GENETIC ANALYSES OF DISPERSAL, HARVEST MORTALITY, AND RECRUITMENT FOR REMNANT POPULATIONS OF LAKE STURGEON, *ACIPENSER FULVESCENS*, IN OPEN-WATER AND RIVERINE HABITATS OF LAKE MICHIGAN

by

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A THESIS

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

MASTER OF SCIENCE

Fisheries and Wildlife

2006

ABSTRACT

GENETIC ANALYSES OF DISPERSAL, HARVEST MORTALITY, AND RECRUITMENT FOR REMNANT POPULATIONS OF LAKE STURGEON, *ACIPENSER FULVESCENS*, IN OPEN-WATER AND RIVERINE HABITATS OF LAKE MICHIGAN

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Lake sturgeon, *Acipenser fulvescens,* have been severely impacted by anthropogenic disturbance and are a conservation priority throughout their range. Restoration efforts are impeded by a lack of information regarding patterns of recruitment and habitat use during non-spawning periods. Using the significant spatial genetic structuring of remnant populations of lake sturgeon throughout the Lake Michigan basin, combined with mixed-stock analyses and individual assignment, I examined (1) population-specific occupancy of open-water habitats and (2) the population composition of a sport fishery on the Menominee River. Also, by combining estimates of population of origin with estimates of age, along with the uncertainty in each of these estimates, I estimated recruitment within lake sturgeon populations since 1975. I found evidence for non-random habitat occupancy in northeast and western portions of Lake Michigan, suggesting that natal river and forage availability may affect habitat occupancy. Significant heterogeneity in habitat occupancy was also observed throughout Green Bay. Mixture analysis of a year of harvest from the downriver section of the Menominee River suggests that 17% of individuals harvested were not of Menominee River origin. A retrospective analysis of recruitment provided evidence for similar patterns in recruitment across Lake Michigan populations, despite varying historical population size.

ACKNOWLEDGEMENTS

Samples for this project came from a variety of sources. The majority of sample collections were coordinated by Robert F. Elliott (USFWS). Samples were provided by Tracy Galarowitcz and Rick Damstra (Central Michigan University), Edward A. Baker (MiDNR) and Nancy A. Auer (Michigan Technological University), Steve Lenart and others (Little Traverse Bay Band of Ottawa Indians), and Marty Holtgren and others (Little River Band of Odawa Indians), as well as commercial fishers around Green Bay. Baseline samples were collected by members of the WiDNR, MiDNR, and USFWS. Menominee hook-and-line samples were collected by Gregory Kornely, Michael Donofrio, and other WiDNR staff. All of these collaborators (and others) helped keep my work and analysis relevant with on-the-ground/in-the-river knowledge.

Funding for meetings, research, and more was provided by: the Michigan State Department of Fisheries and Wildlife (FW), the FW Graduate Student Organization, the College of Agriculture and Natural Resources, the MSU Graduate School, the Midwest chapter of the American Fisheries Society, and the Great Lakes Fishery Trust. Additional support came from the Department of Fisheries and Wildlife, the United States Fish and Wildlife Service, the Wisconsin Department of Natural Resources, and the Michigan Department of Natural Resources. The Great Lakes Fishery Trust provided the majority of the funding for this project.

For help with lab and programming troubleshooting, paper and presentation reviews, and general support, thank you to the members of the Molecular Ecology Lab, particularly Kristi Filcek, Scot Libants, and Rainy Shorey. Michael Hamlin and Ashok Ragavendran helped with computer programming, Ashok Ragavendran assisted with

some statistical matters, and Andrew McAninch created all included maps. Also, a nod to my fellow sturgeon folks, Andrea Drauch (Purdue University), and, from MSU, James Crossman and Patrick Forsythe.

None of this would have been possible without the support of my committee, Dr. Guilherme J.M. Rosa, Dr. Andrew G. McAdam, Mr. Robert F. Elliott, and Dr. Kim T. Scribner. Special thanks to Rob for being perennially-available for questions and clarification, and also for helping me arrange a field trip to visit field sites in and around Green Bay. And, of course, special recognition and thanks to my advisor and committee chair, Dr. Kim T. Scribner for all of his help and support.

Finally, a large “gracias” to the people who have supported me in a variety of ways throughout this degree: members of Genesee Garden Cohousing, the MSU chapter of Graduate Women in Science, and the women’s division of the MSU Cycling Team; also, Esther Deblacam, Tom Gore, Danielle Kaltz, Adam Keith, Nick Merz, Sergio Pierluissi, Mercedes Ramirez, Dr. Fred “Mother” Utter, Lozang Yonten, and Erin Zettle. For incredible support and encouragement, glasses raised to Sara Hughes, Andrew McAninch and Adam Misenheimer. A nod to Dr. Doug Eifler and Dr. Paul Spruell for showing me that science is fascinating and encouraging me to chase things. For teaching me that I can do things that I think are impossible, Tim Arrington, Brooke Nelson, Amanda Rosenberger and the rest of the RMRS/Boise crew. And, more than anyone else, my family, who have supported me in numerous ways throughout the past three years.

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INTRODUCTION

The order Acipenserformes, sturgeon (Acipenseridae) and paddlefish (Polyodontidae), represent an ancient lineage of fishes that has been present since the early Cretaceous period (135 million years before present, Birstein 1993). Twenty-five extant species of sturgeon and two species of paddlefish are found in Europe, Asia, and North America (Birstein 1993). These species exhibit a number of ancient characteristics, including a cartilaginous endocranium, heterocercal tail and ganoid scales (Birstein 1993).

Over the past 200 years, acipenserforms have experienced marked declines in abundance and distribution because of anthropogenic disturbances affecting migration and adult mortality rates (Birstein et al. 1997). In addition, the pollution of waters associated with nursery and spawning habitats has negatively affected recruitment (Auer 1999a, Birstein et al. 1997). Sturgeon use different habitats at different ages and different times of the year (Birstein et al. 1997). Given the diversity and distribution of suitable habitats, it is important to consider the quality of non-spawning habitat when assessing threats to these unique species.

A number of life history characteristics are shared among sturgeon species, including iteroparity (multiple years of spawning) and longevity (Bemis and Kynard 1997). Delayed onset of reproductive maturity is common among acipenserforms (Bemis and Kynard 1997). In all species, spawning occurs in water of low salinity, although there is variation in water temperature and flow during spawning (Bemis and Kynard, 1997). Successful spawning is habitat-dependent, with optimal substrate varying from gravel to large, creviced boulders (Bemis and Kynard 1997). Recruitment is highly variable, and dependent on water flows (Bemis and Kynard 1997). Spawning site fidelity is another common characteristic, although the mechanism for this fidelity is unknown (Bemis and Kynard 1997)

Genetic studies of sturgeon

Genetic techniques are important tools used to understand sturgeon biology and to further conservation efforts. Molecular markers have been applied to a number of population-level questions for several sturgeon species (e.g., Doukakis et al. 1999, Walsh et al. 2001, Wan et al. 2003, Zhang et al. 2003, Israel et al. 2004, Dugo et al. 2004). Three primary research emphases of genetics research among all sturgeon species are examining polyploidy, evaluating relatedness among species and species groups, and applying genetic techniques to management/conservation questions.

All acipenserfoms are polyploid, with putative ploidy levels of 4n, 8n, and 16n (Birstein 1993). Polyploidy in vertebrates is seen primarily in fishes, reptiles, and amphibians (Venkatesh 2003). Within fishes, polyploidy is observed in many lineages, including catostomids (suckers), salmonids (salmon, trout, whitefish), acipenserids (sturgeon, paddlefish), and some cyprinids (carps) and cyprinodontids (live-bearers) (Venkatesh 2003).

The evolutionary significance of polyploidy is unclear, and it has been proposed that polyploidy is a somewhat frequent event with minimal phenotypic effects and little evolutionary significance (Otto and Whitton 2000). Alternately, polyploidy may allow species more opportunity for adaptation, potentially yielding a faster evolutionary response (Otto and Whitton 2000). The degree of polyploidy has been investigated in many sturgeons (e.g., Blacklidge and Bidwell 1993, Fontana et al. 1999, Ludwig et al.

2001). Regardless of evolutionary significance, polyploidy can complicate genetic analysis, as many genetic models assume a disomic mode of inheritance. In polyploid organisms, all loci are replicated, but alleles at some loci may co-segregate, precluding unambiguous interpretation of allelic state (Ludwig et al. 2001).

Phylogenetic research can be used to better understand sturgeon evolution. For example, Birstein and Bemis (1997) reviewed molecular evidence for many sturgeon species using both karyotypes and an analysis of mitochondrial DNA (mtDNA) sequences. Despite low variability present in available mtDNA sequences, the authors were able to revise existing phylogenetic hypotheses regarding major groups of *Huso* spp., *Acipenser* spp*., Scarphirynchus* spp, and *Psuedoscaphirhynchus* spp., which had been formulated based on morphological data. Specifically, within the genus *Acipenser*, the authors found evidence for 17 distinct species, with two species, *A. oxyrhynchus* and *A. baerii,* featuring distinct subspecies. Within North America, the authors found many genetic similarities between the Pacific (*A. transmontanus* and *A. medirostris*) sturgeon species and Atlantic (*A. fulvescens* and *A. oxyrhynchus*) sturgeon species.

Phylogenetic information may also have conservation applications. Campton et al. (2000) examined genetic relationships among pallid, shovelnose, and Alabama sturgeon (*Scaphirhynchus albus*, *Scaphirhynchus platorynchus*, *Scaphirhynchus suttkusi*, respectively) using mtDNA. Genetic affinities among nominal species can be used to help guide management actions. The degree of genetic uniqueness, such as the definition of evolutionary significant units (ESUs, Waples 1991), has been used to provide species protection under the Endangered Species Act (ESA). Waples (1991) incorporated a number of factors, including degree of genetic differentiation among populations, to define distinct population segments. Genetic differentiation across sturgeon species, such as described by Campton et al. (2000), can also be used for forensics purposes such as identifying species of origin for caviar specimens. Species-specific identification provides an important tool for regulating trade and distinguishing poached species from legal take (Birstein et al. 2000).

In another example of genetics applied to management, de la Herran et al. (2004) examined genetic differences between sympatric species of sturgeon (*A. neccarii* and *A. sturio*) in the Mediterranean. Based on three nuclear DNA sequences and two mtDNA sequences, the authors found that previous range definitions for *A. neccarii* (endemic to the Adriatic Sea) were not correct, and that this species is found throughout the Mediterranean. In addition, some individuals had DNA consistent with both *A. neccarii* (nuclear sequences) and *A. sturio* (mtDNA sequences), suggesting introgression, which has also been observed in other sturgeon species. These findings are in contrast to evidence presented in an earlier review paper (Birstein and Bemis 1997), which suggested that all populations of beluga sturgeon in the Mediterranean region represent a single species. The work of de la Herran et al. (2004) have implications for the conservation of these fish, as the results may enable managers to more effectively design and implement restoration efforts based on new knowledge of the range of these species.

Genetic information may also be examined with species placed into a geographic context. For example, Brown et al. (1996) examined a mtDNA size polymorphism in four sturgeon species (*Acipenser transmontanus, A. medirostris, A. fulvescens, A. oxyrhynchus*) and noted a difference in heteroplasmy between the species currently present on the western (*A. transmontanus* and *A. medirostris*) and the eastern (*A.*

fulvescens and *A. oxyrhynchus)* sides of the continental divide. This study is significant in that it provides information about the relationships among North American sturgeons. The same mtDNA size polymorphism was used by DeHaan (2003) and DeHaan et al. (2006) to evaluate differences among remnant populations of lake sturgeon in the Laurentian Great Lakes.

Phylogeographic research has been used to make inferences about a number of North American sturgeon species. Waldman et al. (2002) analyzed mtDNA differences within shortnose, *A. brevirostrum*, Gulf, *A. oxyrinchus desotoi*, and Atlantic sturgeon, *A. oxyrhynchus*, populations to examine stock structure, haplotype diversity, and inferred levels of gene flow within remnant populations. Analyses were conducted both within a single species and across all three species. The authors found evidence for homing fidelity and different levels of gene flow in all three species. Wirgin et al. (2000) examined mtDNA sequence data within Atlantic sturgeon and found that diversity fell along a latitudinal cline consistent with glaciation events. More diversity was present in southern populations, suggesting that monomorphism in two Canadian populations was the result of a founder effect.

Applied questions such as the definition of management units can also be resolved by phylogeographic studies. Wirgin et al. (2002) used data from both mtDNA and microsatellites to examine the degree of homing fidelity and stock structure in Atlantic sturgeon, *A. oxyrhynchus*. The authors found concordance between marker types (nuclear and cytoplasmic) and found that most populations had high levels of genetic diversity. Similar to Wirgin et al. (2000), the authors found that diversity decreased from south to north, with monomorphism observed in Canadian samples. Based on their results, the authors recommended that the studied populations be divided into a minimum of six management units. Similarly, Grunwald et al. (2002) examined mtDNA control region sequence data in shortnose sturgeon, *A. brevirostrum*, and found evidence for population structure and a low level of gene flow. Based on their results, the authors recommended that all populations in their study be treated as separate management units.

