GENETIC ANALYSIS OF PUTATIVE WALLEYE AND SAUGEYE IN RIVERS NEAR FORT WAYNE, INDIANA

by

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To my parents,

who nourished within me a relationship with the Creator

from which my love for Creation springs forth.

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ABSTRACT

A saugeye is the progeny of a female walleye (*Sander vitreus)* and male sauger (*Sander canadensis)*. In the United States, hybrid saugeyes are considered important for recreational fisheries and as a potential food source. Saugeyes grow exceptionally faster than their non-hybrid parents and are more tolerant of a broader range of water conditions. They are also of interest to anglers due to their increased growth rate and ease to catch. Rather unexpectedly, biologists have recently observed fish that they believe to be saugeye in the Fort Wayne Rivers even though only walleye have been stocked in the area. The fish in Hurshtown Reservoir are believed to be walleye and the identification of those in the Three Rivers is unknown. A potential source for saugeye in the Fort Wayne Rivers is St. Marys State Fish Hatchery in Ohio. This research aims to determine if the fish found in the Fort Wayne Rivers are walleye or saugeye using microsatellite analysis. Microsatellites at seven loci were genotyped for 20 reference walleye, sauger, and saugeye as well as 21 unknown fish caught near Fort Wayne. Of the fish caught near Fort Wayne, three are from Hurshtown Reservoir and 18 are from the Three Rivers. Assignment tests of genotypes were completed using model and non-model based cluster analysis. Genotypic variation clearly resolved the two parent species from their hybrid offspring. Sixteen of eighteen *Sander* (unknown species) caught in Fort Wayne Rivers between 2018 and 2019 were determined to be first generation saugeye. The other two were walleye found in the Maumee River downstream of Hosey Dam. The three *Sander* caught in Hurshtown Reservoir were verified to be walleye. Sauger have never been stocked in the Fort Wayne Rivers and connecting waterways. Therefore, it is not likely that the saugeye found in the analysis are from natural reproduction. It is speculated that saugeye are swimming to Fort Wayne from hatcheries within the Maumee watershed. There are many potential sources for walleye in the Fort Wayne Rivers.

CHAPTER 1. INTRODUCTION

1.1 Background

The Fort Wayne, Indiana area is centered on a unique river system. Within the city limits, two major rivers come together. The St. Joseph River originating in Michigan cuts through the northwest corner of Ohio before flowing to Fort Wayne draining 280,852 ha of land (Rocha et al., 2008). There it meets the St. Marys which originates in northwest Ohio and flows northeast to Fort Wayne draining 220,149 ha of land (Phillips, 1984). These two rivers combine to form the Maumee River which flows towards Toledo, Ohio and empties into Lake Erie draining a total of 1,711,464 ha (Greeman et. al, 1994).

1.1.1 Three Rivers History

The present-day Three Rivers surface-water is different now than it was historically. About 14,800 years ago when the last glaciation period was ending, ancestral Lake Maumee covered the area. The lake retreated in stages to what we now know as Lake Erie (Sommers, 1977). As the lake retreated, the drainage course of the Maumee River was established. The Maumee River generally follows the course of a previous sub-glacial channel. It was not until later that silt accumulation recaptured the St. Joseph and St. Marys rivers to the Maumee watershed. (Greeman et. al, 1994) The Three Rivers were then surrounded by marshes and wetlands filled with dense forests. The frequent flooding of the rivers and poor drainage made the soil fertile for growing trees. Early settlers often called the area names such as Great Black Swamp, Maumee Swamp, The Big Swamp, the Lake Plains, and the Dismal Swamp. While the difficult conditions discouraged early settlement of the area, the streams drew Europeans to Indiana as highways for expansion to the west. (Hallett, 2011) Out of necessity for building materials, the timber industry became the first to harvest natural resources in the Three Rivers area. As the land was cleared, settlers began to realize the soil that sustained expansive forests would also be good for growing crops. Massive amounts of tilling and ditching, to break up the clay and drain the soil, transformed the landscape from swamp to farmland.

1.1.2 Fish Community Timeline

While researchers such as Milton Trautman (Ohio State University) have surveyed the Maumee River over time, finding data specific to the Three Rivers confluence in Fort Wayne is a little more challenging (Trautman, 1981). The earliest known records of the area were written in the summer of 1893 (Kirsch, 1894). The St. Marys, St. Joseph, Maumee Rivers, and many of the tributaries and lakes in the watershed were surveyed near Fort Wayne. Descriptive observations of the habitat were recorded as well as records of the fish, mollusks, crawfish, amphibians, and reptiles observed. Among the three rivers, there were 41 fish species (Appendix A) found in Fort Wayne in 1893 and none of them were walleye or saugeye (Kirsch, 1894).

The next survey was done in 1945 by Shelby D. Gerking. Compared to the previous study, increased turbidity had restricted the distribution of clear-water species (Gerking, 1945). Among the three rivers, 47 species (Appendix A) were collected in 1945. No walleye nor saugeye were found in the survey.

Additional surveys were done in 1972, 1977, and 1979. ~~Each of~~ these surveys did not detect walleye or saugeye in the Three Rivers. In 1980, the first reputed walleye was detected in the Three Rivers. In 1989, a survey (Pearson, 1990) examined the fish populations in the St. Joseph River. At the time of the survey the water level in the St. Mary and Maumee Rivers was too low to sample. Among four collection sites, a total of 2,551 fish were collected representing 36 species (Appendix

A)(Pearson, 1990). Reputed walleye were found in the St. Joseph River. This is one of the first records of multiple reputed walleye in the Fort Wayne Rivers.

An incident in 1994 involving Phelps Dodge Magnet Wire Company had a large impact on fish communities in the Fort Wayne Rivers. Phelps Dodge Magnet Wire Company was located on the east side of Fort Wayne. The company manufactured magnet wire. On Monday, September 12, 1994 about 450 gallons of phenol leaked from a holding tank at the property and a portion of the spill entered a drainage ditch connected to the Maumee River. Phenol is toxic to fish, and this spill caused a significant fish kill. Among the species identified as impacted by the phenol leak were reputed walleye and saugeye. As mitigation for the spill, the Phelps Dodge Company paid for the stocking of smallmouth bass, channel catfish, and walleye.

In 1995, stocking of walleye began as part of the Maumee River Fish Restoration Project in response to the fish kill. The first year, Phelps Dodge paid for 5,000 walleye (2-4 inches) to be stocked near the public access ramp at Kreager Park on the Maumee River. In 1996, another 5,000 walleye (2-4 inches) purchased by Phelps Dodge were stocked at the same location. Additionally, the DNR stocked 50,000 walleye (1 inch) distributed among eight locations between Decatur, Indiana and St. Joe, Indiana. This included four locations within Fort Wayne city limits. Walleye were stocked on the St. Mary River at Foster Park and Guldlin Park and on the St. Joseph River at Johnny Appleseed Park and Shoaff Park. In 1997, Phelps Dodge again paid for 5,000 walleye (2-4 inches) to be stocked at Kreager Park on the Maumee River. The DNR also stocked 50,000 walleye (1 inch) again, this year dividing them amongst the four sites within Fort Wayne. In 1998, Phelps Dodge paid for spring and fall stockings at Guldlin Park and Johnny Appleseed Park. In April, 8,000 walleye (4-6 inches) were distributed between these sites on the St. Mary and St. Joseph Rivers. In September, 10,000 walleye (2-4 inches) were distributed between these sites.

From 1995-1998, Indiana DNR stocked a total of 133,000 walleye in and around the Fort Wayne area. This number includes the walleye paid for by Phelps Dodge. The walleye purchased by Phelps Dodge were obtained from Brookcrest Fisheries, a hatchery in Cedar Grove, Wisconsin. This hatchery is no longer in business. The walleye stocked by the DNR were obtained from the St. Marys Fish Hatchery in St. Marys, Ohio. Overlapping with the stocking efforts were three years of sampling efforts to check for survival. Sampling was completed using boat electrofishing. In 1996, 24,633 seconds of effort yielded a total of 69 walleye. In 1997, 12,161 seconds of effort yielded 38 walleye. In 1998, 3,972 seconds of effort found 25 walleye. Among the 132 walleye found in these three surveys, only 10 were shorter than 14 inches. Among those ten, only a few were small enough to possibly be recapture of stocked walleye. The rest of the walleye captured in the surveys were speculated to be saugeye hybrids. Experimental stocking of walleye in the Three Rivers was considered unsuccessful (Pearson, 1998).

The most recent survey was done in 2009 by the IDNR. In this survey, each river was sampled at two locations using a boat electrofisher. A total of 32 species (Appendix A) of fish were found. Reputed walleye were found in the St. Marys River and reputed saugeye were found in the St. Joseph and Maumee Rivers. In total, the survey found one walleye and 17 hybrid saugeye. The DNR has also assisted Purdue Fort Wayne faculty by providing students with hands on electrofishing experience at Johnny Appleseed Park on the St. Joseph River. In 2004, one walleye was caught. In 2011 – three, 2014 - two, 2017 – two, 2018 – eight, and in 2019 - one saugeye was caught at Johnny Appleseed Park.

1.2 Sander Characteristics

1.2.1 Walleye

The scientific name for walleye is *Sander vitreus.* Their species name comes from the root vitrea meaning glassy. This is in reference to their large, silver colored eyes. Their lateral body shape is elongated and vertically compressed. They have a forked caudal fin and terminal mouth. Walleye mean length at maturity is 42.9 cm. They have been found as long as 106.9 cm. The maximum age recorded in a walleye is 29 years old (Hugg, 1996). They have been found to live this long at their Northern extent, while life expectancy in their most southern extent can be three - five years (Bozek, Baccante, and Lester, 2011). They have 13-17 dorsal spines, 18-22 dorsal soft rays on two separate dorsal fins, two anal spines, 11-14 anal soft rays and 83-104 scales along their lateral line. (Scott, 1973)

Walleye can be found in lakes, pools, backwaters, and runs of medium to large rivers. They are often found in clear water (Page and Burr, 1991). When in shallow water, they prefer high turbidity (Frimodt, 1995 and Etnier and Starnes, 1993). They feed at night, mainly consuming insects and fishes. Among fishes, they have a preference to yellow perch and freshwater drum. When fish and insects are scarce, they are known to feed on crayfish, snails, frogs, mudpuppies, and small mammals (Scott, 1973). In rivers, walleye have been found spawning in areas of faster current (Nelson and Walburg, 1977) as well as slower current (Chalupnicki et al., 2010). They have been seen using cobble and small boulder substrate to spawn over (Paragamian, 1989) as well as spawning over cattail beds and sedges (Priegel, 1970). Spawning occurs in small groups of two to three males per female. At night the group moves to the shallows chasing in circular swimming patterns with fin erection. When the female is ready she rolls on her side and disperses her eggs.

