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A Genetic Evaluation of the Impacts of Dams on Sauger Movement Patterns in the Arkansas River

Emily R. Jonagan
Arkansas Tech University

A GENETIC EVALUATION OF THE IMPACTS OF DAMS ON SAUGER *SANDER CANADENSIS* MOVEMENT PATTERNS IN THE ARKANSAS RIVER

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Emily Jonagan

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Thesis Approval

This thesis, "A genetic evaluation of the impacts of dams on Sauger *Sander canadensis* movement patterns in the Arkansas River" by Emily Jonagan, is approved by:

Thesis Chair:

_____

John Jackson, Ph.D.
Interim Dean, College of Natural and Health and Sciences
Professor of Fisheries Science

Thesis Committee:

_____

Susan Colvin, Ph.D.
Assistant Professor of Fisheries Science

_____

Nicholas Feltz
District 10 Fisheries Management Biologist, Arkansas Game and Fish Commission

_____

Tsunemi Yamashita, Ph.D.
Professor of Biology

Program Director:

_____

Tom Nupp, Ph.D.
Interim Department Head of Biological Sciences; Fisheries and Wildlife Science Program Director; Professor of Wildlife Science

Graduate College Dean:

_____

Sarah Gordon, Ph.D.
Interim Dean of Graduate College

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Abstract

Decreased habitat connectivity as a result of damming can lead to genetic isolation in fish communities, especially in highly migratory species. Sauger *Sander canadensis* is a migratory freshwater species native to the Arkansas River. Sauger are highly sought after by anglers during their annual spawning migration in late winter. In order to investigate the impacts Arkansas River dams on Sauger populations, fin clips were collected in the winters of 2019, 2020, and 2021 below eight dams in the McClellan-Kerr Arkansas River Navigational System (MKARNS). Fin clips were also collected from two reservoirs in Kansas to serve as distinct reference populations. DNA samples were processed and genotyped using nine microsatellite loci. Genetic differentiation (F_{ST}), allelic richness (A_R), and heterozygosity were evaluated to determine differences among and between populations. It was found that there was moderate genetic differentiation (F_{ST}=0.06) between Pools 9 and 15 and between Pools 10 and 15 of the Arkansas River across five out of the nine loci. There were also signs of moderate differentiation between Pools 9 and 10 (F_{ST}=0.05). These results indicate that, despite the relatively recent construction of MKARNS, genetic differences are detectable in Sauger in some pools of the Arkansas River.

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I. Introduction

Human-induced habitat destruction has considerably altered the function of freshwater ecosystems. It is estimated that ~50% of the world's river volume is altered by human controlled flow regulation or fragmentation (Grill et al. 2015; Barbarossa et al. 2020). That percentage is expected to increase to 93% with the planned construction of ~3,700 major hydroelectric dams (Barbarossa et al. 2020). Habitat fragmentation due to barriers like dams has altered flow dynamics, nutrient cycling, sedimentation, water quality and overall ecosystem function (Seibert et al. 2018). Dams present a significant obstacle to fish passage especially for highly migratory species. Fish movement in a riverscape is vital for access to rearing and spawning habitats and spatially variable food resources (Baumgartner et al. 2014). There has been evidence to suggest that even smaller barriers such as under-road drainage culverts can limit passage during certain times of the year for many stream fishes (Wang et al. 2017; Briggs and Galarowicz 2018; Jones and Hale 2020). Structures such as mechanical lifts and gates and many other engineered designs have been implemented to assist fish with moving past dams. However, many of these structures do not fully resolve the issues caused by barriers and some are only effective for species that are strong enough swimmers to maneuver through passage mechanisms (Bunt et al. 2012).

One of the long-term consequences of fragmentation in fish communities is a loss of genetic diversity. This ultimately results in decreased fitness and possible extinction in the future without major conservation action. It was found that genetic diversity of White Spotted Char *Salvelinus leucomaenis* was consistently lower in populations above a dam

when compared to below dam sites in three fragmented river basins in Japan (Yamamoto et al. 2009). A theoretical study on river damming and its impacts on White Sturgeon *Acipenser transmontanus* found similar results in that genetic diversity and population sizes decreased as the number of dams increased (Jager et al. 2001). An Australian study recommended urgent assisted gene flow to help the Macquarie Perch *Macquaria australasica* recover in areas that have been significantly fragmented due to recent droughts. (Pavlova et al. 2017)

Time since barrier construction is important to consider when evaluating genetic changes. A recent study evaluated genetic impacts of a 104-year-old dam in Wisconsin on six highly migratory species. They found that the dam has not had significant genetic effects, but they hypothesized that the time period since construction of the dam may be too short to detect significant changes in genetic structure. Based on fish passage simulations using future migration rate estimates, better fish passage designs were recommended in this scenario to avoid significant genetic impacts in the future (Ruzich et al. 2019). Analysis of eight microsatellite loci in Bull Trout *Salvelinus confluentus* did find significant genetic variations among populations sampled above and below a dam built in 1952, indicating that genetic differentiation can be seen in shorter time frames (Neraas and Spruell 2001). These findings suggest that genetic response to habitat fragmentation may be species and system specific. Genetic monitoring of potentially fragmented populations can help determine an appropriate and timely response from conservation agencies.

My study focused on migratory Sauger *Sander canadensis* and investigated how the lock and dam system on the Arkansas River may influence their population genetics.

Sauger is a species of freshwater game fish distributed throughout North American rivers and reservoirs (Robinson and Buchanan 1992; Pegg et al. 1997). Sauger are in the Percidae family, which includes darters, freshwater perches, and zander, though they are a much larger species and more migratory species than many other members (Sloss et al. 2004). They are characterized by a long, streamlined body, with dark dusky saddles that extend down their sides. Average adult Sauger will reach 457 mm in total length and weigh about 1 kg. Sauger prefer deep, turbid waters in rivers and shallow waters in lakes (Bozek et al. 2011). They generally select habitats with strong currents along riprap banks or at the ends of rock dikes (Robinson and Buchanan 1992).

Sauger spawning season begins in late spring and stretches into midsummer, and they begin to move to spawning locations in late winter (Bozek et al. 2011). Some adults will travel several hundred kilometers to reach spawning grounds, (Scott and Crossman 1973; Collette et al. 1977; Bellgraph et al. 2008; Kuhn et al. 2008; Bozek et al. 2011). Optimal water temperature for spawning is approximately 7.7ºC. The spawn are broadcast in gravel and cobble substrates. Eggs incubate for 9 days when water temperature is at 12.8ºC, and 21 days at 8.7ºC. Sauger reach sexual maturity between 2 to 4 years old and have an average lifespan of 7 years. Longevity increases in the northernmost areas of their range (Bozek et al. 2011). Sauger are native to many rivers in Arkansas including the White River, Strawberry River, St. Francis River, Mississippi River, Saline River, Eleven-Point River, and the Arkansas River (Robison and Buchanan 1992).