Microsatellite markers have been developed for a number of sturgeon species. Since microsatellite regions are highly conserved, many of the makers that are developed for given a species can be used for many species (e.g., McQuown et al. 2000, King et al. 2001, Zane et al. 2002). When considering molecular markers for use in sturgeon, it is important to examine the ploidy levels of loci. For example, Pyatskowit et al. (2001) examined the inheritance of four microsatellites in lake sturgeon and found evidence for different ploidy levels at different loci (diploid and tetraploid). Studies in other sturgeon species (e.g. white sturgeon, *A. transmontanus*; Rodzen and May 2002) have also found varying ploidy levels (2N, 4N, 8N) at different loci.

Lake sturgeon

Lake sturgeon, *Acipenser fulvescens*, are endemic to North America, with a distribution spanning 18 states and 5 provinces, including the Hudson Bay, Great Lakes, and Mississippi River drainages (Houston 1987). Figure 1 shows the historical and contemporary distribution of lake sturgeon populations throughout the Great Lakes.

Lake sturgeon, like their congeners, have experienced marked declines in the past century, largely because of habitat loss and degradation, pollution, loss of habitat connectivity and overexploitation through harvest. Throughout the historical range, some populations have been extirpated and others remain with low numbers of individuals in the population. Some researchers estimate that lake sturgeon are present in approximately 1% of their historical abundance (Hay-Chmielewski and Whelan 1997).

Like other acipenserforms, lake sturgeon exhibit delayed sexual maturation, ranging between 15 to 25 years (Houston 1987). While a range exists for age at maturity for both sexes, males generally mature at younger ages than females. After maturation, the interval between spawning events varies by sex, with males spawning every one to four years and females spawning every three to seven years depending on location (Auer 1999b, Beamish et al. 1996). Lake sturgeon are potadromous, migrating from freshwater lakes to rivers to spawn. Lake sturgeon reproduce by broadcast spawning with no nest construction or post-ovulatory parental care. Fertilized eggs adhere to riverine substrate and incubate for a relatively short period of five to fourteen days (Kempinger 1988, Houston 1987). Once hatched, larvae may be present in the vicinity of the spawning site for one to three weeks before dispersing downstream (Auer 1999a, Kempinger 1988). Larval activity and movement vary with changes in light levels and water temperatures (Benson et al. 2005). Larvae are most vulnerable to natural predation before the formation of their scutes during the first year (Houston 1987).

Lake sturgeon population persistence is partially dependent on habitat quality. By the nature of their potadromous life history, lake sturgeon require connectivity between lakes and rivers. If spawning habitat is altered, sturgeon may not spawn successfully or may not spawn at all. For example, changes in water flow can change habitat characteristics (e.g., causing siltation or new algal growth) such that eggs may not adhere to rocks and may not hatch. Changes in temperature caused by industrial discharge or impoundment are also of concern to long-term population persistence (Auer 1996a; Auer

1996b), particularly because it is suspected that temperature is a cue for spawning activity.

Lake sturgeon are found in their natal streams while staging for spawning and during spawning runs, but habitat use during other times of the year is variable, including movement among and within lakes and river mouths. In habitats on the scale of the Great Lakes, individuals may be able to migrate great distances between spawning runs, especially since spawning does not occur annually. It is important to know how different stocks may be using open-water habitats, as this could lead to different management actions if protecting a particular stock is of interest. Also, patterns of habitat use and movement may make certain stocks more susceptible to anthropogenic or environmental processes. This too is an important consideration in conservation efforts.

One conservation concern for lake sturgeon is the impact of harvest on stocks. Harvest from fishing, via both legal takes and poaching, has been a major factor in declines of lake sturgeon and congeners worldwide (Birstein 1993, Boreman 1997). Sturgeon were once considered an annoyance within the Great Lakes because their large size and sharp scutes damaged fishing nets (Auer 1999a, Hay-Chmielewski and Whelan 1997) but have since become valued for both their meat and their eggs (caviar). Globally, the caviar trade has been a major factor in the decline of sturgeon species (e.g., Cohen 1997), and the lake sturgeon is no exception (Houston 1987).

Although lake sturgeon populations have declined in both abundance and distribution, declines have not been consistent across populations (Holey et al. 2000). Some lake sturgeon populations are extirpated and many others are present in low numbers relative to historic levels. However, other lake sturgeon populations are present at levels such that managers have deemed harvest an appropriate management action. Many of these still-harvested populations are in the Mississippi River drainage, but some populations within the Great Lakes drainage are also harvested. Some populations are harvested in the winter by spearing (e.g., Black Lake, MI and Lake Winnebago, WI) and others by hook-and-line fishing in the fall (e.g., Menominee River, WI/MI) and also commercial netting (e.g. Canadian waters of Lake Huron).

The differential status of lake sturgeon populations can be an impediment to conservation efforts, because lake sturgeon do not have a range-wide consistent conservation status. Welsh (2004) reviewed the status of lake sturgeon throughout its range and discussed the difficulties of creating conservation policies for a species with varying numerical abundance across its range, particularly when that range spans both national and international boundaries. Although lake sturgeon are not listed as an endangered species under the United States Endangered Species Act, they do receive protection to varying degrees throughout their range at the state and provincial levels, listed as threatened, endangered, or of special concern (Welsh 2004). As an example of differential status, in Michigan lake sturgeon are listed as threatened, while in Wisconsin they are on unofficial watch, and in Ontario, Canada, they are considered a sensitive species (Welsh 2004).

Lake sturgeon genetics

Recent work represents the first significant population-level genetics study of lake sturgeon throughout the Great Lakes (DeHaan 2003, DeHaan et al. 2006). Studies using tag return data (Stabile et al. 1996) suggest that philopatry may be present in some sturgeon species. Fidelity to natal streams has been suspected in lake sturgeon and was confirmed by research (DeHaan 2003, DeHaan et al. 2006) which provided support for significant spatial population structuring in 11 remnant lake sturgeon populations in the Great Lakes based on eight microsatellite loci and mtDNA sequence data. DeHaan et al. (2006) provide evidence for significant structure present at the level of both Great Lake basins and tributaries within those basins (Figure 2).

In an investigation into the mating system of lake sturgeon, DeHaan (2003) also conducted parentage analysis on individuals from the Black Lake system in northern Michigan. He examined aspects of lake sturgeon reproductive ecology such as variance in reproductive success and disproportionate contribution of individuals to larval production. Using multilocus genotypes of spawning adults and outmigrating larvae, larvae were assigned to putative parents. Parentage analyses indicated that there was both polyandry and polygyny present within the Black Lake/Black River population. There was also evidence for high variance in reproductive success for both sexes (DeHaan 2003).

As part of an examination of population differentiation, DeHaan (2003) and DeHaan et al. (2006) also used an 82 base-pair mtDNA size polymorphism (see Brown et al. 1996). This sequence differed in the number of copies (1,2,3,4) present in remnant populations of lake sturgeon within the Great Lakes. Both the mtDNA and microsatellite data showed more variation between lake basins than within lake basins. Because of its maternal mode of inheritance, mtDNA has an effective population size one-quarter that of nuclear markers and is therefore more sensitive to genetic drift. DeHaan (2003) and DeHaan et al. (2006) found evidence for genetic drift in one population (Menominee River) based on the presence of only one haplotype. Genetic drift in the Menominee

River population may be accelerated by the combination of impoundment and harvest on this river.

Problem statement

While there has been extensive research into aspects of lake sturgeon population dynamics and biology, using both genetic and non-genetic methods, the majority of research on adult movement and habitat use has been focused on spawning adults. During the spawning period, the location of individuals is fairly predictable (as a result of natal philopatry) and individuals are present in high densities relative to non-spawning periods. The disproportionate focus on this relatively brief event results in a limited understanding of the habitat occupancy by individuals during non-spawning periods. Without a more complete understanding of the biology of lake sturgeon, including that of pre-reproductive adults and non-spawning adults, efforts to conserve and restore lake sturgeon may not be designed effectively. Two notable areas in need of investigation are patterns of recruitment and the distribution of individuals in open-water habitat relative to their population of origin.

In this study, I investigated aspects of lake sturgeon biology from natality (recruitment) to mortality (harvest) as well as open-water habitat use. By incorporating genetic, habitat, and population information for remnant breeding populations throughout Lake Michigan, this study provides new insight on lake sturgeon populations, including patterns of recruitment over time and habitat occupancy during non-spawning periods. The knowledge gained through this work will help guide future management actions and restoration efforts for this unique species of significant conservation concern.

CHAPTER ONE : POPLULATION ASSIGNMENT OF LAKE STURGEON OF UNKNOWN ORIGIN FROM OPEN-WATER HABITATS IN LAKE MICHIGAN

Introduction

Examining habitat occupancy and movement by organisms has been a focus within fisheries science and wildlife management, as such examinations allow populations and their movements to be monitored. One method of observing habitat occupancy is through direct methods such as mark-recapture studies (Labonne and Gaudin 2005) and direct observation (Trenham et al. 2000). Other recent studies have combined direct (tagging, mark-recapture, observation) and indirect (genetic) approaches. For example, Wilson et al. (2004) used both direct and indirect methods to examine brook charr, *Salvelinus fontinalis*, movement patterns across two populations in Newfoundland. Some authors suggest moving toward indirect methods as a surrogate for direct methods (e.g., mark-recapture, Pearce et al. 2001).

Using genetic methods in addition to, or in place of, direct methods to better understand habitat use and dispersal may be of utility in certain situations. For example, direct methods may not yield sufficient information or may be too labor-intensive if species inhabit inaccessible habitats or are found in low densities across large areas of habitat. Given genetic data on potential source populations, one method that has been used to elucidate questions of habitat use and dispersal is mixed stock analysis (MSA, e.g. Pella and Masuda 2001)

MSA examines groups of individuals of unknown, and assumed multiple, origins and estimates the proportional contributions of putative source populations based on genetic characteristics (allelic frequencies and multilocus genotypes). Application of this method has included a number of management- and conservation-related research questions in fisheries, specifically for sympatric species subject to harvest. For species that co-occupy habitats and are subjected to harvest, studies have examined the contribution of individual salmonid populations to total salmonid harvest both within U.S. waters (Shaklee et al. 1999) and throughout the northern Pacific Ocean (Beacham et al. 2006). Investigations of population differentiation, including MSA analyses, have also been used to guide management of declining sockeye salmon, *Oncorhynchus nerka*, fisheries in British Columbia (Nelson et al. 2003). Application of MSA has also involved issues outside of harvest management, including how environmental changes from the *Exxon Valdez* oil spill differentially affected spawning populations of sockeye salmon contributing to a common habitat (Seeb et al. 2000).

The Laurentian Great Lakes are home to a diversity of fish species, many of which share habitat but form distinct breeding populations within the same species. In addition to representing a vast area of freshwater habitat, the Great Lakes also provide examples of an variety of human-mediated environmental changes. MSA techniques have been applied within the Great Lakes to better understand the effect of a number of different anthropogenic actions on fish populations. To evaluate the success of various stocking and its effects on lake trout, *Salvelinus namaycush,* populations in the Great Lakes, Page et al. (2003) used MSA to examine recruitment of three lake trout hatchery strains to open-water populations. The results from this analysis showed that different strains (types) of hatchery fish had varying degrees of stocking success. Using MSA to evaluate walleye, *Stizostedion vitreum,* populations in Lakes Erie and Huron, McParland et al. (1999) found that not all populations contributed equally to harvest within the

Huron-Erie corridor. These examples illustrate the utility of MSA for research focusing on fisheries populations for conservation, management, and monitoring.

Within the Great Lakes, one species of great conservation focus is the lake sturgeon, *Acipenser fulvescens*. Lake sturgeon are of conservation priority throughout their range (Welsh 2004), which historically spanned 18 states in the U.S. and 5 provinces in Canada (Houston 1987). Since the colonization of the Great Lakes region by Europeans more than 200 years ago, lake sturgeon have experienced marked declines in numerical abundance, especially over the last half of the 19th (late 1800s) century. These declines have been attributed to habitat loss and degradation, pollution, loss of habitat connectivity and overexploitation. Recent estimates indicate that lake sturgeon currently exist in approximately 1% of their historical abundance; where populations remain, they are numerically depressed relative to historic levels (Hay-Chmielewski and Whelan 1997).