The group of two to three males quickly fertilize the scattered eggs (Scott, 1973). Eggs hatch in 12-18 days and the ensuing larvae are pelagic (Balon, 1990).

1.2.2 Sauger

The scientific name for Sauger is *Sander canadensis.* Their body shape is nearly identical to walleye, elongated and compressed. They also have a forked caudal fin and terminal mouth. Their mean length at maturity is 33.0 cm. This is 9.9 cm less than walleye. The longest sauger recorded was 75.9 cm (Page and Burr, 2011). The oldest sauger was reported at 18 years (Hart, 1928). The sauger has 13-14 dorsal spines and 11-13 dorsal soft rays.

Saugers can be found in sand and gravel runs, muddy pools, and backwaters of small to large rivers. Sauger are less adaptive to lake conditions than walleye (Page and Burr, 2011). Sauger and walleye have both evolved physiology and behavior to excel in low light, turbid, and nocturnal conditions (Ali and Anctil, 1977). Both species have scotopic vision due to the retinal tapetum lucidum in their eyes. This is a layer in their eye that reflects light to increase retinal sensitivity (Bozek, Haxton, and Raabe, 2011). Between the two species, saugers are more negatively phototaxic because they have more reflecting material that is more evenly distributed around the retina. Because of this, sauger are more likely to select darker, deeper, and more turbid environments than walleye. Sauger are especially suited for habitat where clay minerals remain suspended in the water column for long durations (Ali and Anctil, 1977).

1.2.3 Saugeye

A saugeye is the progeny of a female walleye and male sauger. The reciprocal hybrid is possible, though known to not survive well. In the United States, hybrid saugeyes are considered important for recreational fisheries and a potential food source. Hybrid saugeyes grow exceptionally quicker than their non-hybrid parents. Growth is similar between the hybrid and their parents for the first two years, but after that, hybrids grow faster than walleye or sauger (Johnson et al., 1988). They have also been found to be tolerant of a wider range of water conditions and are more tolerant than walleye to warm and eutrophic conditions (Bozek et. al., 2011).

1.3 Need for Molecular Analysis

An evaluation of identifying *Sander* by visual characteristics found that it is impossible to visually distinguish between *Sander* on specimen under 10 cm. Older specimens can be identified by distinguishing characteristics (Flammang and Willis, 1993). There are three main characteristics that help determine walleye from sauger in the field. Hybrids will display some combination or intermediate of their parents' traits. First, the coloration of the two parent species is different from each other. Saugers have a dark brown coloration of blotches or saddles that come down well below the lateral line. Walleye have a lighter green color with a vertical banding pattern. This lighter pattern rarely extends below the lateral line. Secondly, the patterning on the dorsal fin is different between walleye and saugers. Saugers have black spots on their dorsal fin, while walleye have a smeared pattern to their dorsal fin. In addition, walleye have a black membrane between the last few spines on their first dorsal fin. Sauger lack this black colored membrane. Finally, walleye have a white spot on the lower portion of their caudal fin. This spot is commonly lacking in sauger. Occasionally, sauger may have a smaller spot on the tip of their caudal fin. If they do, it is yellowed relative to the bright white spot on walleye.

Hybrid saugeye may show degrees of smearing on dorsal fin spots or differing shades and size of the caudal fin tip. Hybrid body coloration may be more similar to walleye or sauger. Some hybrids may show traits mimicking one species or the other. A guide showing examples of each species and the hybrid is attached in Appendix B. The variation of saugeye makes the identification of hybrids in the field difficult. (Summerfelt, Johnson, and Clouse, 2011)

A good amount of research has been done on assessing the accuracy of *Sander* field identification and species determination through molecular analysis. In the field, identification of walleye, sauger, and saugeye relies heavily on the external pigmentation patterns. As stated earlier, walleye are distinguished by a white spot on the lower tip of the caudal fin. Sauger can be distinguished by dark saddles that extend below the lateral line. Sauger also have more spotted patterns on the dorsal fin. In general, sauger exhibit brown pigments compared to the greenish pigment of walleye (Stroud, 1948 and Nelson, 1968). Saugeye show a combination of these physical traits and sometimes look so similar to a parent species that field identification of hybrids can be speculative.

To assess the usefulness of using external traits to identify walleye and saugeye in the field, Flammang and Willis (1993) compared field identification to electrophoretograms of the supernatant form of phosphoglucomutase. The researchers sampled in Lakes Mina and Richmond, South Dakota. Of the 47 fishes collected in Lake Mina, 53% were correctly identified in the field. Of the 23 fishes collected in Lake Richmond, 78% were correctly identified in the field based upon PGM-1 phenotypes (Flamang and Willis, 1993).

1.4 Development of Molecular Techniques

The technique to accurately identify *Sander* species through genetic analysis has evolved over time. For decades scientists used malate dehydrogenase (MDH) to distinguish walleyes, saugers, and saugeyes by the process of starch gel electrophoresis (Clayton et al., 1971, Clayton et al., 1973, Todd, 1991, and Ward, 1992). In 1973, Clayton expressed concern in interpreting those isozyme phenotypes. In 1991, Todd suggested the use of the supernatant form of

Phosphoglucomutase-1 to avoid concerns of interpretation with MDH. In 2005, a study was published that used multiple methods to examine population structure and accuracy of field identification of walleye. The researchers used two analyses of restriction fragment length polymorphism of mitochondrial DNA, allozyme analysis of nuclear DNA, and microsatellite analysis of nuclear DNA to document genetic variation and species identification of walleye in the Ohio River (White et al., 2005). From this study, we can see the usefulness of different techniques. Microsatellite techniques exemplify their sensitivity to changes in populations over a relatively short amount of time because of their higher mutation rates.

In 2012, researchers used microsatellites to determine the genetic population structure and hybridization of walleye and sauger in the Upper Missouri River drainage. The analysis of their work identified 11 loci that can distinguish 100% of all first and second generation hybrids from *Sander* parental genotypes (Bingham et al., 2011). These same 11 loci were effective in determining genetic groups statistically similar to those of an allozyme analysis done on the same population in 2006 (Billington et al., 2006). In 2014, another study developed 18 microsatellite loci in walleye (Coykendall et al., 2014). Among these 18 loci, 11 were diagnostic to determine walleye from sauger. These microsatellites were created based off the genome of walleye from Lake Superior and Lake Erie.

1.5 Summary

Extensive surveys were done on the Three Rivers in 1893, 1945, 1972, 1977, and 1979. None of the three species - walleye, sauger, or saugeye - were captured in these surveys. Starting in 1980, surveys detected reputed walleye and they have been present in surveys ever since. Beginning in the 1990s, report discussions indicate that among the recorded walleye numbers – some are reputed saugeye. Even though some *Sander* are reputed saugeye; there is no record of stocking hybrid saugeye or sauger in the rivers. Even if some of the walleye stocked in the mid to late 1990s did survive, the lack of sauger in the system for them to hybridize with limits the possibility of natural hybridization. These circumstances beg the question, "Are *Sander* present in the system today walleye or saugeye?" Because it is so difficult to distinguish saugeye from either parent species using physical characteristics, molecular techniques have been developed to identify them. In 2011, microsatellites were effective at distinguishing between *Sander* species in the Missouri River. In 2014, eleven diagnostic (walleye/sauger) loci were developed using the genome of walleye geographically closer to Fort Wayne. These loci were originally tested on a sample of 35 walleye and 4 sauger. This research will test the same eleven diagnostic loci on 20 identified walleye from Lake Erie, 20 identified sauger from the Ohio River, and 20 saugeye raised at Grand Lake St. Marys Hatchery in Ohio. To find whether *Sander* present in the Three Rivers near Fort Wayne today are walleye or saugeye, the microsatellites of 21 unknown *Sander* from the Fort Wayne area will be compared to these reference populations.

1.6 Importance

Species identification of *Sander* in the Fort Wayne Rivers is incredibly important to researchers, managing biologists, and invested entities. Documenting the presence and species of *Sander* provides the framework of information needed for research ichthyologists to further study the *Sander* present in Fort Wayne. For example, if both walleye and saugeye are present in Fort Wayne Rivers, researchers could do a comparative study between them. Additionally, research could be done on reproduction between and within walleye and saugeye populations. These research questions on walleye and saugeye informs managing biologists.

Accurate identification through genetic analysis is crucial information for biologists making management decisions because there are physiological differences between walleye and saugeye. These differences affect their viability in the ecosystem. Research specific to the Fort Wayne population leads to best management practices in the region. Managing the resource is important because *Sander* are popular game species among anglers. Their wide appeal is due to their size and popularity as a food source.

This popularity among anglers is especially relevant to invested entities such as the City of Fort Wayne. Currently, there is a significant riverfront development project underway in Fort Wayne. A goal of the project is to attract people to the rivers. Because of *Sander* popularity, this research is valuable information to riverfront development leaders and city officials looking to entice more visitors to the rivers. Management of *Sander* would increase fishing opportunities in Fort Wayne. Additionally, this research is valuable to hatchery managers from which the known parent species are selected. The analysis will verify the genetic identification of fish used as broodstock at the hatchery.

CHAPTER 2. METHODS

2.1 Research Strategy

River sampling, molecular analysis, and statistical analyses were conducted to find what species of *Sander* are present in the Fort Wayne Rivers. During the fall of 2018 and the spring and summer of 2019, *Sander* were sampled from the St. Marys, St. Joseph, and Maumee Rivers. Additionally, *Sander* were collected from Hurshtown Reservoir to serve as reference walleye. Scales were collected from wild caught fish for ageing. Known walleye, sauger, and saugeye samples were obtained for cluster analysis comparison. Known walleye from Lake Erie, sauger from the Ohio River, and saugeye from St. Marys State Fish Hatchery were collected in the spring of 2019. DNA was extracted from fin tissue to be genotyped using microsatellite loci. Statistical analyses of the resulting genotypes were conducted using model and non-model based cluster analysis to assign fish as one of the parent species or as a hybrid.

