MICROSATELLITES REVEAL THE POPULATION GENETIC STRUCTURE OF YELLOW PERCH IN MINNESOTA

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Abstract*—When analyzing a population's genetic makeup, looking at variation of individuals and populations is a crucial factor. The management of yellow perch will be aided by this information particularly in regard to stocking or relocation. Microsatellite loci were used to analyze the genetic population structure of yellow perch *Perca flavescens* within and among 29 sampling areas throughout Minnesota's basins. Seven microsatellite DNA loci were evaluated, with heterozygosities ranging from 0.41 to 0.78. The Lower Mississippi basin samples showed higher numbers of alleles per locus and greater expected heterozygosities compared to other basins, with Lake Pepin (PEP) exhibiting the highest average expected heterozygosity at 0.78. Pfeiffer Lake (PFE) also contained unique alleles. Genetic population structure analysis revealed diverse ancestral compositions among populations, with some, like BRS, TNB, and PEP, showing dominant ancestral clusters, while most others had weaker differentiation. Neighbor-joining tree analysis indicated little genetic separation within Rainy River basin populations but significant differences between Rainy River and Minnesota River basin populations. PCoA and FST values suggested four distinct genetic groups: a central/southern Minnesota & Upper/Lower Mississippi basin cluster, a highly divergent Red River basin group, and tightly clustered Rainy River and Great Lakes basin groups. This study provides valuable insights into the genetic structure, diversity, and connectivity of yellow perch populations in Minnesota. The observed variations highlight the importance of considering regional differences in genetic makeup for effective fisheries management. Understanding these genetic distinctions will inform future conservation strategies, stocking decisions, and translocation efforts to preserve locally adapted gene pools and enhance the long-term resilience of yellow perch populations in the face of environmental changes.

I. INTRODUCTION

Understanding the variation of individuals and populations is essential when researching a population's genetic structure. Deep comprehension of physical distribution, mating behaviors, dispersion, and life history can improve fitness and lead to versatile populations. For instance, an insect mating behavior study showed that females gain offspring with an increased lifespan when mating with multiple partners (Arnqvist and Nilsson 2000). In another study, fruit fly populations were predicted to have a higher fitness when of a polymorphic population rather than two monomorphic populations (Takahashi et al. 2018). That knowledge, in turn, can be used as a tool for directing efficient restoration, conservation, and management strategies. There is an abundance of equipment accessible to physically monitor a species, however, utilizing traditional techniques has limited the capacity of acquiring genetic variation data. An effective remedy for this issue is observing tandem repeats.

In eukaryotic genomes, repetitive sequences of DNA bases are frequently observed within a chromosome. Represented in the non-coding and coding regions of the DNA and ranging in lengths from one to several thousand base pairs, these repetitive sequences are identified as tandem repeats. Tandem repeats are classified based on the length of their repeated motifs and consist of microsatellite DNA, minisatellite DNA, variable number of tandem repeats, and simple sequence repeats (Marina 2020). Two to six nucleotide base pairs in repeating length distinguishes microsatellite DNA. Appearing in great abundances, manifesting high variability rates, and providing rapid data results, microsatellites are heavily utilized in contrast to any other subcategory of tandem repeats (O'Connell and Wright 1997). Proving enormously useful in studies of population structure, genetic mapping, and evolutionary processes, microsatellite DNA serves as a genetic marker for fisheries scientists to research population and community ecology.

Population structure in many species is dynamic and subject to ongoing refinement through genetic research. The yellow perch *Perca flavescens* is one such species for which population structure has been actively investigated. Previous studies have primarily focused on populations from the East Coast to the Midwest regions of the United States (Leary and Booke 1982; Kapuscinski and Miller

2003; Grzybowski et al. 2010). Miller (2003) reported that spawning groups in Green Bay were genetically distinct from those in Lake Michigan and various inland locations. Similarly, Kapuscinski and Miller (2000) identified significant allelic differentiation among three examined populations. Grzybowski et al. (2010) further revealed greater levels of genetic differentiation than previously documented, based on analysis of 17 populations across the Midwest and East Coast. These studies highlight substantial population structure within yellow perch across its range. Ongoing genetic investigations are necessary to fully characterize the species' genetic diversity and patterns of connectivity, particularly in geographically underrepresented areas.