The Arkansas River is the sixth longest river in the United States, spanning 2,364 km from its source in the Colorado Rocky Mountains to its mouth in the Mississippi

River in southeast Arkansas. It ranks ninth in drainage area with a total drainage area of 259,100 km^2 (Kammerer 1990). The 716 km stretch of river from Catoosa, Oklahoma to the Mississippi-Arkansas River confluence has been significantly altered to create a navigation channel for barges transporting goods up and down river. Riverbanks were dredged to create a minimum nine-foot-deep channel that allowed for barges to haul goods up and down river. This navigation system, completed in 1970, is known as the McClellan-Kerr Arkansas River Navigational System (MKARNS). The MKARNS consists of a series of 18 locks and dams operated by the United States Army Corp of Engineers (USACE) (Figure 1). USACE operators monitor barge traffic, water levels, and hydroelectric power stations at several locations. There are currently no fish passage structures or plans for passage structure on the MKARNS. Movement studies on the American Paddlefish *Polyodon spathula* and the American Eel *Anguilla rostrata* have shown that some movement is possible through locks and over smaller barriers in Arkansas River tributaries. American Eels have even been known to crawl on land to pass smaller dams (Balch 2019) and Paddlefish were recorded traveling through three dams in the Arkansas River basin in Oklahoma (Long et al. 2017). A river corridor project was proposed in 2007 that would add low-head dams to benefit migratory fish in the Arkansas River near Tulsa, just before the river flows into the navigation system. The process has been temporarily delayed due to a lack of funding and public support. Pre-construction and engineering designs are still in progress, but the completion of the project is still highly dependent on the distribution of federal funds (Tulsa County, 2021).

The construction of dams throughout river systems in North America has raised concerns about the ability for movement of migratory fish like Sauger. Sauger

populations have declined in abundance in many systems throughout their native range (Bozek et al. 2011b; Pegg et al. 1997; Bellgraph et al. 2008). Declines have been attributed to lack of connectivity to spawning and rearing habitat, increased exploitation, and hybridization with Walleye (Maceina et al. 1996; Pegg et al. 1997; Amadio et al. 2005). More recently, Leonard et al. 2019 investigated the impacts of dams on both movement and exploitation of Sauger in Pools 9 and 10 of the Arkansas River. In addition to acting as an obstacle to migration, dams have led to an increase in artificial aggregations of Sauger in the turbid waters below spillways during spawning season. These aggregations may face higher angler pressure (Maceina et al. 1996; Pegg et al. 1997). Leonard et al. 2019 found through tracking tagged fish via acoustic telemetry, that 78% of Sauger stayed in the pool of the river that they were tagged in, suggesting that the dams could be an impediment to movement. However, 22% of tagged fish were still able to navigate out of pools, including one fish that traveled through two locks during the study period.

Hybridization is a possible concern for Sauger because they can breed with their very close relative the Walleye *Sander vitreus.* The resulting fertile offspring known as Saugeye can backcross with either parent species (Billington and Heidinger 1996). Walleye habitat requirements are very similar to Saugers', and their ranges overlap in the northern portion of Arkansas. The major distinctions between the two are that Walleye tend to prefer colder water (19.6 ºC as opposed to 22.5 ºC for Sauger), spawn earlier, and generally will grow larger as adults (Bozek 2011b). The introduction of Walleye in areas where they are not native has caused a disruption in Sauger populations leading to an increase in hybrids and a subsequent decrease in Sauger (White et. al 2005).

Hybridization can also have implications for hatchery operations that are now required to screen for Saugeye in their Walleye and Sauger brood stock (Billington et. al 1996).

Little is known about population genetics of Sauger in the Arkansas River since completion of the MKARNS. Phylogenetic assessments have revealed that both Sauger and Walleye are native to Arkansas (Haponski and Stepien 2013). However, Walleye are only native to northern Arkansas and have been introduced in southwest Arkansas (see Figure 2). Introduction of Walleye in some areas and the construction of the MKARNS dams have generated interest in conducting a genetic evaluation of Sauger in the Arkansas River. Genetic evaluation is listed as a current research need in the most recent Arkansas Game and Fish Commission (AGFC) Walleye, Sauger, and Saugeye Management Plan (Adams et al. 2017). A genetic investigation will provide more information regarding genetic diversity and migratory patterns of Sauger in the Arkansas River that will be valuable for continued conservation and management of this species. My objectives in this study were to (1) evaluate genetic diversity and structure of Sauger in the Arkansas River and (2) use genetic measures to evaluate if dams are barriers to Sauger movement in the Arkansas River. I used microsatellite markers to determine the structure and genetic diversity of the populations sampled across nine different loci. I predicted that genetic differentiation and isolation would increase as distance between sampling sites and the number of barriers increased. I also predicted that populations in adjacent pools would be more similar and have a low level of genetic differentiation in comparison to the non-adjacent pools.

II. Methodology

Study Area

Sauger were sampled from Pools 1, 4, 7, 8, 9, 10, 12 and 15 of the Arkansas River (Figure 3). These pools are all created by locks and dams in the MKARNS. Pool 1 is the first pool of the system, starting near the confluence of the Mississippi and Arkansas Rivers near Dumas, AR. Pool 4 begins at the Emmett Sanders Lock and Dam in Pine Bluff, AR and ends at Lock and Dam 5 near England, AR. The next five pools listed are consecutive navigational pools. Pool 7 begins at the Murray Lock and Dam north of Little Rock, AR and ends at the Toad Suck Ferry Dam in Conway, AR. Pool 8 extends from Conway to the Arthur V. Ormond Lock and Dam in Morrilton, AR. Pool 9 begins in Morrilton and ends at the Dardanelle Lock and Dam. Lake Dardanelle is commonly recognized as Pool 10, but it is split into two pools, encompassing Pool 11 as well. I will refer to it as Pool 10 going forward. Pool 12 starts at the Ozark-Jeta Taylor Lock and Dam, west of Lake Dardanelle in Ozark, AR and extends to the James W. Trimble Lock and Dam near Fort Smith, AR. Samples from Pool 15 were collected downstream from the Webbers Falls Dam near Muskogee, OK by the Oklahoma Department of Wildlife Conservation (ODWC). I will refer to Pools 1, 4, 7, 8, 9, 10, 12 as the Arkansas or AR Pools. Samples from Pools 1, 4, and 15 were collected in order to have data from sections of the river that are further removed from the four consecutive pools. The Kansas Department of Wildlife, Parks, and Tourism (KDWPT) collected samples from Banner Creek and Perry Lake reservoirs to serve as representatives of what should be more genetically distinct populations and habitats with fewer barriers to movement (Figure 2).

Sample Collection

Experimental monofilament gillnets were used in the AR Pools. Nets were 45m long, 2.5 m tall and consisted of three 15 m sections, each with a different mesh size (51mm, 64mm, and 76mm) in order to avoid size bias. Gillnetting took place in the months of November through February of 2019, 2020, and 2021. In late fall and winter there is increased activity below dams as Sauger begin to migrate upstream to spawn (Maceina et al. 1996; Pegg et al. 1997). Nets were set approximately 500 meters from dam spillways when flow generated from the dam was low. When flow from the dam was greater than 1133 m^3/s, nets were set perpendicular to the lock wall or behind navigational rock walls downstream from the dam known as wing dikes. Netting occurred in the evenings between 1600 and 2300 when Sauger are most active and angler activity is decreased (Cobb 1960). Most nets were fished for two hours each except for two nets that needed to be left out overnight in Pool 10 due to the lock opening and releasing an unsafe amount of water that would have capsized the sampling boat. The KDWPT used paneled gill nets with eight different mesh sizes (9mm, 25mm, 32mm, 38mm, 44mm, 51mm, 57mm, and 64mm). All KDWPT nets were set overnight. The ODWC used boat electrofishing in Pool 15. Electrofishing was also used in Pool 10 to collect additional fish when the flow coming from the Dardanelle Lock and Dam was too high to safely set nets. Pulsed direct current (DC) was used for all electrofishing, operating at a range of 60-120 pulses per second.