Lake sturgeon are long-lived fish (Baker 1980), with a delayed onset of sexual maturity (between 15 and 25 years, depending on sex; Houston 1987). After maturation, the interval between spawning events varies by sex, with males spawning every one to four years and females spawning every three to seven years depending on location (Auer 1999b, Beamish et al. 1996). Lake sturgeon are potadromous, migrating from freshwater lakes to rivers to spawn (Houston 1987).

As a result of their life history, reproductively mature lake sturgeon are found in their natal streams during the brief spawning period, remaining in rivers for 1-2 weeks, although some individuals may stage in the river for months. Habitat use during other (non-spawning) periods, and by non-spawning individuals, is variable and less well characterized. Considering the late maturation and intermittent spawning of these fish, the spawning period is a small fraction of the life of a sturgeon. Individuals present in potentially shared habitats during non-spawning periods could originate from any population. Therefore, groups of individuals present in common habitats and/or at non-spawning times cannot be used to evaluate population size or population-specific dispersal patterns, regardless of proximity to a given potential source population. Individuals may travel great distances between spawning runs, especially since spawning does not occur annually.

Relying solely on direct methods of population assessment, such as mark-recapture and tagging studies, it is difficult to characterize breeding populations of lake sturgeon sufficiently because of their life history. In order to identify population of origin with high confidence, in the absence of genetic information it would be necessary to capture, tag, and monitor spawning individuals. As juveniles may spend up to 20 years in open-water habitats before returning to spawn, and adults 2-7 years, it is likely that there would be an extensive interval of time between tagging an individual and gathering data on that individual upon its return. Even if individuals bred more frequently (shorter interval between spawning events), it would take a number of years of sampling to characterize a breeding population, as only a fraction of the individuals originating from a given river will spawn, and therefore be susceptible to capture, in any one year.

In contrast to direct methods, genetic approaches have the potential to yield information on population of origin for all individuals captured at any time and location. Lake sturgeon provide an excellent system in which to apply MSA approaches to address issues relevant to conservation. Previous research (DeHaan 2003, DeHaan et al. 2006) identified regions of significant genetic discordance among breeding populations of lake sturgeon in the Great Lakes **(**Figure 2). Spatial variance in allelic frequencies were shown to be apportioned between lake basins (Lake Superior vs. Lake Michigan and Lake Huron) and among rivers within each basin. Breeding populations in tributaries to Lake Superior are highly differentiated from all other populations in the upper Great Lakes (significant bootstrap iterations = 990/1000), and there is also significant genetic differentiation between populations in western Lake Michigan when compared to populations in Lake Superior and Lake Huron (significant bootstrap iterations = 907/1000). Within Lake Michigan, populations in tributaries on the eastern shore (Muskegon and Manistee Rivers) were shown to be genetically differentiated from populations in tributaries to Lake Huron (significant bootstrap iterations = 830/1000). Significant spatial structure was also found within the western basin of Lake Michigan, with significant divergence among populations in the Menominee, Wolf, Fox, Oconto, and Peshtigo Rivers. As there is not evidence for significant differentiation between populations in the Oconto and Peshtigo Rivers, populations in these rivers were paired as the Oconto/Peshtigo Rivers in all analyses. Similarly, the Fox and Wolf River systems are not significantly genetically differentiated. In all analyses, the Fox River was used to represent this system. On the eastern side of Lake Michigan, the Manistee and Muskegon Rivers are not highly genetically differentiated, so the Manistee River was used to represent the eastern basin of Lake Michigan, as it is the larger of these two remnant populations (Elliott 2003).

Successful restoration efforts for lake sturgeon populations require a fundamental understanding of life-history patterns, including habitat occupancy during non-spawning periods, which can be elucidated using MSA. The results from this work provide information not previously available to research and recovery efforts, as patterns of population presence can now be examined across shared habitats. Such information is crucial because little is known about adult or subadult distributions and movements in open-water habitats during non-spawning periods. Information on habitat use can be implemented to predict risks to populations. For example, if individuals from distinct breeding populations are concentrated in different habitats, populations may be differentially affected by incidental harvest or environmental catastrophe.

This study focuses on open-water sites sampled throughout Lake Michigan over multiple years in order to examine patterns of population presence and abundance throughout the lake basin. If fish are dispersing at random throughout all available habitat, it is expected that the probability of encountering individuals from breeding populations will not differ significantly throughout the basin. Heterogeneity in estimates of population contribution at different open-water sampling locales across the basin would provide strong evidence for non-random dispersal of individuals from specific breeding populations.

The life history of lake sturgeon precludes the use of more traditional, direct methods of observing movements within open-water habitats. By using genetic information, I was able to examine habitat use and dispersal during non-spawning periods, which represent important, yet not fully understood, aspects of lake sturgeon biology. Knowledge of how populations contribute to open-water habitats, and particularly how those contributions change as a function of distance, season (time of year), bathymetric features, and other factors, will help inform more effective conservation and management actions for lake sturgeon.

Methods

Sample collection: baselines

As part of ongoing research on lake sturgeon throughout Lake Michigan (e.g. DeHaan 2003, Gunderman and Elliott 2004) spawning adult lake sturgeon were captured by cooperating agencies and institutions from five different remnant populations that breed in tributaries throughout Lake Michigan: the Fox (n=71), Peshtigo and Oconto Rivers (n=91) (Wisconsin), the Manistee River (n=80) (Michigan) and the Menominee River (n=41) (Michigan/Wisconsin) (Figure 3). Individuals were measured (length, girth, weight), and implanted with internal (passive integrated transponder, PIT) and external (Floy) tags for future identification. Samples of fin tissue (approximately a 1 cm^2 clip of the dorsal fin) were also collected, allowed to dry, and stored in scale envelopes at ambient temperature (Gunderman and Elliott 2004, DeHaan et al. 2006).

Sample collection: mixtures

Sampling of lake sturgeon (population of origin not known) was concentrated throughout the waters of Green Bay, Lake Michigan, from 2002 to 2006 (Figure 4). In Green Bay, lake sturgeon were sampled by USFWS, Michigan Technological University, Michigan Department of Natural Resources (MiDNR), and Wisconsin Department of Natural Resources (WiDNR) field researchers working with commercial fishers using trap nets and gill nets. Nets varied slightly in dimensions but all commercial lake whitefish, *Coregonus clupeaformis,* trap nets had leads of approximately 1000 ft and pot mesh of 4.5 inches. Commercial gill nets varied in mesh size depending on the targeted species (lake whitefish, ~4.5 inches; perch, *Perca flavescens,* ~2.5 inches). Samples were collected throughout the calendar year, with the majority of samples from collections in the spring and early summer in most areas of the lake and additional samples collected during the fall and winter in other areas. All individuals were marked with an external Floy tag and an internal (PIT, passive integrated transponder) tag (Gunderman and Elliott 2004). Samples also were collected from individual lake sturgeon captured from several locations in open-water around Lake Michigan (Figure 3). All fin tissue samples (approximately a 1 cm^2 clip of the dorsal fin) were allowed to dry, and stored in scale envelopes at ambient temperature.

Sampling within Green Bay represents a combination of logistical limitations and opportunity. Specific areas were targeted based on results of prior telemetry surveys and previous fishing that indicated the presence of adult lake sturgeon (R. F. Elliott, personal communication). Large commercial-size trap nets were used because of their efficiency in collecting large numbers of sturgeon in open water habitats. However, the use of trap nets was limited to water deeper than approximately shallower than 30 ft.

Additional incidental collections of lake sturgeon by commercial fishers were influenced by the species they were targeting at the time. For example, in the far southern portion of Green Bay, commercial fishing targeted perch, *Perca flavescens***,** and whitefish, *Coregonus clupeaformis,* where only whitefish were targeted in the central and northern waters of the bay. Elsewhere around Lake Michigan, fin tissue samples were collected from a number of sources, including incidental catches by commercial fishers and fish found dead on lakeshores. Other samples were obtained by additional researchers throughout Lake Michigan (e.g., Little Traverse Bay Band of Odawa Indians, Grand Traverse Band of Chippewa Indians, Little River Band of Ottawa Indians) using trap nets and gill nets.

Genetic Analyses

MSA requires information from multilocus genotypes for both individuals of known origin (baselines) and individuals of unknown origin (mixtures). DNA was extracted from lake sturgeon fin tissue samples using QIAGEN DNeasy® kits (QIAGEN Inc.) according to manufacturers' protocols. All samples were diluted to a consistent DNA concentration of 20 ng/ul for use in PCR reactions.

Individuals were genotyped at 8 microsatellite loci including *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001) *AfuG9, AfuG63, AfuG74, AfuG112*, (Welsh et al. 2003). PCR reactions were conducted in 25 µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M MgCl2, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), additional MgCl2 as determined by optimizations (1.0ul: *LS-68, Afu68B, Spl120, AfuG112*; 1.5ul: *AfuG9*), 2mM of each dNTP, 10 pmol of forward and reverse primer and 0.5 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 (*AfuG9, AfuG63, AfuG112:* 48C; *AfuG74*: 50C; *Aox27:* 53C; *LS68, Afu68B:* 56C; *Spl120: 62C)* and 72° C for 1 minute. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIOII scanner. Allele sizes were determined using commercially available size standards (MapMarkerTM, BioVentures Inc.) and based on standard samples of known genotype. To minimize error, all genotypes were independently scored by two experienced lab personnel and verified again after data were entered into electronic databases.

Statistical Analyses

Background

Mixed stock analysis (MSA) uses differences in allelic and genotypic frequencies among baseline (breeding) populations to estimate proportional contributions of baseline populations to a given mixture sample. In general, this method is more accurate as more polymorphic markers are added to baselines, assuming that there are differences in allelic frequencies among baseline populations (Kalinowski 2004**)** and that allele frequencies are estimated with minimal error (Guinand et al. 2004).

Mixture analysis was conducted using the Statistical Program for the Analysis of Mixtures, version 3.7b (SPAM; Debevec et al. 2000, ADFG 2003). SPAM uses a Bayesian approach, incorporating information from baseline populations as priors to inform population allocation of mixtures to putative populations of origin. In contrast to other (frequentist) approaches, the Bayesian method incorporates information about the genetic similarities across stocks in allocating mixtures to populations of origin, which increases both accuracy and precision of estimates (Pella and Masuda 2001). Unlike other estimators, this Bayesian method is accurate even in the presence of rare alleles (in mixtures or baselines) and when composition estimates begin to differ greatly across baselines (Pella and Masuda 2001). In this study, individuals from open-water habitats (population of origin is not known) were assigned to populations of most probable origin by repeated resampling (over a series of Monte Carlo Markov Chain [MCMC] iterations) of both baselines (individuals of known origin) and mixtures (individuals of unknown origin).

The first step of analysis in SPAM is to treat each baseline as a set of unknown individuals and proportionally assign individuals from each baseline population to potential populations of origin. The purpose of these simulations is to determine accuracy, precision, and allocation bias when assigning individuals from a mixture sample (admixed population) to source populations. In a dataset with completely accurate population allocation, 100% assignment would occur to the same baseline population and assignments to the other baseline populations would be 0%. Deviations from 100% allocation indicate where, and in what direction, misassignments are likely to occur in empirical mixtures of unknown composition.

Misallocation may be due to two factors: populations that are not sufficiently characterized (e.g., low sample size and imprecision in estimates of allele frequency), or as a result of genetic similarities across populations **(**Kalinowski 2004). In the second case, the magnitude of misallocation will reflect degrees of genetic differentiation among baseline populations **(**Kalinowski 2004). Baseline populations characterized by higher misallocation are likely to be genetically similar to other baseline populations.

Given a sufficient degree of accuracy in baseline assignments, based on simulation analysis, empirical mixtures are subsequently analyzed (estimation analysis) using the same statistical techniques as the simulations described above. In this study, baselines populations are remnant spawning populations of lake sturgeon across the Lake Michigan basin.

Because of the limited number of possibilities of resampling within a small sample size, SPAM can have very low power when working with a small sample size. When a small sample size is of interest, individual assignment (IA; Paetkau et al. 1995) is a more effective method of classification. Additionally, IA analysis allows the examination of population of origin for specific individuals of interest.

While there is utility in examining individual assignments, there are also limitations to IA. The confidence with which an individual may assign to a population may be very low, indicating a high degree of uncertainty in assignment. In an effort to be somewhat conservative in IA analysis, an individual was only considered “assigned” if the posterior probability of assignment was >90%. This value was chosen as a balance between two factors: (1) the commonly accepted error rate of 5% (Gotelli and Ellison 2004) which in this case corresponds to a posterior probability of assignment >95%, and (2) the nature of work within interconnected source populations, as gene flow across populations can decrease the accuracy of assignment (Hansen et al. 2001).

