DEMOGRAPHIC AND LIFE HISTORY CHARACTERISTICS OF REMNANT LAKE STURGEON POPULATIONS IN THE UPPER GREAT LAKES BASIN: INFERENCES BASED ON GENETIC ANALYSES

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Fisheries and Wildlife

2003

ABSTRACT

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While once abundant throughout the Great Lakes, lake sturgeon (*Acipenser fulvescens*) have experienced dramatic population declines over the past 100 years. Declines are due mainly to anthropogenic activities including over-harvest, blockage of spawning habitat by dams, and loss of spawning habitat due to sedimentation and pollution. While the need for rehabilitation has been recognized, knowledge of many fundamental aspects of this species life history are currently lacking. The objectives of this study were to employ molecular genetic markers to 1) assess the degree of genetic variability and assess levels of population structure present between remnant lake sturgeon populations in the Upper Great Lakes Basin 2) to determine parentage and describe the lake sturgeon mating system 3) to examine the genetic consequences of a supportive breeding program designed to rehabilitate a declining lake sturgeon population. The results of this study show that a significant degree of population structure exists among remnant lake sturgeon populations. Using genetic determination of parentage, this study describes the lake sturgeon mating system and documents a high degree of polygyny and polyandry as well as variance in male and female reproductive success. Results of this study also indicate that a supportive breeding program for lake sturgeon has led to an overall increase in relatedness. Results and implications of these data are discussed in light of declining population numbers.

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Introduction

The lake sturgeon, *Acipenser fulvescens*, is found in three major drainages throughout North America; The Great Lakes, Hudson Bay, and the Mississippi River. This distribution spans 18 states and 5 Canadian provinces (Houston 1987). Lake sturgeon are members of the order Acipenseriformes, which includes 25 species of sturgeon and two species of paddlefish (Birstein and DeSalle 1998). Species in the order Acipenseriformes are often referred to as "living fossils" because they have existed since the Upper Cretaceous period, over one hundred million years ago. Modern day Acipenseriformes still posses many primitive characteristics, including a cartilaginous skeleton, a heterocercal tail, and five rows of bony scutes along their body (Auer 1999a, Birstein 1993). Acipenseriformes also posses the unique genetic characteristic of polyploidy and have chromosome numbers of 4n, 8n, or 16n (Blacklidge and Bidwell, 1993).

Lake sturgeon have a life history strategy that is common among members of the genus *Acipenser*. Lake sturgeon are mostly potamodromous fishes and spend most of their lives in fresh waters between 4 and 9 meters deep (Houston 1987). In the spring, lake sturgeon migrate up large river systems where they spawn in groups in areas characterized by fast moving water and rocky substrate (Auer 1999a, Beamesderfer and Farr 1997). Lake sturgeon do not construct any type of nest for egg deposition. Lake sturgeon spawn once water temperatures are between 10 and 20° C (Auer 1996, Kempinger 1988, Houston 1987). After the female releases her eggs and they are fertilized by the male, the adhesive eggs stick to rocks and gravel in the river, where they

incubate for 5 to 14 days (Kempinger 1988, Houston 1987). Lake sturgeon typically leave spawning grounds after spawning and make no parental investment in their offspring. After hatching, larval sturgeon remain in the river for 1 to 3 weeks before drifting downstream to larger bodies of water (Auer 1999a, Kempinger 1988).

During the first year of life, lake sturgeon experience rapid growth. In subsequent years, however, growth slows considerably (Harkness and Dymond 1961). Although lake sturgeon have a slow growth rate, they typically grow to sizes between 1 and 2 m and weights of 5 to 40 kg. Lake sturgeon reach sexual maturity in fifteen to twenty-five years; longer than any other freshwater fish (Auer 1999a, Houston 1987). Males generally reach sexual maturity before females (Threader and Brousseau 1986). Once mature, lake sturgeon do not spawn every year, males may spawn every 1 to 4 years and females may spawn every 3 to 7 years (Auer 1999b, Beamish et al. 1996). Both growth rate and age at maturity seem to be inversely related to latitude, which implies a direct relation to temperature (Beamish et al. 1996). Lake sturgeon are one of the longest-lived freshwater fish, often reaching ages of over 100 years (Auer 1999a, Houston 1987).

Historically lake sturgeon were abundant throughout their native range including the Great Lakes. In the late 1880s lake sturgeon were among the five most abundant commercial fishes in the Great Lakes with catches in the millions of kilograms annually (Auer 1999a). While their longevity has historically buffered lake sturgeon and other Acipenseriformes to changes in their environment, in recent years life history characteristics such as delayed maturity, periodicity of spawning and low survivorship of offspring have made lake sturgeon extremely vulnerable to increasing anthropogenically-mediated environmental changes such as increases in pollution and loss of spawning

habitat (Beamesderfer and Farr 1997, Houston 1987). Auer (1999a) identified the three greatest threats to sturgeon populations worldwide as: 1) physical impacts on spawning and nursery habitat, 2) barriers to migration, and 3) effects of fishing. Lake Sturgeon are vulnerable to predation for only a short period of time during the first year of life before their scutes have fully formed (Houston 1987). During this time juvenile lake sturgeon may spend much of their time in rivers or near river mouths after migration. These areas often contain high levels of pollutants from upstream industrial activities. Other environmental stresses, such as water level manipulation by hydroelectric facilities, can also be harmful to juvenile lake sturgeon. In the late 1800s and early 1900s many dams were built on Great Lakes tributaries that prevented lake sturgeon from accessing historic spawning grounds. Erosion due to deforestation and other anthropogenic activities has further degraded many historic lake sturgeon spawning grounds (Auer 1999a, Houston 1987).

Overfishing has been the greatest cause for decline not only for lake sturgeon, but for all species of sturgeon worldwide (Birstein et al. 1997). Prior to the late 1800s, lake sturgeon were largely viewed as a nuisance species because their sharp scutes would often damage or destroy fishing nets (Auer 1999a, Hay-Chmielewski and Whelan 1997). However, around 1860 the value of smoked sturgeon flesh was realized and the annual harvest in the Great Lakes was over a million kilograms before the turn of the century. Sturgeon are also valued for their eggs (caviar) and increased demand for caviar has also contributed significantly to their decline (Houston 1987). Due to drastic declines in lake sturgeon populations throughout their historic range, commercial fishing for lake sturgeon has been closed in the United States but still exists in some areas in Canada

(Auer 1999a). Some recreational fisheries still exist for lake sturgeon in areas with relatively large, self-sustaining populations (Bruch 1999, Thuemler 1997).

Today lake sturgeon populations are believed to be at less than 1% of their former size and very few remnant spawning populations exist in the Great Lakes (Hay-Chmielewski and Whelan 1997). Currently the lake sturgeon is classified as either endangered, threatened, or a species of "special concern" in all of its 18 native states (Auer 1996) and it is considered a species of regional concern by the United States Fish and Wildlife Service (USFWS). Because of the threatened status of the lake sturgeon, the Michigan Department of Natural Resources (MDNR) has developed a lake sturgeon rehabilitation strategy (Hay-Chmielewski and Whelan 1997) which lists its goal as "To conserve and rehabilitate self-sustaining populations of lake sturgeon to a level that will permit delisting as a threatened species under the Michigan Endangered Species Act." Several other states including Wisconsin, Ohio, and New York have developed similar rehabilitation plans for lake sturgeon.

Plans to help rehabilitate lake sturgeon populations in the Great Lakes should be based on an understanding of the species biology and life history. Due to low population numbers, delayed maturity, long life span, and infrequent spawning, little information is known about many aspects of lake sturgeon life history that are critical to the development of effective rehabilitation plans. Genetic information represents one source of data that can be used to help elucidate many of the unknown aspects of lake sturgeon biology and life history. Genetic markers have become increasingly popular for use in fisheries management. Wirgin and Waldman (1994) cite several examples of the utility of genetic data in fisheries management including, stock identification, taxonomic

investigation, identification of hybrids, discrimination of wild and hatchery reared fish, restoration of extinct populations, and forensic analysis.

My thesis utilizes genetic markers to assess different demographic and life history traits that are important to the conservation and management of remnant lake sturgeon populations in the Great Lakes. The first chapter of my thesis focuses on the use of microsatellite and mitochondrial DNA markers to assess the level of genetic diversity and population structuring among remnant lake sturgeon populations in the upper Great Lakes basin and the historical, ecological and anthropogenic forces influencing levels of population structure. Based on this information, decisions regarding stocking can be made which will maintain current levels of population structure and conserve genetic diversity of these remnant stocks.

The second chapter of my thesis examines lake sturgeon reproductive ecology based on genetic determination of parentage. Using microsatellite markers, parentage is determined for a group of juvenile lake sturgeon from the Black River in Michigan. Using this data, estimates of the number of mates per male and female and male and female variance in reproductive success can be made. Based on this behavioral data, an estimate of effective numbers of breeders is calculated as a means to infer the conservation status of this declining population.

The final chapter of my thesis examines the effects of hatchery supplementation of declining lake sturgeon populations. Using genetic markers I assess the levels of relatedness (r_{xy}: Queller and Goodnight 1989) in two groups of lake sturgeon. The first group was composed of fish that spawned naturally in Black Lake, MI during the spring of 2001. The second group was composed of individuals from supportive breeding efforts

in which very few adults were spawned to produce large numbers of offspring. Using data on levels of relatedness, along with demographic data on the Black Lake lake sturgeon population and the lake sturgeon mating system, I identify the factors that will lead to an increase in the amount of inbreeding in this population and the effects that increased inbreeding will likely have on the long-term viability of this population.

CHAPTER I

Genetic Population Structure of Remnant Lake Sturgeon Populations In the Upper Great Lakes Basin

Introduction

Prior to the 1900s lake sturgeon were abundant throughout all of the Great Lakes. Historic numbers of lake sturgeon were estimated to be in the hundreds of thousands in the Great Lakes prior to the 1880s and tens of thousands more in inland lakes and rivers in Canada alone (Houston 1987). In the early 1880s, increased demand for sturgeon flesh and caviar led to the development of a commercial fishery for lake sturgeon in the Great Lakes. Lake sturgeon quickly became one of the five most abundant commercial species in the Great Lakes by the late 1880s with harvests totaling in the millions of kilograms annually (Auer 1999a).

By the early 1900s, however, the lake sturgeon fishery rapidly declined as a result of over-exploitation (Auer 1999a). By 1929 most Great Lakes states had closed their lake sturgeon fisheries. Today commercial fisheries for lake sturgeon only exist in Canadian waters (Auer 1999a). Overfishing, along with the construction of dams that blocked access to historic spawning grounds, and the loss of suitable spawning habitat due to pollution and sedimentation have led to continual and drastic declines in lake sturgeon population numbers throughout the Great Lakes (Auer 1999a, Houston 1987, Harkness and Dymond 1961). Today, lake sturgeon numbers in the Great Lakes are thought to be less than 1% of historic numbers (Hay-Chmielewski and Whelan 1997).

Because of this dramatic decline in population numbers, lake sturgeon have become a species of conservation concern throughout their native range. Many states

have drafted management strategies for lake sturgeon that list stocking of hatchery-reared individuals as a primary means for rehabilitation (WDNR 2000, Hay-Chmielewski and Whelan 1997). Sound management plans for remnant populations and plans to repopulate extirpated populations should be based on a fundamental understanding of the population structure of remnant lake sturgeon populations.

Most organisms exhibit some degree of genetic differentiation between populations. Genetic structuring can often be partitioned hierarchically across macro and micro-geographic scales. For example, populations within the Great Lakes may be structured by lake basin, by river drainage or even by tributaries within river drainages. Genetic population structure of lake sturgeon in the Great Lakes is likely to be influenced by historical, ecological, and anthropogenic factors.

The most significant historical process that influenced the population structure of native fish species in the Great Lakes was the Wisconsin Glaciation. During the Wisconsin ice age, the Great Lakes were covered by ice until approximately 15,000 years before the present time (Mandrak and Crossman 1992, Underhill 1986). During this time, Great Lakes fish species either persisted in low numbers in glacial refugia or became extirpated (Underhill 1986). Species that persisted in glacial refugia were likely to have reduced population sizes as well as reduced levels of genetic diversity (Bernatchez and Wilson 1998). As glacial ice sheets receded, species that persisted in glacial refugia were able to recolonize the Great Lakes via a series of proglacial lakes and their associated drainages (Mandrak and Crossman 1992, Underhill 1986). Several different recolonization routes were utilized by fish species, and this in turn helped to shape present day population genetic structure (Mandrak and Crossman 1992). In a study on

fish species that re-colonized previously glaciated areas, Bernatchez and Wilson (1998) found that past glacial events have had a significant influence on levels of genetic diversity and population structuring in various species of Great Lakes fishes.

Population structure of lake sturgeon in the Great Lakes is also likely to be significantly influenced by ecological and evolutionary forces. Gene flow is one evolutionary force that can influence population structure. Lake sturgeon are capable of migrating large distances (Auer 1999b). Typically, populations with high dispersal rates show reduced levels of population structure due to an increase in migration of individuals among populations (Stabile et al. 1996). Species that exhibit natal philopatry should show a higher degree of spatial population structure. While tag return data on lake sturgeon are limited due to infrequency of spawning, data suggest that lake sturgeon do exhibit some degree of natal philopatry, where the same individuals are repeatedly captured spawning in the same stream (Auer 1999b, R. Elliott, USFWS, personal communication). Other species of sturgeon have also been found to exhibit some degree of natal phiolopatry based on tag return data (Smith et al. 2002, Stabile et al. 1996). Given the broad geographic range occupied by lake sturgeon (Houston 1987), presumably populations further apart geographically should show a greater degree of divergence in allele frequency from one another than populations in close geographic proximity.

Anthropogenic factors are likely to have affected the degree to which remnant lake sturgeon populations are genetically structured as well. Lake sturgeon population numbers have declined substantially over the past 100 years (approximately 5 sturgeon generations) largely due to anthropogenic forces (Auer 1999a, Hay-Chmielewski and Whelan 1997). Organisms that have experienced drastic reductions in population size are

likely to show a strong degree of population structure due to an increase in genetic drift (Avise 1994). While current population sizes are unknown for most populations of lake sturgeon in the Great Lakes, it is believed that the majority of the remnant populations in the Great Lakes are relatively small (i.e. less than 200 spawning adults annually) (Elliott 2003, Holey et al. 2000).

Previous lake sturgeon genetics studies have primarily utilized mitochondrial DNA. These studies found levels of genetic variation to be low in lake sturgeon populations across a broad geographic range (Ferguson and Duckworth 1997, Ferguson et al. 1993). These studies concluded that highly variable nuclear DNA markers would be needed to detect and adequately characterize levels of genetic variation within populations and to detect structuring among lake sturgeon populations. Recently, polymorphic microsatellite markers have been developed for lake sturgeon that are effective tools for assessing population structure (Welsh et al. 2003, McQuown et al. 2002, McQuown et al. 2000, May et al. 1997). King et al. (2001) found that significant differences in allele frequencies existed among Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) populations based on microsatellite DNA data. Other studies have also demonstrated the efficacy of using mitochondrial DNA markers, primarily direct mtDNA sequencing, as a means of inferring population structure in shortnose (*Acipenser brevirostrum*), Atlantic and Gulf of Mexico sturgeon (*Acipenser oxyrinchus desotoi*) (Grunwald et al. 2002, Waldman et al. 2002, Stabile et al. 1996). Brown et al. (1996) and Ludwig et al. (2000) have also identified an 82 base pair repeated region in the lake sturgeon mtDNA control region that could also be used to assay for population-level genetic differences.

Given the magnitude and duration over which historical, ecological and anthropogenic forces that have affected lake sturgeon populations, it is likely that lake sturgeon exhibit some degree of genetic population structure. The objective of this study, therefore, was to use genetic markers to assess the amount of genetic variability and to examine the degree of population structure among remnant populations of lake sturgeon in the Upper Great Lakes basin. Through the use of new and more polymorphic genetic markers, this study is likely to be able to document genetic variability on a finer scale than previous studies and to answer previously unknown questions regarding population structuring of Great Lakes lake sturgeon populations. This information is critical for the development of sound management plans and effective restoration strategies for extirpated populations and remnant lake sturgeon populations across the Great Lakes.

Methods

Sample Collection

Lake sturgeon were captured during spring spawning migrations in 10 different Great Lakes tributaries (Figure 1) during 1999, 2000, 2001 and 2002. Two of the populations sampled, Black Lake (MI) and the Wolf River (WI), are presently isolated due to the construction of dams that prevent out-migration to the Great Lakes. Sturgeon were captured using large dip nets, gill nets, setlines, electrofishing and trawls. Fin clips were taken from the caudal or dorsal fin from all lake sturgeon captured. Tissue samples were preserved in either tissue storage buffer (4M Urea, 0.2M NaCl, 0.1M Tris-HCl, 5%

Sarcosine, 10mM EDTA) at -70° C or dried and placed in scale envelopes and stored at ambient temperatures. The number of samples per locale ranged from 9 to 111 (Table 1).

