	–	14.54 (12.92–16.07)
Catchment area (CatchArea)	Drainage area of the catchment (km ²)	NHDHRDV2 ^c	+	8.01 (1.12–42.87)
Mean August precipitation (AugPrecip)	30-year (1981–2010) average monthly precipitation (mm) in the catchment; proxy for potential low-flow barriers	PRISM ^b	+	105.71 (95.17–118.62)
Impervious surface (Impervious)	Percentage of the catchment classified as impervious surface	NLCD ^d	–	2.37 (0.03–19.84)
Surficial coarseness (SurfCoarse)	Percentage of the catchment that is “sandy” or “gravelly”; proxy for groundwater inputs	NRCS ^e	+	2.94 (0.00–42.95)
Nonnative salmonids (NonNative)	Presence of nonnative salmonids in the patch (binary)	EBTJV ^f	–	NA
Patch complexity (PatchComplexity)	Number of confluences per stream kilometer	NHDHRDV2 ^c	+	0.34 (0.00–0.86)
Patch area (PatchArea)	Patch area (km ²)	NHDHRDV2 ^c	+	15.18 (0.71–60.08)

^aU.S. Fish and Wildlife Service (USFWS) National Wetlands Inventory (sourced from the Spatial Hydro-Ecological Decision System [SHEDS]).

^bPRISM Climate Group (sourced from SHEDS).

^cNational Hydrography Dataset High-Resolution Delineation version 2 (NHDHRDV2; sourced from SHEDS).

^dNational Land Cover Database (NLCD; sourced from SHEDS).

^eU.S. Department of Agriculture, Natural Resources Conservation Service (NRCS), Soil Survey Geographic Database (sourced from SHEDS).

^fEastern Brook Trout Joint Venture (EBTJV) patch layer.

Patch Modification

The modified patches were smaller in size (mean = 2 km²; range = 0–10 km²) compared to the original EBTJV patches (mean = 8 km²; range = 0–110 km²). After patch modifications, 7 of 19 patches no longer had multiple catchments due to reductions in patch size and were subsequently removed from the analysis. Compared to the original patch delineation, there was less genetic structuring observed within the modified patches (Figures 3, 4; Tables S1, S2). The percentage of significant tests decreased from 30% (46/152) to 14% (13/96) for GD, from 7% (38/532) to 3% (11/336) for LD, and from 9% (13/152) to 7% (7/96) for HWE; mean intrapatch F_{ST} was reduced from 0.039 (range = –0.015 to 0.139) to 0.026 (range = –0.012 to 0.092).

In addition to the reduction in overall genetic structuring, the structure in the modified patches had a stronger relationship to Distance (i.e., isolation by distance) compared to Impervious and riparian tree canopy cover. Although there were multiple top competing models across the four different model sets, Distance was the last

variable to be included in all four stepwise regressions (Tables 4, S5). Additionally, Distance was a significant explanatory variable in at least one of the GD, LD, and HWE models with ΔBIC values less than 2.00 (Table 4). There was also a positive relationship observed between Distance and F_{ST} (Figure 4), although Distance was not a significant predictor in the top supported model ($P > 0.05$). The lack of a significant relationship was likely due to the outlier for patch 19 ($F_{ST} = 0.09$; Figure 4), which had the lowest sample size ($N = 12$) after siblings were removed.

Genetic Diversity

The presence of nonnative salmonids was significantly related to patch area; patches with nonnatives had a higher mean average area (21.25 km²) compared to patches with allopatric Brook Trout populations (10.08 km²; Welch's $t_{51.95} = -3.62$, $P < 0.001$). We therefore chose to omit the nonnative salmonid variable from the models. All other explanatory variables were retained in subsequent analyses.