All species captured in AR pools were recorded. Fin clips were taken in the field from all Sauger using surgical scissors washed with 90% ethanol between samples. Weight in grams and total length in millimeters were taken for Sauger at all sampling

locations. Measuring boards were cleaned and sanitized using ethanol before and after each Sauger fin clip was taken to prevent DNA cross contamination among samples. Fin clips were placed in 90% ethanol in 1.7 μL microcentrifuge tubes and stored in the ATU lab -20ºC freezer until genomic DNA extraction. All other by-catch species were identified in the AR pools and this data was used to examine composition of catch and compare richness based on location using a Kruskal-Wallis one-way analysis of variance. The ODWC and KDWPT did not report other species captured in their sampling. Catch per unit effort (CPUE) for Sauger, measured as netting hours per fish, was calculated for all gillnetting sampling in order to compare abundance across sample sites. Median length and weight were used to determine differences in Sauger size using a Kruskal-Wallis one-way analysis of variance with Dunn's post hoc test using a Bonferroni correction. All test results were considered significant using an alpha level of 0.05.

DNA Extraction and Sample Processing

Genomic DNA was extracted from fin clips using Qiagen DNeasy Blood and Tissue Kits (QIAGEN, Hilden, Germany) following the manufacturer's protocol and eluted in nuclease-free water (MilliporeSigma, St. Louis, MO). DNA samples were stored at -20ºC for further use. Samples were quantified using a Qubit 4 Fluorometer (Invitrogen, Waltham, MA) and normalized to ~10 ng/μL. A set of ten microsatellite loci (Table 1) previously developed for use with Sauger and Walleye was selected to use on all samples (Ruzich et al. 2019; Hammen 2009, Wirth 1999). The initial polymerase chain reaction (PCR) used was denaturation at 95ºC for 10 minutes, 94ºC for 50 seconds, annealing at 50ºC for 50 seconds, extension at 72ºC for 50 seconds, repeating the previous three steps for 30 cycles, and 72ºC for a 10-minute final extension. This PCR

protocol resulted in large amounts of artifact bands that were not in the size range of the desired loci due to non-specific annealing of primers. A touchdown PCR was ultimately used that begins with a more stringent annealing temperature of 60ºC and decreases with each cycle. Specifically, the parameters below were used as described in (Schanke 2012):15 min at 95°C (1 cycle), 45 s at 94°C, 45 s at 60°C, and 45 s at 72°C (repeat for 20 cycles decreasing annealing temperature by 0.8°C/ cycle), 45 s at 94°C, 45 s at 55°C, and 45 s at 72°C (repeat for 10 cycles), 10 min at 72°C (final extension).

One locus, Svi 17, was not used because it showed non-specific annealing, leaving a total of nine loci for further analysis. All PCR cycles were run on either an Eppendorf 5431 (Eppendorf, Hamburg, Germany) or a Bio-Rad T100 (Bio-Rad, Hercules, CA) thermal cycler. The reagent mixture used consisted of 14.5 µL of Bullseye 2X Taq DNA Polymerase Master Mix (MidSci, St. Louis, MO), 2 µL of 10 µM forward and reverse primer, 0.25 µL of 30% BSA, 0.25 µL DMSO, 0.25 µL of formide, and 5 µL of DNA for total reaction volume of 24.25 µL. Primers were tagged with fluorescent dyes (6-FAM, NED, PET, or VIC, Applied Biosystems, Waltham, MA) on the 5’ (forward) end so that allele bands produced could be properly visualized after fragment analysis. Primers were multiplexed together, two per sample well, based on dye color, fragment size, and approximate annealing temperatures. Primers with the same dye color or dye colors with similar wavelengths were not mixed to avoid confusion in allele calls and to avoid interference that can be caused when using similar wavelength dyes in the same sample. PCR products were verified using gel electrophoresis. Any samples with non-specific bands or not in the expected allele size range were re-run. PCR amplicons were diluted 1:10 then sent to the DNA Core Facility at the University of Missouri for analysis

using capillary electrophoresis on an ABI 3730xl DNA Analyzer (Life Technologies, Carlsbad, California) to determine allele sizes of fluorescently tagged fragments. DNA Core staff added Genescan 600 LIZ to each sample. This size standard ranges from 20 base pairs (bp) to 600 bp and serves as a reference to more accurately genotype alleles.

It was determined that purification was required for the majority of the PCR products sent for fragment analysis due to sample contamination. This was done using Nanosep® centrifugal devices with Omega™ 30k membranes (Pall Corporation, Port Washington, NY). Products were loaded into individual Nanosep tubes, purified water was added to reach 500 µL, and tubes were centrifuged once at 5,000 x g for 10 minutes. Purification yielded 15-20 µL of product in the retentate cups. 2 µL of these filtered products was loaded into wells in 96-well plates and shipped again to the University of Missouri. Approximately 25% of PCR's were re-run to resolve the issue with contamination, confirm proper amplification, and reduce error in the PCR protocol. DNA analyzer outputs were reviewed using PeakScanner™ (Applied Biosystems, Waltham, MA) to manually score allele sizes for each sample. Manual genotyping was tested for scoring errors due to non-specific annealing, also called stuttering or stutter bands, using MICRO-CHECKER. This program also checks for the presence of null alleles and large dropout of alleles in a population by measuring excess homozygosity at each locus, assuming Hardy-Weinberg equilibrium (HWE) (van Oosterhout et al. 2004; Tomke 2020). Null alleles occur when one allele does not properly amplify at a locus and this gene is then labeled as a homozygote due to the presence of only one band or peak in the electropherogram. MICRO-CHECKER is programmed to recognize patterns of excess homozygosity that point to the presence of null alleles.

Initially, a restriction site-associated DNA (RAD or RADseq) method was used on all samples to investigate Sauger population genetics in the Arkansas River. RADseq protocols are generally associated with Next Generation Sequencing (NGS) methods that have become more popular in the last decade. NGS techniques are much more efficient and cost-effective and provide higher resolution population data (Behjati and Tarpey 2013). Specifically, a "3RAD library preparation" procedure developed by researchers at the University of Georgia (UGA) was used to tag each individual with unique adapter and primer combinations that are compatible with Illumina sequencing (Bayona-Vásquez et. al2019). The procedure was not successful in producing DNA fragments large enough to be sequenced and further analyzed. (See **Appendix A** for further description)

Measures of Genetic Diversity, Isolation, and Population Structure

GenAlEx was used to enter genotypes and estimate measures of genetic diversity that included allelic richness (A_R), number of alleles per locus (A), and observed (H_O) and expected (H_E) heterozygosity. GenePop v4.7 (Rousset 2008) was used to run Fisher's exact tests to check for deviations from Hardy-Weinberg Equilibrium (HWE) and to test for non-random association of alleles at all loci, known as linkage-disequilibrium (LD). A population is in HWE when mating is random and little selection or inbreeding is occurring. Both HWE and LD will determine allele association within these populations and show if there is a large amount of inbreeding, indicative of isolated populations. Default Markov Chain Monte Carlo (MCMC) parameters were used for both HWE and LD. These consisted of 1000 dememorizations, 100 batches, and 100,000 iterations per batch, per recommended default parameters (Porras-Hurtado et. al 2013). GenAlEx was used to identify the number of alleles that are only present in one population among other

populations known as private alleles (P_A) and the genetic differentiation between populations (F_{ST}) (White et al. 2021). F_{ST} values typically are evaluated based on three ranges: F_{ST} <0.05=little genetic difference, F_{ST} ≥ 0.05, ≤ 0.15=moderate genetic difference, F_{ST} >0.25=great genetic differentiation (Hartl and Clark 1997). An analysis of molecular variance (AMOVA) using 10,000 permutations was performed in GenAlEx as an additional method to assess distance and what factors contribute the most to genetic differentiation. AMOVAs can be sensitive to a small number of populations within a sample group, so 10,000 permutations were used to increase power of the analysis. *P*-values were considered significant at an alpha-level less than 0.05.

