IDENTIFYING GENETIC DIVERSITY AND POPULATION STRUCTURE IN BROOK TROUT (*Salvelinus fontinalis*) POPULATIONS OF THE UPPER HUDSON RIVER AND ITS TRIBUTARIES

A Final Report of the Tibor T. Polgar Fellowship Program

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ABSTRACT

As anthropogenic impacts accelerate changes to landscapes across the globe, understanding how genetic population structure is influenced by habitat features and dispersal is key to preserving evolutionary potential at the species level. Furthermore, knowledge of these interactions is essential to identifying potential constraints on local adaptation and for the development of effective management strategies. These issues were addressed for Brook Trout (*Salvelinus fontinalis*) populations residing in the Upper Hudson River watershed of New York State by investigating the spatial genetic structure of over 300 fish collected from 14 different sampling locations encompassing three river systems (the Upper Hudson, the Boreas, and the Schroon). The results of this work suggest that fish in the area (i) exhibit varying degrees of introgression from State-directed stocking activities (ii) exhibit genetic population structure at the level of individual tributaries as well as the larger river systems where they are found, dictated by migration and influenced by habitat connectivity, and (iii) demonstrate comparatively similar measures of genetic diversity but varied measures of effective population size based on sampling location. These findings represent a significant contribution to the current literature surrounding Brook Trout migration and dispersal, especially as it relates to larger interconnected systems. Finally, this study is concluded with a discussion about how the methods used here may aid in the development of other species-focused conservation plans that incorporate an evolutionary perspective.

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INTRODUCTION

A major goal of conservation biology is to determine how anthropogenic influences are shaping wild populations and their genetic structure under altered habitat regimes (Bushar et al. 2014; Cornille et al. 2015; Inoue et al. 2015). Among the many fish species found in the upper Hudson River watershed, wild Brook Trout (*Salvelinus fontinalis*) are among the most likely to be negatively impacted by habitat alterations (Merriam et al. 2017). Understanding these patterns of biogeographic structure is essential to maintaining viability and evolutionary potential in changing landscapes (Abdul-Muneer 2014; Bruce et al. 2017).

Over 300 lakes and ponds in New York State are actively managed as Brook Trout habitat, with records of wild reproduction in over a hundred (Baker et al. 1990). Despite this fact, the number of wild, self-sustaining populations in New York is considered to be far lower, and is projected to make up only 5% of the total number of water bodies that have been sampled (Baker et al. 1990). Many New York Brook Trout populations have been manipulated with supplemental stocking, populations have been disconnected, and water quality declines have reduced habitat; therefore, sorting out present-day genetic structure has become exceedingly complex (Perkins et al. 1993a, b; Bruce et al. 2017). In addition, Brook Trout require highly oxygenated water at temperatures below 22°C, within a pH range of 5.0 - 7.5, making them acutely sensitive to acid deposition and warming waters (Smith 1985; Xu et al. 2010). Based on current climate projections, estimates by the United States Environmental Protection Agency (EPA) have predicted a 50-100% decline in Brook Trout abundance for the region by the year 2100 (EPA 2015). The degree to which Brook Trout populations have been, and are

predicted to be, impacted by direct and indirect anthropogenic factors makes it critical that regions exhibiting heightened levels of effective population size and comparatively distinct genetic structure be distinguished and preserved (Ficke et al. 2007; Gao et al. 2012).

In the upper Hudson River watershed, three main river systems act as the major hydrological feeders to the downstream Hudson River. These rivers include the Boreas River, the Schroon River and the upper Hudson itself. The river systems in the upper Hudson drainage are unique in that they are fed by a number of tributaries that possess ideal native Brook Trout habitat (DeWeber and Wagner 2015). In addition, the upper Hudson drainage has been subjected to decades long state-sanctioned stocking activities in the main stem of all three of these systems, with additional proximal stocking in nearby lakes and ponds for recreational angling that can be attributed to the State, the County, and a number of private hatcheries that stock on property owned by fish and game clubs in the region. Little is currently known about Brook Trout population genetic structure in this region (but see Perkins et al. 1993a, b; Bruce et al. 2017). In addition, a number of recent studies have shown relatively short dispersal distances for Brook Trout in streams with limited water flow (Hudy et al. 2005; Kanno et al. 2011; Kanno et al. 2014), but little information is available on rates and magnitude of dispersal and/or gene-flow between Brook Trout populations that are potentially connected by such larger riverine systems.

The overall goal of this study was to characterize the genetic structure of Brook Trout currently found in the upper Hudson River drainage of New York State. More specifically, this study sought to address three key questions concerning Brook Trout

genetic structure throughout the upper Hudson River drainage: (i) Have any of the Brook Trout in the area experienced introgression from known strains stocked by the New York State Department of Environmental Conservation (NYSDEC), and if so, to what extent? (ii) What level of population structure is present in the area and is ongoing migration occurring between populations, or do the river systems act as a barrier to gene flow? (iii) How do levels of effective population size and genetic diversity compare between sampling sites throughout the region? Thus, the goals of this study were to 1) compare genetic data from wild collected samples to those of various stocked strains to estimate any reciprocal ancestry that may be present due to introgression; 2) assess population structure in the area by employing current microsatellite techniques for population genetics; 3) use diversity indices to quantify effective population size and the level of genetic diversity present in the area, such as heterozygosity and allelic richness. In conclusion, the management implications of this work is discussed as well as how the methods used here may help to inform future conservation strategies related to stream and river dwelling fish species.

