ADVANCING BROODSTOCK SELECTION TO OPTIMIZE BROOK TROUT STOCKING IN WISCONSIN

By

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My master’s thesis is dedicated to my grandmother who peacefully passed away on May 29th, 2024. She was selfless beyond measure, and she will be deeply missed.

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CHAPTER I: GENETIC DIVERSITY, SPATIAL GENETIC STRUCTURE, AND DOMESTIC STRAIN INTROGRESSION OF WISCONSIN BROOK TROUT

Abstract - Brook trout *Salvelinus fontinalis* is the only native stream-dwelling salmonid to many parts of central and eastern North America and has been intensively stocked across its native range. As wild brook trout stocking programs gain momentum, information is needed detailing endemic patterns of genetic structure and stocking influence to aid in the conservation of endemic wild strains. My objectives were to determine how measures of genetic diversity, inbreeding, and effective population size vary for wild brook trout populations across Wisconsin. Moreover, I aimed to identify and characterize patterns in genetic structure on the landscape and quantify the influence of domestic strain stocking for each survey population. Brook trout were genotyped from 63 wild populations across Wisconsin and one domesticated hatchery strain at 68 microsatellite loci. Measures of genetic diversity, inbreeding, and effective population size were estimated for each survey population. Discriminant analysis of principle components (DAPC) was used to assign survey populations to native or domestic lineages, yielding estimates of domestic strain introgression. Spatial genetic structure was assessed using genetic distances, neighbor-joining trees, DAPC, and STRUCTURE. Genetic diversity and effective population size showed positive relationships with latitude, and overall levels of domestic strain introgression were low. Six major groups were resolved on a neighbor-joining tree. Distinct groups of genetically similar populations were identified in central and northeastern Wisconsin, but spatial clustering was weak elsewhere. This study found higher levels of genetic diversity and effective

population size in northern Wisconsin, low overall levels of domestic strain introgression that generally coincide with available stocking records, and evidence of spatial genetic structure, particularly in central and northeastern Wisconsin. This study includes information on endemic genetic structure of brook trout populations in the midwestern United States, providing insights that may help inform conservation, management, and stocking practices of brook trout in Wisconsin and beyond.

Introduction

The propagation of hatchery-reared fish is a management strategy commonly used to improve harvest opportunity and restore self-sustaining populations; however, fish stocking can alter the genetic composition of resident wild populations, potentially undermining local adaptation and genetic structure (Ferguson 1990; Currens and Busack 1995; Trushenski et al. 2018). When stocked fish interbreed with wild fish, they introduce their genetic material into the wild population (i.e., introgression), which may include maladaptive alleles that are present due to directional selection associated with different environmental conditions (Miller and Kapuscinski 2003; Claussen and Philipp 2023). Fisheries managers may benefit from information describing the degree to which wild fish populations have been influenced by stocking, as it can aid in the conservation of endemic populations and genetic diversity through improved stocking practice (e.g., selection of genetically appropriate broodstock).

Consequences associated with introgression can occur from stocking fish that are of hatchery origin (domesticated) or non-local origin (wild parentage), as both actions can introduce maladaptive alleles into wild populations. The degree that stocked fish introgress into wild populations is likely influenced by several factors, including stocking intensity (Marie et al. 2010; Lamaze et al. 2012), domestication intensity (Araki et al. 2007b, 2008), habitat characteristics (Bruce et al. 2020), angling exposure (Harbicht et al. 2014a), and geographic distance from stocking location (Bruce and Wright 2018). Some resident brook trout populations have shown the ability to resist hatchery introgression despite intense stocking (Kazyak et al. 2018; White et al. 2018; Beer et al. 2019; Lehnert et al. 2020; Erdman et al. 2022), which may be explained by selective forces purging non-native alleles from the resident populations (Harbicht et al. 2014b). Nevertheless, when stocking to augment native fish populations, best practices support using genetically appropriate broodstock to avoid the introduction of deleterious alleles (Miller and Kapuscinski 2003).

High levels of introgression resulting from widespread stocking of a single strain can homogenize the genetic structure of a region and reduce the genetic diversity within and among populations (Perkins et al. 1993; Marie et al. 2010; Erdman et al. 2022). This homogenization breaks down local adaptation and decreases resiliency (Williamson and May 2005). Moreover, populations with low genetic diversity are at a greater risk for inbreeding depression, which occurs when reproduction within family groups results in reduced survival and fertility of offspring (Reed et al. 2002). Inbreeding depression increases risks for local extirpation and can be hastened by declines in genetic diversity, effective population size (N_e), or a combination of the two (Frankham 1995; Brook et al. 2002). Effective population size is an approximation of the number of successfully breeding adults in a population and can be an indicator of a population's susceptibility to inbreeding depression and genetic drift (i.e., loss of genetic diversity), making it a practical metric for the management of fish populations (Wright 1931; Waples et al. 2014). The stocking of hatchery-reared fish (both wild parentage and domestic origin) has been found to reduce the N_e of resident wild fish populations (Christie et al. 2012; Gossieaux et al. 2019). Similarly to genetic diversity, N_e has been correlated with fitness in wild populations (Reed and Frankham 2003). Therefore, to conserve the genetic integrity of contemporary wild fish populations, fisheries management would likely benefit from information that describes the influence of past stocking events on current genetic diversity, population structure, and N_e.

Effective stocking practices and fisheries management more broadly may benefit from knowledge of the genetic structure and diversity on the landscape. For instance, spatial genetic structure can help inform the appropriate spatial scale for management of wild fish populations. Under natural conditions (i.e., without human intervention), hydrological connectivity should be expected to dictate spatial genetic structuring among fish populations, as hydrological connectivity permits fish movement and gene flow. Fish populations that are hydrologically isolated are expected to genetically differentiate from one another over time due to genetic drift, differing selective pressures, and the absence of gene flow (Wright 1943). However, the degree of genetic structuring can vary due to several factors including current and historical hydrological connectivity, movement patterns, N_e, habitat availability, and human intervention.

In Wisconsin, hatchery-based stocking and translocation have been used as brook trout management and restoration tools since the late 19th century (Krueger and Menzel 1979). Early history of brook trout propagation in Wisconsin was poorly documented, and stocking records detailing strains are only available post-1972. From about 1925 to 1975, the Wisconsin Department of Natural Resources (WDNR) primarily stocked fish derived from a domesticated broodstock reared at the Osceola State Fish Hatchery in northwestern Wisconsin (Hunt 1979). Although domesticated, it is possible that this Osceola strain was originally native to Wisconsin (Hughes 2008). In the 1970s, WDNR state hatcheries transitioned from the Osceola strain toward the use of strains native to eastern North America to resolve issues with disease and to produce larger, fastergrowing brook trout (Hunt 1979). Eastern-origin strains included a domesticated New Hampshire strain, the Assinica strain from Quebec, and the Owhi strain from the White Sulfur Springs hatchery in West Virginia (Hoxmeier et al. 2015). Additional strains originating from Rome, New York and North Attleboro, Massachusetts were also briefly used (Hoxmeier et al. 2015). The domesticated New Hampshire strain, which became most widely stocked, was obtained from the Nashua National Fish Hatchery and eventually became known as the St. Croix Falls strain due to it being maintained at the St. Croix Falls State Fish Hatchery starting in 1973 (Hunt 1979; Hughes 2008; Hoxmeier et al. 2015). The domestic St. Croix Falls strain is still stocked in many of Wisconsin's Class II and Class III trout waterbodies (WDNR 2019).

In 1995, the WDNR started the Wild Trout Stocking Program, which aimed to improve post-stocking survival by stocking first-generation (F1) hatchery trout rather than domestic strain trout (WDNR 2019). Production quantities of F1 trout were found to be limited by the abundance of the wild broodstock source, so the WDNR later began producing second-generation (F2) hatchery trout that were crosses between F1 broodfish derived from different source streams. From 1999 to 2014, Ash Creek in southwestern Wisconsin was used as the only broodstock source for the Wild Trout Stocking Program. Following 2017, Ash Creek was eliminated from use as a broodstock source due to declines in brook trout abundance and high levels of gill lice *Salmincola edwardsii* infection (Mitro 2016). Ash Creek was later discovered to not be a genetically suitable wild broodstock source, as the population has close relation to the domestic St. Croix Falls strain (Erdman et al. 2022). In place of Ash Creek, an annual rotation of populations believed to reflect remnant native brook trout genetics has been used as wild broodstock in southwest Wisconsin. This rotation is collectively named the Southwest Feral strain and has included the use of several wild populations, including South Fork Hay River, Lowery Creek, Melancthon Creek, Cady Creek, and West Branch Mill Creek. A Northwest Feral strain has been developed for use in northwestern Wisconsin; however, South Fork Hay River is the only wild broodstock source that has been selected for this strain to date. The complex history of brook trout propagation and lack of regional genetic data has limited the WDNR’s ability to infer the ancestry of many populations and subsequently identify suitable native broodstock sources in other areas of the state.

Erdman et al. (2022) delineated genetic structure of midwestern United States brook trout with 5-7 microsatellite markers (5 markers for the full dataset, 7 for the dataset containing populations from Wisconsin). That study focused on the Driftless Area of Wisconsin, Iowa, and Minnesota, finding high rates of genetic differentiation among nearby populations and little evidence of hydrological population structure (Erdman et al. 2022). These results were primarily attributed to the intensive stocking and translocation of various brook trout strains disrupting the natural genetic structure. While this study provided important data to inform hatchery practices and fisheries management in Wisconsin, it was limited by a low number of markers, potentially reducing the ability to identify patterns of ancestry. Additionally, the regions of Wisconsin currently lacking a genetically suitable wild broodstock rotation were not broadly assessed. I aimed to fill this knowledge gap by focusing on many populations that have little recorded stocking history, assessing populations from relatively unstudied regions, and increasing the number of genetic markers analyzed by nearly an order of magnitude.

Microsatellites (also known as short tandem repeats or simple sequence repeats) are a widely used genetic marker in the field of conservation genetics. Single nucleotide polymorphisms (SNPs) have begun to replace microsatellites for many applications; however, microsatellites remain valuable because they can be highly polymorphic, making them more information dense per locus than biallelic SNPs (Allendorf 2017). For example, about twice as many SNP markers are required to achieve the same statistical power as microsatellites for parentage analysis (Fernández et al. 2013). Much of the previous conservation genetics research involving microsatellites has used capillary electrophoresis (CE) genotyping, a labor-intensive and time-consuming genotyping method (Gruenthal and Larson 2021). Amplicon sequencing is an advancement from CE methods because it allows for simultaneous genotyping of large numbers of microsatellites (Zhan et al. 2017). Microsatellite-based amplicon sequencing has been successfully used to evaluate genetic diversity, N_e, spatial genetic structure, and perform parentage assignments in several species (Bradbury et al. 2018; Tibihika et al. 2019; Layton et al. 2020; Lehnert et al. 2020; Prystupa et al. 2021).

With this study, I used a refined amplicon-based microsatellite panel to characterize the genetic diversity, N_e, spatial genetic structure, and domestic strain introgression for wild brook trout populations across Wisconsin. I intended to investigate how measures of genetic diversity and N_e vary spatially across the state, and I aimed to determine whether natural spatial genetic structure remains in a species that has been highly influenced by anthropogenic disturbance, translocation, and stocking. Moreover, I set out to quantify the degree to which wild populations have been influenced by stocking and compare the results with similar studies. I also intended to integrate my dataset with genotype data from Erdman et al. (2022) to produce a larger, combined dataset that could be evaluated using the aforementioned objectives where possible. I hypothesized that the survey populations would show lower levels of St. Croix identity than those assessed by Erdman et al. (2022) due to lower levels of recorded stocking history in our surveyed watersheds. For the same reason, I expected more pronounced spatial genetic structuring of the study populations relative to the findings of Erdman et al. (2022) and typical of brook trout populations elsewhere in their native range (Hoxmeier et al. 2015; Kazyak et al. 2018, 2022; Beer et al. 2019; Lehnert et al. 2020; Mamoozadeh et al. 2023). This chapter may provide guidance for the selection of genetically appropriate broodstock sources, aid in allocation of management resources, as well as provide a spatially dense characterization of the species' spatial genetic structure.

Methods

Sample Collection

I selected 63 brook trout populations for analysis based on a history of limited stocking, existing sample availability, and geographic distribution in areas less well surveyed by Erdman et al. (2022; Table 1.1). Populations with minimal recorded stocking history were prioritized to limit the potential influence of past stocking events on my observations of natural genetic structure (61.9% of the survey populations have no record of direct stocking, 15.9% have 1-3 recorded direct stocking events, and 22.2% have >3 recorded direct stocking events). Survey populations were sampled using either barge or backpack electrofishing. Twenty-one populations were sampled during surveys conducted from 2015 to 2021 and 42 populations were sampled in 2022. Caudal fin clips from up to 30 brook trout (>75 mm total length) were collected from each population and were individually stored in 95% ethanol pending DNA extraction.

Laboratory Methods

DNA was extracted from caudal fin clip samples using a Chelex DNA extraction procedure (Bootsma et al. 2020). For each 96-sample extraction, a 10% Chelex solution was prepared using 0.1% NP-40, 0.1% Tween 20, in 10 µL Tris low EDTA (TLE). This solution was heated for 30 minutes at 50°C, rotated overnight at room temperature, and stored at 4°C. In a pipet basin, 12 mL of the 10% Chelex solution (warmed to room temperature) was mixed with 5 mL TLE, and 150 µL of this mixture was added to each well of a 96-well plate. A 3 mm biopsy punch was taken from each fin clip sample and added to each well, except for one well used as a no-template control. Plates were placed in a thermocycler for 15 minutes at 95°C, followed by 4°C for 2 minutes. Plates were vortexed and centrifuged before transferring 50 µL of solution from each sample into the corresponding wells of a new plate that was then stored at -20°C pending library preparation.

The first step of library preparation was polymerase chain reaction (PCR), which multiplies the copies of each microsatellite locus so they can be better detected for sequencing. A mixture was created using 371 µL Qiagen Plus Master Mix and 159 µL panel specific GT-seq primers. Each primer corresponds to a microsatellite locus. Then 5 µL of the mixture was distributed into each well of a new 96-well plate labeled "PCR1". DNA was transferred from each well of the DNA extraction plate into each corresponding well of the PCR1 plate, and the PCR1 plate was placed in the thermocycler to run a PCR (~2 hrs for 15 cycles).

The second step of library preparation was ligating unique barcodes to each sample, which provides a means of individual-level identification once the DNA has been pooled and sequenced. I used i05 barcodes to differentiate samples within a plate, and i07 barcodes to differentiate samples among plates. First, the amplified DNA was diluted by transferring 3 µL of DNA from each well of the PCR1 plate into new 96-well plate, then adding 37 µL of nuclease free H_2O to each well. An i07 barcoding mixture was created by combining 106 µL of the desired i07 index [10 µM] with 530 µL Qiagen Plus Master Mix. Then 75 µL of the i07 barcoding mix was distributed into each well of an 8-well strip tube before aliquoting 6 µL into each well of a new 96-well plate labeled "PCR2". Next, 2 µL of i05 barcode was added to each well of the PCR2 plate, and 2 µL from each well of the diluted DNA plate was transferred to the corresponding wells of the PCR2 plate. The PCR2 plate was then placed in the thermocycler for a second round of PCR (~40 minutes for 10 cycles) that annealed the i05 and i07 barcodes to the DNA.

The third step of library preparation was SequelPrep Normalization, which is important for equalizing the concentrations of DNA libraries before multiplexing. The DNA generated by PCR2 was bound to the normalization plate by adding 10 µL of SequelPrep Binding Buffer and 10 µL of barcoded DNA from PCR2. The normalization plate was then sealed, vortexed, centrifuged, and left to incubate at room temperature for at least 1 hour. The liquid was removed from the SequelPrep Normalization Plate by aspirating, as the DNA was bound to the sides of the wells. Then 50 µL of SequelPrep Wash Buffer was dispensed into each well, and an aspirator was used again to remove the liquid. Normalization was concluded by eluting the well-bound DNA using 20 µL of SequelPrep Elution Buffer and incubating at room temperature for 5 minutes.

The fourth step of library preparation was pooling and concentrating the normalized DNA to ensure that enough DNA from each sample is present for sequencing. From each well of the SequelPrep Normalization plate, 10 µL was pooled into a single 5 mL tube. A Qiagen PCR cleanup/gel extraction kit was then used to concentrate the DNA. First, 2.88 mL of buffer QG and 960 µL of 100% isopropanol were added to the tube of pooled DNA. A spin column and vacuum manifold were used to suction filter all the liquid from the tube. Then 600 µL of buffer PE was vacuumed through to wash the spin column twice over. The spin column was then inserted into a new 1.5 mL tube. Lastly, 100 µL of TLET was added to the spin column and was centrifuged for 2 minutes twice over to pull the DNA out of the spin column and into the tube containing liquid.

To complete library preparation, AMPure XP beads were used to perform size selection and remove the DNA sequences that were too long or too short. First, 120 µL of beads were transferred into the tube of concentrated DNA. This must be mixed well, while avoiding the formation of bubbles, followed by incubation at room temperature for 10 minutes. With the cap open, the tube was then placed on a magnetic stand and let sit for 5 minutes. The supernatant was fully transferred to a new tube, leaving the beads behind which were then discarded. Next, 40 µL of AMPure beads were added to the supernatant and mixed well, avoiding the formation of bubbles again. This mixture was incubated at room temperature again for 10 minutes. The tube was then placed on the magnetic stand to sit for 5 minutes. The supernatant was fully removed and discarded, as the DNA was then bound to the beads. While the tube containing the beads was still on the magnetic stand, it was washed with 500 µL of 80% ethanol for 30-60 seconds. The ethanol was removed, and this wash was repeated once more. Lastly, 40 µL of TLET was added to the tube containing the beads. The tube was mixed thoroughly, incubated at room temperature for 2 minutes, then placed back onto the magnetic stand. After 5 minutes, 40 µL of the supernatant was transferred to a new 1.5 mL tube. This tube was then labeled with its respective i07 designation, as this was the final product. This tube contained normalized, barcoded DNA from the specific loci of interest from 95 different brook trout.

Once the library preparation was complete, the DNA concentrations were verified using the Qubit HS assay. A mixture was created by combining 1 µL of Qubit reagent and 199 µL of Qubit dsDNA Buffer. Then 198 µL of the Qubit mixture was combined with 2 µL of DNA in a 0.5 mL tube. This mixture was analyzed with the Qubit HS assay to estimate the final DNA concentration. The DNA was sent to the Biotechnology Center at the University of Wisconsin - Madison for sequencing the Illumina NovaSeq platform to generate 150 basepair paired-end sequence reads.

Microsatellite Genotyping

I used an amplicon sequencing panel to genotype an initial set of 91 microsatellite loci. Data processing began by stitching forward and reverse reads together (i.e., joining at an overlapping portion) using FLASH v1.2 (Magoc and Salzberg 2011). Samples were then genotyped using MEGAsat v1.0 (Zhan et al. 2017) which uses a workflow that first sorts input reads by locus, then determines the microsatellite genotypes by individual based on rules concerning relative number of reads for various repeat sizes (Zhan et al. 2017). I used the default MEGAsat allele calling rules. MEGAsat parameters were set to allow two base pair mismatches with a minimum depth of three reads per allele. Once MEGAsat called genotypes and allele sizes, I generated histogram plots of read depth versus allele size for each locus using random subsets of fish to evaluate reliability of the MEGAsat automated genotype calling approach (i.e., peak morphology plots; Figure 1.1).

Genotyping Panel Refinement

The initial genotyping panel contained 91 microsatellite loci that were developed from several other studies (Angers et al. 1995; King et al. 2012; Lien et al. 2016; Bradbury et al. 2018; Christensen et al. 2018). Loci containing low confidence genotyping calls (e.g., excessive allele stuttering) were removed from the panel. Next, I estimated null allele frequencies and removed loci with estimated frequencies greater than 0.15 PopGenReport (Adamack and Gruber 2014). I also tested for deviations from

Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD) for each locus. Deviations from HWE can occur if genotype frequencies do not match expectations of neutrality, which may be caused by natural selection or genotyping errors. The presence of LD would indicate that allele frequencies are not independent across loci. Hardy-Weinberg equilibrium and LD were evaluated using exact tests in Genepop (Weir and Cockerham 1984), with resulting alpha levels corrected for multiple comparisons using the false discovery rate approach (Benjamini and Hochberg 1995) and loci with a q-value less than 0.05 were removed.

Genetic Diversity Statistics

Within population diversity was assessed with four statistics, each calculated using the R package *hierfstat* (Goudet 2005). Allelic richness (A_r) was estimated as the average number of alleles per locus per population and was adjusted to the minimum sample size using the rarefaction method (n = 8; Kalinowski 2004). A Welch two sample t-test was performed to assess whether A_r differs between the hydrologic unit code (HUC) 2 Upper Mississippi and Great Lakes regions. I also estimated observed (H_o) and expected (H_e) heterozygosity and averaged them across individuals to estimate population-level estimates of H_e and H_o. I estimated inbreeding coefficients (F_{IS}) which range from -1 to 1, with larger values suggesting inbreeding. Single-sample contemporary N_e was estimated for each population using the LD method (Waples and Do 2010) with a critical value of 0.02 and jackknife (95%) confidence intervals in the program NeEstimator V2.1 (Do et al. 2014). Linear regressions were performed to evaluate the relationships between latitude and each of the aforementioned genetic diversity statistics including N_e.

Spatial Genetic Structure

Brook trout population structure and differentiation were first evaluated by calculating pairwise genetic distances (Nei's G_{ST}; Nei 1973) and building neighbor-joining (NJ) trees. Pairwise comparisons of genetic distance were estimated using the R package *mmod* (Winter 2012) and were visualized with a NJ tree using the R package *ggtree* (Yu et al. 2017). The relationship between Euclidean geographic distance measured using the R package *geosphere* (Hijmans 2022) and genetic distance was evaluated by constructing a Mantel correlogram test using the R package *vegan* (Oden and Sokal 1986; Dixon 2003).

Spatial genetic structure was characterized using discriminant analysis of principle components (DAPC) and model-based Bayesian clustering (STRUCTURE; Kanno et al. 2011). DAPC is a multivariate method that partitions populations into K clusters by minimizing the variation within groups and maximizing the variation among groups. I used the Bayesian Information Criterion to determine the most parsimonious number of genetic clusters in our dataset (Figure 1.2). DAPC was performed using the R package *adegenet* (Jombart 2008). I performed Bayesian clustering analyses using the program STRUCTURE (Pritchard et al. 2000). Bayesian clustering provides estimates of Q-scores that describe the estimated proportional ancestry (i.e., admixture proportion) of each fish. Bayesian clustering also attempts to identify major cutoffs between genetic groupings. The most supported number of genetic clusters (K) was determined using the Evanno method (Figure 1.3; Evanno et al. 2005). Population level admixture proportions were spatially depicted using the R package *mapmixture* (Jenkins 2024).

Domestic Strain Introgression

To describe the degree to which each survey population was influenced by the stocking of domesticated hatchery strain fish, I use the term “St. Croix identity” referring to the domestic St. Croix Falls brook trout strain. The St. Croix Falls strain was used to evaluate levels of domestic strain introgression for two reasons; the strain is historically the most widely stocked domesticated strain in Wisconsin, and it was the only domestic hatchery strain that was available for analysis. To estimate levels of St. Croix identity, I first simulated two reference populations; a domestic hatchery population (n = 100) representing the domestic St. Croix Falls brook trout strain and a “native” population (n = 100) intended to represent Wisconsin native brook trout (White et al. 2018). The domestic reference group was simulated by pooling three different cohorts of domestic St. Croix Falls strain brook trout (n = 450 total, see Chapter II), dividing them randomly into two groups, and hybridizing them using the hybridize function in the R package *adegenet* (Jombart 2008). To create the native reference group, populations were subset to only include those with no recorded history of brook trout stocking in their subwatershed (HUC 12). Pairwise genetic distances (Nei’s G_{ST}) were calculated between the hatchery reference group and each of the subset survey populations. Brook trout with the top 25% greatest G_{ST} value from any given fish within the domestic reference group (n = 209) were then randomly divided into two groups and again hybridized *in silico* to simulate the native reference group.

After the domestic hatchery and native reference groups were simulated, a two-cluster DAPC model was created. Reassignment probabilities were calculated for each simulated fish back to its true reference group. All survey populations were then introduced to the model using the predict.dapc function, yielding assignment probabilities for every wild fish back to the domestic hatchery reference group and the native reference group. Assignment probabilities were averaged at the population level and multiplied by 100 to estimate mean St. Croix identity (%) for each population.

Integration with Erdman et al. (2022)

Genotypes were combined for 4,132 brook trout from 86 populations from Erdman et al. (2022) at loci that were also genotyped in our dataset to generate a spatially dense analysis of genetic diversity and population structure. Brook trout samples from Erdman et al. (2022) were sequenced using capillary electrophoresis, whereas I used amplicon sequencing. Allele calls from capillary electrophoresis and amplicon sequencing are not directly comparable, so scaling factors needed to be identified for each of the seven microsatellite loci that were used in both studies (Gruenthal and Larson 2021). Scaling factors were determined by reprocessing 92 samples using both capillary electrophoresis and amplicon genotyping to identify a standard shift in size for each allele. Only loci with consistent scaling factors were retained to ensure confident genotype conversion. Using a small number of loci to quantify allele frequencies may cause spurious conclusions driven by only a single locus. Therefore, I calculated the correlation between the reduced and 68-locus datasets for all samples used in this study to quantify precision and bias of pairwise genetic distance (Nei's G_{ST}), A_r, H_o, and H_e. For each metric, a linear regression model was used to compare the estimates calculated using the reduced set of loci and the estimates calculated using all 68 loci. A simple linear regression was also performed to evaluate the relationship between latitude and A_r.

Results

Sequencing and Panel Performance

A total of 1,823 brook trout were sequenced from 63 wild populations. The final genotyping panel included 68 microsatellite loci, and the number of alleles per locus ranged from 2 to 21 (mean = 10.3; Table 1.2). Genotype missingness across individuals averaged 5.46%.

Genetic Diversity & N_e

Surveyed populations in northern Wisconsin generally exhibited higher levels of genetic diversity than elsewhere in the state (i.e., A_r, H_o, and H_e). A_r ranged from 1.28 to 1.5 with a mean of 1.43 (Figure 1.4A), H_o ranged from 0.28 to 0.5 with a mean of 0.41 (Figure 1.4C), and H_e ranged from 0.28 to 0.50 with a mean of 0.44 (Figure 1.4D). Latitude was positively correlated with A_r ($R^2_{adj} = 0.191$, p-value = 0.0002), H_o ($R^2_{adj} = 0.188$, p-value = 0.0002), and H_e ($R^2_{adj} = 0.189$, p-value = 0.0002). Populations in the Upper Mississippi Region showed significantly higher A_r than populations in the Great Lakes Region (p-value = $1.429e^{-5}$), however the effect size was slight (difference in means = 0.008). F_{IS} ranged from -0.004 to 0.11 with a mean of 0.05. F_{IS} was variable among populations; however, no spatial trends were observed (Figure 1.4B). N_e estimates ranged from 2 to 4,794 with two populations yielding infinite estimates (Lepage Creek and Lunch Creek; Table 1.1). Infinite N_e estimates typically indicate that the sample size was not large enough relative to the true N_e for the LD method to generate an N_e estimate (Waples and Do 2010). Median N_e was 96, with two populations showing notably large N_e estimates: Wisconsin Creek (N_e = 4,794) and Buena Vista Creek (N_e = 1,333; Figure 1.5, Table 1.1).

Spatial Genetic Structure

Estimates of pairwise genetic distance (Nei's G_{ST}) ranged from 0 to 0.23 with a mean of 0.07 (Figure 1.6). Schuett Creek (0.147), Chase Creek (0.140), and Trout Creek (0.127) had notably greater mean pairwise genetic distances than other populations. Larger clades based on Nei's G_{ST} did not coincide with geography (Subregion; HUC 4); however, several smaller groupings (~2-6 populations each) can be seen that do share geographic proximity (Figure 1.7). Geography was a weak but statistically significant predictor of genetic differentiation at distances up to approximately 75 km (Figure 1.8).

Genetic clustering generally did not coincide with specific hydrological (HUC) boundaries for STRUCTURE or DAPC analyses (Figures 1.9 and 1.10). The DAPC model indicated support for K = 7 genetic clusters (Figure 1.2). When comparing discriminant functions 1 and 2 from the DAPC model, Clusters 2, 3, 5 were most distinct (Figure 1.11A). Clusters 2, 5, and 7 distinguish themselves when comparing discriminant functions 2 and 3 (Figure 1.11B). STRUCTURE analyses yielded three supported clustering configurations: K = 3 (Figure 1.12), K = 6 (Figure 1.13), and K = 9 (Figure 1.14). Subbasins (HUC 8) containing more than one survey population generally failed to exhibit uniform or distinct genetic clustering. Two clear exceptions to this were the Brule and Menominee subbasins, which both showed predominant assignment to Cluster 2. These two subbasins provided the strongest evidence of hydrological population structure; however, the Upper Fox, Wolf, and Castle Rock subbasins also presented some hydrological population structure, generally showing assignment to Clusters 3 and 9. To investigate the genetic differentiation among the genetic clusters from the K = 7 DAPC, K = 6 STRUCTURE, and K = 9 STRUCTURE configurations, individual fish were subset to only include those with at least 75% assignment to a given cluster. The subset fish were then grouped by their assigned cluster and depicted on NJ trees using pairwise estimates of Nei's G_{ST} (Figure 1.15). The K = 9 (STRUCTURE) NJ tree is arranged into two major clades: one containing Clusters 2 (blue), 3 (green), 4 (purple), 5 (orange), and 9 (grey), and another containing Clusters 1 (St. Croix Falls Strain; red), 6 (yellow), 7 (brown), and 8 (pink) (Figure 1.15C). The first clade (containing Clusters 2, 3, 4, 5, and 9) does not show any overall geographic affinity; however, the second clade (containing Clusters 1, 6, 7, and 8) is primarily found in the Driftless Area (except for Cluster 6; Figure 1.15C).