The purpose of this study was to describe the intra- and inter-population genetic variation in yellow perch populations across the major hydrologic basins of Minnesota. By utilizing highly polymorphic microsatellite DNA markers, this study aims to provide insights into the genetic structure, diversity, and connectivity of yellow perch populations at a regional scale. Such information is critical for informing fisheries management decisions, particularly with regard to stocking, translocation, and habitat restoration efforts. Understanding population differentiation can help managers avoid unintended genetic homogenization, preserve locally adapted gene pools, and enhance long-term population resilience. Additionally, this study contributes to the broader understanding of how landscape features, hydrologic connectivity, and historical management practices may have shaped the genetic structure of yellow perch in Minnesota. Ultimately, the findings will support evidence-based strategies for conserving, maintaining, and potentially expanding yellow perch populations in the face of environmental change and anthropogenic pressures.

II. Methods

Sample collections. — In the summer of 2022, 20 state natural resource agency personnel collected yellow perch scales from any life stage at random sampling locations. Collections were obtained from 40 sampling locations within Minnesota: Six from Red River of the North basin, five from Rainy River basin, five from Great Lakes basin, five St. Croix River basin, six from Upper Mississippi River basin, six from Lower Mississippi River basin, five from Minnesota River basin, and two from Missouri River basin (Figure 1). The scales were air-dried and stored in envelopes for genetic analysis. Sample sizes from each location ranged from 7 to 38. A total of 29 out of the 40 sampling locations were included in the analysis, as each location had a sample size of 25 individuals or more (Table 1 and 2).

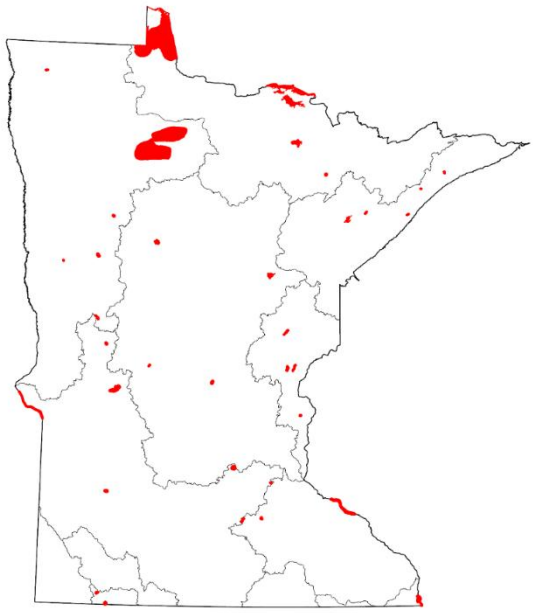

Figure 1. Map of the 40 sampling locations across Minnesota collected by state natural resource agencies in the summer of 2022.

Genetic analysis. — DNA was extracted from scale samples using 300 µL of 5% Chelex (BioRad Research Co., Hercules, California) solution. One to two scales were used for each preparation, lysed overnight, then boiled at 400 ºC for 8 minutes.

Sixteen microsatellite loci previously developed from walleye *Sander vitreus* and yellow perch were evaluated: *Svi3, Svi7* from Eldridge et al. (2002), *Svi4* and *Svi17* from Borer et al. (1999), *Pfla-L2*, *Pfla-L4*, *Pfla-L5*, and *Pfla-L6* from Leclerc et al. (2000), *MPf-2*, *MPf-4*, *MPf-6*, and *MPf-7* from Grzybowski et al. (2010), and *YP6*, *YP13*, *YP16*, and *YP79* from Li et al. (2006). Microsatellite amplification via the polymerase chain reaction (PCR) was performed in a 96-well plate. Each 15 µL PCR reaction contained 0.15 µL of the forward and 0.50 µL of the reverse primers, 5 µL of Chelex extraction as the DNA template, 0.1 µL of GoTaq G2 DNA polymerase, 3 µL of 5X colorless GoTaq reaction buffer, and 0.3 µL of a 0.10 mM deoxynucleotide triphosphate mixture was used for one locus. One primer of each pair was labeled with a fluorescent dye (FAM, PET, VIC, or NET). The following protocol for amplification was used in an Applied Biosystem 2720 thermal cycler: 3 min initial denaturation at 95 ºC; 34 cycles of 30 s denaturation at 95 ºC, 30 s annealing at 50 ºC, and elongation at 72 ºC for 45 s; and a final elongation of 5 min at 72 ºC.

Table 1. Summary of study lakes across the 6 major hydrologic basins in Minnesota. Each entry includes the major basin designation, associated DNR office location, lake name, unique Minnesota Department of Natural Resources (MNDNR) lake identification number, and a corresponding site acronym using throughout the study.

