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ARTICLE

Genetic Structure of Maryland Brook Trout Populations: Management Implications for a Threatened Species

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Abstract

Brook Trout *Salvelinus fontinalis* have declined across their native range due to multiple anthropogenic factors, including landscape alteration and climate change. Although coldwater streams in Maryland (eastern United States) historically supported significant Brook Trout populations, only fragmented remnant populations remain, with the exception of the upper Savage River watershed in western Maryland. Using microsatellite data from 38 collections, we defined genetic relationships of Brook Trout populations in Maryland drainages. Microsatellite analyses of Brook Trout indicated the presence of five major discrete units defined as the Youghiogheny (Ohio), Susquehanna, Patapsco/Gunpowder, Catoctin, and Upper Potomac, with a distinct genetic subunit present in the Savage River (upper Potomac). We did not observe evidence for widespread hatchery introgression with native Brook Trout. However, genetic effects due to fragmentation were evident in several Maryland Brook Trout populations, resulting in erosion of diversity that may have negative implications for their future persistence. Our current study supplements an increasing body of evidence that Brook Trout populations in Maryland are highly susceptible to multiple anthropogenic stresses, and many populations may be extirpated in the near future. Future management efforts focused on habitat protection and potential stream restoration, coupled with a comprehensive assessment framework that includes genetic considerations, may provide the best outlook for Brook Trout populations in Maryland.

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Conservation of native fishes is a critical component of contemporary fisheries management, especially with the advent of modern analytical techniques to evaluate the genetic structure of fish populations (Warren et al. 2000; Habera and Moore 2005; Hauser and Seeb 2008; Bruce et al. 2019, 2020). Effective fisheries management for a salmonid species, such as the Brook Trout *Salvelinus fontinalis,* is dependent on a wide array of biological, physical, social, political, and economic data with a strong emphasis on integration of these factors to develop appropriate adaptive strategies (Habera and Moore 2005; Heft et al. 2006; Shepard et al. 2019; Pelletier et al. 2020). For Brook Trout, fishery management generally focuses on habitat (physical), water quality (chemical), population and community dynamics (ecological), and human interactions (social, political, and economic) to derive a workable adaptive system (Shepard et al. 2019; Pelletier et al. 2020).

For Brook Trout (a member of the North American char complex), it is a vital requirement to have both cold and clean water (Power 1980); however, this dual concept has been expanded to include the increasing importance of both connected and complex habitat for all trout species (Gresswell et al. 1997; Haak and Williams 2013; Hauer et al. 2016; Muhlfeld et al. 2019). These four factors together are widely promoted to support not only Brook Trout, but also other salmonid conservation efforts worldwide (Muhlfeld et al. 2019). Because of their widespread native range, keystone position in coldwater streams, importance as a fishery resource, and charismatic attributes as being the major freshwater salmonid in eastern North America (Scott and Crossman 1973; Behnke 1980; Power 1980), Brook Trout are now the focus of numerous rangewide conservation efforts (Hudy et al. 2006; King et al. 2012; Aunins et al. 2015; Muhlfeld et al. 2019).

Population genetics is another key but often underappreciated factor that may have major impacts on conservation outcomes since it serves as a framework for local adaptation and as a reservoir of future evolutionary potential (Fraser et al. 2011, 2014; Hutchings 2011). For salmonids, an understanding of population genetics is important in order to develop management strategies, especially for Brook Trout (Habera and Moore 2005; Heft et al. 2006; Whitely et al. 2019). There are substantial current and future threats that make it important to document relationships among populations and to characterize any potential threats to the overall genetic integrity of extant populations.

Brook Trout genetic data are useful for conservation prioritization and planning (Nathan et al. 2018, 2020; Bruce et al. 2019; Merriam et al. 2019), reintroductions (Bruce et al. 2020), and potentially genetic rescue (Robinson et al. 2017; Bell et al. 2019; Wells et al. 2019), particularly when populations occur across diverse landscapes with divergent evolutionary histories. In addition, there are significant regional efforts to ascertain any potential influences of past and present hatchery releases on population structure (Kazyak et al. 2018; White et al. 2018) and to protect heritage populations of Brook Trout (Perkins et al. 1993; Hayes et al. 1996; Bruce et al. 2018).

Maryland's diverse landscapes support distinct populations of Brook Trout throughout its varied geography and geology (Vokes 1957). Based on previous work using mitochondrial DNA (mtDNA), we know that Maryland harbors three distinct mtDNA lineages (clades) separated for long time periods (Danzmann et al. 1998; Hall et al. 2002). However, there is no information known about any potential Maryland Brook Trout population substructure within these three clades, especially those occurring eastward of the eastern continental drainage divide. Brook Trout populations in Maryland presently occur in many fragmented patches that may have been isolated for extended periods of time with the potential to develop local adaptations. Given threats to the persistence of these Brook Trout populations, it is critical to understand fine-scale genetic patterns to provide a foundation for both conservation prioritization and potential restoration (Kelson et al. 2015; Printz et al. 2018).

There are numerous and substantial pressures that make it critical to document relationships among salmonid populations and to characterize potential threats to the genetic integrity of extant populations, especially for Brook Trout. With increasing interest in Brook Trout, numerous studies have addressed eastern Brook Trout genetics (e.g., Hayes et al. 1996; Morgan and Danzmann 1997, 1998; Danzmann et al. 1998; Hall et al. 2002; Stauffer and King 2014; Aunins et al. 2015; Kazyak et al. 2015, 2016; Buonaccorsi et al. 2017; Bruce et al. 2018; Nathan et al. 2018; Pregler et al. 2018; Weathers et al. 2018). However, key spatial gaps in genetic structure remain unaddressed throughout the native range of eastern Brook Trout, particularly in the mid-Atlantic region, consisting of New York; New Jersey; Pennsylvania; Delaware; Maryland; Washington, D.C.; Virginia; and West Virginia.

Using microsatellite analyses, we examined the genetic structure of Maryland Brook Trout, including genetic diversity population structure and potential management options for this species in the future. We examined two key questions: (1) what is the genetic diversity and population structure within Maryland Brook Trout, and (2) are there any detectable effects of past or present hatchery introductions?

METHODS

Study area.— In Maryland, native Brook Trout occur from the higher elevations of the Allegany Plateau to the fall line of the Piedmont (Figure 1). Brook Trout

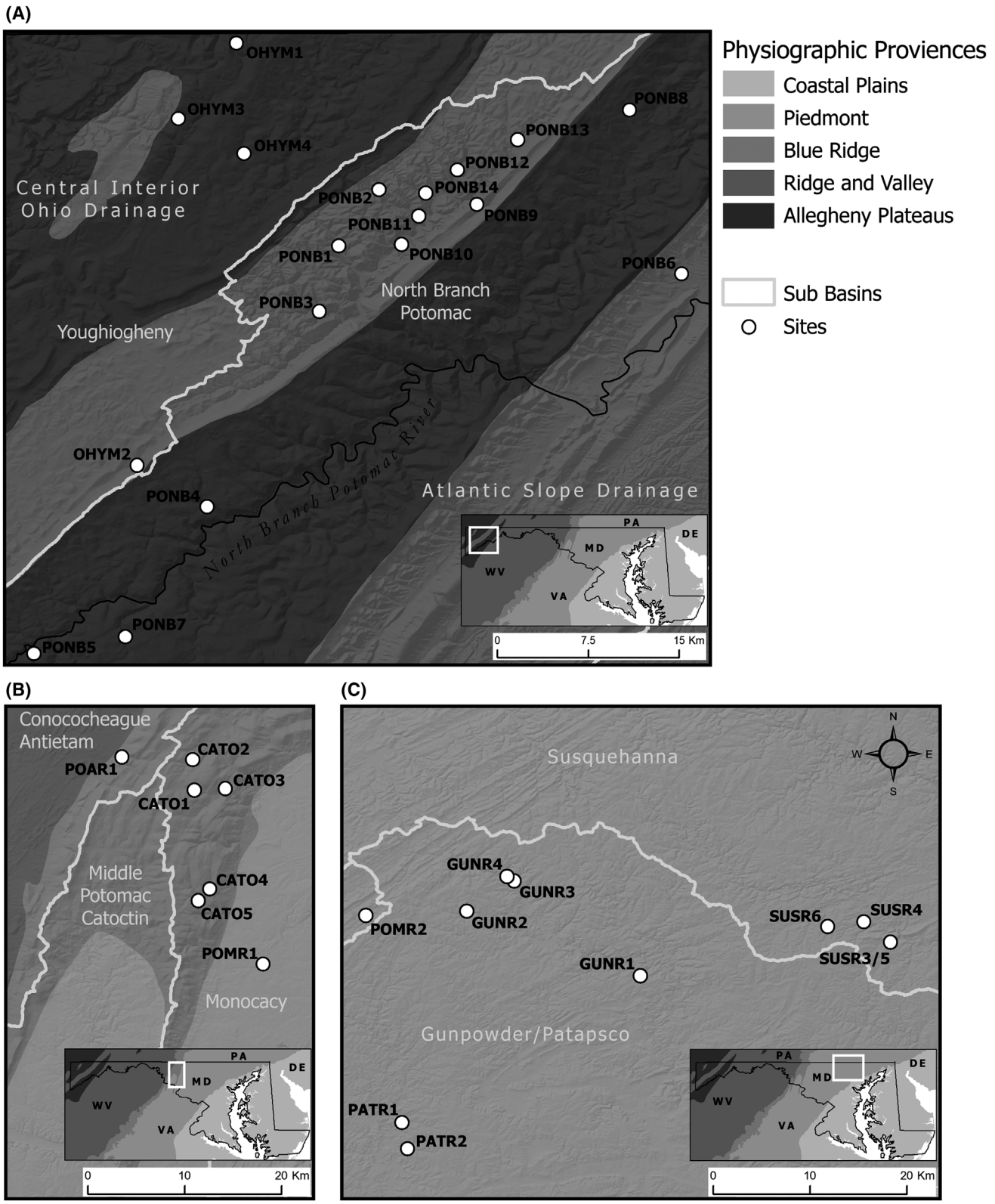

FIGURE 1. Distribution of Brook Trout collection sites across Maryland, including two West Virginia streams flowing into the North Branch Potomac River. Sites were in **(A)** western Maryland, **(B)** central Maryland throughout the Catoctin Mountain region, and **(C)** eastern Maryland in the Piedmont province. Brook Trout collections were obtained from three watersheds: the Patapsco River, Gunpowder River, and Susquehanna River.

populations are found within three river drainages in eastern Maryland: the Susquehanna, Gunpowder, and Patapsco, all located within the Piedmont province (Vokes 1957; Heft et al. 2006). Brook Trout populations in central Maryland are found in stream complexes on both sides of the Blue Ridge province (Catoctin Mountains), with highly isolated populations present throughout the western Piedmont and the Great Valley of the Ridge and Valley province (Vokes 1957; Heft et al. 2006).