METHODS

Study Sites and Sample Collection

Upper caudal fin clippings (n = 337) were taken from Brook Trout in the upper Hudson River drainage (Figure 1) in the summer of 2016 and 2017. Sample sites were chosen based on accessibility and relevance to the study goals. All fish were captured using a Model 12-B Smith-Root backpack electrofishing unit across an approximately 50-100 m stretch of stream with the exception of the fish from the Dix-Elk watershed, which were

collected in 2014 from multiple tributaries within the system and have previously been shown to be a single panmictic population (Bruce et al. 2017). Fin clippings were taken using sterile technique and stored in 95% ethanol. In addition to fin clip samples, GPS coordinates were recorded for each individual sample site, as well as date and time. Also included in this study are six additional previously collected and genotyped reference groups, acquired between 2015 and 2016 from State run hatcheries (n = 185), in this study. These populations were specifically bred for stocking purposes by the New York State Department of Environmental Conservation and are commonly used for supplementation in the study area.

DNA Extraction and Genotyping

All DNA extraction and quantification for this study was carried out at the New York State Museum in Albany, NY. DNA for each individual fish was extracted following the tissue protocol included with the QIAGEN DNeasy tissue kit (QIAGEN, Inc., Valencia, California). Thirteen autosomal microsatellite loci were genotyped using the same procedures for all individuals. Polymerase Chain Reaction (PCR) was used to amplify loci using primer pairs created specifically for Brook Trout (*SfoB52*, *SfoC24, SfoC28, SfoC38, SfoC79, SfoC86, SfoC88, SfoC113*, *SfoC115*, *SfoC129, SfoD75, SfoD91, SfoD100*; T. L. King 2006). The forward primers were fluorescently labeled with HEX, FAM, or NED dye for downstream electropherogram analysis. PCR-related methods resulted in five 20 ul multiplex PCR reactions and one single 20 ul PCR reaction for each individual fish. PCR amplification was carried out in the Research & Collections Molecular Laboratory at the New York State Museum using two Bio-Rad T100 thermal