2.1.1 Sampling

The study area is focused on the three rivers in Fort Wayne, IN and can be divided into three extents. There is a central, northern, and southern extent. The central extent focused around the confluence of the St. Marys and St. Joseph Rivers within Fort Wayne City limits. *Sander* were sampled in the St. Marys River from the confluence of the Three Rivers to Foster Park. On the St. Joseph River, sampling occurred from the confluence to Johnny Appleseed Dam. Sampling continued upstream of Johnny Appleseed Dam heading North until the Interstate 469 overpass. On the Maumee River, sampling went from the confluence to the downstream side of Hosey Dam (Figure 1).

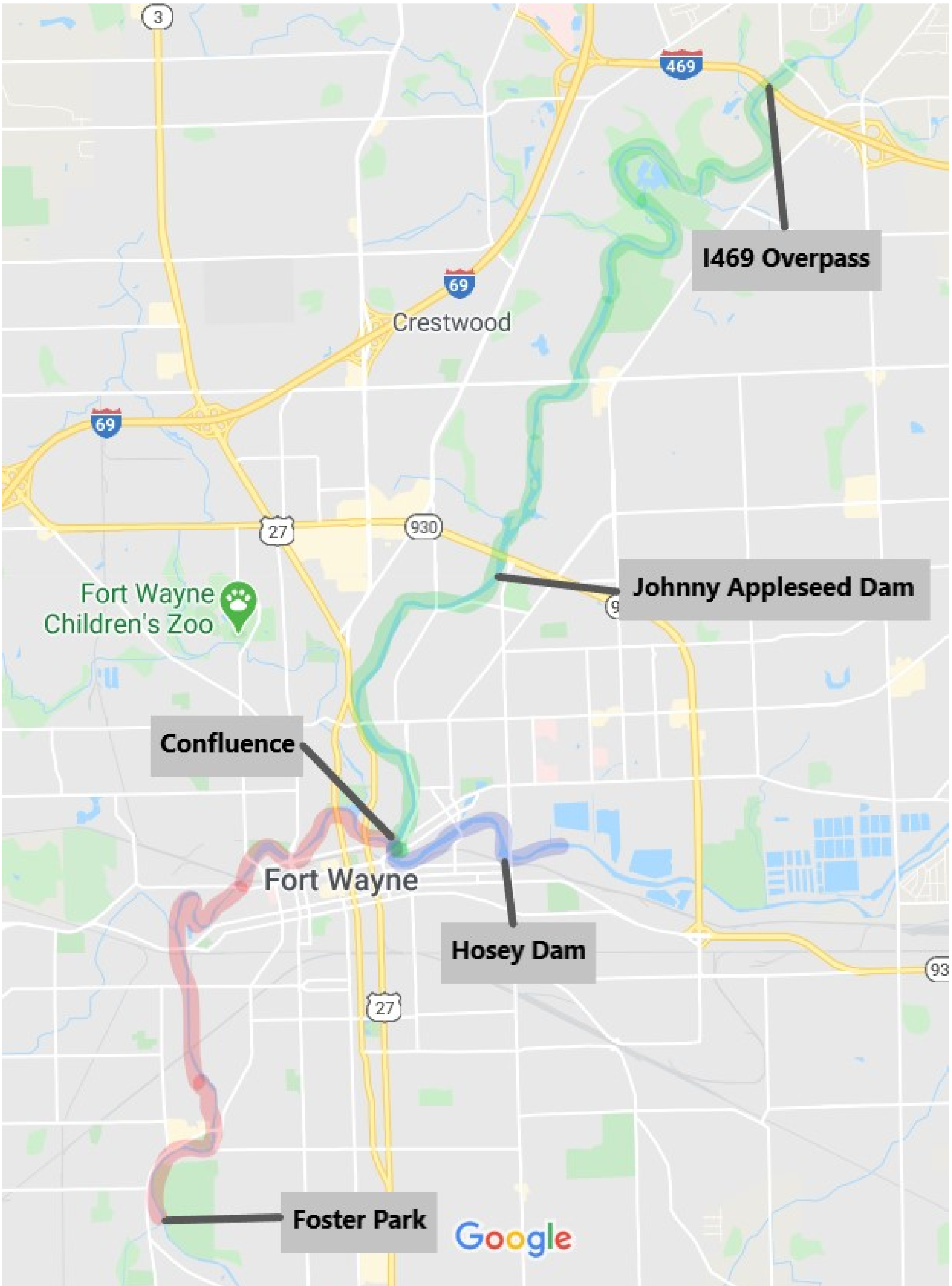

Figure 1. Map of *Sander* sampling extent in the Fort Wayne Rivers. Red – St. Marys River, Green – St. Joseph River, and Blue – Maumee River.

In the northern extent of the study area, sampling continued further upstream on the St. Joseph River. Sampling continued at the Leo-Cedarville Reservoir progressing up to Spencerville, IN. The most upstream extent of sampling on the St. Joseph River is just 3 river miles shy of the stocking extent of walleye in the late 1990s. On the north side of Fort Wayne, Hurshtown Reservoir was also sampled. (Figure 2).

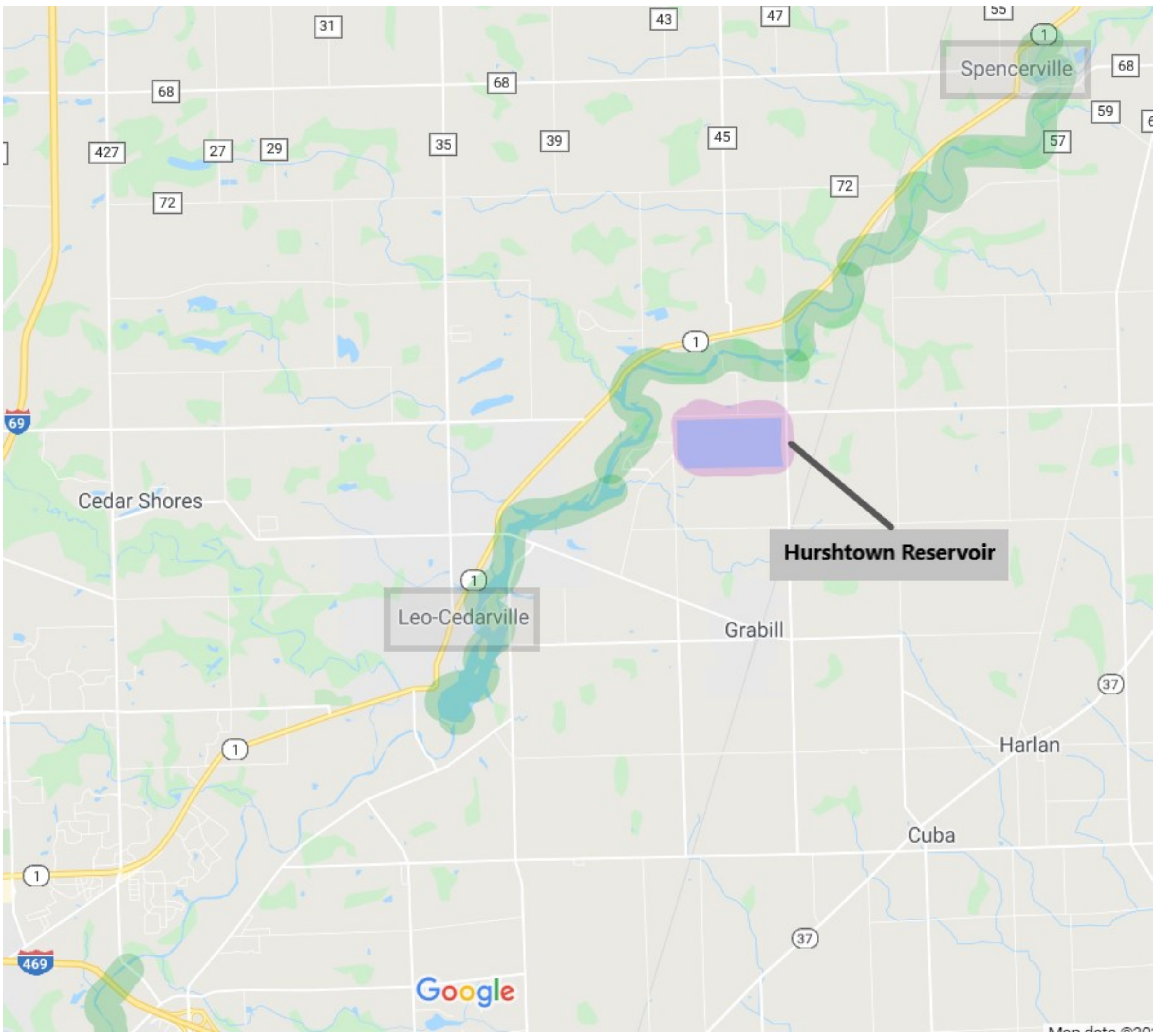

Figure 2. Map of *Sander* sampling extent north of Fort Wayne on the St. Joseph River and Hurshtown Reservoir. Green – St. Joseph River, Pink – Hurshtown Reservoir.

In the southern extent of the study area, sampling continued upstream on the St. Marys River near Decatur, IN (Figure 3). This section of the river is the most upstream extent of walleye stocking on the St. Marys River in the 1990s. This location is approximately 33 river miles upstream of the confluence on the St. Marys River.