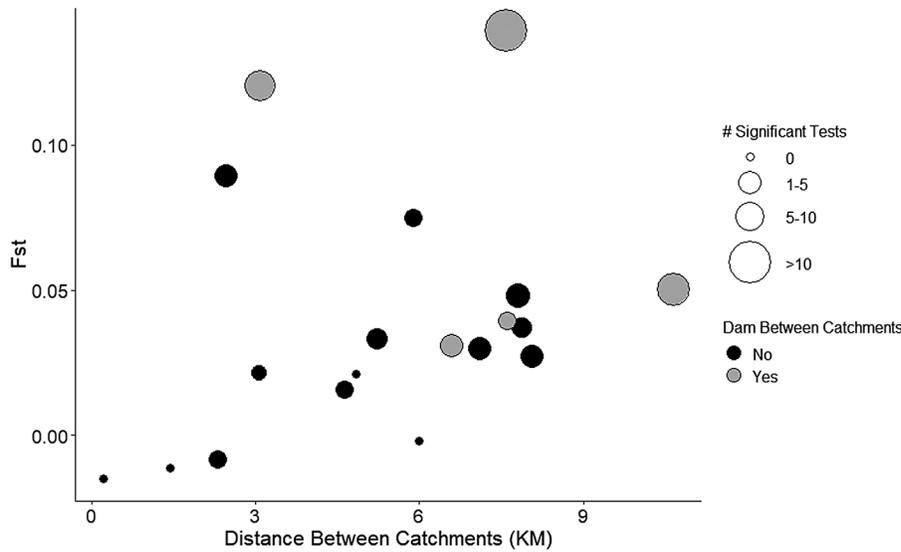


FIGURE 3. Intrapatch genetic structuring (genetic differentiation index F_{ST}) in original Eastern Brook Trout Joint Venture patches, organized by distance between catchments. Circle size represents the total number of significant tests (Hardy–Weinberg equilibrium, linkage disequilibrium, and genetic differentiation) based on a Benjamini–Yekutieli false discovery rate correction.

TABLE 3. Results of backward stepwise model selection for genetic structuring in original Eastern Brook Trout Joint Venture patches (F_{ST} = genetic differentiation index; GD = genic differentiation; HWE = Hardy–Weinberg equilibrium; LD = linkage disequilibrium; BIC = Bayesian information criterion; Δ BIC = BIC difference; BIC_{wt} = BIC weight; R^2_{adj} = adjusted R^2). Only models with Δ BIC less than 2.00 are shown; detailed results are presented in Table S4.

Metric	Variables	BIC	Δ BIC	BIC_{wt}	R^2	R^2_{adj}	Variables with $P < 0.05$
F_{ST}	Dams	-65.51	0.00	0.61	0.29	0.25	Dams
GD	Distance + Dams + Impervious	68.83	0.00	0.75	0.69	0.62	Dams, Impervious, Distance
HWE	Dams + Impervious	41.79	0.00	0.43	0.57	0.52	Dams
HWE	Dams	42.09	0.30	0.37	0.39	0.35	Dams
LD	Dams + Impervious	70.66	0.00	0.69	0.65	0.60	Dams, Impervious

The linear model results identified catchment area and patch area as important variables that were positively related with increased genetic diversity. The model that included only patch area and catchment area was the top-ranked model, with a BIC weight of 0.73, and it was the only model with a Δ BIC value less than 2.00 (Table 5). Both patch area ($\beta = 0.24$, $t = 3.25$, $P < 0.01$) and catchment area ($\beta = 0.24$, $t = 3.08$, $P \leq 0.01$) were significant in the model ($F_{2, 83} = 12.25$, $P < 0.001$; multiple $R^2 = 0.27$; adjusted $R^2 = 0.25$). Overall model fit was relatively low, suggesting a high degree of unexplained variance in catchment genetic diversity.

DISCUSSION

In this study, we evaluated the ability of a GIS-derived patch layer to predict contemporary genetic structuring of Brook Trout populations in Connecticut. We demonstrated

that the EBTJV patch layer can accurately predict most population structuring, therefore representing spatially explicit management units that could be used to inform Brook Trout conservation strategies. Patches with dams and high levels of upstream development had increased genetic structuring, however, indicating that modifications may be needed to represent contemporary structuring more accurately. The modified patches produced by our delineation algorithm were able to capture extant genetic structuring better than the original EBTJV patch layer, and the remaining observed genetic structuring was due to isolation by distance as opposed to barriers. Habitat area at the patch and catchment scales was the most important variable predicting catchment-scale genetic diversity. Catchments that were part of larger patches and those with larger upstream drainage areas typically had greater genetic diversity. These results highlight the importance of maintaining connectivity among headwater streams, even if