Population structure was evaluated using the Bayesian clustering program STRUCTURE (Pritchard 2000). The number of genetic clusters (K) was run from 1 to 10 for 10 iterations. A burn-in period of 100,000 was used and 100,000 MCMC replications were performed. The admixture model was used, and allele frequencies were assumed to be correlated to increase power. The LOCPRIOR option was also selected. LOCPRIOR incorporates sampling location information like the population of origin to inform the estimated number of population clusters. This method is very useful in situations where the population structure signal is weak, such as low sample sizes and or low number of loci. A final K was selected using the **Δ**K method described by Evanno et al. (2005). This method of determining K evaluates the rate of change in the log probability of data between successive K values. It is a more accurate method than the default STRUCTURE algorithm because it is better at detecting non-homogenous populations. The clusters were then visualized using STRUCTURE Harvester (Dent et al. 2012)

III. Results

Sample Collection

A total of 27 species were recorded across the seven gillnet sites in the AR pools (n=7; median=12). The minimum number of species captured was 7 in Pool 1 and a maximum of 19 in Pool 10 (Table 1). There were three species that were collected using electrofishing in Pool 10 that were not seen in gillnets. These were Redear Sunfish *Lepomis microlophus*, Bluegill *Lepomis macrochirus*, and River Redhorse *Moxostoma carinatum*. Sauger CPUE ranged from 0.44 in Pool 3 to 11.5 in Pool 12 (n=7; median=2). Electrofishing effort was excluded from CPUE because relatively few Sauger were captured using this gear type (three in Pool 10, 16 in Pool 15). Two hundred and twenty Sauger were sampled across all sites including Pool 15 in OK and the two KN reservoirs. Total length in Sauger ranged from 120 to 472mm (n=220; median=365mm) while total weight ranged from 44 to 1130g (n=220; median=469). Species richness and total length for Sauger were compared across the AR Pools using a Kruskal-Wallis one-way test of variance. There was no significant difference in species richness across the AR Pools (X^2=6, df=6, P=0.42). There were significant differences in Sauger total length across all sample locations (X^2=37.1, df=9, P<.001). A Dunn's post-hoc test with Bonferroni correction revealed that Pool 15 had a significantly larger median when compared against all other sites.

Sample Preparation and Microsatellite Scoring

Several steps were performed to verify DNA sample quality and microsatellite genotype scoring. DNA quality was visualized using gel electrophoresis in addition to

Qubit quantification. Ultimately, 32 individuals were removed due to poor quality and quantity (>5 ng/μL in concentration) of DNA resulting in 188 individuals for further analysis. One hundred and twenty of these 188 samples were used in PCR. Many samples were not producing distinguishable peaks in the expected size range in the electropherogram output from the ABI 3730xl. Nearly 25% of PCR products were not injected into gel lanes of the DNA analyzer due to high salt contamination, even after adding the Nanosep purification step. PCR was run on a set of samples excluding BSA, DMSO, and formide from the PCR mix to determine if these reagents were the source of contamination. There were little to no differences in DNA analyzer outputs in this set of samples and the contamination source was never determined. Fragment analysis results did show a pattern in which loci were producing peaks that could not be genotyped. Svi4, SviL9, Svi7, and Svi33 were excluded from analysis due to repeated failed runs and the five remaining loci were examined across 52 individuals from five different sample sites. These sites were Pool 8 (n=15), Pool 9 (n=5), Pool 10 (n=24), Pool 15 (n=6), and Banner Creek (n=3).

MICRO-CHECKER tests for null allele frequencies and scoring errors revealed that there was an excess in homozygosity at locus Svi26 for the Pool 10 population. This suggests that there may be null alleles at this locus due to lack of amplification during PCR. However, there were no signs of allele dropout or scoring errors due to stuttering across all loci for four populations. The three individuals from Banner Creek reservoir were removed due to a sample size that was too low to accurately run in MICRO-CHECKER, leaving Pools 8, 9, 10, and 15 for genetic diversity and population structure analysis. Null allele frequency averaged lower than 5% across all populations, which is

the commonly accepted threshold for analyses of genetic difference (Chapuis and Estoup 2007; Dąbrowski et al. 2015). Typically, loci with suspected null alleles would be removed from a dataset. However, considering the low null allele frequency at Svi 26 across other populations, it was kept in (Table 4)

Measures of Genetic Diversity, Isolation and Population Structure

The total number of alleles present in each population, or A, ranged from 12 (n=6) in Pool 15 to 22 in Pool 10 (n=24). All five loci across the four sites were 100% polymorphic, meaning that more than one allele could be detected in at least one of each locus for each population. The mean number of alleles per locus, known as allelic richness (A_R), across all sites was 3.40 (SD=0.29). All loci excluding Svi 26 ($P<0.001$) were in HWE ($P>0.05$) according to the Fishers exact test results across all populations. At the population level, Pool 10 was not in HWE due to the excessive homozygosity at locus Svi26. Locus Svi 6 was also not in HWE in Pool 10 (P=0.01). There was no significant non-random association of alleles across populations (LD) (Table 6). No private alleles were present among these four populations. Pairwise F_{ST} values ranged from 0.02 to 0.07. The pairs that showed moderate genetic variation were between Pools 9 and 10, Pools 10 and 15, and Pools 8 and 15. The AMOVA reported a global F_{ST} of 0.03, indicating overall low genetic differentiation. The AMOVA revealed that variation within individuals contributed to 97% of the overall molecular variance and 3% was due to variation among populations.

IV. Discussion

Overall genetic differentiation among Pools 8, 9, 10 and 15 was low considering the global F_{ST} of 0.03 from the AMOVA (Table 7) and variation was not associated with population groupings. This result is somewhat counterintuitive when working with a highly fragmented system like the MKARNS. As other studies have shown, some barriers may be too recent to reveal discernable genetic differences in fish populations. A Wisconsin study showed overall F_{ST} estimates for Sauger and five other migratory species were well below 0.01when evaluating the impacts of a dam built 104 years before the study took place (Ruzich et. al 2019). Researchers in southern California sampled 20 populations of Rainbow Trout *Oncorhynchus mykiss* in several drainage basins above and below dams that have been built within the last century. They found that any genetic variation between population pairs could not be attributed to dams, likely because of their recent construction. (Clemento et al. 2009). It is reasonable to assume that not enough time has passed since the completion of MKARNS in 1970 to dramatically influence overall population structure of Sauger in the Arkansas River. However, including Sauger captured in the remaining 14 navigational pools that were not evaluated in this study could further elucidate these patterns. Horreo et al. (2011) found that genetic differentiation in Brown Trout *Salmo trutta* sampled in four different river systems increased as the number of dams increased in the system. As populations become more fragmented, it becomes more difficult for local populations to maintain genetic diversity as population abundance decreases and inbreeding increases.

Moderate genetic differences were found in some of the pairwise population comparisons in my study. Pools 8 and 10 were moderately different when independently

compared to Pool 15 (F_{ST}=0.06 for both pairs). Genetic differentiation between Pools 9 and 10 was also considered moderate with an F_{ST} of 0.05. The differences between Pools 8 and 10 and Pool 15 may be related to distance considering the Weber Falls Dam in Oklahoma is approximately 250 km from the Ozark-Jeta Taylor Dam at the start of Pool 10 and 320 km the Arthur V. Ormond Dam at the start of Pool 8. Moderate genetic differentiation between Pools 9 and 10 signals that separation by the Dardanelle Dam may be influencing gene flow between these pools. Pool 10, Lake Dardanelle, is unique because it is technically split into two pools according to navigation charts but there is not a barrier that separates these pools. The larger size of this location and more lacustrine habitat may have contributed to genetic differences. However, genetic differences have been found between adjacent sites separated by a barrier in other studies. A study in Brazil found two morphometrically distinct populations of a native characid, one above a dam and one below the same dam (Esguícero and Arcifa 2010). A study on Rainbow Trout in the Columbia River found consistently lower genetic diversity in above-dam sites in comparison to below-dam sites in three different drainages (Winans et al. 2018). These results all indicate low migration rates across dams. Low interpool movement in some sections of the MKARNS may be starting to influence genetic variation as indicated by the moderate differentiation seen in this study.