To examine the concordance between STRUCTURE and DAPC clustering, I compared the configurations that had similar number of clusters between the two methods: K = 7 for DAPC and K = 6 for STRUCTURE (Figures 1.16 and 1.13). Both methods found Cluster 2 to be primarily located in northern Wisconsin and Cluster 3 to be primarily located in central Wisconsin (Figures 1.9B and 1.10). The two methods had very similar groupings of Clusters 5 and 6. The primary source of dissonance between the methods arose from Cluster 4, as the DAPC model more commonly assigned fish to that cluster. In general, the two methods found disagreement when assigning fish to Clusters 1, 3, or 4. This may be due to the low genetic differentiation between clusters 1 and 4 (Figure 1.15B) and between clusters 3 and 4 (Figure 1.15A).

Domestic Strain Introgression

Levels of St. Croix identity were generally low with an overall mean of 4.7% (SD = 19.3%; Figure 1.17). Population-level estimates of mean St. Croix identity (i.e., assignment probability to St. Croix Falls strain) ranged from 0 to 75.9%, with nearly half of the surveyed populations showing no evidence of influence from the St. Croix Falls strain. Populations with higher levels of St. Croix identity were more commonly found located in the Driftless Area of southwestern Wisconsin (Figure 1.18). Willow Creek (Richland County) had the greatest St. Croix identity (75.9%). This result aligned with my expectation, considering many of the survey populations had little recorded history of stocking. Group A on the NJ tree showed a higher mean St. Croix identity than any other group (mean = 16%, SD = 33.2%; Table 1.3).

Integration with Erdman et al. (2022)

Three out of the seven common loci showed inconsistent or systematic variation in allele calls, so they were not used. The remaining four loci exhibited strong concordance in allele calls, allowing for confident application of scaling factors (L_SFOC113, L_SFOC28, L_SFOC88, and SfoC38). The number of alleles for these loci ranged from 7 to 14 (mean = 10.75). For CE genotypes, L_SFOC113 allele calls were reduced by 79, L_SFOC28 allele calls were reduced by 115, L_SFOC88 alleles calls were reduced by 114, and SfoC38 allele calls were reduced by 58. The combined dataset contained 6,285 samples after merging my samples with the Erdman et al. (2022) data at the remaining four shared loci. Estimates of H_o and H_e produced using four loci were systematically higher and yielded weak correlations with the estimates produced by all 68 loci, therefore heterozygosity could not confidently be estimated for the combined dataset (H_o: $R^2_{adj} = 0.190$, p-value = 0.0002; H_e: $R^2_{adj} = 0.346$, p-value = $2.953e^{-7}$; Figures 1.19C and 1.19D). Estimates of A_r produced using the four loci were systematically higher than estimates produced using all 68 loci; however, the correlation between estimates was high enough to warrant evaluation of A_r for the combined dataset ($R^2_{adj} = 0.525$, p-value

= $1.694e^{-11}$; Figure 1.19B). Estimates of Nei's G_{ST} also showed a moderate correlation between the two sets of loci, supporting the evaluation of genetic distance for the combined dataset ($R^2_{adj} = 0.494$, p-value = $2.2e^{-16}$; Figure 1.19A).

For the combined dataset (148 populations, 2 hatchery collections, 4 loci), A_r ranged from 2.08 to 4.91with a mean of 4.08. Estimates of A_r were plotted on a heatmap of Wisconsin to depict spatial trends in genetic diversity (Figure 1.20). Similarly to my pre-combined dataset (63 populations, 1 hatchery collection, 68 loci), genetic diversity was typically lowest in the Driftless Area of southwestern Wisconsin. A latitudinal gradient in genetic diversity was less obvious for the combined dataset; however, a weak but statistically significant relationship between latitude and A_r was found ($R^2_{adj} = 0.059$, p-value = $2.2e^{-16}$). A NJ tree was produced using pairwise genetic distances and several major clades were resolved (Figure 1.21). Manchester Fish Hatchery and the St. Croix Falls strain were located adjacent to one another on the NJ tree, suggesting close relatedness between the two hatchery collections.

Discussion

Localized extirpations, reintroductions, and widespread stocking of various strains has obscured the endemic patterns of genetic diversity and spatial genetic structure throughout many areas of the brook trout's range (Marie et al. 2010; Hoxmeier et al. 2015; Kazyak et al. 2018; Erdman et al. 2022; Mamoozadeh et al. 2023). In Wisconsin, warming stream temperatures, artificial waterway barriers, and habitat degradation have led to decreased connectivity of wild brook trout populations. At the same time, widespread and intensive brook trout stocking has occurred across the state. By focusing on many brook trout populations with little recorded history of stocking, this study aimed to determine whether endemic genetic structure may remain. Broad latitudinal gradients in genetic diversity were observed, with the greatest levels found in northern Wisconsin (Figure 1.4). Regions containing populations of similar genetic ancestry were identified, such as in central and northeastern Wisconsin (Figure 1.9). Populations in the Driftless Area of southwestern Wisconsin generally showed lower levels of genetic diversity, higher levels of St. Croix identity, and heterogeneous genetic clustering in the region. Overall, I identified genetic groupings and summarized the genetic characteristics of 63 wild brook trout populations using measures of genetic diversity, N_e, domestic strain introgression, genetic differentiation, and spatial genetic structure.

Genetic Diversity & N_e

Latitude was found to be a positive predictor of genetic diversity (A_r, H_o, and H_e), with brook trout populations in northern Wisconsin generally showing higher levels of genetic diversity (Figure 1.4). Although the absolute values for genetic diversity cannot be compared with other studies due to differing microsatellite panels, others have similarly observed spatial trends in genetic diversity. On a comparable geographic scale to this study, Erdman et al. (2022) found greater A_r in brook trout populations in the Great Lakes Region (HUC 2) compared to those in the Upper Mississippi Region of Wisconsin. I found the opposite to be true, but the difference in mean A_r between the two regions was very small. Similarly to our latitudinal trend, genetic diversity in brook trout populations from the southern Appalachian Mountains was generally lower than those from the northern Appalachian Mountains (Kazyak et al. 2022). In my study, genetic diversity was less spatially variable than N_e; however, populations that showed larger N_e estimates were typically found in central or northern Wisconsin (Figure 1.5). The higher levels in genetic diversity and N_e found in northern Wisconsin may be attributable to greater waterbody connectivity in northern Wisconsin, permitting greater levels of gene flow. Moreover, seasonal water temperatures can be more variable in rivers and streams of northern Wisconsin compared to other areas of the state, which may lead to greater brook trout movement and gene flow. I generally observed larger N_e estimates (range: 2-4,794, median = 92) in Wisconsin as compared to brook trout populations in the Appalachian Mountains (range: 1-2,777, median = 55.1; Kazyak et al. 2022), Tennessee (range: 1.3-548, median = 12.5; Hargrove et al. 2022), and the Midwest USA (range = 2.6-infinity, median = 70.6; Erdman et al. 2022). I typically found smaller N_e estimates than those from a collection of 14 small streams in Nova Scotia (range: 48.6-595, median = 142; Ruzzante et al. 2016). My population-level estimates of N_e showed less uncertainty than those in the aforementioned brook trout studies, likely due to my use of a greater number of loci. Using a 100-locus microsatellite panel including many of the loci used here, Lehnert et al. (2020) generally observed larger N_e estimates from 12 river systems in Nova Scotia (range = 10.1-3,188.5, median = 168.3). Populations with large N_e and high levels of genetic diversity may be favorable broodstock source candidates. However, increases in genetic diversity can also occur due to mixing of wild and stocked individuals from recent stocking events. I found F_{IS} to be the most spatially variable genetic diversity parameter (Figure 1.4B). High F_{IS} could indicate long-term isolation or a lack of disturbance (e.g., stocking influence) for some wild populations, particularly those that have persisted at a relatively low abundance.

Spatial Genetic Structure

Little concordance was found between hydrological boundaries and genetic clustering; however, a few geographical areas of Wisconsin were identified that may reflect natural genetic structure (Figure 1.9C). To describe patterns in spatial genetic structure from here on, I refer to the K = 9 configuration from STRUCTURE (Figures 1.9C and 1.14). This configuration identified a genetic grouping primarily located in northeastern Wisconsin, as all survey populations in the adjacent Brule and Menominee subbasins (HUC 8) showed majority assignment to Cluster 2 (blue). In addition to this geographic grouping, these populations generally had minimal recorded stocking history and low estimated St. Croix identity (Figure 1.17). For these reasons, I propose that predominant assignment to Cluster 2 may be indicative of remnant native ancestry. However, there were a few populations (Cady Creek, Maple Creek, Swan Creek, Mt. Pelee Creek) with strong assignment to Cluster 2 that are located west of the HUC 2 hydrological boundary, suggesting that this lineage may have been distributed westward via undocumented stocking or translocation (assuming the grouping of populations in northeast Wisconsin reflects native ancestry). Cluster 3 (green) was particularly prevalent in central Wisconsin; however, populations with predominant assignment to Cluster 3 occur on either side of the Mississippi River-Great Lakes regional (HUC 2) boundary (Figure 1.9C). Under natural conditions I would expect genetic clustering to conform to this hydrological boundary, as observed in Wisconsin smallmouth bass *Micropterus dolomieu* (Euclide et al. 2020). This non-conformity to the HUC 2 hydrological boundary may be indicative of human influence. It is possible that Cluster 3 reflects a lineage that was endemic to one side of the HUC 2 boundary and propagated (e.g., stocked or translocated) to the other side. Alternatively, Cluster 3 could reflect an unknown non-native strain that was stocked across central Wisconsin. Little spatial genetic structure was observed in other areas of the state. For example, several genetic clusters and fine-scale genetic structuring were observed in the Driftless Area of southwestern Wisconsin (Figure 1.9C). This may be attributable to past human activities. Beginning around the 1850s, detrimental agricultural practice practices, overexploitation, and stocking of non-native brown trout *Salmo trutta* led to the extirpation of most brook trout populations in the Driftless Area (Thorn et al. 1997). Populations that persisted likely experienced bottlenecks and drastic reductions in N_e, subsequently resulting in high rates of genetic drift that may have led to the observed differentiation. Other brook trout populations were reintroduced, potentially with non-native strains. This study and Erdman et al. (2022) both found fine-scale genetic structure, low genetic diversity, and high St. Croix identity in the Driftless Area relative to other regions of Wisconsin. Based on our observations, I propose that northeastern Wisconsin is a better representation of remnant native brook trout genetics.

Spatial genetic structure has been observed for brook trout at spatial scales similar to my observations in several areas of North America. Brook trout from Maryland exhibited spatial genetic structure that clearly coincided with five different geographic regions, and little evidence of homogenization from hatchery sources (Morgan et al. 2021). Stott et al. (2010) similarly observed geographically concordant genetic clustering in brook trout populations from Isle Royale, three Minnesota rivers, and Lake Nipigon, Ontario. Despite observing fine-scale spatial genetic structure, genetic clustering was found to consistently align with hydrological boundaries in brook trout populations of Nova Scotia as well (Lehnert et al. 2020). At an even broader geographic scale, Kazyak et al. (2022) found distinct spatial genetic groupings across the southern Appalachian Mountains, northern Appalachian Mountains, and northeastern states. Moreover, that study evaluated four populations from Wisconsin (Melancthon Creek, Ash Creek, Parfrey's Glen Creek, and Soper Creek) and found shared clustering assignments with eastern populations and hatchery collections, suggesting those populations may have been influenced by non-native stocking (Kazyak et al. 2022). Mamoozedah et al. (2023) resolved five distinct genetic groupings of brook trout at the continental scale using a newly developed panel of SNPs with samples from that dataset and others. Although broad spatial patterns in genetic structure have been detected in many of these similar studies, natural genetic structure may be less intact for the midwestern brook trout, and specifically brook trout in the Driftless Area of Minnesota, Iowa, and Wisconsin (Hoxmeier et al. 2015; Erdman et al. 2022). The results from my study align with prior findings of little concordance between hydrological boundaries and genetic structuring for brook trout populations in this region (Erdman et al. 2022).

Spatial genetic structure for other regional fishes is typically more geographically defined than observed in brook trout. Unlike our study, northern Wisconsin populations of johnny darter *Etheostoma nigrum* and rock bass *Ambloplites rupestris* were found to have distinct genetic groupings that closely aligned with watershed boundaries (Westbrook 2012). Similarly, distinct genetic lineages were identified for smallmouth bass between the Upper Mississippi and Lake Michigan regions of Wisconsin (Euclide et al. 2020). Because these three species have minimal stocking histories in Wisconsin, they may provide insight into endemic genetic structure for other species, such as brook trout. muskellunge *Esox masquinongy* and walleye *Sander vitreus* have been stocked intensively in Wisconsin (although still less than brook trout), altering the species' natural genetic structure in some areas of the state (Turnquist et al. 2017; Hammen and Sloss 2019; Bootsma et al. 2021). Because brook trout require colder water than muskellunge and walleye, they are more sensitive to anthropogenic disturbance and subsequent genetic consequences (e.g., reduced N_e, genetic drift, bottlenecks, extirpation). For example, many Driftless Area brook trout populations have been highly impacted by anthropogenic disturbance (e.g., poor land use practices, waterway barriers, introduction of non-native brown trout), leading to declines in abundance, fragmentation, and extirpation (MacCrimmon and Campbell 1969; Erdman et al. 2022). The fine-scale spatial genetic structure observed in the Driftless Area may be evidence of reductions in N_e and rapid genetic drift ultimately resulting from these anthropogenic disturbances (Kelson et al. 2015). In addition to the intensive stocking of brook trout across Wisconsin, genetic drift has likely contributed to their comparatively irregular genetic structure.

Tree Groupings

Survey populations were divided into six groups (A-F) based major breaks in the neighbor-joining (NJ) tree (Figure 1.7) and cluster assignments from the K = 9 STRUCTURE configuration (Figure 1.14). There were some discrepancies between the NJ tree and genetic clustering produced by STRUCTURE, so it should be noted that there are exceptions to each of the defined groups. Group A reflects close ancestry to the St. Croix Falls strain, as populations within this group have the lowest genetic differentiation with the St. Croix Falls strain. The populations within Group A (n = 17) are spatially variable with many of them located in the Driftless Area or north-central Wisconsin. Populations within Group A generally had higher estimates of St. Croix identity (mean = 16.0%, SD = 33.2%; Table 1.3) and intensive stocking histories. Several populations in this group including Allequash Creek, Upper Pine River, Bearskin Creek, Little Willow Creek, and Chambers Creek had no recorded history of direct stocking; however, each of them are connected to one or more waterbodies that have received stocking, suggesting nearby stocking events may have had a spillover effects on the genetic composition of these populations (Bruce and Wright 2018). Two populations in Group A, Venison Creek and Little Deerskin River, have no recorded history of direct or adjacent stocking, which may be indicative of undocumented stocking or translocation. Fish from populations in Group A typically assigned to Clusters 1 (St. Croix Falls strain), 6, 7, or 8 (Figure 1.14). Unsurprisingly, these four clusters make up one of the two major groupings on the K = 9 cluster tree (Figure 1.15C), suggesting that fish with predominant assignment to these four clusters likely share some degree of non-native common ancestry.

Group B likely reflects remnant native ancestry. Populations in Group B (n = 16) were primarily located in northern Wisconsin. Only two populations in Group B, Tributary to Smokey Hollow and Spring Brook, had any record of direct stocking. About half of the populations in Group B are connected to a nearby waterbody that has received stocking, yet these populations showed little to no sign of hatchery influence. Spring Brook has shown notable resilience to stocking, as it has been intensively stocked with fish of unknown origin and St. Croix Falls strain, yet it showed St. Croix identity of 0%. This could be due to poor fitness of stocked fish, lack of immigration from stocked fish, or the selective purging of maladaptive alleles (Harbicht et al. 2014b). Regardless, most populations in Group B appear to be genetically unaltered with minimal estimates of St. Croix identity (mean = 0.4%, SD = 5.1%; Table 1.3). Fish from populations in Group B predominantly assigned to Cluster 2 or Cluster 4. The primary outlier in this group was Tributary to Smokey Hollow, as it showed strong assignment to Cluster 6 (similarly to two populations in Group A). Tributary to Smokey Hollow has also been subject to direct and nearby stocking efforts and is located in the Driftless Area (unlike all other populations in this group). Because of these characteristics, I would have expected this population to be placed within Group A on the NJ tree. I believe the remaining populations in Group B likely represent remnant native ancestry; thus, Group B may contain candidate broodstock sources that could be genetically appropriate for watersheds in northern Wisconsin.

Group C is comprised of just two populations, Lowry Creek and Lowery Creek. Both populations are located in the Driftless Area and showed predominant assignment to Cluster 4 (Figure 1.14). Lowery Creek was previously suggested to be of native ancestry

(Erdman et al. 2022), and has been used in the broodstock rotation for the Southwest Feral strain. Lowery Creek has been stocked once with fish of unknown origin, and Lowry Creek has no recorded history of direct stocking. Additionally, both populations had 0% estimated St. Croix identity, suggesting that Group C may reflect native ancestry.

Group D consists of populations that had unclear origins and may have been influenced by propagation (n = 5). Fish from the populations in this group commonly had mixed cluster assignments, except for Little Scarboro Creek which showed predominant assignment to Cluster 3 (Figure 1.14). Populations in Group D were geographically variable, exhibiting fine-scale spatial genetic structure. Unnamed tributary to Maple Dale Creek, Tamarack Creek, and Little Scarboro Creek are tributaries to waterbodies that have been stocked with brook trout of St. Croix Falls strain and/or unknown origin. Populations in this group had 0% estimated St. Croix identity, apart from Fancy Creek (3.4%) which has been intensively stocked with various strains (mean = 0.7%, SD = 8.1%). Although populations in Group D generally show little resemblance to the St. Croix Falls strain, it is likely they have been influenced by the stocking of other strains due to their mixed ancestries and geographic variability (Figure 1.9C).

Group E showed spatial genetic structure that could be natural, as all four populations in this group are all located in central Wisconsin and on the east side of the HUC 2 hydrological boundary (Great Lakes Region). Group E is comprised of Tagatz Creek, Caves Creek, Spranger Creek, and Flume Creek. These populations showed no indication of hatchery influence from the St. Croix Falls strain, as their mean St. Croix identity was 0% (SD = 0%). They all showed predominant assignment to Cluster 3, except for Tagatz Creek which also showed considerable assignment to Cluster 4. Each of these populations has been stocked with fish of unknown origin three or fewer times, apart from Flume Creek which has been intensively stocked with fish of unknown origin. It is possible that these populations were all stocked with the same unknown strain, resulting in their genetic similarity. If this is true, these populations may not reflect remnant native ancestry. It is difficult to determine whether these populations were genetically influenced by the stocking of unknown origin fish, but if they were able to resist such influence, they may yet reflect remnant native genetics.

Group F is complicated to interpret, as populations in this group are geographically variable (n = 19). Most fish from populations in these groups have predominant assignment to Clusters 3, 5, or 9. Most populations in Group F have a record of just three or fewer direct stocking events; however, four populations in Group F have received intensive stocking from various strains: South Fork Hay River, Plover River, Byrds Creek, and Tenmile Creek – South Branch. These four populations show predominant assignment to Clusters 3 or 9 and are located on the west side of the HUC 2 hydrological boundary (Upper Mississippi Region). Three populations in Group F have been used in the broodstock rotation for the Southwest Feral strain: Melancthon Creek, South Fork Hay River, and Cady Creek. All three of these population have previously been suggested to be of native ancestry (Erdman et al. 2022). However, clustering analyses performed by Kazyak et al. (2022) indicated that Melancthon Creek may be related to populations or hatchery collections from the eastern United States. For this reason, further comparison to non-native strains would be advisable to verify the ancestry of Melancthon Creek and other closely related populations (those with predominant assignment to Cluster 5 such as Schuett Creek and Laxey Creek). Overall, populations in

Group F showed minimal influence from the St. Croix Falls strain, as mean St. Croix identity was just 0.1% (SD = 2.1%). Many populations from Group F have predominant assignment to Cluster 3 and occur on either side of the HUC 2 hydrological boundary. This makes it very difficult to infer whether Cluster 3 reflects a remnant native lineage that has been propagated across the hydrological boundary or a widely propagated non-native lineage. For this reason, I cannot confidently claim that Group F reflects remnant native genetics.

Domestic Strain Introgression

Estimates of domestic strain introgression (St. Croix identity) were generally low, with little similarity in allele frequencies to the St. Croix Falls strain reference group (Figure 1.17). These results are consistent with my expectation, as many survey populations had little recorded stocking history. However, some of the surveyed populations with a history of stocking of the St. Croix Falls strain showed little evidence of the strain's persistence, as observed in some other Wisconsin populations (Erdman et al. 2022). Low levels of domestic strain introgression despite widespread stocking efforts have also been observed in eastern United States and Canadian brook trout populations (Kazyak et al. 2018, 2022; Beer et al. 2019; Lehnert et al. 2020; Morgan et al. 2021). This could be due to poor fitness of stocked fish, genetic drift, or the selective purging of hatchery-derived alleles (Harbicht et al. 2014b). Populations I analyzed that showed high levels of St. Croix identity no longer represent remnant native brook trout genetics, thus would not be genetically appropriate broodstock sources (e.g., Chase Creek, Willow Creek, Little Willow Creek, Knapp Creek, Bergen Creek). One notable survey population was Chase Creek which had the second highest estimate of St. Croix identity (46.8%;

Table 1.1). Chase Creek has three recorded brook trout stocking events, all of which used the Southwest Feral (Ash Creek) strain. Chase Creek contained no brook trout prior to these stocking events, suggesting that the population is completely derived from Ash Creek. The high levels of St. Croix identity observed in Chase Creek subsequently provide strong support for previous claims that Ash Creek shares common ancestry with the St. Croix Falls strain (Erdman et al. 2022).

Estimates of domestic strain introgression were limited to the St. Croix Falls strain. Although the St. Croix Falls strain has historically been the most widely stocked brook trout strain in Wisconsin, there are many other strains that have been used. Some survey populations may have ancestry derived from non-native sources that I could not identify. Additionally, it is possible that the St. Croix Falls strain and wild populations have genetically changed over time, as both hatchery and wild populations are dynamic and subject to genetic drift and varying selective forces. This could ultimately result in lower levels of St. Croix identity than past stocking would suggest being present. It is also important to note that survey populations with little recorded stocking history were targeted for this study, therefore our St. Croix identity estimates are not likely to be representative of more general patterns in Wisconsin. My estimates of domestic strain introgression were generally lower than those of Erdman et. al (2022), that observed an overall mean domestic hatchery identity of 12.5% (SD = 14.5%) for inland Wisconsin brook trout populations. However, Erdman et. al (2022) and my study both found domestic strain introgression to be greatest in the Driftless Area.

Genotyping Panel

Microsatellite loci have been the primary tool for conservation geneticists for nearly two decades (Goldstein and Pollock 1997; Allendorf 2017). A shift to single nucleotide polymorphism (SNP) markers has been underway for several years (Mamoozadeh et al. 2023); however, the advent of amplicon-based microsatellite genotyping techniques provides an opportunity for continued use of these allele-rich markers despite the phasing out of the CE platforms typically used for microsatellite genotyping (Gruenthal and Larson 2021; Marcy-Quay et al. 2023). Genotyping of microsatellites via amplicon sequencing will allow for existing datasets to be leveraged into greater sample sizes as I demonstrated here and help address challenges in cross-laboratory replicability of locus-sizing that were common when genotyping microsatellites using CE. With this study, a brook trout microsatellite panel was optimized for amplicon sequencing that incorporates legacy and more recently developed loci. This 68-locus panel extends the work of Lehnert et al. (2020) by including legacy loci that have been used in numerous past studies as well as stringent filtering of the Lehnert et al. (2020) loci to only include those with allele sizes that can be confidently assigned through the automated scoring of MEGAsat (Table 1.2). The inclusion of additional legacy loci in our amplicon sequencing panel will help ensure that such legacy CE microsatellite datasets remain comparable and expandable.

Management Implications

These findings may provide useful guidance for management actions such as prioritizing populations for resource allocation (e.g., project funding and management action), identifying candidate brook trout reserves (i.e., designated places where brook trout have the best chance of enduring environmental consequences associated with climate change; WDNR 2019), selecting wild broodstock sources, and developing an appropriate spatial scale for the genetic management of wild brook trout populations. Measures of genetic diversity, inbreeding, and N_e can help identify populations that are at risk for local extirpation. Populations with low levels of genetic diversity, high levels of inbreeding, or small N_e could be considered for additional conservation effort to avoid irretrievable losses in genetic diversity. In contrast, populations with high levels of genetic diversity and large N_e may be candidates for brook trout reserve status (WDNR 2019), as they are more likely to have adaptive potential and genetic resiliency that managers may be interested in conserving. These populations may also warrant additional conservation efforts, as they have displayed a greater likelihood of long-term persistence.

Estimates of genetic diversity, inbreeding, and N_e can be used in combination with hatchery introgression and clustering results to identify and select new genetically appropriate broodstock sources. When selecting new wild broodstock sources, there are several genetic criteria that should be considered. First, a wild broodstock source should have adequate levels of genetic diversity and subsequently low levels of inbreeding. Genetic diversity is often thought of as a surrogate for fitness and adaptive potential, both of which are desirable traits for recipient populations (Reed and Frankham 2003). Moreover, populations that have undergone considerable inbreeding are more likely to pass on deleterious alleles (Claussen and Philipp 2023). Second, a wild broodstock source should have a relatively large N_e to better withstand the removal of many spawning adults during broodstock collection (Miller and Kapuscinski 2003; Hughes 2008). Additionally, the likelihood of collecting and spawning related individuals declines as the number of wild breeders (N_e) increases. Third, a wild broodstock source should have minimal evidence of past stocking of domesticated lineages (Araki and Schmid 2010). One of the primary reasons for using wild broodstock is to avoid distributing potentially deleterious hatchery-adapted alleles into wild populations. Lastly, a wild broodstock source should be genetically representative of the hydrological region to be stocked (i.e., of remnant native/endemic ancestry; Miller and Kapuscinski 2003). Stocking locally with endemic broodstock reduces the likelihood of consequences that can arise from introgression with stocked fish (e.g., outbreeding depression, significant changes to a population's genetic composition; Claussen and Philipp 2023). This strategy can also result in greater stocking success (e.g., increased post-stocking survival), as endemic fish are more likely to have advantageous local adaptation than fish of non-local origin. Remnant native ancestry is the most challenging of these genetic criteria to identify due to the convoluted history of brook trout propagation in Wisconsin.

These four criteria provide a framework for selecting genetically appropriate sources of broodstock, with this study providing a frame of reference for identifying candidate populations. Populations that meet these criteria could further be investigated for other necessary metrics such as accessibility for streamside spawning operations and overall population health. I suggest that Groups B and C on the NJ tree (Figure 1.7) are most likely to contain candidate broodstock sources that could be genetically appropriate due to their possible remnant native ancestry. It should be noted that these genetic criteria may not be of importance when stocking waterbodies that do not support natural reproduction or interannual survival of brook trout (Wisconsin's Class III trout waterbodies that are not connected to any Class I or Class II trout waterbodies). The identification of new broodstock sources also supports the growing emphasis among fisheries managers to stock trout that are of wild parentage (e.g., WDNR’s Wild Trout Stocking Program). Trout of wild parentage (F1 and F2) have been used to improve the interannual survival of stocked trout and to reintroduce endemic genetic variation to populations that have been genetically altered due to introgression with domesticated hatchery fish (Mitro 2004). Lastly, the findings from the Mantel correlogram test suggest that spatial genetic structure begins to break down at distances greater than approximately 75 km for our survey populations, indicating that 75 km may be an appropriate spatial scale for the genetic management of brook trout populations in the study region (Figure 1.8).

Conclusion

As wild brook trout populations face mounting pressure from warming stream temperatures, non-native species, habitat degradation, and fragmentation, the conservation of natural genetic structure and diversity has gained increasing management attention. With this chapter, I produced detailed genetic information to collectively summarize the population genetics of 63 wild brook trout populations in Wisconsin. Moreover, the refined genotyping panel used here unveiled population ancestries that could not otherwise be characterized. Patterns in spatial genetic structure were detected that could be indicative of remnant native genetics in central and northeastern Wisconsin, and a “body of evidence” approach was used to classify populations into groupings that describe their possible ancestry. Collectively, this chapter may aid fisheries management as future efforts are made to preserve the genetic integrity of wild brook trout populations and avoid the irretrievable loss of endemic genetic variation.

Literature Cited

Adamack, A. T., and B. Gruber. 2014. PopGenReport: Simplifying basic population genetic analyses in R. Methods in Ecology and Evolution 5(4):384–387.

Allendorf, F. W. 2017. Genetics and the conservation of natural populations: Allozymes to genomes. Molecular Ecology 26(2):420–430.

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for Brook Charr reveal strong population subdivision on a microgeographic scale. Journal of fish biology 47(sA):177–185.

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1(2):342–355.

Araki, H., B. Cooper, and M. S. Blouin. 2007. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318(5847):100–103.

Araki, H., and C. Schmid. 2010. Is hatchery stocking a help or harm? Aquaculture 308:S2–S11.

Beer, S. D., S. Cornett, P. Austerman, B. Trometer, T. Hoffman, and M. L. Bartron. 2019. Genetic diversity, admixture, and hatchery influence in Brook Trout (*Salvelinus fontinalis*) throughout western New York State. Ecology and Evolution 9(13):7455–7479.

Benjamini, Y., and Y. Hochberg. 1995. Controlling the false discovery rate: A practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological) 57(1):289–300.

Bootsma, M. L., K. M. Gruenthal, G. J. McKinney, L. Simmons, L. Miller, G. G. Sass, and W. A. Larson. 2020. A GT-seq panel for Walleye (*Sander vitreus*) provides important insights for efficient development and implementation of amplicon panels in non-model organisms. Molecular Ecology Resources 20(6):1706–1722.