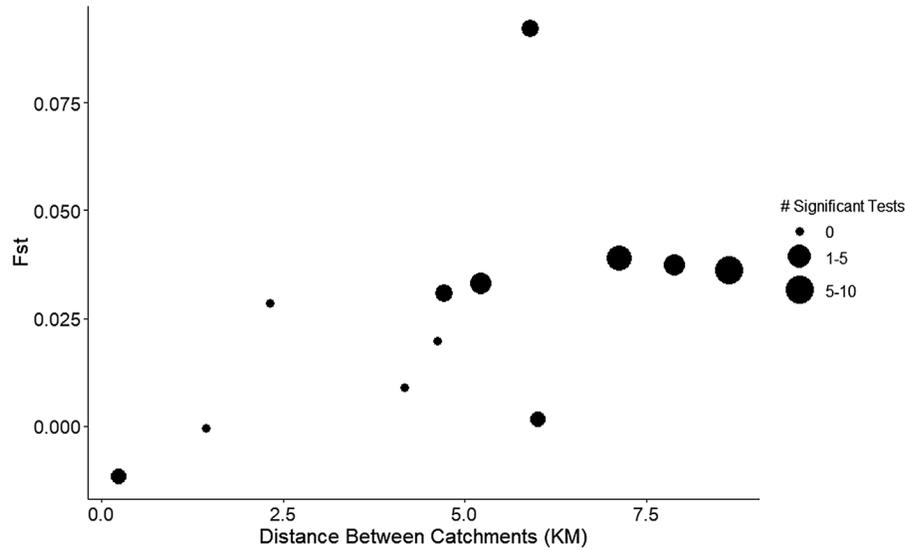


FIGURE 4. Intrapatch genetic structuring (genetic differentiation index F_{ST}) in modified Eastern Brook Trout Joint Venture patches, organized by distance between catchments. Circle size represents the total number of significant tests (Hardy–Weinberg equilibrium, linkage disequilibrium, and genetic differentiation) based on a Benjamini–Yekutieli false discovery rate correction.

TABLE 4. Results of backward stepwise model selection for genetic structuring in modified Eastern Brook Trout Joint Venture patches (F_{ST} = genetic differentiation index; GD = genic differentiation; HWE = Hardy–Weinberg equilibrium; LD = linkage disequilibrium; BIC = Bayesian information criterion; Δ BIC = BIC difference; BIC_{wt} = BIC weight; R^2_{mult} = multiple R^2 ; R^2_{adj} = adjusted R^2). Only models with Δ BIC less than 2.00 are shown; detailed results are presented in Table S5.

Metric	Variables	BIC	Δ BIC	BIC_{wt}	R^2_{mult}	R^2_{adj}	Variables with $P < 0.05$
F_{ST}	Distance + Impervious	–52.47	0.00	0.50	0.5	0.40	None
F_{ST}	Distance	–50.81	1.66	0.22	0.3	0.24	None
F_{ST}	Distance + Riparian + Impervious	–50.57	1.90	0.19	0.5	0.36	None
GD	Distance + Riparian + Impervious	28.65	0.00	0.40	0.8	0.71	Distance, Impervious
GD	Distance + Impervious	28.93	0.28	0.35	0.7	0.58	Distance
GD	Distance	29.56	0.91	0.25	0.6	0.52	Distance
HWE	Intercept only	25.16	0.00	0.38	0.0	0.00	NA
HWE	Distance	25.21	0.05	0.369	0.4	0.34	Distance
HWE	Distance + Riparian	26.88	1.72	0.16	0.5	0.33	None
LD	Distance + Riparian	22.83	0.00	0.38	0.9	0.91	Distance
LD	Distance + Riparian + Impervious	23.03	0.20	0.34	1.0	0.98	Distance
LD	Distance	23.44	0.61	0.28	0.8	0.73	Distance

separated by unsuitable main-stem habitat (e.g., Figure 1A). The modified EBTJV patches in this study could be used to identify conservation actions at the watershed scale. Specifically, because the patches can be used to predict genetic structuring, they could be used to identify potentially fragmented populations. The patches could then be used to target opportunities for reconnecting patches that are fragmented by anthropogenic influences, thereby increasing habitat area to increase genetic diversity and long-term adaptive potential of headwater stream populations.

Eastern Brook Trout Joint Venture Patch Structuring

Despite not incorporating genetic information in the delineation algorithm, the habitat patches based on historical data and physical barriers (Whiteley et al. 2013, 2014; Coombs and Nislow 2015) aligned with most of the observed contemporary genetic structuring. However, we found higher levels of structuring in the present study compared to previous evaluations of the EBTJV patches. Using the same eight microsatellites and sibling removal strategies, Whiteley et al. (2013) reported 2 of 110 (1.82%)

TABLE 5. Results of backward stepwise model selection for nested genetic diversity models (BIC = Bayesian information criterion; Δ BIC = BIC difference; BIC_{wt} = BIC weight; R^2_{mult} = multiple R^2 ; R^2_{adj} = adjusted R^2). Variable codes are defined in Table 2.