Overall, genetic diversity was somewhat low across four sites. This could be a result of genetic isolation or an indicator of small sample sizes in the microsatellite analysis due to PCR complications. Specifically, the excessive homozygosity at locus Svi 26 in Pool 10 was likely due to the presence of null alleles. Manual genotyping using PeakScanner™ did reveal low error in scoring, but this method is not very robust in

addressing small sample sizes (Tomke 2020). An issue with using software to detect null alleles is that many of these programs cannot accurately determine the difference between true missing data due to null alleles and PCR failure or poor DNA quality. It would ultimately be best to use another program that attempts to address this distinction like ML-NullFreq in combination with MICRO-CHECKER and re-amplification of loci to reduce error (Kalinowski and Taper 2006). There could be a correlation between high homozygosity in Pool 10 and genetic isolation, but a larger sample size and multiple error-checking steps would help to discern this relationship.

Managers must consider if it is beneficial to increase habitat connectivity through facilitating passage or if it best to manage each population segment as a distinct group. Little research specifically assesses the application of passage structures for Sauger in the MKARNS. There is evidence to support that Sauger can swim through structures with relative ease at velocities lower than 97 cm/s, suggesting that there are designs of fish passage that could work for Sauger. Although they are typically found in turbid waters in rivers, this study on Sauger swimming ability determined that high turbulence and higher velocities deterred Sauger from attempting to pass the barrier in a lab setting (Dockery et al. 2017). Fish migration simulations could reveal how dams will impact highly migratory species like Sauger if passage structures are not implemented in the near future. Based on the data from my study, it is not yet conclusive when or if Sauger will need fish passage structures in the Arkansas River or if any navigational pools need individual management. Unfortunately, sample sizes were too low for STRUCTURE to accurately infer distinct population clusters. However, considering the relatively recent completion of the MKARNS and the moderate rate of movement between pools (22%)

observed in recent Sauger telemetry studies (Leonard 2019), it could be expected that the number of distinct population clusters may be lower than predicted.

In conclusion, it was determined that overall genetic differentiation was low for Sauger among four Arkansas River sites separated by dams. There were moderate pairwise differences between Pools 8 and 15, Pools 10 and 15, and Pools 9 and 10. These results provide a glimpse of what can be expected as far as genetic structure and diversity of Sauger in the Arkansas River. There are several avenues that should be taken going forward to expand upon my study. Extended telemetry studies into other pools of the Arkansas River could generate more data to compare to genetic methods. Additional telemetry surveys could also provide habitat use and location data in other pools of the river to increase efficiency in sampling techniques. The current method of sampling directly below dams during spawning season is highly influenced by seasonal timing and water levels and increases size bias. Success with other gear types like electrofishing could reduce time waiting for ideal water conditions. Expanding sampling into tributaries and other river systems in Arkansas where Sauger are found could provide interesting lineage data and possibly act as reference of genetic separation due to distance and more natural barriers.

Collection of Walleye is vital for a complete genetic evaluation of Sauger in this system. Fin clips could be provided by local hatcheries like the AGFC Andrew H. Hulsey State Fish Hatchery in Hot Springs, AR to reduce sampling effort. It is important to analyze hybridization in these populations to ensure that hybrid introgression is not correlated with significant decreases in abundance of Sauger or decreases in genetic diversity in Sauger in the Arkansas River. Completing a genetic evaluation of Sauger in

the Arkansas River is also directly dependent upon the success and optimization of lab techniques. PCR should be re-optimized adding in other suites of primers to produce a more reliable and informative set of loci for Sauger.

In general, molecular methods are shifting towards the genomic approach used in NGS techniques like RADseq (Daw et al. 2005). There are also methods in development that provide less markers than those generated by RADseq but are more than sufficient to answer population structure and genetic diversity questions. Genotyping-in-Thousands (GT-seq) is an NGS method that uses panels ranging from 50 to 500 single-nucleotide polymorphism (SNP) markers. Microsatellite markers typically range from 10 to 100 markers while RADseq can generate hundreds of thousands. GT-seq offers a middle-ground option that is cost effective and requires relatively simple library preparation (Campbell et al. 2015). GT-seq panels have been successfully developed for Walleye to identify genetic stock origin in 23 inland lakes across Wisconsin and Minnesota (Bootsma et al. 2020). Developing a GT-seq panel in addition to better optimization of microsatellite PCR may be the best way forward to determine further genetic structure of Sauger in Arkansas. Although the dataset for my study was limited, it provides important results to inform future studies on barriers to movement for Sauger and other migratory species. A continued extensive genetic investigation will not only reveal more about the relationships between habitat fragmentation and genetic isolation but can provide updated and detailed Sauger status reports within the Arkansas River system for managers and stakeholders.

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Appendix A. Adapterama 3RAD Library Preparation

Adapterama 3RAD is a genomic library preparation method developed by researchers at the University of Georgia (Bayona-Vásquez 2019). The protocol uses three restriction enzymes that are compatible with cut sites on genomes of most living organisms. I used Design 1 enzymes XbaI, EcoRI-HF, and NheI-HF. The third enzyme, NheI-HF in this case, is known as the dimer cutting enzyme that prevents the sheared DNA fragments from ligating back together after the enzyme digestion. The enzymes, CutSmart® Buffer (New England Biolabs, Ipswich, MA), and unique iTru adapter tags for identification of samples after sequencing, were added to 10μL of Sauger DNA, normalized to ~10ng/μL and aliquoted in 96-well plates. The digestion reactions were placed in the thermal cycler for one hour at 37ºC. Next, the ligation mix was added to the digested DNA mixture from the previous step. The mix included, T4 DNA Ligase, T4 DNA Ligase Buffer, and rATP. The ligation reactions were incubated in the thermal cycler at 22°C for 20 min., 37°C for 10 min., 22°C for 20 min., 37°C for 10 min., 80°C for 20 min., and then held at 10ºC until the next step. After the ligation process, 10μL of the ligated fragments from the previous step were added to the iTru PCR mix. The iTru PCR mix contained primers compatible for Illumina sequencing equipment that are designed to attach to opposite ends of the fragmented DNA. These primers anneal to the iTru adapter tags that were ligated to the restriction-digested DNA in the previous step. The diversity of these primers is robust enough so that they can serve as an additional tagging system for later identification of samples after the individuals are pooled together. The following PCR conditions were used: 98°C for 40 sec.; then, 12 cycles of:

98°C for 20 sec., 60°C for 30 sec., 72°C for 60 sec.; followed by 72°C for 5 min. Hold at 15°C.

Samples were pooled together based on similar concentrations verified through gel electrophoresis. The pooled samples were shipped to the University of Georgia Environmental Health and Science lab. Their team determined using a PippinPrep size selection instrument (Sage Science, Beverly, MA) that the fragments produced were far too small for Illumina sequencing. I was able to successfully digest the Sauger DNA with the restriction enzymes, but ligation of the iTru adapters to the restriction enzyme digested DNA before PCR was not successful. The iTru primers then had nothing to anneal to and the PCR cycles amplified primer and adapters instead of the genomic DNA. This issue is still unresolved, however, ATU undergraduate students have been working to further troubleshoot the process.