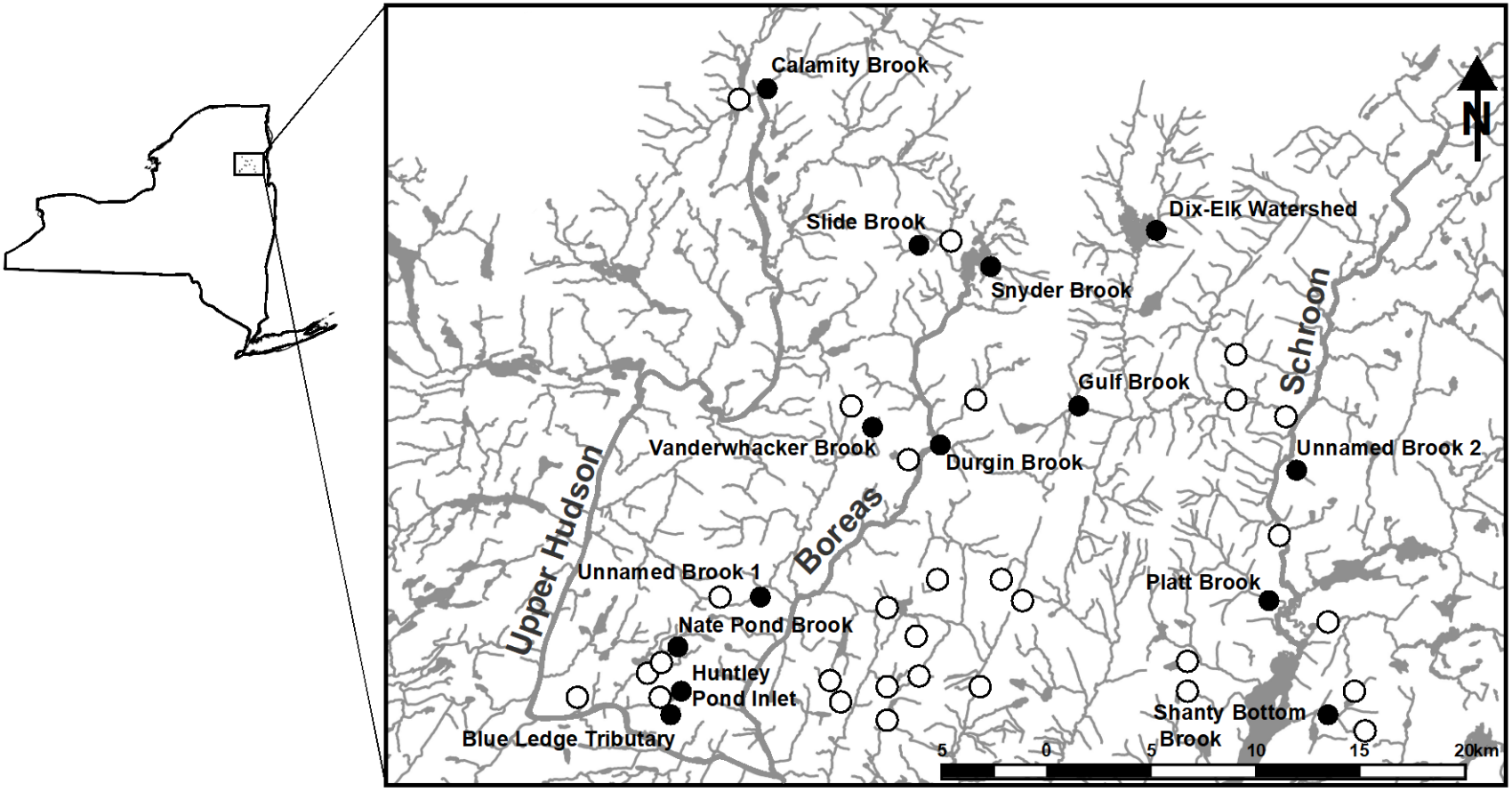

Figure 1: **Map of New York State with inset of study area. Labeled sample site locations represent the geographic center of sampling, while open circles demarcate proximal documented stocking locations. Hydrology is demarcated in grey while the major river systems are also labeled in grey.**

cyclers. Fragment analysis using an internal size standard (Liz600, Applied Biosystems) was performed at the University at Albany Center for Functional Genomics using an Applied BioSystems 3730XL DNA Analyzer. The automated scoring of genotypes was carried out using the Geneious 4.0 (Biomatters Ltd.) software package. All automated genotype calls were confirmed by eye.

Neutrality Testing and Summary Statistics

All thirteen microsatellite loci included in this study were subjected to outlier tests of neutrality using the LOSITAN workbench (Antao et al. 2008). An initial simulation was run to remove potentially non-neutral loci before computing the genomic mean F_{ST} for downstream analysis, while also running a bisection algorithm over repeated simulations to approximate a desired F_{ST}. Each sampling location was treated as a putative population employing a drift with migration model, assuming stepwise mutation across one million simulations. In addition, all samples were subjected to exact tests of Hardy-Weinberg equilibrium (Guo and Thompson 1992) using the ARLEQUIN 3.5 software package (Excoffier and Lischer 2010). Deviations from Hardy-Weinberg equilibrium were tested against 1,000,000 random permutations. Tests for Linkage Disequilibrium were also carried out using the log-likelihood ratio test as implemented in the program GENEPOP (Rousset 2008), between all pairs of loci, for all sample groupings.

Summary statistics were also calculated, including observed and expected heterozygosity using the ARLEQUIN 3.5 software package. In addition, the inbreeding

coefficient (F_{IS}; Weir and Cockerham 1984) was calculated using GENEPOP and measures of allelic richness were calculated across all sampling locations while correcting for differences in sample size using the FSTAT 2.9.3.2 software package (Goudet 2001). Effective population size (N_e) with 95% parametric confidence intervals were also calculated using NeEstimator V2.01 (Do et al. 2014). Finally, pairwise F_{ST} tests (Hardy Weinberg Equilibrium not assumed) were performed, using Weir and Cockerham's unbiased estimator of F_{ST} (theta) using the FSTAT 2.9.3.2 software package.

Cluster Analysis and Migration Rates

All individuals in this study were initially subjected to cluster analysis to infer any potential introgression from hatchery fish commonly stocked in the region by the NYSDEC. Inferred ancestry for each sampling locale was determined using the Bayesian clustering approach executed by the program STRUCTURE (Version 2.3; Pritchard et al. 2000). STRUCTURE estimates the shared population history of individuals based on their genotypes and is therefore able to assign individuals to one of K populations based on the probability associated with its genotype, or to multiple populations if an individual's genotype appears mixed (Pritchard et al. 2009). Each sampling locality was run individually with all six strains used for supplemental stocking, assuming an admixture model with correlated allele frequencies. The analysis was run with a burn-in step of 50,000 Markov Chain Monte Carlo (MCMC) iterations, followed by 300,000 MCMC iterations. Five replicates for each K-value were performed assuming K=4 through K=8 to examine population structure across potentially different groupings.

Sampling areas that appeared to be significantly influenced by stocked strains were then removed from downstream analysis to avoid potential errors in determining natural population genetic structure and migration rates across the landscape.

Cluster analysis was then performed a second time, excluding the strains used for supplemental stocking as well as two sampling locales that exhibited heightened levels of hatchery ancestry (Q-value $\leq$ 0.30). The analysis was again run with a burn-in step of 50,000 Markov Chain Monte Carlo (MCMC) iterations, followed by 300,000 MCMC iterations with five replicates for each K value, but this time assuming K=1 through K=15 to examine population structure across the wild-collected sample set. The program CLUMPAK (Clustering Markov Packager Across K) (Kopelman et al. 2015) was then used to identify sets of highly similar runs and permute clusters identified by STRUCTURE across independent runs for the purpose of producing bar plots for visualization. In order to assess the number of distinct groupings across all of the scenarios tested, the Evanno method (Evanno et al. 2005) was used to evaluate the best supported K value, as well as the value where the mean likelihood Ln(K) plateaus across increasing K (Evanno et al. 2005, Earl and vonHoldt 2012) using the web program STRUCTURE HARVESTER (Earl and vonHoldt 2012).