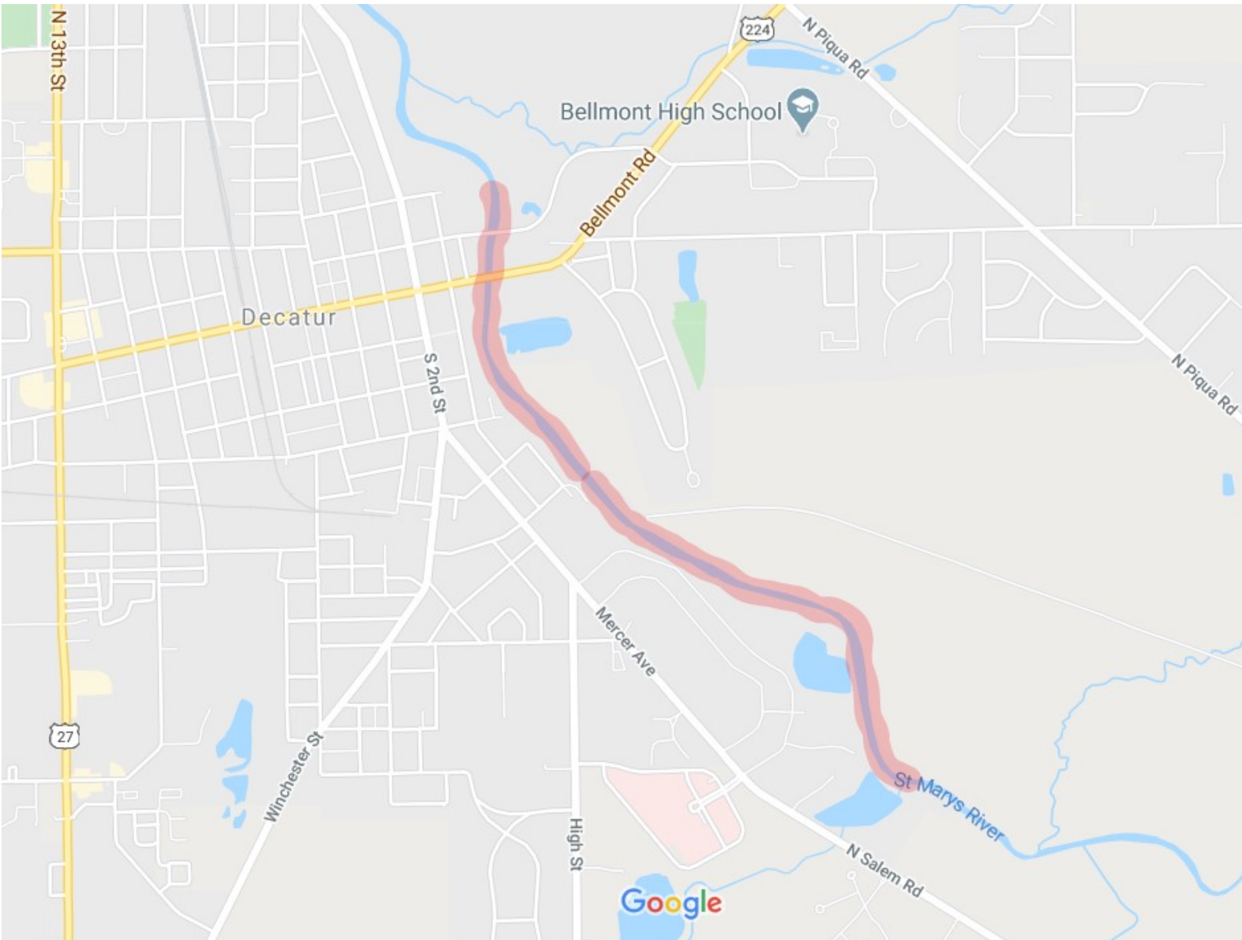

Figure 3. Map of *Sander* sampling extent south of Fort Wayne upstream on the St. Marys River. Red – St. Marys River.

For genetic analysis, the right pelvic fin was clipped and stored in non-denaturing alcohol. Scales were collected for age estimates of each fish. Length was recorded. Unknown *Sander* were collected in Fort Wayne using boat electrofishing and hook and line methods. Electrofishing was done on the St. Marys River starting at Guldlin Park and proceeded downstream as far as the confluence and upstream as far as Jefferson Boulevard. Within three separate days, seven hours of electrofishing were completed in this stretch of waterway. Electrofishing was also done on the St. Joseph River starting at the Johnny Appleseed Dam and moving towards the confluence. Within three days, eight hours of electrofishing were done in this section of river. In order to revisit these sites and explore the presence of *Sander* in the other stretches, hook and line surveying was implemented. Trolling was used initially to detect where *Sander* were present. Then, sampling intensified where fish had been caught, seen, or landed by local fisherman. Fishing efforts concentrated at the dams in Fort Wayne because *Sander* congregate in deeper pools in the summer and fall. Casting was used on the downstream side of Hosey Dam and Johnny Appleseed Dam. Casting was also used to sample Spy Run Creek, a smaller tributary to St. Marys River.

Scales were collected from each fish using a pocket knife and stored in a paper pouch until analysis. Scales were pressed onto acetate slides to make scale impressions. A heated press was used. Pictures of the impressions were taken using a dissecting microscope. Annuli were counted on each scale and the mode was recorded.

Known walleye used as broodstock at Grand Lake St. Marys Hatchery were collected near the mouth of the Maumee River at Lake Erie. Boat electrofishing was used to collect 52 walleye as they were swimming upstream to spawn. Known sauger collected from the Ohio River were being kept at London State Fish Hatchery. These fish were also used as broodstock at Grand Lake St. Marys Hatchery in the production of saugeye. Twenty-five sauger were chosen at random from the available stock. It was noted at the time of collection that some sauger showed signs of hybridization so pictures were taken to compare to results. Once the progeny of the known walleye and sauger had reached about ¾ inch, 25 whole body samples were collected. Each fish was euthanized before being preserved in non-denaturing alcohol. Saugeye samples were pulled from two of the ponds at Grand Lake St. Marys Fish Hatchery.

2.1.2 Molecular Analysis

DNA was extracted from fin samples using the DNeasy Blood and Tissue Kit following manufacturer protocol. A microspectrophotometer (Nanodrop) was used to estimate DNA concentration before DNA samples were frozen.

Microsatellite loci were chosen for this study due to their relatively high mutation rate and allelic diversity making them useful in distinguishing between groups. Seven diagnostic loci were used to distinguish between species (Coykendall et al, 2014). These microsatellite loci were based off the genome of walleye from Lake Erie and Lake Superior. Because all of the loci were of similar size range and in order to save on costs, universal primers for fluorescent labelling were used with two fluorophores. A universal primer 15 bp long was attached to the forward primer at each locus to allow attachment of fluorescent dyes (HEX and FAM). Thirty ng of DNA was added to the master mix. The PCR master mix included 2.5mM of $MgCl_2$, 0.2mM of dNTPs, 0.075uM forward primer, 0.15 uM reverse primer, 0.075 uM labeled universal tail, 1 x GoTaq Flexi buffer (Promega), and 0.05 units/ul 1X GoTaq Flexi in a final volume of 15 ul. PCRs were run under the following thermocycling conditions: 95 °C for 2 minutes, 35 cycles each of 95 °C for 1 minute, 58 °C for 1 minute, 72 °C for 1 minute, followed by a final extension of 72° for 5 minutes (Coykendall et al, 2014). PCR products were visualized by gel electrophoresis, diluted, and sent to the DNA Analysis Facility on Science Hill at Yale University for genotyping. Alleles at each locus were called using Geneious (v.11.1.5).

Results were analyzed and organized into Excel recording two alleles at each loci. Results were transferred into the Excel add-on GenAlEx. GenAlEx is useful for reformatting data for other software programs. Genepop 4.7 (Rousset, Raymond and Rousset 2008, 1995) and GenAlEx 6.5 (Peakall and Smouse 2006, 2012) were used to test for conformance to Hardy-Weinburg Equilibrium and measure heterozygosity and polymorphism.

GenAlEx, STRUCTURE2.3.4 (Pritchard, Stephens, and Donelly, 2000), and NewHybrids1.1 (Anderson and Thompson, 2002) were used to assign individuals to species through cluster analysis. These three programs use different approaches to address the same question. GenAlEx is a frequency based cluster analysis while STRUCTURE and NewHybrids employ models to identify clusters. All three programs were run to increase confidence in results. GenAlEx population assignment is a frequency based cluster analysis. It operates by calculating the allele frequencies of predetermined groups and then calculating the Log likelihood of each individual to belong to each of these groups. It also allows for some samples to be treated as 'unknowns' meaning they are not included in cluster allele frequency calculations. Likelihoods were changed to positive values for interpretation. The lowest value indicates the most likely cluster an individual belongs to. STRUCTURE was created as a Bayesian approach for distinguishing hybrids. It can calculate the posterior probability (q) of an individual to belong to one of two clusters (k=2). The q-value ranges from 0 to 1 with a value of 1 indicating walleye and 0 for sauger, with hybrids to falling between these values. The threshold q-value for hybrids was chosen between 0.2 and 0.8 (Rohde et al., 2015). These values have been shown in previous studies to be a conservative estimate for hybridization. The admixture model was run with a burn-in period of 10^4 followed by 10^5 Markov chain Monte Carlo simulations. NewHybrids takes the model-based approach in STRUCTURE a step further by calculating the posterior probability (0 to 1) that each individual belongs to either parent species or one of four hybrid classes. These hybrid classes are first and second generation hybrids or a backcross to either parent species. The outputs from

GenAlEx, STRUCTURE, and NewHybrids are all useful in determining the identification of each fish.

CHAPTER 3. RESULTS

Known walleye (WA) and sauger (SA) populations from the Maumee River near Lake Erie and the Ohio River were in Hardy Weinberg equilibrium. Both the saugeye (SE) from the hatchery and the unknown (UK) caught in Fort Wayne were out of equilibrium. One of the seven loci was fixed in the sauger population (Table 1). Table two shows the number of fish tested, number of alleles, and observed and expected heterozygosity for each population at each locus. The total rows show all populations combined at that locus (Table 2).

Table 1. Mean population values of HWE, Allelic Richness, Heterozygosity, and Polymorphism.

	Hardy-Weinburg Equilibrium	No. Alleles and Heterozygosity			Polymorphic Loci
Pop	**P-Value**	**Na**	**Ho**	**He**	**%P**
WA	>0.05	6.9	0.62	0.62	100%
SA	>0.05	2.7	0.21	0.22	85%
SE	<0.001	7.1	0.97	0.70	100%
UK	<0.001	6.9	0.91	0.72	100%

Table 2. Data for each loci shown for each population separately and combined. N-number of fish amplified, Na – number of alleles, Ho – observed heterozygosity, He – expected. heterozygosity.