Analyses of baseline populations: simulation analyses

To examine precision and accuracy of assignment, multilocus genotypes from known spawning individuals from four Lake Michigan tributaries— the Fox River, “Michigan” (the eastern basin of Lake Michigan, as represented by the Manistee River), the Menominee River and the Oconto River/Peshtigo River— were used in 100% simulation analyses using SPAM 3.7b (ADFG 2003). Individuals from the Peshtigo and Oconto Rivers were combined into a single composite baseline population because of similarities in allelic frequency (DeHaan 2003, DeHaan et al. 2006). Simulation analyses were based on 1000 MCMC replicates, with both the mixtures and the baselines re-sampled at every iteration. Each iteration was checked for sufficient convergence (defined as a guaranteed percent achievement of the maximal likelihood, GPA, of 95% or more) to ensure that simulations were converging on the true global maximum.

Analyses of mixture composition: estimation analyses

Using multilocus genotypes of individuals from samples collected throughout the open-waters of Lake Michigan (focused on Green Bay), analyses were conducted to estimate the contribution of baseline populations to mixture samples. These analyses are based on 1000 MCMC replicates, with both the mixtures and the baselines re-sampled at every iteration. Again, iterations were checked for convergence (see above).

Lake sturgeon collected from open-water habitats were formed into groups based on proximity of collection locales, seasons of collection and collection gear type with additional consideration of bathymetric features that might influence fish movement. For example, the Peshtigo River Area (PRA), Young's Reef Area (YRA), Peshtigo Reef Area (PRF), and Oconto River Area (ORA) collection locales span regions of Green Bay in close geographic proximity to each other (Figure 4). However, within this region of the lake, there are bathymetric features that might cause habitat to be more or less permeable to fish movements, potentially affecting occupancy of different regions. Also, differences in season of collection and gear were considered in forming groups. Some groups were entirely collected by trap net (e.g. Peshtigo Reef Area, PRA) and others largely by gill net (e.g. Little Sturgeon Area-south, LSS), and others a mixture of trap nets and gill nets (e.g. Little Sturgeon Area-north, LSN). Some collections were made during winter/spring months (e.g. Dykesville Area, DYK) with others conducted largely in the summer months (e.g. Pensaukee Area, PEN). In total, 13 groups were formed from samples collected throughout Green Bay (Figure 4**).** Mixture analysis was then conducted on these 13 groups (data not shown).

After analyzing population contribution to these small-scale groups, these 13 groups were further combined, as the discriminatory power of MSA is lower with small sample sizes. With a goal of a sample size of at least 50 individuals per sample group, collection locales were combined based on geographic proximity and similar bathymetric features, being careful not to combine locales that may represent physically distinct areas of the lake. Point estimates of mixture composition from initial mixture analysis were used to further inform groupings but, again, physical aspects of collection sites were prioritized in an attempt to avoid combining collection points that might span dissimilar habitats. Based on these criteria, six groups were formed for mixture analysis: 1) SGB/DYK/PEN/ORA, 2) PRA/YRA, 3) PRF/MAR, 4) LSS/LSN, 5) CRA, 6) WAI/DCO (Figure 4).

Analyses of mixture composition: homogeneity tests

Estimates of proportional population contribution, from both simulation and estimation analyses, are provided by SPAM as a point estimate of contribution and 90% confidence intervals. Some MSA studies have examined difference between samples by comparing the point estimates of contribution and confidence intervals (e.g., Seeb and Crane 1999**).** However, this approach leads to higher rates of Type I and Type II errors, as a result of not correcting for multiple tests and examining marginal (not joint) summary statistics (Reynolds and Templin 2004).

Reynolds and Templin (2004) describe a method of examining homogeneity across mixtures using likelihood ratio tests (LRT), which allows the assessment of heterogeneity across mixtures without increasing Type I and Type II error rates. This is a two-stage calculation. First, the observed likelihood ratio must be calculated (from empirical data). The likelihood of the null model (homogeneity, all samples come from a single mixture) is first calculated, and then the likelihood of the alternative model (heterogeneity, all samples do not come from a single mixture) is calculated. Second, a likelihood ratio distribution is calculated, given that the null model (homogeneity) is true. The observed likelihood ratio, the ratio of the null to the alternative (step one) is calculated, and this is compared to likelihood ratios that are expected under the null hypothesis (step two). Monte Carlo P-values are approximated using the method of Davison and Hinkely (1997), where $p=(1+Q)/(1+R)$ with Q=the number of log likelihood ratios greater than or equal to the observed log likelihood ratio, and R= the number of simulation replicates.

In an effort to decrease the probability of Type I and Type II error rates in estimations, homogeneity of mixtures was examined within the study area using the LRT described in Reynolds and Templin (2004), implemented in SPAM 3.7b (ADFG 2003). Homogeneity analysis involves a series of simulations to create observed and null distributions representing each mixture. All simulations were conducted using 5000 MCMC replicates, and all output files were checked for convergence (defined as guaranteed percent achievement of the maximal likelihood, GPA $\geq$95%, and random seeds consistent across simulations) to ensure validity of all results (ADFG 2003).

After sequential grouping of samples from different areas of Lake Michigan, as described for estimation analyses, tests for homogeneity were conducted within and across the larger groups that were created for the initial estimation analyses. First, LRTs were conducted within each of the six larger groupings to ensure that these mixtures were homogeneous. Given homogeneity within these groups, mixtures of neighboring groups were formed to examine patterns of homogeneity in a given area. (For example, given homogeneity within the WAI/DCO mixture, homogeneity was tested within a mixture of WAI/DCO/CRA.) By conducting a series of homogeneity tests, it was possible to make final comparisons between and among the homogeneous groups that were formed using a combination of statistical and geographic criteria.

Individual assignments

A non-zero allocation of a baseline population to a mixture provides support for the presence of fish from that baseline population. However, if the confidence interval of this estimate includes zero, there is no statistical support for presence of fish from that population. In certain situations, researchers may be interested in the support for the assignment of population of origin for individual fish. In this investigation, it was of particular interest to examine the degree of support for presence of individual fish where the confidence interval from mixture analysis included zero.

Individual assignments (IA) were conducted using a Bayesian-based analysis (program STRUCTURE; Pritchard et al. 2000). STRUCTURE provides a posterior probability for each individual's assignment to each potential source population. For the purposes of this study, the posterior probability (Bayesian approach) is more desirable than methods that estimate a most likely population (e.g., GENECLASS2; Piry et al. 2004), as it is of interest to incorporate uncertainty in assignments and be able to gauge the support for each individual's assignment decision. Both the baseline and the mixture datasets used in these analyses were the same as used for the mixture analyses described above.

Results

Analysis of baseline populations: simulation analyses

Results from simulation analyses show fairly high accuracy and precision for estimates of proportional contribution to population of origin (diagonal values, Table 1). With the exception of the misassignments from the Oconto/Peshtigo Rivers to the Fox River (point estimate=0.054), all of the confidence intervals surrounding the point estimates include zero, indicating that the misallocations do not statistically differ from zero.

Misassignments reflect degrees of current population genetic structure. For example, simulations (Table 1) revealed a greater probability of misassignment from the Fox River to the Oconto/Peshtigo Rivers (point estimate=0.055) than would be expected from the Fox River to the Menominee River (point estimate=0.009), reflecting genetic affinities among populations (i.e., the Fox and Oconto/Peshtigo Rivers are more similar genetically). Asymmetry in estimates of population misclassification may also suggest unidirectional migration between populations. For example, if the Peshtigo and Oconto baselines are examined separately (data not shown), a greater number of fish of Oconto River origin assign to the Peshtigo River than fish of Peshtigo River origin assign to the Oconto River, suggesting that individuals may be moving from the Peshtigo River (a larger population) to the Oconto River (a smaller population).

Analyses of mixture composition: estimation analyses

Green Bay represents a fairly small area of habitat within Lake Michigan. However, results from estimation analyses provide evidence for significant differences in mixture composition across sample sites (Table 2). Estimates of spatial heterogeneity can be examined by comparing mixture composition from south to north across the bay. In the southernmost sample group (SGB/DYK/PEN/ORA), the Oconto/Peshtigo Rivers compose the majority of the mixture samples (point estimate=0.683), followed by the Fox River (point estimate=0.287), with contributions from both the Menominee River and Michigan not significantly different from zero.

Patterns of population presence change as more northern habitats are examined. In contrast to results from the southern group, the one of the mid-bay groups (PRF/MAR), was characterized by a higher contribution from the Fox River population (point estimate=0.327) and a non-zero contribution from both Michigan (point estimate=0.332) and Menominee River (point estimate=0.221) populations, with only a small contribution from the Oconto/Peshtigo Rivers (point estimate=0.120). Nearby collection sites (PRA/YRA) were dominated by a contribution from the Oconto/Peshtigo Rivers (point estimate=0.580) with lower, but significant levels of contribution from the Fox (point estimate=0.244) and Menominee Rivers (point estimate=0.176). On the eastern shore of Green Bay (LSS/LSN), sample sites were dominated by a contribution from the Oconto/Peshtigo Rivers (point estimate=0.531) with additional significant contributions from the Fox River (point estimate=0.268) and Menominee River (point estimate=0.165) baseline populations.

Further north in Green Bay (CRA), the mixture sample is comprised of higher proportions of Menominee River fish (point estimate=0.477) and similar contributions from the Oconto/Peshtigo Rivers and Fox River populations (point estimates= 0.214 and 0.207, respectively) and a small but statistically significant contribution from the eastern Lake Michigan basin (represented by the Manistee River baseline, point estimate=0.102).

Additional collections in the northern portion of the bay (WAI/DCO) are dominated by contributions from the Oconto/Peshtigo Rivers (point estimate=0.326) and Fox River (point estimate=0.315). Also, a collection of fish from the Little Traverse Bay area (eastern basin of Lake Michigan, northern lower peninsula of the state of Michigan) was examined, and all fish within this collection assigned to the eastern Lake Michigan basin (point estimate=0.888), with no statistically significant contribution from any other population.

From these data, it is possible to make statements about general trends of population presence throughout the bay. Individuals from the Fox River and Oconto/Peshtigo Rivers are found throughout the bay, while individuals from the Menominee River are encountered infrequently in some areas of the bay. Also, while fish from the eastern side of Lake Michigan are seen in significant numbers within Green Bay, there is no evidence for the presence of fish from the western portion of the Lake Michigan basin in waters of the eastern Lake Michigan shore (as represented by the Little Traverse Bay sample).

Analysis of mixture composition: homogeneity tests

Patterns of homogeneity were initially examined within geographically distant groups, beginning with the northern and southern extremes of Green Bay. An initial series of homogeneity tests revealed no significant difference between the composition of the most northern collection points (CRA and WAI/DCO). Similarly, no significant difference was seen in the composition of the southernmost collection points (SGB/DYK/PEN/ORA). Given evidence for homogeneity, these groups were used as “North” and “South” groups for further analysis. Homogeneity in these groups suggests that (1) season and (2) gear type may not cause bias in collection, as (1) the DYK group was collected in the winter months and PEN, ORA, SGB largely in the summer and (2) the ORA group was collected mostly using trap nets and the SGB, PEN, DYK groups were collected using gill nets. A lack of gear bias is also supported by homogeneity across the Little Sturgeon Area/north (LSN) and Little Sturgeon Area/south (LSS) groups, as the southern group was collected primarily by gill nets and the north primarily by trap nets.

After confirming homogeneity within the northern and southern groups, homogeneity was examined in the middle portion of Green Bay. Within the middle bay, homogeneity tests revealed no significant differences between collection locales on the east shore of the bay (LSS, LSN). On the western side of the bay, no significant differences were seen between mixture compositions from PRA and YRA (“Middle A”) or between MAR and PRF (“Middle B”), although significant differences were seen between “Middle A” and “Middle B”. When tested against the east shore grouping (LSS/LSN), there were no significant differences found between the eastern shore and “Middle A” (PRA/YRA). However, significant differences were found between the east-shore and “Middle B” (MAR/PRF). Subsequently, the east shore (LSS/LSN) group was combined with “Middle A” (PRA/YRA) to form the “Middle A” group for all further analysis.

Tests for homogeneity across all samples within the middle of the bay, as well as in both northern and southern extremes, provided evidence for homogeneity within four sample groups— “North” (CRA/WAI/DCO), “South” (ORA/PEN/SGB/DYK), “Middle A” (LSN/LSS/PRA/YRA), and “Middle B” (PRF/MAR) (Figure 5). Given this information, questions of mixture homogeneity were examined over larger geographic scales and across all groups (Table 3), beginning with (1) homogeneity across all four groups and (2) between “North” and “South.” Subsequently, patterns of homogeneity were examined between the “Middle A” and “Middle B,” as well as between each central group (“Middle A” and “Middle B”) and each of the more distant groups (“North” and “South”).