Major Basin	Office Location	Lake Name	Lake ID	Acronym
Great Lakes	Duluth	Bassett	69004100	BAS
Great Lakes	Duluth	Whiteface	69037500	WIT
Great Lakes	Finland	Lax	38040600	LAX
Great Lakes	Finland	Thunderbird	38003100	TNB
Great Lakes	Grand Maris	White Pine	16036900	WPL
Lower Mississippi	Lake City	Lake Pepin	25000100	PEP
Lower Mississippi	Waterville	Gorman	40003200	GOR
Lower Mississippi	Waterville	Volney	40003300	VON
Minnesota River	Glenwood	Minnewaska	61013000	MIN
Minnesota River	Glenwood	Moses	21024500	MOS
Minnesota River	Glenwood	Pelican	61011100	PEL
Minnesota River	Ortonville	Big Stone	06015200	BSN
Minnesota River	Spicer	Tyson	87001900	TYS
Rainy River	Baudette	Lake of the Woods	39000200	LOW
Rainy River	International Falls	Kabetogama	69084500	KAB
Rainy River	Rainy Lake	Rainy Lake	69069400	RNY
Rainy River	Tower	Pfeiffer	69067100	PFE
Red River	Baudette	Bronson	35000300	BRS
Red River	Detroit Lakes	Lee	14004900	LEE
Red River	Detroit Lakes	Rock	03029300	ROK
Red River	Detroit Lakes	Roy	44000100	ROY
St. Croix River	Hinkley	Cross	58011900	CRS
St. Croix River	Hinkley	South Pine	00010001	SP
Upper Mississippi	Atkin	Big Sandy	01006200	BS
Upper Mississippi	Little Falls	Little Sauk	77016400	LLS
Upper Mississippi	Little Falls	Mayhew	05000700	MAY
Upper Mississippi	Park Rapids	Mantrap	29015100	MTP
Upper Mississippi	Shakopee	Crystal	27003400	CRY
Upper Mississippi	Shakopee	Waconia	10005900	WAC

PCR products were visualized in two ways. In order to verify amplification and approximate product size, the products were displayed on a 14 × 16 cm nondenaturing 8% acrylamide gel and stained with ethidium bromide. To score alleles, the results of each individual PCR reaction were pooled and forwarded to the Azenta Commercial sequencing facility for fragment analysis. Genotypes were determined using Geneious Prime software (Biomatters, Boston, MA). Each plate contained a negative control without DNA to identify possible PCR contamination.

Data analysis. — The intra-population genetic variation, using seven polymorphic loci found during the first screening, was quantified as observed

heterozygosity (H_o), expected heterozygosity (H_e), and allelic richness , the number of alleles standardized to a common sample size (A_r). Exact tests were used to assess conformity with the Hardy-Weinberg assumptions (Guo and Thompson 1992).

To reduce the potential for false detections of deviations from Hardy-Weinberg equilibrium while conducting multiple comparisons, a sequential Bonferroni correction was applied to Hardy-Weinberg exact test statistics (Rice 1989). A significance level (α) of 0.05 was used across 202 pairwise comparisons among 29 samples (k).

Spatial genetic structure interpopulation was examined using a Bayesian clustering approach in the program STRUCTURE (Pritchard et al. 2000) to identify distinct populations. STRUCTURE was run with 50,000 iterations of burn-in followed by 200,000 iterations to evaluate 1-25 possible populations.

To depict the genetic organization of populations, a neighbor-joining tree was created based on genetic distances in the software program Populations (Langella 1999). Results were visualized using FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

In order to represent inter-object similarity in a Euclidean space, a Principal Coordinates Analysis (PCoA) was performed (Gower 1966). The PCoA accounted for the similarity of allele frequencies to locate populations in multi-dimensional space. The add-in software tool GenAlEx (Peakall and Smouse 2012), on Excel 2010, was utilized to perform the PCoA analysis.

III. Results

Microsatellite Variation. — In the preliminary trials, 13 of the 16 microsatellites amplified yellow perch DNA. Out of the 13, 5 loci had monomorphic or minimal variance genetic makeup (*Svi3*, *YP6*, *YP13*, *YP16*, and *YP79*) and one fluorescently colored locus was incompatible due to overlapping size ranges *(Pfla-L2).* The remaining 7 loci (*Svi4*, *Svi7*, *Pfla-L4*, *Pfla-L6*, *MPf-4*, *MPf-6*, and *MPf-7*) were used to evaluate the genetic variation of the samples.

The sample collections revealed polymorphisms for all seven loci. The one exception included locus *Svi7* at Thunderbird (TNB), which was monomorphic. From the samples in the Lower Mississippi basin, the average number of alleles per locus was 9.0 (range: 6.3–11.9). The average expected heterozygosity per locus was 0.69, with a range of 0.61 to 0.78. These numbers exceeded those from the Red River basin by a wide margin. The Lower Mississippi basin samples showed more alleles per locus and greater predicted heterozygosities than samples from all other basins (Table 2). An exception was observed in the Minnesota River basin, where the number of alleles was comparable, but heterozygosity was slightly lower. In comparison to samples from the Upper Mississippi basin, those from the Minnesota River basin had a similar number of alleles but consistently lower heterozygosity. Lake Pepin (PEP) had a higher level of heterozygosity at all seven loci, with an average expected heterozygosity of 0.78, the highest among all sampled populations.