Laboratory Analyses

Two classes of genetic markers were utilized, microsatellite and mitochondrial DNA. Microsatellites are bi-parentally inherited regions of non-coding nuclear DNA that contain short (2-5 base pairs) repeated motifs (i.e. $(GATA)_n$) and are generally characterized by a high degree of polymorphism (Scribner et al. 1996). Microsatellites offer many advantages over other genetic markers including a greater level of variation and the ability to sample using non-lethal and non-invasive techniques. Further, they can often be used in different species of similar taxa (Welsh et al. 2003, King et al. 2001, McQuown et al. 2000, May et al. 1997, Scribner et al. 1996). Microsatellites have been widely employed in population level studies of several organisms and have shown to be an effective tool for determining population structure for many species of fishes, including sturgeons (Nielsen and Sage 2002, King et al. 2001, Scribner et al. 1996).

The other class of genetic markers used was mitochondrial DNA (mtDNA). Mitochondrial DNA is a closed circular molecule that is inherited exclusively maternally (Brown 1985). The most variable region of the mtDNA molecule is the control region or D-Loop. This region serves as the origin of mtDNA replication and variations are usually comprised of base pair substitutions (Brown 1985). Mitochondrial DNA has also been shown to be a useful tool for identifying population structure in fish species including sturgeons (Grunwald et al. 2002, Stabile et al. 1996).

Total DNA was extracted from fin clips using either the Puregene® (Gentra Systems) or DNeasy® (QIAGEN Inc.) protocols. Final re-suspensions were in 50µl TE buffer for Puregene extractions and 50µl AE buffer for QIAGEN extractions. DNA was quantified using fluorometry. For microsatellite analysis, DNA was amplified at 8 loci, *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001) *AfuG9*, *AfuG63*, *AfuG74* and *AfuG112* (Welsh et al. 2003). Microsatellite Polymerase Chain Reactions (PCR) were conducted in 25µl volumes and consisted of 100ng template DNA, 10X PCR2 Buffer (1M Tris-HCl, 1M $MgCl_2$, 1M KCl, 10% Gelatin, 10% NP-40, 10% Triton-X), 2mM of each dNTP, 10pmol of fluorescently labeled forward and reverse primer, and 0.3 µl Taq polymerase. PCR reactions were conducted using Stratagene Robocycler® 96 (Stratagene Inc.) and Perkin Elmer 9600 thermocyclers. PCR conditions were as follows: 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, 1 minute for primer specific annealing temperature, and 72° C for 1 minute, and finally a 2.5 minute final extension at 72° C. Microsatellite PCR products were run on 6% denaturing polyacrylamide gels and visualized using the Hitachi FMBIO® II scanner (Hitachi Corp) and associated software. Allele sizes were determined using a commercially available size standard (MapMarker™, BioVentures Inc.) and using standard samples of known genotype.

Size polymorphisms due to an 82 base pair repeat in the control region as described in Ludwig et al. (2000) was also assayed for the examination of population structure. For this assay the, primer tPro123 (Brown 1996) was used and a new primer, SturgD1R1 (5' CAT AGA GAT AAT GGT GTG GAT 3'), was developed that flanked this repeated region. This primer was developed using lake sturgeon mtDNA control

region sequence available from the National Center for Biotechnology Information website (accession # U32309). PCR reactions for this primer were conducted in 50 µl volumes and consisted of 100ng template DNA, 10X Kocher buffer (1M Tris, 1M $MgCl_2$, 10% Tween 20), 10mM dNTPs, 50pmol tPro123, 50pmol SturgD1R1, and 0.5µl Taq polymerase. PCR conditions were as follows: 94° C for 2 minutes, followed by 40 cycles of 94° C for 30 seconds, 50° C for 30 seconds and 72° C for 1 minute, and a final extension cycle of 72° C for 5 minutes. PCR reactions were conducted in Robocycler® 96 thermocyclers (Stratagene Inc.). PCR products were then run on 1% agarose gels with 5µl of ladder. Gels were stained with ethidium bromide and visualized using UV trans-illumination. The number of repeated units was counted and recorded and each individual was checked for heteroplasmy (presence of multiple mitochondrial genomes).

Statistical Analyses

Estimates of microsatellite allele frequencies and tests for Hardy-Weinberg equilibrium were calculated using the program Fstat v. 2.9.3 (Goudet 2001). Tests for genotypic disequilibrium for each pair of loci were also calculated using Fstat. Fstat was also used to calculate Weir and Cockerham's (1984) F-statistics and pair-wise F_{st} values. P-values associated with pair-wise F_{st} values were adjusted for multiple comparisons using a Bonferroni correction (Rice 1989). Estimates of genetic variability including mean number of alleles per locus, and observed and expected heterozygosities were calculated for each population using the program BIOSYS-1 (Swofford and Selander 1981). A neighbor-joining tree based on Cavalli-Sforza and Edwards chord distance over

2000 bootstrapping replicates was produced using the program "NJBP" (Cornuet, unpublished).

Hierarchical F-statistics were calculated using the program GDA (Lewis and Zaykin 2001) to determine how variation was partitioned. Populations were grouped according to their basin of origin (Lake Michigan, Lake Superior, or Lake Huron). Estimates of F_{is}, F_{it}, θ_s (variance among populations within basins) and θ_p (variance among basins) were generated for all loci. Confidence intervals for F_{is}, F_{it}, θ_s and θ_p were generated based on 1000 bootstrapping replicates over the 8 loci used.

The program Contribute (Petit et al. 1998) was used to calculate allelic richness to adjust estimates of allelic diversity for population differences in sample size. The total contribution each population made to the overall gene diversity (CT) was also estimated using Contribute. Each population's contribution to the overall diversity was partitioned into two components; C_s, the contribution of that population to the overall diversity based on its own diversity, and C_d, the contribution of a population to the overall diversity based on differentiation from other populations.

Analysis of molecular variance for mtDNA was performed to calculate Φ-statistics using the program AMOVA (Excoffier et al. 1992). Hierarchical analyses of molecular variance were performed by grouping populations together based on their basin of origin (Lake Michigan, Lake Superior, Lake Huron) in order to estimate Φ_{st} (variance within populations), Φ_{sc} (variance among populations within basins) and Φ_{ct} (variance among basins). Incidences of heteroplasmy (multiple mtDNA genomes) were documented for each population. In incidences of heteroplasmy, individuals were assigned to the haplotype that appeared more intense when viewed on agarose gels.

Individuals that exhibited equal intensity of more than one haplotype were omitted from the analysis.

Results

Levels of Genetic Variation Within Populations

Allelic richness for the 10 populations in this study ranged from 2.3 for the Manistee River to 2.7 for the St. Clair River. Observed heterozygosity across the 10 populations ranged from 0.541 for the Manistee River to 0.681 for the Oconto River (Table 1). All populations except the Black River and the Manistee River were in Hardy Weinberg equilibrium. These two populations deviated from Hardy-Weinberg equilibrium at a single locus (*LS-68)*. Tests for genotypic disequilibrium showed that none of the 8 loci used in this study were linked.

Four different size polymorphisms were observed in the mtDNA control region as described by Ludwig et al (2000). Polymorphisms reflected variable numbers of an 82 base pair repeated segment. Individuals were assigned to haplotypes based on the number of repeated units they possessed. The Menominee River population was the only population that exhibited only 1 haplotype. The Wolf River population had a relatively high frequency of haplotype 2 (frequency = 0.680). The two populations from Lake Superior, the Bad and Sturgeon Rivers, exhibited high frequencies of haplotypes 1 and 3, however, haplotype 2 was not observed in any individuals from Lake Superior and haplotype 4 was found at a relatively low frequency in the Bad river (0.129) and was not found in any individuals in the Sturgeon River (Appendix 1).

Heteroplasmy was observed in 11.9% (35 out of 293) of all the individuals assayed for the mtDNA size polymophism. The frequency of heteroplasmic individuals was relatively low (<0.10) in all but three of the populations. Two of these populations, the Bad and the Sturgeon River, are located in Lake Superior. The other population with a relatively high frequency of heteroplasmy was the isolated Black Lake population. Heteroplasmy was not observed in any individuals in the Menominee River or the Oconto River populations.

Not all populations contributed equally to the overall gene diversity (Figure 2). The populations from lake Michigan contributed less than average to the total diversity (indicated by negative CT values) and the populations from Lakes Superior and Huron contributed the most to the total diversity. High relative contributions from populations in the Lake Superior Basin were based primarily on these populations differentiation from the other populations. In the populations from Lake Huron high contributions to total gene diversity are based mostly on the diversity within these populations.

Levels of Genetic Differentiation Among Populations

Mean values for F_{it}, F_{is}, and F_{st} for all populations over all loci were 0.114, 0.059, and 0.059 respectively. All values were found to be statistically significant ($P < 0.05$). The majority of the pair-wise F_{st} values were found to be statistically significant (Table 2). The exceptions were the pair-wise F_{st} values between the Oconto River and the other Green Bay tributaries to Lake Michigan (Menominee, Peshtigo, Fox, and Wolf Rivers) and the pair-wise F_{st} between the Fox and Wolf Rivers.

Mean Φ_{st} (variance among populations) was estimated to be 0.201 ($P<0.001$). Pairwise estimates of Φ_{st} (Table 2) showed that the Menominee River exhibited a relatively high degree of variance from all of the other populations in the study. The Wolf River also showed a high degree of variance from all other populations in the study except for the Fox River. Similar to microsatellite analyses, a low degree of variance was found between the Oconto River and the rest of the Green Bay tributaries (Fox, Peshtigo, Wolf) except for the Menominee River.

The hierarchical analysis of variance for microsatellite DNA showed that more variance exists among populations within basins (mean $\theta_s = 0.065$; $P<0.05$) than among basins (mean $\theta_p = 0.021$; $P<0.05$). Hierarchical analysis of mtDNA variance showed similar results. The majority of the mtDNA variance, 80.45% was partitioned within populations, with 21.38% of the variance being partitioned among populations ($\Phi_{sc} = 0.201$, $P<0.001$). Variance partitioned among basins was negligible (Table 3).

The relationships among populations are shown in a neighbor-joining tree that shows three distinct population assemblages (Figure 3). One assemblage consists of the Bad and Sturgeon Rivers from Lake Superior. Strong genetic divergence was observed between the other two population assemblages (84% bootstrap support; Figure 3). One of these assemblages consisted of the populations from the Green Bay Basin in western Lake Michigan (the Menominee, Peshtigo, Oconto, Fox, and Wolf Rivers). The other population assemblage consisted of populations from eastern Lake Michigan (Manistee River) and Lake Huron (Black Lake and St. Clair River).

Discussion

Lake sturgeon in the Great Lakes have been subject to historical, ecological, and anthropogenic forces which have influenced the amount of genetic variation present within populations as well as the degree of genetic structuring among remnant populations. Previous population level genetic studies focused exclusively on mitochondrial DNA and revealed that lake sturgeon exhibited low levels of genetic diversity (Ferguson and Duckworth 1997, Ferguson et al.1993). By focusing on more polymorphic regions of the mtDNA genome as well as highly variable microsatellite markers, this study addresses previously unanswered questions regarding levels of genetic variation and the degree of genetic population structure. The results of this study indicate that lake sturgeon exhibit higher levels of genetic variability than previously detected and that lake sturgeon populations in the Upper Great Lakes Basin are spatially genetically structured.

Description of Population Structure

Significant levels of overall F_{st} (0.059) and Φ_{st} (0.210), as well as significant pair-wise levels of F_{st} and Φ_{st} values (Table 2) provide evidence that remnant lake sturgeon populations in the upper Great Lakes are genetically structured. The neighbor- joining tree indicates that populations from the same basin are more similar to one another than to populations from other basins. This tree shows three distinct population assemblages corresponding to Lake Superior (Bad and Sturgeon Rivers), tributaries from the Green

Bay basin (Fox, Oconto, Peshtigo, Menominee, and Wolf Rivers) and populations from eastern Lake Michigan (Manistee) and Lake Huron (Black and St.Clair).

The two hierarchical analyses of variance based on microsatellite DNA and mitochondrial DNA showed similar results as to how genetic variability is partitioned (Table 3). The microsatellite DNA data showed that there is more variance among populations within basins than there is among basins ($\theta_s = 0.065$ vs. $\theta_p = 0.021$). Similarly the mtDNA data shows that there is a higher variance among populations within basins than there is among basins ($\Phi_{sc} = 0.210$ vs. $\Phi_{ct} = 0.0$). While variance does exist at multiple levels, both microsatellite and mtDNA analyses show that the majority of this variance is partitioned among populations within basins. Data indicating significant levels of population structure along with data on partitioning of variance suggest that remnant lake sturgeon populations in the Great Lakes do not exist as a single mixed population.

The pair-wise F_{st} values for the Oconto River and the other tributaries from the Green Bay Basin (The Fox, Wolf, Peshtigo, and Menominee Rivers) were not found to be significant. This is likely due to the small size of this population and the subsequent small sample size (n = 9) from this population used in this study. Since the Oconto River represents the smallest remnant population in this study and one of the smaller populations in the Green Bay Basin (Elliott 2003, Holey et al. 2000), it is conceivable that individuals that may have historically spawned in the Oconto River are searching for more favorable spawning opportunities in other nearby river systems.

The pair-wise F_{st} between the Fox and Wolf Rivers was also not found to be significant. In addition, the pair-wise Φ_{st} for the Fox and Wolf Rivers was relatively low.

Although lake sturgeon were once abundant in the Fox River, until recently their numbers during spawning periods had been low (Cochran 1995). In past years, however, lake sturgeon numbers in the Fox River have been increasing with over 50 adults seen in spawning areas in past years. Approximately 5% of the adult lake sturgeon captured in the Fox River since 2000 have been tagged previously in the Lake Winnebago/Wolf River system (R. Elliott, USFWS, personal communication). While it is not likely that fish can ascend the Fox River to Lake Winnebago and the Wolf River due to the presence of multiple dams, fish from Lake Winnebago and the Wolf River do seem to be traveling downstream over dams. This downstream movement of fish is a likely explanation for the low levels of pair-wise F_{st} and Φ_{st} observed between these two populations.

The results of this study are similar to studies on other species of sturgeon that demonstrate a significant amount of population structure. To date, one other study has employed nuclear microsatellite markers to examine population structure in a sturgeon species. King et al. (2001) observed a significant degree of population structure in Atlantic sturgeon based upon microsatellite markers. Pair-wise values of F_{st} for Atlantic sturgeon were found to range from 0.023 to 0.278 (compared to 0.0 to 0.146 in this study). Similar to lake sturgeon, this study found regional genetic differences to exist between populations of Atlantic sturgeon. Several studies using mitochondrial DNA markers have also shown sturgeon populations to be significantly genetically structured (Waldman et al. 2002, Grunwald et al. 2002, Stabile et al. 1996). While the degree of genetic population structure ranged from species to species, mtDNA markers documented a significant degree of regional genetic differences in shortnose, Atlantic, and Gulf of Mexico sturgeon.

One interesting result of this study was the observation of heteroplasmy (the presence of multiple mitochondrial DNA genomes) in the mtDNA control region. Heteroplasmy was observed in 11.9% of the individuals that were assayed for mtDNA size polymorphism. This represents a much higher incidence of heteroplasmy than observed in previous studies, Brown et al. (1996) found no incidence of heteroplasmy in 21 lake sturgeon and Ludwig et al (2000) found heteroplasmy in only 0.064% of the lake sturgeon they assayed. Heteroplasmy has been observed in other species of sturgeon as well. Grunwald et al. (2002) observed heteroplasmy in approximately 1/3 of the shortnose sturgeon assayed and Miracle and Campton (1995) observed heteroplasmy in 18.5% of Gulf of Mexico sturgeon they assayed. The ability to detect heteroplasmy is dependant on the ability to visually detect bands of low copy numbers of DNA. Because of this, heteroplasmy may be underestimated in this study.

Factors Influencing Population Structure

Past Glacial events have been found to influence population structure in many species of fishes (Bernatchez and Wilson 1998). The northern range of lake sturgeon was extensively glaciated during the Wisconsin Ice Age and therefore populations in this study must have originated from glacial refugia (Mandrak and Crossman 1992, Underhill 1986). Previous studies have suggested that lake sturgeon persisted in multiple refugia with one glacial refugium re-colonizing the Great Lakes and another re-colonizing the Hudson/James Bay area (Fergeson and Duckworth 1997).

The results of this study seem to support a multiple refuge hypothesis as well. These results indicate the possibility that multiple refugia contributed to populations

within the Great Lakes. A fundamental divide in the neighbor-joining tree suggests that the populations in Lake Superior are greatly different from the other populations sampled in the Great Lakes. This differentiation is further supported by pair-wise F_{st} values, pair-wise Φ_{st} values, and the fact that Lake Superior populations exhibit a high degree of gene diversity based on differentiation from other populations. Populations in Lake Superior were also found to exhibit a high degree of heteroplasmy compared to other populations in the Great Lakes and one of the mtDNA haplotypes, haplotype 2, which was found in other Great Lakes populations was not observed in either of the Lake Superior populations. These data suggest that populations in Lake superior have been isolated considerably longer than other Great Lakes populations, possibly the result of these populations originating from a different glacial refugium than other populations in the Great Lakes.