Locus	Group	N	Na	Ho	He
	WA	20	6	0.80	0.78
	SA	20	4	0.25	0.34
Svit038	**SE**	20	8	1.00	0.73
	UK	21	6	0.95	0.77
	Total	81	8	0.75	0.66
	WA	19	4	0.37	0.46
	SA	20	2	0.05	0.05
Svit048	**SE**	20	5	1.00	0.63
	UK	20	6	0.90	0.69
	Total	79	8	0.58	0.46
	WA	20	7	0.70	0.67
	SA	20	2	0.05	0.05
Svit054	**SE**	20	6	1.00	0.67
	UK	21	5	0.95	0.70
	Total	81	8	0.68	0.52
	WA	20	5	0.55	0.55
	SA	20	4	0.60	0.57
Svit055	**SE**	20	7	0.95	0.80
	UK	21	7	0.86	0.77
	Total	81	8	0.74	0.67
	WA	20	12	0.60	0.69
	SA	20	4	0.45	0.47
Svit060	**SE**	20	10	0.85	0.76
	UK	20	8	0.90	0.77
	Total	80	13	0.70	0.67
	WA	20	9	0.90	0.86
	SA	20	1	0.00	0.00
Svit106	**SE**	20	10	1.00	0.71
	UK	21	11	0.95	0.8
	Total	81	14	0.71	0.59
	WA	20	5	0.40	0.35
	SA	19	2	0.05	0.05
Svit109	**SE**	20	4	1.00	0.58
	UK	21	5	0.86	0.58
	Total	80	8	0.58	0.39

GenAlEx has a frequency-based population assignment test (Paetkau et al. 1995, 2004). This test allowed prior population source information for the three known populations. The test treated the Fort Wayne *Sander* group as unknown. The test agreed 100% with the prior grouping of known walleye, sauger, and saugeye. The test assigned each of the unknowns to one of those groups (Table 3). The most likely population each fish belongs to is displayed in Figure 4.

Table 3. GenAlEx broodstock and unknown species assignment. Species assigned based off of Log genotype likelihood for walleye, sauger, and saugeye.

	GenAlEx Analysis						
	Broodstock			Unknown			
	Lake Erie	Ohio River	Hatchery	Hurshtown	St. Joe	St. Mary	Maumee
Walleye	20	0	0	3	0	0	2
Sauger	0	20	0	0	0	0	0
Saugeye	0	0	20	0	12	1	3

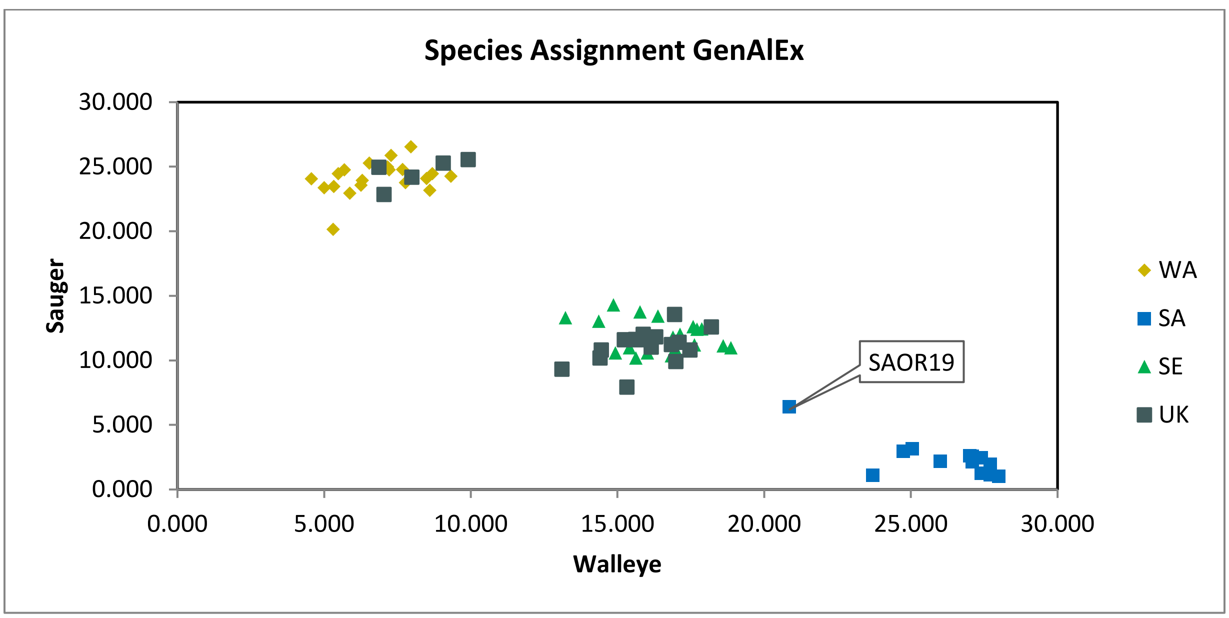

Figure 4. GenAlEx frequency-based species assignment. –Log likelihoods shown as positive. Lowest value indicates the most likely species. WA-Walleye, SA-Sauger, SE-Saugeye, UK-Unknown. SAOR19 log likelihood values: SA(6.400) SE(8.158). Unknown group treated as unknown.

The species assignment of broodstock and known hybrids did not agree 100% with prior information (Table 4). One of the sauger (SAOR19) was identified as a hybrid. The unknown group was assigned the same way as they were by GenAlEx. A summary of the q-values assigned by STRUCTURE can be seen in Figure 5. Each fish appears as a green bar in the histogram. The y-axis displays the q-value and the x-axis labels each group: 1 – known walleye, 2 – known sauger, 3 – known saugeye, and 4 – unknown

Table 4. STRUCTURE broodstock and unknown species assignment. Q-value thresholds to assign hybrids at 0.8 and 0.2.

	Structure Analysis						
	Broodstock			Unknown			
	Lake Erie	Ohio River	Hatchery	Hurshtown	St. Joe	St. Mary	Maumee
Walleye (q>.8)	20	0	0	3	0	0	2
Sauger (.8>q>.2)	0	19	0	0	0	0	0
Saugeye (q<.2)	0	1	20	0	12	1	3

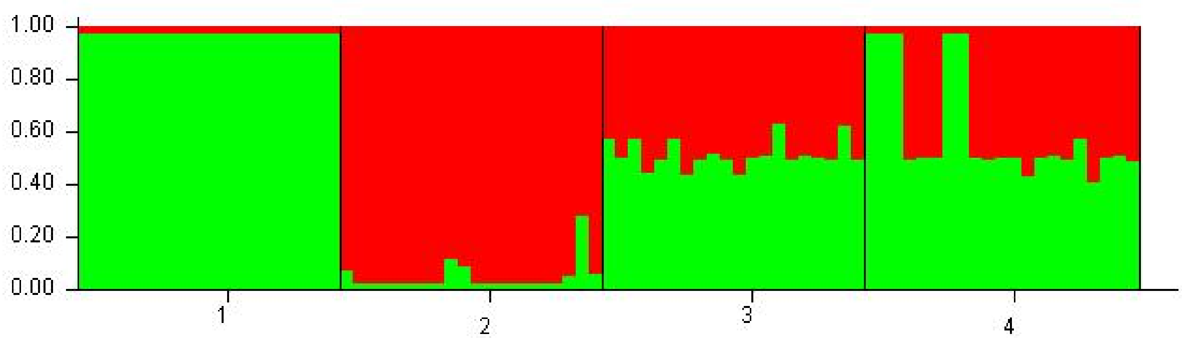

Figure 5. STRUCTURE q-value sorted by population on the x axis: 1 – known walleye, 2 – known sauger, 3 – known saugeye, 4 – unknown. Q-values shown as green bars. Q-values displayed on the y axis.

NewHybrids 1.1 (Anderson and Thompson, 2002) is designed to take a Bayesian approach to not only identify hybrids, but to assign hybrids to a class. The program can determine between first and second generation hybrids and between hybrid backcrosses to either parent species.

Posterior probabilities were assigned after a burn-in period of 10^4 iterations and 10^5 iterations of Markov chain Monte Carlo. The NewHybrids analysis confirms the STRUCTURE analysis and identifies sauger SAOR19 as a backcross hybrid to sauger (Table 5). The analysis of the unknown population found that all the previously identified walleye were pure walleye and all of the previously identified hybrids were first generation hybrids (Table 5).

Table 5. NewHybrids broodstock and unknown species and hybrid assignment.

	NewHybrids Analysis						
	Broodstock				Unknown		
	Lake Erie	Ohio River	Hatchery	Hurshtown	St. Joe	St. Mary	Maumee
Walleye	20	0	0	3	0	0	2
BC Walleye	0	0	0	0	0	0	0
F1 Hybrid	0	19	20	0	12	1	3
F2 Hybrid	0	0	0	0	0	0	0
BC Sauger	0	1	0	0	0	0	0
Sauger	0	0	0	0	0	0	0

The primary objective of the research is to identify what species of *Sander* are present in the Fort Wayne Rivers. A comparison of the three analyses for broodstock and known hybrids are displayed in Appendix D. GenAlEx, STRUCTURE, and NewHybrids analyses are in agreement for each of the parent species and known hybrids except for individual SAOR19 (Tables 6 and 7). GenAlEx identified this fish as a sauger while the other two analyses identified it as a hybrid. The GenAlEx results do make sense in light of the NewHybrids results indicating this fish as a backcross hybrid to sauger. SAOR19 is a fish originally identified as a known sauger caught in the Ohio River and kept at the London State Fish Hatchery to be used as broodstock. While GenAlEx identified the fish as a sauger, it was evident through GenAlEx this fish was more similar to hybrids than the other known sauger (Figure 4). This result makes sense in light of the fact that walleye also occur in the Ohio River. Hybridization and backcrossing can occur. Before tissue samples were collected at London State Fish Hatchery, an Ohio DNR fisheries biologist removed many other sauger that showed physical traits of hybridization. Examining a photo of individual SAOR19, there are some hybrid traits (Appendix C). On the first dorsal fin there is smearing of the spots normally distinct on sauger. There is also a white spot at the tip of the caudal fin indicating hybridization.

Table 6. Summary of broodstock indentification in GenAlEx, STRUCTURE, and NewHybrids. N_{WA}-number of walleye, N_{SA}-number of sauger, and N_{SE}-number of saugeye.