Table 2. Sample information and genetic diversity measures for 29 locations of yellow perch populations from Minnesota. For each location, values are given for the following: sample size (N), observed heterozygosity (H_o), expected heterozygosity (H_e), and allelic richness standardized to a sample of 29 (A_r).

Sample	ID	N	H_o	H_e	A_r
BAS	1	27	0.59	0.63	4.7
WIT	11	14	0.69	0.64	5.2
LAX	18	35	0.69	0.69	5.6
TNB	20	25	0.41	0.40	3.2
WPL	17	15	0.67	0.58	4.1
PEP	25	29	0.78	0.78	8.5
GOR	3	21	0.65	0.68	6.9
VON	22	27	0.59	0.66	5.4
MIN	5	27	0.64	0.61	6.0
MOS	16	26	0.64	0.66	6.3
PEL	23	27	0.61	0.64	5.9
BSN	19	29	0.66	0.66	6.8
TYS	24	16	0.56	0.56	4.0
LOW	9	23	0.62	0.62	4.9
KAB	29	16	0.69	0.67	5.7
RNY	8	22	0.63	0.63	5.2
PFE	7	24	0.62	0.63	4.5
BRS	15	27	0.57	0.52	3.9
LEE	21	27	0.63	0.63	5.5
ROK	27	21	0.63	0.65	5.7
ROY	13	21	0.58	0.57	4.9
CRS	10	23	0.66	0.66	6.0
SP	14	26	0.64	0.61	4.8
BS	2	21	0.64	0.67	6.1
LLS	4	14	0.68	0.69	6.7
MAY	26	21	0.69	0.68	6.4
MTP	6	15	0.63	0.62	6.1
CRY	12	28	0.67	0.69	5.7
WAC	28	25	0.69	0.66	6.9

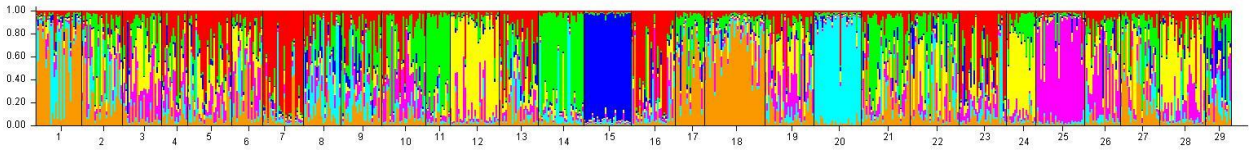

Figure 2A. The individual admixture proportions for 29 distinct populations, generated from a Bayesian clustering analysis without any prior information on sample locations. Each vertical line represents a single individual, and the different colored segments within each line indicate the estimated proportion of that individual's genome assigned to each of the inferred ancestral genetic clusters. The analysis utilized data from seven loci.