Bootsma, M. L., L. Miller, G. G. Sass, P. T. Euclide, and W. A. Larson. 2021. The ghosts of propagation past: Haplotype information clarifies the relative influence of stocking history and phylogeographic processes on contemporary population structure of Walleye (*Sander vitreus*). Evolutionary Applications 14(4):1124–1144.

Bradbury, I. R., B. F. Wringe, B. Watson, I. Paterson, J. Horne, R. Beiko, S. J. Lehnert, M. Clément, E. C. Anderson, N. W. Jeffery, S. Duffy, E. Sylvester, M. Robertson, and P. Bentzen. 2018. Genotyping-by-sequencing of genome-wide microsatellite loci reveals fine-scale harvest composition in a coastal Atlantic Salmon fishery. Evolutionary Applications 11(6):918–930.

Brook, B. W., D. W. Tonkyn, J. J. O'Grady, and R. Frankham. 2002. Contribution of inbreeding to extinction risk in threatened species. Conservation Ecology 6(1).

Bruce, S. A., Y. Kutsumi, C. Van Maaren, and M. P. Hare. 2020. Stocked-fish introgression into wild Brook Trout populations depends on habitat. Transactions of the American Fisheries Society 149(4):427–442.

Bruce, S. A., and J. J. Wright. 2018. Estimates of gene flow and dispersal in wild riverine Brook Trout (*Salvelinus fontinalis*) populations reveal ongoing migration and introgression from stocked fish. Ecology and Evolution 8(23):11410–11422.

Christensen, K. A., E. B. Rondeau, D. R. Minkley, J. S. Leong, C. M. Nugent, R. G. Danzmann, M. M. Ferguson, A. Stadnik, R. H. Devlin, R. Muzzerall, M. Edwards, W. S. Davidson, and B. F. Koop. 2018. The Arctic Charr (*Salvelinus alpinus*) genome and transcriptome assembly. PLoS ONE 13(9):e0204076.

Christie, M. R., M. L. Marine, R. A. French, R. S. Waples, and M. S. Blouin. 2012. Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity 109(4):254–260.

Claussen, J. E., and D. P. Philipp. 2023. Assessing the role of supplementation stocking: A perspective. Fisheries Management and Ecology 30(6):583–591.

Currens, K. P., and C. A. Busack. 1995. A framework for assessing genetic vulnerability. Fisheries 20(12):24–31.

Dixon, P. 2003. VEGAN, A package of R functions for community ecology. Journal of Vegetation Science 14(6):927–930.

Do, C., R. S. Waples, D. Peel, G. M. Macbeth, B. J. Tillett, and J. R. Ovenden. 2014. NeEstimator v2: Re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources 14(1):209–214.

Erdman, B., M. G. Mitro, J. D. T. Griffin, D. Rowe, D. C. Kazyak, K. Turnquist, M. Siepker, L. Miller, W. Stott, M. Hughes, B. Sloss, M. T. Kinnison, C. C. Wilson, and W. Larson. 2022. Broadscale population structure and hatchery introgression of midwestern Brook Trout. Transactions of the American Fisheries Society 151(1):81–99.

Euclide, P. T., J. Ruzich, S. P. Hansen, D. Rowe, T. G. Zorn, and W. A. Larson. 2020. Genetic structure of Smallmouth Bass in the Lake Michigan and Upper Mississippi River drainages relates to habitat, distance, and drainage boundaries. Transactions of the American Fisheries Society 149(4):383–397.

Evanno, G., S. Regnaut, and J. Goudet. 2005. Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. Molecular Ecology 14(8):2611–2620.

Ferguson, M. M. 1990. The genetic impact of introduced fishes on native species. Canadian Journal of Zoology 68(5):1053–1057.

Fernández, M. E., D. E. Goszczynski, J. P. Lirón, E. E. Villegas-Castagnasso, M. H. Carino, M. V. Ripoli, A. Rogberg-Muñoz, D. M. Posik, P. Peral-García, and G. Giovambattista. 2013. Comparison of the effectiveness of microsatellites and SNP panels for genetic identification, traceability and assessment of parentage in an inbred Angus herd. Genetics and Molecular Biology 36:185–191.

Frankham, R. 1995. Conservation Genetics. Annual Review of Genetics 29:305–327.

Goldstein, D. B., and D. D. Pollock. 1997. Launching microsatellites: A review of mutation processes and methods of phylogenetic inference. Journal of Heredity 88(5):335–342.

Gossieaux, P., L. Bernatchez, P. Sirois, and D. Garant. 2019. Impacts of stocking and its intensity on effective population size in Brook Charr (*Salvelinus fontinalis*) populations. Conservation Genetics 20(4):729–742.

Goudet, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5(1):184–186.

Gruenthal, K. M., and W. A. Larson. 2021. Efficient genotyping with backwards compatibility: Converting a legacy microsatellite panel for Muskellunge (*Esox masquinongy*) to genotyping-by-sequencing chemistry. Conservation genetics resources 13(2):151–159.

Hammen, J. J., and B. L. Sloss. 2019. Walleye genetic characterization in the northern ceded territory of Wisconsin: Implications for stocking using conservation strategies. North American Journal of Fisheries Management 39(4):693–704.

Harbicht, A. B., M. Alshamlih, C. C. Wilson, and D. J. Fraser. 2014a. Anthropogenic and habitat correlates of hybridization between hatchery and wild Brook Trout. Canadian Journal of Fisheries and Aquatic Sciences 71(5):688–697.

Harbicht, A., C. C. Wilson, and D. J. Fraser. 2014b. Does human-induced hybridization have long-term genetic effects? Empirical testing with domesticated, wild and hybridized fish populations. Evolutionary Applications 7(10):1180–1191.

Hargrove, J. S., D. C. Kazyak, B. A. Lubinski, K. M. Rogers, O. K. Bowers, K. A. Fesenmyer, J. W. Habera, and J. Henegar. 2022. Landscape and stocking effects on population genetics of Tennessee Brook Trout. Conservation Genetics 23(2):341–357.

Hijmans, R. J. 2022, November 15. geosphere: Spherical Trigonometry. R.

Hoxmeier, R. J. H., D. J. Dieterman, and L. M. Miller. 2015. Brook Trout distribution, genetics, and population characteristics in the Driftless Area of Minnesota. North American Journal of Fisheries Management 35(4):632–648.

Hughes, M. S. 2008. Genetic impacts of broodstock selection strategies for Wisconsin's Wild Brook Trout Stocking Program. Thesis M.S., University of Wisconsin - Stevens Point.

Hunt, R. L. 1979. Exploitation, growth and survival of three strains of domestic Brook Trout. Madison, Wisconsin, Research Report 99, Dept. of Natural Resources.

Jenkins, T. L. 2024. MAPMIXTURE: An R package and web app for spatial visualisation of admixture and population structure. Molecular Ecology Resources:e13943.

Jombart, T. 2008. *adegenet*: A R package for the multivariate analysis of genetic markers. Bioinformatics 24(11):1403–1405.

Kalinowski, S. T. 2004. Counting alleles with rarefaction: Private alleles and hierarchical sampling designs. Conservation Genetics 5(4):539–543.

Kanno, Y., J. C. Vokoun, and B. H. Letcher. 2011. Fine-scale population structure and riverscape genetics of Brook Trout (*Salvelinus fontinalis*) distributed continuously along headwater channel networks. Molecular Ecology 20(18):3711–3729.

Kazyak, D. C., B. A. Lubinski, M. A. Kulp, K. C. Pregler, A. R. Whiteley, E. Hallerman, J. A. Coombs, Y. Kanno, J. M. Rash, R. P. Morgan, J. Habera, J. Henegar, T. C. Weathers, M. T. Sell, A. Rabern, D. Rankin, and T. L. King. 2022. Population genetics of Brook Trout in the southern Appalachian Mountains. Transactions of the American Fisheries Society 151(2):127–149.

Kazyak, D. C., J. Rash, B. A. Lubinski, and T. L. King. 2018. Assessing the impact of stocking northern-origin hatchery Brook Trout on the genetics of wild populations in North Carolina. Conservation Genetics 19(1):207–219.

Kelson, S. J., A. R. Kapuscinski, D. Timmins, and W. R. Ardren. 2015. Fine-scale genetic structure of Brook Trout in a dendritic stream network. Conservation Genetics 16(1):31–42.

King, T. L., B. A. Lubinski, M. K. Burnham-Curtis, W. Stott, and R. P. Morgan. 2012. Tools for the management and conservation of genetic diversity in Brook Trout (*Salvelinus fontinalis*): Tri- and tetranucleotide microsatellite markers for the assessment of genetic diversity, phylogeography, and historical demographics. Conservation Genetics Resources 4(3):539–543.

Krueger, C. C., and B. W. Menzel. 1979. Effects of stocking on genetics of wild Brook Trout populations. Transactions of the American Fisheries Society 108(3):277–287.

Lamaze, F. C., C. Sauvage, A. Marie, D. Garant, and L. Bernatchez. 2012. Dynamics of introgressive hybridization assessed by SNP population genomics of coding genes in stocked Brook Charr (*Salvelinus fontinalis*): SNP population genomics in Brook Charr. Molecular Ecology 21(12):2877–2895.

Layton, K. K. S., B. Dempson, P. V. R. Snelgrove, S. J. Duffy, A. M. Messmer, I. G. Paterson, N. W. Jeffery, T. Kess, J. B. Horne, S. J. Salisbury, D. E. Ruzzante, P. Bentzen, D. Côté, C. M. Nugent, M. M. Ferguson, J. S. Leong, B. F. Koop, and I. R. Bradbury. 2020. Resolving fine-scale population structure and fishery exploitation using sequenced microsatellites in a northern fish. Evolutionary Applications 13(5):1055–1068.

Lehnert, S. J., S. M. Baillie, J. MacMillan, I. G. Paterson, C. F. Buhariwalla, I. R. Bradbury, and P. Bentzen. 2020. Multiple decades of stocking has resulted in limited hatchery introgression in wild Brook Trout (*Salvelinus fontinalis*) populations of Nova Scotia. Evolutionary Applications 13(5):1069–1089.

Lien, S., B. F. Koop, S. R. Sandve, J. R. Miller, M. P. Kent, T. Nome, T. R. Hvidsten, J. S. Leong, D. R. Minkley, A. Zimin, F. Grammes, H. Grove, A. Gjuvsland, B. Walenz, R. A. Hermansen, K. Von Schalburg, E. B. Rondeau, A. Di Genova, J. K. A. Samy, J. Olav Vik, M. D. Vigeland, L. Caler, U. Grimholt, S. Jentoft, D. Inge Våge, P. De Jong, T. Moen, M. Baranski, Y. Palti, D. R. Smith, J. A. Yorke, A. J. Nederbragt, A. Tooming-Klunderud, K. S. Jakobsen, X. Jiang, D. Fan, Y. Hu, D. A. Liberles, R. Vidal, P. Iturra, S. J. M. Jones, I. Jonassen, A. Maass, S. W. Omholt, and W. S. Davidson. 2016. The Atlantic Salmon genome provides insights into rediploidization. Nature 533(7602):200–205.

MacCrimmon, H. R., and J. S. Campbell. 1969. World distribution of Brook Trout, *Salvelinus fontinalis*. Canadian Journal of Fisheries and Aquatic Sciences 26(7):1699–1725.

Magoc, T., and S. L. Salzberg. 2011. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27(21):2957–2963.

Mamoozadeh, N. R., A. R. Whiteley, B. H. Letcher, D. C. Kazyak, C. Tarsa, and M. H. Meek. 2023. A new genomic resource to enable standardized surveys of SNPs across the native range of Brook Trout (*Salvelinus fontinalis*). Molecular Ecology Resources (00):1–20.

Marcy-Quay, B., C. C. Wilson, C. A. Osborne, and J. E. Marsden. 2023. Optimization of an amplicon sequencing-based microsatellite panel and protocol for stock identification and kinship inference of Lake Trout (*Salvelinus namaycush*). Ecology and Evolution 13(4):e10020.

Marie, A. D., L. Bernatchez, and D. Garant. 2010. Loss of genetic integrity correlates with stocking intensity in Brook Charr (*Salvelinus fontinalis*). Molecular Ecology 19(10):2025–2037.

Meek, M. H., and W. A. Larson. 2019. The future is now: Amplicon sequencing and sequence capture usher in the conservation genomics era. Molecular Ecology Resources 19(4):795–803.

Miller, L., and A. Kapuscinski. 2003. Genetic guidelines for hatchery supplementation programs. Population Genetics: Principles and Applications for Fisheries Scientists:329–355.

Mitro, M. G. 2004. Stocking trout of wild parentage to restore wild populations: An evaluation of Wisconsin's Wild Trout Stocking Program. Proceedings of Wild Trout XIII 8:255–264.

Mitro, M. G. 2016. Brook Trout, Brown Trout, and ectoparasitic copepods (*Salmincola edwardsii*): Species interactions as a proximate cause of Brook Trout loss under changing environmental conditions. Transactions of the American Fisheries Society 145(6):1223–1233.

Morgan, R. P., D. C. Kazyak, T. L. King, B. A. Lubinski, M. T. Sell, A. A. Heft, and J. W. Jones. 2021. Genetic structure of Maryland Brook Trout populations: Management implications for a threatened species. North American Journal of Fisheries Management 41(4):1097–1119.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proceedings of the National Academy of Sciences 70(12):3321–3323.

Oden, N. L., and R. R. Sokal. 1986. Directional autocorrelation: An extension of spatial correlograms to two dimensions. Systematic Zoology 35(4):608–617.

Perkins, D. L., C. C. Krueger, and B. May. 1993. Heritage Brook Trout in northeastern USA: Genetic variability within and among populations. Transactions of the American Fisheries Society 122(4):515–532.

Pritchard, J. K., M. Stephens, and P. Donnelly. 2000. Inference of population structure using multilocus genotype data. Genetics 155(2):945–959.

Prystupa, S., G. R. McCracken, R. Perry, and D. E. Ruzzante. 2021. Population abundance in arctic grayling using genetics and close-kin mark-recapture. Ecology and Evolution 11(9):4763–4773.

Reed, D. H., D. A. Briscoe, and R. Frankham. 2002. Inbreeding and extinction: The effect of environmental stress and lineage. Conservation Genetics 3(3):301–307.

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17(1):230–237.

Ruzzante, D. E., G. R. McCracken, S. Parmelee, K. Hill, A. Corrigan, J. MacMillan, and S. J. Walde. 2016. Effective number of breeders, effective population size and their relationship with census size in an iteroparous species, *Salvelinus fontinalis*. Proceedings of the Royal Society B: Biological Sciences 283(1823):20152601.

Thorn, W. C., C. S. Anderson, W. E. Lorenzen, D. L. Hendrickson, and J. W. Wagner. 1997. A Review of Trout Management in Southeast Minnesota Streams. North American Journal of Fisheries Management 17(4):860–872.

Tibihika, P. D., M. Curto, E. Dornstauder-Schrammel, S. Winter, E. Alemayehu, H. Waidbacher, and H. Meimberg. 2019. Application of microsatellite genotyping by sequencing (SSR-GBS) to measure genetic diversity of the East African *Oreochromis niloticus*. Conservation Genetics 20(2):357–372.

Trushenski, J. T., G. E. Whelan, and J. D. Bowker. 2018. Why keep hatcheries? Weighing the economic cost and value of fish production for public use and public trust purposes. Fisheries 43(6):284–293.

Turnquist, K. N., W. A. Larson, J. M. Farrell, P. A. Hanchin, K. L. Kapuscinski, L. M. Miller, K. T. Scribner, C. C. Wilson, and B. L. Sloss. 2017. Genetic structure of Muskellunge in the Great Lakes region and the effects of supplementation on genetic integrity of wild populations. Journal of Great Lakes Research 43(6):1141–1152.

Waples, R. S., T. Antao, and G. Luikart. 2014. Effects of overlapping generations on linkage disequilibrium estimates of effective population size. Genetics 197(2):769–780.

Waples, R. S., and C. Do. 2010. Linkage disequilibrium estimates of contemporary N_e using highly variable genetic markers: A largely untapped resource for applied conservation and evolution. Evolutionary Applications 3(3):244–262.

WDNR. 2019, March. Wisconsin Inland Trout Management Plan 2020-2029. Wisconsin Dept. of Natural Resources, Bureau of Fisheries Management.

Weir, B. S., and C. C. Cockerham. 1984. Estimating f-statistics for the analysis of population structure. Evolution 38(6):1358–1370.

Westbrook, L. J. 2012. Genetic structure of Rock Bass and Johnny Darters: Implications for gamefish management in Wisconsin. Thesis M.S.--University of Wisconsin--Stevens Point.

White, S. L., W. L. Miller, S. A. Dowell, M. L. Bartron, and T. Wagner. 2018. Limited hatchery introgression into wild Brook Trout (*Salvelinus fontinalis*) populations despite reoccurring stocking. Evolutionary Applications 11(9):1567–1581.

Williamson, K. S., and B. May. 2005. Homogenization of fall-run Chinook Salmon gene pools in the central valley of California, USA. North American Journal of Fisheries Management 25(3):993–1009.

Winter, D. J. 2012. mmod: an R library for the calculation of population differentiation statistics. Molecular Ecology Resources 12(6):1158–1160.

Wright, S. 1931. Evolution in Mendelian Populations. Genetics 16(2):97–159.

Wright, S. 1943. Isolation by distance. Genetics (Austin) 28(2):114–138.

Yu, G., D. K. Smith, H. Zhu, Y. Guan, and T. T.-Y. Lam. 2017. ggtree: An R package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods in Ecology and Evolution 8(1):28–36.

Zhan, L., I. G. Paterson, B. A. Fraser, B. Watson, I. R. Bradbury, P. Nadukkalam Ravindran, D. Reznick, R. G. Beiko, and P. Bentzen. 2017. MEGASAT: Automated inference of microsatellite genotypes from sequence data. Molecular Ecology Resources 17(2):247–256.

Tables

TABLE 1.1. Table displaying the latitude (Lat), longitude (Long), hydrologic unit code (HUC) 8 subbasin, and sample size (N) for each brook trout *Salvelinus fontinalis* survey population. Also shown are estimates for effective population size (N_e), allelic richness rarefied to the minimum sample size of 8 (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), inbreeding coefficient (F_{IS}), and St. Croix identity for each survey population. N_e values within parentheses indicate the 95% upper and lower jackknife confidence intervals. St. Croix identity is defined as the mean assignment (%) to the domestic St. Croix Falls strain.

Waterbody	Lat	Long	Subbasin (HUC 8)	N	N_e (95% CI)	A_r	H_o	H_e	F_{IS}	St. Croix identity (%)
Devils Creek	46.320	-90.653	Bad-Montreal	30	325 (42 - ∞)	1.443	0.418	0.444	0.044	0
Alvin Creek	45.969	-88.874	Brule	30	72.7 (31.3 - ∞)	1.449	0.431	0.450	0.050	0
Elvoy Creek	46.039	-88.939	Brule	24	406.2 (111.9 - ∞)	1.452	0.406	0.453	0.085	0
Wisconsin Creek	45.994	-88.588	Brule	30	4793.8 (161.8 - ∞)	1.440	0.411	0.440	0.065	0
Buena Vista Creek	44.389	-89.549	Castle Rock	30	1332.5 (94.5 - ∞)	1.403	0.378	0.404	0.061	0
Chester Creek	44.229	-89.866	Castle Rock	30	75.4 (41 - 274)	1.432	0.413	0.432	0.057	0
Fourmile Creek	44.338	-89.550	Castle Rock	30	327 (83.2 - ∞)	1.404	0.371	0.405	0.109	0
Lowry Creek	44.106	-90.490	Castle Rock	30	174.5 (17.9 - ∞)	1.433	0.425	0.433	0.002	0
Plover River	44.987	-89.276	Castle Rock	30	172.2 (37.4 - ∞)	1.440	0.421	0.440	0.059	0
Tenmile Creek - South Branch	44.272	-89.549	Castle Rock	30	88 (44 - 638.9)	1.418	0.398	0.419	0.068	0
Unnamed trib to Dell Creek (a)	43.542	-89.838	Castle Rock	30	54.3 (27.6 - 247.1)	1.464	0.429	0.465	0.073	11.6

Waterbody	**Lat**	**Long**	**Subbasin (HUC 8)**	**N**	**N_e (95% CI)**	**A_r**	**H_o**	**H_e**	**F_{IS}**	**St. Croix identity (%)**
Unnamed trib to Dell Creek (b)	43.547	-89.873	Castle Rock	30	49.5 (32.3 - 92.3)	1.459	0.446	0.460	0.021	28.0
Little Scarboro Creek	44.513	-87.628	Door-Kewaunee	30	88.6 (41.2 - 2812.7)	1.449	0.429	0.450	0.054	0
Allequash Creek	46.033	-89.608	Flambeau	30	55.1 (16.2 - ∞)	1.465	0.455	0.466	0.019	4.9
Chase Creek	42.843	-91.069	Grant-Little Maquoketa	30	13.5 (7.7 - 26)	1.361	0.342	0.362	0.069	46.8
Unnamed trib to Maple Dale Creek	43.559	-90.827	Kickapoo	30	159.4 (71.6 - ∞)	1.454	0.421	0.455	0.067	0
Spring Brook	45.059	-89.249	Lake Dubay	30	545.9 (142.6 - ∞)	1.441	0.414	0.441	0.063	0
Spring Creek	45.165	-90.060	Lake Dubay	30	468.3 (123 - ∞)	1.499	0.482	0.499	0.024	0.2
Cady Creek	44.841	-92.176	Lower Chippewa	30	122 (40.5 - ∞)	1.407	0.396	0.408	0.011	0
Byrds Creek	43.241	-90.562	Lower Wisconsin	30	20.7 (8.8 - 117.1)	1.390	0.388	0.390	-0.004	0
Fancy Creek	43.456	-90.498	Lower Wisconsin	30	61.4 (36.8 - 147.3)	1.450	0.423	0.451	0.042	3.4
Knapp Creek	43.229	-90.637	Lower Wisconsin	30	19.4 (13.2 - 30.8)	1.403	0.380	0.404	0.050	18.7
Lowery Creek	43.114	-90.058	Lower Wisconsin	30	51 (31.2 - 112.2)	1.441	0.421	0.441	0.035	0
Marshall Creek	43.424	-90.472	Lower Wisconsin	30	36.9 (21.7 - 84.9)	1.482	0.457	0.483	0.048	0.9
Marshall Creek - West Branch	43.430	-90.479	Lower Wisconsin	30	148.2 (12.3 - ∞)	1.466	0.422	0.467	0.073	3.7
Melancthon Creek	43.534	-90.355	Lower Wisconsin	30	54.4 (23.4 - 5484.2)	1.411	0.396	0.412	0.039	0

Waterbody	**Lat**	**Long**	**Subbasin (HUC 8)**	**N**	**N_e (95% CI)**	**A_r**	**H_o**	**H_e**	**F_{IS}**	**St. Croix identity (%)**
Tributary to Smokey Hollow	43.062	-90.324	Lower Wisconsin	30	92 (52.1 - 290.8)	1.455	0.455	0.455	0.002	0.1
Trout Creek	43.028	-89.929	Lower Wisconsin	30	12.2 (5.1 - 37.8)	1.345	0.329	0.345	0.062	0
Willow Creek	43.432	-90.243	Lower Wisconsin	30	15.1 (8.4 - 31.2)	1.440	0.410	0.440	0.061	75.9
Schuett Creek	43.860	-87.926	Manitowoc-Sheboygan	30	3.2 (2.4 - 6.4)	1.283	0.281	0.283	0.038	0
Tisch Mills Creek	44.328	-87.637	Manitowoc-Sheboygan	30	52.5 (31.9 - 117.1)	1.423	0.401	0.423	0.070	0.3
Lepage Creek	45.864	-88.175	Menominee	30	∞ (253.7 - ∞)	1.432	0.419	0.433	0.041	0
Woods Creek	45.831	-88.389	Menominee	30	159.5 (75.7 - ∞)	1.458	0.442	0.458	0.036	3.3
Chambers Creek	43.639	-88.044	Milwaukee	29	12.8 (8 - 21.7)	1.418	0.402	0.418	0.048	0.4
Bergen Creek	46.156	-91.865	Namekagon	30	52.3 (34.8 - 94.5)	1.428	0.398	0.428	0.073	26.8
Cap Creek	46.186	-91.196	Namekagon	30	408.7 (60.9 - ∞)	1.480	0.457	0.481	0.040	0
Laxey Creek	42.960	-90.220	Pecatonica	30	26.1 (12.4 - 100.6)	1.336	0.310	0.337	0.082	0
North Otter Creek	45.604	-88.774	Peshtigo	30	181.2 (37.8 - ∞)	1.489	0.455	0.490	0.058	0.2
South Fork Hay River	45.135	-92.098	Red Cedar	30	221.4 (110.4 - 3830.3)	1.443	0.421	0.444	0.050	0
Foulds Creek	45.824	-90.098	South Fork Flambeau	30	692.9 (154.5 - ∞)	1.402	0.373	0.403	0.077	0
Mt. Pelee Creek	45.768	-90.512	South Fork Flambeau	23	83.4 (33.6 - ∞)	1.464	0.438	0.464	0.045	2.1
Becky Creek	45.553	-91.312	Upper Chippewa	30	73.7 (29.6 - ∞)	1.477	0.466	0.478	0.022	0

Waterbody	Lat	Long	Subbasin (HUC 8)	N	N_e (95% CI)	A_r	H_o	H_e	F_{IS}	St. Croix identity (%)
Maple Creek	45.698	-91.266	Upper Chippewa	30	201.8 (55 - ∞)	1.464	0.440	0.465	0.051	0
Swan Creek	45.668	-91.236	Upper Chippewa	30	146.1 (38.4 - ∞)	1.471	0.451	0.464	0.024	0
Venison Creek	45.965	-91.051	Upper Chippewa	30	360.9 (88.3 - ∞)	1.407	0.404	0.407	0.003	7.0
Caves Creek	43.929	-89.515	Upper Fox	30	96.1 (41.2 - ∞)	1.407	0.360	0.408	0.101	0
Little Pine Creek	44.012	-89.344	Upper Fox	30	339 (108.4 - ∞)	1.438	0.396	0.439	0.110	0
Lunch Creek	44.052	-89.365	Upper Fox	13	∞ (57.2 - ∞)	1.420	0.381	0.422	0.086	3.7
Tagatz Creek	43.960	-89.523	Upper Fox	30	30 (13.1 - 226)	1.441	0.416	0.441	0.047	0
Bearskin Creek	45.645	-89.687	Upper Wisconsin	30	183.9 (95.2 - 1383.1)	1.460	0.432	0.461	0.059	9.4
Jennie Creek	45.563	-89.561	Upper Wisconsin	30	36.4 (18.4 - 132.2)	1.446	0.440	0.446	0.012	0.6
Little Deerskin River	45.987	-89.134	Upper Wisconsin	30	528.8 (126.5 - ∞)	1.484	0.460	0.484	0.061	3.1
Little Willow Creek	45.758	-90.035	Upper Wisconsin	30	192.1 (96.1 - 2667.8)	1.471	0.444	0.472	0.056	26.4
Noisy Creek	45.522	-89.394	Upper Wisconsin	30	493.5 (186.1 - ∞)	1.450	0.397	0.451	0.102	6.7
Tamarack Creek	46.111	-89.325	Upper Wisconsin	30	136.2 (58.1 - ∞)	1.466	0.448	0.467	0.027	0
Bruce Creek	44.060	-89.159	Wolf	8	2.2 (1.2 - 8.5)	1.388	0.379	0.389	0.050	0
Evergreen River	45.128	-88.795	Wolf	30	174.9 (83.6 - 135536.2)	1.467	0.466	0.467	0.004	0
Flume Creek	44.598	-89.267	Wolf	30	261.4 (123.8 - ∞)	1.455	0.433	0.456	0.040	0

Waterbody	**Lat**	**Long**	**Subbasin (HUC 8)**	**N**	**N_e (95% CI)**	**A_r**	**H_o**	**H_e**	**F_{IS}**	**St. Croix identity (%)**
Little Silver Creek	44.136	-89.092	Wolf	30	32.6 (18.8 - 76.2)	1.380	0.367	0.380	0.021	0
Spranger Creek	44.791	-89.204	Wolf	30	124.7 (52.3 - ∞)	1.434	0.405	0.435	0.042	0
Tomorrow River	44.556	-89.354	Wolf	16	56 (22.1 - ∞)	1.424	0.425	0.424	0.013	0
Upper Pine River	44.180	-89.252	Wolf	30	90.6 (46.5 - 524.9)	1.427	0.408	0.428	0.072	1.9
Walczak Creek	45.481	-89.073	Wolf	30	35.8 (23.1 - 65.7)	1.423	0.408	0.424	0.040	0

TABLE 1.2. Microsatellite loci (n = 68) for brook trout *Salvelinus fontinalis*. Oligos sequences are 5’-3’. The number of alleles (N) and null allele frequencies (NAF) are listed for each locus.