Variables	BIC	Δ BIC	BIC_{wt}	R^2_{mult}	R^2_{adj}	Variables with $P < 0.05$
CatchArea + PatchArea	182.98	0.00	0.73	0.27	0.25	PatchArea, CatchArea
CatchArea + AugPrecip + PatchArea	185.21	2.23	0.24	0.29	0.26	PatchArea, CatchArea
PatchArea	187.30	4.32	0.08	0.19	0.18	PatchArea
CatchArea + AugPrecip + SurfCoarse + PatchArea	187.52	4.54	0.08	0.31	0.27	PatchArea, CatchArea
Waterbodies + CatchArea + AugPrecip + SurfCoarse + PatchArea	191.42	8.44	0.01	0.31	0.27	PatchArea, CatchArea
Waterbodies + CatchArea + AugPrecip + SurfCoarse + PatchComplexity + PatchArea	195.71	12.73	0.00	0.31	0.26	PatchArea, CatchArea
Waterbodies + CatchArea + AugPrecip + Impervious + SurfCoarse + PatchComplexity + PatchArea	200.09	17.11	0.00	0.31	0.25	PatchArea, CatchArea
Intercept only	201.08	18.10	0.00	0.00	0.00	NA
Waterbodies + Temp + CatchArea + AugPrecip + Impervious + SurfCoarse + PatchComplexity + PatchArea	204.50	21.52	0.00	0.31	0.24	PatchArea, CatchArea

significant HWE tests and 2 of 379 (0.53%) significant LD tests in eight EBTJV patches in northern Virginia. In this study, 9% of HWE tests and 7% of LD tests produced significant results following the same sibling removal strategy and corrections for multiple comparisons. The difference was likely due to the presence of dams within the patches and upstream development, which were the two variables most strongly related to genetic structuring. Although dams were included in the original patch delineation algorithm (Coombs and Nislow 2015) and the same database was used in this study, the underlying catchment layers used in the delineations differed in spatial resolution (see Methods). The difference in resolution allowed us to delineate patches based on dams, with greater accuracy (Figure 1C, E).

Revising the EBTJV patch layer using dams and greater than 3% impervious surface as barriers led to reduced genetic structuring, and the remaining structuring was due to isolation by distance. This result supports previous analyses with the same data set that identified isolation by distance as the primary pattern of genetic differentiation in an eastern Connecticut watershed (Nathan et al. 2019). By modifying the patch algorithm to better incorporate dams and landscape-scale influences, our modified patches better represent contemporary genetic structuring of Brook Trout, which can be used as a guide to identify conservation opportunities to reconnect small patches and protect large, intact patches. Future versions of rangewide patches may benefit from (1) finer-resolution data, such as the future NHD High-Resolution catchments, which are comparable in size to those of SHEDS and cover the full extent of the EBTJV range (J. Coombs, University of Massachusetts–Amherst, personal communication); or (2) additional validation by local conservation organizations. It is important to note that our

analyses were restricted to Connecticut, which may not be representative of the entire eastern Brook Trout’s range. In particular, the northern extent of the range (e.g., Maine) is characterized by larger, more intact patches, and structuring may be influenced by different riverscape characteristics. Future work could expand these analyses to other states within the range to determine the validity of the revised patches.

Riverscape Influences on Genetic Diversity

As predicted, habitat area at the patch and catchment scales was the best predictor of the stream-scale genetic diversity observed in this study. Our results support previous evaluations of the EBTJV patches that have documented positive relationships between patch size, N_b , and genetic diversity (heterozygosity; Whiteley et al. 2014). The relationship between patch area and genetic diversity has been well documented in several species of stream salmonids (Neville et al. 2006; Kovach et al. 2015; Buonaccorsi et al. 2017). Larger patches of salmonid habitat can lead to an increase in N_b (Whiteley et al. 2013, 2014), decreased differentiation between patches (Meeuwig et al. 2010), and increased genetic diversity (Kovach et al. 2015). What makes this analysis unique is that we focused on modeling *stream-scale* genetic diversity as a function of patch area. Therefore, results from this study indicate that the observed increases in genetic diversity are not simply the result of sampling individuals over a large spatial extent. Smaller catchments located in larger patches tended to have higher genetic diversity, indicating that gene flow within patches likely supports increased genetic diversity in smaller catchments. Furthermore, because our patches often contained main-stem river habitat, which does not typically support Brook Trout populations year-round (Figure 1A), our results emphasize that gene flow

occurs among headwater streams and such dynamics are important for Brook Trout conservation.