Levels of contemporary gene flow are also likely to influence genetic population structure. Species that are highly migratory typically have high levels of gene flow between populations that in turn leads to a reduction in differences between populations and a reduction in population structure (Stabile et al. 1996, Chakraborty and Leimar 1987). While Lake sturgeon migrate large distances in non-spawning periods (Auer 1999b), adults appear to exhibit a high degree of natal philopatry given the high degree of population structure observed. This hypothesis is further substantiated by tag return data that has yet to find adults straying between populations during spawning periods (R. Elliott, USFWS, personal communication). High levels of population structure have been attributed to a high level of natal philopatry in other sturgeon species as well (Waldman et al. 2002, Grunwald et al. 2002, King et al. 2001, Stabile et al. 1996).

Anthropogenic forces have also significantly influenced the genetic structure of remnant lake sturgeon populations in the Great Lakes. Populations that have experienced significant declines and a subsequent decrease in effective population size are likely to experience an increase in genetic drift (Lynch 1996). As genetic drift increases, the genetic differences between populations will become accentuated and as a result the degree of population structure will increase (Avise 1994). Lake sturgeon populations in the Great Lakes have been drastically reduced as a result of anthropogenic forces over the past 100 years (Hay-Chmielewski and Whelan 1997). Currently the majority of populations in the Great Lakes, including the majority of the populations in this study, have annual spawning runs of 200 or fewer adult fish (Elliott 2003, Holey et al. 2000).

Mitochondrial DNA data provides evidence of genetic drift in lake sturgeon populations in the Great Lakes. Because of its rapid rate of evolution, mtDNA can often provide a better view of the degree of genetic differences between populations than nuclear markers (Ferris and Berg 1987). Because of its maternal mode of inheritance mitochondrial DNA experiences approximately 4 times higher levels of genetic drift than would be expected for nuclear DNA (Avise 1994). The Menominee River represents one population that seems to be experiencing high levels of genetic drift. Only 1 mtDNA haplotype (haplotype 1) was observed in the Menominee River, whereas 4 haplotypes were observed in only 9 individuals sampled in the nearby Oconto River. The Menominee River is one of the few populations that remains subject to harvest. The exploitation rate in the un-impounded section of the river was found to be 18.7%, which is higher than any other section of the river that contains sturgeon (Thuemler 1997). Spawning habitat for sturgeon in the Menominee River returning from Green Bay and

Lake Michigan is also significantly reduced due to the construction of a dam 3.9km upstream from the mouth of the Menominee River. It is likely that the combination of exploitation and habitat loss in this system have accelerated the process of genetic drift and subsequently reduced the effective population size. This population provides an example of the relatively short time period required for anthropogenic effects to cause an increase in levels of genetic drift and subsequently cause lake sturgeon populations to become highly differentiated.

Conclusion

Remnant lake sturgeon populations in the upper Great Lakes exhibit a high degree of population structure. This structure appears to be influenced by historical, ecological, and anthropogenic factors. Past glacial events and a strong degree of natal philopatry have led to significant genetic differences among populations within basins. The dramatic reduction in lake sturgeon population size in the Great Lakes over the past 100 years has further accentuated these differences through the process of genetic drift. These results have important implications for conservation efforts. Based on the fact that significant genetic differences do exist between remnant lake sturgeon populations in the upper Great Lakes, lake sturgeon in the Great Lakes should not be managed as a single population. Furthermore, genetic differences between populations should be considered when utilizing hatcheries as a means to rehabilitate declining and extirpated populations.

CHAPTER II

Lake Sturgeon Reproductive Ecology and Conservation Status Based on Genetic Determination of Parentage

Introduction

Fishes have evolved many different reproductive strategies. These strategies have evolved to maximize fitness under a wide variety of conditions including varying population densities and habitats (Avise et al. 2002). Fishes that reside in high densities, including many species of sunfish, have evolved reproductive strategies in which larger bourgeois males build and guard nests from younger cuckolding males who attempt to sneak mating opportunities (Avise et al. 2002, Mackiewicz et al. 2002, Taborsky 2001, DeWoody et al. 1998). Certain salmonids exhibit ontogentic shifts in reproductive strategies in which males may spawn as either freshwater parr or as fully developed adults returning from marine environments (Avise et al. 2002, Moran et al. 1996, Gross 1991). Other reproductive strategies that have evolved include group spawning, oral incubation, sex-role reversals, and live bearing of young (Avise et al. 2002).

Direct observation of fish mating behavior can be difficult given that fishes may spawn in areas with high turbidity or at depths that make observation difficult. Different spawning behaviors (i.e. large group spawning, multiple extra-pair matings) may also make observation of fish mating systems difficult. Recent advances in genetic techniques have made it possible to uncover many aspects of fish mating systems that have previously been difficult to observe (Avise et al. 2002). To date, most genetic studies of fish mating systems involve cases where one parent is known and the other parent is determined through the process of excluding all but one of the potential unknown parents

(Avise et al. 2002). Due to their highly variable nature and their Mendelian mode of inheritance, microsatellite markers are well suited for examining mating systems in fishes (Avise et al. 2002, Mackiewicz et al. 2002, DeWoody et al. 2000, DeWoody et al. 1998, Colbourne et al. 1996, Queller et al. 1993). Highly variable microsatellite markers allow for exclusion of potential parents when the genotypes of potential parents are inconsistent with offspring genotypes (Dewoody and Avise 2001).

Genetic studies of mating systems in fishes can provide valuable information for conservation efforts. As populations of many fish species continue to decline, the use of genetic markers can provide data on numbers of spawning adults, skews in sex ratios, variance in reproductive success and reductions in effective population sizes (Bekkevold et al. 2002, Garant et al. 2001, Moran and Garcia-Vazquez 1998). As spawning habitat is modified and/or lost, fishes are likely to exhibit changes in their mating behavior including increased hybridization (Scribner et al. 2001) and shifts in mating strategies (Gross 1991). These changes in mating behavior may lead to a subsequent loss of genetic diversity. Species that are unable to adapt their mating behavior to changes and reductions in spawning habitat, and are experiencing declining population sizes and low reproductive outputs are likely to be at an increased risk of extinction as spawning habitat continually declines.

Information on mating systems can be used to assess conservation status including estimation of effective population size, N_e. The effective population size of a population refers to the size of an idealized population that would have the same genetic characteristics (i.e. rate of genetic drift, increase in inbreeding, decrease in heterozygosity) as the population under consideration (Kimura and Crow 1963). Multiple

methods, parameterized using different demographic and genetic variables, exist for calculating effective population size (Frankham 1995). Three main factors that significantly influence effective population size are unequal sex ratios, variance in reproductive success, and fluctuations in population size (Frankham 1995). Ideally ratios of N_e/N should be close to 1, however this is not typically the case. Anthropogenic factors such as overharvest and loss of habitat can skew sex ratios and increase variance in reproductive success which in turn decreases N_e. Populations with a low N_e are likely to lose genetic diversity more rapidly and experience higher rates of extinction (Newman and Pilson 1997).

Small populations with low effective population sizes are likely to be subject to an Allee effect. The Allee effect has been defined as a decrease in population growth rate when population size becomes low (Saether et al. 1996). Principles of the Allee-effect can be applied to fish species and fish mating systems in multiple ways. Fishes that exhibit group behavior such as schooling or group spawning are likely to be less successful at defending themselves from predators when group sizes are low (Stephens and Sutherland 1999, Saether et al. 1996). When population sizes are low, fishes that exhibit group spawning behavior may also experience difficulties finding mates or may be forced to pair with poor mates, resulting in lower reproductive success and ultimately a reduction in population growth rate (Stephens and Sutherland 1999, Saether et al. 1996).

Observational studies have shown that lake sturgeon have evolved a group spawning system. Lake sturgeon are broadcast spawners and congregate in large groups during spawning periods (Bruch and Binkowski 2002, Kempinger 1988). Unlike other

species of fishes, sturgeon construct no nest, they do not protect their eggs after deposition, and they make no parental investment in their offspring (Kempinger 1988). The unprotected lake sturgeon eggs and juveniles are prone to high rates of predation. Lake sturgeon have developed compensatory mechanisms to minimize predation levels on eggs and offspring. Lake sturgeon eggs incubate rapidly (5-14 days) (LaHaye et al. 1992, Kempinger 1988, Houston 1987), which helps to reduce predation on the unprotected eggs. Lake sturgeon are capable of producing large numbers of offspring, a single female lake sturgeon can produce over 100,000 eggs in one spawning period (Houston 1987). The large number of offspring produced also helps to ensure that adequate numbers of lake sturgeon survive.

Lake sturgeon migrate up their natal river to spawn in the spring. Lake sturgeon do not spawn annually; males may spawn every 1 to 4 years, and females may spawn every 3 to 7 years (Auer 1999b, Houston 1987). The tendency for males to spawn more frequently than females leads to male-biased sex ratios during spawning periods (Kempinger 1988). Sturgeon spawning grounds are characterized as having rocky substrate and high flow rates (LaHaye et al. 1992, Harkness and Dymond 1961). Lake sturgeon spawning is temperature dependent with spawning observed primarily between 10 and 15° C (LaHaye et al. 1992, Kempinger 1988, Houston 1987). Often, a drop in temperature during spawning periods will cause lake sturgeon to cease spawning and leave spawning areas until temperatures increase, at which time spawning will resume. (Bruch and Binkowski 2002, Kempinger 1988, E. Baker, MI DNR, personal communication).

Several male sturgeon have been observed to spawn with one female simultaneously. Kempinger (1988) observed between 6 and 8 males spawning with one female, and Bruch and Binkowski (2002) observed similarly skewed spawning sex ratios. Prior to egg release, female lake sturgeon have been observed to swim upstream, presumably to attract potential mates. Immediately before the female lake sturgeon releases her eggs, males compete to gain the closest possible position to females (Bruch and Binkowski 2002). Lake sturgeon eggs incubate for 5 to 14 days before they hatch. Soon after hatching the larval lake sturgeon drift downstream to larger bodies of water (LaHaye et al. 1992, Kempinger 1988, Houston 1987).

One remnant lake sturgeon population that still has relatively large annual spawning migrations is in Black Lake, MI (Figure 4). Historically sturgeon could migrate from Lake Huron to the Upper Black River to spawn. Following the construction of the Alverno Dam on the Lower Black River in 1903, sturgeon became isolated in Black Lake and immigration from Lake Huron was cut off (Baker and Borgeson 1999). Sturgeon in Black Lake still spawn in the Upper Black River, however, construction of the Kleber Dam on the Upper Black River has reduced the amount of available spawning habitat. Adult lake sturgeon in Black Lake spend relatively little time in the upper Black River during spawning periods. Typically fish will remain in the river for a few days and leave the river immediately following spawning. Recent population estimates suggest that there are approximately 1,250 individuals currently in the Black Lake population, with about 550 of those being fish of reproductive age (Baker and Borgeson 1999). Comparing their data to previous studies, these authors found evidence for a substantial reduction in

population size over approximately 25 years. These authors also found recruitment in this population to be low (Baker and Borgeson 1999).

The objective of this study, therefore, was to use microsatellite markers to determine parentage in the remnant population of lake sturgeon in Black Lake. Based on this information, inferences can be made in regards to many aspects of the lake sturgeon mating system about which little information is currently available, including the number of mates each male and female is spawning with, variation in reproductive success, and the underlying mechanisms responsible for variance in reproductive success. These data will also be used to estimate the effective number of breeding adults. These data have important conservation implications for this declining population as well as other populations across the species range.

Methods

Sample Collection

Field sampling was conducted during the spring and summer of 2001. Adult lake sturgeon were captured during spring spawning migrations in the Upper Black River in the 11km section below the Kleber Dam. Three periods of spawning activity were observed between April 28 and May 26 2001, in the Upper Black River. Many fish were observed to return to Black Lake between periods of peak spawning activity. A total of 114 adult lake sturgeon (70 males and 44 females) were captured in the Upper Black River using large dip nets. All adults were measured, weighed, and sexed based on the

presence of eggs or milt. A small tissue sample was taken from the upper lobe of the caudal fin and dried and stored at ambient temperatures.

Once spawning behavior had been observed, sampling also began for out-migrating larval sturgeon. D-frame drift nets were used to capture larval lake sturgeon downstream of spawning grounds in the Upper Black River. Sampling occurred during the peak drift hours, between 10:00pm and 2:00am. Three peaks were observed in the numbers of drifting larvae, presumably coinciding with the 3 peaks in adult spawning activity. All larval lake sturgeon that were captured were transported to the Michigan DNR Wolf Lake State Fish Hatchery. Juvenile lake sturgeon were divided into three groups at the hatchery based on the three periods of larval drift.

Lake sturgeon were reared in the hatchery and released the following fall back into the Upper Black River. All hatchery mortalities (n = 779) were preserved in 95% non-denatured ethanol for genetic analyses. Prior to their release into the Upper Black River, fin clips were taken from all juvenile lake sturgeon (n = 912) and preserved in scale envelopes at ambient temperatures.

Laboratory Analyses

DNA was extracted from 111 adult tissue samples using DNeasy® kits (QIAGEN Inc.) according to the manufactures protocol. DNA was extracted from 576 juvenile lake sturgeon also using the DNeasy® protocol. 192 juvenile lake sturgeon were selected from each of the three out-migrating groups for genetic analyses. Juvenile lake sturgeon were sampled proportionally from the hatchery mortalities and the fish that were released back into the Upper Black River. From the first group of juveniles samples from 48 alive and

144 dead individuals were used. From the second group samples from 176 alive and 16 dead fish were used. From the third group samples from 106 alive and 86 dead fish were used. DNA was quantified using a Beckman DU® 7400 spectrophotometer.

DNA was amplified at 8 microsatellite loci, *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001) *AfuG9, AfuG63, AfuG74 and AfuG112* (Welsh et al. 2003). PCR reactions were conducted in 25 µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M $MgCl_2$, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), 2mM of each dNTP, 10 pmol of forward and reverse reverse primer and 0.3 µl Taq polymerase. PCR conditions were as follows; 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, 1 minute at primer specific annealing temperatures, and 72° C for 1 minute, and a final extension at 72° C for 2.5 minutes. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIO II scanner. Allele sizes were determined using commercially available size standards (MapMarker™, BioVentures Inc.) and based on standard samples of known genotype.

Statistical Analyses

Estimates of allele frequencies, mean number of alleles per locus, and observed and expected heterozygosityies were calculated using the computer program CERVUS v2.0 (Marshall et al. 1998). Chi-squared goodness of fit tests were performed using CERVUS for each locus to test for Hardy Weinberg equilibrium. CERVUS was also used to generate probabilities of excluding unrelated individuals from parentage assignment.

All adults that were captured in the Upper Black River prior to spawning were considered as potential parents of each offspring. Parentage assignments were made using a maximum likelihood-based approach (Meagher 1986, Thompson 1975) employed by the program CERVUS. Parental pairs were assigned based on total exclusion of all other potential parents or when CERVUS assigned parents at either 95% (strict) or 80% (relaxed) probabilities relative to other parental pairs. Parentage was assessed by first determining maternity then paternity, and then validated by performing the analysis in reverse order. Confidence levels associated with each parental pair were derived based on population allele frequencies and representative levels of tolerance of false parentage (see Marshall et al. 1998 for details). In cases in which only a male parent or female parent could be identified for a particular offspring based on total exclusion of all other males or females, maternity or paternity only was assigned.

Mean and variance for numbers of mates and numbers of offspring produced were estimated from the sample. To determine if size-related assortive mating was taking place, the correlation between the total length of males and females that spawned with one another was estimated using SAS (SAS Institute Inc.). In order to determine the factors underlying reproductive success, the correlation between male and female total length and the number of offspring produced was estimated using SAS. The correlation between the number of mates and the number of offspring produced was also calculated using SAS to examine the number of mates as another potential factor responsible for variance in male and female reproductive success. Relationships were considered significant when $P<0.05$.

Because only a subset of the adult lake sturgeon population in Black Lake spawns each year, it is not possible to estimate a true effective population size based on parentage data from only 1 spawning season. Instead, an effective number of breeders (N_b) was estimated based on the parentage data from 2001. To account for unequal sex ratio and variance in reproductive success, the effective number of breeders was calculated as in Ballou and Foose (1996):

$$Nb = \frac{4NefNem}{(Nef + Nem)} \qquad (1)$$

$$Nef = \frac{Nfk - 1}{[k - 1 + (Vk / k)]} \qquad (2)$$

$$Nem = \frac{Nm - 1}{[k - 1 + (Vk / k)} \qquad (3)$$

N_{ef} represents the effective number of females, with mean family size k and variance in family size V_k and N_{em} represents the effective number of males with mean family size k and variance in family size V_k.