Locus name	**Left oligo**	**Right oligo**	**N**	**NAF**	**Source**
Sfo-18	TGGTGTATCCTGCTCCTG	TGGAATGTGTGTCTGTTTTCT	14	0.043	Angers et al. (1995)
L-SFOC113	GGGGAGCCCAGACTATATTGA	TCATCATTGCCATCAGGGTA	11	0.036	King et al. (2012)
L-SFOC24	AACACTGGAGCCGTTGAAGT	AGAGATGGGGTGATGCCTAA	9	0.016	King et al. (2012)
L-SFOC28	CAGTTGAAGTGATTGGGTTAGC	CACACCACACACACAAAAGC	11	0.052	King et al. (2012)
L-SFOC88	GGGAGAACCCAGTGTTTCTTT	CTGAACCCTGATTGTGAACG	8	0.02	King et al. (2012)
L-SFOD129	GTGCAGGCACTAACTGGACA	CCAGGGAATCCTCATCTTCA	8	0.019	King et al. (2012)
SfoC38	GTTGTGTTGCTTTGGTTTCAG	AATTTTGGACTGGTAACTAGTAGC	4	0.134	King et al. (2012)
SfoC79	CACTGGCCTGGTTTAGTAGG	CTGCTAGCCCCATACATCAC	2	0.138	King et al. (2012)
SfoC86	ACCGATGGCCTTCAACAC	ATAGGCCCCTACCTCAAACC	5	0.153	King et al. (2012)
SfoD100	AGGTGGACCGGTCTGG	GTGCAACGTGGCGTTG	11	0.044	King et al. (2012)
SfoD75	AAGAGAAAGAGGGAGAAGAAGAG	TTGTTGTCTTCCCTCGGTC	20	0.053	King et al. (2012)
SfoD91	GCAACGTGGAAATTGTTGTG	CTTTGGTAATATTGTGTGTAGCTATG	21	0.034	King et al. (2012)
L-Ssa-1.14	TCGTATTTGTCAAGGATGTGCC	AGATGCCCATTGTATTGCCC	8	0.028	Lien et al. (2016)
L-Ssa-1.7	AGAACACAACAGAACCAGGTAC	CTCGAACACACTTCCAACCC	20	0.068	Lien et al. (2016)
L-Ssa-10.3	TGATGGGTCTTGGTGTAGGG	TCAACGGTGAAGCCCAGG	3	0.017	Lien et al. (2016)
L-Ssa-11.1	AGAGCTCCGACACACATTCG	CATAGAGCTAGGCCGGTGC	3	0.009	Lien et al. (2016)
L-Ssa-12.11	GTCTGAGGAAGCTGGCTCTG	TGTCCAGCAGCAACAACG	17	0.042	Lien et al. (2016)
L-Ssa-12.2	ACTGGTAGGTCATTGTTCTGTG	CCTTATGTGTGTTCTCGGTGC	5	-0.011	Lien et al. (2016)
L-Ssa-12.4	TGCTTGCACCTAATAGTCCTAC	CTTGCGAGACATTGATTCAGC	3	0.014	Lien et al. (2016)
L-Ssa-13.6	GCTGTTCCTCTGGCCTCAC	AGCACCTCAACACTGTACTATC	2	0.106	Lien et al. (2016)

Locus name	Left oligo	Right oligo	N	NAF	Source
L-Ssa-14.10	GGGAACGTGTGGAAGATTCAC	AAGGTATGGAGGGTGATGCC	7	0.017	Lien et al. (2016)
L-Ssa-15.1	TTTCTTTGTGTGTTGTGCCC	CAGCTGTGGTTCCTCTGGG	2	0.022	Lien et al. (2016)
L-Ssa-15.7	GATGTGATGGCAGTGCTATG	CAGCAACAAGGTCAATCTCC	2	0.014	Lien et al. (2016)
L-Ssa-15.9	ATACTACCTGTTCAGGCGGC	CCTGTGATGACTCTCCTCCC	6	0.042	Lien et al. (2016)
L-Ssa-16.2	GTTTACGTCACCTGCAGCTG	GCAGTAGATGTTAAGCCCTCG	3	0.003	Lien et al. (2016)
L-Ssa-20.3	GGAGGGAGTGTAGAGGCTTTC	CACACACCCATCTCTGCTAC	3	0.014	Lien et al. (2016)
L-Ssa-21.5	CACTCCCTAACTCCATGGTC	TCATGGATGTCGTCACTGTG	2	0.014	Lien et al. (2016)
L-Ssa-23.9	ACGGATACAGAGAGACGCAC	ACAGCGAGGAGGACAAAGTC	6	0.03	Lien et al. (2016)
L-Ssa-27.1	TCCATGAGTACACGCCACTG	GTTCTCCACTACTCTACCCTGG	4	0.029	Lien et al. (2016)
L-Ssa-27.d07	GATTTCACAAAGCAGCGCG	CAGCATGTTCTGTCGCGAG	3	0.069	Lien et al. (2016)
L-Ssa-27.d19	GGAATACTGTCTCATTGCGCC	AGGCAATCAAAGGTTGTAGGTG	3	0	Lien et al. (2016)
L-Ssa-28.d08	TCTGACCTACACACAACAATGG	GTGTGTGCTCGCTCAGAATG	3	0.067	Lien et al. (2016)
L-Ssa-29.2	GGCACAGCACACCAGTTG	ACAGCGTTCCAAGATGTTCC	3	0.003	Lien et al. (2016)
L-Ssa-4.9	AGAATCTCTAGCCCACACAAC	GCAGGGTTGAGATGTGAGC	5	0.093	Lien et al. (2016)
Salv-1_Di-05-1487	CTTCATGCTCATCACTGGCC	CATGCACCAAACACCACTCC	4	0.004	Christensen et al. (2018)
Salv-1_Di-08-1675	ACACAGCTTCAAGTGTAGAGTG	TGGGTGACAAGAGACCGTG	3	0.022	Christensen et al. (2018)
Salv-1_Di-30-1164	GCTTGATTCGCTGTTGTCTC	AAATCCATGTTTACAGTCACGC	10	0.071	Christensen et al. (2018)
Salv-1_Di-31-422	CACCATTAGCCAGCGGTG	ACCTTGTTCTGCTCCATCAC	6	0.027	Christensen et al. (2018)
Salv-1_Di-32-188	AGGAGTTTGTCTGGGCACAC	GACAGACAGTAACCATCCATAGC	9	0.038	Christensen et al. (2018)
Salv-1_Di-33-1038	TCGGTTTCAGTTGACTCCCAC	CCTGCCTCCTGTTCTTTCAAG	2	0.051	Christensen et al. (2018)
Salv-1_tri-01-26	ATGGCTAACTTGCGCAATGTC	ACTTTATCGACCACTGCAACG	16	0.038	Christensen et al. (2018)

Locus name	Left oligo	Right oligo	N	NAF	Source
Salv-1_tri-11-1	GTGGCGGCATGTACTTCTAAC	GATACAGGGTTTGACGGACTC	8	0.07	Christensen et al. (2018)
Salv-1_tri-11-16	CCGTTGGCAAGGTGATTAAC	ATCAAGGTCAATGCAACGGTC	3	0.014	Christensen et al. (2018)
Salv-1_Tri-12-2	ATGAGAGTGCAACCCAACAAC	AACACAGACTTCCTCCCAGAC	10	0.067	Christensen et al. (2018)
Salv-1_tri-14-6	CTAATGTGGCTGAGTCAATTGC	TCGTCGTTCATGTCCTTATCATC	12	0.09	Christensen et al. (2018)
Salv-1_tri-16-9	CCTGCAGCAGTAAGAGAGGAC	GTAGATCATGGCCCAGGGTG	5	0.008	Christensen et al. (2018)
Salv-1_tri-18-13	ATCCCAGCGGTTGTTGAC	CATCTGTAGCCGCCGATTAC	6	0.031	Christensen et al. (2018)
Salv-2_tri-02-5	AGACTACTTCAAGCACCAGAC	AGAGAAAGGGAGTGAGCTTAAC	6	0.017	Christensen et al. (2018)
Salv-2_tri-05-4	GCAAATGACTTCCCTCTCTGC	CTTCTATCTCTCTCGGCCGG	2	0.014	Christensen et al. (2018)
Salv-2_tri-07-12	CGACATGAAGGAAGCTGCAC	CTTCAGTCTGTTCAGTGGAGC	5	0.056	Christensen et al. (2018)
Salv-2_tri-20-30	CTGGACCGGCATAATACAGAAG	TTCACGTTTGTTCCTTTCCCG	5	0	Christensen et al. (2018)
Salv-2_tri-20-5	TGGCCACATATGCTCCCG	CTTGTGATCGCCAACCACC	3	0.01	Christensen et al. (2018)
Salv-2_tri-23-5	GCGCTTCTGTTCTATCCTCAC	AGCTCGACTTGCAGGACTC	4	0.036	Christensen et al. (2018)
Salv-2_tri-28-9	CGGCCAATAGTCCTGTTCATC	ACTTTCCTAAACAAGCACCATTG	7	0.168	Christensen et al. (2018)
Salv-2_tri-31-5	AACAGCCTGAGTGTTGTTGTC	ATAGTCCTCCTCACAGACACG	2	0.003	Christensen et al. (2018)
Sfon-6_Di-01-402	ATGTGCATTTGTGTACCTCCG	AGTTCACTCCCGTCCACAC	6	0.013	Christensen et al. (2018)
Sfon-6_Di-07-134	TGAGAACAAACACACAGCTGC	CTCCGCTGTTTGAGATGTGC	3	0.008	Christensen et al. (2018)
Sfon-6_Di-24-298	AATGCTCTAACGGATGCTGTG	GTAAGAGACTTGGGCTTGTGTG	3	0.175	Christensen et al. (2018)
Sfon-6_Di-36-592	TGGTTTCACTCACTCCCAAG	ACTTGTGGTCATAGCCCACTG	7	0.044	Christensen et al. (2018)
Sfon-6_Di-4q-1577	CTCGCATATGTTTATTGTGGTGC	ACACTGAAACCGCTTACCTAG	10	0.129	Christensen et al. (2018)
Sfon-6_Di-6.1-1018	CTGCTCATTACTGGTGCTCTC	TAGAATGATCGAATGTGCGCG	3	0.11	Christensen et al. (2018)
Sfon-6_tri-01-18	GGAGGCCCAAACTTGTATATGG	CCACCAATATGCCCTTGAAATAG	4	-0.004	Christensen et al. (2018)

Locus name	Left oligo	Right oligo	N	NAF	Source
Sfon-6_tri-16-3	AACATGGTCACTGGAGGACTC	CTCAGGAAGTTTAGCCATTCATC	9	0.046	Christensen et al. (2018)
Sfon-6_tri-20-20	CATGTTGCTATGGAGGAGGG	CCTCTGCCTTTGAATTTGCTC	3	0.004	Christensen et al. (2018)
Sfon-6_tri-32-0	TAATGGACATCTGAGCCTCCG	GGCTGGCTTCATATGTTCTGC	2	0.004	Christensen et al. (2018)
Sfon-6_tri-33-10	TTGTTCTGCTCGTCTTTCTGC	AAAGGCAGTCAAGGAGGAGAG	4	0.077	Christensen et al. (2018)
Sfon-6_tri-4p-3	TGCCAACTGTACACATGATTCC	ATGAAGTGGTGATGTGGCATG	3	0.028	Christensen et al. (2018)
Sfon-6_tri-4q-0	GTTCTGAGTTTAGGACACGCC	TGTGGTAGAACTGTGTGGATATG	2	0.024	Christensen et al. (2018)

TABLE 1.3. Mean domestic strain introgression (St. Croix identity) and standard deviation for each group depicted on the NJ tree (Figure 3). Overall mean St. Croix identity was 4.7% (standard deviation = 19.3%).

Group	**Mean St. Croix identity (%)**	**Standard deviation (%)**
A	16.0	33.2
B	0.4	5.1
C	0	0
D	0.7	8.1
E	0	0
F	0.1	2.1

Figures

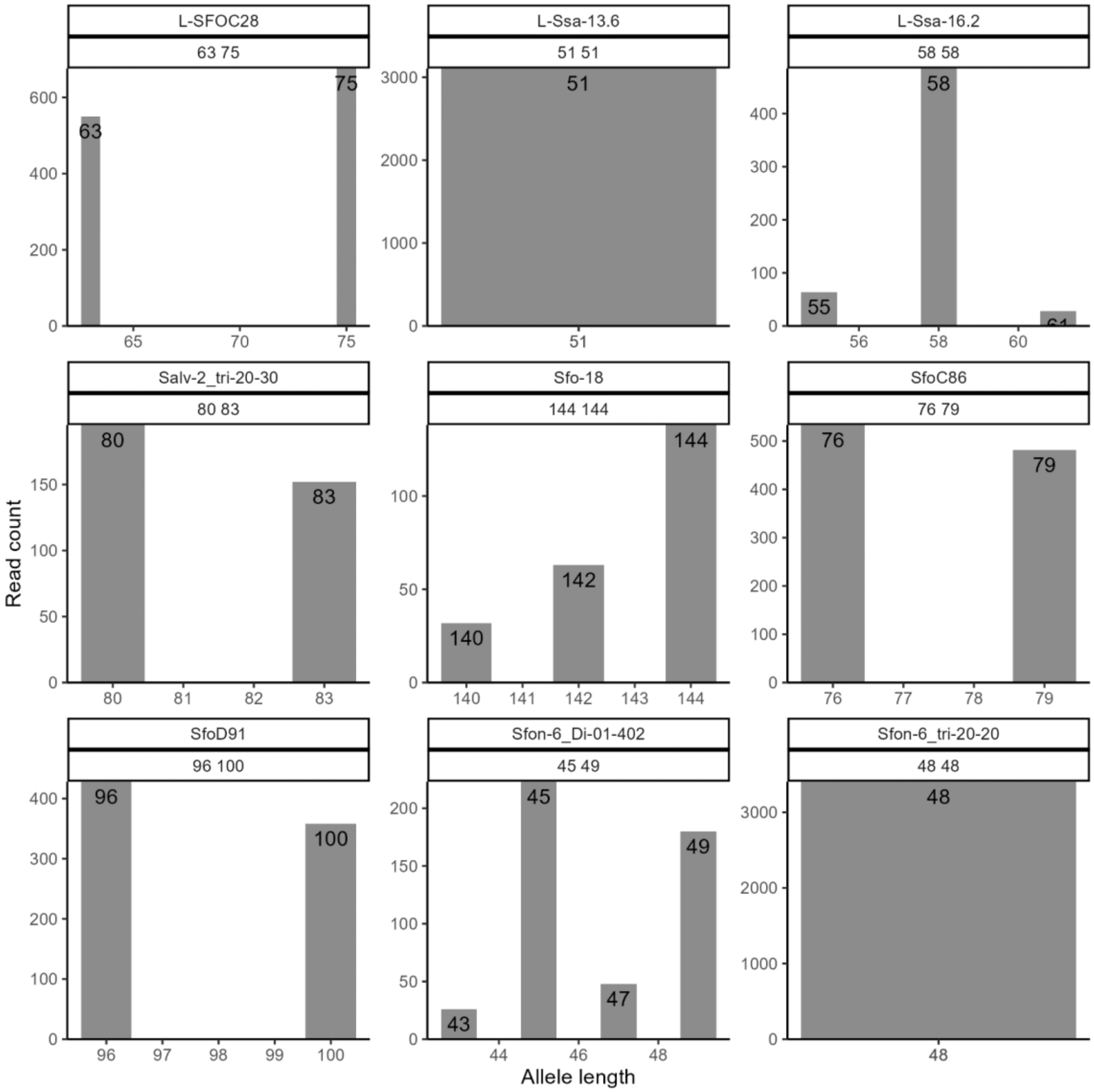


FIGURE 1.1. An example of peak morphology plots from one brook trout *Salvelinus fontinalis* at nine microsatellite loci. Each peak morphology plot depicts the read counts for each allele at each locus. The locus names and allele calls from MEGAsat are shown above each plot. For each locus, alleles with read counts less than or equal to 5% of the greatest read count are not shown.

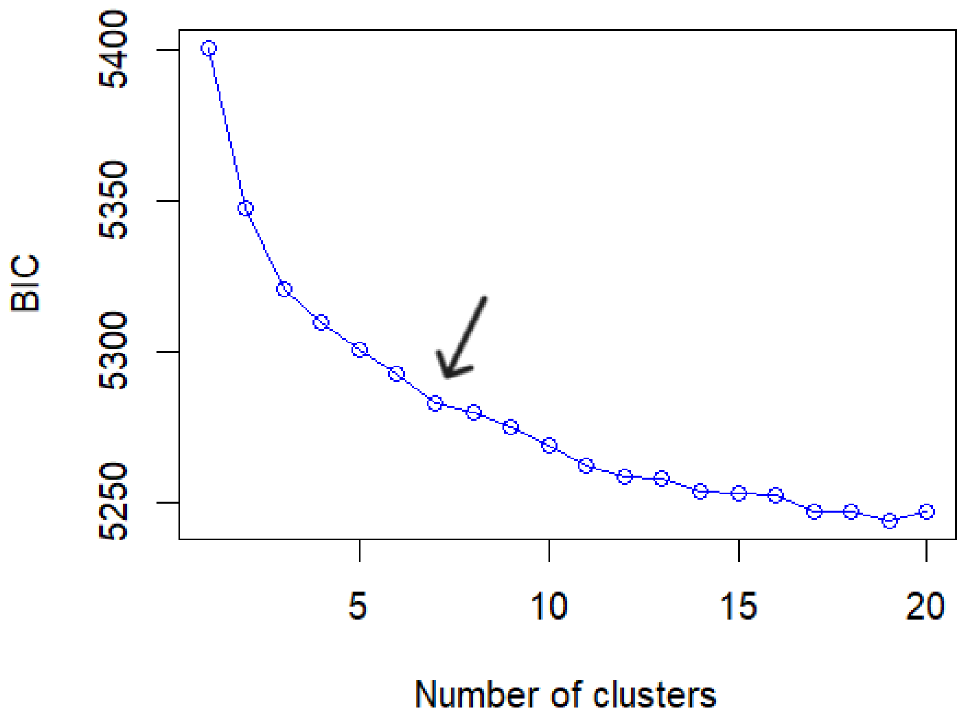


FIGURE 1.2. Plot displays the Bayesian information criterion (BIC) for each number of K clusters from the DAPC model. K = 7 was selected, as it appears to be a slight inflection point in the values (indicated by the arrow).

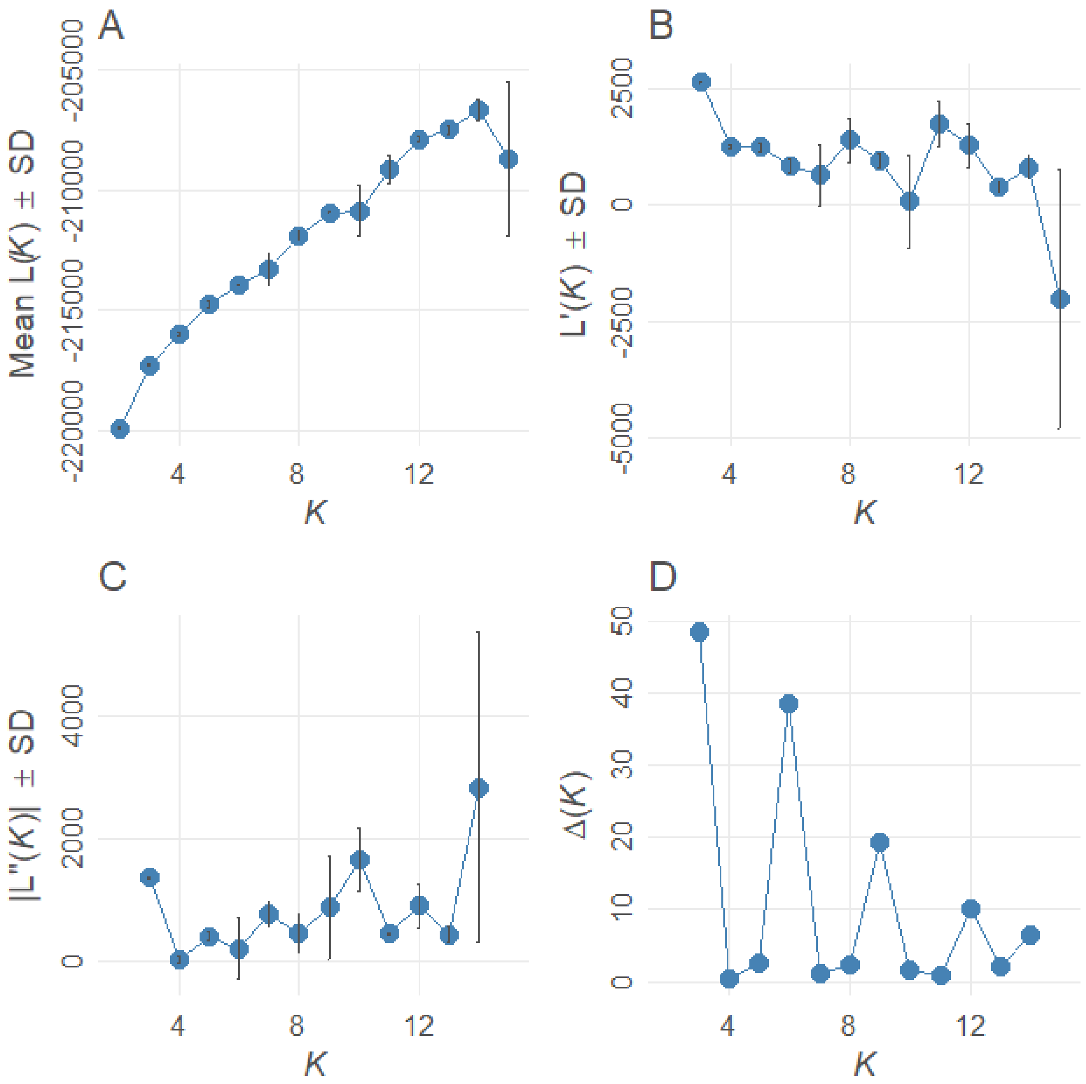


FIGURE 1.3. Plots display (A) mean L(K), (B) L'(K), (C) |L''(K)|, and (D) ΔK for each number of K clusters. Plots were produced using the Evanno method to evaluate the best number of K clusters from the STRUCTURE results.

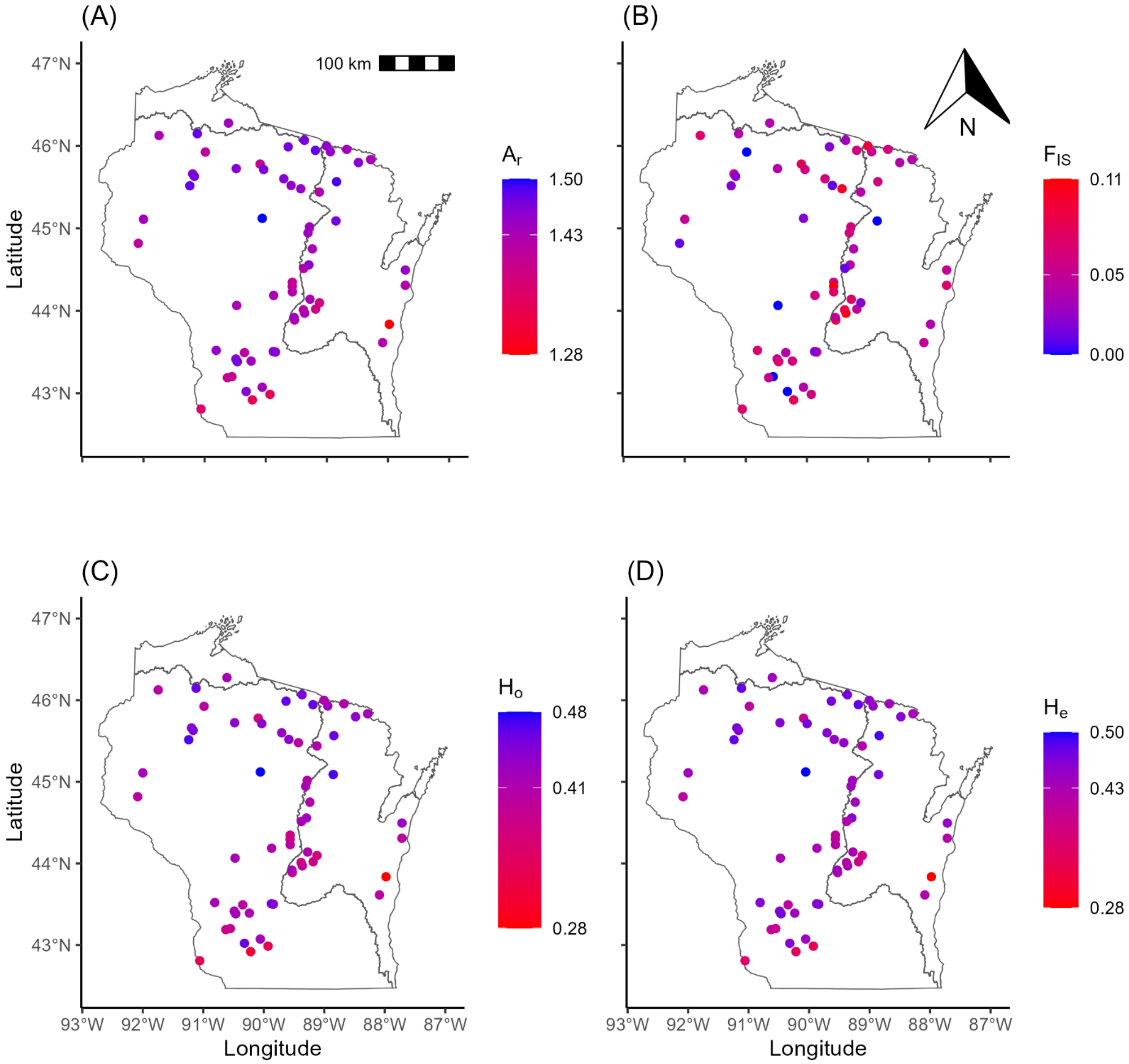


FIGURE 1.4. Maps displaying (A) allelic richness (A_r), (B) inbreeding coefficient (F_{IS}), (C) observed heterozygosity (H_o), and (D) expected heterozygosity (H_e) for each brook trout *Salvelinus fontinalis* survey population (dots). The upper and lower values within each legend indicate the range, while the middle values represent the mean. The color of each dot corresponds to that population's parameter estimate. Dark grey lines indicate regional boundaries at the hydrologic unit code (HUC) 2 level.

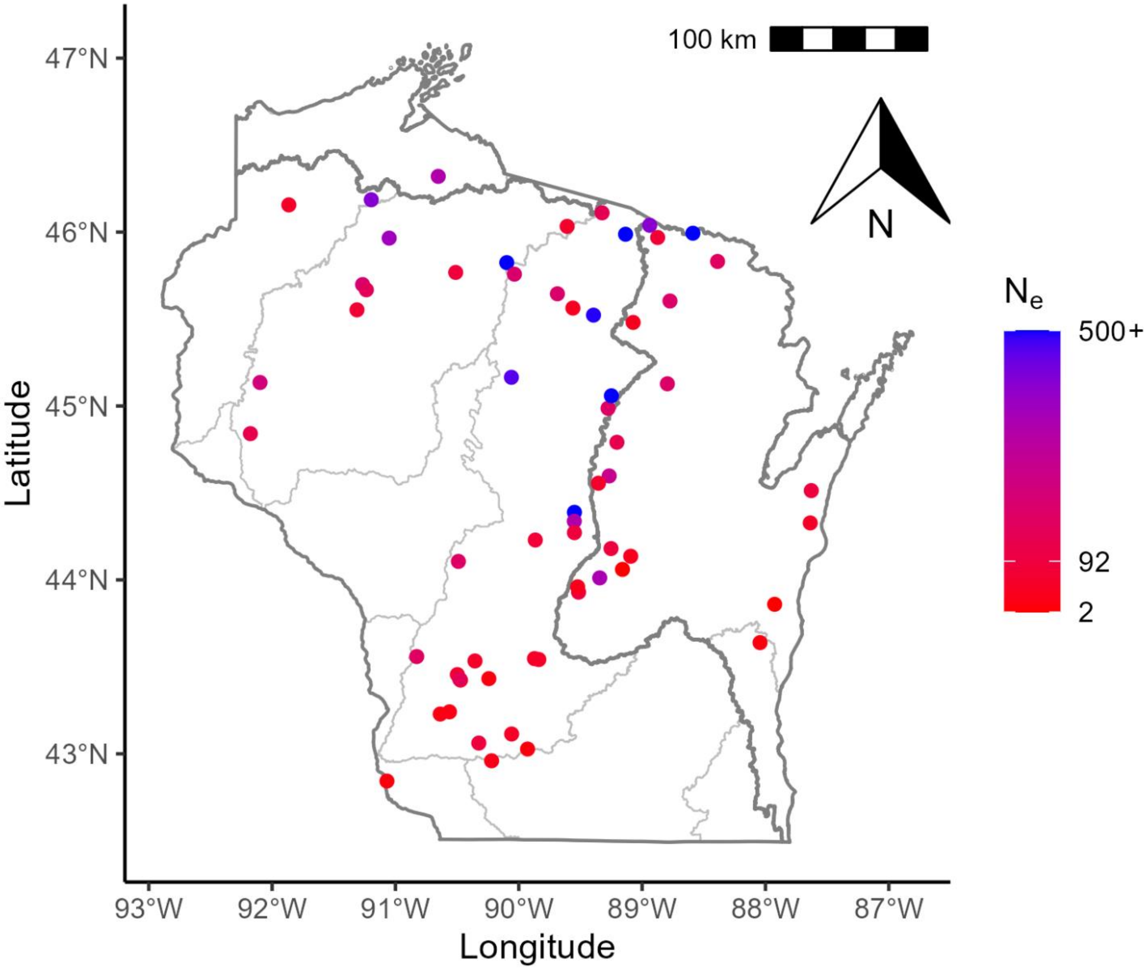


FIGURE 1.5. Heatmap displaying estimates of effective population size (N_e) for each brook trout *Salvelinus fontinalis* survey population. Populations with infinite estimates were not plotted (n = 2), and populations with N_e estimates greater than 500 were truncated to 500 for improved visualization (n = 5). Dark grey lines indicate regional boundaries at the hydrologic unit code (HUC) 2 level, and light grey lines indicate subregion (HUC 4) boundaries. The upper and lower values in the legend indicate the range, while the middle value represent the median.