Significant differences were found across all four groups (p=0.0002), providing strong evidence for heterogeneity within Green Bay. Significant differences were also seen between ”North” and “South” (p=0.0002), “South” and “Middle A” (p=0.0002), and “Middle A” and “Middle B” (p=0.0002). No evidence for heterogeneity was found between the following groups: “North”/”Middle A” (p=0.0655), “North”/”Middle B” (p=0.4235), and “South”/”Middle A” (p=0.2580).

Results from mixture analysis show that not all populations are equally represented in collections throughout the bay. Not all individuals remain in close proximity to their natal stream, as can be seen in the significant presence of Fox River fish found throughout the bay. However, some populations are represented in certain regions of the bay and not in others. For example, individuals from the Menominee River were not present in the southernmost portion of the bay. Results from homogeneity tests support significant heterogeneity across Green Bay, and in some cases (seen between “Middle A” and “Middle B”), significant heterogeneity across collections that are in close geographic proximity.

Individual assignments

IA data were used to examine support for population assignments where the confidence interval for a given population allocation included zero. For the largest groupings, this was evidence for a eastern basin (Michigan) contributions to the YRA/PRA (point estimate= 0, CI = 0-0.065) group, and the Menominee contribution to the SGB/DYK/PEN/ORA group (point estimate= 0.007, CI= 0-0.0115). For the YRA/PRA group, no fish were assigned to Michigan waters (represented by the Manistee River) with a posterior greater than 0.90. For the SGB/DYK/PEN/ORA group, one individual was assigned to the Menominee River with a posterior probability greater than 0.90.

Discussion

The results of this study support a pattern of non-random fish dispersal across available habitat, suggesting that population of origin affects where individuals are likely to be found throughout the lake. In general, results from mixture analysis show that fish tend to be found in greater abundance close to natal streams even during non-spawning periods while in open-water habitats. Within Green Bay, individuals tend to be found in greater abundance near their natal streams, but there is also evidence for the significant presence of individuals from breeding populations in the eastern side of the Lake Michigan Basin. This suggests that Green Bay may be an important area of habitat for individuals originating from tributaries both within and outside of Green Bay. Finally, tests for homogeneity across mixtures revealed significant heterogeneity across Green Bay, further supporting non-random habitat use.

Even within the fairly small area of Green Bay, there is evidence for statistically significant differences in mixture composition. Based on mixture analyses, spawning populations were not contributing equally to open-water collections. Across sample sites (mixtures), the contribution of a single river changed over the sampled space. For example, the abundance of fish from the Menominee River changed across Green Bay. In the southernmost sample location, the estimated contribution of the Menominee River was small and with a confidence interval (CI) that includes zero (SGB/DYK/PEN/ORA, est=0.007 , CI=0-0.115). Conversely, in the northernmost location in the bay, the estimated contribution from the Menominee River dominated the sample (CRA, est=0.477, CI=0.319-0.634). Other rivers show significant contributions to all mixture samples (e.g., the Fox River). However, the estimated level of that contribution varies across sampling locations. When discussing the contribution of the Fox River to mixtures in Green Bay, one should note that the Fox River population is likely affected by downstream migration from Lake Winnebago through the Wolf and Fox River systems. Since fish from these locales are not genetically differentiated (DeHaan 2003), it is not possible to identify whether an individual (or proportion of a mixture) that appears to be from the Fox River is a product of reproduction in the Fox River itself or reproduction in Lake Winnebago and subsequent migration downstream.

In order to interpret patterns across the bay, the contribution of particular breeding populations to mixtures can be examined on a north-south gradient. Not only does the estimated contribution from each population change, but populations also change in their rank order of contribution to mixtures. For example, the southernmost sampling sites (SGB/DYK/PEN/ORA) exhibited a lack of significant contribution from the Menominee River and fish from the Michigan baseline, and were dominated by individuals from the Oconto/Peshtigo River grouping. The northernmost sampling site

(CRA) was dominated by individuals of Menominee River origin with significant contributions from all baseline populations. Within the center of the bay (YRA/PRA, PRF/MAR, LSS/LSN), differences are seen in contribution across sites that are in fairly close geographic proximity. For example, the PRA/YRA mixture shows a low level of contribution from the Menominee River and no significant presence of fish from Michigan waters. The neighboring PRF/MAR grouping is dominated by fish of Menominee River origin and shows a contribution from Michigan waters of over 20%.

Simply comparing confidence intervals and point estimates of contribution across a number of mixtures can lead to inflated error rates (Reynolds and Templin 2004). In order to avoid inflated error rates, this study applied a statistically more rigorous approach to compare population contribution across mixtures. Given this approach, the ability to reject the hypothesis of homogeneity throughout Green Bay provides particularly strong support for a non-random movement of individuals from contributing populations.

Within the bay, significant patterns of heterogeneity, as well as mixture estimates at given locales (see above) can be partially explained by examining the results on a north-south gradient. In the southern portion of the bay, homogeneity is seen across mixtures composed of multiple sampling locales. Similarly, in the north, homogeneity is seen across mixtures from the northern portion of Green Bay and the western shore of Lake Michigan. Within the middle of the bay, however, significant differences in mixture composition are present, likely dictated by physical features (e.g. Peshtigo shoal on the western shore of Green Bay, near PRA and PRF) and water circulation patterns (counterclockwise within the bay, US EPA 1990). Across all sample groupings throughout the bay there is also significant evidence for heterogeneity.

Such significant variation in population presence within a small area suggests that habitat use may be dictated by bathymetric features or other aspects of lake habitat. This is further supported by evidence for a non-zero contribution of fish from Michigan tributaries in the waters of Green Bay, and that Green Bay may be attracting individuals from throughout the lake. To estimate the occurrence of individuals from eastern Lake Michigan (Manistee River/"Michigan" baseline) in mixtures in Green Bay, the point estimate for contribution from Michigan waters can be multiplied by the sample size for a given collection group. For example, the WAI/DCO group (n=47) shows a contribution of approximately 9 individuals (point estimate(Michigan)=0.194), the CRA group (n=75) approximately 7-8 individuals (point estimate=0.102), and the PRF/MAR group (n=80) approximately 17 individuals (point estimate 0.221). If these numbers are considered in the context of the estimated spawning population sizes for tributary populations in the eastern Lake Michigan Basin (e.g. Muskegon+Manistee Rivers, combined estimate <75 individuals, Elliott 2003), it can be seen that there is not only a statistically significant presence of eastern basin Lake Michigan lake sturgeon in Green Bay, but also a significant number of individuals relative to the estimated sizes of the eastern basing Lake Michigan spawning populations.

Conversely, within mixtures on the eastern shore of Lake Michigan (e.g., Little Traverse Bay), which were collected across all seasons, there is no significant (non-zero) contribution of fish from the western basin of Lake Michigan. The lack of reciprocity in assignments across Lake Michigan may be due to individuals migrating to Green Bay, as it is a shallow and productive habitat. Given that lake sturgeon are benthivores, habitat productivity or other characteristics may be a significant factor influencing movements.

Habitat characteristics have been found to affect sturgeon movement in other species and other regions. Researchers working in the Rainy River/Lake of the Woods system (Ontario, CA/Minnesota, USA) tracked movements of individual lake sturgeon within both riverine and lacustrine habitats over a three-year period (Rosak and Mosindy 1997). Results from this work suggest that many movements were dictated by foraging behavior, as fish congregated at appropriate feeding habitat. Similarly, work with the Gulf sturgeon, *Acipenser oxyrhynchus desotoi*, found that sturgeon tended to prefer specific microhabitats within river habitats (Wooley and Crateau 1985). Results from a study of subadult Atlantic sturgeon, *Acipenser oxyrinchus*, suggest that non-natal habitats are important for the persistence of sturgeon populations, although the function of these habitats (feeding, protection, staging) is unknown (Savoy and Pacileo 2003).

Studies of lake sturgeon habitat occupancy in other regions have found support for a variety of patterns. Haxton (2003) examined movements in four individual lake sturgeon via radio-telemetry tracking in the Ottawa River, Canada, and found that individuals did not exhibit high dispersal within the river. Conversely, Auer (1999a) examined patterns of habitat use and movement by adult lake sturgeon in Lake Superior after spawning in the Sturgeon River. Individuals inhabited a variety of water depths throughout the lake and traveled throughout the southern portion of the lake (70-280 km). The lack of concordance across studies suggests that any observed patterns of habitat use and movement may not be generalizable to all remnant populations of lake sturgeon inhabiting such different habitats (i.e. riverine vs. lacustrine populations). Characteristics of habitat may be driving dispersal patterns, rather than dispersal occurring in similar patterns irrespective of habitat. For example, Knights et al. (2002) observed movement within lake sturgeon populations in the upper Mississippi River. Individual fish were observed returning to certain habitat areas with common characteristics, which the authors hypothesize may provide optimal feeding habitat. Also, groups of fish tended not to overlap in the geographic range of their movements, suggesting that even when movements occur, all available habitats are not used equally by all individuals.

Given the amount of time sturgeon spend in open-water habitats, certain habitats may be occupied by a relatively high density of lake sturgeon while other habitat is vacant. Based on results from Green Bay, differences in mixture composition may be partially explained by physical features. A pattern of non-random habitat occupancy may help managers prioritize habitats for restoration or protection, particularly if the reasons for that non-random occupancy pattern can be explained. In Green Bay, the significant presence of more distant populations (i.e., Michigan) may be explained by productivity patterns, suggesting that protecting feeding habitat should be a priority in restoration efforts.

Results from this study offer previously unavailable insights into lake sturgeon biology in the area of habitat occupancy. However, these findings are also significant in a number of contexts, including managerial applications such as risk assessment. If a portion of habitat (e.g., Green Bay) is somehow degraded (e.g., regional environmental pollution event), these results offer some indication of which populations may be most affected. In the case of Green Bay, the results from this study suggest that fish from populations across the entire lake may be affected by habitat changes in Green Bay. In the context of risk assessment, results from this work suggest that proximity of a given tributary population to degradation, pollution, or other environmental change does not necessarily represent the population that is most likely to be affected.

Further research is needed to understand patterns of lake sturgeon habitat occupancy. Within the study area, additional years of sampling with increased sample sites will help illustrate dispersal patterns across a larger amount of habitat. While this study was focused within the waters of Lake Michigan, populations more proximal to Lake Superior and Lake Huron also merit attention. Previous studies of sturgeon movements (Auer 1999a) have provided some insight on patterns of dispersal within these systems, but using an MSA approach may allow a more extensive examination of movement patterns, partially because of increased sample sizes. Spawning populations in tributaries to these rivers have been characterized (DeHaan 2003, DeHaan et al. 2006), providing the first step toward additional MSA studies. Given significant spatial genetic structuring in populations, MSA analyses should be conducted across larger spatial scales and across a variety of habitats to elucidate what variables (e.g., habitat complexity, productivity) may affect habitat occupancy.

The results from this work provide information that is significant in both ecological and managerial contexts. The results of this study have management implications not only for lake sturgeon populations, but also for other sturgeon species. Given significant population structure (a byproduct of natal philopatry), it may be possible to better understand habitat occupancy in other sturgeon species, such as those inhabiting marine waters and spawning in coastal tributaries.

This research is the first MSA study of lake sturgeon anywhere throughout its range. The results from this study provide strong evidence for heterogeneity in habitat use by lake sturgeon relative to population of origin, adding important information to a poorly understood area of sturgeon biology. While other studies (e.g., Stabile et al. 1996, Rusak and Mosindy 1997, Auer 1999a, Knights et al. 2002, Haxton 2003) have examined patterns of lake sturgeon movement, the methods used in this study allow analysis of sample sizes far greater than those that are possible via direct methods, with samples taken over a span of time that would be difficult if using direct methods. The strength of the statistical approaches employed in this study, combined with extensive sampling, provides strong support for the observed results. The patterns observed in this examination of habitat occupancy of lake sturgeon throughout the Lake Michigan basin make possible a more full understanding of movement and habitat use during non-spawning periods. Such understanding is crucial in order to design more effective restoration efforts for populations of this unique and ancient fish.

CHAPTER TWO : AN INVESTIGATION OF COHORT-SPECIFIC DIFFERENTIAL RECRUITMENT OF REMNANT POPULATIONS OF LAKE STURGEON THROUGHOUT THE LAKE MICHIGAN BASIN

Introduction

Recruitment and environmental variation

Stock recruitment relationships have been a central focus in fisheries research, and a number of models exist to calculate recruitment. Historically, models have been based on the assumption that future recruitment is dependent on current stock size, as in the classic Ricker equation (Ricker 1975):

$$R_t = S_t * e^{[a - bS_t + e_t]}$$

where R recruits are produced from spawners S at time t, a is the number of recruits per spawner at low spawner abundance, b represents density dependence in recruitment (e.g., juvenile survival rate) and e_t is a normally distributed random error term.