Results

None of the 8 loci were found to significantly deviate from Hardy-Weinberg equilibrium. The mean number of alleles per locus was 6.63 and the observed heterozygosity ranged from 0.448 for the locus *Aox27* to 0.818 for the locus *AfuG9* (Table 4). Overall exclusion probability was 0.967. For 146 offspring a single parental

pair was assigned. 103 of these assignments (70.5%) were at the 95% confidence level, 33 of the parentage assignments (22.6%) were at the 80% confidence level and 10 of the parentage assignments (6.9%) were below the 80% confidence level. All of the cases where parentage assignments were made below 80% confidence represented situations where parentage was assigned based on total exclusion. Additionally a single parent only was assigned for 139 of the offspring in the study based on total exclusion.

Mating System

Multiple incidences of polygyny and polyandry were observed. Estimates of the number of males spawning with each female ranged from 0 to 11 (mean = 3.10; variance = 6.19) (Figure 5a). The number of females spawning with each male ranged from 0 to 5 (mean = 1.93; variance = 2.20) (Figure 5b). Three females and 8 males were not assigned as potential parents to any of the offspring sampled. Low correlation (0.068; $P>0.436$) was observed between the total length of males and females that spawned with one another (Figure 6). Females whose offspring were found in only one of the groups of drifting larvae spawned with an average of 1.86 males, whereas females whose offspring were found in more than one group spawned with an average of 4.16 males. Similarly, males whose offspring were found in only one of the groups of drifting larvae spawned with an average of 1.25 females and males whose offspring were found in multiple groups spawned with an average of 2.74 females.

Reproductive Success

Reproductive success varied considerably among males and females. Estimates of the number of offspring each female produced ranged from 0 to 22 (mean = 5.05; variance = 21.02) (Figure 7a). The number of offspring produced per male ranged from 0 to 17 (mean = 3.16; variance = 8.02) (Figure 7b).

The numbers of offspring that each adult produced in each of the three out-migrating groups of juveniles varied considerably (Figure 8a, b). Fourteen females and 24 males produced offspring in a single out-migrating group. The mean number of offspring produced by adults that produced offspring in only 1 group was 2.21 for females and 1.75 for males. Alternatively, 25 females and 38 males produced offspring in 2 or 3 different groups. The mean number of offspring produced by adults that produced offspring in multiple groups was 7.24 for females and 4.71 for males.

Overall, correlation between total length and number of offspring produced was low ($r = 0.064$, $P>0.520$) (Figure 9). For males, correlation between total length and number of offspring produced was –0.093 ($P>0.05$) and for females correlation between total length and number of offspring produced was 0.180 ($P>0.05$). In contrast, a high degree of correlation was found between the number of known mates and the number of offspring produced ($r = 0.888$, $P<0.0001$) (Figure 10). For males, correlation between the number of mates and the number of offspring produced was found to be 0.810 ($P<0.0001$). For females correlation between the number of mates and the number of offspring produced was 0.927 ($P<0.0001$).

Effective Number of Breeders

Based on equations (2) and (3) above, the effective number of females and males was estimated to be 20.71 and 31.72 respectively. Substituting these estimates of N_{ef} and N_{em} into equation (1) produced an estimate of 50.12 effective breeders for 2001.

Disscussion

To date, studies of the lake sturgeon mating system have focused exclusively on observational data (see Bruch and Binkowski 2002, Kempinger 1988). Observational studies have provided insight into some of the spawning behaviors lake sturgeon exhibit and have observed polygyny and polyandry, however, many lingering questions regarding the mating system of lake sturgeon exist. Estimates presented in this study represent the first attempt to examine the mating system of any sturgeon species using genetic markers. This study also represents one of the few studies in which no prior information is known about either of the parents of any given offspring.

Mating System

Because lake sturgeon spawn in large groups and are broadcast spawners, it is difficult to assess visually which males are spawning with which females and vice versa. The fact that lake sturgeon often leave spawning grounds due to changes in water temperature and then return later (Bruch and Binkowski 2002, Kempinger 1988), further complicates observational studies. This study allows a much more accurate method of

assessing the lake sturgeon mating system. The results of this study confirm direct observations of the lake sturgeon mating system but also provide evidence for many aspects of the lake sturgeon mating system which were previously unknown.

Previous observational studies on the lake sturgeon mating system have observed likely evidence for polygyny and polyandry. Kempinger (1988) observed 6 to 8 males spawning with one female and Bruch and Binkowski (2002) observed between 2 and 8 males spawning with a single female and also observed males spawning with multiple females. The results of the genetic analyses in this study found polygyny and polyandry to be widespread. Whereas females in the Lake Winnebago system were observed to be spawning with an average of 5-6 males (Bruch and Binkowski 2002), genetic analyses showed that female lake sturgeon in the Black River were observed to spawn with an average of 3.10 males. This discordance is likely to be a result of more heavily skewed sex ratios during spawning periods in the Winnebago system (1:5.7 females to males (Bruch and Binkowski 2002) vs. 1:1.6 females to males in this study). It is important to consider that since the number of mates was based on successful assignment of parentage, the number of mates per male and female in this study represents a conservative estimate. The low correlation between the total length of males and females that spawned with one another in Black Lake indicates that assortive mating based on size is not occurring place in this population.

Eleven of the adults in this study, 3 females and 8 males, were not assigned as potential parents to any of the 576 offspring. One possible explanation for this is that the offspring from these adults were not represented in the sub-sample used in this study. Another possible explanation is that these individuals were present in the Black River

during spawning periods but did not spawn. Given the large number of offspring that were surveyed, it is likely that these individuals did not successfully spawn during 2001. This may be attributed to a low number of potential mates while these individuals were in spawning areas or the fact that these individuals were less aggressive for mating opportunities.

Reproductive Success

A high level of variation in reproductive success was found among both male and female lake sturgeon. Multiple studies have utilized genetic markers to examine variance in male and female reproductive success in other fish species and have found similar trends whereby males and females exhibited a high degree of variance in reproductive success (Bekkevold et al. 2002, Fuimera et al. 2002, Garant et al. 2001, Moran et al. 1996). One potential factor underlying this variation in reproductive success is the size of the fish. Since larger sturgeon can hold greater numbers of gametes it is possible that larger sturgeon may have higher reproductive success. In a study on cod, Bekkevold et al. (2002) found that on average, larger males did indeed sire higher numbers of offspring. This does not appear to be the case in lake sturgeon, however. In this study the correlation between total length and number of offspring produced was very low (0.064). Garant et al. (2001) similarly found a low correlation between the body size of Atlantic salmon and the number of offspring produced.

A significantly higher correlation was found between the numbers of mates a male or female had and the number of offspring produced (0.888). Data suggest that for lake sturgeon, where multiple males mate with a single female simultaneously and a high

degree of physical competition exists between males to obtain optimal spawning positions, distributing gametes among several mates rather than concentrating reproductive efforts on just one or two mates maximizes reproductive success. Garant et al. (2001) also observed a high degree of correlation between the number of mates and the number of offspring produced in Atlantic salmon.

A high degree of variation was also observed in the numbers of offspring that each male and female produced in each of the three groups of out-migrating juveniles. These data, coupled with data on the numbers of mates adults had, suggests that two different mating strategies exist in Black Lake. One group of sturgeon appear to be migrating up the Black River, releasing all their gametes at once and then returning to Black Lake. On average these individuals have fewer mates and produce fewer offspring. Another group of the adult population in Black Lake appear to be releasing a portion of their gametes at one time, leaving the stream and then returning at a later time and releasing the remainder of their gametes. These individuals on average had more mates and greater numbers of offspring. The mating strategy whereby males and females apportion gametes among multiple spawning events appears to lead to higher reproductive success. Incidences of lake sturgeon leaving spawning areas and then returning at a later time have been documented previously and are thought to be a result of fluctuations in temperature (Bruch and Binkowski 2002, Auer 1999b, Kempinger 1988). Based on this information, the decision to apportion gametes over a single versus multiple spawning episodes may be random event in response to environmental conditions rather than a conscious decision.

Effective Number of Breeders

The effective number of breeders for the 2001 adult spawning population in Black Lake was found to be lower than the census number of adults observed in the Upper Black River ($N_b/N = 0.45$). Frankham (1995) reviewed reported estimates of effective population size for 102 different species from several taxa and found that the three most important factors influencing effective population size were fluctuations in population size, variance in family size, and sex ratio. Lake sturgeon in Black Lake exhibit male biased sex ratios during spawning periods as well as a high variance in reproductive success. The combination of these two factors along with the declining nature of this population has contributed to a reduction in the effective number of breeders in the Black Lake population.

The low number of effective breeders raises conservation concerns for this population. Populations that experience reduced effective population sizes are likely to experience an increase in the loss of genetic diversity as well as an increase in the rate of extinction (Newman and Pilson 1997). The size of the lake sturgeon population in Black Lake was found to have significantly declined in a span of approximately 25 years (Baker and Borgeson 1999). Many other populations of lake sturgeon in the Great Lakes have population sizes much lower than Black Lake (Holey et al. 2000). Populations that have experienced more severe declines than Black Lake will likely have much more heavily skewed sex ratios during spawning periods. Because of this it is reasonable to assume that many populations around the Great Lakes are currently experiencing effective population sizes much smaller than in Black Lake, and thus are at a much higher risk of extinction.

The lake sturgeon mating system evolved at a time when lake sturgeon were much more abundant than they are presently. Mechanisms including rapid incubation of eggs and the production of large numbers of offspring likely evolved to counter the effects of predation on eggs and offspring and to help increase reproductive fitness. Currently lake sturgeon numbers in the Great Lakes are significantly reduced. Given the aggregate nature of their spawning behavior and the fact that juvenile lake sturgeon drift in large groups after hatching, low population sizes are likely to lead to a decrease in population growth. Low population numbers will make it difficult for lake sturgeon to find adequate breeding opportunities to ensure long-term population viability.

Conclusion

This study offers the first analysis of the lake sturgeon mating system from a genetic viewpoint. The genetic data presented in this study confirm the results of previous observational studies and also provide new insight into aspects of lake sturgeon reproductive ecology about which little information had been known. The lake sturgeon mating system evolved at a time when populations had much greater numbers of individuals than they do presently. Currently, lake sturgeon experience high variance in reproductive success and skewed sex ratios, factors that have been shown to significantly reduce effective population sizes. Other remnant populations of lake sturgeon that have fewer individuals and more heavily skewed sex ratios than the population in Black Lake are likely to experience an even greater variance in reproductive success and in turn much lower effective population sizes. Historically this mating system served as an effective

means of preserving population viability, however, given the reduced size of most populations of lake sturgeon and the factors causing lake sturgeon effective population sizes to be low, the viability of this mating system is greatly reduced.

Chapter III

Genetic Consequences of Supportive Breeding For a Declining Population of Lake Sturgeon

Introduction

As lake sturgeon populations in the Great Lakes continue to decline, it is likely that hatchery supplementation will be increasingly used to rehabilitate declining and extirpated populations (Hay-Chmielewski and Whelan 1997). While hatchery programs have been a popular management and conservation strategy for many species of fishes, such programs have also received much critical attention (Ford 2002, Meffe 1992, Krueger and May 1991). Production goals for lake sturgeon hatchery programs may be easily met by spawning relatively few females given their high fecundity (Houston 1987). The development of lake sturgeon hatchery programs that successfully accomplish conservation goals of maintaining genetic diversity will be difficult, however, due to small sizes of potential source populations (Holey et al. 2000) and limited opportunities to capture large numbers of adults in spawning condition due to infrequency of spawning (Auer 1996) and male-skewed sex ratios during spawning periods (Bruch and Binkowski 2002). In addition to obtaining suitable numbers of adults, hatchery programs for lake sturgeon will also need to focus on choosing donor stocks that are genetically similar to those populations that are targeted for rehabilitation in order to preserve the genetic diversity of wild populations (Meffe 1995).

One remnant lake sturgeon population that has received hatchery supplementation in the recent past is a landlocked population in Black Lake, MI. This population represents one of the few remnant lake sturgeon populations that still has a relatively

large number of individuals. Recent population estimates found that there were approximately 550 fish of reproductive age in this population (Baker and Borgeson 1999). Comparisons of recent population estimates to estimates from approximately 25 years prior have shown that the size of this population is declining and recruitment is low (Baker and Borgeson 1999).

Anthropogenic factors are largely responsible for this decline. This population has been isolated from other populations of lake sturgeon in the Great Lakes for the past 100 years due to the construction of the Alverno Dam on the Lower Black River (Figure 4). Additionally the construction of the Kleber Dam on the Upper Black River has caused a significant decline in the amount of available spawning habitat. This population also supports one of the few sport fisheries for lake sturgeon. Currently the fishery is restricted to a harvest of five fish annually, however, less conservative harvest regulations historically likely contributed to the decline of this population as well (Baker and Borgeson 1999).

Hatchery supplementation was utilized in Black Lake as a means to supplement this declining population and to sustain the sport fishery. In the spring of 1983, 1984 and 1988, male and female lake sturgeon in spawning condition were captured in the Black River and gametes were taken for supplemental breeding. Each year gamete takes were performed, eggs were taken from 1 or 2 females, the exact number of females used each year is not known. The exact number of males used each year for gamete takes is also unknown, though it is likely to be no more than 2 males per year (D. Borgeson, MI DNR, personal communication). Each year gametes were taken, eggs were fertilized and juvenile lake sturgeon were reared in a hatchery prior to their release the following fall

into the Black Lake system. During 1983, 1984, and 1988, 1,187, 6,698 and 3,587 juvenile lake sturgeon were released respectively. The probability of survival of these juveniles was greatly increased given the fact that the time period when these fish were most vulnerable to natural mortality and predation was spent in captivity (Houston 1987). A 1997 population estimate of the Black Lake sturgeon population showed that a large number of individuals in the population fit a size range that was consistent with length at age data for cohorts from 1983, 1984 and 1988 (Baker and Borgeson 1999) (Figure 11). Based on the delayed maturation of lake sturgeon (Houston 1987, Harkness and Dymond 1961), it is expected that these cohorts will shortly be recruiting into the adult spawning population in Black Lake. Given the large proportion of the total breeding population that these offspring will shortly represent and the high levels of coancestry present due to the extremely small number of adults used to produce these individuals, it is likely that some level of inter-breeding among these cohorts will take place.

The practice of breeding wild caught individuals in a captive environment and then returning their offspring to their native environment, as was the case in Black Lake, has commonly been referred to as supportive breeding (Ryman and Laikre 1991). Supportive breeding programs favor the reproduction rate of a small segment of the population by increasing the probability of survival of the offspring of captively bred individuals (Ryman and Laikre 1991). When one segment of the population experiences much higher reproductive success, there is an overall increase in the variance in family size for the population, a factor that has been shown to significantly reduce effective population size (Frankham 1995). The use of supportive breeding as a means of population restoration often results in a tradeoff between numbers of individuals in the

population and the population's effective population size (N_e). This phenomenon has previously been referred to as a the Ryman and Laikre effect and can be represented by the equation:

$$\frac{1}{Ne} = \frac{x2}{Nc} + \frac{(1-x)2}{Nw}$$

where N_w represents the number of effective parents reproducing in the wild, N_c represents the number of effective parents bred in captivity and x is the relative contribution of offspring from captive parents and (1 - x) the relative contribution from wild offspring. This tradeoff is accentuated in species that have high reproductive outputs such as sturgeons (Ryman and Laikre 1991).

Another potential consequence associated with supportive breeding programs is an increase in the number of related individuals in a population. Supportive breeding programs for fishes often rely on small numbers of adults to produce large numbers of offspring (Wang et al 2002). Depending on the numbers of parents used for mating and the number of offspring produced and released back into the wild population, this practice can result in a disproportionate amount of individuals in a population that are closely related (i.e., full or half siblings). As the number of related individuals in a population increases, the likelihood of inbreeding in future generations increases.

The term inbreeding generally refers to the mating between two individuals who share one or more common ancestors (Templeton and Read 1994). Inbreeding is likely to occur to some degree in all natural populations. In fish species the level of inbreeding can be influenced by factors including reductions in effective population sizes, variance in reproductive success, dispersal rates and natal fidelity (Lynch 1996, Bekkevold et al.

2002, Gerlach et al. 2001, Wang et al. 2002). Low effective population size in wild populations will lead to a reduction in genetic diversity and an increase in genetic drift (Keller and Waller 2002, Wang et al. 2002). Inbreeding may also lead to an increase in the expression of deleterious alleles in a population (Keller and Waller 2002). Increased levels of inbreeding in salmonid populations have been shown to have a negative effect on phenotypic traits including body weight and juvenile survivorship (Wang et al. 2002). High levels of inbreeding are also likely to increase the probability of extinction, particularly in small populations (Bijlsma et al. 2000).