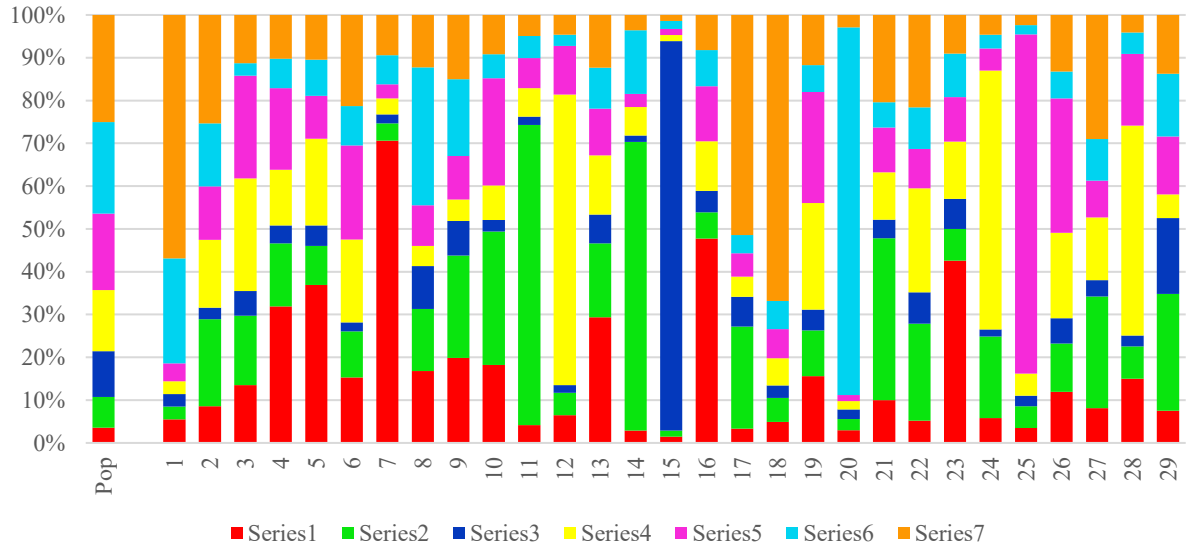

Figure 2B. Illustrates the estimated ancestral proportions for the 29 distinct populations derived from a Bayesian clustering analysis performed without prior location information. Each bar represents a single population, and the colored segments within each bar denote the average proportion of each of the seven inferred ancestral clusters contributing to that population's genetic makeup. The analysis was based solely on genetic data from seven loci, aiming to identify underlying genetic clusters among these distinct populations.

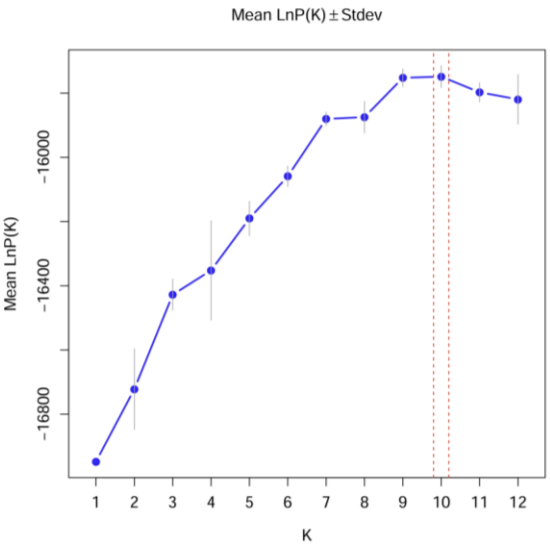

Figure 2C. The mean log-likelihood LnP(K) (± standard deviation) as a function of K, the number of inferred genetic clusters. The analysis was performed using a Bayesian clustering approach in STRUCTURE without a location prior. The line plateaus at 7 showing a plausible K value in accordance with Pritchard et al. (2010).

Only 12 of the alleles in the samples from the 29 samples were specific to a single population. In the Lake Pepin (PEP) sample, *MPf-6* had the greatest frequency of a particular allele at 80.0%. With frequencies ranging from 0.03 to 0.80, the Lake Pepin sample had the most distinct alleles (63 in total). The sample from Lake Pepin contained numerous rare or high-frequency alleles not found all throughout Minnesota. With the exception of *MPf-4* (the locus with the least variation), Pfeiffer Lake (PFE) had alleles at frequencies of 0.16 that were completely absent or only appeared at frequency of 0.01 or less in any sample from the Minnesota River basins. Every basin sample possessed at least one of these common 198 bp alleles.

After sequential Bonferroni correction for multiple tests ($\alpha = 0.05$, $k = 202$ [7 loci × 29 samples]), all loci in all samples complied with Hardy-Weinberg predictions. Eleven percent of the individual tests yielded statistically significant results ($P < 0.05$), and four remained significant after applying Bonferroni correction for multiple comparisons.

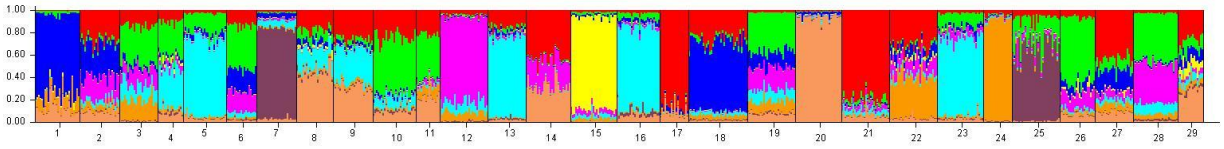

Figure 3A. Individual admixture proportions for the 29 populations (labeled 1-29) inferred using a Bayesian clustering approach in STRUCTURE with a location prior. Each vertical line within a population block represents an individual, and the colored segments indicate the proportion of that individual's genome assigned to each of the inferred genetic clusters. Distinct colors represent different genetic clusters. The analysis was conducted using nine microsatellite loci.