Brook Trout populations in western Maryland occupy two major river drainages: the Youghiogheny (Allegheny Plateau of the Appalachian province) and the North Branch Potomac River (Atlantic Slope), as well as other minor stream drainages in the western Valley and Ridge province (Vokes 1957). The Youghiogheny River drainage flows northward to the Ohio River, whereas the North Branch Potomac River flows along the Atlantic Slope towards the Chesapeake Bay via the Potomac River (Vokes 1957).

Sample sites.— A total of 1,638 individual Brook Trout representing 38 discrete collections were taken from 1999 to 2018 for microsatellite analyses from Maryland streams (Figure 1; Tables 1, A.1.1). These collections were distributed across the native range of Brook Trout in Maryland, including each major watershed. Sixteen Brook Trout collections were from the Savage River/North Branch Potomac River stream complex of the Allegheny Mountains (Atlantic Slope). Hind Leg and Difficult Creek samples were from two West Virginia streams that flow into the upper North Branch Potomac River. Eight collections were from central Maryland (Catoctin Mountains of the Blue Ridge province), with another 10 collections from Brook Trout streams found throughout the Piedmont province of eastern Maryland. Finally, four Brook Trout collections were made from the Youghiogheny River watershed, west of the eastern continental drainage divide (Figure 1; Tables 1, A.1.1). Two Brook Trout collections from the same stream were obtained over a long time span. Poplar Lick was sampled 15 years apart. Because of a small sample ($n = 12$) in 2000, Kellogg Branch was resampled in 2008 after the population rebounded.

Hatchery collections.— Fin clip collections were also obtained from 17 major fish hatcheries throughout the eastern United States to examine any potential introgression of hatchery-reared Brook Trout stocking into native populations (Kazyak et al. 2018). Based on historic stocking records in Maryland (Heft et al. 2006), eight hatcheries were selected to be analyzed, including Pequest, New Jersey (2005; $N = 20$); Phillips, Maine (2005; $N = 60$); Sandwich, Massachusetts (2003; $N = 37$); Hyde Park strain, New York (2005; $N = 35$; initially obtained from the Temiscamie River [Quebec, Canada]); Bellefonte, Pennsylvania (2004; $N = 31$); Edray, West Virginia (2003; $N = 21$; presumed to be from an unknown Maine source [Hall et al. 2002]); Berlin, New Hampshire (2008; $N = 50$); and Milford, New Hampshire (2008; $N = 50$).

Sample collection.— Brook Trout were collected by electrofishing (generally in summer and fall), with an effort to collect multiple cohorts and to spread out collections in stream reaches. Adipose fin clips were obtained nonlethally, with samples preserved individually in cold 95% ethanol in the field, held for 2 d at 4°C, and then stored at room temperature. All Brook Trout collecting techniques followed approved sampling protocols using guidelines established by the University of Maryland Institutional Animal Care and Use Committee. Several Brook Trout collections were made by personnel from the Maryland Department of Natural Resources and other university and governmental entities.

Sample preparation and analyses.— Brook Trout fin clip samples were transferred to the U.S. Geological Survey (USGS) Leetown Science Center, Kearneysville, West Virginia for all molecular analyses. Whole genomic DNA was extracted from fin clips using the Puregene Kit (Gentra Systems, Minneapolis, Minnesota). Processed DNA samples were screened for 13 microsatellite loci specifically designed for Brook Trout: *SfoB52*, *SfoC24*, *SfoC28, SfoC38*, *SfoC79*, *SfoC86*, *SfoC88*, *SfoC113*, *SfoC115*, *SfoC129*, *SfoD75*, *SfoD91*, and *SfoD100* (King et al. 2012). Protocols for the master mix composition, thermal cycling parameters, and multiplexing conditions followed King et al. (2012) and Kazyak et al. (2018).

Polymerase chain reaction amplifications were completed on PTC-200 and PTC-225 thermal cyclers (Bio-Rad Laboratories, Hercules, California), with allelic sizes of microsatellites characterized on ABI 3100 or ABI 3130xl genetic analyzers (Applied Biosystems, Foster City, California). GeneScan and Genotyper fragment analysis software (Applied Biosystems) were employed to score, bin, and output all allelic data characterized on the ABI 3100, and Genemapper software (Applied Biosystems) was used to score data generated on the ABI 3130xl (Kazyak et al. 2018). Positive and negative control samples were included with each plate of samples for PCR amplification and electrophoresis. In addition, five duplicate samples were blindly submitted to the USGS Leetown Science Center as an additional positive control, all of which were identified by their matching multilocus genotypes (Table 1). The Microsatellite Toolkit Microsatellite for Microsoft Excel (Park 2001) identified null alleles, scoring errors, and/or large allelic dropout within the data set.

Microsatellite analyses.— Previous studies have shown that Brook Trout typically exhibit limited dispersal (Hudy et al. 2010). Consequently, we screened for family structure within collections prior to genetic characterization of population structure to avoid samples being dominated by a few families, possibly leading to incorrect estimations of true population structure (Allendorf and Phelps 1981).

TABLE 1. Summary of basic genetic data for Brook Trout collections. The identification code for each sample (ID code) is given. Genetic data include sample size analyzed (N), number of full sibling families (N_{FSF}), number of alleles per locus (N_A), unbiased expected heterozygosity (H_E), observed heterozygosity (H_O), inbreeding coefficient (F), effective number of alleles (A_E), effective population size (N_{EPS}) with the 95% CI, allelic richness (A_R), genetic differentiation (F'_{ST}), the proportion of loci with significant deviations from Hardy–Weinberg equilibrium expectation (HWE; $P = 0.0038$, determined using Fisher's method), and the proportion of pairwise comparisons among loci with significant linkage disequilibrium (LD; $P = 0.00064$). Significant levels for both the HWE and LD tests were adjusted using a sequential Bonferroni correction (Rice 1989) with diversity measures averaged over 13 Brook Trout microsatellite loci (King et al. 2012). Five samples (denoted with asterisks) with $N = 49$ represent collections with duplicate fin clips for blind quality assurance/quality control samples (all five correctly identified); UT = unnamed tributary.

Collection location	ID code	N	N_{FSF}	N_A	H_E	H_O	F	A_E	N_{EPS} (95% CI)	A_R	F'_{ST}	HWE	LD
Big Run*	PONB1	49	49	7.77	0.71	0.71	−0.011	4.44	54 (42–71)	7.04	0.39	0.000	0.013
Poplar Lick (1999)*	PONB2	49	49	6.85	0.67	0.66	0.023	3.80	188 (104–710)	6.47	0.41	0.000	0.000
Middle Fork Crabtree Creek	PONB3	50	50	7.54	0.72	0.69	0.028	4.39	113 (74–212)	6.99	0.38	0.077	0.013
Lostland Run*	PONB4	49	49	5.23	0.60	0.58	0.042	2.83	33 (22–52)	4.74	0.58	0.154	0.013
Hind Leg	PONB5	48	48	3.08	0.35	0.36	−0.061	1.77	8 (4–13)	2.97	0.78	0.077	0.064
Mill Run	PONB6	50	50	3.31	0.49	0.54	−0.083	2.14	18 (12–30)	3.12	0.62	0.077	0.013
Difficult Run	PONB7	30	30	7.08	0.71	0.71	−0.025	4.48	109 (53–1913)	7.04	0.44	0.000	0.013
Sand Spring Run	PONB8	29	28	5.46	0.68	0.63	0.030	3.55	12 (8–16)	5.43	0.49	0.077	0.038
Little Savage River	PONB9	50	49	7.38	0.69	0.69	−0.010	4.05	95 (66–160)	6.85	0.38	0.000	0.000
Main-stem Savage River	PONB10	50	50	7.85	0.70	0.69	0.006	4.00	596 (193–∞)	7.09	0.38	0.000	0.026
Poplar Lick (2014)	PONB11	50	50	7.08	0.67	0.65	0.020	3.82	229 (116–1830)	6.61	0.41	0.000	0.000
Blue Lick	PONB12	50	50	7.85	0.68	0.68	−0.004	4.03	163 (103–350)	7.07	0.38	0.000	0.013
Mud Lick	PONB13	30	29	6.77	0.68	0.67	−0.014	3.75	67 (35–260)	6.68	0.39	0.000	0.013
Elk Lick	PONB14	30	30	6.15	0.69	0.66	0.030	3.76	98 (38–∞)	6.09	0.40	0.000	0.000
Black Lick	PONB15	29	27	6.39	0.70	0.73	−0.072	3.76	24 (12 – 83)	6.38	0.37	0.154	0.000
Bear Pen Run	PONB16	29	27	7.08	0.70	0.72	−0.046	4.29	45 (19 – 2447)	7.04	0.38	0.000	0.026
Warner Hollow Run	POAR1	50	50	4.23	0.56	0.58	−0.048	2.74	12 (8–16)	4.04	0.62	0.000	0.115
Big Hunting Creek	CATO1	47	47	4.62	0.63	0.61	0.011	2.84	31 (23–45)	4.46	0.61	0.000	0.013
Owens Creek	CATO2	49	49	4.92	0.64	0.65	−0.022	3.15	54 (36–96)	4.73	0.54	0.000	0.013
Still Creek	CATO3	32	32	5.54	0.72	0.73	−0.039	3.97	15 (12–19)	5.49	0.49	0.000	0.038
Right Fork Fishing Creek	CATO4	38	38	6.46	0.70	0.71	−0.032	3.93	76 (52–129)	6.23	0.47	0.000	0.013
Left Fork Fishing Creek	CATO5	48	48	6.15	0.69	0.69	−0.014	3.49	219 (94–∞)	5.85	0.51	0.000	0.000
Tuscarora Creek	POMR1	47	47	6.00	0.67	0.68	−0.025	3.44	59 (39–102)	5.68	0.52	0.077	0.013
UT to Big Pipe Creek	POMR2	54	53	2.85	0.45	0.46	−0.072	3.88	14 (7 – 26)	2.83	0.68	0.077	0.051
Norris Run	PATR1	8	8	3.15	0.52	0.52	−0.03	2.58	11 (3–∞)	3.15	0.59	0.000	0.000
Timber Run	PATR2	5	5	2.62	0.44	0.42	−0.03	1.93	142 (10–∞)	2.62	0.65	0.000	0.000
Panther Branch	GUNR1	50	49	6.38	0.68	0.71	−0.056	3.84	20 (16–24)	5.99	0.44	0.000	0.244

TABLE 1. Continued.