While current stock biomass is certainly a factor in future recruitment, stock-recruitment relationships are also affected by environmental factors (e.g., King et al. 2000, Koster et al. 2002, Sinclar and Crawford 2005). Because of the multiple environmental factors that may be affecting a region or population simultaneously, accounting for environmental variation can be difficult. Studies have found significant relationships between stock recruitment and environmental variables such as water temperature during larval drift in herring, *Clupea harengus* (Fiksen and Slotte 2002). Holt and Peterman (2004) used multiple regression and covariation analyses to examine the effects of a number of biological (e.g., abundance and body size of recruits) and environmental (e.g., ocean-basin-wide changes in climate, sea-surface temperature) variables on recruitment of sockeye salmon, *Oncorhynchus nerka*. The authors found significant relationships between environmental variables and stock recruitment, although the collinearity of environmental variables made inferring causation difficult. Within the Great Lakes, a recent study (Madenjian et al. 2005) examined factors affecting recruitment variability of alewives within Lake Michigan. Both environmental (severity of winter, summer-spring temperatures) and ecological (lake productivity, predation level) factors were considered. The authors concluded that alewife, *Alosa pseudoharengus*, recruitment is largely affected by salmonid predation but is also affected by environmental factors (specifically spring-summer temperatures).

A number of studies have examined the relationship between stock recruitment and environmental variables using a variety of specific modeling methods. Modeling provides researchers the ability to manipulate variables of interest (e.g. aspects of environmental stochasticity) and evaluate the magnitude of the effect of those variables on aspects of populations (e.g. recruitment). Allen and Miranda (2001) examined interactions between crappie, *Poxomis* spp., populations and environmental variation and found the effect of environment differed with respect to population size. Cardinale and Arrhenius (2000) used a generalized additive model to examine the effects of both biotic and abiotic factors on Baltic cod, *Gadus morhua callarias*, populations.

Another modeling approach used in the examination of environment-recruitment relationships is so-called fuzzy logic models (Chen et al. 2000, Chen 2001, Chen and Hare 2006), which may incorporate uncertainty more systematically than traditional approaches. Modeling studies such as these provide a theoretical background for research that analyzes the potentially-complicated relationship between stock recruitment and environmental variation. Researchers have also employed simple linear models (e.g. Pitchford et al. 2005), which may be more applicable and/or accessible to managers, to examine the effects of environmental stochasticity on recruitment. In this case, the authors found a significant positive relationships between environmental variables and the probability of individual recruitment, and they cite field studies as well as other modeling studies with concordant results.

Environmental changes that affect recruitment may be natural variation (e.g. changes in weather) during a critical time period, such as spawning or during early juvenile life history stages. Recruitment may also be affected by human-mediated environmental changes. Throughout the Great Lakes, lake sturgeon, *Acipenser fulvescens*, populations have declined because of a variety of anthropogenic disturbances, including habitat loss and degradation, the effects of impoundment, pollution, and overexploitation (Auer 1999b). Many of these factors are not distributed uniformly across tributaries. Some rivers may have sources of intense pollution, such as the Fox River (high in PCB levels; Barron et al. 2000), or dams that have blocked access to traditional spawning habitat and altered flow regimes. Additionally, overexploitation, a major cause of sturgeon declines in the Great Lakes (Baldwin et al. 1979), may not have equally impacted all breeding populations.

Given such heterogeneity of anthropogenic impacts across populations, it is not surprising that there are differences in abundance across lake sturgeon populations. Holey et al. (2000) identified remnant populations of lake sturgeon throughout the Great Lakes as small, medium, and large. While there are differences in particular legal status (e.g., threatened, endangered, of special concern) lake sturgeon are consistently a high conservation priority throughout their range (Welsh 2004).

In addition to their significance in a conservation context, lake sturgeon provide a unique opportunity to study recruitment patterns and how recruitment relates to environmental variables, particularly in a species with some unique life history characteristics, including a long life span, late reproductive maturation, and low mortality upon reaching maturity (Houston 1987). In addition to these characteristics, lake sturgeon are highly philopatric, returning to their natal waters to spawn (DeHaan et al. 2006). When examining recruitment, the implications of this philopatry are significant, as recruitment realized within a given spawning population is largely due to reproductive contributions from adults outmigrating from that stream.

Evaluating the effects of environmental variation on populations can be complicated when working with a long-lived, late-maturing species such as lake sturgeon, as recruitment to adult stages does not occur for almost 20 years. Despite the difficulties of such analyses, understanding the relationship between environmental variation and recruitment is important, as that relationship will help inform and guide future management actions. A more complete understanding of the lake sturgeon, particularly their unique biology, is a key element in designing effective conservation efforts.

Low levels of recruitment, or no successful recruitment, has been suggested as a factor preventing recovery of a number of sturgeon species, including white sturgeon, *Acipenser transmontanus* (Paragamian et al. 1996); pallid sturgeon, *Scaphiryhchus albus* (Tews and Gardner 2001); Gulf sturgeon, *A. oxyrinchus desotoi* (Peterson et al. 1999);

and Atlantic sturgeon, *A. oxyrhinchus* (Secor and Waldman 1999). The role of environmental variation in observed low levels of recruitment in sturgeon species is not fully understood. A simulation study based on white sturgeon populations in the Snake River found the most limiting factors to recruitment to be environmental variation, specifically in terms of water pollution and water quality (Jager et al. 2001). Results from this study also suggest that environmental variation does not act on single populations if populations are physically connected.

Age estimation in lake sturgeon

In lake sturgeon, pectoral fin rays give a metric of age that is based on the same principles as aging methods using other structures, such as scales and otoliths, used in other fish. Pectoral fin ray analysis as an aging method has been critically examined by a number of studies (e.g., Rossiter et al. 1995, LeBreton et al. 1999, LeBreton and Beamish 2000). Results support pectoral fin ray analysis as a method of aging, given that investigators account for reader error and the effects of environmental variation on growth patterns. However, pectoral fin ray samples are labor-intensive to collect and may have detrimental impacts on the individual fish after sampling. A representative sample of fish was aged using cross-sections of pectoral fin rays to determine an age-size relationship for the Green Bay population (Gunderman and Elliott 2004) (Figure 6). Estimation of approximate age based on size reduces the need to remove spines from all of the sampled fish. Size-age relationships allow adult lake sturgeon to be placed with quantifiable confidence to an approximate cohort year.

Genetic structure in lake sturgeon

A recent examination (DeHaan 2003, DeHaan et al. 2006) of the genetic characteristics of lake sturgeon throughout the Great Lakes found evidence for significant spatial genetic structuring among remnant populations (Figure 2). The degree of genetic differentiation across remnant populations is sufficient to distinguish lake basin of origin (e.g., Superior, Michigan) as well as region (eastern and western Lake Michigan) and river of origin in many cases. Specifically, breeding populations in tributaries to Lake Superior are highly differentiated from all other populations in the upper Great Lakes (significant bootstrap iterations = 990/1000), and there is also significant genetic differentiation between populations in western Lake Michigan and populations in Lake Superior and Lake Huron (significant bootstrap iterations = 907/1000). Significant spatial structure was also found within the western basin of Lake Michigan, with significant divergence among the Menominee, Wolf, Fox, Oconto, and Peshtigo Rivers. As there is not evidence for significant differentiation between the Wolf and Fox Rivers, or the Oconto and Peshtigo Rivers, these rivers were combined as the Wolf/Fox system and Oconto/Peshtigo Rivers in all analyses.

Problem statement

Using a combination of age and location assignment techniques, I examined patterns of recruitment by population over time, using individuals sampled throughout greater Green Bay over a multi-year sampling period (1999-2006). Additionally, I used a novel method to incorporate uncertainty into analyses by using a probabilistic approach in both age and location assignments. By incorporating, rather than ignoring, the issue of uncertainty, this study was able to provide results that are more representative of patterns in lake sturgeon populations. Because recruitment patterns in lake sturgeon are not well-understood, data generated from this research will be helpful in guiding further restoration efforts, particularly by identifying forces that most affect recruitment, such as a basin-wide environmental factor or more local (river-level) environmental variation.

Given the ability to assign age and population of origin for individuals sampled throughout Green Bay, this study investigated how patterns of recruitment varied for tributary (baseline) populations across time. If recruitment is independent of population of origin, recruitment may be influenced primarily by basin-wide abiotic factors (e.g., rainfall). However, if recruitment is not independent of population of origin, recruitment may be primarily influenced by local anthropogenic variables (e.g., changes in dam discharge, pollution levels).

Methods

Sampling methods: baseline populations

In order to assign individual fish to populations of most probable origin, it was necessary to sample potential source populations and characterize these populations genetically. As part of ongoing research on lake sturgeon throughout Lake Michigan (e.g. DeHaan 2003, Gunderman and Elliott 2004) spawning adult lake sturgeon were captured by cooperating agencies and institutions from five different remnant populations that breed in tributaries throughout Lake Michigan: the Fox (n=71), Peshtigo and Oconto Rivers (n=91) (Wisconsin), the Manistee River (n=80) (Michigan) and the Menominee River (n=41) (Michigan/Wisconsin) (Figure 3). Individuals were measured (length, girth, weight), and implanted with internal (passive integrated transponder, PIT) and external (Floy) tags for future identification. Samples of fin tissue (approximately a 1 cm^2

clip of the dorsal fin) were also collected, allowed to dry, and stored in scale envelopes at ambient temperature (Gunderman and Elliott 2004, DeHaan et al. 2006).

Sampling methods: open-water collections

While lake sturgeon were captured from several locations in open water around Lake Michigan, the majority of sampling was conducted throughout the waters of Green Bay, Lake Michigan, from 2002 to 2006 (Figure 4). In Green Bay, lake sturgeon were sampled by USFWS and Wisconsin Department of Natural Resources (WiDNR) field researchers and commercial fishers using trap nets and gill nets. Nets varied slightly in dimensions but all commercial lake whitefish, *Coregonus clupeaformis*, trap nets had leads of approximately 1000 ft and pot mesh of 4.5 inches. Commercial gill nets varied in mesh size depending on the targeted species (lake whitefish, *Coregonus clupeaformis*, ~4.5 inches; perch, *Perca flavescens*, ~2.5 inches). Nets were deployed throughout the calendar year, although the majority of samples came from collections in the spring and early summer, with a few from the fall. All individuals were labeled with an external (Floy) tag and an internal (PIT, passive integrated transponder) tag (Gunderman and Elliott 2004). Also, samples of fin tissue (approximately a 1 cm^2 clip of the dorsal fin) were collected from all individuals, allowed to dry, and stored in scale envelopes at ambient temperature.

Laboratory methods

In order to assign individuals to populations of origin, it is necessary to characterize genetically both individuals of unknown origin (mixture) and individuals of known breeding individuals from populations (baseline). DNA was extracted from fin tissue for both mixture and baseline individuals using QIAGEN DNeasy kits (QIAGEN

Inc.) according to manufacturer's protocols. DNA was quantified using a Beckman DU 7400 spectrophotometer. All samples were diluted to a common DNA concentration (20 ng/ul) for use in PCR reactions.

Individuals were genotyped at 8 microsatellite loci including *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001), *AfuG9, AfuG63, AfuG74, AfuG112* (Welsh et al. 2003). PCR reactions were conducted in 25 µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M MgCl2, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), additional MgCl2 as determined by optimizations (1.0ul: *LS-68, Afu68B, Spl120, AfuG112*, 1.5ul: *AfuG9*), 2mM of each dNTP, 10 pmol of forward and reverse primer and 0.5 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 (*AfuG9, AfuG63, AfuG112:* 48C; *AfuG74*: 50C; *Aox27:* 53C; *LS68, Afu68B:* 56C; *Spl120:* 62C*)* and 72° C for 1 minute. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIOII scanner. Allele sizes were determined using commercially available size standards (MapMarkerTM, BioVentures Inc.) and based on standard samples of known genotype. To minimize error, all genotypes were independently scored by two experienced lab personnel and verified again after data were entered into electronic databases.

Statistical methods

Population assignment

Program STRUCTURE (Pritchard et al. 2000) was used to assign individuals to population of most probable origin. STRUCTURE quantifies the uncertainty of population assignments on an individual-by-individual basis, using a Bayesian method. STRUCTURE provides a posterior probability of assignment for each individual and each population. Since this study focuses on individual fish (rather than groups of fish) as data points, the ability to incorporate uncertainty in classification decisions is a necessary piece of information for analysis.

Within STRUCTURE, populations of origin can either be defined by the user or inferred by the program, based on genetic structure. As population of origin is known for baseline (spawning) individuals, baseline populations were defined *a priori*. Both individuals of unknown origin (open-water collections) and baseline populations (known spawning individuals) were analyzed for population of most probable origin.