Appendix B. Tables

TABLE 1. Scientific names of all species captured in AR pools using gillnets and the percent of total catch that each species contributed. All fish were caught in November-February of 2019, 2020, and 2021.

	Percent of catch per site						
Species scientific name	Pool 1	Pool 4	Pool 7	Pool 8	Pool 9	Pool 10	Pool 12
Pomoxis nigromaculatis	0	0	0	0	0	0.67	0
Ictaurus furcatus	15.5	0	2.85	0.95	17.3	4.69	0
Lepomis macrochirus	0	0	0	0	0	10.7	0
Cyprinus carpio	0	0	0	0	0	0.33	0
Ictalurus punctatus	0	0	7.14	1.90	3.33	4.36	0
Aplodinotus grunniens	15.5	0	0	9.52	9.33	8.67	0
Dorosoma cepedianum	20.7	0	11.4	0.95	4.00	9.06	1.61
Carpiodes velifer	3.45	0	0	2.85	0	0	0
Micropterus salmoides	0	0	0.71	0	0	1.34	0
Lepomis megalotis	1.72	0	0	0	0	0.33	0
Lepisosteus osseus	8.62	46.5	0	0	12.0	2.68	1.61
Lepomis microlophus	0	0	0	0	0	0.67	0
Moxostoma carinatum	0	0	0	0	0	0.33	0
Carpiodes carpio	0	0	0	0.95	0	0	1.61
Sander canadensis	5.17	9.30	13.6	36.2	19.3	13.1	74.2
Moxostoma macrolepidotum	1.72	0	1.43	3.81	0	0	0
Lepisosteus platostumus	3.45	14.0	7.86	8.57	0.67	0.33	0

Scaphirhynchus platorynchus	0	0	0	0.95	0	0	0
Alosa chrysochloris	0	20.9	2.14	8.57	12.7	11.4	4.84
Micropterus dolomieu	0	0	0.71	0.95	0	0	0
Ictiobus bubalus	0	0	0.71	0	0	2.35	0
Micropterus punctulatus	0	2.32	4.29	4.76	3.33	1.678	0
Minytrema melanops	0	0	0.71	0	3	0.33	0
Morone saxatilis	0	0	0.71	4.76	0.67	3.69	3.22
Dorosoma petenense	0	0	0	0	0	2.35	0
Sander vitreus	0	0	0	0	0	0	1.61
Morone chrysops	1.72	4.65	41.4	0.95	11.3	14.4	8.06
Pomoxis annularis	1.72	0	0.71	0	0	1.01	0
Morone chrysops x *Morone saxatillis*	0	0	0.71	3.81	0	1.67	0
Morone mississippiensis	0	2.32	2.14	0.95	4	8.05	3.22

TABLE 2. Sauger microsatellite loci name, primer sequence, allele size range, and references.

Locus	Primer Sequence (5' to 3')	Allele size (bp)	Reference
Svi2	F:CAA CCA GAC CCA ATC CCT TG R:GGG CCG AGT ATA TCA GTT AAC	192-208	Hammen et al. 2009
Svi4	F:ACA AAT GCG GGC TGC TGT TC R:GAT CGC GGC ACA GAT GTA TTG	102-118	Hammen et al. 2009
Svi6	F:AGT CGA CAT ATT ATG TAG AGT GC R:GAT CAA CTG TGG AGG ATG AGC	136-173	Hammen et al. 2009
Svi7	F:GAA ACC TTA CAA AAG CCT GG R:TTA TCT GCA CTT CTA CAG GC	163-173	Hammen et al. 2009
SviL9	F:TAC TGT TCA CTT ATC TAT CC R:TGT ATG TGT GTG TGT TCA TGT	243-297	Hammen et al. 2009
Svi17	F:GCG CAC TCT CGC ATA GGC CCT G R:CGT TAA AGT CCT TGG AAA CC	101-113	Hammen et al. 2009
Svi20	F:CAA GTG CGC AAT GGT GCA TTA C R:GAA TGA AGA AAT GCA CCC ATG C	144-193	Hammen et al. 2009
Svi26	F:CGA ACT ACT TAT CTT CTG GC R:GTA AGT GTG AAT CAG CCA GAC	156-189	Hammen et al. 2009
Svi33	F:CAG GAC TGC TGT GTA TAG ACT TG R:GAT ATA GCT TTC TGC TGG GGT C	90-102	Hammen et al. 2009
SviL5	F: CAT ATCC TACT GTA GTA TGG R: CAA ATC CCA TTT ACA CCC AC	188-224	Wirth et al. 1999

TABLE 3. A summary of genomic DNA extraction success and microsatellite PCR success.

Number of individuals with DNA extracted	Number of individuals used in microsatellite PCR	Number of individuals that generated results for five loci
202	121*	49

*79 individuals were not used in PCR due to either poor DNA quality (17 individuals) or time and resource constraints (62 individuals)

TABLE 4. van Oosterhoot null allele frequencies estimated by MICRO-CHECKER for 5 loci across sample sites. Null alleles occur when one allele does not properly amplify at a locus and this gene is then incorrectly labeled as a homozygote due to the presence of only one band or peak in the electropherogram. Values in bold indicate significant presence of null alleles.

	Locus				
Sample site	Svi2	SviL5	Svi6	Svi20	Svi26
Pool 8	-0.15	-0.15	-0.08	-0.01	0.00
Pool 9	-0.37	-0.55	-0.15	-0.37	-0.11
Pool 10	-0.09	0.01	-0.07	0.11	**0.17**
Pool 15	-0.42	-1.00	-0.56	-0.18	0.05

TABLE 5. Summary of genetic diversity measures for Sauger in four Arkansas River pools across 5 microsatellite loci. N=sample size, A=total number of alleles per sample group, P_A=number of private alleles, H_o=observed heterozygosity, H_e=expected heterozygosity, F_{IS}=inbreeding coefficient. Values in bold indicate moderate levels of inbreeding.

Sample site	N	A	A_R	P_A	H_o	H_e	F_{IS}
Pool 8	14	21	4.2	0	0.66	0.60	-0.10
Pool 9	5	13	2.6	0	0.72	0.54	-0.38
Pool 10	24	22	4.4	0	0.53	0.58	**0.06**
Pool 15	6	12	2.4	0	0.77	0.519	-0.49

TABLE 6. Linkage Disequilibrium results for each locus combination across four Arkansas River pools. Values calculated in GenePop v4.7 using Fisher's exact tests. *P*-values greater than 0.05 indicate no significant Linkage Disequilibrium between locus pairs.

Locus pair	X^2	*df*	*p*-value
Svi2-SviL5	5.01	6	0.54
Svi2-Svi6	1.77	8	0.99
SviL5-Svi6	1.16	6	0.99
Svi2-Svi20	5.27	8	0.73
SviL5-Svi20	1.14	6	0.98
Svi6-Svi20	5.76	8	0.67
Svi2-Svi26	3.67	8	0.89
SviL5-Svi26	3.34	6	0.76
Svi6-Svi26	3.83	8	0.87
Svi20-Svi26	5.01	8	0.76

TABLE 7. Summary results of Analysis of Molecular Variance (AMOVA) using allelic distance matrix as input.