Collection location	ID code	N	N_{FSF}	N_A	H_E	H_O	F	A_E	N_{EPS} (95% CI)	A_R	F'_{ST}	HWE	LD
Indian Run	GUNR2	50	50	3.77	0.53	0.55	−0.038	2.45	17 (12–25)	3.69	0.64	0.077	0.013
Silver Run	GUNR3	50	50	4.54	0.60	0.60	−0.009	2.84	34 (25–47)	4.39	0.61	0.000	0.000
Walker Run	GUNR4	50	50	4.54	0.55	0.56	−0.019	2.68	60 (37–122)	4.34	0.66	0.077	0.000
Kellogg Branch (2000)	SUSR3	12	12	5.15	0.69	0.65	−0.008	3.35	18 (11–38)	5.15	0.52	0.000	0.000
Rock Hollow Run	SUSR4	100	98	6.69	0.66	0.66	0.003	3.82	55 (42–75)	5.79	0.53	0.231	0.064
Kellogg Branch (2008)	SUSR5	50	50	6.00	0.66	0.68	−0.037	3.60	35 (26–48)	5.63	0.49	0.077	0.038
Spooners Creek	SUSR6	50	50	5.46	0.63	0.66	−0.063	3.25	22 (17–28)	5.17	0.55	0.000	0.013
Puzzley Run	OHYM1	49	49	6.85	0.66	0.66	−0.011	3.73	89 (55–193)	6.33	0.68	0.000	0.000
Black Run*	OHYM2	49	48	7.08	0.74	0.75	−0.026	4.56	114 (75–213)	6.66	0.57	0.000	0.000
Little Bear Creek*	OHYM3	49	49	7.38	0.58	0.58	0.024	3.55	235 (115–3,741)	6.62	0.72	0.000	0.013
Upper Casselman River	OHYM4	30	30	6.23	0.70	0.72	−0.044	4.15	41 (28–69)	6.18	0.55	0.000	0.013
Mean (SE)		43.1 (2.6)	42.8 (2.6)	5.8 (0.53)	0.63 (0.015)	0.64 (0.014)	−0.019 (0.0051)	3.5 (0.1)	85 (17)	5.5 (0.2)	0.52 (0.018)	0.032 (0.009)	0.024 (0.007)

Fish collections dominated by one or a few families may lead to a false interpretation of an entire population being out of Hardy–Weinberg equilibrium (HWE; Rodriguez-Ramilo and Wang 2012). Unidentified family structure may also be a problem for the detection of obscured population structure using Bayesian clustering programs like STRUCTURE. However, recent work by Waples and Anderson (2017) suggests that any benefits of purging siblings are often offset by the loss of information, which may lead to skewed inferences. Consequently, we chose to examine our collections for family structure and report the number of full sibling families observed, but still include all sampled individuals in our statistical analyses.

We used the program COLONY v2.0 to determine any full sibling families represented in each collection of Brook Trout (Wang and Santure 2009). For COLONY analyses, program settings included an assumption of male and female polygamy, no genotyping error per locus, a lack of inbreeding, a long run length with the full likelihood analysis method, a high likelihood of precision, no updates of allelic frequency and no prior sibship. Brook Trout were analyzed as offspring without any candidate male or female parents since these data were not available. While family relationship inference is weakened without sex, age, relational information, and assumption of polygamy for both sexes, family predictions made using COLONY are expected to be more accurate as compared to pairwise relationship estimates (Wang and Santure 2009).

We quantified genetic diversity of all Brook Trout collections using GenAlEx 6.5 (Peakall and Smouse 2012) to calculate allelic frequencies, the number of alleles per locus (N_A), the effective number of alleles (A_E), the observed heterozygosity (H_O), the unbiased expected heterozygosity (H_E), and an inbreeding coefficient (F). We also calculated rarefied allelic richness (A_R) in HP-rare (Kalinowski 2005), which enables comparisons of allelic numbers across all collections with divergent sample sizes. Using HP-rare, the sample size was set to 56 genes (equivalent to 28 diploid individuals) for all rarefaction calculations. In those Brook Trout collections with fewer than 28 individuals, it was assumed that all alleles were observed.

For each Brook Trout collection, we employed exact tests in Genepop (Raymond and Rousset 1995) to test all genotypes observed at each locus for conformance to HWE. Multilocus tests of HWE conformance for each population were completed using Fisher's method in Genepop. We also tested linkage disequilibrium (LD) for all locus pairs using contingency tables in Genepop. All HWE and LD tests in Genepop used the default Markov chain parameters, with significance levels adjusted for HWE and LD tests using a simple Bonferroni correction (Rice 1989).

Statistical analyses.— We employed a hierarchical analysis of molecular variance (AMOVA), implemented with the "pegas" package within R (Paradis 2010; R Core Team 2015), in order to examine the geographic structure of Brook Trout. There were three hierarchical levels considered: drainage (Mississippi or Atlantic), watershed, and collection. The five Maryland watersheds were defined as follows: Ohio (Youghiogheny), North Branch, Gunpowder and Patapsco, Catoctin, and Susquehanna (Figure 1).

Genetic differentiation among all Brook Trout populations was examined using pairwise F'_{ST} values (Meirmans and Hedrick 2011) as calculated with the "diveRsity" package in R (Keenan et al. 2013; R Core Team 2015). In addition, we calculated the mean pairwise F'_{ST} value for each collection using the "diveRsity" package. The effective population size (N_{EPS}) for each collection was estimated based on LD using NeEstimator v2.1 with a rare allele cutoff of 0.02 and jackknifed confidence intervals (Do et al. 2014).

We used STRUCTURE (Pritchard et al. 2000), a Bayesian clustering software, to further examine the genetic relationships among Brook Trout populations from Maryland. We used a burn-in period of 200,000 steps followed by 200,000 iterations for data collection. The model was run without admixture and collection location was not considered as prior information. Five replicate models were run with a unique random seed to evaluate each potential K value, ranging from $K = 1$ through $K = 38$. We used STRUCTURE HARVESTER (Earl and vonHoldt 2012) to evaluate model results across potential K values based on likelihoods. Repeated model runs for selected K values were aligned using the default settings of CLUMPAK (Kopelman et al. 2015) and implemented using StructureSelector (Li and Liu 2018).

We visualized relationships among all Brook Trout collections from different drainages throughout Maryland by constructing a neighbor-joining (NJ) tree (Saitou and Nei 1987; Tamura et al. 2011) using POPTREEW (Takezaki et al. 2014). For microsatellite genotypes, only D_{SW} (Shriver et al. 1995) and $(\delta\mu)^2$ (Goldstein et al. 1995) genetic metrics were applicable. Employing 13 microsatellite loci from Brook Trout, we constructed an NJ tree using D_{SW} in POPTREEW, with bootstrapping performed for 1,000 and 5,000 replications. Both bootstrapping efforts were essentially identical, with between-tree percent concordance = 99.3%.

Additionally, we employed D_A to examine trees since it is considered to be more efficient in developing the correct microsatellite tree topology (Takezaki and Nei 2008). However, the D_A tree generated was identical to the tree generated by D_{SW} with similar bootstrap values at each node (nodal percent concordance between the D_A and D_{SW} trees was 99.1%). Although sample size was small for three eastern Maryland collections, these were included in the tree to visualize relationships among all Brook Trout populations (Tables 1, A.1.1). All other field collections

exceeded the lower 25 individual cutoff point as suggested by Hale et al. (2012), while two of the eight hatchery collections were below 25 individual Brook Trout (Pequest [$N = 20$] and Edray [$N = 21$]).

For additional perspectives on relationships among both native and hatchery Brook Trout, we performed a principal coordinates analysis (PCA) using GenAlEx 6.5 (Peakall and Smouse 2012) and drew inferences into the extent of potential hatchery introgression across Maryland based on observed relationships among collections in visually-based PCA analyses.

RESULTS

Genetic Analyses

Thirty-eight Brook Trout collections ($N = 1{,}638$ individuals) were analyzed with sample sizes ranging from 5 at Timber Run (PATR2) to 100 in Rock Hollow Run (SUSR4), with a mean sample size of 43.1 ± 2.6 SE (Table 1). The mean number of full sibling families (N_{FSF}) was 42.8 (SE = 2.6), indicating a lack of large family units in the data set and a low prevalence of full-sibling relationships in all sample collections (Table 1). Where they occurred, inferred full-sibling families were small and included two to three individuals.

For each Maryland Brook Trout population sampled (Table 1), the number of alleles (N_A) per locus ranged from 3.1 in Hind Leg (PONB5) to 7.9 in the main-stem Savage River (PONB10), with a mean of 5.8 (SE = 0.3). Concomitantly, the effective number of alleles (A_E) ranged from 1.8 in Hind Leg (PONB5) to 4.6 at Black Run (OHYM2), with a mean of 3.5 (SE = 0.1). Unbiased expected heterozygosity (H_E) averaged 0.63 (SE = 0.02), ranging from a low value of 0.35 in Hind Leg (PONB5) to a high value of 0.74 at Black Lick (OHYM2) with observed heterozygosity (H_O) ranging from 0.36 at Hind Leg (PONB5) to 0.75 in Black Run (OHYM2) and a mean of 0.64 (SE = 0.01).

Most collections conformed to HWE and showed little evidence of LD. However, there were a few collections showing signs of deviation from the expectations of a large, well-mixed population (e.g., Panther Branch [GUNR1] and Walker Run [SUSR4]).

Among all Brook Trout collections (Table 1), the mean inbreeding coefficient (F) was estimated as −0.019 (SE = 0.005), with the highest value (0.042) found at Lostland Run (PONB4) and the lowest value (−0.083) at Mill Run (PONB6). Estimates of the effective population size (N_{EPS}) for Brook Trout ranged from 8 in Hind Leg (PONB5) to 596 in the main-stem Savage River (PONB10), with a mean N_{EPS} of 85 (SE = 17). The mean rarefied allelic richness (A_R) was 5.5 (SE = 0.2), ranged from 2.6 at Timber Run (PATR2) to 7.1 in the main-stem Savage River (PONB10). For all Brook Trout collections, F'_{ST} values ranged from 0.37 at Black Lick (PONB15) to 0.78 in Hind Leg (PONB5), with mean $F'_{ST} = 0.52$ (SE = 0.02).