Finally, recent migration rates between wild populations that showed minimal influence from stocked strains were estimated using the program BAYESASS (Wilson and Rannala 2003). BAYESASS employs a Bayesian statistical framework to estimate rates of immigration using individual multilocus genotypes. The Markov chain Monte Carlo technique implemented by the program is used to estimate joint posterior probability distributions of parameters. BAYESASS considers migrants up to two

generations back and can be applied to dynamic populations that do not meet standard expectations for Hardy–Weinberg or genetic equilibrium. The Dix-Elk population was excluded from this analysis since it was the only population not collected in the 2016-2017 timeframe. BAYESASS was run for 2,000,000 iterations with a burn-in of 1,000,000 iterations, sampling every 200 iterations after burn-in to estimate parameters.

RESULTS

Results of the neutrality testing using the LOSITAN workbench suggested that all loci were selectively neutral and therefore suitable for further analysis. Out of 182 tests across all sampling sites, a single locus tested positive for Hardy Weinberg disequilibrium in the Shanty Bottom Brook population (following Bonferroni correction $\alpha = 0.05$, initial nominal P value = 0.002). Tests for linkage disequilibrium resulted in three significant pairwise occurrences: two between loci from Snyder Brook and one between loci from Unnamed brook 1 out of 1,092 pairwise tests (following Bonferroni correction $\alpha = 0.05$, initial nominal P value = 0.00005).

Genetic diversity indices did show variation between sampling locales, but estimates were generally in the same range across the putative populations sampled (H_E: 0.51–0.69; A: 43.70–71.13; Table 1). The highest levels of genetic diversity were exhibited by Huntley Pond Inlet ($H_E = 0.69$, $A = 71.13$) whereas some of the lowest estimates of genetic diversity were attributed to both Gulf Brook ($H_E = 0.56$, $A = 43.70$) and Slide Brook ($H_E = 0.51$, $A = 58.33$).

River system	Sampling location	N	H_E	H_O	A	F_{IS}	N_e
Hudson	Calamity Brook	29	0.63	0.64	60.39	-0.004	27.2(19.0–42.4)
	Nate Pond Brook	26	0.68	0.70	69.67	-0.028	44.8(29.0–85.7)
	Huntley Pond Inlet	30	0.69	0.71	71.13	-0.029	67.4(41.8–147.1)
	Blue Ledge Tributary	16	0.63	0.64	59.88	-0.012	14.8(9.4–26-2)
Boreas	Slide Brook	17	0.51	0.51	58.33	0.019	75.6(27.2–∞)
	Snyder Brook	28	0.55	0.51	59.62	0.083	33.0(21.6–58.8)
	Durgin Brook	27	0.60	0.57	67.70	0.040	114.8(50.9–∞)
	Vanderwhacker Brook	17	0.62	0.58	60.58	0.062	17.3(10.8–32.9)
	Unnamed Brook 1	30	0.61	0.63	64.80	-0.045	315.3(80.3–∞)
Schroon	Dix-Elk Tributaries	25	0.60	0.58	62.72	0.039	254.3(67.3–∞)
	Gulf Brook	17	0.56	0.53	43.70	0.043	74.6(26.3–∞)
	Unnamed Brook 2	13	0.61	0.57	64.00	0.065	46.4(18.1–∞)
	Platt Creek	29	0.56	0.57	59.40	-0.012	60.7(33.2–200.7)
	Shanty Bottom Brook	27	0.62	0.60	64.24	0.041	60.7(33.9–185.9)

Table 1: Sample sizes and summary statistics for all wild caught individuals. N = Number of specimens, H_E = Mean expected heterozygosity, H_O = Mean observed heterozygosity, A = Total allelic richness (based on a minimum sample size of 13 individuals), F_{IS} = Wright's inbreeding coefficient, N_e = Effective population size, with 95% confidence intervals.

Measures of F_{IS} for Upper Hudson River localities all exhibited estimates slightly less than zero (suggesting possible population expansion), whereas all other sampling locales with the exception of Unnamed Brook 1 and Shanty Bottom Brook exhibited F_{IS} measures slightly elevated from zero (suggesting possible population contraction). Tests to determine the presence of heterozygote excesses or deficits produced no significant

results. Measures of effective population size varied widely between sampling locales. Estimates of effective population size were lowest for the Blue Ledge Tributary and Vanderwhacker Brook (N_e = 14.8 and N_e =17.3, respectively), whereas the highest measures of effective populations size were attributed to Unnamed Brook 1 and the combined Dix-Elk Tributaries (N_e = 315.3 and N_e =254.3, respectively).