Age assignment using a bivariate approach

It is possible to age lake sturgeon to an approximate year of recruitment based on annuli of the pectoral fin ray. Recent research (Gunderman and Elliott 2004) aged a subset of 102 fish, representative of the size distribution (based on length and weight) of individuals caught in the open waters of Green Bay during 2003-2003. The data of Elliott et al. (2004) were used to estimate age/cohort assignments in a retrospective analysis of recruitment. Using individuals of known age (as determined by pectoral fin ray analysis), a scatter plot of log(age) and length was constructed (r=0.91, Figure 7). A Box-Cox transformation of age indicated that a log transformation was the most appropriate for transforming the residuals to normality.

To estimate age given length, a linear regression was used in program SAS. However, as neither age nor length was a fixed constant, a bivariate normal model was used, which is more appropriate than the standard conditional regression model (as neither variable is a fixed constant). In the bivariate normal model, the data (x_1, y_1), (x_2, y_2)... are assumed to be realization of a random vector (X_1, Y_1), (X_2, Y_2)... with the joint distribution of the random vectors (X_I, Y_I) assumed to be bivariate normal. (For more details, see Casella and Berger 1990, p 167)

Specifically, this study assumed:

$$(\log(\text{age}), \text{length}) \sim N(\mu_{\log(\text{age})}, \mu_{\text{length}}, \sigma^2_{\log(\text{age})}, \sigma^2_{\text{length}}, \rho)$$

Maximum likelihood estimates of above parameters were used in the prediction of age from length as described below. Given length, the conditional distribution of log(age) was calculated as

$$\mu_{\log(\text{age})|\text{length}}=\mu_{\log(\text{age})}+\sqrt{(\sigma^2_{\log(\text{age})}/\sigma^2_{\text{length}})}*\rho_{\text{length},\log(\text{age})}\ (\mu_{\text{length}} - \text{length})$$

$$\sigma^2_{\log(\text{age})|\text{length}} = \sigma^2_{\log(\text{age})} * (1 - \rho^2)$$

Combining population and age assignments

Based on the conditional normal distribution of age given length, estimated as described above, a SAS macro was created to assign individuals to age bins. Bins were created based on quantiles of the normal distribution. Bins were expanded until a sufficient number of individuals per cell was reached, allowing a chi-squared analysis to be performed. Because of the annual spawning of lake sturgeon (only one cohort is possible per year), bins were restricted to a minimum of one year in size. The binning scheme allowed the highest level of resolution, allowing the examination of recruitment on a year-by-year basis for most years in this analysis. Given length, the conditional distribution for age of each individual was estimated and the probability of belonging to an age bin was calculated as

$$\Pr\ (\mathrm{bin}_{j-1} < \mathrm{age} \leq \mathrm{bin}_{j})$$

given the conditional distribution of age for each individual. Thus, each individual was partitioned across all age bins based on the probability of the conditional normal distribution for that particular individual. To create age bins, the age distribution was portioned onto equally-spaced probability intervals in the probability density function (PDF) of the age distribution. Assuming independence between the location and age assignments, the product of the two probabilities summed over all individuals, and age and location categories was used to create a location*age contingency table.

Loglinear Analysis

A log-linear model was used to assess significant association within the *location*×*age* table using PROC GENMOD (SAS). Log-linear models are used to model associations within contingency tables when there is no clear indication as to which variable is the response variable. For multinomial sampling with probabilities in an $I \times J$ contingency table, the null hypothesis being tested is $H_o: \pi_{ij} = \pi_{i+}\ \pi_{j+}$, where individual cell probabilities are a product of the marginal probabilities. A chi-squared test of independence (χ^2) with $(I\text{-}1)(J\text{-}1)$ degrees of freedom can be used as to test for independence. The likelihood of the chi-squared statistic ($-2 log\Lambda$), is denoted by G^2 and equals

$$G^2 = -2 log\Lambda = -2\ n_{ij} \log\ (n_{ij}/\mu_{ij})$$

where μ_{ij} is an estimate equal to $(n_{i+}*n_{j+})/n$ and G^2 is summed over all values of I and J. The larger the values of the G^2 and the χ^2statistics, given the degrees of freedom, the more evidence against independence between the two variables, age and location.

Results

Genetic Assignment

The ability to correctly assign individuals to baseline population of origin varied by population (Table 4). Accuracy increased with increasing degree of population genetic differentiation. For example, the degree of misassignment between individuals from the Fox River and the eastern basin of Lake Michigan (represented by the Manistee baseline) was far less than the degree of misassignment between the Fox River and the Oconto/Peshtigo Rivers.

Overall, the probability of correctly assigning individuals to populations of origin (estimated using baseline samples) was fairly high, especially considering the close proximity of the majority of the populations (breeding populations in tributaries to Green Bay). The lowest mean posterior probability of correct population assignment was 0.874, seen in the Menominee River population. Individuals from the Manistee River (representing the eastern basin of Lake Michigan) showed the highest posterior probability of correct assignment (0.968) (Table 4).

Age Assignment

Given data on known ages based on pectoral fin ray analysis, the maximum likelihood estimate of the correlation coefficient between log(age) and length was estimated (r=0.91). A plot (Figure 8) of the joint distribution of log(age) of any length (based on the joint probability density function of log(age) and length) illustrates the assignment probability of ages given length of an individual. As contour lines (representing probabilities of age assignment) become more distant from each other, the probability of assignment across all age classes becomes more equitable. Where probability contours are found in close proximity, probabilities of correct age assignment given length are less homogenous. Given the known age data, estimates of age are likely to be less biased for median ages, where there is more data in the known distribution, and also because the log(age) and length relationship is anchored at the mean.

Combining genetic assignment and age assignment : log-linear analysis

A chi-squared test for location (χ^2 = 30.41, df = 3) was significant ($p<0.0001$). This result indicates support for different population sizes for all four baseline population groupings. Based on these data, the Fox and the Menominee rivers historically (e.g., 1975 and earlier cohorts) were more numerous, which is consistent with historical and current data on the populations (R. F. Elliott, personal communication).

However, based on the loglinear model there was no significant effect of recruitment year (χ^2 = 4.15, df = 19, p=0.9999), meaning that overall patterns of recruitment across years were not significantly different (Figure 9). While all populations had different levels of estimated historical population size, all populations showed similar patterns of change over time. Breeding populations exhibiting a larger number of adults initially (inferred based on estimated cohorts $\geq$1975) subsequently contributed to proportionately higher levels of recruitment in those same populations.

There was not significant evidence to reject the null hypothesis of independence between the effects of year and location on recruitment. Recruitment across years is independent of population of origin. Therefore, it was not possible to proceed with analyses of location*year interactions, to elucidate trends in recruitment due to different locations (populations). Results suggest that there are likely larger-scale abiotic factors affecting recruitment rather than river-specific factors.

Discussion

The unique life history of the lake sturgeon calls for unique approaches when examining patterns of recruitment. It is not possible to sample individuals extensively during spawning bouts, particularly in large river systems where individuals may be dispersed throughout available habitat. Also, since fish do not spawn annually (Lyons and Kempinger 1992, Auer 1999a) and initially spawn between 12 and 33 years of age (Roussow 1957, Harkness and Dymond 1961), direct assessment methods would not yield information on large segments of current populations. Finally, lake sturgeon are long-lived (Baker 1980), which causes considerably more uncertainty in age assignment than in other more short-lived species. For these reasons, this work sought to construct a method that would identify most probable age (based size and validated using samples aged by pectoral fin ray analysis) and population of origin (based on genetic data) for individuals, and subsequently incorporate uncertainty in both age and location assignment in order to examine recruitment patterns across populations over time.

The non-significant result of the chi-squared test for a year effect provides support for similar patterns in recruitment across all years examined in this analysis. Results suggest that, regardless of all local perturbations in the environment, lake sturgeon recruitment did not vary significantly across rivers over the time period examined in this study (1975 to 2006). Evidence for a significant location effect provides support for different historical population levels. These results combined (non-significant effect of year, significant effect of location) suggest that while overall trends in recruitment are not independent across populations, the numerical abundance of recruits differs across populations as a function of differences in the numerical abundance of adults. The lack of significant variation in recruitment across years is in contrast to a recent study (Adams et al. 2006) which supported variation in recruitment across year-classes of lake sturgeon in the Rainy Lake system. The authors also found no significant effect of annual or monthly environmental variation on recruitment, save temperature in the month of June.

The failure to reject the null hypothesis of independence for effects of location and year precludes the identification of a river-specific environmental or anthropogenic effect that may be driving recruitment. Assuming that the data are sufficient, and the methods employed appropriate, there are two main explanations for similarities in recruitment trends across populations. One, that recruitment in populations is not affected by river-specific effects, such as differing degrees of water pollution, impoundment or localized harvest. Alternatively, populations may be affected by river-specific effects, but the impact of these local factors on recruitment is overridden by larger-scale environmental factors.

Given the demonstrated importance of local environmental factors on recruitment within populations (e.g., King et al. 2000, Fiksen and Slotte 2002, Koster et al. 2002, Holt and Peterman 2004, Sinclar and Crawford 2005, Lobón-Cervía and Rincón 2005, Madenjian et al. 2005) it is possible that some aspect of this approach limited the ability to identify patterns in recruitment across populations over time. If so, there are four areas that may be the source of the inability to detect patterns: insufficient certainty in (1) population or (2) age assignment, (3) a violation of assumptions or (4) some aspect of the statistical approach. For example, if population and/or age assignments carry a high level of uncertainty, probabilities of assignment for individuals will be more equally-distributed across population and/or age categories. More equitable distributions of assignment probabilities will cause overall trends in recruitment to be smoothed, instead of the boom-and-bust years that can characterize recruitment patterns (Anderson 2002).

The results of similarities in patterns of recruitment across populations could also be a byproduct of assumptions made in this study. There are four main assumptions made in this study: that it is possible to (1) age individuals, and also (2) assign them to populations of origin, with some certainty, (3) that there is no effect of sampling location or gear type, and (4) that mortality is consistent across ages and populations.

One of the assumptions made in this study is that it is possible to correctly age individuals with some certainty. Other researchers have investigated the accuracy of aging lake sturgeon via pectoral fin rays.   For example, LeBreton et al. (1999) compared aging results, based on pectoral fin ray analysis, across three different populations of lake sturgeon. The authors conclude that variation in growth in sturgeon is affected by population-level environmental factors. In addition, LeBreton and Beamish (2000) concluded that growth rings do provide a valid method for aging sturgeon, despite the potential confounding effects of environment on growth patterns. In this study, the effect of environmental variation (e.g. growth varying by stream of origin) on aging estimate accuracy should be minimized, as the fish that were used for establishing the known age baseline are a representative sample from a common habitat which is inhabited by individuals from all breeding populations (Gunderman and Elliott 2004, chapter one).

With respect to genetic assignments, the sample sizes used in this study are likely sufficient to characterize each population genetically. The baselines used in this study are equal to, and in some cases greater than, the baselines used by DeHaan et al. (2006) to describe population structure for lake sturgeon throughout the Great Lakes. In addition to increased sample sizes, accuracy in genetic assignment may also be increased by incorporating more loci into the analysis, depending on the allele-frequency differences across populations and the among of admixture across populations (Pritchard et al. 2000). However, of the microsatellite markers currently available for lake sturgeon, the most polymorphic loci that do not have known null alleles were used.

Two different classes of sampling gear, gill nets and trap nets, were used in this study. Gill nets used by commercial fishers to target lake whitefish, *Coregonus clupeaformis,* tend to be more successful at catching smaller fish, while trap nets are more likely to retain larger individuals (R. F. Elliott, personal communication). Nets were not distributed randomly around the bay. Gill nets were concentrated in the southernmost collection points and the LSS/LSN locales, while trap nets were the predominant gear used at most other collection points. In this retrospective analysis of recruitment, it is assumed that there is no bias in the results of this study as a result of sampling gear. Homogeneity across sample groups (see chapter one) in these groups suggests that (1) season and (2) gear type may not cause bias in collection. For example, within the southernmost portion of Green Bay (collection sites: Southern Green Bay (SGB), Oconto River Area (ORA), Pensaukee Area (PEN), and Dykesville Area (DYK), Figure 4), the ORA group was collected mostly using trap nets and the SGB, PEN, DYK groups were collected using gill nets. A lack of gear bias is also supported by homogeneity across the Little Sturgeon Area/north (LSN) and Little Sturgeon Area/south (LSS) groups, as the southern group was collected primarily by gill nets and the north primarily by trap nets. However, a t-test conducted on size of individual (based on total length in cm) grouped by class of collection gear (trap net and gill net, both irrespective of specific mesh size) strongly supported ($p<0.001$) a gear effect on the size of individuals targeted by gear. This suggests that the effect of gear type on results (e.g. as a source of bias) may merit further investigation.