TABLE 2. Hierarchical analysis of molecular variance (AMOVA) for 38 wild Brook Trout collections from across Maryland and neighboring West Virginia streams. (SSD = sum of squared deviations in AMOVA).

Source of variation	SSD	Variance explained (%)
Among drainages	601,992	7.2
Among watersheds within drainages	1.47×10^6	17.7
Among collections within watersheds	2.51×10^6	30.2
Among individuals within collections	3.73×10^6	44.8
Total	8.31×10^6	100.0

Based on AMOVA, we observed that most genetic variation in Maryland Brook Trout occurred at small spatial scales (Table 2). Approximately half of the observed genetic variation was attributed to differences among individuals within fish collections (44.8%). Most of the remaining genetic variation was among collections within watersheds (30.2%). Smaller amounts of genetic variation were associated with differences among watersheds (17.7%) and between the Atlantic Slope and Ohio (interior) drainages (7.2%).

Generally, rarefied allelic richness (A_R) increased as a curvilinear function ($A_R = 2.31 + 1.81 \cdot \log_{10} N_{EPS}$; $r = 0.44$) of the effective population size (N_{EPS}) for Brook Trout (Figure 2). Eleven collections with a high rarefied allelic richness ($A_R \geq 6.0$) consisted of Brook Trout contained within the upper Savage River watershed. Four collections from the Youghiogheny River watershed also had an $A_R \geq 6.0$ (Figure 2). Although some Brook Trout collections in the upper Savage River had relatively small effective population sizes, they nonetheless maintained high A_R values, presumably reflecting genetic exchange among this tributary network. It should be noted that the observed relationship between N_{EPS} and A_R was steepest at smaller N_{EPS} and became much more gradual at larger N_{EPS} (with an estimated inflection point at N_{EPS} of approximately 50–60 Brook Trout).

In addition to the relationship of A_R with N_{EPS} (Figure 2), we found that populations with higher levels of allelic richness were less differentiated (based on their population-specific mean F'_{ST}) from other populations ($R^2 = 0.56$; Figure 3). Eighteen populations had A_R values > 6 with a $F'_{ST} < 0.17$, while 20 populations had A_R values < 6 and elevated F'_{ST} values (Figure 3).

High levels of genetic differentiation (F'_{ST} mean = 0.52; range = 0.01–0.88; Table A.2.1) were found among many

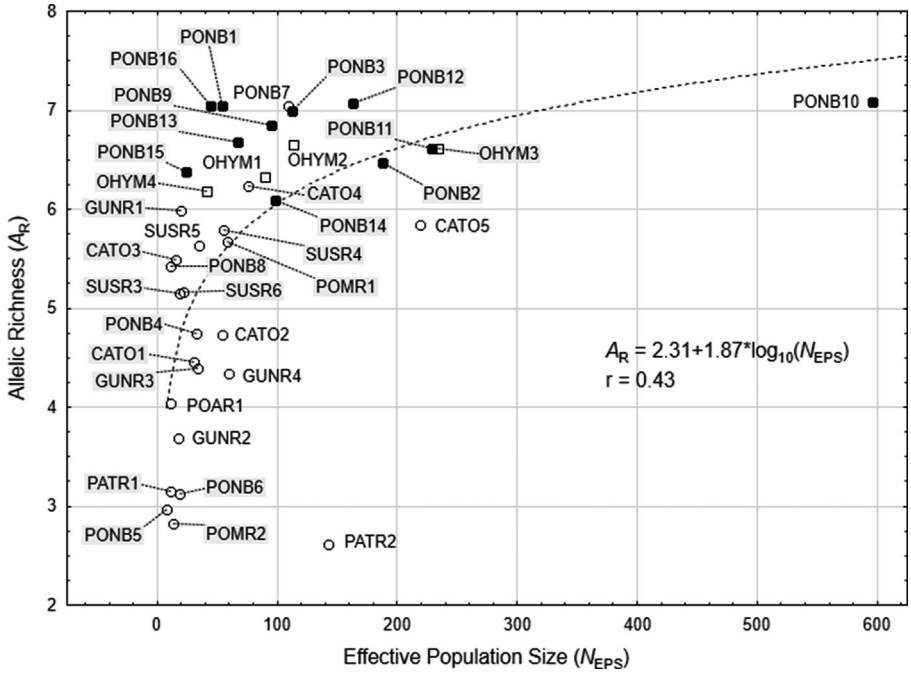

FIGURE 2. Relationship of rarefied allelic richness (A_R) versus the effective population size (N_{EFS}) for 38 Maryland Brook Trout collections. Solid circles denote collections from the upper Savage River stream complex, open squares represent collections from the Youghiogheny (Ohio) stream complex, and open circles denote collections from the rest of Maryland and two West Virginia streams flowing into the North Branch Potomac River.

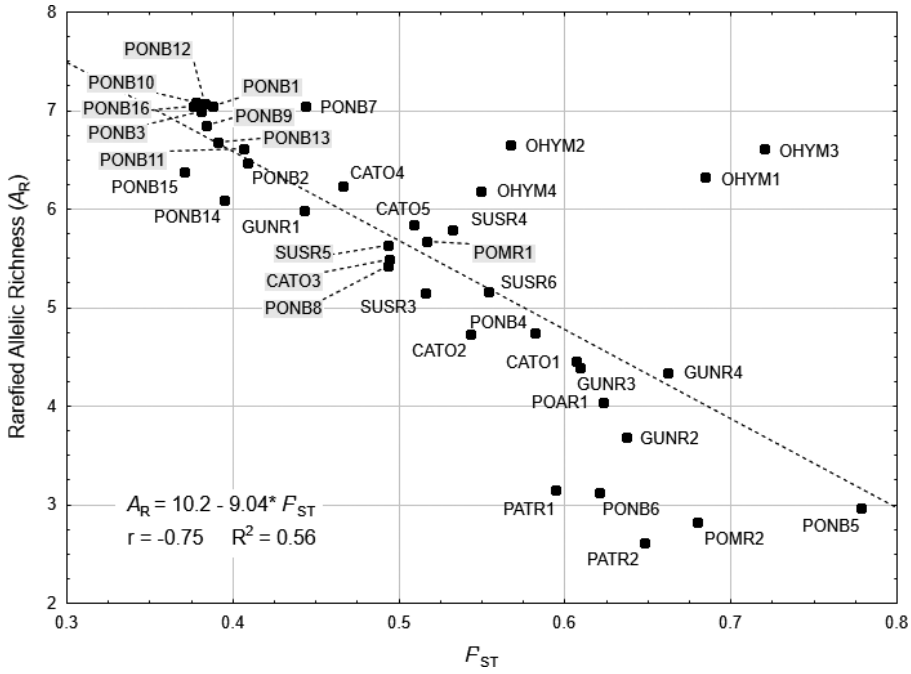

FIGURE 3. Relationship of rarefied allelic richness (A_R) versus the mean population-specific F'_{ST} for 38 Maryland Brook Trout collections.

Maryland Brook Trout, suggesting that little to no gene flow is occurring among most populations and that any past hatchery stocking has not had any homogenizing influence on native Brook Trout collections (Figure 4). The largest pairwise F'_{ST} values typically included very small, highly isolated Brook Trout populations.

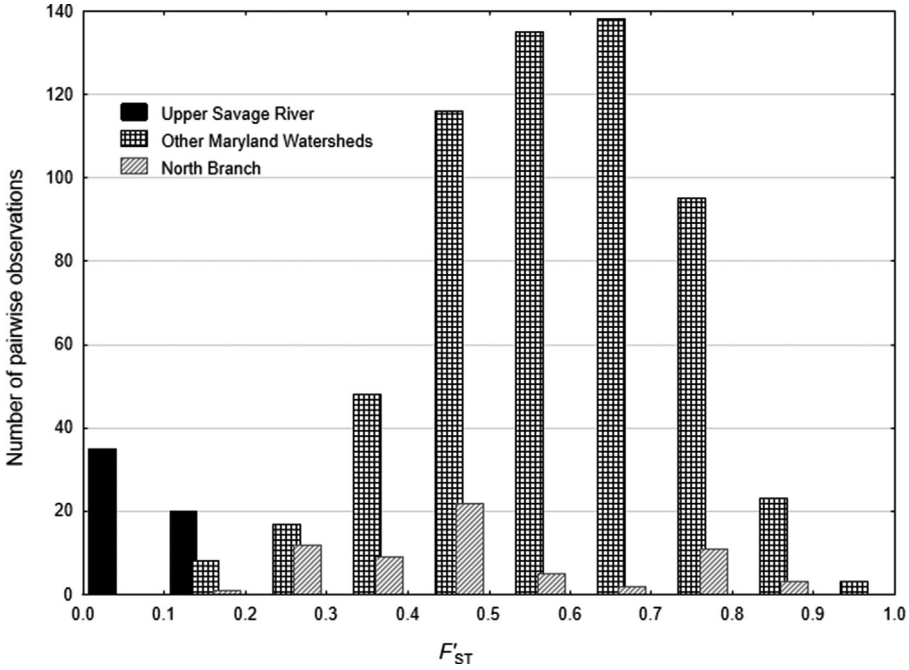

FIGURE 4. Histogram of pairwise genetic differentiation F'_{ST} values among 38 wild Brook Trout collections from Maryland and neighboring West Virginia.

The upper Savage River watershed was a notable exception to this overall trend of isolation, as can be clearly seen in the bimodal F'_{ST} distribution (Figure 4). Comparatively small F'_{ST} values (mean = 0.08; range = 0.01–0.17) suggested recent and/or ongoing gene flow among Brook Trout populations within this system. The main-stem Savage River showed very little differentiation among several tributaries, suggesting either the presence of a metapopulation or a large interbreeding population. It should be noted that the upper Savage River Brook Trout complex has been effectively isolated from the remainder of the Potomac watershed since the construction of the Savage River Dam in 1952.

We were concerned about possible changes in the genetic structure of Brook Trout collections sampled over a long time span (Table A.1.1). Brook Trout collections taken 8 years apart on Kellogg Branch in 2000 (SUSR3) and 2008 (SUSR5) had a $\Delta F'_{ST}$ of 0.11, while collections taken 15 years apart on Poplar Lick in 1999 (PONB2) and 2014 (PONB11) had a $\Delta F'_{ST}$ of 0.04. Poplar Lick, with larger N_{EPS} estimates, was more stable between samples than Kellogg Branch.