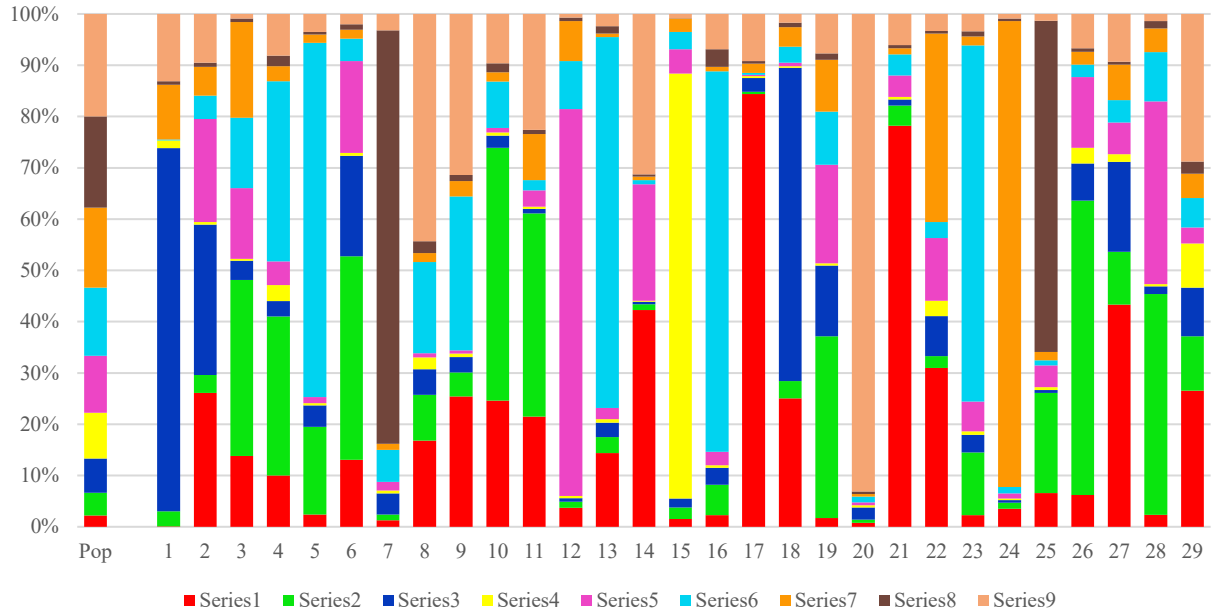

Figure 3B. Each bar represents the average profile for a single population with the colored segments within each bar indicate the average proportion contributed by each of the nine inferred genetic clusters.

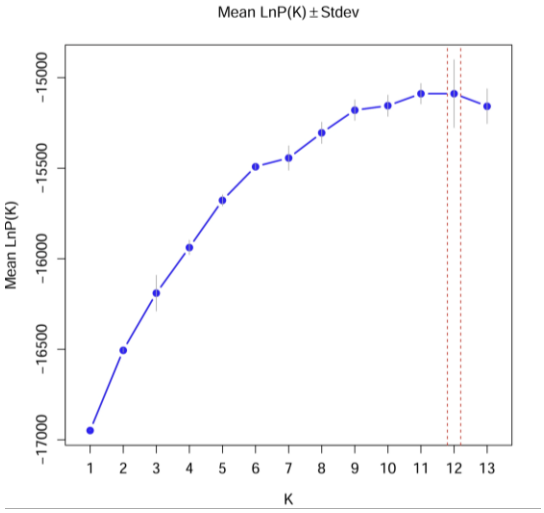

Figure 3C. The mean log-likelihood LnP(K) (± standard deviation) as a function of K, the number of inferred genetic clusters with location prior. The analysis was performed using a Bayesian clustering approach in STRUCTURE. The line plateaus at 9 showing a plausible K value in accordance with Pritchard et al. (2010).

None of the significant deviations originated from the same location or population, and no consistent pattern was observed. There is no evidence that any specific locus or population is systematically out of Hardy Weinberg equilibrium and all data was retained.

Genetic Population Structure. — Populations exhibit diverse ancestral compositions with and without incorporating prior information about the geographic locations of the samples (Figure 2A, 2B, 2C, 3A, 3B, 3C). A handful of populations contain dominant ancestral or population specific clusters (e.g., 15 (BRS), 20 (TNB), and 25 (PEP)), while the majority of the populations contain relatively weak differential structure that was too weak for the amount of markers used to distinguish. No major similarities within watershed populations are observed.

The construction of the tree indicates how similar two groups of samples are genetically (Figure 4). Little genetic separation existed between the Rainy River basin populations. On the tree diagram, the locations within the group were close together (Figure 4).