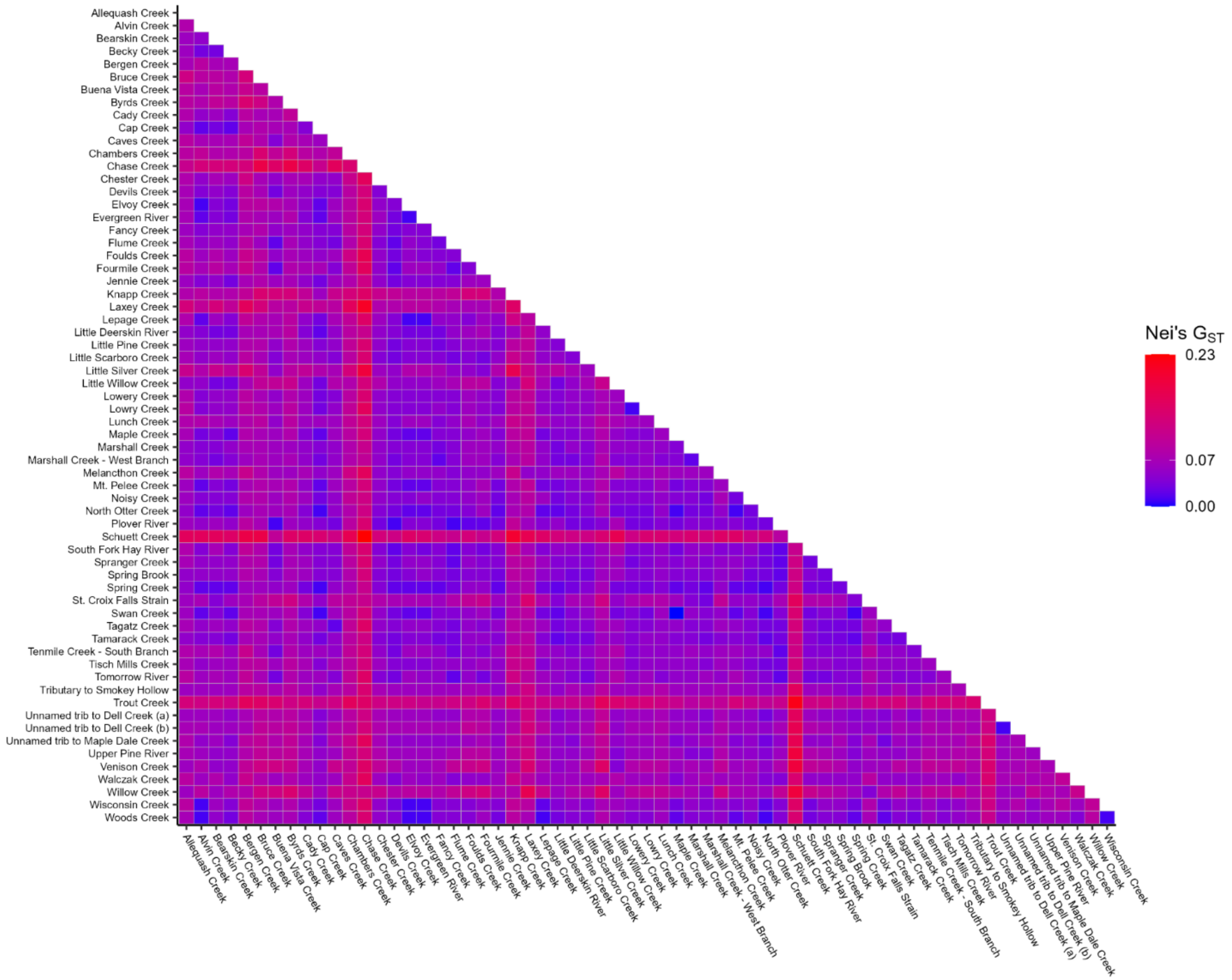


FIGURE 1.6. Pairwise genetic distance (Nei's G_{ST}) for each brook trout *Salvelinus fontinalis* survey population. Red squares indicate high genetic distance and blue squares indicate low genetic distance. The upper and lower values within the legend indicate the range, while the middle value is the mean. Note that the St. Croix Falls strain has been included.

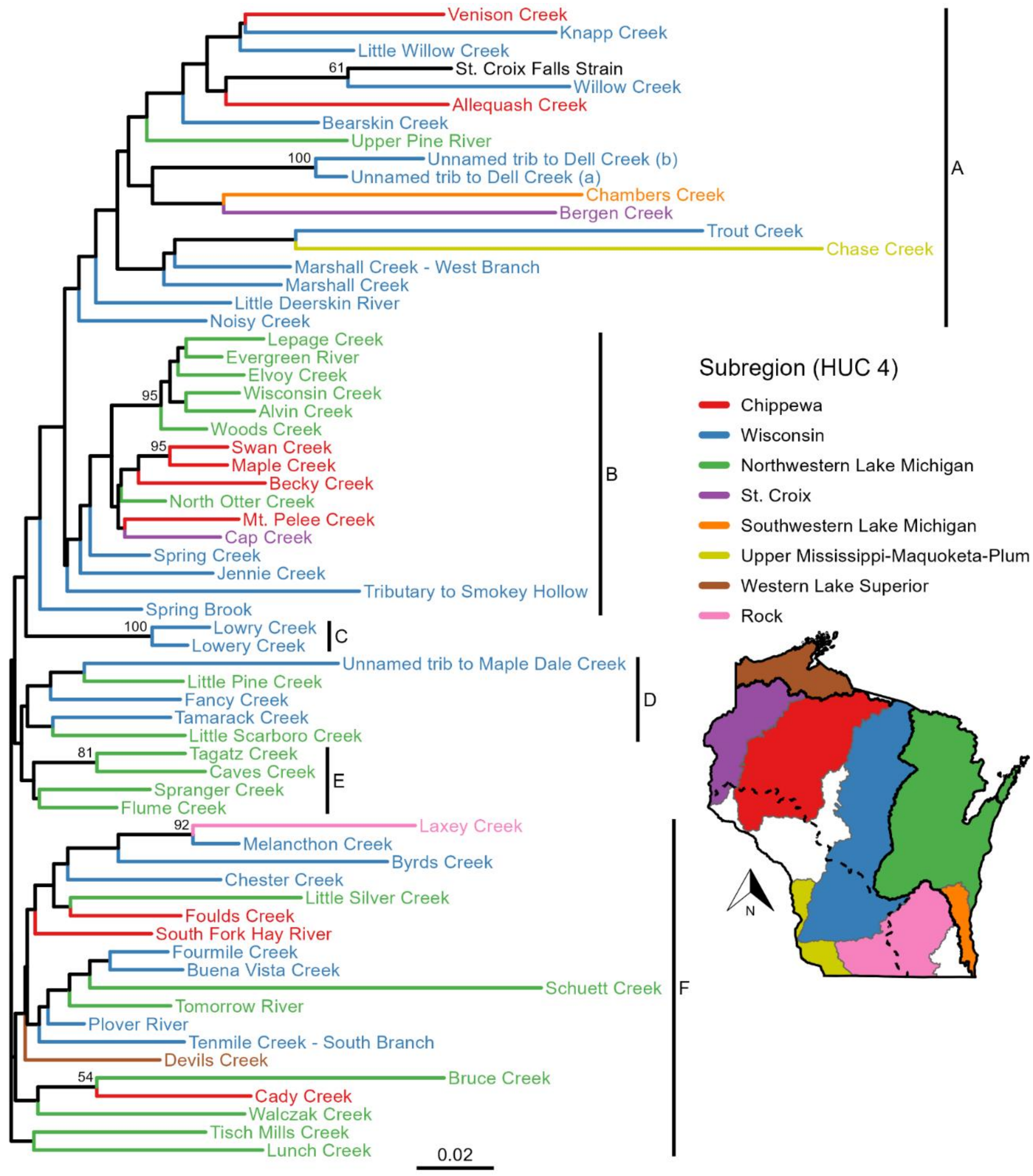


FIGURE 1.7. Neighbor-joining tree displaying the genetic differentiation of each surveyed brook trout *Salvelinus fontinalis* population. Group A reflects ancestry to the St. Croix Falls strain. Groups B and C may reflect remnant native ancestry. Groups D, E, and F reflect mixed or unclear ancestry. Branch lengths indicate genetic distance (Nei's G_{ST}). The St. Croix Falls Strain is shown in black. Bootstrap values below 50 were omitted. Populations are color-coded by their subregion (HUC 4). A map of Wisconsin is also color-coded by subregion, with black lines indicating regional boundaries at the hydrologic unit code (HUC) 2 level and dashed lines indicating the extent of the Driftless Area of southwestern Wisconsin. White areas on the map reflect unsampled subregions.

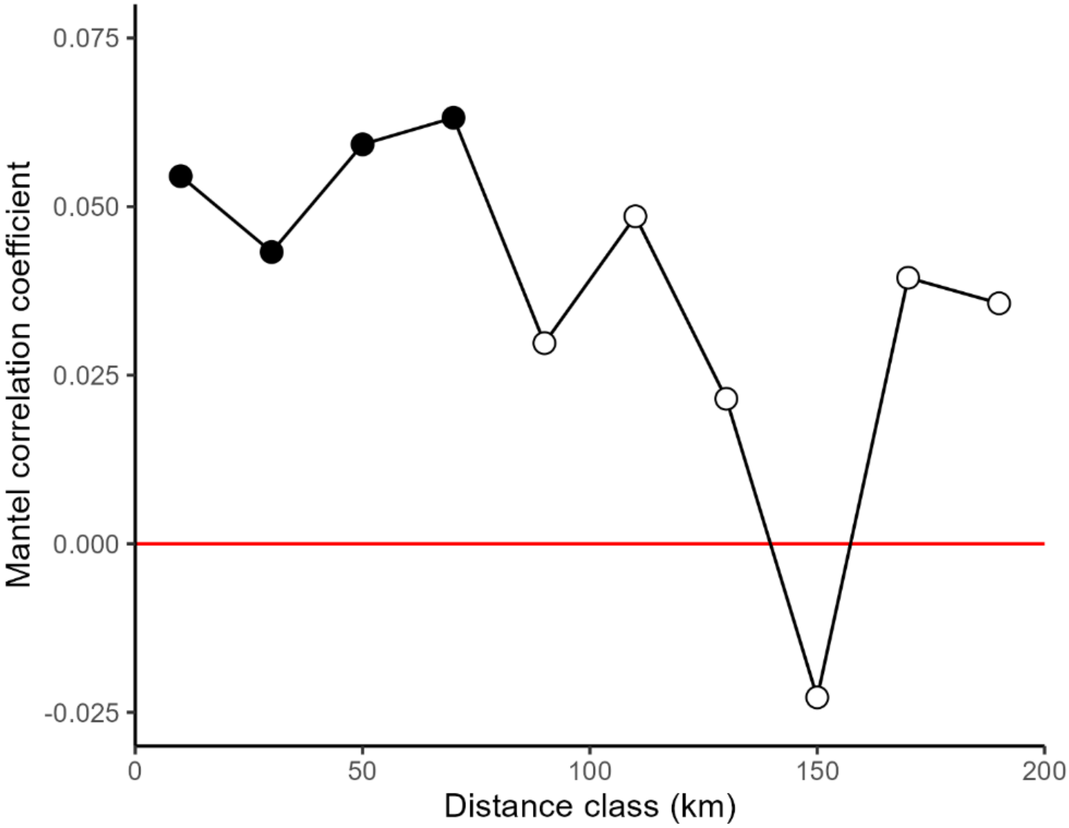


FIGURE 1.8. Mantel correlogram illustrates the relationship between geographic distance (kilometers) and genetic distance (Nei's G_{ST}). Black dots indicate values that are significantly different from zero (p-value < 0.05).

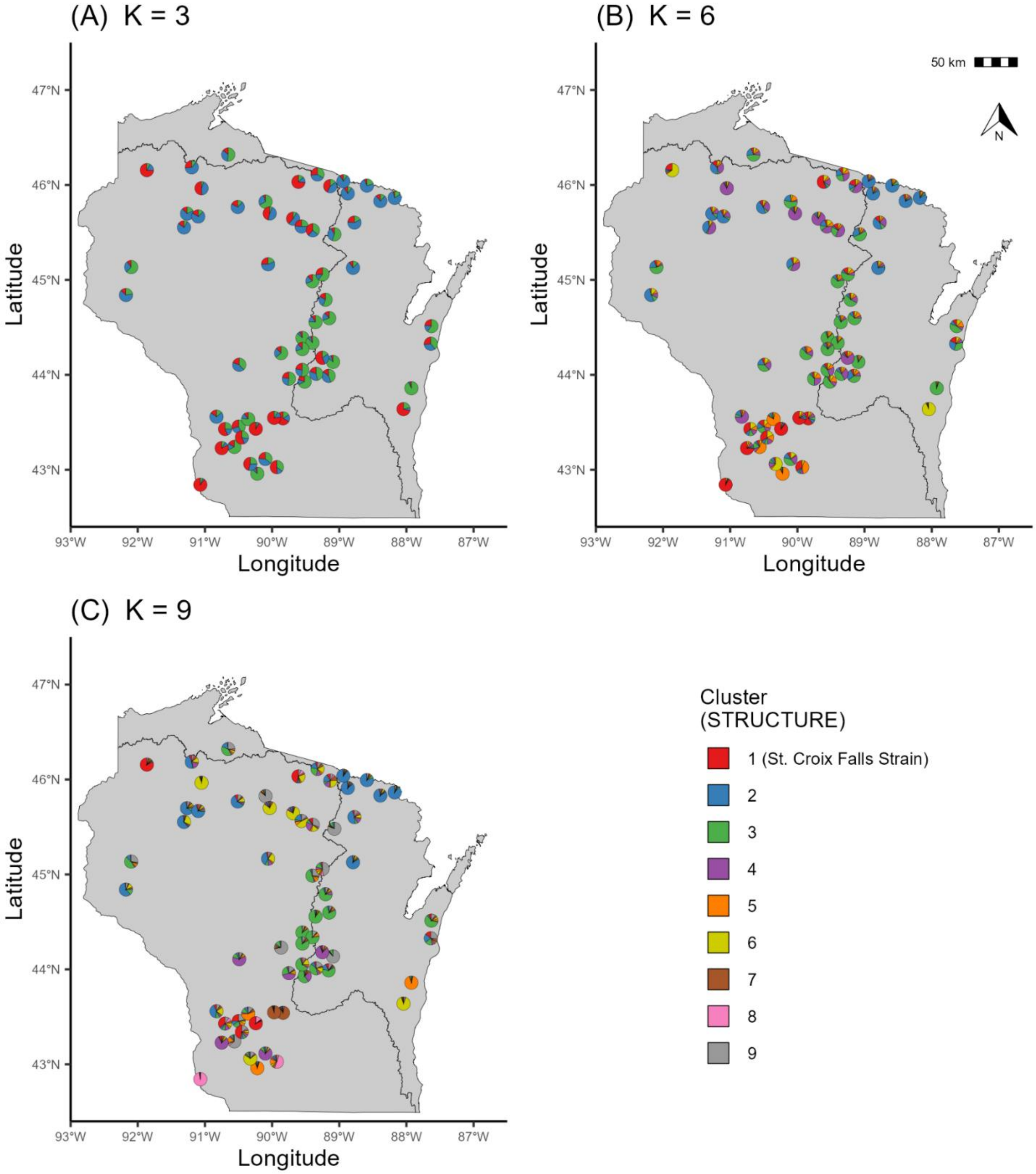


FIGURE 1.9. Spatial trends in admixture proportion for the (A) K = 3, (B) K = 6, and (C) K = 9 STRUCTURE configurations. Pie charts display the proportion of cluster assignment (admixture proportions) for each brook trout *Salvelinus fontinalis* population. Dark grey lines indicate regional boundaries at the hydrologic unit code (HUC) 2 level.

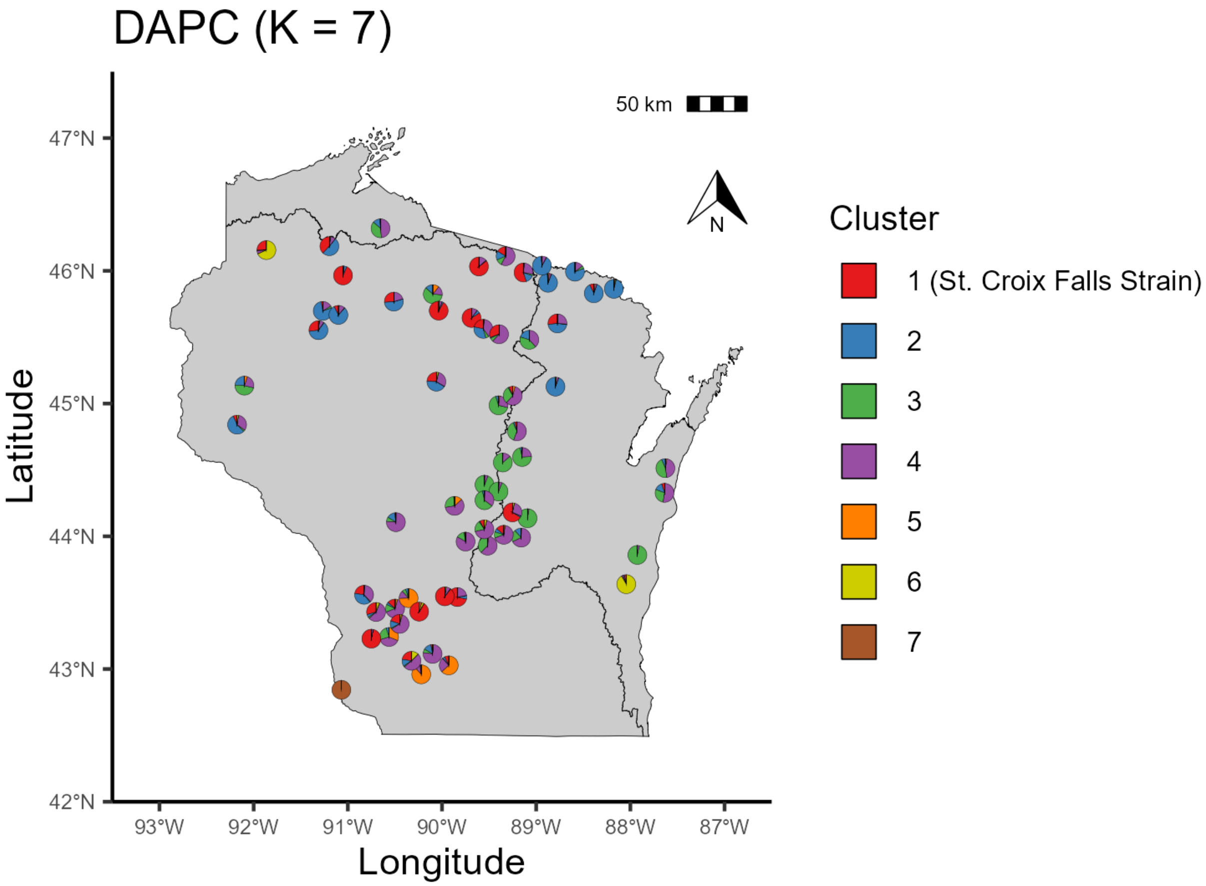
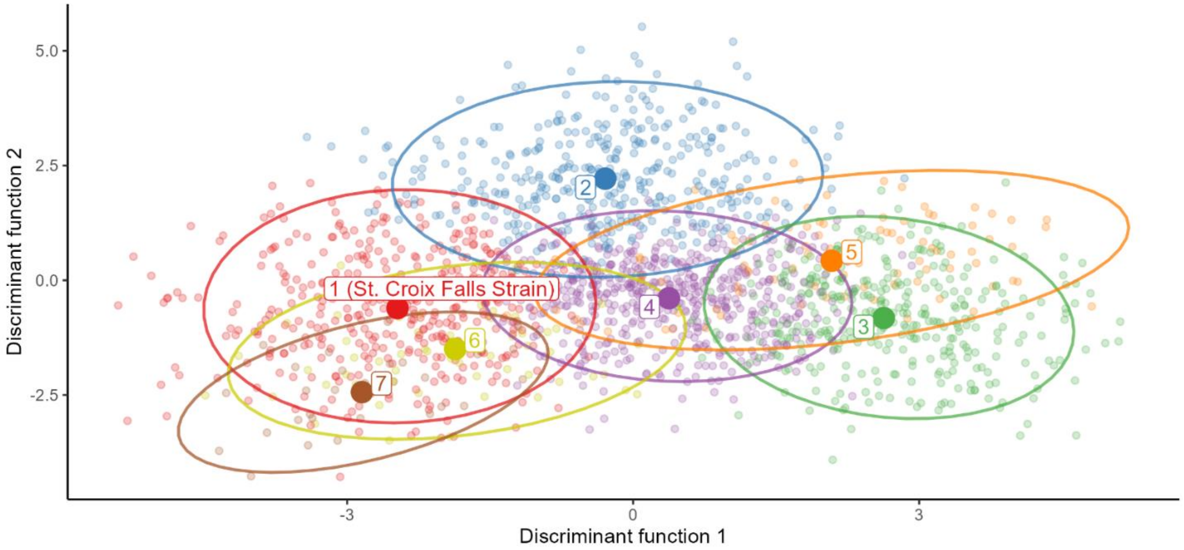


FIGURE 1.10. Spatial trends in DAPC genetic clustering (K = 7). Colors indicate the admixture proportions of each survey population to each cluster. Dark grey lines indicate regional boundaries at the hydrologic unit code (HUC) 2 level.

(A) DAPC discriminant functions 1 & 2



(B) DAPC discriminant functions 2 & 3

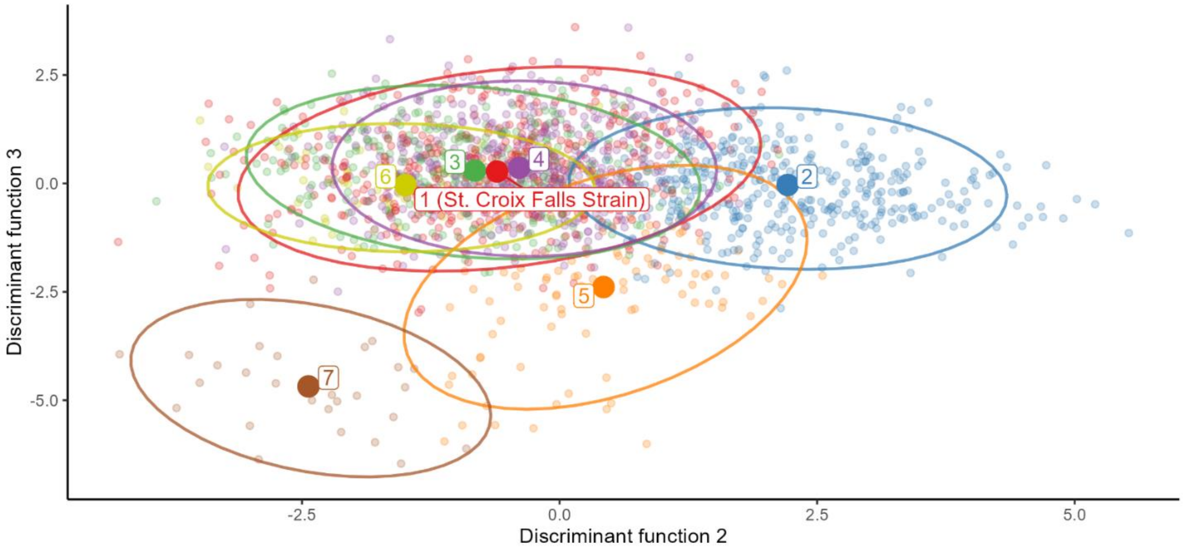


FIGURE 1.11. Ordination plots displaying (A) the first and second discriminant functions and (B) the second and third discriminant functions of the DAPC model (K = 7). Small dots correspond to individual fish. Large dots indicate the centroid for each cluster. Colors correspond with the numbered clusters. Ellipses represent the 95% confidence interval for each cluster.

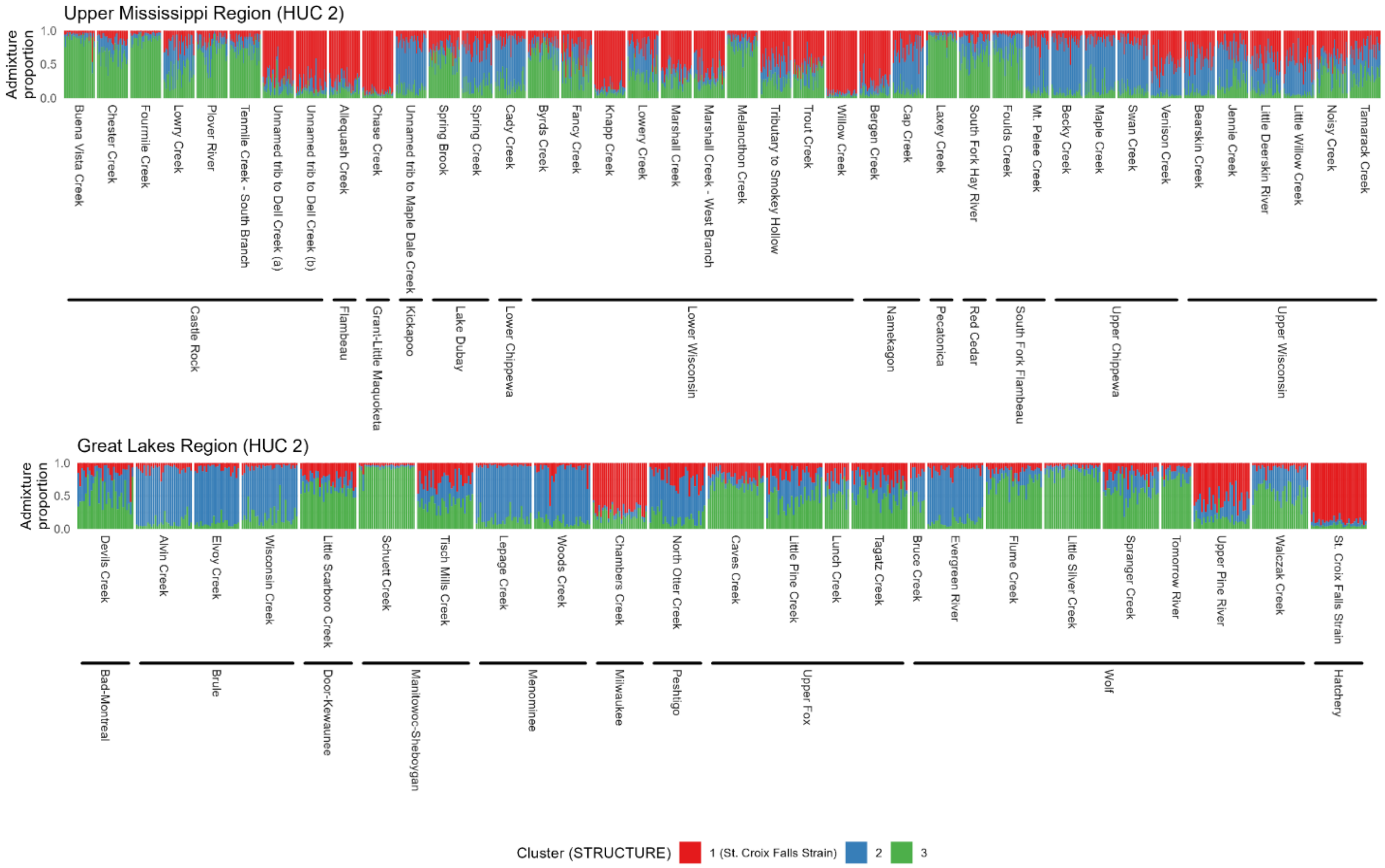


FIGURE 1.12. Barplots displaying admixture proportions (assignment probabilities) to STRUCTURE genetic clusters (K = 3). Populations are organized at the hydrologic unit code (HUC) 2 level and by subbasin (HUC 8). Colored bars correspond to individual brook trout *Salvelinus fontinalis* and their proportional assignment to each genetic cluster.

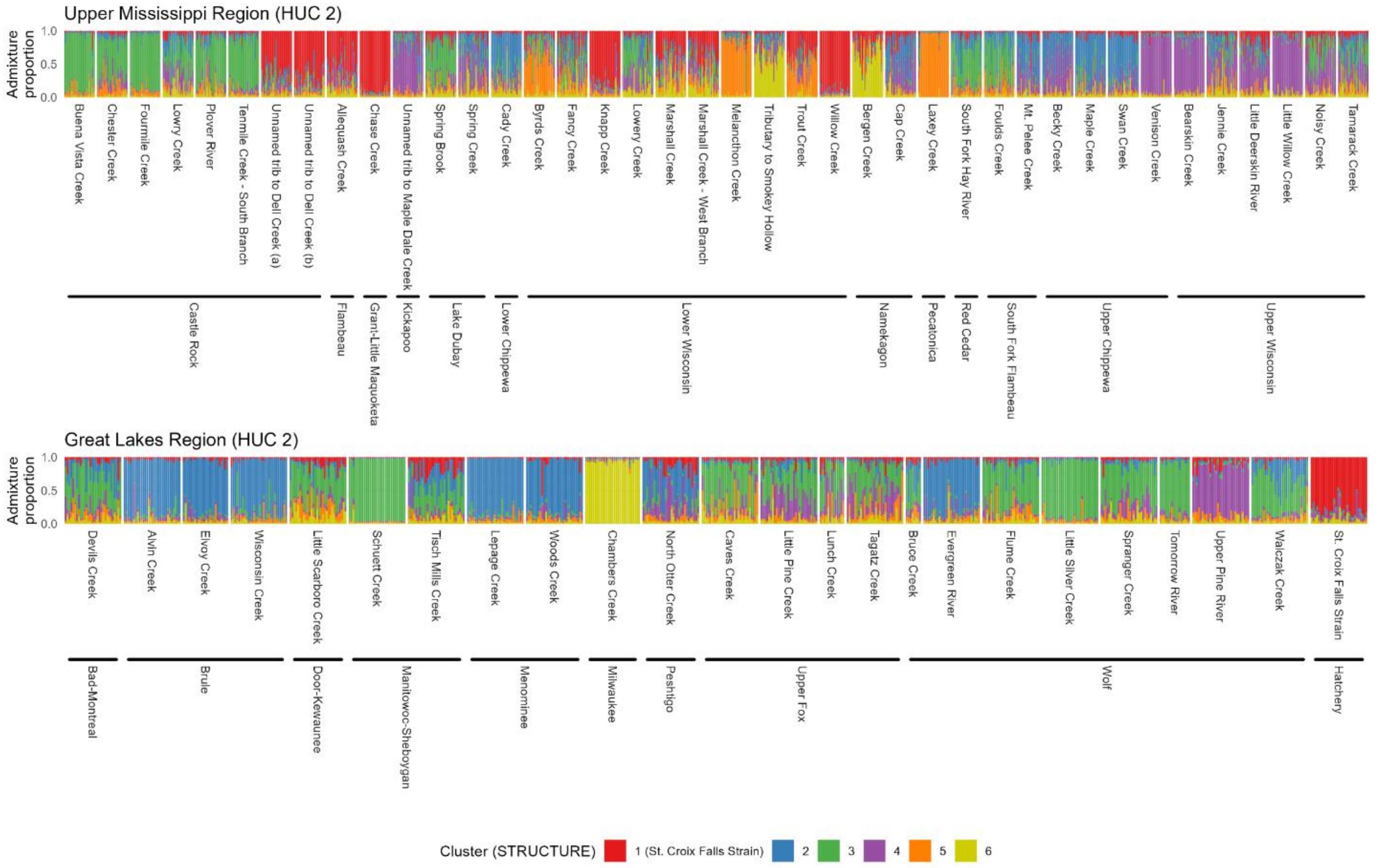


FIGURE 1.13. Barplots displaying admixture proportions (assignment probabilities) to STRUCTURE genetic clusters (K = 6). Populations are organized at the hydrologic unit code (HUC) 2 level and by subbasin (HUC 8). Colored bars correspond to individual brook trout *Salvelinus fontinalis* and their proportional assignment to each genetic cluster.