Spatial Structure

Bayesian clustering analysis using STRUCTURE showed a clear inflection point in likelihood scores at $K = 5$ (Appendix Figure A.3.1). Visualization of these model runs clearly revealed spatial genetic structure corresponding to five distinct geographic areas of the state: the Susquehanna, Upper Potomac, Catoctin, Patapsco/Gunpowder, and Ohio (Youghiogheny) drainages (Figure 5). At $K = 5$, the only exceptions to this pattern occurred at Hind Leg (PONB5) and an unnamed tributary to Big Pipe Creek (POMR2), both highly isolated Brook Trout populations.

At the higher STRUCTURE level of $K = 15$, additional genetic structuring was evident with many collections cleanly breaking out into discrete patterns (Figure 5). Both Hind Leg (PONB5) and the unnamed tributary to Big Pipe Creek (POMR2) displayed the same pattern as at $K = 5$. However, Lostland Run (PONB4) clearly broke out as a distinct Brook Trout population at $K = 15$, as did Sand Spring Run (PONB8). These are both highly isolated populations in the North Branch Potomac River drainage, with lower Lostland Run (PONB4) disconnected from the North Branch Potomac River main stem due to a severe stream elevation change in its lower reach, thus limiting any potential upstream movement of Brook Trout. Big Hunting Creek (CATO1) displayed a mixed signature, with Tuscarora Creek (POMR1) showing a distinct STRUCTURE pattern from the other Catoctin Brook Trout at $K = 15$. Panther Branch (GUNR1) continued to display a discrete STRUCTURE pattern at $K = 15$ as it did at $K = 5$.

We also did not observe any strong evidence of past hatchery influences on Brook Trout in Maryland after running STRUCTURE using only the Pequest hatchery brood fish from New Jersey, assumed to be the most recent and major source for stocking from Maryland hatcheries. This observation indicated that hatchery

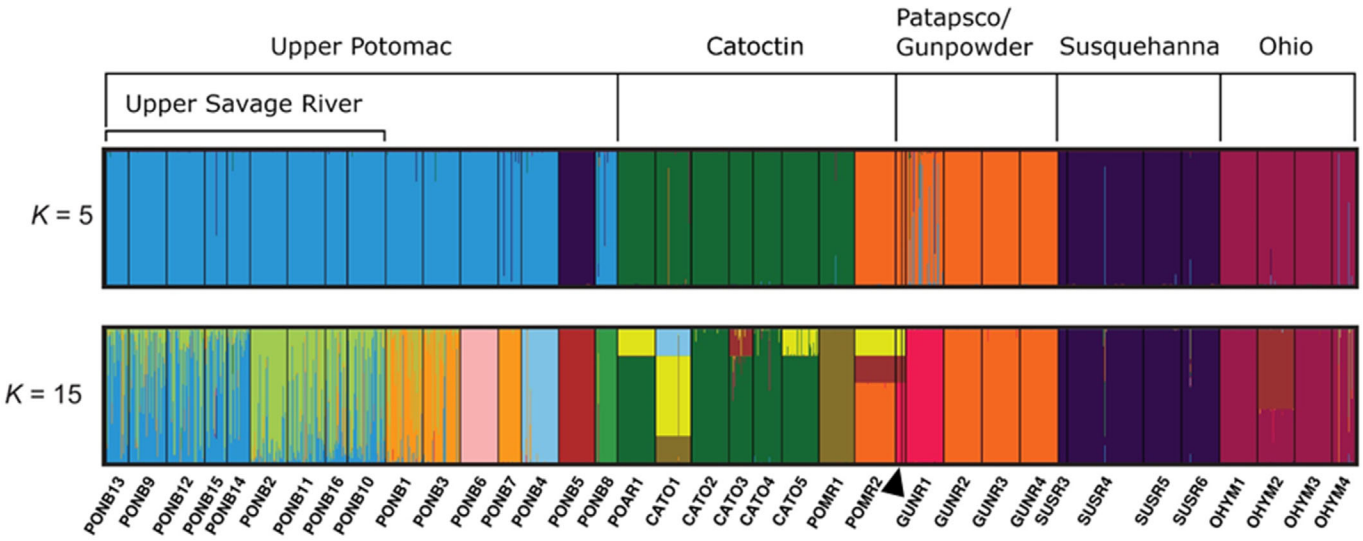

FIGURE 5. Bar plots from a STRUCTURE analysis showing clustering results for 38 collections of Brook Trout from five geographic regions across Maryland and two West Virginia streams. Each vertical bar corresponds to an individual, and the colors represent the posterior probabilities of belonging to each modelled STRUCTURE cluster at $K = 5$ and $K = 15$. Ohio represents Brook Trout collections from the Youghiogheny River. The black triangle denotes two populations (PATR1 and PATR 2) with small sample sizes from the Patapsco River.

introgression has not had any strong, widespread impact across Maryland streams. First, we observed clear watershed structuring of Brook Trout using STRUCTURE, where no aberrant patterns were found at either $K = 5$ or $K = 12$. Second, there was a high level of differentiation among most wild Brook Trout populations despite past stocking, which would be expected to homogenize populations.

The initial Brook Trout tree analysis produced an NJ tree having both strongly and weakly supported nodes. Upon examination of the STRUCTURE analyses, especially at $K = 15$, the data set was trimmed to 28 populations by the selective removal of 10 populations (Table 3), followed by construction of a NJ tree using 5,000 bootstrap iterations (Figure 6). The NJ tree with the trimmed data set still generated some smaller bootstrap values, but five major Maryland clusters were determined, which was consistent with STRUCTURE results. Overall, the tree structure of all five major groupings was consistent between the trimmed and untrimmed trees.

The PCA indicated similar patterns among the Maryland populations as determined by STRUCTURE, with divergent populations corresponding to these observations (Figure 7). For example, the unnamed tributary to Big Pipe Creek (POMR2) showed an affinity between the Gunpowder and Patapsco drainages, while Panther Branch (GUNR1) displayed a close relationship to Susquehanna Brook Trout. All other collections, except for a few of the highly fragmented populations (PONB4–6 and PONB8), agreed with grouping from STRUCTURE (Figure 5).

More importantly, plotting PCA axis 1 versus PCA axis 2 (Figure 7) showed a clear separation of eight selected hatcheries compared to native Brook Trout. The Pequest hatchery trout were distinct from all Maryland collections, an important observation in that Pequest trout were extensively used in Maryland hatcheries as broodstock (Heft et al. 2006). However, a more detailed assessment of hatchery introgression, as well as the effects of any undocumented stocking, is beyond the scope of this current effort due to the inability of obtaining samples from all past state, federal, and private hatchery sources (Heft et al. 2006).

DISCUSSION

Based on genetic analyses of Maryland Brook Trout, we found that (1) five distinct genetic groups were observed, (2) most fragmented populations were highly differentiated from one another—with the exception of a larger interconnected network in the upper Savage watershed that appears to harbor a metapopulation, and (3) there was no evidence of widespread hatchery introgression. In our analysis, 90.9% of comparisons among populations had pairwise F'_{ST} scores ≥ 0.2, which is similar to the F'_{ST} distribution observed by Kazyak et al. (2018) in North Carolina Brook Trout. However, the upper Savage River watershed was a unique exception to this overall trend of isolation (Figure 4). Pairwise comparisons here showed the lowest levels of differentiation observed anywhere in Maryland (16 pairs of collections had F'_{ST} scores < 0.05). These low levels of genetic differentiation observed in the upper Savage River

TABLE 3. Rationale for removal of 10 Brook Trout collections from cluster analysis based on results from STRUCTURE (see Figure 5) analysis at $K = 5$ and $K = 15$ (following Morgan et al. 2004; Heft et al. 2006).

Collection location	Code	Rationale for collection removal based on stressors
Big Run	PONB1	Disconnected from other Savage River populations due to the presence of the Savage River reservoir (dam constructed in 1952).
Middle Fork Crabtree Creek	PONB3	Disconnected from other Savage River populations due to the presence of the Savage River reservoir.
Lostland Run	PONB4	Fragmented population due to severe acid mine drainage in the North Branch Potomac River as well as steep gradient in lower stream reach.
Hind Leg	PONB5	Fragmented population due to severe acid mine drainage.
Mill Run	PONB6	Fragmented population due to severe acid mine drainage, industrial effluent, and sewage discharges.
Difficult Run	PONB7	Fragmented population due to severe acid mine drainage.
Sand Spring Run	PONB8	Fragmented population isolated to headwater, highly isolated due to mining, forestry, limited agriculture, sewage inputs, and urbanization.
Big Hunting Creek	CATO1	Fragmented population due to small hydroelectric dam constructed in 1908. Also potentially affected by stocking of native and exotic trout, forestry, dam, and road effects.
Tuscarora Creek	POMR1	Disconnected from other east-slope Catoctin populations due to agriculture, forestry, and urbanization.
Panther Branch	GUNR1	Separated from other Gunpowder River populations by the presence of an upper dam built in 1932 and a lower dam built in 1914.

watershed exemplify genetic signatures expected in systems with large effective population sizes and interconnected habitats facilitating gene flow among populations (Holland et al. 2017; Jenkins and Stevens 2018).

Interestingly, even in the interconnected reaches of the upper Savage River watershed, there was some evidence for spatial genetic structure. For example, at $K = 15$, tributaries to the northern end of the watershed were distinguishable from those closer to the Savage Reservoir (Figure 5). The main stem of the upper Savage Reservoir (PONB10) appeared to contain some individuals from both clusters, which is consistent with observations from a previous telemetry study that found migratory individuals from both tributary groups occupying the main stem seasonally (Sell et al. 2014).

We observed that A_R increased as a function of N_{EPS} and decreased as a function of mean population-specific F'_{ST} (Figure 2, 3). Brook Trout collections with an $A_R \leq 5.0$ represented isolated populations with a lack of connectivity with any nearby populations. While this finding is consistent with theoretical expectations of isolation by both distance and genetic drift (Frankham et al. 2017), it highlights the apparent intensity of genetic drift and the subsequent loss of genetic diversity in Brook Trout populations in Maryland, especially given the relatively short time frames since some of the populations have become isolated (Figure 5). Genetically diverse Brook Trout populations were seldom observed where effective population sizes were less than 60 (Figure 2). This aligns well with recommendations of Franklin (1980) and Soulé (1980), who suggested that an N_{EPS} of 50 is required to avoid harmful impacts in the short term (see Frankham et al. 2014 for other perspectives).