Direct negative effects of supportive breeding have been previously documented in other species of fishes. Tessier et al. (1997) found that that a supportive breeding program for Atlantic salmon caused a significant change in allele frequencies between wild and F1 generation hatchery fish. This supportive breeding program also had negative impacts on effective population sizes of the populations receiving stocked individuals. Hedrick et al. (2000) similarly found that in certain years supportive breeding practices for winter run Chinook salmon led to a reduction in effective population size when non winter run individuals were used as parents.

Reductions in effective population size and increases in levels of inbreeding will ultimately lead to a reduction in population size. As population size decreases, the effects of genetic drift become more significant (Lynch 1996). As the amount of genetic drift increases in a population, the rate of accumulation of deleterious mutations will also increase, causing populations to decline even further. This phenomenon has been referred to as a mutational meltdown (Lynch 1996). Mutational meltdowns may be further exacerbated in situations where supportive breeding is used. Mutations that are

deleterious in the wild may be rendered neutral in captive environments (such as fish hatcheries) that provide more favorable environmental conditions. This can lead to a much more rapid accumulation of these deleterious mutations which will presumably be incorporated into the wild population once individuals from captive environments are released into the wild (Lynch 1996).

Given the potentially harmful effects of the supportive breeding program for lake sturgeon in Black Lake, the objective of this study was to examine the effect that hatchery supplementation had on levels of relatedness (r_{xy}) among lake sturgeon in Black Lake. Levels of relatedness were assessed for two groups of lake sturgeon in Black Lake. The first group was composed of wild adult fish that spawned in the spring of 2001. The second group was composed of individuals that were likely from the 1983, 1984 and 1988. This study examines how aspects of the lake sturgeon mating system along with low numbers of breeding adults will influence the likelihood of mating between closely related individuals. A conceptual model is used to examine the ways in which aspects of the lake sturgeon mating system as well as the declining nature of the Black Lake lake sturgeon population will influence the amount of inbreeding.

Methods

Sample Collection

Two groups of lake sturgeon were used for this study. The first group of sturgeon in this study was composed of 114 adult fish collected during spring 2001 spawning migrations (see chapter 2 for collection methods). These individuals represented a sample

of the population that was produced by natural matings between wild adults. The second group of fish was composed of individuals that, based on length at age data, were likely to be from the 3 cohorts stocked in 1983, 1984 and 1988. The second group of sturgeon was collected during the summer of 2002 by personnel from the Michigan DNR using large mesh gill nets. These individuals fit a size range of 104-130cm. All fish captured were measured, weighed, tagged and fin clips were taken from the upper lobe of the caudal fin for genetic analyses and stored at ambient temperatures in scale envelopes.

Laboratory Methods

DNA was extracted from all sturgeon sampled in the summer of 2002 that fit into this size range (n= 53). All DNA extractions were performed using the DNeasy® (QIAGEN Inc.) protocol with final re-suspensions in 50µl AE buffer. DNA was quantified using a Beckman DU® 7400 spectrophotometer.

DNA was amplified at 8 microsatellite loci, *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001) *AfuG9, AfuG63, AfuG74 and AfuG112* (Welsh et al. 2003). Microsatellite PCR reactions were conducted in 25 µl volumes containing 100ng DNA, 10X PCR2 Buffer (1M Tris-HCl, 1M $MgCl_2$, 1M KCl, 10% Gelatin, 10% NP-40, 10% Triton-X), 2mM of each dNTP, 10 pmol of forward and reverse primer and 0.3µl Taq polymerase. PCR conditions were as follows; 94° C for 2 minutes, followed by 30 cycles of 94° C for 1 minute, 1 minute at primer specific annealing temperatures, and 72° C for 1 minute, and a final extension at 72° C for 2 minutes and 30 seconds. All PCR reactions were conducted using Stratagene Robocycler® 96 (Stratagene Inc.) thermocyclers. PCR products were run on 6%

denaturing polyacrylamide gels and visualized on a Hitachi FMBIO II scanner. Allele sizes were determined using commercially available size standards (MapMarker™, BioVentures Inc.) and using samples of known genotype.

Statistical Analyses

The program GENEPOP v3.1 (Raymond and Rousset 1995) was used to generate estimates of allele frequencies and to perform exact tests of Hardy-Weinberg Equilibrium using a Markov Chain method. A Bonferroni correction (Rice 1989) was performed to correct nominal alpha levels for multiple comparisons for tests of Hardy Weinberg. The program BIOSYS-1 (Swofford and Selander 1984) was used to estimate measures of genetic diversity including observed and expected heterozygosities and mean numbers of alleles per locus for individuals in the two groups. A Chi-squared contingency test was used to determine if observed heterozygosity and number of alleles per locus were significantly different between the two groups of sturgeon in this study.

Levels of relatedness among individuals were inferred using estimates of coefficients of relatedness (r_{xy}) calculated as in Queller and Goodnight (1989) using the formula:

$$r_{xy} = \frac{\sum(Py - P^*)}{\sum(Px - P^*)}$$

where P_x represents the frequency of a particular allele in individual x, P_y represents the frequency of the same allele in individual y and P^* represents the frequency of the allele in the entire population. Coefficients of relatedness were calculated for both groups of lake sturgeon using the program Kinship v2.1 (Goodnight and Queller 1999). Proportions

of individuals significantly related at the half and full sibling level in each of the two groups of sturgeon were estimated using likelihood methods employed using the program Kinship. A Wilcoxon test was performed using the program SAS to test for differences in distributions of r_{xy} values between the two groups.

A conceptual model was used in order to determine some of the demographic and life history characteristics of the Black Lake lake sturgeon population that would effect the level of inbreeding in this population and how these different factors would effect inbreeding. This model accounted for factors including sex ratios, recruitment of hatchery individuals into the adult population as well as senescence/ mortality of the wild adult population and declining nature of this population.

Results

Levels of genetic diversity differed for the two groups of sturgeon used in this study. Estimates of allele frequencies showed that 8 alleles present in the 2001 spawning population were not present in the group of sturgeon that were likely derived from supportive breeding (Table 5). Additionally 2 alleles that were not present in the 2001 spawning group were present in the fish sampled in 2002. In the 2001 spawning group all but one locus (*LS68*) were in Hardy Weinberg equilibrium. In the group of individuals that were likely derived from supportive breeding, 4 of the loci deviated from Hardy Weinberg equilibrium (*LS68, Aox27, AfuG9, and AfuG63*). The mean number of alleles per locus was greater in the group that spawned in 2001 (6.0) than the group composed of suspected stocked individuals (5.3). A chi-squared contingency test showed that the

number of alleles per locus was not significantly different between the two groups (P = 0.497). Observed and expected values of heterozygosity were slightly lower for the group of sturgeon believed to be derived from supportive breeding (Table 6). The Chi-squared test showed that differences in heterozygosity between the two groups of sturgeon were not significantly different (P = 0.99)

Mean r_{xy} values were –0.020 for the 2001 spawning adults and 0.061 for the group of sturgeon that were likely derived from supportive breeding. While both groups of sturgeon appear to have r_{xy} values that are skewed to the right (Figure 12), the group of individuals likely representing the stocked cohorts shows a much greater skew. The Wilcoxon test showed that the distribution of r_{xy} values for the two groups significantly differed (Test statistic = 6372571.5; P<0.0001).

In the group of adult sturgeon that spawned in spring 2001, estimates showed that 90.4% of the pair-wise comparisons among individuals were not indicative of relatedness at the level of half or full siblings, 7.5% of comparisons were indicative of individuals significantly related at the level of half siblings (P<0.05), and 2.1% of the comparisons were indicative of individuals significantly related at the level of full siblings (P<0.05). In the group of sturgeon that were likely the progeny produced from supportive breeding, estimates showed that 84.5% of the pair-wise comparisons were indicative of individuals that were not significantly related at the level of half or full siblings, 10.2% of the comparisons were indicative of individuals were significantly related at the level of half siblings (P<0.05) and 5.3% of the comparisons were indicative of individuals that were significantly related at the level of full siblings (P<0.05).

Discussion

The remnant lake sturgeon population in Black Lake was targeted for supportive breeding to counteract population decline and to support a sport fishery. 11,472 lake sturgeon were stocked into Black Lake and the immediate goal of increasing the population size was accomplished. As is evidenced by recent population estimates and size distributions of the Black Lake lake sturgeon population, individuals from these stocking events currently represent a large portion of the lake sturgeon population in Black Lake (Baker and Borgeson 1999), and will shortly begin to recruit into the adult population. While the short-term goals of the supportive breeding efforts in Black Lake (increasing population size) were met, this action does not represent a viable management strategy for this population.

One of the primary goals of a hatchery program should be to maintain genetic characteristics (i.e., levels of heterozygosity) of the wild source population in the hatchery stock (Allendorf and Ryman 1987). The use of genetically suitable source populations represents one method in which this goal can be accomplished (Meffe 1995). While the source population used in the Black Lake supportive breeding program was identical to the target population, the goal of effectively capturing the genetic characteristics of the wild population was clearly not met. Due to the fact that only a small portion of the adult population in Black Lake was used for this supportive breeding program, several rare alleles that were present in the wild population were lost in the hatchery individuals and there was a decrease in the mean number of alleles per locus. Additionally, significant departures from Hardy Weinberg equilibrium were also

observed in the group of sturgeon that were likely to be the progeny produced by supportive breeding efforts. These data clearly illustrate that the genetic characteristics of the progeny of the supportive breeding efforts do not represent those of the wild source population. Data in this study clearly illustrates the need for captive mating scenarios to focus on factors besides simply genetic compatibility of source and recipient populations.

It is likely that supportive breeding has also led to a decline in the effective population size of the Black Lake lake sturgeon population. Ryman and Laikre (1991) showed that as the relative captive contribution to a population increased, the effective population size decreased proportionally, primarily the result of an increase in the variance in family size. Reductions in effective population size due to supportive breeding programs can occur rapidly as was the case in Atlantic salmon when a supportive breeding program was predicted to cause significant reductions in effective population size in a single generation (Tessier et al. 1997). Proper use of supportive breeding in which steps are taken to maximize effective population size and minimize inbreeding can result in a net effect of maintaining N_e (Hedrick et al. 2000). With the case of lake sturgeon in Black Lake, however, these factors clearly were not taken into account. The small number of adults used for this supportive breeding program combined with the large number of offspring produced will significantly influence the variance in family size, which in turn will result in further declines in effective population size in this population which already has a reduced N_e (Chapter 2).

Another consequence of the supportive breeding program was an increase in the number of closely related individuals in the Black Lake lake sturgeon population. This hypothesis is directly evidenced by the increase in both estimated levels of relatedness

among individuals (r_{xy}) in the group of sturgeon likely to be derived from supportive breeding, and by the increase in estimates of proportions of half and full siblings in this group. Even if individuals mate randomly during future spawning events, the increase in the number of related individuals due to the addition of the hatchery cohorts will lead to an increase in the level of inbreeding in the population. More of the progeny of the supportive breeding program will recruit into the adult spawning population coinciding with senescence of wild spawning adults and leading to further increased levels of inbreeding.

When considering the levels of inbreeding in this population there are many aspects of lake sturgeon biology and the nature of the population in Black Lake that are likely to influence levels of inbreeding. The fact that this population has been isolated from all other lake sturgeon populations for approximately 100 years (roughly 5 lake sturgeon generations) significantly increases the likelihood of elevated levels of inbreeding in this population (Keller and Waller 2002). In small isolated populations rates of genetic drift and accumulations of deleterious alleles will be accentuated as the amount of inbreeding increases (Keller and Waller 2002, Lynch 1996). It is likely that when this population became isolated, a number of related juvenile sturgeon were present in Black Lake as well as adult fish that were also related at some level. As time progresses and the population size is reduced this level of relatedness in the population will gradually increase.

The fact that this population is declining is also likely to influence the amount of inbreeding in this population. As the number of adults in this population decreases, the number of potential mates for each male or female will also decrease. As a result,

spawning adults will have a greater chance of mating with individuals with whom they are related at some level. As matings of this type become more common, more closely related offspring will be produced that will in turn recruit into the adult population and will experience some probability of mating with one another.

The nature of the lake sturgeon mating system is also likely to influence inbreeding in this population. Lake sturgeon typically spawn with multiple individuals each spawning season (Bruch and Binkowski 2002, Kempinger 1988, Chapter 2 this document). While mating does appear to be random, in a finite population such as Black Lake, the more mates an individual has, the greater the chances are that one or more of those mates will be a close relative. The fact that lake sturgeon spawn multiple times throughout their life can also affect the amount of inbreeding in this population. While lake sturgeon may not mate with a close relative one year, the chance of mating with a close relative still exists in subsequent spawning years. Lake sturgeon may spawn with a close relative one year and spawn with another close relative in a subsequent year as well.

Lake sturgeon that were produced as a result of supportive breeding efforts in Black Lake will soon be recruiting into the adult spawning population. Each year as the proportion of highly related individuals in this population increases, the chance of mating among closely related individuals also increases. The chance of inbreeding will be further increased each generation as some proportion of the adult population senesces and less opportunities exist for individuals to mate with older individuals with whom they presumably have little close relation.

The manner in which these factors influence the amount of inbreeding in this population is depicted in Figure 13. Adults in Black Lake may spawn with individuals

that they are closely related to, or individuals to whom they have little relation. When matings occur between adults that are not closely related, the increase in inbreeding in the population will be gradual and depend on the background levels of inbreeding in the population. When closely related individuals mate, the increase in the amount of inbreeding in this population will be much more rapid. This rapid increase in inbreeding will be directly influenced by the recruitment of hatchery individuals into this population coupled with the loss of some proportion of the adult spawning population due to senescence and mortality. Male biased sex ratios will further influence this decline. As the number of mates per female increases, females have more mates and the likelihood of one of those mates being a close relative also increases. Population decline will also cause inbreeding to increase more rapidly. As the number of potential mates in the population declines, more individuals will be forced to mate with close relatives. Both the rapid and gradual increase in the amount of inbreeding in this population will cause a reduction in population fitness that over time will lead to an increased likelihood of extinction.

Conclusions

Past supportive breeding efforts for lake sturgeon in Black Lake are likely to have had serious negative conservation implications for this declining population. Supportive breeding in this population has failed to capture the genetic characteristics of the wild population in the hatchery-reared offspring and has also significantly increased the number of closely related individuals in this population. As closely related individuals

begin to recruit into the adult spawning population in Black Lake and the current spawning population senesces and experiences mortality, levels of inbreeding will increase in this population, effective population size will become further reduced and an increase in deleterious mutations will also likely occur. Inbreeding is likely to be influenced by several of the biological characteristics of lake sturgeon as well as the declining nature of this population. It is likely that levels of inbreeding in this population will cause population fitness to be reduced to the point that this population is no longer self-sustaining. This situation clearly illustrates the need to consider more information than the source population used for supportive breeding in order to develop biologically sound management strategies.