F_{ST} values for all sample sites are shown in Table 2. All pairwise F_{ST} comparisons between sampling locations were statistically significant following adjustments for multiple comparisons (following Bonferroni correction $\alpha = 0.05$, initial nominal P value = 0.00055). The lowest estimated F_{ST} values were attributed to Durgin Brook and Snyder Brook (F_{ST} = 0.012), although this measure was followed closely by comparisons between Durgin Brook and Slide Brook (F_{ST} = 0.024) as well as Durgin Brook and Unnamed Brook 1 (F_{ST} = 0.026). Overall, sampling sites within the Boreas river system exhibited the lowest pairwise F_{ST} values calculated (mean F_{ST} = 0.037). The highest F_{ST} values were exhibited between Huntley Pond Inlet and Slide Brook (F_{ST} = 0.184) followed by Huntley Pond Inlet and Gulf Brook (F_{ST} = 0.179). The overall highest F_{ST} values were calculated between comparisons involving three sites (Huntley Pond Inlet, the Blue Ledge Tributary and Shanty Bottom Brook; mean combined F_{ST} = 0.115).

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Calamity (1)	0	0.078	0.105	0.098	0.104	0.108	0.054	0.088	0.082	0.079	0.134	0.032	0.047	0.111
Nate (2)		0	0.035	0.051	0.141	0.118	0.083	0.112	0.100	0.111	0.125	0.062	0.081	0.072
Huntley (3)			0	0.050	0.184	0.159	0.11	0.146	0.135	0.138	0.179	0.109	0.124	0.086
Blue Ledge (4)				0	0.169	0.155	0.108	0.151	0.119	0.116	0.150	0.094	0.109	0.069
Slide (5)					0	0.052	0.024	0.068	0.041	0.079	0.105	0.074	0.087	0.147
Snyder (6)						0	0.012	0.030	0.049	0.072	0.103	0.061	0.086	0.121
Durgin (7)							0	0.021	0.026	0.036	0.082	0.031	0.051	0.090
Vanderwhacker (8)								0	0.047	0.064	0.095	0.079	0.079	0.116
Unnamed 1 (9)									0	0.059	0.094	0.048	0.056	0.105
Dix-Elk (10)										0	0.084	0.045	0.043	0.102
Gulf (11)											0	0.083	0.054	0.145
Unnamed 2 (12)												0	0.016	0.090
Platt (13)													0	0.116
Shanty Bottom (14)														0

Table 2: **Pairwise F_{ST} values between all sample sites, calculated using Weir and Cockerham's unbiased estimator of F_{ST} (theta).**

When comparing the ΔK measures in unison with the *Ln(K)* measures produced by STRUCTURE HARVESTER for the runs that included the stocked strains, all sampling locales were identified as genetically distinct from the supplementation strains (K = 6). Bar plots produced by STRUCTURE and processed with CLUMPAK, which examined each sampling site individually with these strains, suggested that the majority of sampled individuals were relatively unaffected by long term stocking in the area, showing minimal signs of admixture associated with the New York State stocked strains (Figure 2). Individuals from Huntley Pond Inlet and the Blue Ledge Tributary were the exceptions, exhibiting mean ancestry associated with stocked strains at levels greater than two times that of all other populations examined, 70% (Q-value $\geq$ 0.70); these individuals were therefore removed from further analyses. Gulf Brook and Snyder Brook exhibited the lowest level of ancestry associated with the New York stocked strains (Q-value $\leq$ 0.05). All other populations exhibited mean hatchery ancestry at levels that ranged between 5% and 35% (Q-value; 0.05 – 0.35), with the majority of populations exhibiting estimates in the 10% or less range (Q-value $\leq$ 0.10), potentially suggesting this level of ancestry may be associated with hierarchal population structure and genetic clines rather than true admixing at the landscape level, especially given that all of New York's supplementation stocks are at least partially derived from New York populations.