The modeling results presented here also identified upstream catchment drainage area as an important predictor of local genetic diversity. This finding is distinct from the relationship with total habitat area, as indicated by the low correlation between catchment area and patch area ($r = 0.20$). Within the same patch, downstream catchments had 7% higher genetic diversity compared to upstream catchments, indicating that location within the watershed influences genetic diversity. Movement in stream networks is often biased in the downstream direction for young individuals because dispersal is aided by the flow of water and movement upstream can be impeded by physical barriers. Our results support theoretical predictions of higher genetic diversity lower in the watershed (i.e., larger upstream area; Thomaz et al. 2016) as well as empirical studies of stream salmonid genetics (Angers et al. 1999; Narum et al. 2008). It is therefore important to consider sampling locations in the watershed when evaluating local genetic diversity, particularly if samples are obtained from a single stream reach to represent stream- or patch-scale genetics.

There were no observed influences of stream-scale habitat variation on genetic diversity, despite documented influences of habitat quality and landscape characteristics on Brook Trout population dynamics. Watershed development was expected to have an influence on genetic diversity based on reduced habitat suitability (i.e., occupancy; Stranko et al. 2008; Kanno et al. 2015b) and genetic connectivity (Nathan et al. 2019). Variability in environmental characteristics, such as streamflow and temperature, also influences growth (Xu et al. 2010b; Letcher et al. 2015), survival (Xu et al. 2010a; Letcher et al. 2015; Kanno et al. 2015a), and seasonal movement and densities (Petty et al. 2005) of Brook Trout in headwater streams. Although we did not observe any significant relationships with our proxies for local habitat covariates, such as precipitation and temperature, studies on other species have found habitat quality to be important for influencing genetic diversity (Kovach et al. 2015) and differentiation (Hand et al. 2016). It is possible that our habitat covariates (e.g., mean maximum annual temperature) presented a potential spatial scale mismatch between the exploratory and response variables given the variation that can be observed across different metrics (Arismendi et al. 2013). Future work with the EBTJV patches could explore a broader range of variables to identify additional influential environmental factors for genetic diversity. Alternatively, the lack of a significant influence of habitat or watershed characteristics on genetic diversity may suggest that patch area (i.e., connectivity) is the primary driver of genetic diversity in human-dominated landscapes like those in Connecticut. Therefore, conservation actions focused on reconnecting fragmented populations or habitat at the

watershed scale may be the preferred management strategy when the goal is to maximize genetic diversity.

Conservation Applications

The results of this study support the use of the modified EBTJV patch layer as management units for Brook Trout conservation. Most patches appeared to represent accurate delineations of Brook Trout population structuring, and the observations of increased genetic diversity in streams within larger patches emphasized the importance of within-patch structuring. However, the observed genetic structuring in this study is likely a reduced version of historical connectivity due to anthropogenic fragmentation and habitat alteration. Therefore, the modified patch layer could be used to explicitly target areas of fragmentation (i.e., small patches) for restoration actions or remaining intact habitat (i.e., larger patches) for protection.

Goals of the Brook Trout conservation plan include increasing genetic and life history diversity, access to critical habitats, and long-term population viability (EBTJV 2018). To achieve these goals, conservation efforts could focus on protecting the largest occupied patches and promoting restoration actions that reconnect habitat to form larger patches. Although maximizing reconnected habitat is often a common strategy for prioritizing management actions, landscape-scale factors are typically not included in such prioritizations (see Kemp and O'Hanley 2010 for a summary). To develop the most effective strategies, restoration could recognize all potential factors influencing gene flow, including local physical barriers (e.g., dams and road crossings) as well as more cryptic, watershed-scale influences (e.g., development) that might be reducing connectivity. The EBTJV patches could be used as the basis to make informed decisions about conservation actions using genetic connectivity as a metric for prioritization. In some cases, this type of prioritization scheme may identify barriers in main-stem habitat as the highest priority for removal to reconnect headwater populations. Although this may seem counterintuitive for managing a coldwater species in headwater streams, these types of actions would promote gene flow among streams and would improve long-term population viability.

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SUPPORTING INFORMATION

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