In contrast to the majority of collections that conformed well to HWE expectations and showed few loci in LD, the analysis of 19 of 78 pairs of loci from Panther Branch (GUNR1) revealed a statistically significant LD (Table 1). This observation could reflect (1) a small effective number of breeders, (2) sampling of multiple co-occurring populations or the presence of hatchery and wild trout, or (3) population structure consisting of a mixture of mtDNA clades A and B (Hall et al. 2002). Our estimate for the effective population size was small ($N_{EPS} = 19.8$; 95% CI = 16–24) but not unusually so in comparison to other isolated populations. Our analysis with COLONY identified only one pair of full siblings in the Panther Branch collection (Table 1). Furthermore, STRUCTURE analyses at $K = 15$ indicated that Panther Branch was unique in comparison to all other Brook Trout populations (Figure 5) and showed a mixed assemblage at $K = 5$.

There was an unanticipated example of strong population fragmentation in the Catoctin Mountains. In Big Hunting Creek (CATO1), Brook Trout were effectively isolated from other eastern slope Catoctin populations by installation of a small hydroelectric dam in 1908.

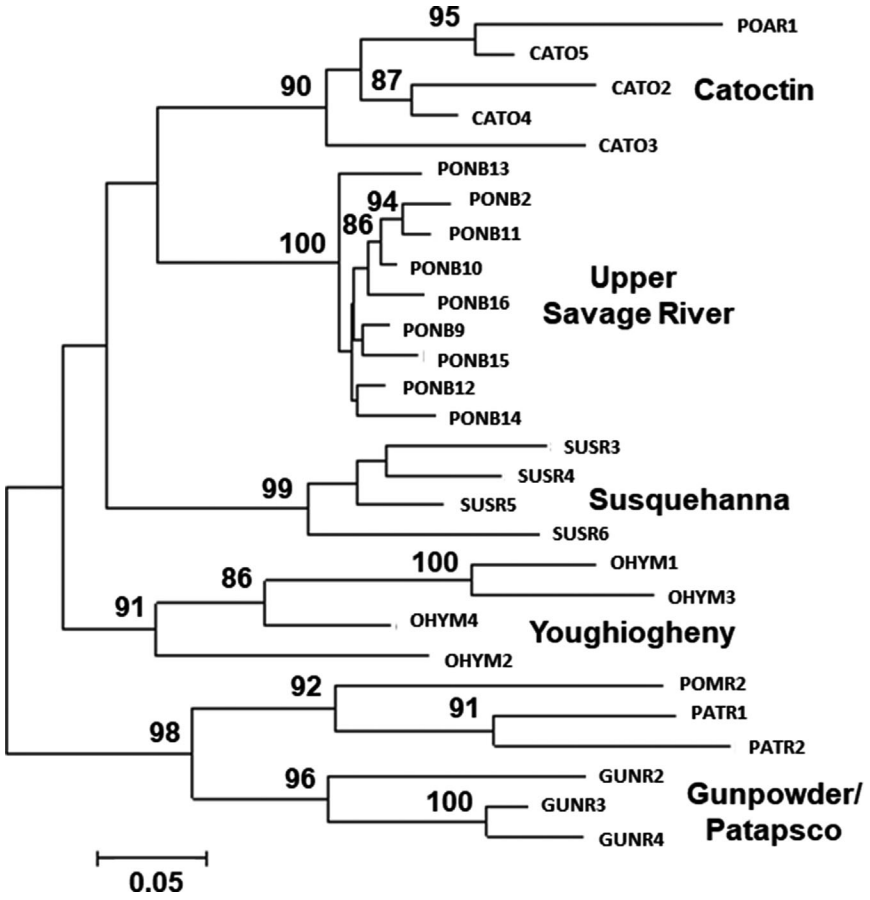

FIGURE 6. Expanded neighbor-joining (NJ) phenogram employing D_{SW} in POPTREEW. The phenogram represents the underlying genetic tree structure observed in the pairwise genetic distance matrix among 38 discrete Brook Trout collections. Numbers indicate percent bootstrap support for nodes based on 5,000 replications using 13 Brook Trout microsatellite loci.

Additional factors, such as road salt, sediment, thermal effects, and stocking of native and nonnative hatchery trout, have driven the Brook Trout genetic structure in Big Hunting Creek (Powell 1967; Heft et al. 2006), thus causing its strong genetic divergence over time (≈100 years) from other east slope Catoctin Mountain populations.

In addition, Brook Trout from an unnamed tributary to Big Pipe Creek (POMR2) showed a strong affinity with the Gunpowder–Patapsco stream complex (Figures 5, 6, and 7), especially Norris Run (PATR1) and Timber Run (PATR2) of the upper Patapsco River. However, Big Pipe Creek drains westward into the Monocacy River. This potentially represents a past stream capture event, with upper Big Pipe Creek either capturing streams in the upper Patapsco or the western Gunpowder drainages (D. Krantz, University of Toledo, personal communication).

There are numerous population genetic models that explain any potential changes in the genetic structure of a fish population (Waples et al. 2016); for example, isolation by distance, fragmentation, selection and genetic drift. For Maryland Brook Trout and possibly other Appalachian fish species (and other stream organisms), isolation by anthropogenic disturbances also may be an alternative genetic model, especially in those areas of rapid urbanization with extensive impervious surface development (Stranko et al. 2008). Snyder et al. (2015) pointed out that habitat fragmentation due to the increasing presence and persistence of thermal barriers in headwater streams inhabited by Brook Trout may adversely affect population variability. For example, several populations of Brook Trout along the North Branch Potomac River display large differences in genetic structure in comparison to more connected populations in the upper Savage River (Figures 5 and 6). Past activities, including deforestation, limited agriculture, and mining, served to isolate these upper Potomac Brook Trout populations (Staubitz and Sobashinski 1983; Gude 1984; Gutheim 1986; Stanton 1993), with acid mine discharges being a significant isolating factor as observed in other Brook Trout populations (Buonaccorsi et al. 2017).

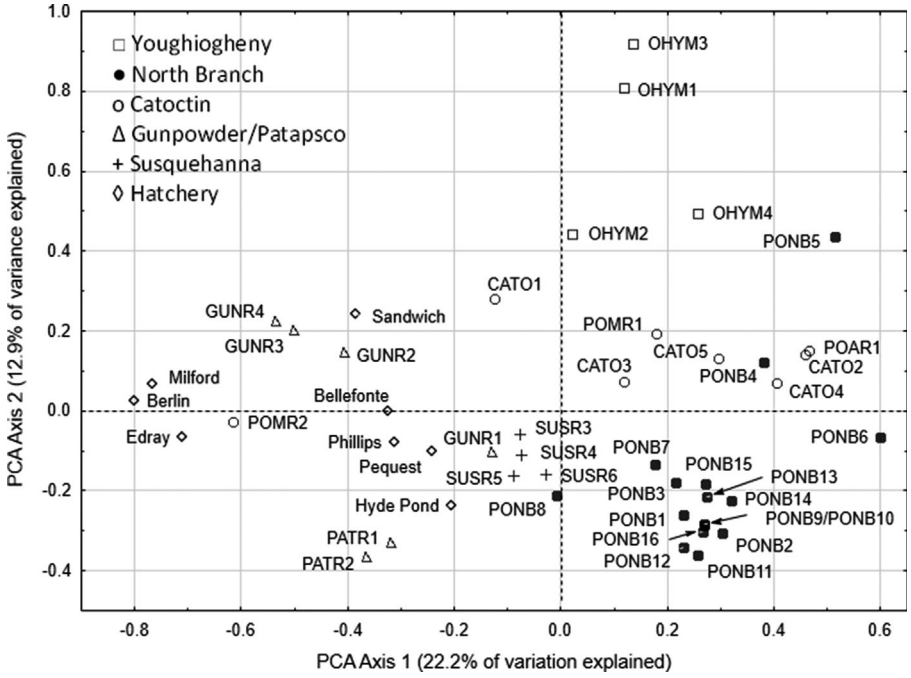

FIGURE 7. Visualization of principal coordinates analysis results (PCA axis 1 versus PCA axis 2) for 38 collections of Brook Trout in Maryland and West Virginia and eight regional hatchery collections.

It is difficult to recreate the genetic structure of a Brook Trout population once it becomes fragmented. Within the upper Potomac River complex, several populations represent Brook Trout effectively isolated since at least 1950 displaying strong genetic drift signatures based on the microsatellite data, similar to genetic drift patterns observed by Zastavniouk et al. (2017). In part, Maryland populations were effectively isolated due to mining influences and other stream pollutants in the upper North Branch Potomac River since 1950 and perhaps even earlier.

Based on the microsatellite comparison of Maryland and eight hatchery collections in both STRUCTURE analysis and PCA, our study suggests that stocking has not had any widespread homogenizing influence on native Brook Trout, as also noted by Hall et al. (2002). In general, other studies on Brook Trout stocking have also noted that hatchery introgression in the eastern United States is not nearly as pervasive as previously thought (Annett et al. 2012; Kazyak et al. 2018; Pregler et al. 2018; White et al. 2018; Beer et al. 2019). This finding is in contrast to the observation that other Brook Trout populations along the Appalachians and Midwest have been influenced by hatchery stocks (e.g., Wisconsin, Krueger and Menzel 1979; southern Appalachians, Hayes et al. 1996; Pennsylvania, Buonaccorsi et al. 2017; New York, Beer et al. 2019). Although Maryland has not stocked hatchery-reared Brook Trout since the early 1980s, there is still a potential for past, and perhaps even present, undocumented translocations and private stocking of Brook Trout (from hatcheries with unknown broodstock) throughout Maryland, with efforts dating back to 1877 and continuing into the 1970s and 1980s (Elser 1961; Powell 1967; Heft et al. 2006).

Future Directions for Maryland Brook Trout Management and Conservation

The future for Brook Trout in Maryland is forbidding, as Maryland now contains far less than 5% of its precolonial Brook Trout numbers (Morgan et al. 2004) based on precolonial statewide population estimates generated by both Roth et al. (1999) and Morgan et al. (2004). Moreover, there are recent surveys providing evidence of continued contraction and extirpation of Brook Trout throughout Maryland (Sell and Heft 2019), with anthropogenic threats continuing to intensify within their native range (Heft et al. 2006; Hudy et al. 2006, 2008; Stranko et al. 2008; Kovach et al. 2019; Muhlfeld et al. 2019). Currently, Brook Trout now occupy only 7.4% of the total stream kilometers west of the Maryland fall line (Sell and Heft 2019). The Maryland Department of Natural Resources is considering restricting Brook Trout harvest in Maryland by proposing a zero-harvest regulation for all Brook Trout populations east of Interstate 81 (COMAR 2020).