Source	df	SS	MS	Estimated variance	% of variance
Among populations	3	7.45	2.49	0.05	3%
Among individuals	45	65.31	1.45	0.00	0%
Within individuals	49	75.50	1.54	1.54	*97%
Total	97	148.26		1.59	100%

*This percentage indicates that genetic variation between four sample sites was mainly attributed to variation within individuals (97%) as opposed to among sample populations (3%)

Appendix C: Figures

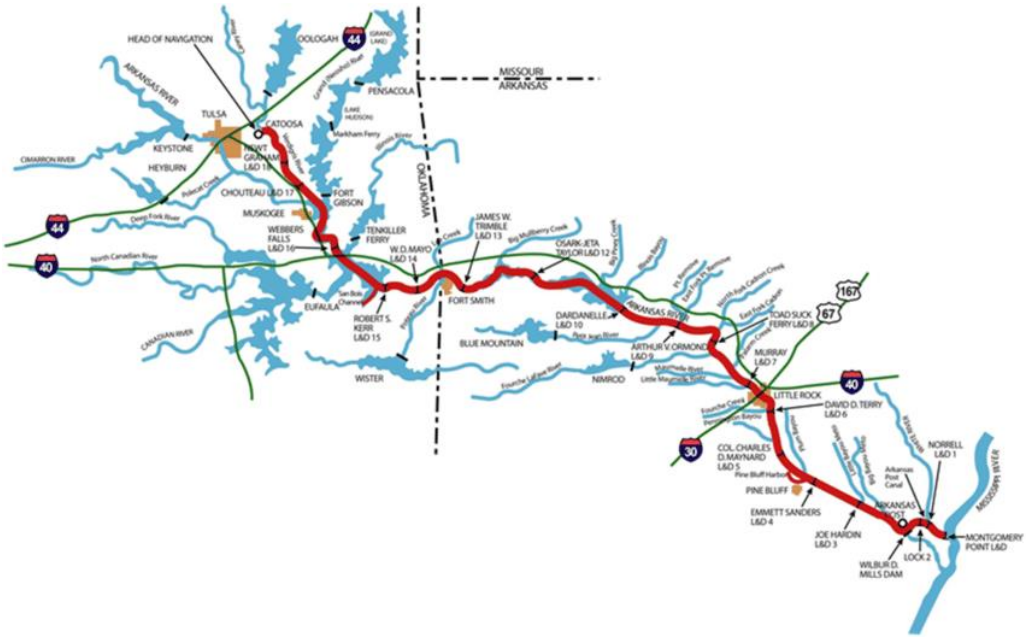

FIGURE 1. Map of the McClellan Kerr Arkansas River Navigation System (provided by the U.S. Army Corp of Engineers (USACE). This map indicates all locks and dams in the system (outlined in red) with black lines across the river. State borders are indicated using dashed lines.

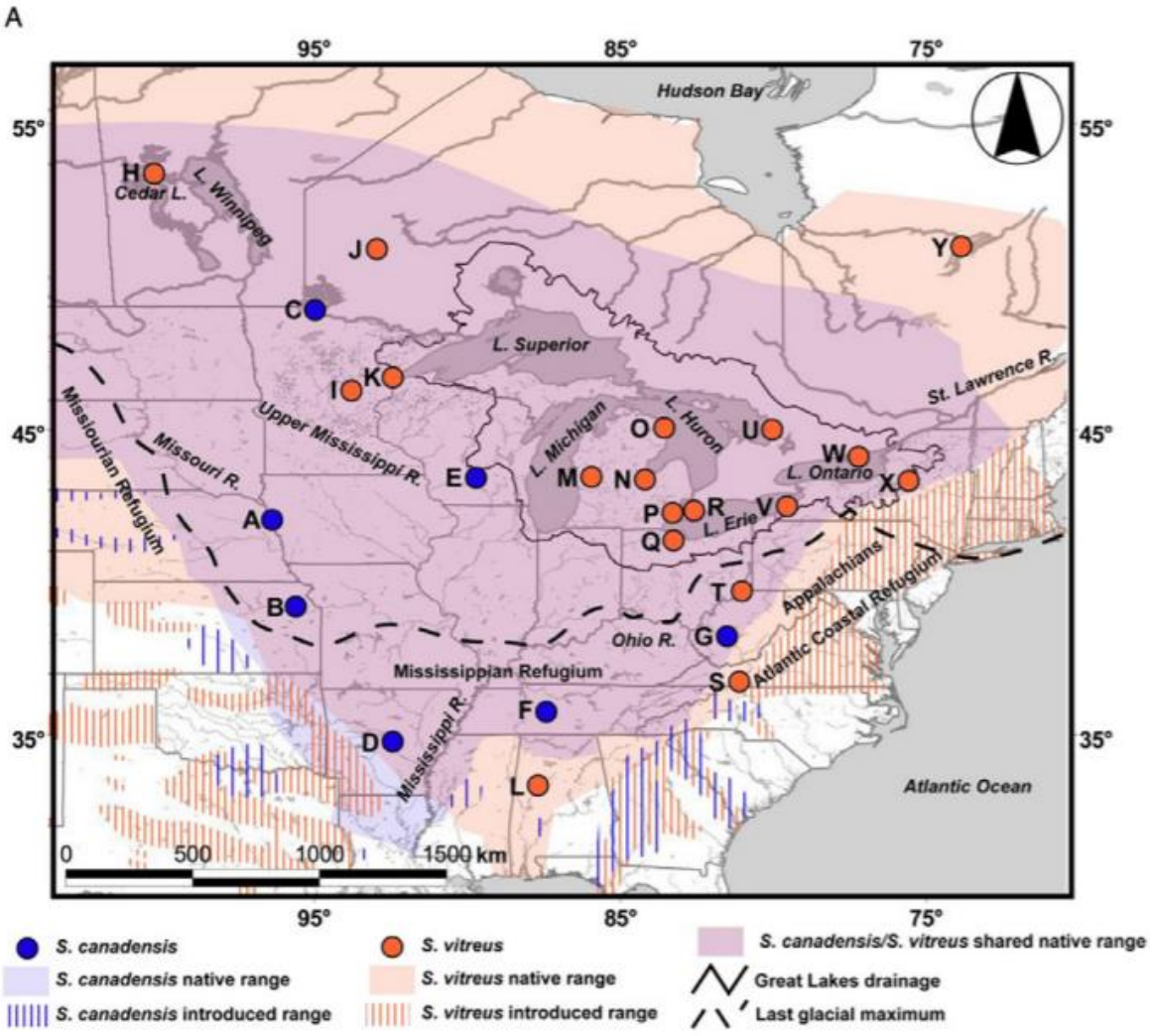

FIGURE 2. Native and introduced range maps determined by phylogenetic analysis for Sauger and Walleye in the United States and Canada (Haponski and Stepien 2013).