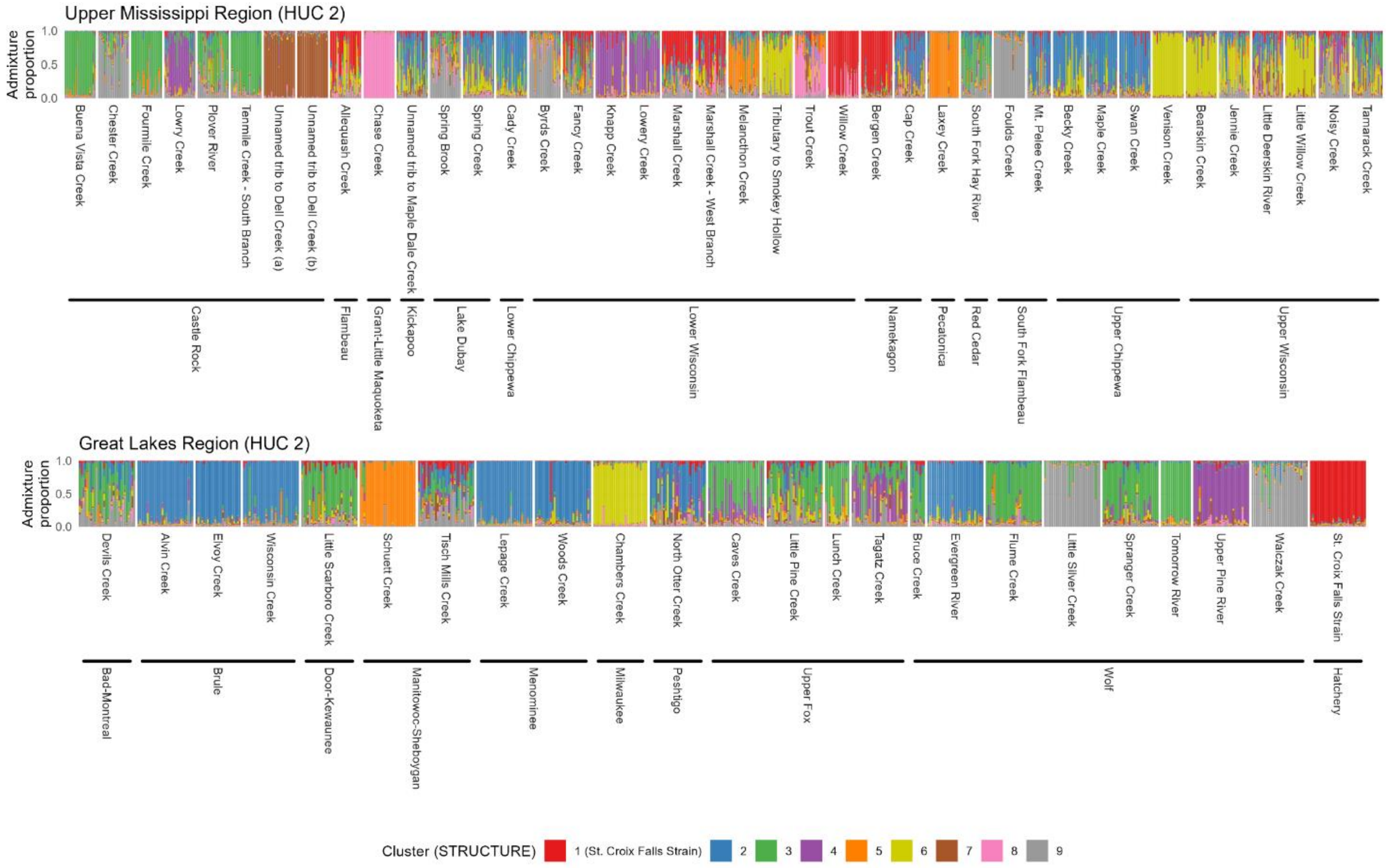


FIGURE 1.14. Barplots displaying admixture proportions (assignment probabilities) to STRUCTURE genetic clusters (K = 9). Populations are organized at the hydrologic unit code (HUC) 2 level and by subbasin (HUC 8). Colored bars correspond to individual brook trout *Salvelinus fontinalis* and their proportional assignment to each genetic cluster.

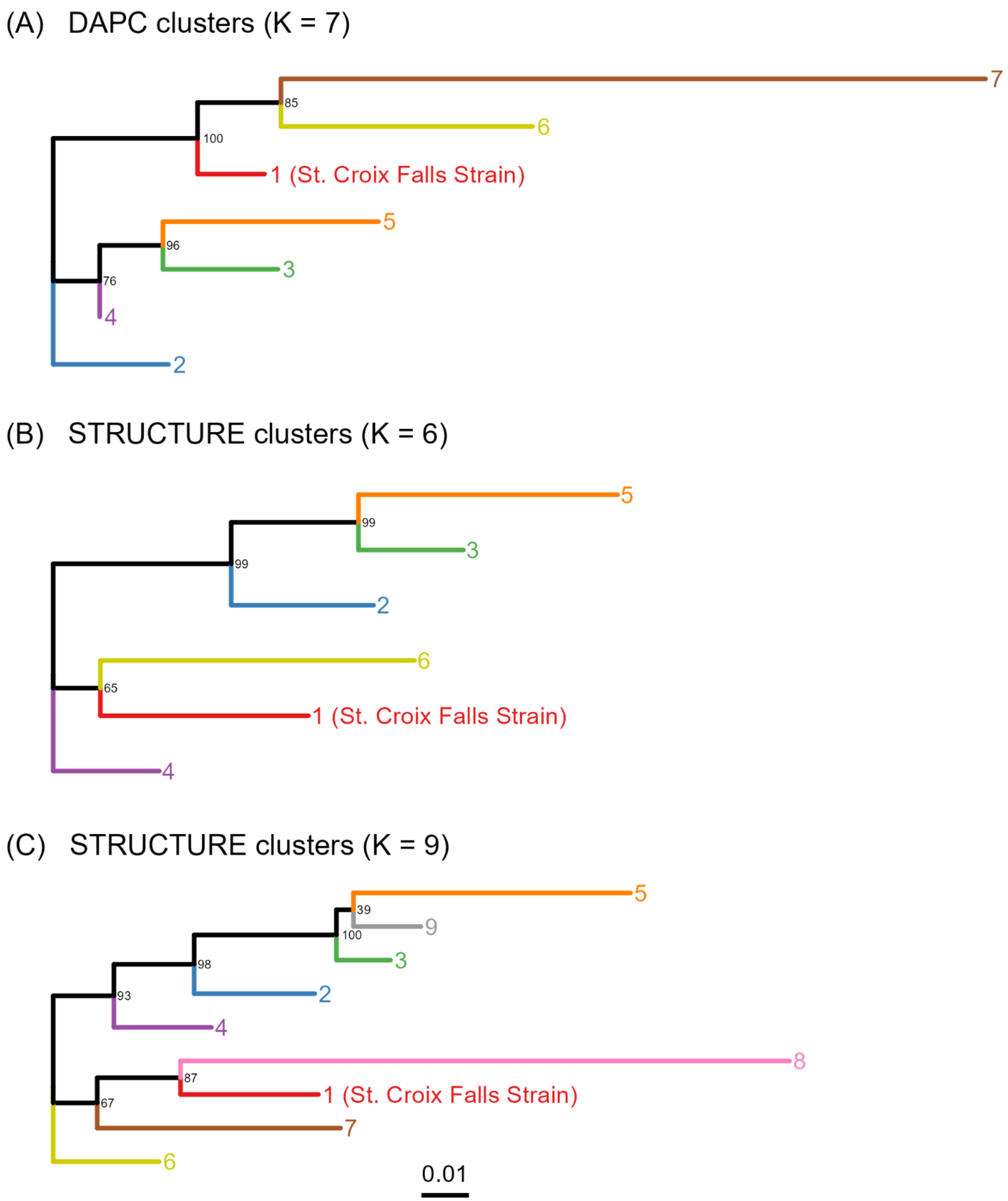


FIGURE 1.15. Neighbor-joining trees displaying genetic differentiation of each genetic cluster from the (A) K = 7 DAPC configuration, (B) K = 6 STRUCTURE configuration, and (C) K = 9 STRUCTURE configuration. Data were subset to only include brook trout *Salvelinus fontinalis* with at least 75% assignment to a cluster. Branch lengths indicate genetic distance (Nei's G_{ST}).

FIGURE 1.16. Barplots displaying admixture proportions (assignment probabilities) to DAPC genetic clusters (K = 7). Populations are organized at the hydrologic unit code (HUC) 2 level and by subbasin (HUC 8). Colored bars correspond to individual brook trout *Salvelinus fontinalis* and their proportional assignment to each genetic cluster.

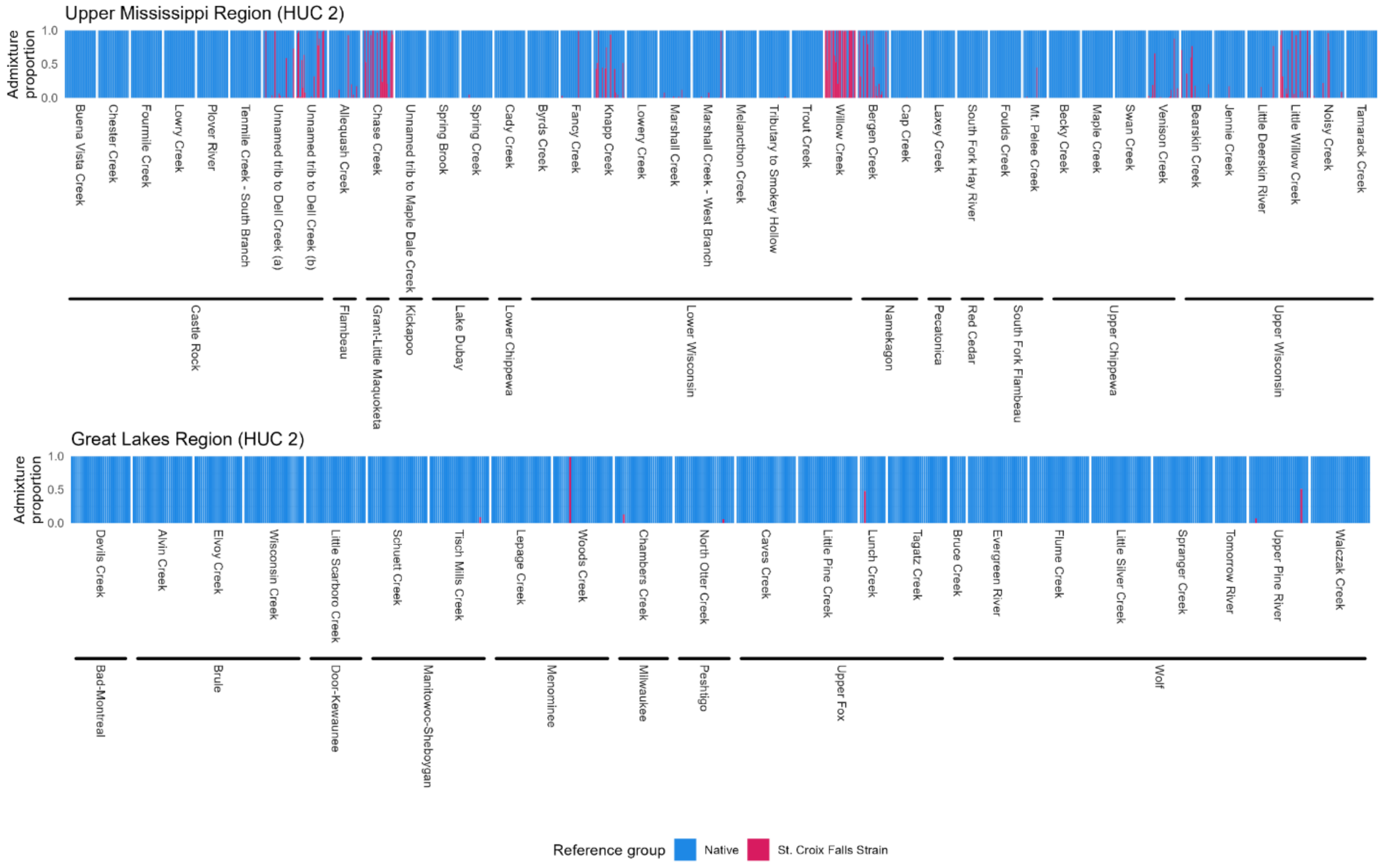


FIGURE 1.17. Barplots displaying admixture proportions (assignment probabilities) to each simulated reference group (native, St. Croix Falls Strain). Brook trout *Salvelinus fontinalis* populations are organized at the hydrologic unit code (HUC) 2 level and by subbasin (HUC 8). Colored bars correspond to individual fish and their proportional assignment to each group.

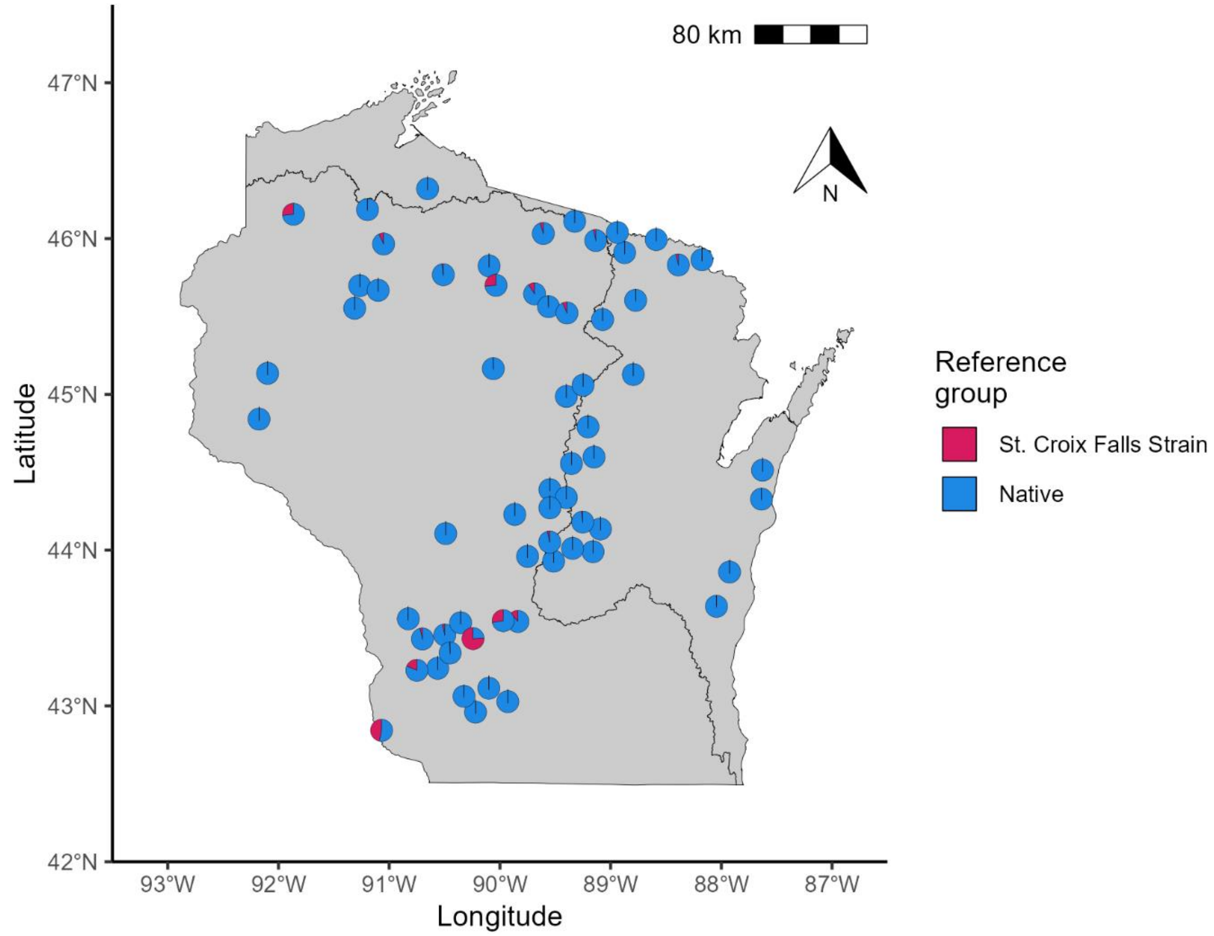


FIGURE 1.18. Spatial distribution of estimated St. Croix identity for each brook trout *Salvelinus fontinalis* survey population (mean assignment to the domestic St. Croix Falls Strain). Red indicates proportional assignment to the domestic St. Croix Falls Strain, while blue indicates proportional assignment to the native reference group.

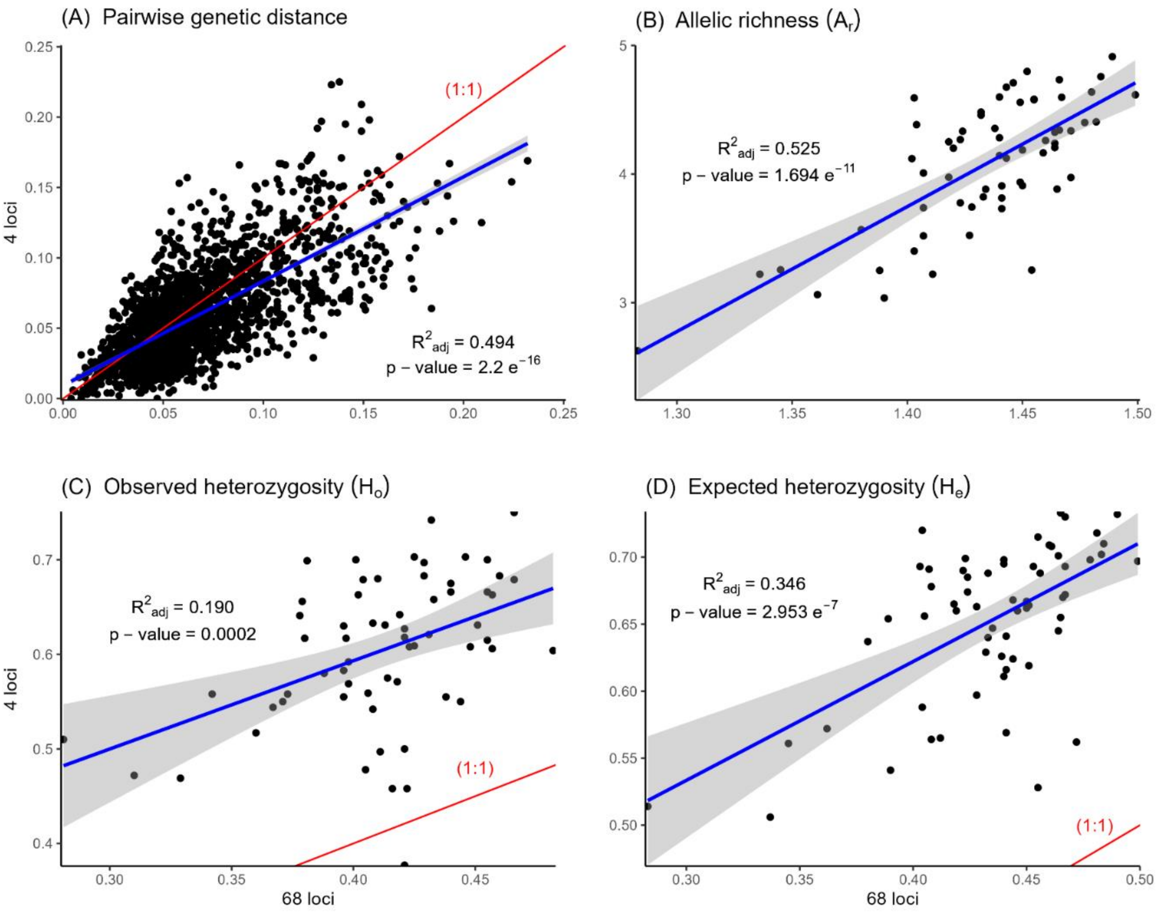


FIGURE 1.19. Linear regression models for (A) pairwise genetic distance (Nei's G_{ST}), (B) allelic richness (A_r), (C) observed heterozygosity (H_o), and (D) expected heterozygosity (H_e). The linear regressions show the correlation between parameter estimates when using 4 microsatellite loci versus 68 microsatellite loci. Red lines indicate perfect agreement (1:1 relationship), whereas blue lines indicate regression lines. The red 1:1 line could not be shown for A_r because it was too low to display on the plot. Correlation coefficients (R^2_{adj}) and p-values are listed for each regression. These regressions were performed simply to verify whether similar conclusions could be drawn from the 4 locus and 68 locus datasets.

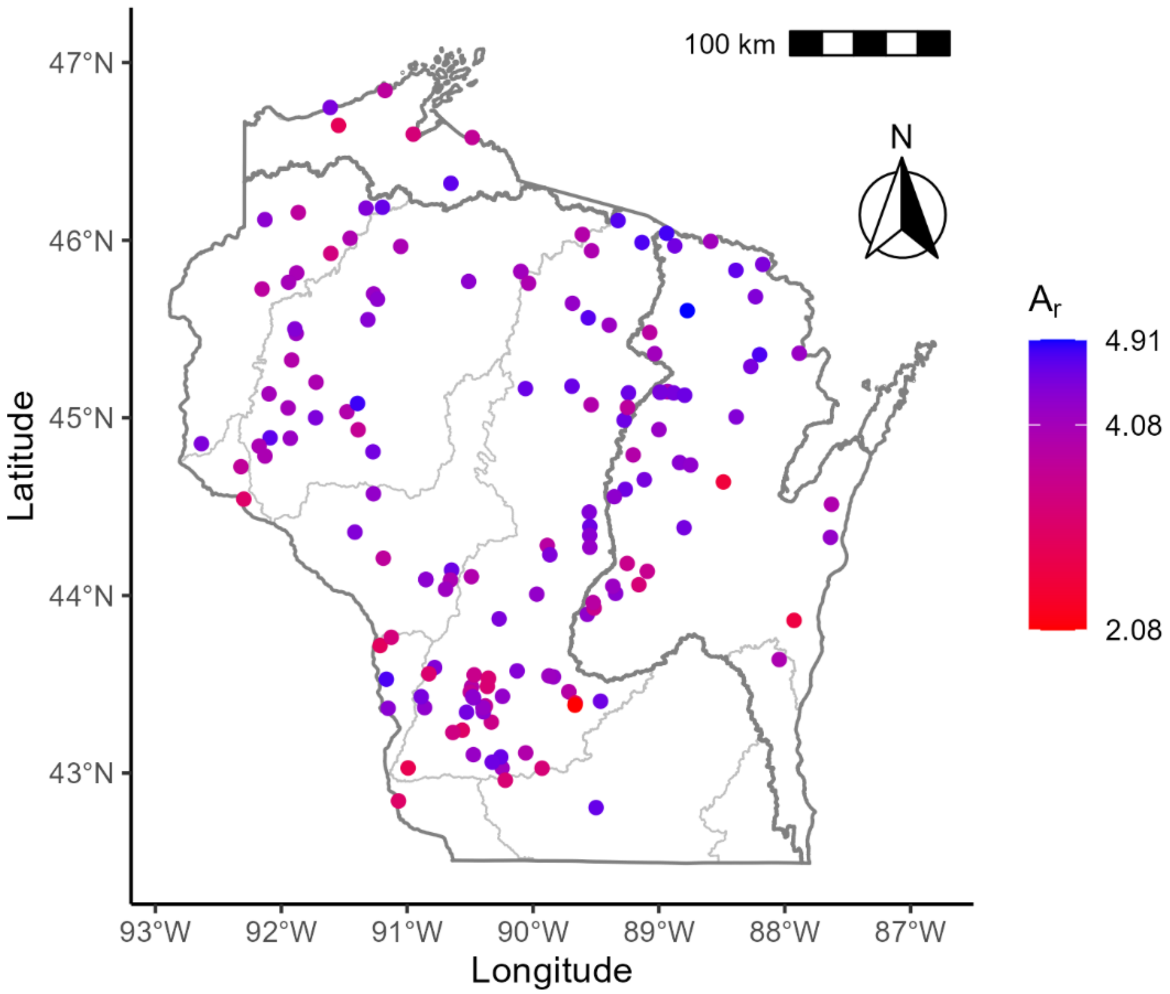


FIGURE 1.20. Heatmap displaying allelic richness (A_r) for each brook trout *Salvelinus fontinalis* survey population (dots) in the combined dataset (four loci). The upper and lower values within the legend indicate the range, while the middle value represents the mean. The color of each dot corresponds to that population's parameter estimate. Dark grey lines indicate regional boundaries at the hydrologic unit code (HUC) 2 level, and light grey lines indicate subregion (HUC 4) boundaries.

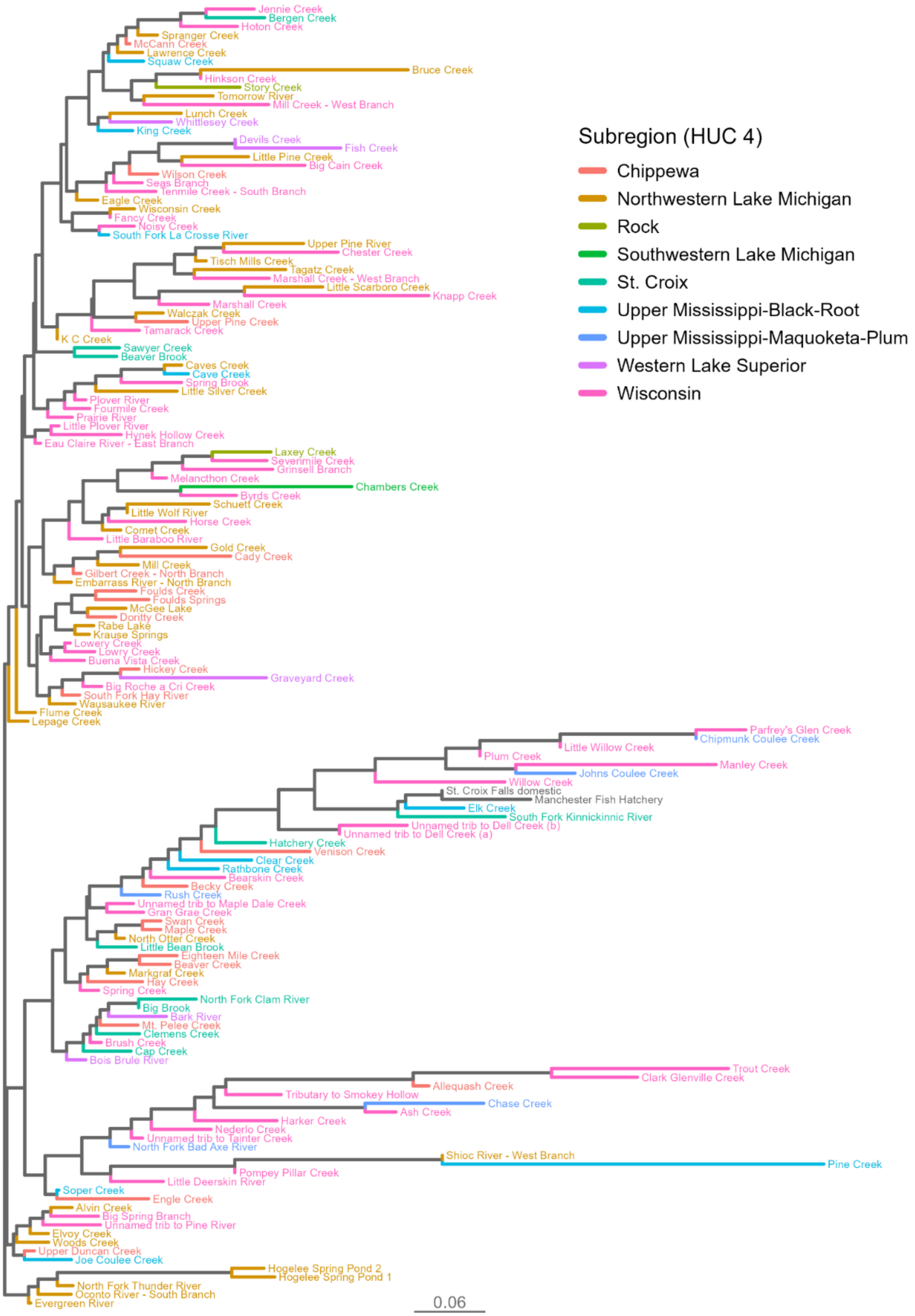


FIGURE 1.21. Neighbor-joining tree displaying the genetic differentiation of each brook trout *Salvelinus fontinalis* population in the combined dataset (four loci). Colors indicate the hydrologic unit code (HUC) 4 subregion. Branch lengths indicate genetic distance (Nei's G_{ST}). The St. Croix Falls and Manchester Fish Hatchery strains are shown in grey.