By the end of this century, Brook Trout may completely disappear from certain critical conservation regions, such as those on the Piedmont, with increasing

stream temperatures (Meisner 1990; Marschall and Crowder 1996; Clark et al. 2001; Curry and MacNeill 2004; Wenger et al. 2011; Trumbo et al. 2014; Briggs et al. 2018; Kovach et al. 2019; see Snyder et al. 2015 for a discussion of fine-grain variability). Based on current climatic predictions for stream temperatures, Brook Trout in the high-elevation Youghiogheny River and Savage River drainages may be the only Maryland Brook Trout populations to potentially not be severely affected by climate change (Rice and Jastram 2015; USGCRP 2018). However, there remains a potential for future thermal bottlenecks that may affect fish size, fecundity, and early life stages (Barneche et al. 2018; Dahlke et al. 2020).

Throughout the course of this genetic study, we made several observations related to the outlook for Brook Trout in Maryland. First, the upper Savage River watershed is very unique relative to other Maryland watersheds and other watersheds to the south. Our estimate of the effective population size was very large compared to most other populations at this latitude (Table 1), and the low levels of differentiation among collections in this area suggest that at least some gene flow is likely ongoing (Figure 4) with the potential existence of a metapopulation. Metapopulations are more likely to persist than isolated populations under challenging environmental conditions, as source–sink dynamics allow habitats to be recolonized and gene flow promotes the maintenance of genetic diversity necessary for future adaptation (Fagan 2002; Fraser et al. 2011).

In contrast, several isolated Brook Trout collections had very small effective population sizes, less genetic diversity, and high levels of differentiation in comparison to other neighboring populations. These biotic characteristics suggest that reproduction is dominated by a few individuals and/or the population is very small and that genetic diversity is being lost from fragmentation and subsequent genetic drift (Whitely et al. 2013). For many of the effective population sizes observed in Maryland Brook Trout, the loss of diversity through genetic drift is expected to occur rapidly and may overwhelm natural selection (Grueber et al. 2013; Frankham et al. 2017). Since standing genetic diversity is thought to be critical for rapid adaptation (Barrett and Schluter 2008; Bitter et al. 2019), these populations could be faced with the prospect of reduced capacity for adaptation during a period of rapid, unprecedented environmental change that has already resulted in numerous extirpations across Maryland (Morgan et al. 2004; Heft et al. 2006; Stranko et al. 2008; Sell and Heft 2019).

Management based on the conservation of evolutionary lineages of fish populations has become a widely utilized and successful conservation strategy (Meffe and Carroll 1994; Waples 1995; Ferchaud et al. 2020). The transitional status of mid-Atlantic Brook Trout populations provides an opportunity to conserve significant amounts of genetic diversity within a relatively small area. The natural history (i.e., stream capture, historical isolation, etc.) of the streams sampled from this region may have made a greater contribution to extant assemblage structure than any anthropomorphic actions (i.e., stocking or translocation).

Consistent management for Brook Trout populations in Maryland may possibly prevent genetic erosion and preserve the ability of populations to respond to future environmental changes. Since our current study suggests that watersheds account for a substantial amount of genetic variation, these represent potential conservation units. However, we also found greater genetic variation among collections within watersheds than at broader geographic scales. Thus, management to conserve genetic diversity may require extensive consideration of fine-scale differences among Brook Trout populations (Kelson et al. 2015), especially with a strong focus on conservation prioritization at the individual population level (Bruce et al. 2019). Developing this level of genetic information for individual and regional populations has direct relevance to fisheries management and conservation activities of state and federal agencies tasked with managing these resources. As an example, the Maryland Department of Natural Resources is developing a classification system for individual statewide Brook Trout populations to direct conservation efforts for those populations that are the most resilient for long-term survival. Brook Trout populations are rated on five criteria overall, one of which is an effective population size (N_{EPS}) of at least 100. The use of N_{EPS} as a criterion highlights the importance of using genetic information for direct relevance to fisheries management and conservation issues (Ferchaud et al. 2020).

Investigation of adaptive genetic variation has not been extended to Brook Trout populations across the extant species range and could further inform management of evolutionary lineages. While the genetic basis for adaptation in Brook Trout remains largely unknown, further understanding of adaptive genetic variation could inform management of populations to conserve their long-term adaptive potential, particularly in relation to adaptation to anthropogenic disturbance and climate change over time (Fraser et al. 2014; Pelletier et al. 2020). Moreover, continued exploration of adaptive and deleterious genetic variation in isolated populations may provide much-needed insight into appropriate management strategies (Ferchaud et al. 2020).

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Appendix 1: Sample Collection Information

TABLE A.1.1. Summary of sample collections made for Maryland Brook Trout from 1999 to 2018. All collections are from Maryland waters unless noted by a state abbreviation. River is abbreviated as "R." in drainage names; North is abbreviated as "N." Null collections are not presented in Appendix 1.

ID code (*N*)	Primary drainage	Collection location and year	Latitude	Longitude
	North Branch Potomac River/upper Potomac River (Atlantic Slope)/middle Chesapeake Bay			
PONB1 (49)	Savage R./N. Branch Potomac R./Potomac R.	Big Run 1999	39.5644	−79.1547
PONB2 (49)	Savage R./N. Branch Potomac R./Potomac R.	Upper Poplar Lick 1999	39.6059	−79.1250
PONB3 (49)	Savage R./N. Branch Potomac R./Potomac R.	Middle Fork Crabtree Creek 2005	39.5157	−79.1689
PONB4 (50)	Savage R./N. Branch Potomac R./Potomac R.	Lostland Run 1999	39.3707	−79.2526
PONB5 (48)	Savage R./N. Branch Potomac R./Potomac R.	Hind Leg (WV) 2002	39.2616	−79.3812
PONB6 (50)	N. Branch Potomac R./Potomac R.	Mill Run 2005	39.5437	−78.8999
PONB7 (30)	N. Branch Potomac R./Potomac R.	Difficult Run (WV) 2006	39.2741	−79.3130
PONB8 (29)	Georges Creek/N. Branch Potomac R./ Potomac R.	Sand Spring Run 2009	39.6650	−78.9386
PONB9 (50)	Savage R./N. Branch Potomac R./Potomac R.	Little Savage River 2014	39.5950	−79.0519
PONB10 (50)	Savage R./N. Branch Potomac R./Potomac R.	Main-stem Savage River 2014	39.5654	−79.1080
PONB11 (50)	Savage R./N. Branch Potomac R./Potomac R.	Lower Poplar Lick 2014	39.5864	−79.0954
PONB12 (50)	Savage R./N. Branch Potomac R./Potomac R.	Blue Lick 2014	39.6206	−79.0667
PONB13 (38)	Savage R./N. Branch Potomac R./Potomac R.	Mud Lick 2018	39.6433	−79.0218
PONB14 (30)	Savage R./N. Branch Potomac R./Potomac R.	Elk Lick 2018	39.6036	−79.0900
PONB15 (29)	Savage R./N. Branch Potomac R./Potomac R.	Black Lick 2018	39.6067	−79.0800
PONB16 (29)	Savage R./N. Branch Potomac R./Potomac R.	Bear Pen Run 2018	39.5631	−79.1121
	Central Potomac River (Atlantic Slope)/middle Chesapeake Bay			
POARI (50)	Little Antietam Creek/Antietam Creek/ Potomac R.	Warner Hollow Run 2005	39.6659	−77.5459
CATO1 (47)	Monocacy R./Potomac R.	Big Hunting Creek 2000	39.6349	−77.4789
CATO2 (49)	Monocacy R./Potomac R.	Owens Creek 2000	39.6632	−77.4804
CATO3 (32)	Monocacy R./Potomac R.	Still Creek 2000	39.6363	−77.4500
CATO4 (38)	Monocacy R./Potomac R.	Right Fork Fishing Creek 2000	39.5432	−77.4647
CATO5 (48)	Monocacy R./Potomac R.	Left Fork Fishing Creek 1999	39.5327	−77.4752
POMR1 (47)	Monocacy R./Potomac R.	Tuscarora Creek 1999	39.4734	−77.4152
POMR2 (54)	Big Pipe Creek/Monocacy R./Potomac R.	UT to Big Pipe Creek 2018	39.6543	−76.9077
	Patapsco River/upper Chesapeake Bay			
PATR1 (8)	Patapsco R./Chesapeake Bay	Norris Run 2001	39.4620	−76.8741
PATR2 (5)	Patapsco R./Chesapeake Bay	Timber Run 2001	39.4376	−76.8689
	Gunpowder River/upper Chesapeake Bay			
GUNR1 (50)	Gunpowder R./Chesapeake Bay	Panther Branch 2001	39.5981	−76.6531
GUNR2 (50)	Gunpowder R./Chesapeake Bay	Indian Run 2001	39.6580	−76.8139
GUNR3 (50)	Gunpowder R./Chesapeake Bay	Silver Run 2011	39.6860	−76.7698
GUNR4 (50)	Gunpowder R./Chesapeake Bay	Walker Run 2011	39.6905	−76.7767
	Deer Creek/Susquehanna River/upper Chesapeake Bay			
SUSR3 (12)	Deer Creek/Susquehanna R./Chesapeake Bay	Kellogg Branch 2000	39.6295	−76.4209
SUSR4 (100)	Deer Creek/Susquehanna R./Chesapeake Bay	Rock Hollow Run 2008	39.6481	−76.4454

TABLE A.1.1. Continued.

ID code (*N*)	Primary drainage	Collection location and year	Latitude	Longitude
SUSR5 (50)	Deer Creek/Susquehanna R./Chesapeake Bay	Kellogg Branch 2008	39.6295	−76.4209
SUSR6 (50)	Little Deer Creek/ Deer Creek/Susquehanna R.	Spooners Creek 2008	39.6438	−76.4790
	Youghiogheny River/Monongahela River/Ohio River/Mississippi River (interior drainage)			
OHYM1 (49)	Youghiogheny R./Monongahela R./Ohio R.	Puzzley Run 1998	39.7148	−79.2305
OHYM2 (49)	L. Youghiogheny R./Youghiogheny R./ Monongahela R./Ohio R.	Black Run 1999	39.4016	−79.3043
OHYM3 (49)	Youghiogheny R./Monongahela R./Ohio R.	Little Bear Creek 1999	39.6588	−79.2739
OHYM4 (30)	Youghiogheny R./Monongahela R./Ohio R.	Casselman River 2003	39.6078	−79.1952

Appendix 2: Pairwise F'_{ST} Values

TABLE A.2.1. Matrix showing pairwise F'_{ST} values among 38 collections of wild Brook Trout from Maryland and West Virginia. Collection site codes are presented in Table 1 and Appendix Table A.1.1.