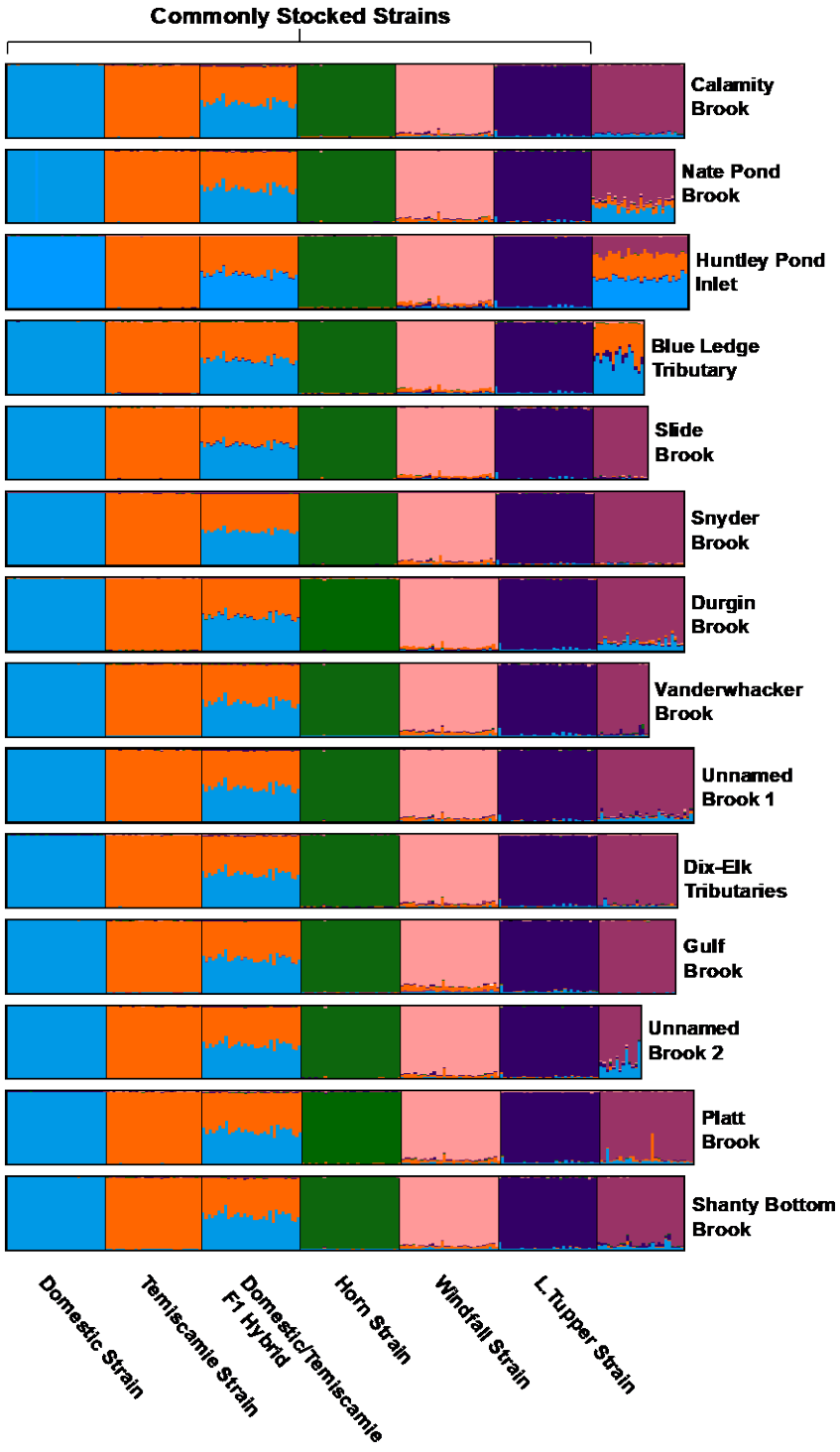

Figure 2: **STRUCTURE bar plots for stocked strains and individual sampling locations (K = 6). Each vertical line represents an individual, and colors represent their inferred ancestry from K ancestral populations.**

When comparing the remaining sampling sites to each other, the ΔK measure and the *Ln(K)* measure produced conflicting results (*ΔK* = 2; *Ln(K)* = 5); therefore, the succession of bar plots in this range were examined simultaneously (Figure 3). The bar plots produced by STRUCTURE and processed with CLUMPAK exhibited a high level of admixture across all sampling sites at all K-values, with the exception of Gulf Brook. When examining the K = 2 bar plot the Upper Hudson samples group with the Shanty Bottom Brook population in the Schroon system, while all other populations group together with some level of reciprocal admixture between groupings. As K increases, the other Schroon sampling sites break out together, followed by the individual Hudson sampling locales breaking out on their own. The Boreas River system remains highly admixed throughout, as do the majority of the Schroon samples (with the exception of Gulf Brook). These results indicate weak population structure at the level of the individual river system and sampling sites, with substantial mixing between individuals at sampling sites both within and between river systems.