APPENDIX I

Estimates of Microsatellite Allele Frequencies and Mitochondrial DNA Haplotype Frequencies from Chapter I

Appendix 1: Estimates of microsatellite allele frequencies and mitochondrial DNA haplotype frequencies from Chapter I

	Peshtigo	Manistee	Fox	Black	Bad	St.Clair	Menominee	Oconto	Sturgeon	Wolf
Locus: LS68										
N	43	86	42	107	35	49	22	9	28	81
108	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000	0.054	0.000
112	0.198	0.308	0.429	0.322	0.457	0.429	0.455	0.333	0.732	0.265
116	0.012	0.000	0.000	0.033	0.000	0.000	0.000	0.000	0.018	0.006
120	0.256	0.052	0.131	0.107	0.029	0.061	0.091	0.222	0.107	0.216
124	0.233	0.430	0.226	0.276	0.000	0.306	0.000	0.167	0.071	0.210
128	0.302	0.116	0.214	0.201	0.014	0.133	0.432	0.278	0.018	0.302
132	0.000	0.029	0.000	0.009	0.071	0.010	0.023	0.000	0.000	0.000
136	0.000	0.058	0.000	0.014	0.186	0.000	0.000	0.000	0.000	0.000
140	0.000	0.006	0.000	0.037	0.143	0.061	0.000	0.000	0.000	0.000
Locus: Afu68b										
N	44	89	42	109	35	50	23	9	28	75
153	0.000	0.039	0.000	0.018	0.000	0.000	0.000	0.056	0.000	0.000
157	0.057	0.000	0.060	0.005	0.100	0.000	0.196	0.111	0.286	0.040
161	0.000	0.017	0.012	0.000	0.000	0.030	0.022	0.000	0.000	0.020
165	0.023	0.017	0.024	0.009	0.000	0.020	0.109	0.000	0.036	0.033
169	0.057	0.292	0.083	0.078	0.029	0.220	0.109	0.000	0.054	0.053
173	0.159	0.337	0.083	0.358	0.243	0.190	0.130	0.000	0.232	0.100
177	0.295	0.034	0.333	0.349	0.057	0.290	0.174	0.444	0.161	0.433
181	0.148	0.011	0.179	0.023	0.029	0.000	0.065	0.167	0.071	0.140
185	0.011	0.079	0.048	0.096	0.314	0.190	0.087	0.000	0.071	0.007
189	0.068	0.107	0.167	0.060	0.000	0.020	0.065	0.167	0.000	0.140
193	0.182	0.067	0.012	0.005	0.229	0.040	0.043	0.056	0.071	0.033
197	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.018	0.000
Locus: Aox27										
N	43	89	45	111	35	50	23	9	28	81
130	0.884	0.860	0.944	0.734	0.686	0.650	0.978	0.944	0.732	0.864
134	0.012	0.051	0.022	0.081	0.214	0.120	0.000	0.000	0.018	0.037
138	0.105	0.090	0.033	0.185	0.100	0.230	0.022	0.056	0.250	0.099
Locus: Spl120										

	Peshtigo	Manistee	Fox	Black	Bad	St.Clair	Menominee	Oconto	Sturgeon	Wolf
N	41	89	40	104	35	50	20	9	28	62
254	0.329	0.635	0.438	0.279	0.214	0.470	0.450	0.500	0.214	0.500
258	0.159	0.140	0.013	0.380	0.400	0.180	0.150	0.056	0.464	0.048
262	0.207	0.062	0.050	0.135	0.186	0.060	0.250	0.222	0.089	0.089
274	0.207	0.062	0.263	0.077	0.029	0.120	0.075	0.167	0.143	0.250
278	0.012	0.000	0.013	0.067	0.157	0.020	0.050	0.000	0.018	0.008
282	0.085	0.101	0.213	0.063	0.014	0.150	0.000	0.056	0.071	0.097
286	0.000	0.000	0.013	0.000	0.000	0.000	0.025	0.000	0.000	0.008
Locus: AfuG9										
N	43	81	43	106	30	46	22	9	23	67
124	0.047	0.037	0.035	0.033	0.433	0.163	0.000	0.056	0.109	0.030
128	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
132	0.012	0.012	0.058	0.052	0.000	0.043	0.023	0.056	0.000	0.015
136	0.000	0.006	0.012	0.052	0.000	0.065	0.000	0.000	0.000	0.015
140	0.512	0.340	0.605	0.264	0.383	0.413	0.364	0.444	0.435	0.537
144	0.256	0.438	0.047	0.278	0.050	0.228	0.341	0.278	0.326	0.142
148	0.151	0.037	0.128	0.104	0.000	0.043	0.182	0.167	0.022	0.194
152	0.012	0.111	0.070	0.160	0.133	0.043	0.068	0.000	0.109	0.045
156	0.000	0.019	0.047	0.052	0.000	0.000	0.023	0.000	0.000	0.022
160	0.000	0.000	0.000	0.005	0.000	0.000	0.000	0.000	0.000	0.000
Locus: AfuG63										
N	44	86	45	110	33	50	22	9	27	78
127	0.295	0.523	0.278	0.409	0.106	0.280	0.295	0.278	0.185	0.224
135	0.045	0.029	0.144	0.000	0.000	0.100	0.045	0.111	0.000	0.103
139	0.318	0.326	0.400	0.332	0.333	0.300	0.432	0.167	0.537	0.506
143	0.341	0.122	0.178	0.259	0.545	0.300	0.227	0.444	0.222	0.167
147	0.000	0.000	0.000	0.000	0.015	0.020	0.000	0.000	0.056	0.000
Locus: AfuG74										
N	44	87	46	110	27	48	23	9	27	73
218	0.841	0.770	0.793	0.623	0.611	0.594	0.761	0.778	0.667	0.664
222	0.080	0.029	0.000	0.091	0.222	0.042	0.196	0.056	0.185	0.041
226	0.080	0.201	0.207	0.286	0.167	0.365	0.043	0.167	0.148	0.295
Locus: AfuG112										

	Peshtigo	Manistee	Fox	Black	Bad	St.Clair	Menominee	Oconto	Sturgeon	Wolf
N	41	82	43	106	21	38	16	9	25	79
240	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000
244	0.244	0.372	0.256	0.349	0.286	0.303	0.625	0.222	0.200	0.215
248	0.000	0.000	0.012	0.005	0.000	0.000	0.000	0.000	0.040	0.013
252	0.073	0.116	0.198	0.170	0.167	0.105	0.188	0.111	0.080	0.241
256	0.415	0.207	0.267	0.250	0.071	0.263	0.156	0.500	0.540	0.272
260	0.061	0.061	0.105	0.151	0.476	0.145	0.031	0.056	0.140	0.114
264	0.207	0.232	0.151	0.075	0.000	0.184	0.000	0.111	0.000	0.146
268	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Haplotype Frequencies										
1	0.053	0.537	0.227	0.143	0.484	0.425	1.000	0.333	0.214	0.000
2	0.125	0.073	0.409	0.071	0.000	0.075	0.000	0.222	0.000	0.680
3	0.219	0.293	0.273	0.536	0.258	0.350	0.000	0.333	0.321	0.260
4	0.063	0.049	0.000	0.000	0.129	0.075	0.000	0.111	0.000	0.000
Heteroplasmic	0.063	0.049	0.091	0.250	0.129	0.075	0.000	0.000	0.464	0.050

APPENDIX II

Tables and Figures

Table 1. Measures of genetic diversity based on 8 microsatellite loci for the 10 populations used in this study.

Population	N	A	H_{obs}	H_{exp}
Peshtigo River	44	2.424	0.609	0.618
Manistee River	89	2.264	0.541	0.588
Fox River	46	2.401	0.588	0.600
Black River	111	2.630	0.606	0.679
Bad River	37	2.373	0.646	0.653
St. Clair	50	2.647	0.664	0.690
Menominee River	23	2.300	0.567	0.580
Oconto River	9	2.458	0.681	0.615
Sturgeon River	30	2.384	0.544	0.613
Wolf River	81	2.440	0.591	0.626

A = Allelic Richness as in Petit et al. (1998)

H_{obs} = Observed Heterozygosity

H_{exp} = Expected Heterozygosity

Table 2. Pair-wise estimates of F_{st} based on 8 microsatellite loci (above the diagonal) and Φ_{st} based on mtDNA (below the diagonal).

	Peshtigo	Manistee	Fox	Black	Bad	St.Clair	Menominee	Oconto	Sturgeon	Wolf
Peshtigo	-	0.064*	0.027*	0.041*	0.116*	0.0434*	0.047*	0.000	0.072*	0.024*
Manistee	0.000	-	0.069*	0.047*	0.147*	0.040*	0.067*	0.069*	0.109*	0.074*
Fox	0.128*	0.163*	-	0.057*	0.128*	0.038*	0.055*	0.010	0.084*	0.006
Black	0.152*	0.130*	0.157*	-	0.079*	0.020*	0.051*	0.037*	0.052*	0.048*
Bad	0.000	0.000	0.220*	0.181*	-	0.073*	0.114*	0.117*	0.081*	0.131*
St. Clair	0.000	0.000	0.140*	0.065*	0.000	-	0.066*	0.030*	0.056*	0.037*
Menominee	0.245*	0.240*	0.490*	0.607*	0.230*	0.319*	-	0.038	0.079*	0.055*
Oconto	0.000	0.000	0.000	0.032*	0.036*	0.000	0.548*	-	0.070*	0.007
Sturgeon	0.074*	0.046*	0.141*	0.000	0.088*	0.000	0.550*	0.000	-	0.087*
Wolf	0.333*	0.378*	0.051*	0.340*	0.439*	0.347*	0.702*	0.188*	0.353*	-

* indicates significance at the level alpha = 0.05 after Bonferroni Correction

Table 3. Hierarchical partitioning of variance based on microsatellite and mitochondiral DNA for the 10 populations used in this study.

		Within individuals	Among individuals within populations	Populations within basins	Among Basins
Microsatellite DNA	Locus	F	f	(θs)	(θp)
	LS68	0.255	0.195	0.074	0.003
	Afu68b	0.103	0.023	0.081	0.009
	Aox27	0.150	0.089	0.068	0.058
	Spl120	0.073	0.000	0.091	0.050
	AfuG9	0.120	0.060	0.064	0.011
	AfuG63	0.100	0.051	0.052	0.008
	AfuG74	0.122	0.082	0.043	0.027
	AfuG112	0.055	0.017	0.039	0.000
	Overall	0.120	0.059	0.065	0.021
mtDNA			Φ_{st}	Φ_{sc}	Φ_{ct}
		N.A.	0.195	0.210	0.000

Table 4. Measures of genetic diversity for the 8 microsatellite loci used in the parentage study.

Locus	Number of Alleles	H_{obs}	H_{exp}
LS68	8	0.517	0.754
Afu68b	11	0.735	0.739
Spl120	6	0.749	0.740
Aox27	4	0.448	0.436
AfuG9	11	0.818	0.812
AfuG63	3	0.585	0.663
AfuG74	3	0.450	0.492
AfuG112	7	0.774	0.781
Mean	6.63	0.630	0.680

H_{obs} = Observed Heterozygosity
H_{exp} = Expected Heterozygosity

Table 5. Allele frequencies based on 8 microsatellite loci for the two groups of sturgeon used in this study. One group was composed of wild adults that spawned in the Spring of 2001 and the other group was composed of individuals likely to be the progeny of supportive breeding events.

Locus	Group		Locus	Group	
		Supportive			Supportive
LS68	**2001 Adults**	**Breeding**	***AfuG9***	**2001 Adults**	**Breeding**
112	0.311	0.387	124	0.035	0.038
116	0.033	0.019	132	0.045	0.009
120	0.108	0.123	136	0.054	0.019
124	0.278	0.255	140	0.252	0.302
128	0.203	0.217	144	0.277	0.189
132	0.009	0.000	148	0.109	0.236
136	0.014	0.000	152	0.168	0.113
140	0.042	0.000	156	0.054	0.000
Afu68b			160	0.005	0.094
133	0.000	0.019	***AfuG63***		
153	0.018	0.085	127	0.408	0.509
157	0.005	0.000	139	0.335	0.274
161	0.000	0.009	143	0.257	0.217
165	0.009	0.000	***AfuG74***		
169	0.078	0.113	218	0.630	0.698
173	0.349	0.274	222	0.088	0.151
177	0.358	0.434	226	0.282	0.151
181	0.023	0.009	***AfuG112***		
185	0.096	0.019	244	0.354	0.198
189	0.060	0.038	248	0.005	0.000
193	0.005	0.000	252	0.161	0.255
Spl120			256	0.254	0.208
254	0.285	0.283	260	0.161	0.075
258	0.370	0.330	264	0.073	0.264
262	0.140	0.094			
274	0.080	0.160			
278	0.060	0.085			
282	0.065	0.047			
Aox27					
130	0.735	0.802			
134	0.084	0.104			
138	0.181	0.094			

Table 6. Measures of Genetic diversity calculated for each of the two groups of lake sturgeon used in this study.

Group	Mean # alleles per locus	Heterozygosity Observed	Heterozygosity Expected
2001 Spawning Adults	6.0	0.624	0.679
1983, 1984, 1988 Stocked Cohort	5.3	0.594	0.656

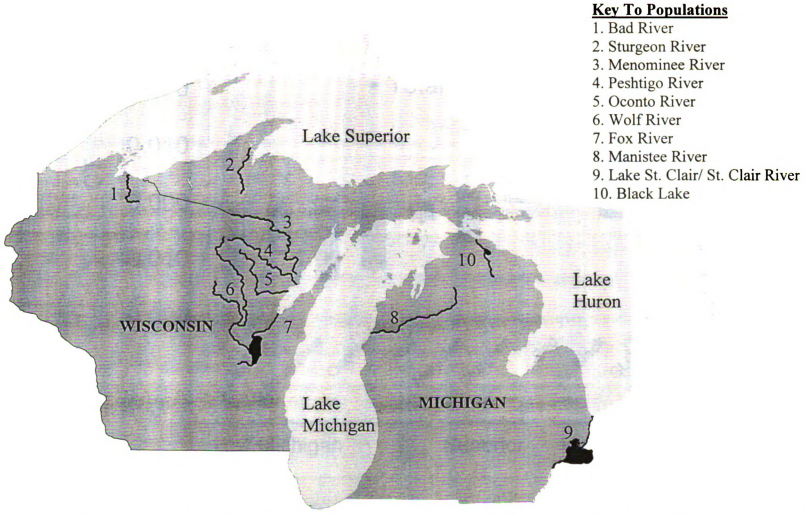


Figure 1. Map showing the locations of the 10 lake sturgeon populations in Michigan and Wisconsin that were sampled for this study.

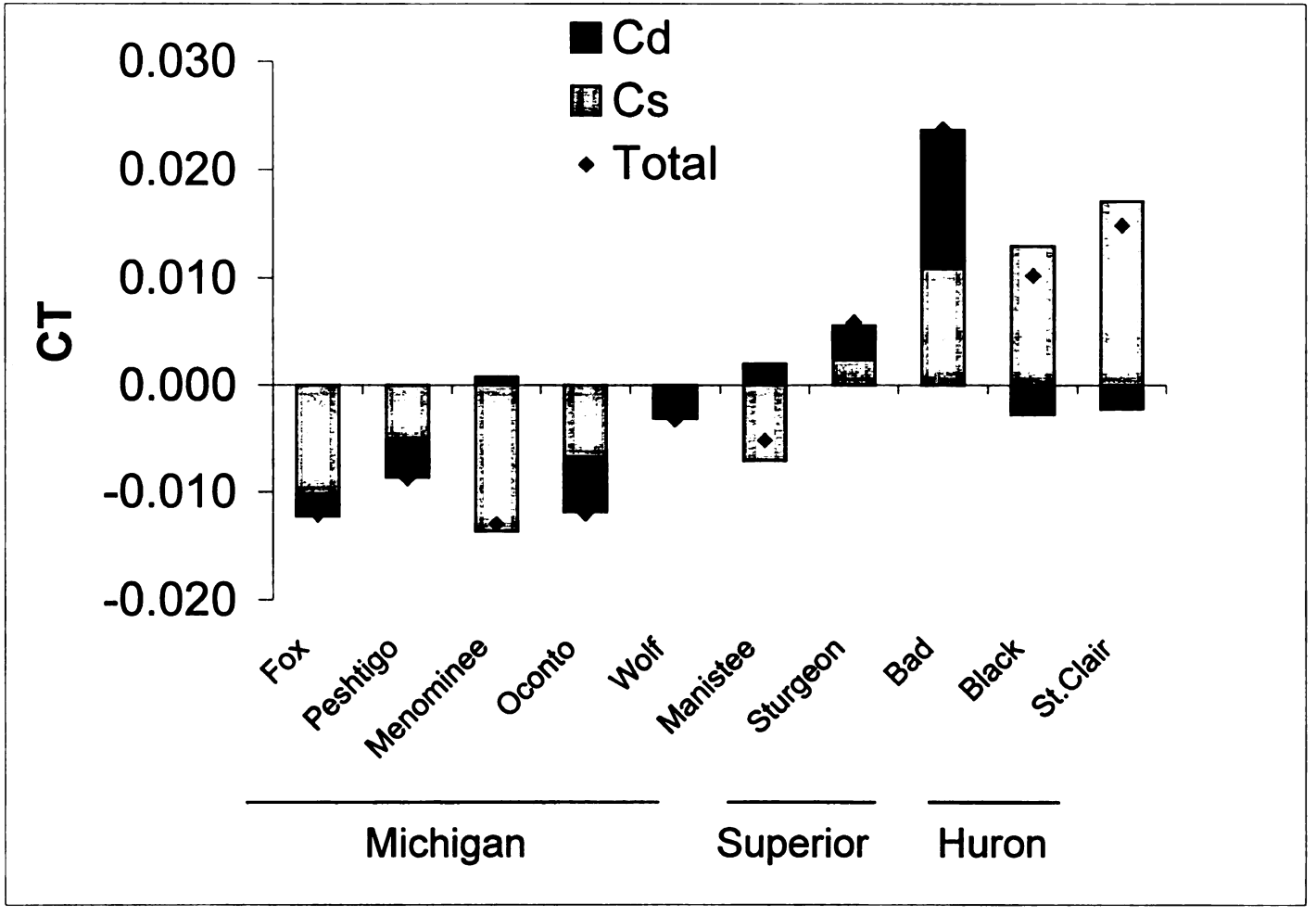


Figure 2. Graph showing the relative contribution of each of the 10 populations to total gene diversity (CT). Gene diversity is broken down into two separate components; Cd, the contribution of each population to the overall diversity based on differentiation from other populations and Cs, the contribution of each population to the overall diversity based on its own diversity.