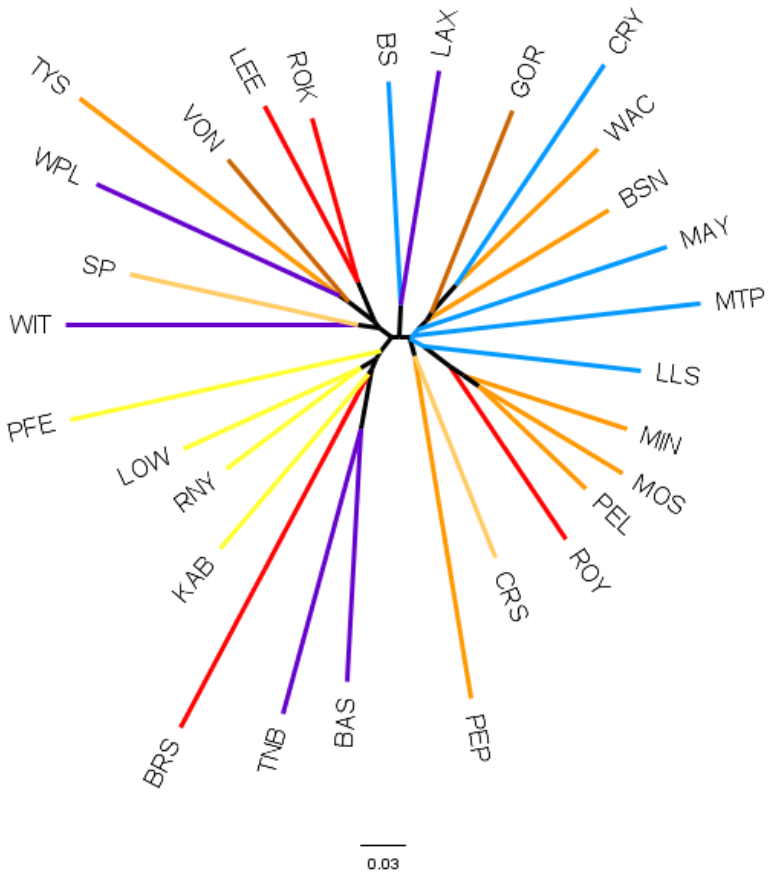

Figure 4. The genetic relationships among the 29 populations, with each line representing a distinct population. The colors of the lines correspond to the major basins from which the populations were sampled, highlighting genetic clustering and potential migratory pathways or divergence patterns associated with geographic origin. The length and angular separation of the lines represent genetic distance, indicating the degree of relatedness and evolutionary divergence between populations.

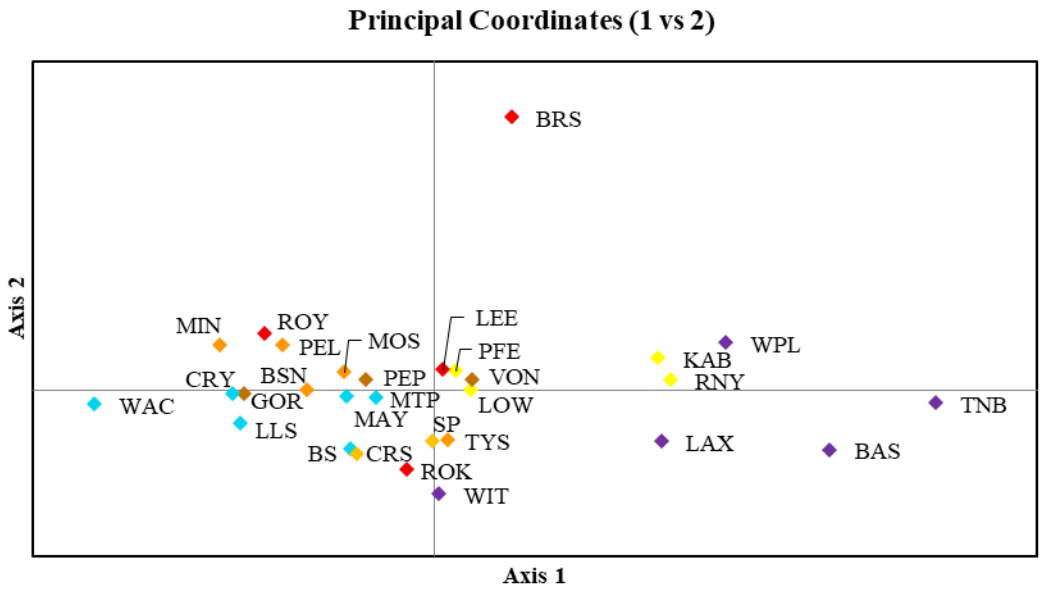

Figure 5. A Principal Coordinates Analysis (PCoA) plot showing the genetic relationships among the 29 populations. The plot is based on the first two principal coordinate axes (Axis 1 vs Axis 2), which explain the largest proportion of the total genetic variation. Each point represents a distinct population, labeled by its acronym and colored according to its major basin of origin. The proximity of points indicates genetic similarity, with more distant points suggesting greater genetic differentiation.