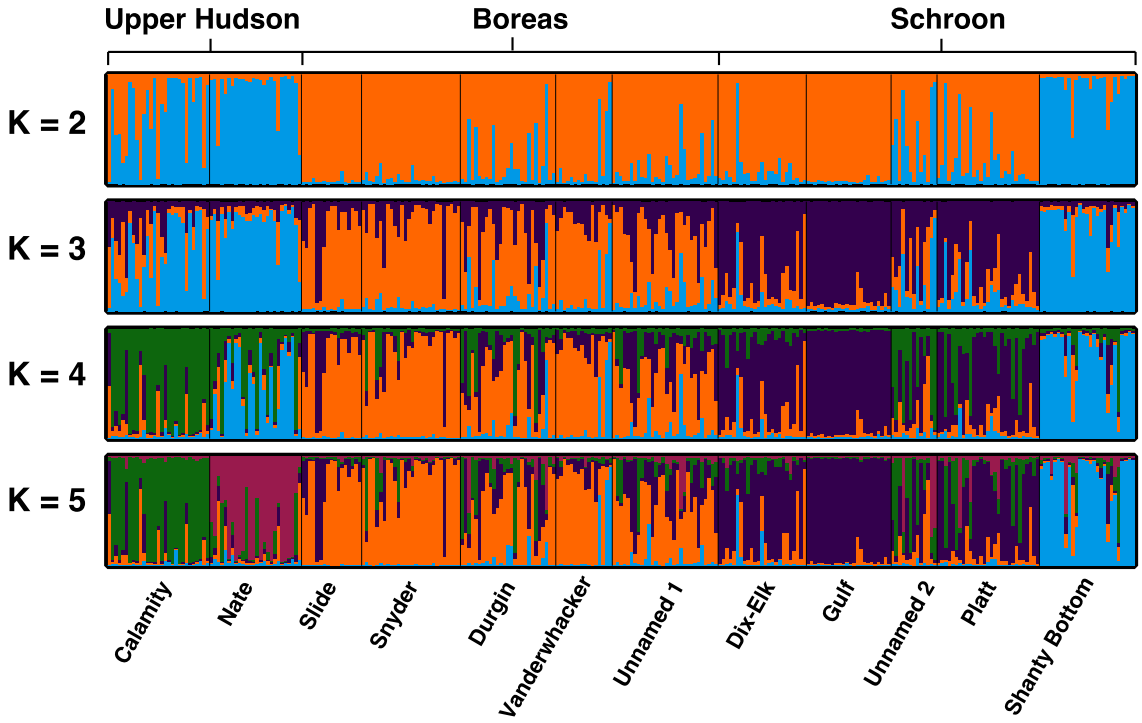

Figure 3: **STRUCTURE bar plots across all sampling locations, excluding Huntley Pond Inlet and the Blue Ledge Tributary for K values 2 through 5. Each vertical line represents an individual, and colors represent their inferred ancestry from K ancestral populations.**

Migration estimates produced using the program BAYESASS mirror the results of the STRUCTURE analysis (Figure 4). While ongoing migration (*M*) was detected to some extent across all sampling sites, it was estimated to be the highest within the Boreas River system (*M*; 16–24%). Estimates of the number of non-migrants (*NM*) for each sampling site within this region were also comparatively low (*NM*; 68-74%). The number of non-migrants exhibited by the Gulf Brook populations was the highest (*NM* = 89%), while both Shanty Bottom Brook and Calamity Brook produced similarly high measures (*NM* = 87% and 86%, respectively). Migration estimates produced by BAYESASS were generally much lower between river systems than within, consistent with the admixture patterns produced by STRUCTURE.

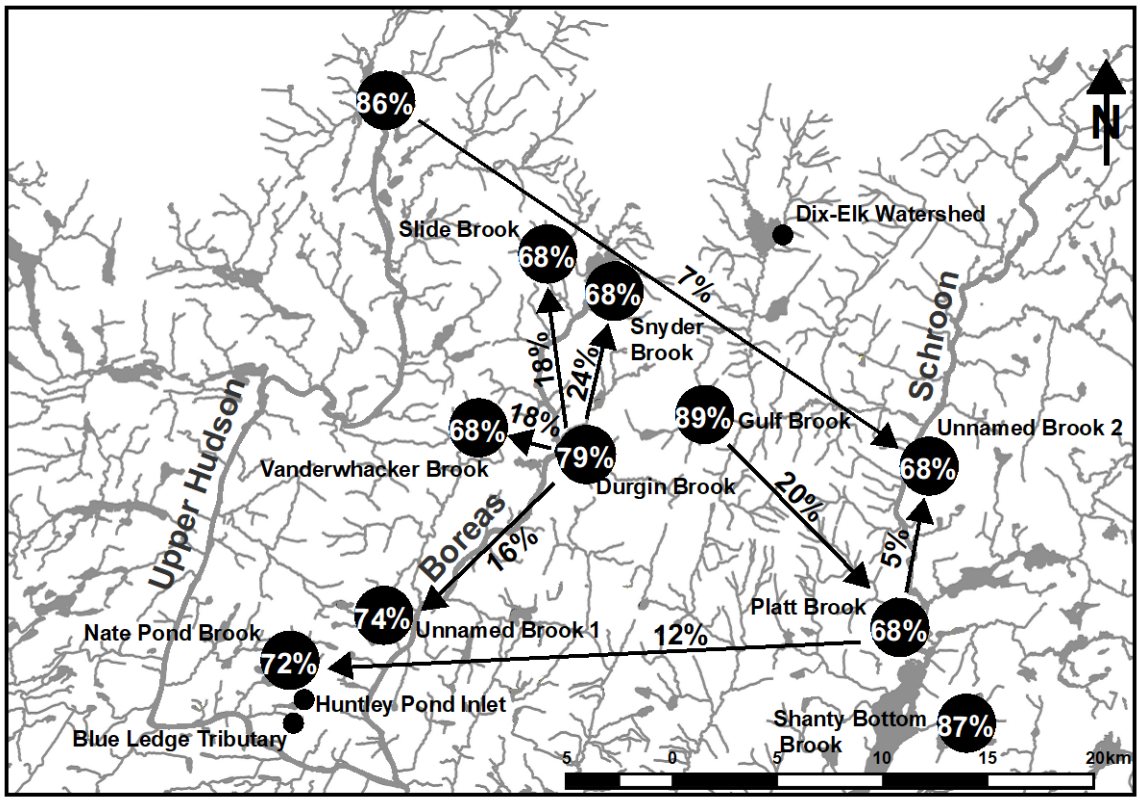

Figure 4: **Recent migration rates (*M*) between sample sites estimated using the program BAYESASS are represented with arrows. The numbers within the circles denote the proportion of non-immigrants (*NM*) within populations. For clarity of presentation *M* values less than 5% are not shown.**

DISCUSSION

Understanding how anthropogenic changes have affected the natural genetic structure of fish communities across the landscape is key to making responsible decisions about the effective management of those species, especially if they are in a state of decline (Schwartz et al. 2007; Laikre et al. 2005). As human induced habitat alterations continue to alter the genetic structure of wild populations, informed consideration should be given to how these challenges may be influencing the livelihood of wild reproducing

fish. Thus, it has become critical to gain a clear understanding of how fish that live in interconnected river systems breed and interact.