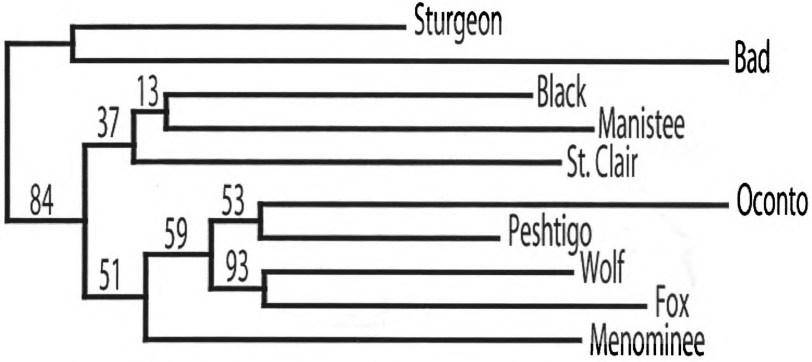


Figure 3. Neighbor joining tree based on Cavali-Sforza and Edwards chord distance showing the genetic structuring of remnant lake sturgeon populations in the Upper Great Lakes. Values indicate bootstrap support over 2000 replicates.

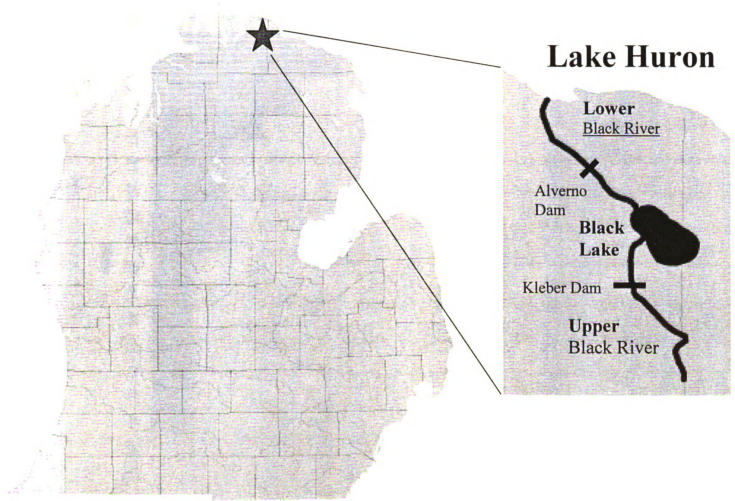
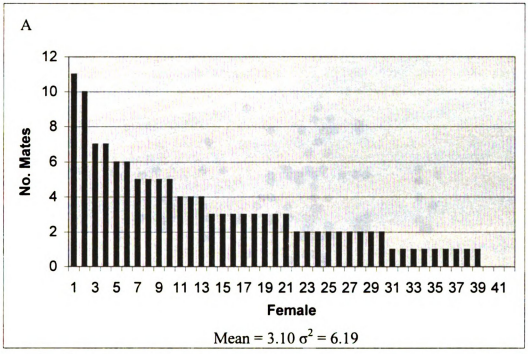


Figure 4. Map showing the Black Lake system including the Lower and Upper Black Rivers and Black Lake.



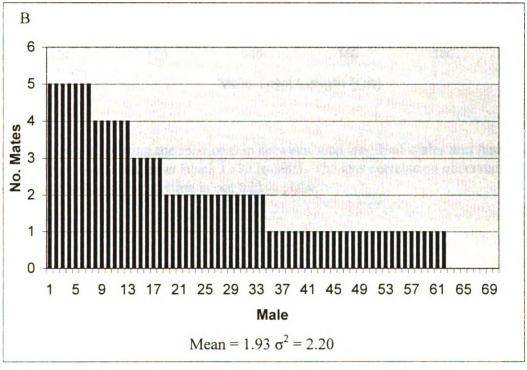


Figure 5. Graphs showing the number of mates per female (5a) and the number of mates per male (5b) for the 111 adult lake sturgeon in this study.

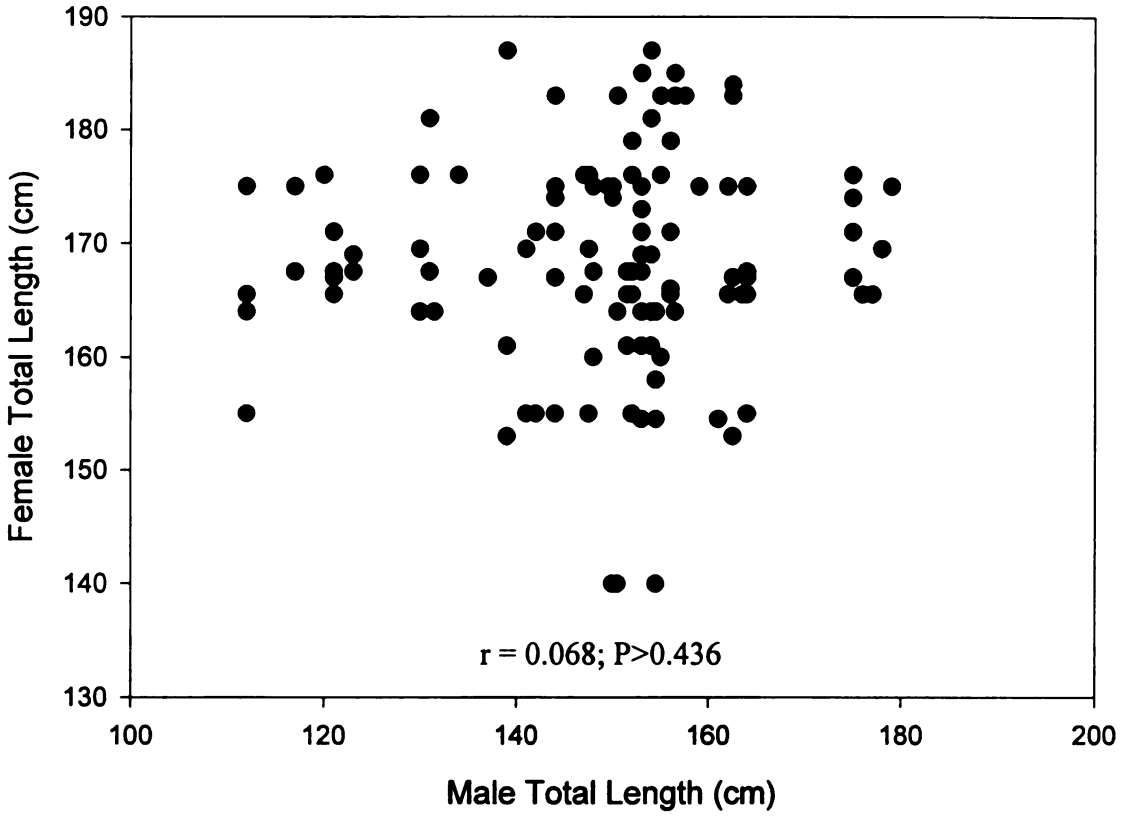
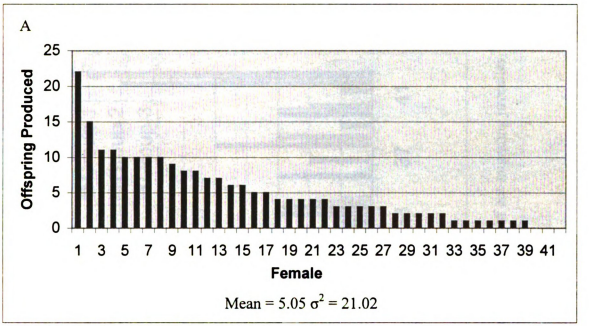


Figure 6. Graph showing the relationship between total length of males and females that spawned with one another in Black Lake in 2001. The low correlation observed suggests that size-based assortive mating is not taking place.



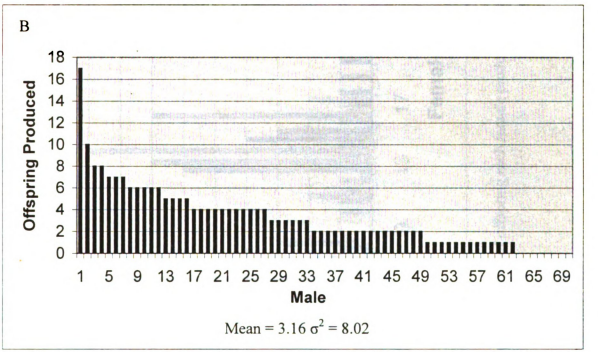


Figure 7. Graph showing the numbers of offspring produced for females (7a) and males (7b) for the 111 lake sturgeon in this study.

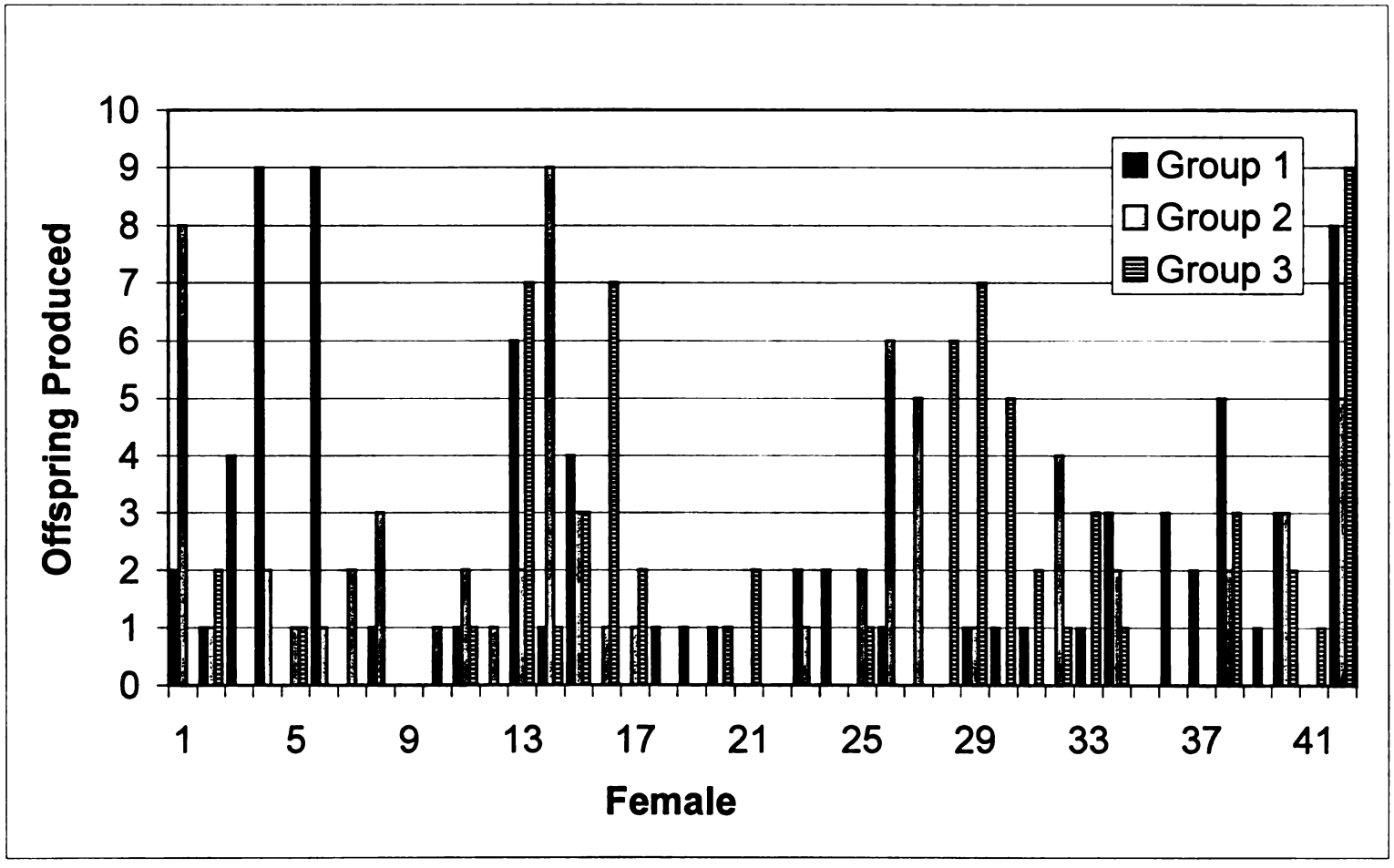


Figure 8a. Graph showing the number of offspring each female produced in each one of the groups of out-migrating juveniles.

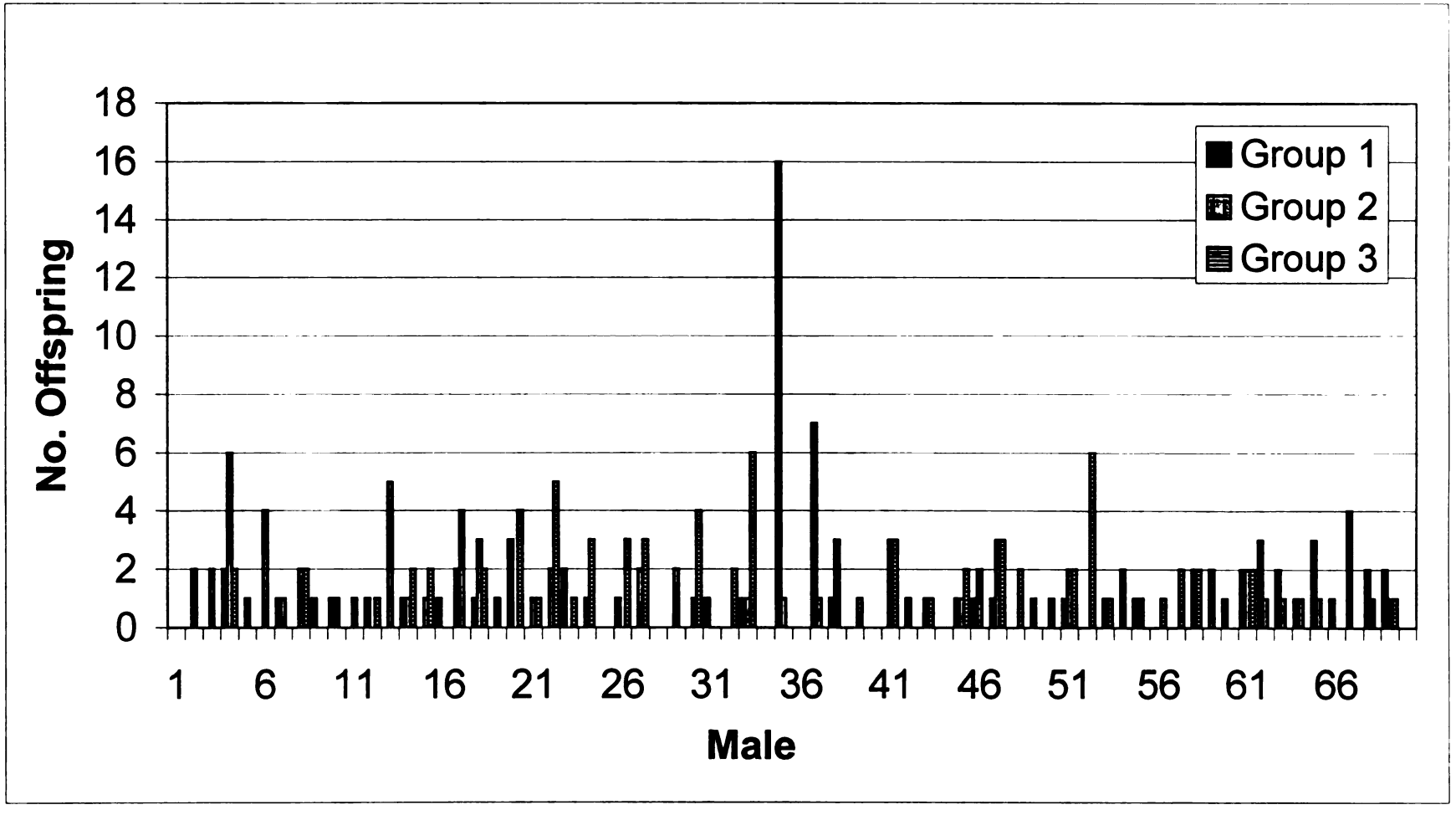


Figure 8b. Graph showing the number of offspring each male produced in each one of the three groups of out-migrating juveniles.