Broodstock Comparison Summary			
Program	N_{WA}	N_{SA}	N_{SE}
GenAlEx	20	20	20
STRUCTURE	20	19	21
NewHybrids	20	19	21

A detailed comparison of the three analyses for unknown Fort Wayne caught fish are displayed in Appendix E. GenAlEx, STRUCTURE, and NewHybrids all agreed on the identification of the unknown *Sander* caught in the Fort Wayne Rivers (Table 7). All three fish caught in Hurshtown Reservoir were identified as walleye. This was the expected outcome for these fish based off of physical characteristics. In the Maumee River, 3 of the 5 fish collected were identified as saugeye. NewHybrids identified them as first generation hybrids. The other two fish from the Maumee River were identified as walleye by all three programs. The rest of the fish caught in the St. Joseph and St. Marys Rivers were identified as saugeye. NewHybrids identified each of these as first generation hybrids.

Table 7. Summary of unknown species identification in GenAlEx, STRUCTURE, and NewHybrids. N_{WA}-number of walleye, N_{SA}-number of sauger, and N_{SE}-number of saugeye.

Unknown Comparison Summary			
Program	**N_{WA}**	**N_{SA}**	**N_{SE}**
GenAlEx	5	0	16
STRUCTURE	5	0	16
NewHybrids	5	0	16

Because some fish were caught in 2018 and some in 2019, results are reported as age when caught and year born (Table 8). None of the fish caught were over three years old. Scales were not collected from one of the walleye in Hurshtown Reservoir because it was deceased. Another fish caught on the Maumee had all regenerated scales collected so an age was not able to be estimated. This fish was the same size as other three year old fish.

Table 8. Age when caught and year born of fish caught in 2018 and 2019.

	Scale Aging Summary						
	Age When Caught			Year Born			
	1	**2**	**3**	**2015**	**2016**	**2017**	**2018**
2018	5	4	3	3	4	5	0
2019	3	3	1	0	1	3	3
Total	8	7	4	3	5	8	3

CHAPTER 4. DISCUSSION

4.1 Species Identification

The primary objective of this research was to determine whether *Sander* currently present in the Fort Wayne Rivers are walleye or saugeye. Of the eighteen *Sander* collected from the Fort Wayne Rivers, sixteen were saugeye and two were walleye. Results from one frequency-based analysis and two model-based analyses of seven microsatellite loci agreed. Of the seven loci that amplified consistently, four were diagnostic and three were informative. Of the informative loci, two had one shared allele and the other loci had two shared alleles. The frequency of shared alleles was very low in sauger, (3-8%) at each locus. This low amount of overlap indicated that these loci were very informative for frequency-based analysis. Of the eighteen *Sander* caught in the Fort Wayne Rivers, one was caught in the St. Marys River, five were caught in the Maumee River, and twelve were caught in the St. Joseph River. The two walleye found in the survey were both in the Maumee River.

4.2 Potential Sources

By combining the results of species identification with known stocking efforts and hatchery operations, I was able to propose potential sources of walleye and saugeye. Surveys done in the 1990s by Indiana DNR biologists speculate that hybrids were coming from a hatchery near the St. Marys River called Grand Lake St. Marys Hatchery. Evidence from this study supports this hypothesis. The source of walleye found in the Three Rivers near Fort Wayne is a bit more speculative. There are five potential sources of walleye for the three rivers of Fort Wayne

Saugeye are most likely coming from Grand Lake St. Marys Hatchery near St. Marys, Ohio. Evidence to support this includes long-term records that show no sauger detected in any of the surveys conducted near the Fort Wayne area. Another piece of evidence against natural hybridization in the rivers is the lack of sauger in these rivers. All saugeye caught for this research were first generation hybrids. The lack of second generation hybrids and backcross progeny suggests that sauger do not exist in this system. Another piece of evidence supporting the "hatchery source" is the timing of saugeye production at the hatchery and when saugeye speculation began in Fort Wayne. Saugeye production at the hatchery began in 1987 and just five years later Fort Wayne regional biologists began to speculate that some of the walleye they were finding in surveys were hybrids. Finally, molecular analyses show that the unknown and reference saugeye from Grand Lake St. Marys hatchery grouped genetically similar to one another.

Saugeye likely escape from the hatchery when the grate is removed to let water out of the pond. It is possible that some fish make it through to the drain, and into the St. Marys River. Once in the St. Marys River, fish would only need to swim over one small boulder dam downstream of the St. Rt. 81 bridge in Willshire, Ohio to reach Fort Wayne.

The potential sources of walleye in the Fort Wayne Rivers are St. Marys Hatchery, the stocking of walleye in the 1990s, Clear Lake, Lake Erie, and Hurshtown Reservoir. If saugeye can swim from the St. Marys Hatchery to Fort Wayne, then why not the walleye too? Walleye production at the hatchery began in 1951 though, and *Sander* were not detected in Fort Wayne surveys until 1980. In spite of 133,000 walleye stocked in the Three Rivers from 1995-1998, there appears to be very little survival and reproduction. However, I cannot exclude the possibility that some walleye did survive and reproduced in the Three Rivers. There is no evidence that *Sander* are reproducing from this study. The walleye caught in this study were younger than 3 years old, younger than the age of maturity for females. At the time of the walleye stockings, regional biologists conducted recapture surveys and considered the stocking efforts unsuccessful. While natural reproduction cannot be excluded as a possibility, it is unlikely based on the recapture surveys in the 1990s and age of fish caught in this study.

Another possible source for walleye found in the Fort Wayne Rivers is Clear Lake in the very Northeast corner of Indiana. Walleye are stocked consistently by Indiana DNR in Clear Lake. The lake is connected to the St. Joseph River through a series of ditches. However, some of these are ephemeral, greatly limiting the passage of walleye. Aerial photographs show that many miles of the connection are very shallow and narrow.

Lake Erie is a potential source of walleye in the Three Rivers. Evidence to support this is that walleye found in the Maumee River were genetically similar to those of reference walleye from Lake Erie. Evidence against Lake Erie is that for fish to swim to the Fort Wayne area from Lake Erie there are two dams to surpass. The first is at Jane Thurston State Park in Grand Rapids, Ohio. The second is Independence Dam northeast of Defiance, Ohio. These are both significant sized dams without fish ladders. The only potential for walleye to overcome these dams would be when flooding conditions overtake the tops of the dams. When water levels are this high though, flow rates are very high which would make the trek difficult. Interestingly, walleye were not found above the Hosey Dam in Fort Wayne. This makes sense if walleye are coming from Lake Erie, although it does not exclude other source possibilities. Finally, there is potential for walleye to be escaping from Hurshtown Reservoir. The walleye in Hurshtown and the Maumee River grouped genetically similar to each other. The reservoir, which is an emergency drinking supply for the city of Fort Wayne gets its water from the St. Joseph River. There is direct drainage from the reservoir back to the St. Joseph as well. To my knowledge, water has not been drained from the reservoir back to the river since its construction. A land mass about 800 feet wide separate the two bodies of water and heron activity is high along the St. Joseph River in this area. There is a slight possibility that walleye could be moved across this land mass by herons and people.

The most likely explanation for walleye and saugeye in the Fort Wayne Rivers is inadvertent escape from an inland hatchery. The swim from St. Marys Hatchery to Fort Wayne is a considerable distance. The St. Marys River is 99 miles from its origin near St. Marys, Ohio to Fort Wayne. The hatchery is located near its origin meaning *Sander* would need to swim nearly all 99 miles. Walleye and sauger are the most migratory species of the Percidae family. *Sander* have traveled distances of 131 mi (Carbine and Applegate, 1946), 175 mi (Ferguson and Derksen, 1971), and 236 mi (Wolfert, 1963) to reach spawning grounds annually. In the Tennessee River, saugers swam over 124 mi in 10 days (Pegg, Bettoli, and Layzer, 1997). In light of the previous research done on the migratory movements of *Sander* species, the movement of walleye and saugeye into Fort Wayne is certainly possible.

4.3 Saugeye Fishery Establishment

Since walleye stocking failed to increase walleye numbers in the Fort Wayne Rivers in the 1990s, could saugeye stocking be an alternative? Afterall, the majority of *Sander* caught in this study were saugeye. From 2011 to 2014, an average of 1.4M walleye and 3.0M saugeye were produced at Grand Lake St. Marys Hatchery each year (Yost, 2015). Roughly a third of all *Sander* produced at the hatchery are walleye yet walleye only made up about 11% of *Sander* caught in the Fort Wayne Rivers. The higher proportion of saugeye may be evidence of hybrid vigor (Lynch et al., 1982 and Malison et al., 1990) or their increased resilience to warm eutrophic conditions (Fiss et al., 1997). The Ohio DNR raises saugeye for put and take fisheries in Ohio Reservoirs where water quality does not support walleye populations. It is very likely that saugeye would perform better than walleye in the Fort Wayne Rivers, however, a stocking program should not begin without carefully considering the consequences.

Saugeye stocked in the Fort Wayne Rivers could potentially swim downstream to Lake Erie where a significant walleye population exists. Saugeye have been found to have differing degrees of reproductive success. This could have significant ramifications on Lake Erie walleye. Saugeye reproduction and backcrossing was documented in Tennessee. After walleye stocking was ineffective, saugeye were stocked in Normandy Reservoir, Tennessee throughout the 1980s intended for a put and take fishery. Of 35 *Sander* collected from the reservoir in 1994, 24 were either backcrossed or F_2 hybrids, 1 was an F_1 hybrid, and 1 was an F_2 hybrid (Fiss et al., 1997). In this case, the potential for saugeye reproduction places the genetic integrity of downstream walleye populations at risk.

Stocking sterile, triploid saugeye could be a safer strategy than stocking diploid hybrids. To assess the effectiveness of stocking diploid and triploid saugeye, equal numbers of 3-6 day old diploid and triploid saugeye were stocked in four different reservoirs in Kansas for three years. After two years of sampling, diploid saugeye comprised approximately 80% of saugeye recruitment among the four reservoirs. Mean length of diploid saugeye was greater than triploids at age 0 with no significant difference between the groups at ages 1 and 2 (Koch et. al, 2018). The survival of diploid saugeye was about four times that of triploid saugeye. The increased recruitment of diploid over triploid saugeye suggests that even though triploid saugeye are the safe option, managers should carefully weigh the potential for genetic integration. Although diploid saugeye are more cost effective, they should only be stocked where the risk of impact to the existing communities is low.