CHAPTER II: RELATIVE POST-STOCKING FITNESS OF F1, F2, AND DOMESTIC STRAIN BROOK TROUT

Abstract - Brook trout *Salvelinus fontinalis* have been stocked throughout Wisconsin since the late 19th century to improve angling opportunities and mitigate population declines. When stocking to reestablish self-sustaining populations, it is important to consider the fitness of fish being released. Many studies have investigated the fitness of hatchery-reared salmonids, generally observing inferior survival and reproductive success when compared to wild-born fish. However, to the best of our knowledge no studies have made such fitness comparisons for hatchery-reared brook trout with varying degrees of wildness. To improve stocking efficacy, the Wisconsin Department of Natural Resources often stocks first-filial generation brook trout (F1; wild parents) or second-filial generation fish (F2; wild grandparents) rather than domestic strain brook trout that have been maintained in hatcheries for many generations. Genetic analyses were used following three years of experimental stocking to estimate relative measures of post-stocking fitness among F1, F2, and domestic strain brook trout. From 2018 to 2020, 500 F1, 500 F2, and 500 domestic brook trout were stocked each year into Strutt Creek (Iowa County, Wisconsin). Strutt Creek supported reproduction by brown trout *Salmo trutta*, but no brook trout were present prior to stocking. Wild-born brook trout collected in subsequent electrofishing surveys (n = 258) were assigned as offspring of F1, F2, or domestic stocked fish using parentage analyses. F1 fish were superior to F2 fish and domestic strain fish, as they were more likely to survive, naturally reproduce, and produce multiple offspring. An overall decline in natural reproduction was observed in

each consecutive year of sampling. An understanding of relative fitness for stocked brook trout may help inform future propagation, management, and restoration strategies.

Introduction

The stocking of hatchery-reared fish is commonly used in fisheries management to improve harvest opportunity, augment wild populations, and support reintroduction efforts (Brown and Day 2002; Trushenski et al. 2018). When management goals are centered on bolstering wild fish populations, it is important that the factors influencing the fitness of stocked fish are well understood. Fitness generally refers to a fish’s ability to survive and naturally reproduce in the wild. One factor that influences the fitness of stocked fish is the degree of “wildness” (i.e., number of generations removed from the wild; Araki et al. 2008). As a result, fish propagation programs are increasingly stocking fish of wild parentage rather than domestic strains (Mitro 2004; Christie et al. 2014). Fish that are three or more generations removed from the wild are typically considered domesticated, whereas wild parentage fish are those that have wild-born parents (first-filial; F1) or wild-born grandparents (second-filial; F2). Here, I use the term “F1” to describe first generation hatchery-reared fish and “F2” to describe second-generation hatchery-reared fish.

Researchers have been studying differences in fitness and genetic integrity between wild and domesticated salmonids for decades. Early research has demonstrated that domesticated trout exhibit poorer survival than wild-born trout (Flick and Webster 1964; Mason et al. 1967; Fraser 1981). F1 brook trout *Salvelinus fontinalis* have similarly been found to display greater survival rates than domestic fish (St. Croix Falls strain; Edlin 1995). In addition to survival, captive rearing can also negatively impact reproductive success, as many studies have found rainbow (steelhead) trout *Oncorhynchus mykiss* to have reduced reproductive success resulting from one or more generations in captivity (Leider et al. 1990; Kostow et al. 2003; McLean et al. 2004; Araki et al. 2007a, 2007b; Berntson et al. 2011; Ford et al. 2016). Even wild-born offspring of hatchery-reared steelhead trout have been found to have poorer reproductive success than wild-born offspring of wild parents (Araki et al. 2009). Several other salmonids have demonstrated reduced post-stocking success associated with hatchery rearing, including coho salmon *Oncorhynchus kisutch* (Thériault et al. 2011), Atlantic salmon *Salmo salar* (Milot et al. 2013), and pink salmon *Oncorhynchus gorbuscha* (Shedd et al. 2022).

Domestication selection, the inadvertent adaptation to an artificial environment (e.g., a hatchery), is one mechanism that likely contributes toward fitness declines in hatchery-reared fish (Vincent 1960; Araki and Schmid 2010). Rearing fish in an artificial setting unintentionally selects for traits and alleles that are beneficial to that setting (e.g., aggression due to high rearing densities; Huntingford 2004; Araki et al. 2008). Moreover, the relaxation of natural selective forces may result in adaptive or behavioral deficits that could reduce survival and reproduction in the wild. For example, captive-reared fish are provided food and sheltered from predators, therefore they may not develop feeding or predator avoidance behaviors that are necessary in the wild (Huntingford 2004). Domestication selection is especially important to consider when making efforts to restore wild populations, as stocked fish lacking necessary adaptations for the wild may not effectively contribute to natural reproduction and recruitment.

Fitness reductions in domestic strain fish are also likely attributable to declines in genome-wide genetic diversity that results from multi-generational captive rearing. Several studies have demonstrated that hatchery stocks have reduced genetic diversity relative to their wild counterparts (Allendorf and Phelps 1980; Christie et al. 2012; Bingham et al. 2014). Further, Araki and Schmid (2010) identified 21 studies that reported fewer alleles in hatchery stocks when compared with wild populations for a variety of species. Similarly, effective population size (N_e) is often smaller in hatchery stocks when compared to wild stocks (Christie et al. 2012; Bingham et al. 2014). As a result, hatchery stocks can be more susceptible to inbreeding and genetic drift. Low levels of genetic diversity and small N_e can partially be explained by the low quantities of fish that hatchery broodstocks often originate from (i.e., founder effects; Miller and Kapuscinski 2003). Many hatcheries periodically introduce new fish into their captive broodstock to reduce the detrimental effects of small N_e (Mobrand et al. 2005). Maintenance of genetic diversity is imperative for hatchery broodstocks, as reductions in genetic diversity can have negative consequences including reduced resistance to pathogens (Ferguson and Drahushchak 1990; Brieuc et al. 2015) and reduced potential for adaptation to environmental change (Reed and Frankham 2003; DeWoody et al. 2021).

Brook trout have been intensively stocked across the state of Wisconsin since the late 1800s. As described in Chapter I, the Wisconsin Department of Natural Resources (WDNR) implemented a wild trout stocking program in 1995. The original goal of this program has been to improve the instream survival of stocked trout, and one of the primary strategies has been to stock wild parentage trout (F1 and F2) rather than domestic strain trout. However, domestic strain brook trout are still stocked in many of Wisconsin's Class II and Class III trout waterbodies (WDNR 2019). Because there are differing logistical and financial costs associated with producing F1, F2, and domestic strain trout, it is important for fisheries managers to have accurate expectations of post-stocking fitness for each propagation strategy. For example, F2 fish can be produced using broodstock reared in a hatchery for one generation (F1 fish), and domestic fish can be produced using broodstock maintained in a hatchery for many generations. Each time F1 fish are produced, new broodstock must first be gathered from the wild making F1 fish more costly. Additionally, it can be more difficult to produce large quantities of F1 fish due to limitations in the number of spawners that can be gathered from a wild broodstock source. However, there may be fitness advantages in F1 or F2 fish that outweigh some of these costs. Providing expectations of post-stocking fitness for wild parentage and domestic strain brook trout may assist fisheries managers with cost-effective decision-making in future stocking efforts.

I aimed to identify parent-offspring relationships from stocked fish and wild-born offspring to quantify relative measures of survival and reproductive success among F1, F2, and domestic strain fish following their stocking in Strutt Creek (Iowa County, WI). I hypothesized that post-stocking fitness would be inversely related to the number of generations removed from the wild. More specifically, I predicted that F1 fish would show the greatest levels of survival and reproductive success, followed by F2 fish, and the lowest levels would be shown by domestic strain fish. Although many studies have examined fitness differences between wild and domesticated salmonids, less is known about how a second generation in captivity (F2) may influence fitness. Therefore, the primary objective of this chapter was to estimate the relative fitness of each type of brook trout stocked by the WDNR (F1, F2, domestic strain), as measured using relative rates of survival and reproductive success.

Methods

Study Site

Strutt Creek is a 3.6-kilometer stream in Iowa County, Wisconsin. Each fall from 2018-2020, the WDNR stocked Strutt Creek with 500 age-0 brook trout from each of F1, F2, and domestic strain rearing strategies (Table 2.1). Prior to being stocked, the fish received fin clips that were distinct by group, with the clipped fin tissue being retained in 95% ethanol for genetic analyses. From 2019-2022, spring and fall electrofishing surveys were conducted by WDNR personnel to monitor survival and reproduction of the stocked fish. Fin clip tissue samples were collected from fish that had no clipped fins, which were assumed to be wild-born offspring of the stocked fish. These fin clips were stored individually in vials of ethanol. Given that Strutt Creek did not contain resident brook trout at the time of these stocking events, natural reproduction from the stocked fish presented an opportunity to estimate the levels of relative survival and reproductive success from each stocked group in the absence of conspecific competition with the aid of genetic-based parentage analyses.

Stocked Strains

The domesticated brook trout used in this study are derived from the St. Croix Falls strain, the most frequently stocked domestic strain since its arrival to Wisconsin in 1973. The St. Croix Falls strain is a non-native strain that was originally acquired from the Nashua National Fish Hatchery in New Hampshire and is still frequently stocked into many waterbodies in Wisconsin. Each year, the stocked F1 fish were derived from a different broodstock source. Several of these streams coincide with those used to produce the Southwest Feral strain described in Chapter I. More specifically, the F1 fish stocked in 2018, 2019, and 2020 were derived from South Fork Hay River, Cady Creek, and Lowery Creek, respectively. The F2 fish stocked in 2018 were a hybrid cross between F1 fish derived from Melancthon Creek and South Fork Hay River. The F2 fish stocked in 2019 were a hybrid cross between F1 fish derived from Melancthon Creek and Lowery Creek. Lastly, the F2 fish stocked in 2020 were a hybrid cross between F1 fish derived from South Fork Hay River and Lowery Creek.

Laboratory Methods & Microsatellite Genotyping

I used the refined sequencing panel of 68 amplicon-based microsatellite loci described in Chapter I, including use of the same laboratory and genotyping methods. For the fish stocked in 2018 and 2020, a random sample of 200 fish was genotyped from each group. Unfortunately, fin clips from only 50 randomly selected F1 and domestic strain brook trout each were retained for analyses in 2019 (zero F2 fin clips from 2019), therefore all were genotyped (Table 2.1).

Parentage Analyses

Parent-offspring assignments were inferred through genetic parentage analyses using the program Colony (Jones and Wang 2010). I used three separate Colony parentage analyses based on the timing of wild offspring capture (Figure 2.1). Each Colony parentage analysis consisted of differing sets of possible parents, allowing me to exclude stocked fish that would not have been reproductively mature in time to have parented those wild-caught offspring (e.g., fish stocked in 2019 could not be parents of wild offspring caught in 2019). The first Colony parentage analysis contained fish stocked in 2018 as potential parents and wild offspring captured in fall 2020 and spring 2021. The second Colony parentage analysis contained fish stocked in 2018 and 2019 as potential parents and wild offspring captured in fall 2021 and spring 2022. The third Colony parentage analysis contained fish stocked in all three years as potential parents and wild offspring captured in fall 2022. Parameters for each Colony parentage analysis included no updating allele frequency (as recommended in the user manual), dioecious (male and female brook trout have separate reproductive organs), no inbreeding (I assume there was not a considerable amount of inbreeding), male and female polygamy (brook trout can have multiple mates), and no sibship size prior (I did not have a prior distribution of sibship for the offspring). Each Colony parentage analysis was medium length with high precision for the full likelihood method. The full likelihood method is favorable over pairwise comparison methods in that it simultaneously infers parent and sibling relationships, and evaluates the likelihood of full pedigree configurations (Jones and Wang 2010).

Parentage Assignment Accuracy

The accuracy of Colony-based parentage assignments was assessed to quantify the likelihood of erroneous assignments and to determine if bias correction would be necessary. To evaluate assignment accuracy, I simulated individuals with known parent-offspring relationships based on our observed allele frequencies, then ran a parentage analysis to assign each simulated offspring back to the known parent fish. This process involved first simulating a breeding matrix (i.e., a matrix in which rows and columns display mothers and fathers, 0 or 1 in the matrix indicates successful or unsuccessful mate pair) using the R package *mater* (Sard et al. 2021). The breeding matrix was simulated using 50 mothers, 50 fathers, and lambda values of 4 for a Poisson distribution. The number of offspring produced per mate pair was defined using a minimum fertility of

250 and maximum fertility of 900 with a uniform distribution (McFadden 1961). The breeding matrix was then randomly subsampled (n = 100 offspring) and introduced to the simulation module in Colony (Wang 2013). For the Colony simulation parameters, I selected no updating allele frequency, dioecious, no inbreeding, male and female polygamy, no sibship size prior, and no known population allele frequency. The simulation was a short length run with low precision for the full likelihood method, and our empirical allele frequencies were used for the required allele frequency distribution. Resulting simulated offspring were then introduced to a Colony parentage parentage analysis to assign them back to the collection of known simulated parent fish using the same parameters described in the aforementioned parentage analyses. Results from this parentage analysis were evaluated using the most supported configuration of parent-offspring relationships.

Relative Fitness Metrics

Because no fin clips were retained from the F2 fish stocked in 2019, a total of 50 fewer F2 fish were genotyped than F1 and domestic strain fish (Table 2.1). This smaller F2 sample size led to a lower overall likelihood of identifying F2 parents than F1 or domestic parents. For our estimates of relative reproductive success, a sample size correction was applied to the F2 group to estimate the expected number of inferred F2 parent fish had the sample sizes been equal. The sample size correction consisted of the total number of F2 fish genotyped (n = 400) divided by the number of F1 or domestic fish genotyped (n = 450). The actual number of F2 inferred parent fish was then divided by the sample size correction (0.889). This correction allowed for equal comparison of reproductive success between the three groups.

Relative reproductive success was estimated using the inferred parent-offspring relationships from each Colony parentage analysis. I define relative reproductive success (RRS) as the likelihood of producing one or more offspring, relative to the group (F1, F2, domestic) with the greatest number of inferred parent fish. Other studies have compared fitness metrics relative to wild fish (Araki and Blouin 2005); however, there were no resident wild brook trout in Strutt Creek for comparison. I estimated RRS by dividing the number of inferred parent fish from each group by the greatest number of inferred parent fish among the groups. RRS was calculated for each group as shown:

$$\text{Eq 1.} \quad \frac{n_{inferred\ parents}}{n_{max\ inferred\ parents}} = RRS$$

The relative likelihoods of producing at least two offspring was also estimated (termed “multiple offspring RRS”). Multiple offspring RRS was estimated for each group by dividing the number of multiple offspring parent fish (i.e., parent fish inferred to have produced two or more offspring) by the greatest number of multiple offspring parent fish among the groups. Multiple offspring RRS was calculated for each group as shown:

$$\text{Eq 2.} \quad \frac{n_{multiple\ offspring\ parents}}{n_{max\ multiple\ offspring\ parents}} = Multiple\ offspring\ RRS$$

Post-stocking recapture data were used to add context to the relative reproductive success estimates. I used raw return rates (fall-recapture counts based on fin clip identification) to estimate relative survival. Because fish were stocked in the fall at age 0, I assumed that fish recaptured in a subsequent fall season reached reproductive maturity. Therefore, I defined relative survival as the likelihood of surviving to reproductive maturity, relative to the group with the greatest number of fall recaptures. By using recapture counts to estimate relative survival, I am making several broad assumptions: First, I assumed that electrofishing was highly efficient at capturing the stocked brook trout. Second, I assumed that catchability was constant for each group of stocked brook trout and across years. Third, I assumed that sampling effort was consistent among years. Fourth, I assumed that the true survival rates were proportional to the fall-recapture rates. Relative survival was estimated by dividing the number of fall-recaptured fish from each group by the greatest number of fall-recaptured fish among the groups. Relative survival was calculated for each group as shown:

Eq 3. $$\frac{n_{fall\ recaptures}}{n_{max\ fall\ recaptures}} = Relative\ survival$$

To account for differential survival among the stocked groups, I also estimated the likelihood of producing at least one offspring if survival were equal among the stocked groups (i.e., “survival independent RRS”). To estimate survival independent RRS, each stocked group’s RRS estimate (Eq 1) was divided by its respective relative survival estimate (Eq 3). The estimates were then scaled such that the group with the largest survival independent estimate was used as the relative control.

Eq 4. $$\frac{RRS}{Relative\ survival} = Survival\ independent\ RRS$$

Reproductive success was also evaluated by comparing the raw quantities of offspring produced per inferred parent fish from each stocked group. Because some inferred parent fish gave rise to large quantities of wild-caught offspring, the distributions of offspring per parent fish were non-normal (Table 2.2). The non-parametric Kruskal-

Wallis test was used to determine if quantities of offspring per parent fish varied significantly among the stocked groups.

The number of successfully reproducing brook trout was estimated for each year of the study using pedigree accumulation analyses conducted with the R package *vegan* (Dixon 2003; Sard et al. 2021). The pedigree accumulation analyses yielded estimates for the number of successfully breeding adult fish (N_s) in each year of observed natural reproduction (2019-2021). The 2019 N_s estimate was produced using relationships inferred from the first Colony parentage analysis, the 2020 N_s estimate was produced using relationships inferred from the second Colony parentage analysis, and the 2021 N_s estimate was produced using relationships inferred from the third Colony parentage analysis. Estimates of N_s were calculated using the Chao method (Chao 1984), a nonparametric species richness estimator that can be effectively applied to pedigree accumulation analyses (Sard et al. 2021). For each stocked group and year of stocking, mean estimates of allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) were produced using the R package *hierfstat* (Goudet 2005). Allelic richness was adjusted to the smallest sample size using the rarefaction method (n = 50; Kalinowski 2004).

Results

Sequencing performance

Of the 4,500 brook trout stocked into Strutt Creek, 1,300 were sequenced (29%) including 450 F1 fish, 400 F2 fish, and 450 domestic strain fish. Additionally, all 258 wild-caught offspring were sequenced. The number of alleles per locus ranged from 2 to 20 (mean = 9.3).

Parentage Assignments

Zero errors were found in the assignment of simulated offspring back to known simulated parent fish, suggesting the microsatellite panel provides sufficient statistical power to accurately resolve parent-offspring relationships. The Colony parentage analyses (from our empirical dataset) inferred a total of 27 distinct successfully reproducing brook trout within our sample, and at least one parent was identified for 155 of the 258 wild-caught offspring (60%). Of the 27 inferred parent fish, 13 were F1, 10 were domestic strain, and 4 were F2. Quantities of offspring produced by the inferred parent fish were variable. Few fish produced large quantities of offspring, while most contributed just one or two offspring (Table 2.2). Overall, the F1 group produced 130 wild offspring, the F2 group produced 8, and the domestic group produced 23 (Table 2.3).

Relative Fitness Metrics

Relative survival was greatest for the F1 fish, followed by F2 fish, and followed very distantly by domestic fish (Figure 2.2A). Relative survival for F2 fish was 71% that of the F1 group. The domestic fish showed very low relative survival at just 5.8% that of F1 fish and 8% that of F2 fish. The likelihood of producing at least one offspring (RRS) was greatest for F1 fish, followed by domestic fish, and lowest for F2 fish (Figure 2.2B). RRS for domestic fish was about 77% that of F1 fish. RRS was for F2 fish was about 35% that of F1 fish and about 45% that of domestic fish. F1 fish also showed the greatest likelihood of producing two or more offspring, as multiple offspring RRS for domestic fish was just 30% that of F1 fish (Figure 2.2C). Multiple offspring RRS was the lowest for F2 fish, at about 23% that of F1 fish and 75% that of domestic fish. The likelihood of producing at least one offspring assuming equal survival (i.e., survival independent RRS) among the stocked groups was greatest for the domestic fish, followed by F1 fish, and lowest for F2 fish (Figure 2.2D). Survival independent RRS for F1 fish was about 7.5% that of domestic fish but about 49% greater than F2 fish. Survival independent RRS for F2 fish was about 3.7% that of domestic fish. Although only four domestic fish were fall-recaptured, 10 domestic fish were identified as parents by the parentage analyses. This mismatch (moderate RRS divided by very low relative survival) resulted in a very high survival independent RRS value for the domestic group.

The F1 stocked group generally produced greater quantities of offspring per inferred parent fish. There were a few inferred parents from the F1 stocked group that gave rise to notably high quantities of offspring, including individuals responsible for 54, 23, and 19 offspring each (Table 2.2). Median values of offspring per inferred parent fish were 3, 1.5, and 1 for the F1, F2 and domestic groups, respectively (Table 2.3). The Kruskal-Wallis test indicated a moderately non-significant difference in offspring per parent fish (p-value = 0.071; Figure 2.3). Mean values of offspring per inferred parent fish were 10, 2, and 2.3 for the F1, F2 and domestic groups, respectively (Table 2.3).

The estimated number of successfully breeding adult fish was greatest in 2019 (N_s = 73.49). N_s declined by approximately 34% from 2019 to 2020 (N_s = 48.87), and N_s declined by approximately 35% from 2020 to 2021 (N_s = 31.95; Figure 2.4). Measures of genetic diversity and inbreeding were variable among the stocked groups and years (Table 2.4). F2 fish stocked in 2020 and F1 fish stocked in 2018 showed higher allelic richness (A_r) than other years/groups. In contrast, domestic strain fish from all three years of stocking showed the greatest levels of observed (H_o) and expected (H_e) heterozygosity. F2 fish stocked in 2018 had the lowest level of inbreeding (F_{IS}), whereas F2 fish stocked in 2020 and domestic fish stocked in 2019 displayed similarly high F_{IS}.

Discussion

As wild brook trout populations face increasing pressures from the influences of climate change, non-native brown trout, and land-use changes, fish stocking may be increasingly relied upon. The development of accurate expectations for brook trout post-stocking fitness can inform cost-effective decision-making. This chapter investigated relative differences in fitness for three groups of stocked brook trout that reflect varying degrees of wildness. Clear differences in performance were observed among F1, F2, and domestic strain stocked groups that aligned with my expectations of greater fitness in fish that are more recently removed from the wild. In general, F1 brook trout had greater post-stocking fitness than F2 and domestic strain brook trout. F1 brook trout were more likely to survive and produce numerous offspring (Figure 2.2). F2 brook trout showed moderate relative survival yet low relative reproductive success. Domestic strain brook trout displayed the greatest likelihood of reproductive success if they survived to reproductive age; however, their relative likelihood of surviving to reproductive maturity was much less, negating this apparent advantage. This chapter highlights the importance of selecting the appropriate type of brook trout rearing strategy (F1, F2, domestic strain) based on the desired outcomes of a stocking program. Overall, these findings suggest that post-stocking fitness is inversely related to the number of generations that brook trout are reared in captivity.

The F1 stocked group displayed the greatest balance of survival and reproductive capability, demonstrating higher per-parent quantities of offspring. The F1 group's strong RRS may be partially attributable to high relative survival, as it could be assumed that greater survival allows for more reproduction to occur (Figure 2.2A). However, the F2 group showed poor RRS (~35% that of F1; Figure 2.2B) despite moderate relative survival (~70% that of F1; Figure 2.2A). In other words, even though F2 fish were surviving, they were less often successfully reproducing. In contrast, the domestic group showed very poor relative survival (~5.8% that of F1; Figure 2.2A) yet moderate RRS (~77% that of F1; Figure 2.2B). Interestingly, more domestic parent fish were identified than were known to survive to reproductive maturity (i.e., fall-recaptured) indicating that some successfully reproducing domestic strain fish were never captured. These findings indicate that if a domestic strain brook trout managed to survive long enough to be recaptured at least one year after being stocked, which was relatively unlikely, that fish was highly likely to successfully reproduce. This relationship between low survival but high reproductive success of surviving domestic fish resulted in the exceptionally high estimate of survival independent RRS (Figure 2.2D). I believe the survival independent RRS estimate for the domestic fish simply shows that they were just as capable of naturally reproducing, but the likelihood of a domestic fish surviving long enough to do so was comparatively abysmal.

The performance of hatchery-reared fish following their release into the wild has been widely studied. One component of post-stocking fitness, survival, has been evaluated in several studies to compare hatchery trout versus wild trout. In two Adirondack Mountain ponds, Flick and Webster (1964) found over-summer survival to be greater for wild strains (F1; 65-76%) than domestic strains (43-53%) in stocked brook trout. Mason et al. (1967) generally found domestic brook trout to exhibit higher first-winter survival, yet lower long-term survival when compared with wild strain (F1) brook trout in several Wisconsin streams. Stocked wild (F1) and hybrid (one domestic parent, one wild parent) strains were also found to have better survival and longevity in several Ontario lakes (Fraser 1981). Further, Edlin (1995) observed greater summer and winter survival of F1 brook trout compared to domestic (St. Croix Falls strain) brook trout in nine streams of southwest Wisconsin. It should be noted that none of these post-stocking survival studies examined the effect of a second captive generation (F2) on survival. My results align with these findings as I found much higher relative survival in F1 (and F2) brook trout compared to domestic strain brook trout (5.8% that of F1; 8% that of F2; Figure 2.2A).

Another component of post-stocking fitness, reproductive success, has typically been compared relative to wild fish (e.g., Araki et al. 2008), with wild-born fish generally displaying greater reproductive success. Although Strutt Creek did not have a resident wild brook trout population for comparison, I would similarly expect wild brook trout to demonstrate greater levels of fitness than each of our evaluated groups. Post-stocking reproductive success has not been studied as thoroughly as survival for brook trout. Steelhead trout have been one of the most prominent study species for post-stocking reproductive success, with several studies finding reduced reproductive success in hatchery steelhead (Leider et al. 1990; Kostow et al. 2003; McLean et al. 2004; Araki et al. 2007a; Berntson et al. 2011; Ford et al. 2016). For example, Araki et al (2007b) observed a 37.5% reduction in reproductive success per captive-reared generation in steelhead trout. In contrast, Dannewitz et al. (2004) found no difference in reproductive success between domestic strain and wild brown trout. However, it was found that male F1 brown trout showed greater reproductive success than male domestic strain brown trout (Dannewitz et al. 2004). Milot et al. (2013) found reproductive success in F1

Atlantic salmon to be about half that of their wild counterparts, indicating that a single generation in captivity can impact post-stocking fitness. I similarly found reduced reproductive success between fish that differed by just one generation in captivity but in our case, it was F1 versus F2 rather than wild versus F1. Overall, our results generally align with previous reports of reduced post-stocking fitness with more generations spent in a hatchery, as I found F1 fish to have greater measures of relative fitness than F2 and domestic strain fish. Unexpectedly, domestic strain fish demonstrated greater relative reproductive success than F2 fish, which conflicted with expectation. One potential explanation for this may be that the F2 fish experienced losses in fitness due to outbreeding depression, which could have resulted from the hybrid crossing of F1 broodfish derived from different source streams. Although unlikely, it is also possible that some of the F2 fish stocked in 2019 had strong reproductive success but went undetected because the samples were not available for genotyping.

There are a few external factors that could have influenced the observed variation in relative reproductive success and survival. For each year, the F1 and F2 stocked groups were derived from different source streams, so it is possible that local adaptation could have provided an advantage or disadvantage for a given stocked group. This is unlikely however, as the F1 fish stocked in 2018 (the best performing stocked group) were derived from South Fork Hay River, which has very different instream habitat compared to Strutt Creek. Other factors that could not be accounted for include conditions within the hatchery such as fish diets, rearing densities, and rearing habitat. It should also be noted that I could only genotype about 29% of the brook trout stocked into Strutt Creek. If a greater number of stocked fish were genotyped, more parent-offspring relationships could have been identified. Genotyping a greater sample size of potential parent fish may also have reduced any random variation that occurred due to small sample sizes. Lastly, replicating this experiment in additional streams could result in different outcomes.

Management Implications

Variation in relative measures of post-stocking fitness was observed suggesting that certain propagation strategies may be more or less suitable for certain management applications. For most fisheries, the desired outcomes associated with fish stocking require that stocked fish survive long enough to either be caught by an angler or contribute toward natural reproduction. Thus, instream survival could be considered a prerequisite for most stocking applications. Because the F1 group exhibited a strong combination of survival and reproductive success, F1 fish are likely to be the most effective choice in most cases, and particularly those in which natural reproduction is a desired outcome. For example, F1 fish are likely the most appropriate choice for supplementing a population with depleted abundance or reintroducing endemic genetics to a hatchery influenced population, as F1 fish demonstrated a greater likelihood of surviving and producing numerous offspring in the wild. In Wisconsin's Class II trout waterbodies, interannual survival is possible and stocked fish are intended to live long enough to be caught/harvested by an angler; however, natural reproduction is generally not of primary importance. F2 fish may be a cost-effective choice for Class II trout waterbodies, as they are less costly to produce than F1 fish and showed far greater relative survival than the domestic fish. The domestic group exhibited relative survival so low, even compared to the F2 group (~8% that of F2), such that domestic strain fish are unlikely to be an effective choice for most stocking applications (Figure 2.2A).

Wisconsin's Class III trout waterbodies may be one of the few appropriate applications for domestic strain brook trout, as interannual survival is not expected to occur. However, it should be noted that Class III trout waterbodies are typically stocked with adult fish rather than age-0 fish (used in this study), as the stocked fish are intended to be caught/harvested in the same season they are stocked. Because all fish were stocked at age-0 for this study, I cannot extend my inferences of post-stocking fitness to brook trout stocked at other life stages. Domestic strain brook trout stocked as adults may be perfectly acceptable for many Class III trout waterbodies; however, connectivity to Class I or Class II trout waterbodies should be carefully considered, as stocked fish can move into nearby tributaries potentially leading to domestic strain introgression (Bruce and Wright 2018).