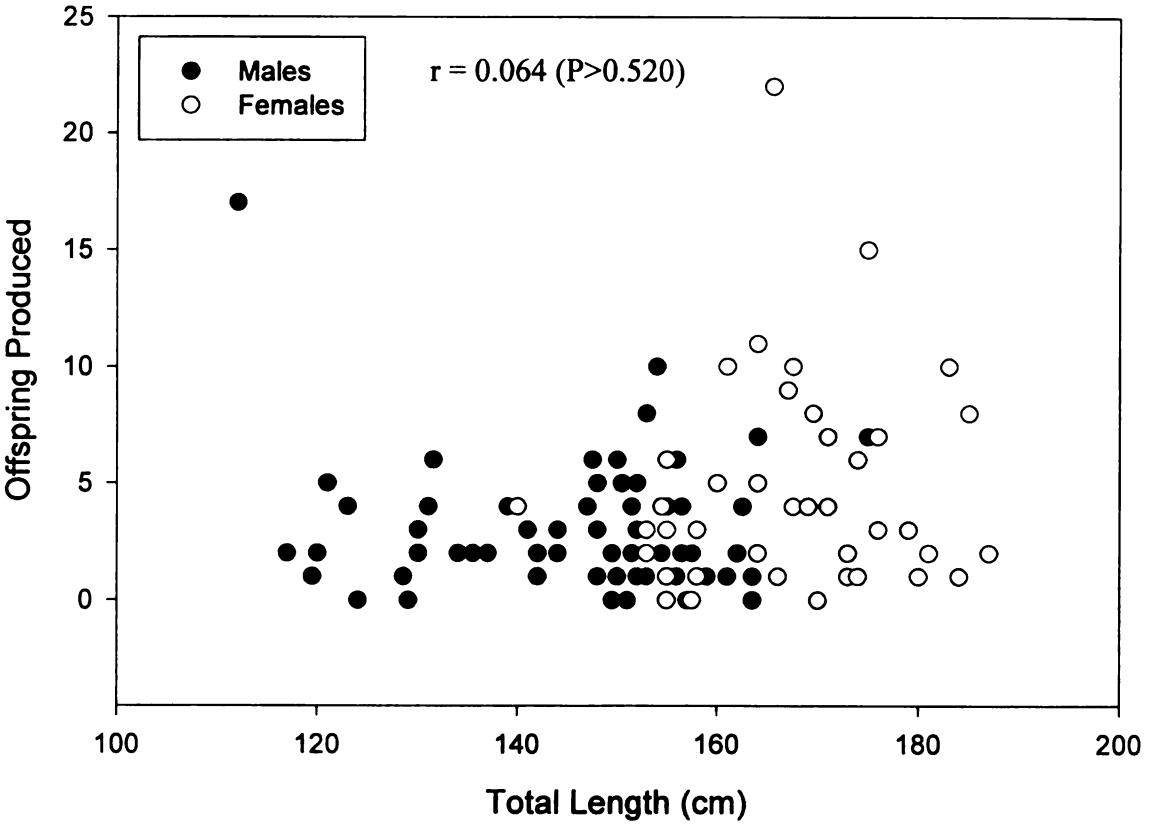


Figure 9. Relationship between total length and the number of offspring produced for each male and female.

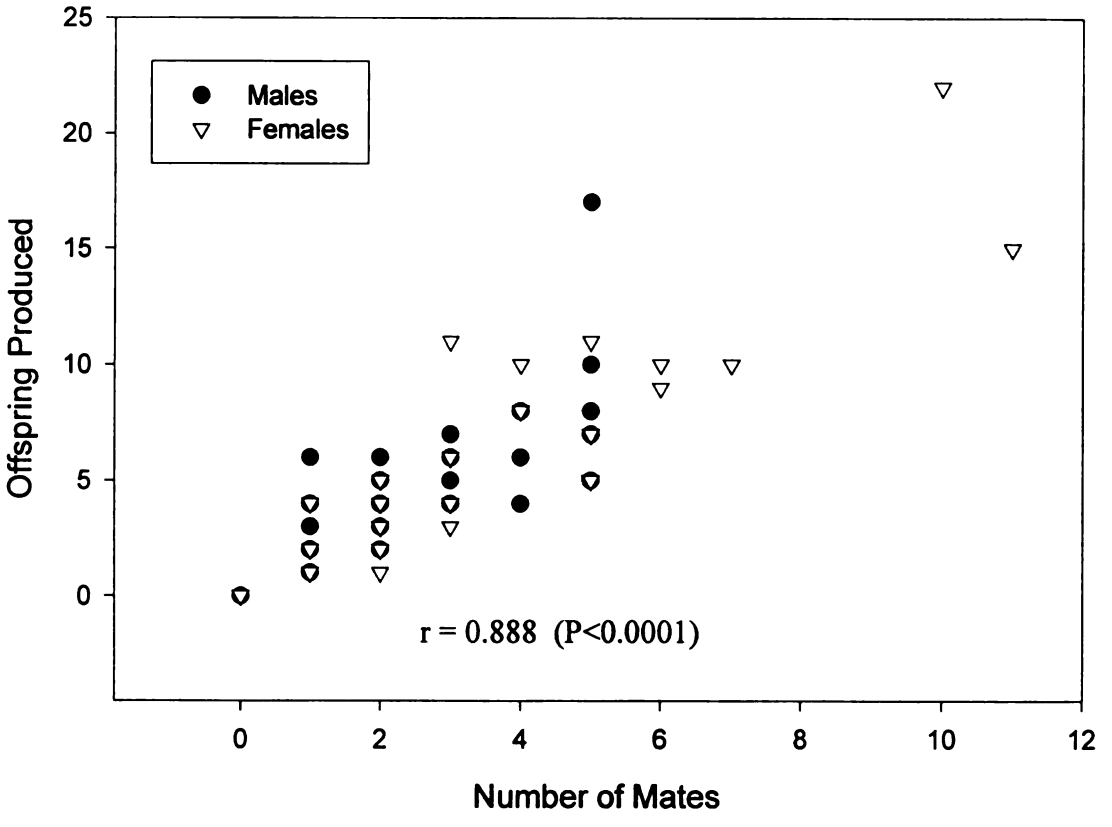


Figure 10. Relationship between the number of mates and the number of offspring produced for male and female lake sturgeon.

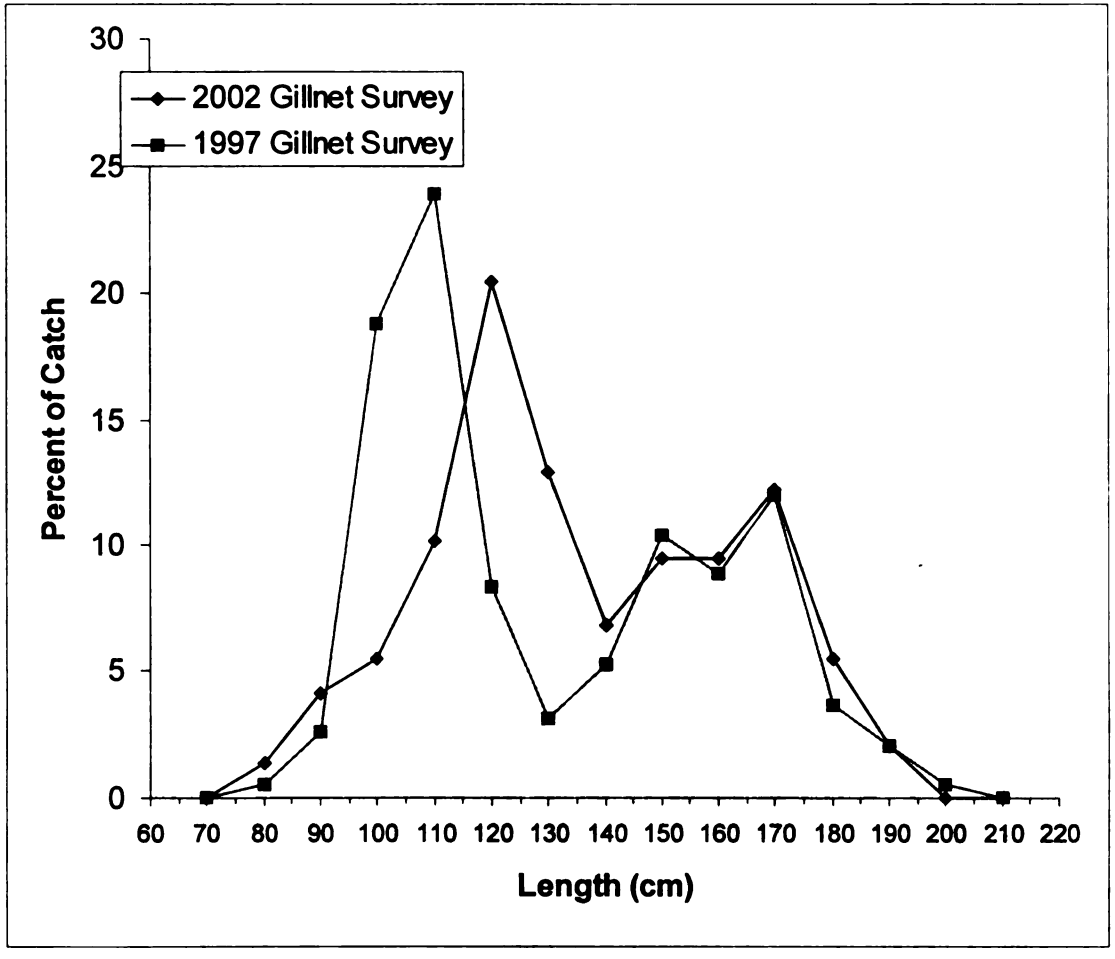


Figure 11. Percent of catch during population estimates in Black Lake composed of individuals of different lengths. The two peaks between 90 and 130 cm in 1997 and 2002 likely represent individuals produced by supportive breeding efforts. 1997 data adapted from Baker and Borgeson 1999, 2002 data adapted from MDNR unpublished data 2002.

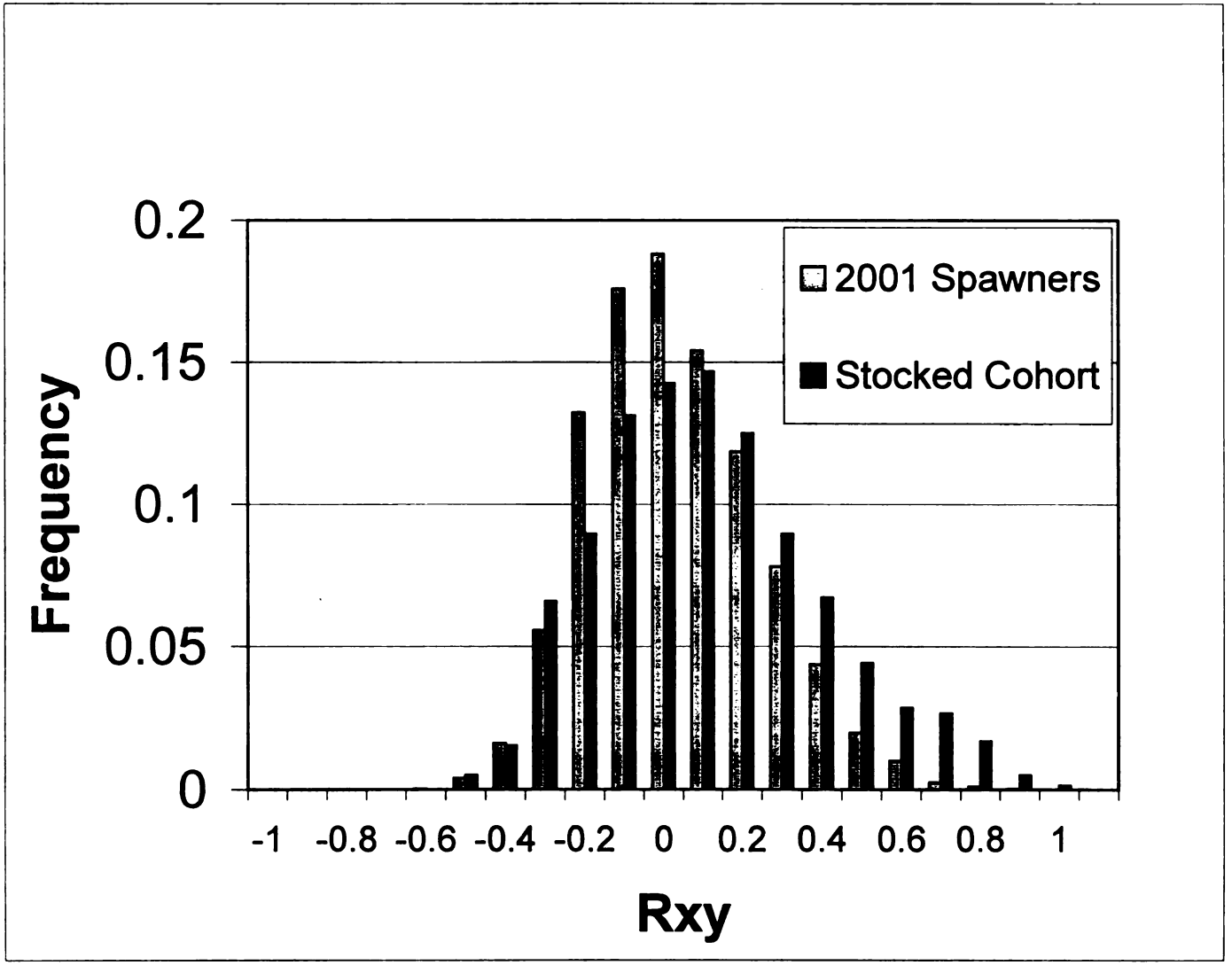


Figure 12. Distribution of levels of relatedness (r_{xy}) for the two groups of sturgeon used in this study.

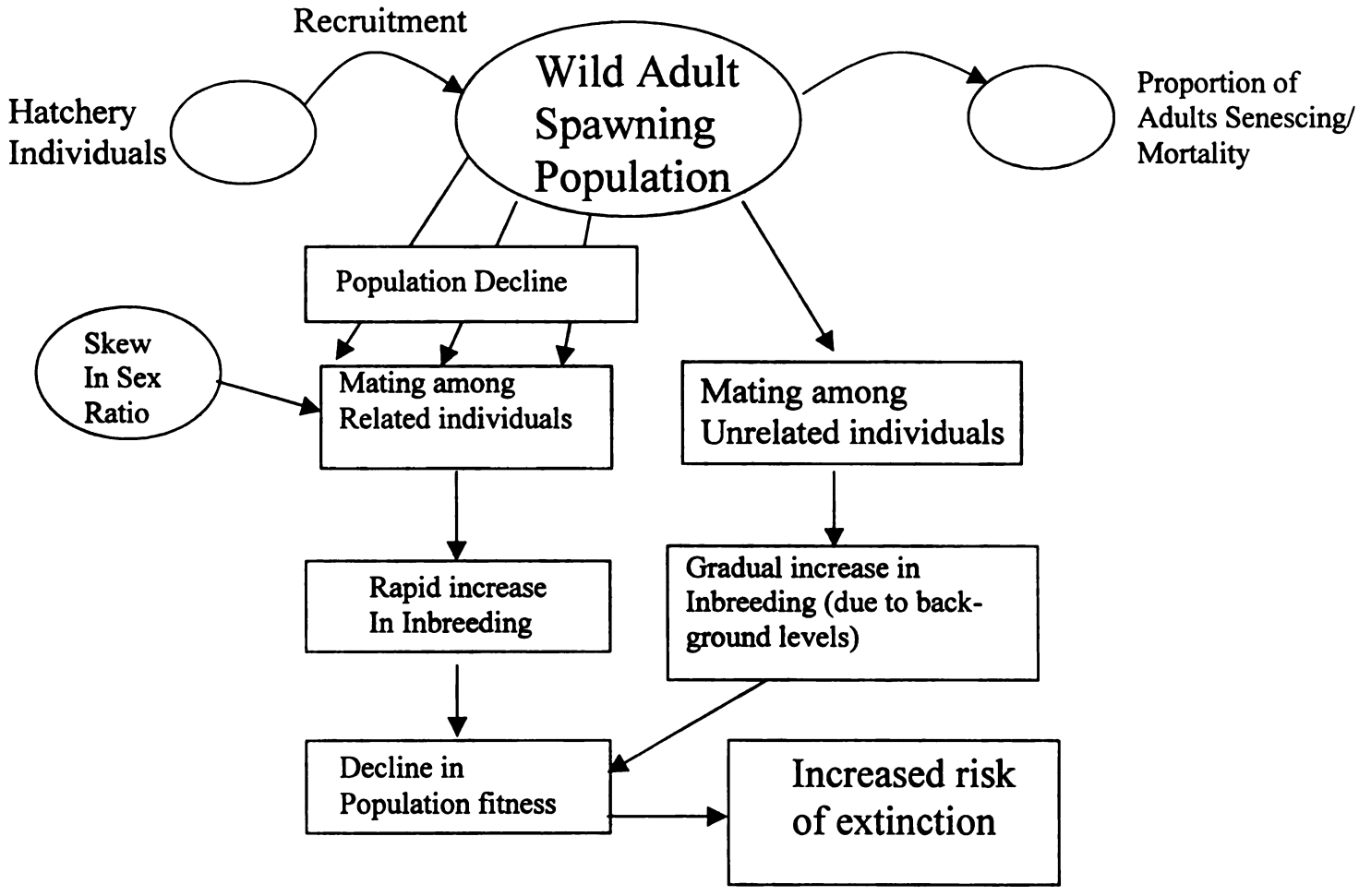


Figure 13. Conceptual model showing the ways in which different aspects of lake sturgeon life history and the demographic characteristics of this population will effect the level of inbreeding in this population. Factors such as population decline, increasing recruitment of hatchery individuals and the nature of the lake sturgeon mating system will all cause levels of inbreeding to increase more rapidly and subsequently cause population fitness to become reduced more rapidly.

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