	PONB13	PONB9	PONB12	PONB15	PONB14	PONB2	PONB11	PONB16	PONB10	PONB1	PONB3	PONB6	PONB7	PONB4	PONB5	PONB8	POAR1	CATO1	CATO2	CATO3	CATO4	CATO5	POMR1	POMR2	PATR1	PATR2	GUNR1	GUNR2	GUNR3	GUNR4	SUSR3	SUSR4	SUSR5	SUSR6	OHYM1	OHYM2	OHYM3
PONB9	0.04																																				
PONB12	0.07	0.03																																			
PONB15	0.07	0.03	0.03																																		
PONB14	0.10	0.06	0.05	0.06																																	
PONB2	0.13	0.08	0.08	0.11	0.15																																
PONB11	0.12	0.08	0.05	0.10	0.10	0.04																															
PONB16	0.08	0.05	0.03	0.05	0.06	0.05	0.03																														
PONB10	0.08	0.01	0.01	0.02	0.06	0.04	0.02	0.02																													
PONB1	0.14	0.12	0.10	0.15	0.17	0.12	0.14	0.09	0.10																												
PONB3	0.17	0.14	0.13	0.14	0.17	0.15	0.15	0.12	0.12	0.09																											
PONB6	0.42	0.49	0.49	0.47	0.47	0.51	0.49	0.46	0.48	0.42	0.41																										
PONB7	0.25	0.24	0.26	0.30	0.29	0.31	0.29	0.25	0.25	0.17	0.24	0.53																									
PONB4	0.45	0.45	0.43	0.40	0.41	0.50	0.50	0.41	0.45	0.43	0.42	0.53	0.47																								
PONB5	0.71	0.75	0.81	0.74	0.79	0.78	0.81	0.78	0.77	0.72	0.73	0.74	0.61	0.75																							
PONB8	0.36	0.37	0.29	0.31	0.36	0.36	0.33	0.29	0.33	0.29	0.31	0.61	0.41	0.58	0.81																						
POAR1	0.56	0.59	0.59	0.57	0.55	0.58	0.62	0.59	0.58	0.54	0.50	0.62	0.55	0.67	0.73	0.65																					
CATO1	0.54	0.58	0.60	0.59	0.55	0.62	0.62	0.59	0.59	0.57	0.55	0.73	0.56	0.65	0.81	0.63	0.62																				
CATO2	0.42	0.42	0.43	0.37	0.38	0.44	0.49	0.43	0.44	0.45	0.40	0.55	0.54	0.50	0.75	0.59	0.46	0.54																			
CATO3	0.43	0.45	0.43	0.38	0.42	0.46	0.47	0.42	0.42	0.42	0.33	0.61	0.39	0.61	0.68	0.42	0.39	0.55	0.45																		
CATO4	0.32	0.32	0.31	0.26	0.29	0.34	0.38	0.34	0.33	0.36	0.29	0.56	0.44	0.48	0.73	0.46	0.32	0.55	0.10	0.31																	
CATO5	0.42	0.42	0.42	0.36	0.41	0.46	0.48	0.43	0.43	0.45	0.40	0.60	0.49	0.57	0.74	0.55	0.23	0.56	0.29	0.27	0.14																
POMR1	0.37	0.39	0.41	0.34	0.43	0.42	0.49	0.43	0.42	0.44	0.35	0.63	0.52	0.55	0.73	0.53	0.55	0.50	0.34	0.49	0.27	0.36															
POMR2	0.65	0.68	0.67	0.69	0.72	0.72	0.70	0.68	0.69	0.62	0.68	0.81	0.60	0.77	0.90	0.62	0.80	0.67	0.79	0.64	0.76	0.72	0.79														
PATR1	0.56	0.52	0.50	0.51	0.56	0.55	0.55	0.50	0.51	0.44	0.51	0.74	0.47	0.66	0.88	0.51	0.71	0.67	0.73	0.57	0.65	0.63	0.71	0.52													
PATR2	0.61	0.59	0.57	0.60	0.62	0.63	0.62	0.59	0.58	0.52	0.57	0.77	0.58	0.78	0.91	0.56	0.69	0.69	0.71	0.61	0.65	0.65	0.72	0.53	0.13												
GUNR1	0.33	0.29	0.29	0.29	0.33	0.33	0.33	0.28	0.31	0.31	0.27	0.66	0.34	0.54	0.80	0.40	0.65	0.53	0.53	0.41	0.43	0.48	0.48	0.51	0.35	0.49											
GUNR2	0.62	0.63	0.62	0.62	0.67	0.63	0.65	0.63	0.62	0.57	0.60	0.84	0.54	0.74	0.83	0.58	0.75	0.65	0.77	0.54	0.70	0.68	0.70	0.47	0.54	0.63	0.50										
GUNR3	0.58	0.58	0.57	0.58	0.60	0.62	0.60	0.60	0.58	0.59	0.55	0.85	0.59	0.78	0.92	0.56	0.84	0.54	0.75	0.60	0.71	0.71	0.67	0.51	0.59	0.66	0.35	0.41									
GUNR4	0.64	0.65	0.65	0.65	0.66	0.67	0.67	0.66	0.65	0.66	0.64	0.88	0.64	0.84	0.90	0.62	0.85	0.53	0.77	0.60	0.74	0.74	0.70	0.59	0.63	0.72	0.44	0.43	0.14								
SUSR3	0.43	0.41	0.42	0.40	0.41	0.45	0.39	0.38	0.42	0.44	0.40	0.70	0.46	0.69	0.77	0.45	0.70	0.55	0.67	0.50	0.57	0.54	0.47	0.67	0.67	0.71	0.43	0.67	0.59	0.67							
SUSR4	0.47	0.45	0.44	0.44	0.48	0.46	0.41	0.41	0.44	0.42	0.42	0.72	0.46	0.71	0.75	0.42	0.68	0.67	0.73	0.49	0.59	0.54	0.54	0.65	0.63	0.68	0.44	0.64	0.60	0.67	0.12						
SUSR5	0.40	0.36	0.37	0.37	0.41	0.40	0.33	0.33	0.35	0.36	0.37	0.68	0.35	0.67	0.72	0.39	0.71	0.59	0.69	0.49	0.60	0.57	0.53	0.60	0.58	0.64	0.39	0.64	0.57	0.64	0.11	0.13					
SUSR6	0.50	0.45	0.46	0.45	0.48	0.46	0.40	0.40	0.44	0.44	0.41	0.70	0.50	0.75	0.79	0.52	0.74	0.70	0.68	0.47	0.60	0.61	0.65	0.67	0.62	0.69	0.44	0.65	0.61	0.67	0.24	0.26	0.25				
OHYM1	0.69	0.71	0.73	0.65	0.70	0.72	0.74	0.70	0.70	0.71	0.68	0.74	0.73	0.62	0.81	0.76	0.77	0.64	0.70	0.72	0.68	0.68	0.61	0.81	0.80	0.86	0.67	0.73	0.67	0.75	0.69	0.75	0.74	0.78			
OHYM2	0.53	0.56	0.56	0.52	0.52	0.55	0.57	0.52	0.55	0.53	0.48	0.65	0.55	0.59	0.76	0.55	0.65	0.55	0.56	0.52	0.51	0.55	0.48	0.70	0.75	0.79	0.54	0.68	0.59	0.66	0.49	0.53	0.52	0.58	0.44		
OHYM3	0.72	0.73	0.76	0.67	0.71	0.76	0.77	0.74	0.72	0.75	0.73	0.78	0.77	0.69	0.84	0.81	0.82	0.76	0.72	0.77	0.71	0.72	0.66	0.84	0.85	0.88	0.70	0.76	0.65	0.73	0.78	0.80	0.78	0.81	0.17	0.49	
OHYM4	0.50	0.49	0.51	0.44	0.44	0.49	0.50	0.48	0.46	0.50	0.48	0.66	0.50	0.52	0.76	0.64	0.62	0.62	0.51	0.55	0.45	0.53	0.48	0.75	0.69	0.73	0.52	0.65	0.64	0.73	0.62	0.64	0.62	0.66	0.27	0.41	0.28

Appendix 3: Comparison of STRUCTURE Model Likelihoods

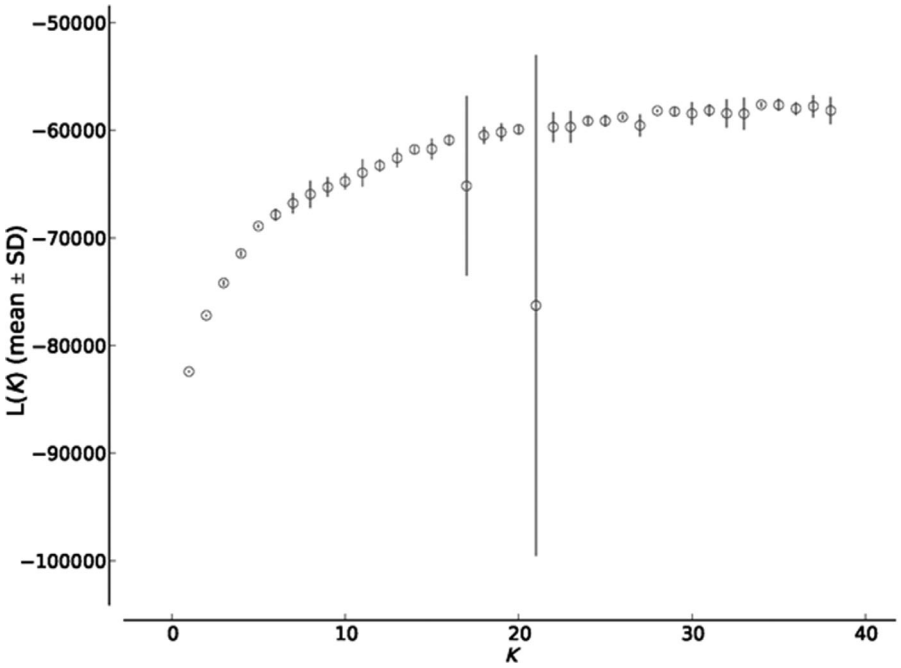

FIGURE A.3.1. Comparison of STRUCTURE model likelihoods for five iterations each of $K = 1$ through $K = 38$.