Without knowing the home range of Fort Wayne saugeye, it is risky to stock saugeye in the Three Rivers. To assess whether or not triploid saugeye would be necessary to preserve the genetic integrity of Lake Erie walleye, radiotelemetry could be used to assess the home range of Fort Wayne saugeye and Lake Erie walleye. River dwelling saugeye and lake dwelling walleye may have differences in reproductive strategies that vary enough to act as a barrier to their genetic integration. Although I was not able to find documentation of this occurring between walleye and saugeye, I did find an example between river dwelling and lake dwelling walleye. This occurred in Claytor Lake, Virginia and its main influent, the New River. Fifty-two walleye were tagged in Claytor Lake and the upper New River for two years. Even though no physical barrier existed between the two populations, they remained distinct. The lake residing walleye spawned separately from river residing walleye (Palmer, Murphy, and Hallerman, 2005). It could be the case that river dwelling saugeye do overlap with Lake Erie walleye but lack introgression because of differences in reproduction timing and preferences.

The state of Ohio has been stocking diploid saugeye in numerous reservoirs in Lake Erie watersheds, including the Maumee watershed, for over thirty years. If saugeye stocked upstream in the Lake Erie watersheds were reaching Lake Erie, surveys done on these rivers should have detected saugeye movement out of the reservoirs. While I could not find research specific to the Lake Erie drainage systems, I could find data on saugeye movement in the Ohio River watershed. The Ohio DNR stocks saugeye in Deer Creek Reservoir, Ohio. Two hundred and three saugeye were tracked for nearly two years. Overall, those saugeye spent 90% of their time remaining in the headwaters, 7-8% of their time just downstream, and only 2-3% of their time 28 miles or more downstream. None of the tagged saugeye reached the Ohio River 96 miles downstream (Spoelstra et. al, 2008). For reference, Lake Erie is more than 100 miles downstream of Fort Wayne. The probability of movement out of the headwaters was highest during high flow, spawning, and low dissolved oxygen levels. Applying these rates to Fort Wayne and the Maumee River would suggest that the movement of saugeye would have a minimal/no effect on Lake Erie populations. The movement of saugeye seemed to be related to the suitability of tailwater over upstream habitat. Because of this, caution should still be used in applying these metrics to other river environments.

Finally, there is no evidence that 30 years of saugeye stocking in Ohio reservoirs has effected the Lake Erie walleye population. Also, in all the research I have done, there is no mention of hybrid saugeye showing up in Lake Erie surveys. With this information it seems very unlikely that saugeye fishery establishment in Fort Wayne will have a negative effect on Lake Erie walleye. Before saugeye fishery stocking is initiated, we need to know the movement of saugeye in the Fort Wayne area. Fort Wayne saugeye home range information will give managers greater confidence in establishing a saugeye fishery in Fort Wayne.

CHAPTER 5. CONCLUSION

Sixteen of the eighteen *Sander* caught in the Fort Wayne Rivers between 2018 and 2019 were first generation saugeye. The other two were walleye found in the Maumee River downstream of Hosey Dam. An additional three *Sander* were caught in Hurshtown Reservoir and verified to be walleye. It is most likely that saugeye are coming from a hatchery at Grand Lake St. Marys which raises both saugeye and walleye. There are many potential sources for walleye in Fort Wayne. I believe they are most likely come from the hatchery via the St. Marys River as well. Other walleye source possibilities include reproduction from the late 1990s stocking, Clear Lake, Lake Erie, and Hurshtown Reservoir.

This information is informative for potential *Sander* fishery establishment in the Fort Wayne Rivers. Walleye and saugeye are evolutionarily equipped to survive in turbid rivarian systems. The attempt to increase walleye numbers in the Three Rivers in the 1990s was unsuccessful. Saugeye are known to be more tolerant of warm, eutrophic, and turbid environments than walleye. Therefore, stocking saugeye may be an alternative to increase fishing opportunities in Fort Wayne. Tracking Fort Wayne saugeye to better understand their home range is valuable information to determine whether diploid or triploid saugeye should be stocked. A saugeye recreational fishery may be of interest to the City of Fort Wayne in light of the Riverfront Development project. In the correspondences I had with anglers I got the impression that *Sander* fishing in Fort Wayne may be one of the best kept secrets. There is an incredible potential for this resource to grow in the City of Fort Wayne.

APPENDIX A. HISTORICAL SURVEYS SUMMARY

Appendix A. List of species collected in the Three-Rivers Area of Fort Wayne, Indiana in 1893, 1945, and 2009

	St. Marys		
Species	1893	1945	2009
Bigeye Chub (Hybopsis amblops)			
Bigmouth Buffalo (Ictiobus cyprinellus)			X
Black Bullhead (Ameiurus melas)		X	
Black Redhorse (Moxostoma duquesnii)			X
Blackside Darter (Percina maculata)		X	
Blackstripe Topminnow (Fundulus notatus)		X	
Black Crappie (Pomoxis nigromaculatus)			X
Bowfin (Amia calva)			X
Bluegill (Lepomis macrochirus)			X
Bluntnose Minnow (Pimephales notatus)	X	X	
Brindled Madtom (Noturus miurus)	X		
Brook Silverside (Labidesthes sicculus)			
Brown Bullhead (Ameiurus nebulosus)	X		
Carp (Cyprinus carpio)			X
Central Stoneroller (Campostoma anomalum)		X	
Channel Catfish (Ictalurus punctatus)	X	X	X
Common Shiner (Luxilus cornutus)			
Creek Chub (Semotilus atromaculatus)		X	
Eastern Sand Darter (Ammocrypta pellucida)	X		
Emerald Shiner (Notropis atherinoides)			
Fathead minnow (Pimephales promelas)		X	
Flathead Catfish (Pylodictis olivaris)			X
Freshwater Drum (Aplodinotus grunniens)			X
Gilt Darter (Percina evides)			
Gizzard Shad (Dorosoma cepedianum)	X		
Golden Redhorse (Moxostoma erythrurum)			X
Golden Shiner (Notemigonus crysoleucas)			
Goldfish (Carassius auratus)			
Greenside Darter (Etheostoma blennioides)	X	X	
Green Sunfish (Lepomis cyanellus)	X	X	X
Grass Pickerel (Esox americanus)	X		
Highfin Carpsucker (Carpiodes velifer)	X		
Hornyhead Chub (Nocomis biguttatus)			
Hybrid Sunfish			
Johnny Darter (Etheostoma nigrum)	X	X	

Largemouth Bass (Micropterus salmoides)	X		X
Logperch (Percina caprodes)	X		
Longear Sunfish (Lepomis megalotis)	X		
Longnose Gar (Lepisosteus osseus)	X		X
Mimic Shiner (Notropis volucellus)			
Mottled Sculpin (Cottus bairdii)			
Northern Hogsucker (Hypentelium nigricans)	X		X
Orangethroat Darter (Etheostoma spectabile)		X	
Orangespotted sunfish (Lepomis humilis)		X	X
Pumpkinseed (Lepomis gibbosus)			
Quillback Carpsucker (Carpiodes cyprinus)			X
Rainbow Darter (Etheostoma caeruleum)			
Redear Sunfish (Lepomis microlophus)			
Redfin Shiner (Lythrurus umbratilis)		X	
River Chub (Nocomis micropogon)	X		
River Redhorse (Moxostoma carinatum)			X
Rock Bass (Amblopites rupestris)	X		X
Rosefin Shiner (Lythrurus ardens)	X		
Rosyface Shiner (Notropis rubellus)			
Sand Shiner (Notropis stramineus)		X	
Saugeye (Sander vitreus x Sander canadensis)			
Shorthead Redhorse (Moxostoma macrolepidotum)	X		X
Silver Shiner (Notropis photogenis)			
Silverjaw Minnow (Notropis buccatus)	X	X	
Silver Redhorse (Moxostoma anisurum)	X	X	X
Smallmouth Bass (Micropterus dolomieu)	X		X
Smallmouth Buffalo (Ictiobus bubalus)			X
Spotfin Shiner (Cyprinella spiloptera)		X	
Spotted Sucker (Minytrema melanops)			X
Steelcolor Shiner (Cyprinella whipplei)	X		
Stoneroller (Garra gotyla)			
Stonecat (Noturus flavus)		X	
Tadpole Madtom (Noturus gyrinus)	X	X	
Texas Shiner (Notropis amabilis)	X		
Walleye (Sander vitreus)			X
Weed Shiner (Notropis texanus)	X		
White Crappie (Pomoxis annularis)			
White Sucker (Catostomus commersonii)	X	X	
Yellow Bullhead (Ameiurus natalis)		X	
Yellow Perch (Perca flavescens)			

	St. Joseph			
Species	1893	1945	1989	2009
Bigeye Chub (Hybopsis amblops)	X	X		
Bigmouth Buffalo (Ictiobus cyprinellus)			X	X
Black Bullhead (Ameiurus melas)			X	
Black Redhorse (Moxostoma duquesnii)				X
Blackside Darter (Percina maculata)		X		
Blackstripe Topminnow (Fundulus notatus)			X	
Black Crappie (Pomoxis nigromaculatus)			X	
Bowfin (Amia calva)				
Bluegill (Lepomis macrochirus)			X	X
Bluntnose Minnow (Pimephales notatus)	X	X	X	
Brindled Madtom (Noturus miurus)	X	X		
Brook Silverside (Labidesthes sicculus)				
Brown Bullhead (Ameiurus nebulosus)	X			
Carp (Cyprinus carpio)		X	X	X
Central Stoneroller (Campostoma anomalum)	X	X		
Channel Catfish (Ictalurus punctatus)	X		X	X
Common Shiner (Luxilus cornutus)		X	X	
Creek Chub (Semotilus atromaculatus)	X	X	X	X
Eastern Sand Darter (Ammocrypta pellucida)	X	X		
Emerald Shiner (Notropis atherinoides)	X			
Fathead minnow (Pimephales promelas)		X		
Flathead Catfish (Pylodictis olivaris)				
Freshwater Drum (Aplodinotus grunniens)			X	X
Gilt Darter (Percina evides)	X			
Gizzard Shad (Dorosoma cepedianum)	X		X	X
Golden Redhorse (Moxostoma erythrurum)		X	X	X
Golden Shiner (Notemigonus crysoleucas)	X		X	
Goldfish (Carassius auratus)			X	
Greenside Darter (Etheostoma blennioides)	X	X		
Green Sunfish (Lepomis cyanellus)	X		X	X
Grass Pickerel (Esox americanus)	X	X		
Highfin Carpsucker (Carpiodes velifer)	X			
Hornyhead Chub (Nocomis biguttatus)		X		
Hybrid Sunfish			X	X
Johnny Darter (Etheostoma nigrum)	X	X		
Largemouth Bass (Micropterus salmoides)		X	X	X
Logperch (Percina caprodes)	X	X	X	X
Longear Sunfish (Lepomis megalotis)		X	X	
Longnose Gar (Lepisosteus osseus)	X		X	
Mimic Shiner (Notropis volucellus)		X		
Mottled Sculpin (Cottus bairdii)		X		