In this study, it was demonstrated that the Brook Trout currently inhabiting the upper Hudson drainage in New York State (i) exhibit varying degrees of introgression from State-based stocking activities, (ii) exhibit genetic population structure at the level of individual tributaries as well as the larger river systems where they are found, (iii) are experiencing on-going migration influenced by habitat connectivity and geographic distance, and (iv) demonstrate comparatively similar measures of genetic diversity but varied measures of effective population size based on sampling location.

When comparing the genetic structure of fish collected on a site by site basis with the strains currently stocked by the NYSDEC, it is apparent that although introgression from stocked fish is minimal, it is present and in discernable amounts in certain areas. The two populations that showed the highest levels of putative hatchery ancestry (Huntley Pond Inlet and the Blue Ledge Tributary) are also in very close proximity to each other. In fact, Huntley Pond is a reclaimed trout pond, which was re-established with a State-bred Brook Trout strain in 1951 (NYSDEC 2010). These results suggest that the fish currently found in that area may still retain a discernable amount of ancestry linked to this event. These findings also suggest that when a pond is reclaimed, the stocked fish have the potential to move into, and mix with, fish in neighboring tributaries. The Boreas, Schroon, and Upper Hudson rivers have all seen regular trout stocking in their main stem (NYSDEC 2017). Surprisingly, the Brook Trout directly connected to these areas show relatively low amounts of ancestry related to stocked Brook Trout strains. This would suggest that stocked fish in these areas are currently making a limited

contribution, if any, to wild reproduction in the region. It is also possible that stocked fish are simply not moving into, or mixing with, wild fish in the river system's feeder tributaries for reasons which are unclear.

Admixture estimates produced by the program STRUCTURE and migration rates produced by the program BAYESASS examining the relatively non-introgressed wild caught samples complement each other and suggest ongoing migration both within and between river systems. This is an important finding, as relatively little work has been done with this species to determine levels of migration in such a network (but see Curry et al. 2002). Given the heightened levels of movement between nearby sampling sites, especially in the Boreas system, considerations related to maintaining connectivity and, by extension, genetic integrity are warranted. More recent studies that have looked at genetic population structure in dendritic stream networks have suggested that Brook Trout in these systems exhibit relatively little movement (Hudy et al. 2005: Kanno et al. 2011). The findings of this study suggest that Brook Trout population dynamics in larger interconnected systems may be far more complex.

When the findings regarding ongoing migration are taken into consideration with the results of pairwise F_{ST} analysis and genetic diversity indices, several populations, such as Gulf Brook and Shanty Bottom Brook, seem to be somewhat cut off from neighboring sites. Gulf Brook in particular showed some of the lowest diversity, admixture, and migration estimates in the study. These are indications that there may be barriers to two-way gene flow, allowing limited movement into these tributaries. In the case of Gulf Brook, this may be a steep elevation gradient in the form of a waterfall or a pitched culvert, whereas in the case of Shanty Bottom Brook, Schroon Lake, which it

drains into, may be difficult to traverse given its large size and occupancy by non-native competitors such as small and large mouth bass and yellow perch (Odell 1932). Regardless of the reasons why, these populations may act as sinks for unique genetic variation, given that they have likely seen relatively little outside influence from either contemporary or historically stocked fish, or the larger Brook Trout assemblage inhabiting the area.

Genetic diversity indices (H_E, A, and F_{IS}) were relatively similar across all sampling sites, while estimates of effective population size (N_e) varied widely. The comparatively large effective populations size of the combined Dix-Elk tributaries (N_e = 254.3) may be a result of sampling across a comparatively wider range (i.e. sampling inconsistencies), but the comparatively large effective population size for Unnamed Brook 1 and Durgin Brook (Ne = 315.3 and 114.8 respectively), taken into consideration with the migration rates for these areas suggest that these tributaries (especially Durgin Brook), may play a central role in reproduction and dispersal throughout the system.

As human induced activities continue to transform Brook Trout habitat across the Northeastern United States, identifying and understanding how hydrology affects dispersal and gene flow is critical to preserving the Brook Trout's ability to adapt. In this study genetic markers were utilized to assess intraspecific interactions and migration in a complex, wide-ranging system, where a traditional radio-tag or mark and recapture study would have been logistically challenging, potentially unable to answer all of the study questions, and likely more costly. There is currently a general consensus among researchers and fisheries managers regarding the need for species-focused conservation plans, although the role of evolutionary applications in such plans continues to be

disputed (Garcia de Leaniz et al. 2007; Allendorf et al. 2010). Nevertheless, the methods presented here can offer insight as to how genetics can answer complex questions related to fisheries conservation not only in New York, but also throughout the world.

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