Overall natural reproduction declined every year after the first spawning season (Fall 2019; Figure 2.4). As additional brook trout were stocked each fall, density dependent factors (e.g., food, spawning habitat, thermal habitat) may have limited the overall survival and/or reproductive success of the stocked individuals. The number of fish stocked into Strutt Creek may have exceeded the stream's carrying capacity. As such, carrying capacity should be considered prior to beginning a stocking program to avoid diminished effectiveness, particularly when the stocking occurs over several consecutive years. It is also possible that poor habitat or variable environmental conditions could have contributed toward repeated reductions in reproductive success.

Prior research suggests that genetic diversity can often be positively related to fitness (Reed and Frankham 2003). As such, our expectation was that whichever stocked group demonstrated the greatest fitness may also display the greatest levels of genetic diversity. Alternatively, it could have been expected that the F2 fish would display the greatest levels of genetic diversity, as they were hybrid crosses from different streams. Neither of these proved to be the case, as variable levels of genetic diversity and inbreeding were found among the stocked groups and years. Even though F1 fish, particularly those stocked in 2018, showed the greatest relative reproductive success, they displayed lower observed and expected heterozygosity than every year of stocked domestic fish. These findings suggest that neutral genetic diversity alone may not be a great predictor of fitness for hatchery-reared brook trout. It is possible that directional selective pressures (i.e., domestication selection) or maladaptive phenotypic plasticity may have been responsible for reduced fitness in the F2 and domestic strain groups. As observed in Atlantic salmon, captive-reared fish can develop phenotypes that are maladapted to wild environments (Stringwell et al. 2014). The domestic fish in this study could have had maladapted phenotypes resulting in their low relative survival. Similarly, the one additional generation of hatchery maintenance for the F2 fish may have been sufficient for domestication selection to occur and negatively impact their performance relative to the F1 fish.

Conclusion

Differential post-stocking fitness was observed among groups of stocked fish that were reared in captivity for varying numbers of generations. First generation hatchery brook trout (F1) showed greater levels of post stocking fitness than second generation hatchery brook trout (F2) and domestic strain brook trout. F1 fish showed greater relative survival than F2 fish and far greater relative survival than domestic fish. F1 fish were also more likely to naturally reproduce and give rise to multiple offspring. This study supports prior findings that post-stocking fitness is positively related to wildness. My findings indicate that F1 fish are superior to F2 and domestic strain fish for most management applications that value natural reproduction. As the WDNR utilizes brook trout stocking to restore populations with depleted abundance and reintroduce remnant native genetics to hatchery-introgressed populations, expectations of post-stocking fitness may be of value. This study may help fisheries managers determine the degree of wildness that would be most appropriate for consideration among other aspects of management goals.

Literature Cited

Allendorf, F. W., and S. R. Phelps. 1980. Loss of genetic variation in a hatchery stock of Cutthroat Trout. Transactions of the American Fisheries Society 109(5):537–543.

Araki, H., W. R. Ardren, E. Olsen, B. Cooper, and M. S. Blouin. 2007a. Reproductive success of captive-bred steelhead trout in the wild: Evaluation of three hatchery programs in the Hood River. Conservation Biology 21(1):181–190.

Araki, H., B. A. Berejikian, M. J. Ford, and M. S. Blouin. 2008. Fitness of hatchery-reared salmonids in the wild. Evolutionary Applications 1(2):342–355.

Araki, H., and M. S. Blouin. 2005. Unbiased estimation of relative reproductive success of different groups: Evaluation and correction of bias caused by parentage assignment errors. Molecular Ecology 14(13):4097–4109.

Araki, H., B. Cooper, and M. S. Blouin. 2007b. Genetic effects of captive breeding cause a rapid, cumulative fitness decline in the wild. Science 318(5847):100–103.

Araki, H., B. Cooper, and M. S. Blouin. 2009. Carry-over effect of captive breeding reduces reproductive fitness of wild-born descendants in the wild. Biology Letters 5(5):621–624.

Araki, H., and C. Schmid. 2010. Is hatchery stocking a help or harm? Aquaculture 308:S2–S11.

Berntson, E. A., R. W. Carmichael, M. W. Flesher, E. J. Ward, and P. Moran. 2011. Diminished reproductive success of steelhead from a hatchery supplementation program (Little Sheep Creek, Imnaha Basin, Oregon). Transactions of the American Fisheries Society 140(3):685–698.

Bingham, D. M., B. M. Kennedy, K. C. Hanson, and C. T. Smith. 2014. Loss of genetic integrity in hatchery steelhead produced by juvenile-based broodstock and wild integration: Conflicts in production and conservation goals. North American Journal of Fisheries Management 34(3):609–620.

Brieuc, M., M. Purcell, A. Palmer, and K. Naish. 2015. Genetic variation underlying resistance to infectious hematopoietic necrosis virus in a Steelhead Trout (*Oncorhynchus mykiss*) population. Diseases of Aquatic Organisms 117(1):77–83.

Brown, C., and R. L. Day. 2002. The future of stock enhancements: Lessons for hatchery practice from conservation biology. Fish and Fisheries 3(2):79–94.

Bruce, S. A., and J. J. Wright. 2018. Estimates of gene flow and dispersal in wild riverine Brook Trout (*Salvelinus fontinalis*) populations reveal ongoing migration and introgression from stocked fish. Ecology and Evolution 8(23):11410–11422.

Chao, A. 1984. Nonparametric estimation of the number of classes in a population. Scandinavian Journal of Statistics 11(4):265–270.

Christie, M. R., M. J. Ford, and M. S. Blouin. 2014. On the reproductive success of early-generation hatchery fish in the wild. Evolutionary Applications 7(8):883–896.

Christie, M. R., M. L. Marine, R. A. French, R. S. Waples, and M. S. Blouin. 2012. Effective size of a wild salmonid population is greatly reduced by hatchery supplementation. Heredity 109(4):254–260.

Dannewitz, J., E. Petersson, J. Dahl, T. Prestegaard, A.-C. Lof, and T. Jarvi. 2004. Reproductive success of hatchery-produced and wild-born Brown Trout in an experimental stream. Journal of Applied Ecology 41(2):355–364.

DeWoody, J. A., A. M. Harder, S. Mathur, and J. R. Willoughby. 2021. The long-standing significance of genetic diversity in conservation. Molecular Ecology 30(17):4147–4154.

Dixon, P. 2003. VEGAN, A package of R functions for community ecology. Journal of Vegetation Science 14(6):927–930.

Edlin, D. G. 1995. Survival and growth of wild and domestic Brook Trout (*Salvelinus fontinalis*) in southwest Wisconsin streams. Thesis M.S., University of Wisconsin - La Crosse.

Ferguson, M. M., and L. R. Drahushchak. 1990. Disease resistance and enzyme heterozygosity in Rainbow Trout. Heredity 64(3):413–417.

Flick, W. A., and D. A. Webster. 1964. Comparative first year survival and production in wild and domestic strains of Brook Trout, *Salvelinus fontinalis*. Transactions of the American Fisheries Society 93(1):58–69.

Ford, M. J., A. R. Murdoch, M. S. Hughes, T. R. Seamons, and E. S. LaHood. 2016. Broodstock history strongly influences natural spawning success in hatchery steelhead (*Oncorhynchus mykiss*). PLOS ONE 11(10):e0164801.

Fraser, J. M. 1981. Comparative survival and growth of planted wild, hybrid, and domestic strains of Brook Trout (*Salvelinus fontinalis*) in Ontario lakes. Canadian Journal of Fisheries and Aquatic Sciences 38(12):1672–1684.

Goudet, J. 2005. HIERFSTAT, a package for R to compute and test hierarchical F-statistics. Molecular Ecology Notes 5(1):184–186.

Huntingford, F. A. 2004. Implications of domestication and rearing conditions for the behaviour of cultivated fishes. Journal of Fish Biology 65(s1):122–142.

Jones, O. R., and J. Wang. 2010. COLONY: A program for parentage and sibship inference from multilocus genotype data. Molecular Ecology Resources 10(3):551–555.

Kalinowski, S. T. 2004. Counting alleles with rarefaction: Private alleles and hierarchical sampling designs. Conservation Genetics 5(4):539–543.

Kostow, K. E., A. R. Marshall, and S. R. Phelps. 2003. Naturally spawning hatchery steelhead contribute to smolt production but experience low reproductive success. Transactions of the American Fisheries Society 132(4):780–790.

Leider, S. A., P. L. Hulett, J. J. Loch, and M. W. Chilcote. 1990. Electrophoretic comparison of the reproductive success of naturally spawning transplanted and wild steelhead trout through the returning adult stage. Aquaculture 88(3):239–252.

Mason, J. W., O. M. Brynildson, and P. E. Degurse. 1967. Comparative survival of wild and domestic strains of Brook Trout in streams. Transactions of the American Fisheries Society 96(3):313–319.

McFadden, J. T. 1961. A population study of the Brook Trout, *Salvelinus fontinalis*. Wildlife Monographs 7:3–73.

McLean, J. E., P. Bentzen, and T. P. Quinn. 2004. Differential reproductive success of sympatric, naturally spawning hatchery and wild steelhead, *Oncorhynchus mykiss*. Environmental Biology of Fishes 69(1):359–369.

Miller, L., and A. Kapuscinski. 2003. Genetic guidelines for hatchery supplementation programs. Population Genetics: Principles and Applications for Fisheries Scientists:329–355.

Milot, E., C. Perrier, L. Papillon, J. J. Dodson, and L. Bernatchez. 2013. Reduced fitness of Atlantic Salmon released in the wild after one generation of captive breeding. Evolutionary Applications 6(3):472–485.

Mitro, M. G. 2004. Stocking trout of wild parentage to restore wild populations: An evaluation of Wisconsin's Wild Trout Stocking Program. Proceedings of Wild Trout XIII 8:255–264.

Mobrand, L. E., J. Barr, L. Blankenship, D. E. Campton, T. T. P. Evelyn, T. A. Flagg, C. V. W. Mahnken, L. W. Seeb, P. R. Seidel, and W. W. Smoker. 2005. Hatchery reform in Washington State: Principles and emerging issues. Fisheries 30(6):11–23.

Reed, D. H., and R. Frankham. 2003. Correlation between fitness and genetic diversity. Conservation Biology 17(1):230–237.

Sard, N. M., R. D. Hunter, E. F. Roseman, D. B. Hayes, R. L. DeBruyne, and K. T. Scribner. 2021. Pedigree accumulation analysis: Combining methods from community ecology and population genetics for breeding adult estimation. Methods in Ecology and Evolution 12(12):2388–2396.

Shedd, K. R., E. A. Lescak, C. Habicht, E. E. Knudsen, T. H. Dann, H. A. Hoyt, D. J. Prince, and W. D. Templin. 2022. Reduced relative fitness in hatchery-origin Pink Salmon in two streams in Prince William Sound, Alaska. Evolutionary Applications 15(3):429–446.

Stringwell, R., A. Lock, C. J. Stutchbury, E. Baggett, J. Taylor, P. J. Gough, and C. Garcia de Leaniz. 2014. Maladaptation and phenotypic mismatch in hatchery-reared Atlantic Salmon *Salmo salar* released in the wild. Journal of fish biology 85(6):1927–1945.

Thériault, V., G. R. Moyer, L. S. Jackson, M. S. Blouin, and M. A. Banks. 2011. Reduced reproductive success of hatchery Coho Salmon in the wild: Insights into most likely mechanisms. Molecular Ecology 20(9):1860–1869.

Trushenski, J. T., G. E. Whelan, and J. D. Bowker. 2018. Why keep hatcheries? Weighing the economic cost and value of fish production for public use and public trust purposes. Fisheries 43(6):284–293.

Vincent, R. E. 1960. Some influences of domestication upon three stocks of Brook Trout (*Salvelinus fontinalis Mitchill*). Transactions of the American Fisheries Society 89(1):35–52.

Wang, J. 2013. A simulation module in the computer program COLONY for sibship and parentage analysis. Molecular Ecology Resources 13(4):734–739.

WDNR. 2019, March. Wisconsin Inland Trout Management Plan 2020-2029. Wisconsin Dept. of Natural Resources, Bureau of Fisheries Management.

Tables

TABLE 2.1. Quantities of brook trout *Salvelinus fontinalis* stocked from 2018-2020 for each stocked group (F1, F2, Domestic) into Strutt Creek (Iowa County, WI). All wild-caught offspring (n = 258) were used for genetic analysis.

Year	**Stocked group**	$N_{stocked}$	$N_{analyzed}$
2018	F1	500	200
	F2	500	200
	Domestic	500	200
2019	F1	500	50
	F2	500	0
	Domestic	500	50
2020	F1	500	200
	F2	500	200
	Domestic	500	200

TABLE 2.2. Table displaying the sample ID number, group, year of stocking, and total quantity of offspring produced by each inferred parent brook trout *Salvelinus fontinalis* (n = 27).

Inferred parent ID	**Group**	**Year stocked**	**$N_{offspring}$**
22-11343	F1	2018	54
22-11341	F1	2018	23
21-09419	F1	2018	19
22-11373	F1	2018	11
22-11841	Domestic	2020	10
21-09476	F1	2018	7
22-11279	Domestic	2018	4
22-11399	F1	2018	4
22-11491	F2	2018	4
22-11318	F1	2018	3
21-09382	Domestic	2018	2
21-09450	F1	2018	2
21-09460	F1	2018	2
22-11344	F1	2018	2
22-11487	F2	2018	2
21-08602	F2	2020	1
21-09241	F2	2018	1
21-09310	Domestic	2018	1
21-09317	Domestic	2018	1
21-09331	Domestic	2018	1
21-09370	Domestic	2018	1
21-09398	Domestic	2018	1
21-09399	Domestic	2018	1
22-11242	Domestic	2018	1
22-11303	F1	2018	1
22-11312	F1	2018	1
22-11348	F1	2018	1

TABLE 2.3. Table displaying the total number of parent fish and total number of offspring produced from each stocked group of brook trout *Salvelinus fontinalis*. Mean, median, and standard deviation are shown for the number of offspring produced per parent fish.

Stocked group	**Total $N_{parents}$**	**Total $N_{offspring}$**	**Mean ($N_{offspring}$ / Parent)**	**Median ($N_{offspring}$ / Parent)**	**Standard deviation**
F1	13	130	10	3	15.0
F2	4	8	2	1.5	1.41
Domestic	10	23	2.3	1	2.87

TABLE 2.4. Table displaying estimates for allelic richness (A_r), observed heterozygosity (H_o), expected heterozygosity (H_e), and inbreeding coefficient (F_{IS}) for each group and year of stocking. DNA samples were not available for the F2 brook trout *Salvelinus fontinalis* stocked in 2019.

Group	**Year stocked**	**A_r**	**H_o**	**H_e**	**F_{IS}**
F1	2018	3.795	0.429	0.450	0.048
	2019	3.266	0.406	0.426	0.037
	2020	3.398	0.418	0.442	0.044
F2	2018	3.049	0.406	0.411	0.017
	2019	-	-	-	-
	2020	3.861	0.432	0.462	0.053
Domestic	2018	3.43	0.464	0.474	0.020
	2019	3.515	0.461	0.481	0.051
	2020	3.422	0.453	0.468	0.034

Figures

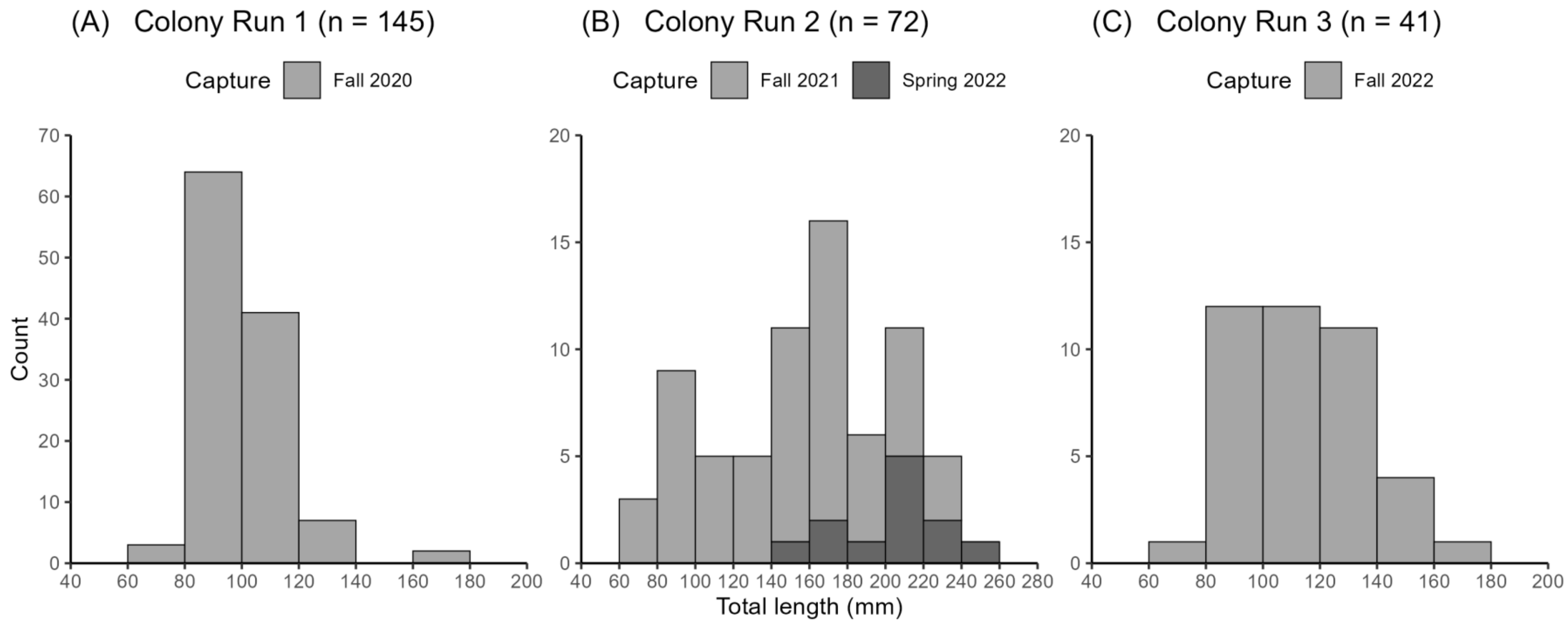


FIGURE 2.1. Length-frequency histograms for the wild-caught offspring included in the (A) first Colony parentage analysis (B) second Colony parentage analysis, and (C) third Colony parentage analysis from Strutt Creek (Iowa County, WI). Brook trout *Salvelinus fontinalis* are grouped into 20mm length bins. Shading indicates the season and year of capture. There are 28 wild-caught offspring included in the first Colony parentage analysis (captured in Spring 2021) that could not be depicted because length data was not available.

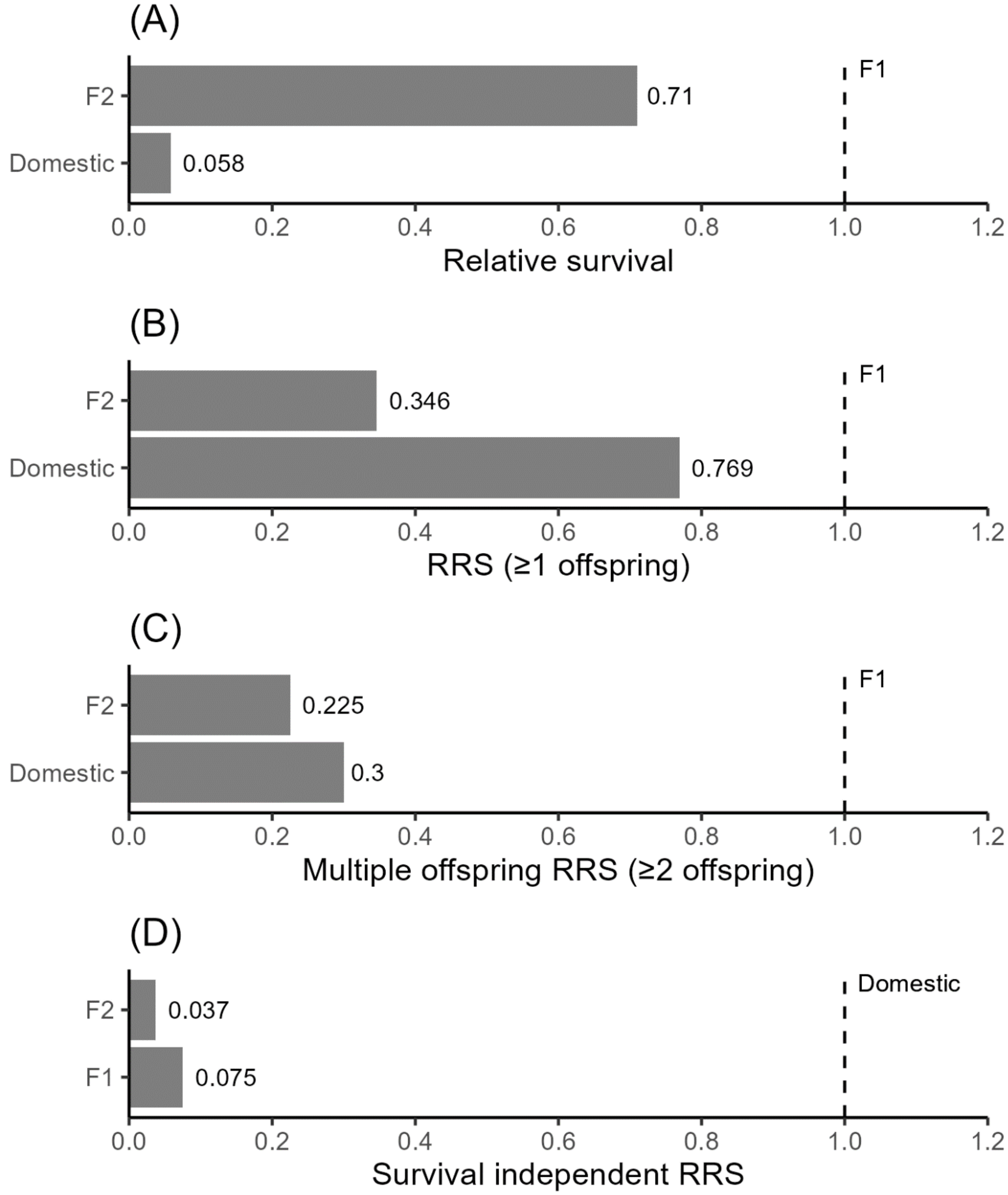


FIGURE 2.2. Bar plots displaying (A) relative likelihood of survival, (B) relative likelihood of producing one or more offspring, (C) relative likelihood of producing two or more offspring, and (D) relative likelihood of producing one or more offspring assuming equal survival for brook trout *Salvelinus fontinalis*. Each estimate is scaled to the largest value, depicted with vertical dashed lines.

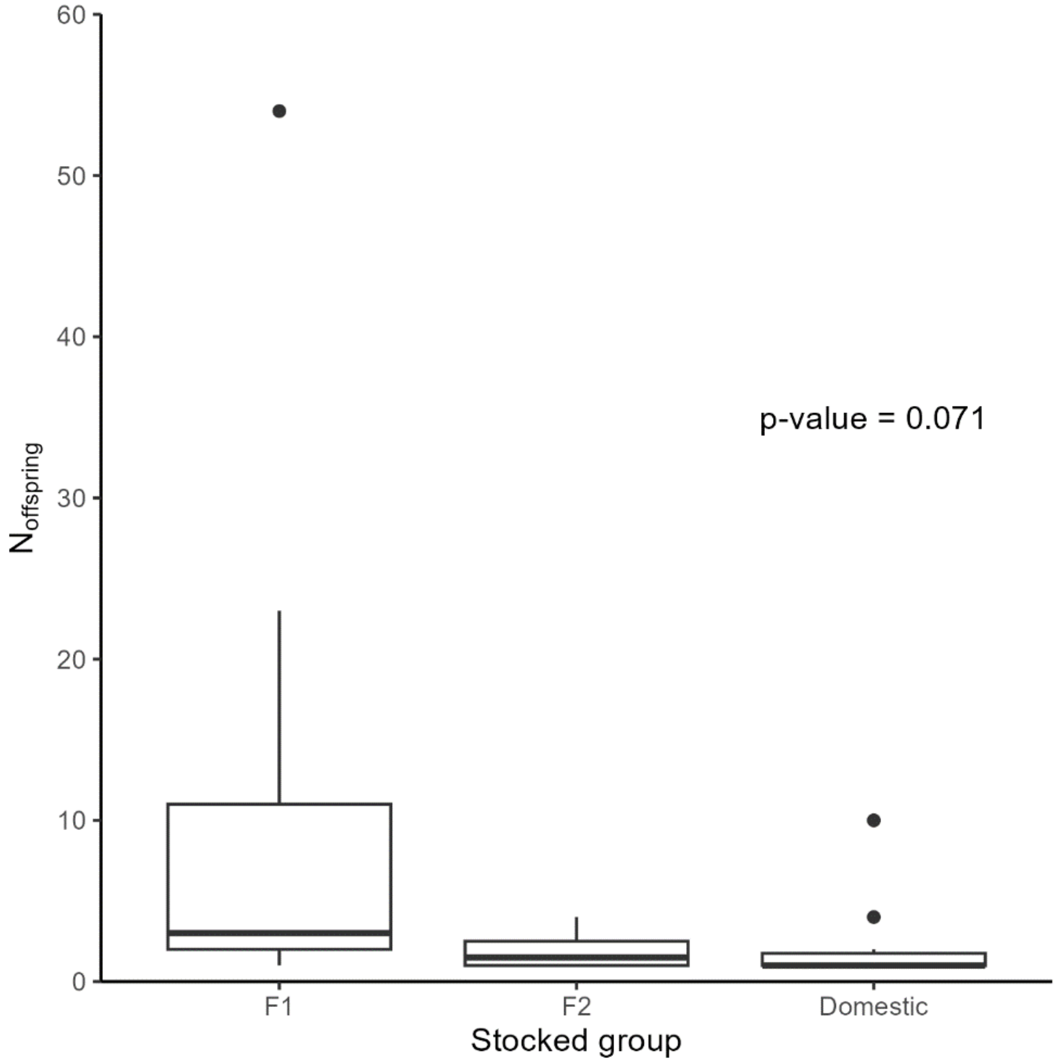


FIGURE 2.3. Boxplot displays quantities of offspring produced by each inferred parent brook trout *Salvelinus fontinalis*. The horizontal line within each box represents the median value. Each box represents the interquartile range. Dots represent outlier values. The p-value listed is associated with a Kruskal-Wallis test, which was used to compare median values among the stocked groups.

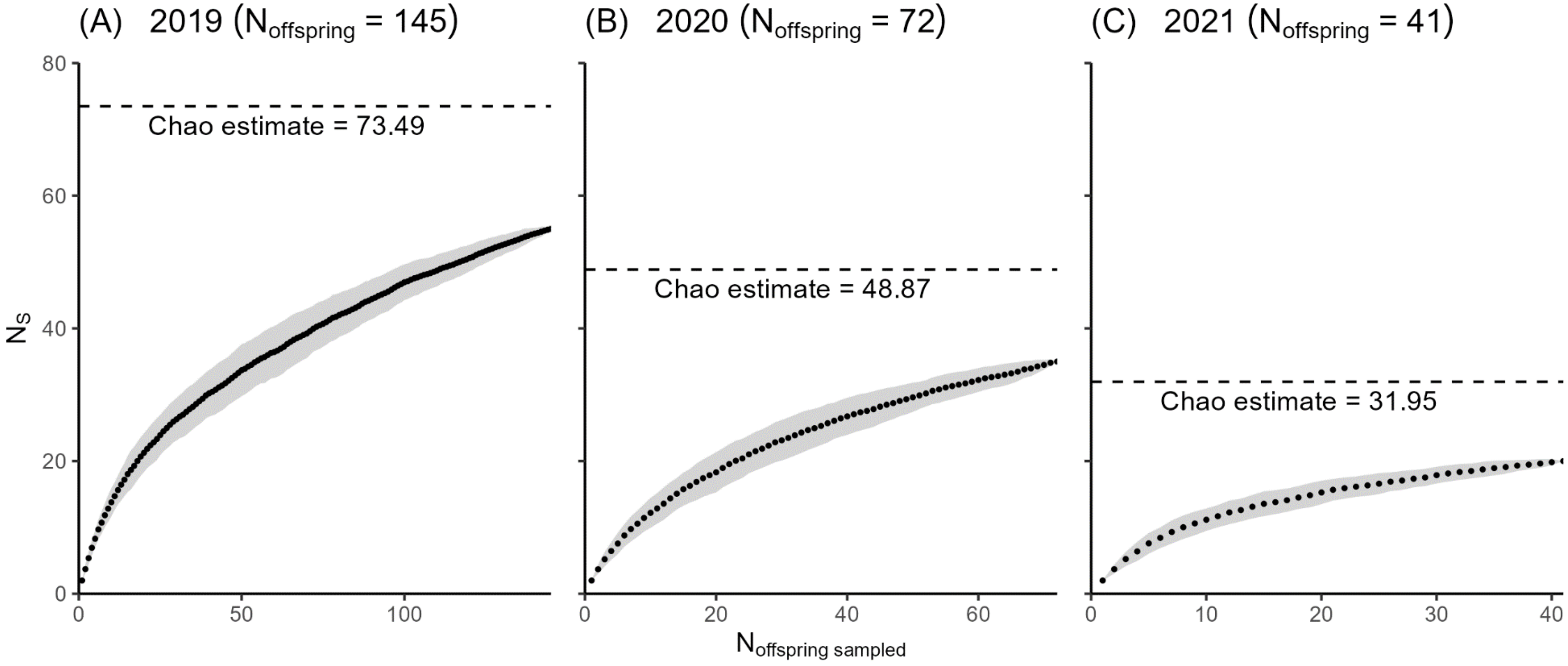


FIGURE 2.4. Pedigree accumulation plots display Chao estimates for the number of successfully breeding adult (N_s) brook trout *Salvelinus fontinalis* corresponding to fall (A) 2019, (B) 2020, and (C) 2021 in Strutt Creek (Iowa County, WI). $N_{offspring}$ refers to the quantity of wild-caught offspring included in each Colony parentage analysis.