Northern Hogsucker (Hypentelium nigricans)	X	X	X	X
Orangethroat Darter (Etheostoma spectabile)				
Orangespotted sunfish (Lepomis humilis)		X	X	X
Pumpkinseed (Lepomis gibbosus)			X	X
Quillback Carpsucker (Carpiodes cyprinus)			X	X
Rainbow Darter (Etheostoma caeruleum)		X		
Redear Sunfish (Lepomis microlophus)			X	
Redfin Shiner (Lythrurus umbratilis)		X	X	
River Chub (Nocomis micropogon)	X			
River Redhorse (Moxostoma carinatum)				X
Rock Bass (Amblopites rupestris)	X	X	X	X
Rosefin Shiner (Lythrurus ardens)	X			
Rosyface Shiner (Notropis rubellus)		X		
Sand Shiner (Notropis stramineus)		X		
Saugeye (Sander vitreus x Sander canadensis)				X
Shorthead Redhorse (Moxostoma macrolepidotum)	X		X	X
Silver Shiner (Notropis photogenis)		X		
Silverjaw Minnow (Notropis buccatus)	X	X		
Silver Redhorse (Moxostoma anisurum)	X			X
Smallmouth Bass (Micropterus dolomieu)	X	X	X	X
Smallmouth Buffalo (Ictiobus bubalus)				
Spotfin Shiner (Cyprinella spiloptera)		X		
Spotted Sucker (Minytrema melanops)	X		X	X
Steelcolor Shiner (Cyprinella whipplei)	X		X	
Stoneroller (Garra gotyla)			X	
Stonecat (Noturus flavus)	X			
Tadpole Madtom (Noturus gyrinus)	X			
Texas Shiner (Notropis amabilis)	X			
Walleye (Sander vitreus)			X	
Weed Shiner (Notropis texanus)	X			
White Crappie (Pomoxis annularis)			X	X
White Sucker (Catostomus commersonii)	X	X	X	
Yellow Bullhead (Ameiurus natalis)				X
Yellow Perch (Perca flavescens)			X	

	Maumee		
Species	1893	1945	2009
Bigeye Chub (Hybopsis amblops)	X		
Bigmouth Buffalo (Ictiobus cyprinellus)			X
Black Bullhead (Ameiurus melas)		X	
Black Redhorse (Moxostoma duquesnii)			
Blackside Darter (Percina maculata)			
Blackstripe Topminnow (Fundulus notatus)			
Black Crappie (Pomoxis nigromaculatus)	X		
Bowfin (Amia calva)			
Bluegill (Lepomis macrochirus)			X
Bluntnose Minnow (Pimephales notatus)	X	X	
Brindled Madtom (Noturus miurus)	X		
Brook Silverside (Labidesthes sicculus)	X		
Brown Bullhead (Ameiurus nebulosus)	X		
Carp (Cyprinus carpio)		X	X
Central Stoneroller (Campostoma anomalum)	X	X	
Channel Catfish (Ictalurus punctatus)	X	X	X
Common Shiner (Luxilus cornutus)			
Creek Chub (Semotilus atromaculatus)	X	X	
Eastern Sand Darter (Ammocrypta pellucida)	X		
Emerald Shiner (Notropis atherinoides)	X		
Fathead minnow (Pimephales promelas)		X	
Flathead Catfish (Pylodictis olivaris)			X
Freshwater Drum (Aplodinotus grunniens)			X
Gilt Darter (Percina evides)	X		
Gizzard Shad (Dorosoma cepedianum)	X	X	X
Golden Redhorse (Moxostoma erythrurum)		X	
Golden Shiner (Notemigonus crysoleucas)	X	X	
Goldfish (Carassius auratus)			
Greenside Darter (Etheostoma blennioides)	X		
Green Sunfish (Lepomis cyanellus)	X	X	X
Grass Pickerel (Esox americanus)	X		
Highfin Carpsucker (Carpiodes velifer)	X		
Hornyhead Chub (Nocomis biguttatus)			
Hybrid Sunfish			X
Johnny Darter (Etheostoma nigrum)	X		
Largemouth Bass (Micropterus salmoides)	X	X	
Logperch (Percina caprodes)	X		X
Longear Sunfish (Lepomis megalotis)	X	X	
Longnose Gar (Lepisosteus osseus)	X		X
Mimic Shiner (Notropis volucellus)		X	
Mottled Sculpin (Cottus bairdii)			

Northern Hogsucker (Hypentelium nigricans)	X		
Orangethroat Darter (Etheostoma spectabile)			
Orangespotted sunfish (Lepomis humilis)			X
Pumpkinseed (Lepomis gibbosus)			
Quillback Carpsucker (Carpiodes cyprinus)		X	X
Rainbow Darter (Etheostoma caeruleum)			
Redear Sunfish (Lepomis microlophus)			
Redfin Shiner (Lythrurus umbratilis)		X	
River Chub (Nocomis micropogon)	X		
River Redhorse (Moxostoma carinatum)			X
Rock Bass (Amblopites rupestris)	X		X
Rosefin Shiner (Lythrurus ardens)	X		
Rosyface Shiner (Notropis rubellus)			
Sand Shiner (Notropis stramineus)		X	
Saugeye (Sander vitreus x Sander canadensis)			X
Shorthead Redhorse (Moxostoma macrolepidotum)	X		X
Silver Shiner (Notropis photogenis)			
Silverjaw Minnow (Notropis buccatus)	X	X	
Silver Redhorse (Moxostoma anisurum)	X		X
Smallmouth Bass (Micropterus dolomieu)	X		
Smallmouth Buffalo (Ictiobus bubalus)			X
Spotfin Shiner (Cyprinella spiloptera)		X	
Spotted Sucker (Minytrema melanops)	X		X
Steelcolor Shiner (Cyprinella whipplei)	X		
Stoneroller (Garra gotyla)			
Stonecat (Noturus flavus)	X		
Tadpole Madtom (Noturus gyrinus)	X		
Texas Shiner (Notropis amabilis)	X		
Walleye (Sander vitreus)			
Weed Shiner (Notropis texanus)	X		
White Crappie (Pomoxis annularis)		X	X
White Sucker (Catostomus commersonii)	X	X	
Yellow Bullhead (Ameiurus natalis)			
Yellow Perch (Perca flavescens)		X	

APPENDIX B. SANDER IDENTIFICATION GUIDE

Sander of Indiana

B.E. Fisher (11/2018)

APPENDIX C. SAOR19 PICTURES

APPENDIX D. SPECIES IDENTIFICATION SUMMARY OF BROODSTOCK AND HATCHERY FISHES

GenAlEx, STRUCTURE, and NewHybrids.

Broodstock and Hatchery Species Assignment			
Individual	GenAlEx	STRUCTURE	NewHybrids
WALE01	WA	WA	WA
WALE02	WA	WA	WA
WALE03	WA	WA	WA
WALE04	WA	WA	WA
WALE05	WA	WA	WA
WALE06	WA	WA	WA
WALE07	WA	WA	WA
WALE08	WA	WA	WA
WALE09	WA	WA	WA
WALE10	WA	WA	WA
WALE11	WA	WA	WA
WALE12	WA	WA	WA
WALE13	WA	WA	WA
WALE14	WA	WA	WA
WALE15	WA	WA	WA
WALE16	WA	WA	WA
WALE17	WA	WA	WA
WALE18	WA	WA	WA
WALE19	WA	WA	WA
WALE20	WA	WA	WA
SAOR01	SA	SA	SA
SAOR02	SA	SA	SA
SAOR03	SA	SA	SA
SAOR04	SA	SA	SA
SAOR05	SA	SA	SA
SAOR06	SA	SA	SA
SAOR07	SA	SA	SA
SAOR08	SA	SA	SA
SAOR09	SA	SA	SA
SAOR10	SA	SA	SA
SAOR11	SA	SA	SA
SAOR12	SA	SA	SA
SAOR13	SA	SA	SA
SAOR14	SA	SA	SA
SAOR15	SA	SA	SA
SAOR16	SA	SA	SA

SAOR17	SA	SA	SA
SAOR18	SA	SA	SA
SAOR19	SA	SE	BCSA
SAOR20	SA	SA	SA
SEHA01	SE	SE	SE
SEHA02	SE	SE	SE
SEHA03	SE	SE	SE
SEHA04	SE	SE	SE
SEHA05	SE	SE	SE
SEHA06	SE	SE	SE
SEHA07	SE	SE	SE
SEHA08	SE	SE	SE
SEHA09	SE	SE	SE
SEHA10	SE	SE	SE
SEHA11	SE	SE	SE
SEHA12	SE	SE	SE
SEHA13	SE	SE	SE
SEHA14	SE	SE	SE
SEHA15	SE	SE	SE
SEHA16	SE	SE	SE
SEHA17	SE	SE	SE
SEHA18	SE	SE	SE
SEHA19	SE	SE	SE
SEHA20	SE	SE	SE

APPENDIX E. SPECIES IDENTIFICATION SUMMARY OF UNKNOWN FORT WAYNE CAUGHT FISHES

	Unknown Species Assignment		
Individual	GenAlEx	STRUCTURE	NewHybrids
UKHU01	WA	WA	WA
UKHU02	WA	WA	WA
UKHU03	WA	WA	WA
UKMA01	SE	SE	SE
UKMA02	SE	SE	SE
UKMA03	WA	WA	WA
UKMA04	WA	WA	WA
UKMA05	SE	SE	SE
UKSJ01	SE	SE	SE
UKSJ02	SE	SE	SE
UKSJ03	SE	SE	SE
UKSJ04	SE	SE	SE
UKSJ05	SE	SE	SE
UKSJ06	SE	SE	SE
UKSJ07	SE	SE	SE
UKSJ08	SE	SE	SE
UKSJ09	SE	SE	SE
UKSJ10	SE	SE	SE
UKSJ11	SE	SE	SE
UKSJ12	SE	SE	SE
UKSM01	SE	SE	SE

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