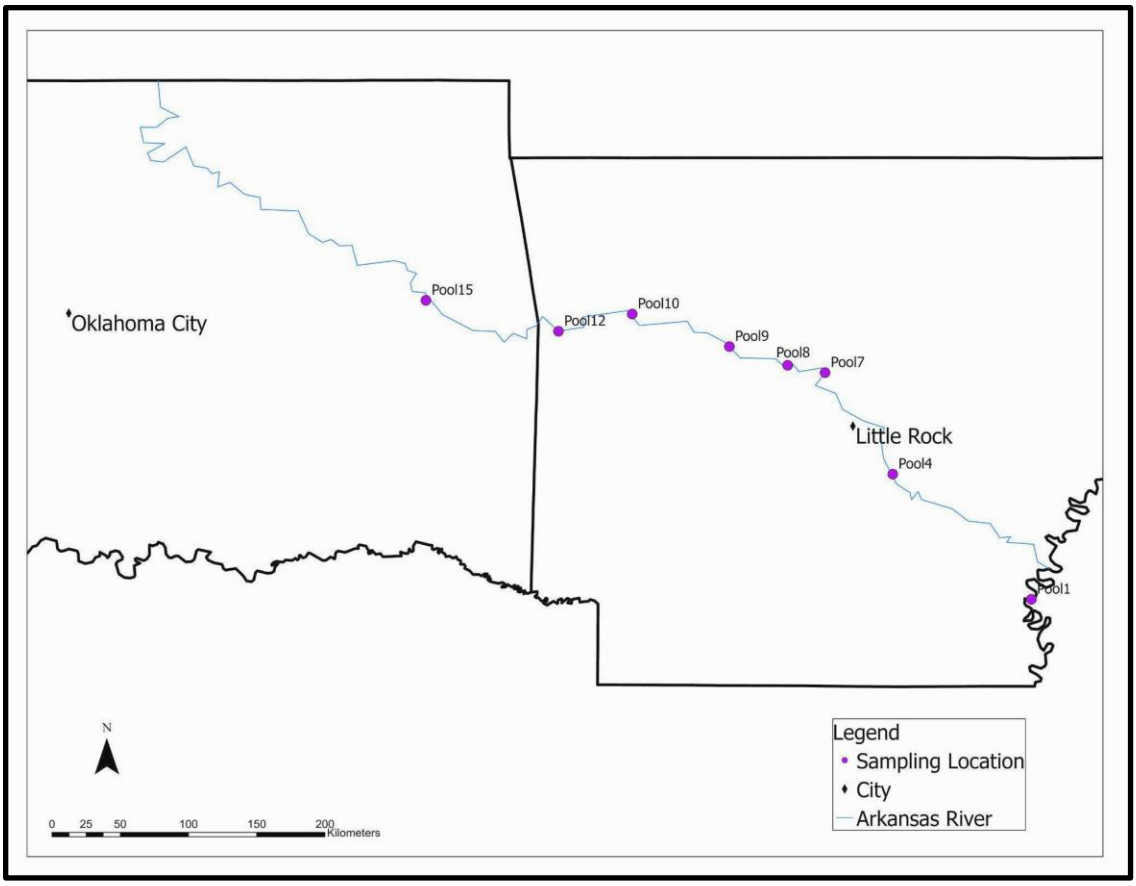

FIGURE 3. Map of study sites on the Arkansas River. Sites were named using navigational pool names designated by USACE. Sites were sampled in the months of November through February of 2019, 2020 and 2021.

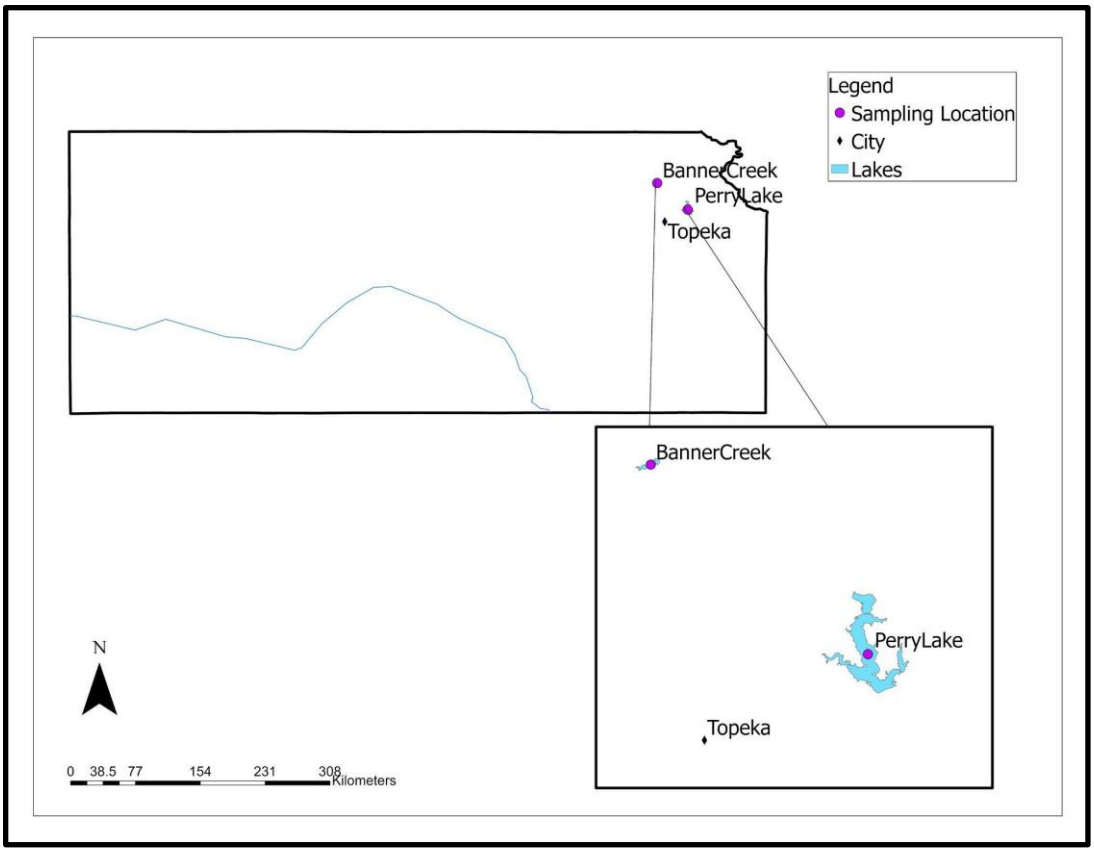

FIGURE 4. Map of study sites in Kansas. These sites were samples by the Kansas Department of Wildlife, Parks, and Transportation in the early spring of 2021. Data from these sites was collected in order to serve as a representative of genetically distinct Sauger.

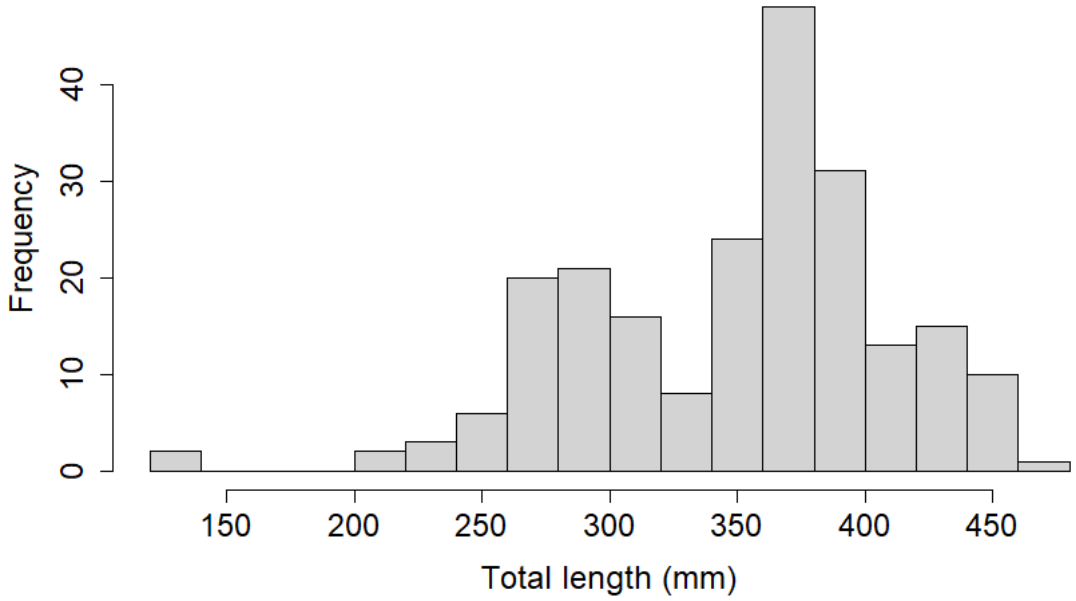

FIGURE 5. Total length frequency histogram for Sauger across all study locations.