However, the samples from Rainy River basin were distinct from those from the Minnesota River basin. The genetic distances between pairs from Rainy River basin and Minnesota River basin (e.g, RNY-MIN = 0.042, RNY-MOS = 0.047) were comparable to those between pairs from Rainy River basin and Great Lakes locales from various drainages (e.g, RNY-WIT = 0.059, LOW-WIT = 0.079). The Rainy River basin sampling locations were grouped on a different branch from the Minnesota River areas on the tree diagram (Figure 4). All samples, including those taken inside and outside of each basin, were divided into two main groups (Figure 4).

The PCoA plot combined with the FST values (not shown) suggests that there are four distinct main genetic groups (Figure 5). One branch includes the large, dense cluster spanning left to central part below Axis 2, which includes samples from the Central/Southern Minnesota and Upper/Lower Mississippi basin locations. These populations exhibit low FST values among themselves representing a major contiguous genetic lineage. The second group observed is the Red River basin which are genetically highly divergent from other populations, with FST values with other basins ranging up to 0.096 to 0.106. The third and fourth groups include the Rainy River basin cluster and the Great Lakes basin cluster which are both tightly clustered and contain low FST values within their own groups.

IV. Discussion

The present study aimed to characterize the intra- and inter-population genetic variation of yellow perch across major hydrologic basins in Minnesota using highly polymorphic microsatellite DNA markers. Our findings reveal significant genetic structuring among yellow perch populations within Minnesota, consistent with the dynamic nature of population structure observed in many species (O'Connell and Wright 1997). The observed differences in allelic richness and heterozygosity among basins, particularly the higher genetic diversity in the Lower Mississippi basin and the unique genetic signature of Lake Pepin (PEP), underscore the importance of regional-scale genetic assessments for effective fisheries management. These insights are crucial for informing decisions related to stocking, translocation, and habitat restoration, ensuring the preservation of locally adapted gene pools and enhancing long-term population resilience against environmental changes (Arnqvist and Nilsson 2000; Takahashi et al 2018).

The substantial population structure identified in Minnesota yellow perch aligns with previous research on *P. flavescens* across its broader range. Miller (2003) reported genetic distinctiveness between spawning groups in Green Bay and Lake Michigan, while Kapuscinski and Miller (2000) found significant allelic differentiation among populations. More recently, Grzybowski et al. (2010) revealed even greater levels of genetic differentiation across Midwest and East Coast populations. Our study extends these findings by demonstrating distinct genetic groups within Minnesota's hydrologic basins, such as a couple of the highly divergent populations within the Red River basin group and tightly clustered Rainy River and Great Lakes basin groups. The relatively weak differentiation observed in some populations, despite the use of highly polymorphic microsatellites (Marina 2020), suggests potential gene flow or historical connectivity that warrants further investigation.

The identified genetic groupings and varying levels of heterozygosity have direct implications for the conservation and management of yellow perch. The presence of distinct genetic lineages, particularly the highly divergent Red River basin populations, suggests these groups may represent unique evolutionary units requiring specific management considerations to prevent genetic homogenization. Conversely, the contiguous genetic lineage observed across Central/Southern Minnesota and Upper/Lower Mississippi basin locations implies a greater degree of connectivity or shared ancestry, which could influence decisions regarding regional stocking programs. Understanding these genetic patterns can help managers avoid practices that might inadvertently dilute local adaptations or reduce overall genetic diversity, thereby supporting the long-term viability of yellow perch populations (Leary and Booke 1982).

While this study provides a foundational understanding of yellow perch genetic structure in Minnesota, certain limitations and avenues for future research exist. The use of seven microsatellite loci, while informative, may not fully resolve all subtle genetic differentiations, particularly in populations exhibiting weak structure. Future studies could benefit from an increased number of highly polymorphic markers, such as single nucleotide polymorphisms (SNPs), to provide higher resolution genetic insights (Yin et al. 2025). Additionally, incorporating environmental variables and landscape features (e.g., riverine connectivity, dam presence, historical stocking records) into spatial genetic analyses could further elucidate the factors driving the observed population structure. Continued genetic monitoring, particularly in geographically underrepresented areas, will be essential to track changes in genetic diversity and connectivity in response to ongoing environmental and anthropogenic pressures.

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