Additionally, in this study, constant age-specific survival across populations is assumed. An evaluation of recruitment is based on the number of subadult and adult lake sturgeon currently present in the open-waters of Green Bay. This assumes a fairly constant mortality rate from juvenile to adult life stages. Previous sturgeon research suggests that larvae are most vulnerable to natural predation before the formation of their scutes during the first year (Houston 1987), providing support for the assumption of constant mortality from juvenile to adult stages. Given the non-random distribution of environmental impacts throughout Green Bay, including pollution (Karasoc et al. 2005, Redder et al. 2005) and harvest (Thuemler 1985), and the non-random distribution of individuals across available habitat (chapter one) the assumption of equal mortality across populations may or may not be valid. However, the analysis of recruitment patterns used here is relatively simple, offered as a nascent and novel approach to an important conservation and management question.

The failure to detect dependence across populations, or a lack of autocorrelation in recruitment, may be a reflection of limitations in data. Further sampling and analysis may change the detected pattern in population recruitment across Green Bay. There is significant spatial genetic structuring in remnant populations of lake sturgeon (DeHaan 2003, DeHaan et al. 2006) and the ability to correctly assign individuals to a population of origin is fairly high (Table 4). As a result of the extensive genetic characterization of these populations, I recommend that future research efforts be focused on increased sampling of known age individuals. A more complete characterization of the age/length relationship, ground-truthed by individuals of known age based on pectoral fin ray analysis will decrease the uncertainty in age assignment, and possibly allow for the examination of other significant factors on lake sturgeon recruitment throughout recent history.

Finally, the analytical methods employed in this study are relatively straightforward, and a more complex approach (e.g., more complex modeling) may provide more insight into recruitment dynamics over time. For example, if harvest records can be examined by approximate location of collection, knowledge can be combined with the results supporting non-random habitat use (chapter one). Given a greater understanding of what populations were historically most affected by harvest activities, patterns may emerge with respect to populations being disproportionately affected. Recent research (reviewed in Birkeland and Dayton 2005) suggests that older, larger individuals are more likely to produce offspring with high survival probabilities, but that these individuals are also more likely to be targeted by fishing efforts. In addition to incorporating information on the likely impacts of past harvest, future models should incorporate information from both river- (e.g. discharge, point-source pollution) and basin-wide (e.g. rainfall) environmental factors. The relationship between environmental variation and stock-recruitment patterns is not necessarily simple or straightforward, and approaches taken to analyze those patterns should be likewise.

Effectively managing and restoring lake sturgeon populations requires consideration of their fairly unique biology and life history. When examining environmental and anthropogenic effects on recruitment over a given time scale, it is important to remember that this is a long-lived species, and that trends in populations may be escaping analysis if done on a shorter time scale. Orensanz et al. (2004) examined age structure to infer patterns of recruitment and the effects of harvest on populations of another long-lived aqautic organism, the geoduck, *Panopea abprupta,* in British Columbia. The authors caution that management assessments and decisions must be made in the context of the organism in question. For long-lived organisms in particular, the authors recommend incorporating data across many temporal scales.

This study takes a novel approach to evaluate recruitment patterns across populations over time by incorporating uncertainty in both age and population assignments. This study is the first of its kind on any sturgeon species in that it specifically accounts for uncertainty when examining recruitment patterns. By using genetic information to identify population of origin, it was possible analyze larger sample sizes, sampled over a shorter time period, than would be available via direct methods (e.g., sampling/tagging spawning adults). Due to the significant degree of spatial genetic structuring in remnant populations of lake sturgeon, it was possible not only assign individuals with high certainty to baseline populations (see Table 4) but also quantify the uncertainty in assignment. Also, it was possible to quantify the uncertainty in age assignment, which is significant as the probability of correctly aging an individual is not constant over an individual's lifetime (Figure 8**)** due to growth patterns in sturgeon.

The results of this study should serve as a first step in understanding recruitment patterns in lake sturgeon in the Great Lakes. More complex models (e.g., incorporating fine-scale environmental factors) may reveal patterns not detected by this analysis. More data, particularly fish of known age across a wider range of ages, may decrease levels of uncertainty in evaluation of trends in recruitment in lake sturgeon across the Great Lakes. Additionally, models incorporating environmental data may be able to detect patterns in recruitment. In a species of such high conservation concern, where little is known about long-term trends in recruitment, it is imperative that a more complete understanding regarding the complex relationships between environmental variables and recruitment patterns is reached.

CHAPTER THREE: MIXED STOCK ANALYSIS OF A LAKE STURGEON HARVEST: THE FALL HOOK-AND-LINE FISHERY ON THE MENOMINEE RIVER

Introduction

Fish species are frequently subdivided and breed in geographically discrete populations but share common habitats during non-breeding periods. Therefore, populations may not be equally affected by impacts on those shared non-breeding habitats as a result of environmental changes (Seeb et al. 2000) or harvest (Seeb et al. 2004). Harvest can represent a significant source of mortality for a single population. Complications arise when multiple populations share a common environment where harvest occurs. Managers often set harvest quotas based on untested assumptions in the absence of information about population susceptibility. Such management decisions can be problematic when populations that share a common environment vary greatly in number, particularly when the numerical abundance of some populations is below levels sufficient to sustain harvest while other populations are numerical abundant.

Managers require techniques that allow them to assess population risk or to assess the magnitude of harvest if populations at risk to a sport or commercial harvest occur in mixtures. If there is sufficient genetic differentiation among breeding populations, it is possible to use mixed stock analysis (MSA) to examine the contribution of spawning populations to a given group of individuals (e.g. Pella and Masuda 2001). This method may be particularly relevant to management of populations if those populations that are spatially segregated and demographically and genetically independent but occur in common environments and are subject to harvest during non-breeding periods. When non-target populations that may be affected by harvest are found in different (e.g., lower) abundances, it is even more crucial to be able to assess the harvest risk.

MSA has been used to assess composition of harvest for numerous fisheries. Beacham and Wood (1999) examined stock differentiation within sockeye salmon, *Oncorhynchus nerka*, and subsequently analyzed population movements and contribution to a harvest from shared habitat. Similarly, Beacham et al. (1999) evaluated population structure in steelhead, *O. mykiss*, and used MSA to analyze the composition of harvests of steelhead that affect spawning populations in both U.S. and Canada. MSA can also be used to quantify changes in composition over time, as seen in an analysis of population of origin for harvested sockeye salmon in the Fraser River (Beacham et al. 2004) that found a change in mixture composition over time.

While there are a number of MSA examples from salmonid fisheries, this technique has also been extended to other species. The composition of a harvest fishery for shad, *Alosa sapidissima*, off of the coast of Virginia was analyzed using information from mitochondrial DNA (mtDNA; Brown et al. 1999). MSA results indicated that harvest composition varied by both year and collection location. Gatt et al. (2003) employed MSA to examine the population contribution of walleye, *Stizostedion vitreum*, populations to fisheries in eastern Lake Erie. Results suggest that walleye populations in the western portion of the basin may be affected by harvest activity in the eastern portion of the basin. MSA has also been used to analyze a harvest of Atlantic sturgeon, *Acipenser oxyrinchus*, in the New York Bight (Waldman et al. 1996). The authors found that all potential source populations did not all contribute equally to this fishery.

Information regarding how different populations contribute to harvest is useful for informed and effective management fisheries.

Lake sturgeon, *Acipenser fulvescens*, is a species of high conservation priority throughout their range (Welsh 2004). Currently, all remnant populations are numerically depressed relative to historic levels, and many populations have been extirpated (Holey et al. 2000). These fish exhibit a number of life history characteristics that make them difficult to study using direct (e.g., mark-recapture, tagging) methods, including a long life span (Baker 1980), and a delayed onset of sexual maturity, with males maturing at approximately age 12-22 and females at age 14-33 (Roussow 1957, Harkness and Dymond 1961). Spawning is intermittent, and spawning frequency varies by sex (females: 3-7 years, males: 1-4 years; Lyons and Kempinger 1992, Auer 1999a).

In lake sturgeon, little is known about adult and subadult habitat use and movements during extended non-spawning periods. Lack of knowledge regarding key aspects of the species' biology presents a significant obstacle to management and restoration efforts. Recent studies (DeHaan et al. 2006) have found evidence for significant spatial genetic structuring in remnant populations of lake sturgeon within the Great Lakes. Levels of differentiation are sufficient to identify probable breeding population of origin for individuals and mixtures from throughout the Great Lakes.

Across all sturgeon species, fishing-induced mortality has been identified as a significant factor associated with declines in sturgeon populations throughout North America (Boreman 1997). Populations of lake sturgeon throughout the Great Lakes have been similarly affected by commercial harvests (Baldwin et al. 1979). However, in areas of abundance, sturgeon populations may be able to sustain additive levels of adult mortality caused by harvest. Within the Great Lakes, few lake sturgeon populations are sufficiently abundant to persist if exposed to harvest. Examples of harvested populations include inland populations in Lake Winnebago, WI and Black Lake, MI which are closely monitored and subject to regulated takes during the winter months, as well as the Canadian waters of Lake Huron (open to regulated commercial netting) (Zollweg et al. 2003).

The Menominee River forms a border between Wisconsin and the Upper Peninsula of Michigan and supports the only sport fishery for lake sturgeon in U.S. waters of the Great Lakes (Zollweg et al. 2003), which was officially opened for harvest by the Wisconsin Department of Natural Resources in 1946. Four sections of this river are open to harvest. Three sections are isolated upstream by dams blocking upstream movement of adult fish. One section lies between the furthest dam downstream on the river and Lake Michigan. Individuals harvested in 2001 on the lowest section of the river (section four) are the focus of this study.

The fall hook-and-line lake sturgeon sport fishery on the Menominee River is managed under the assumption that all the individuals in the lower river are residents of the Menominee River, assumed to be staging during the fall for the spring spawning season. If straying of adults from other populations occurs, it is thought to be in low frequency and therefore would not negatively impact other populations. Without knowledge of lake sturgeon habitat use and movement, it is not possible to evaluate the assumption of low straying rates. If this assumption is not supported, other populations may be exposed to high rates of morality, which will impede their recovery. For example, other individuals from numerically-depressed populations in Lake Michigan may be present in the lower Menominee River in the fall and may be harvested as putative Menominee River residents. As the Menominee River is in close proximity to other remnant lake sturgeon populations of relatively low number (Figure 1; Holey et al. 2000, Gunderman and Elliott 2004), the potential of impacting non-targeted populations may pose a significant obstacle to restoration efforts.

Harvest on the Menominee River has been monitored through mandatory registration of harvested fish since 1983 in Wisconsin and 1986 in Michigan by the respective Departments of Natural Resources. In recent years (from 1986 until present), the focus of effort in this fishery has shifted from the upriver (isolated) sections to the downstream section, which is open to Green Bay, Lake Michigan. While fishing pressure has increased on all sections of this river, pressure has increased most on the lowest section. (M. Donofrio, personal communication)

Because of high levels of genetic divergence documented among remnant population of lake sturgeon within the Great Lakes (Figure 2), it is possible to use statistical techniques such as mixed stock analysis (MSA; Waser and Strobek 1998) and individual assignment (IA; Paetkau et al. 1995) to assign individuals of unknown origin to a population of origin based on genetic data. The goal was to estimate the proportional contributions of different populations in the Menominee River harvest. This study is the first to use MSA methods to examine stock composition of a lake sturgeon harvest.

The null hypothesis of this study was that all individuals targeted in this harvest are individuals originating from the Menominee River. However, since the lowest river section is connected to Lake Michigan and therefore accessible to lake sturgeon from other rivers, it is possible that adults from other populations will be present and subject to harvest. Additional mortality to non-target and numerically-depressed populations would compromise long-term population viability and inadvertently hamper management efforts to restore those populations.

Methods

Sample collection: baselines

As part of ongoing research on lake sturgeon throughout Lake Michigan (e.g. DeHaan 2003, Gunderman and Elliott 2004) spawning adult lake sturgeon were captured by cooperating agencies and institutions from five different remnant populations that breed in tributaries throughout Lake Michigan: the Fox (n=71), Peshtigo and Oconto Rivers (n=91) (Wisconsin), the Manistee River (n=80) (Michigan) and the Menominee River (n=41) (Michigan/Wisconsin) (Figure 3). Individuals were measured (length, girth, weight), and implanted with internal (passive integrated transponder, PIT) and external (Floy) tags for future identification. Samples of fin tissue (approximately a 1 cm^2 clip of the dorsal fin) were also collected, allowed to dry, and stored in scale envelopes at ambient temperature (Gunderman and Elliott 2004, DeHaan et al. 2006).

Sample collection: harvest

Samples of fin tissue (approximately a 1 cm^2 clip of the dorsal fin) were collected from all harvested individuals in the 2001 Menominee hook-and-line recreational fishery by WiDNR personnel. Samples were allowed to dry, placed in scale envelopes, and stored at ambient temperature.

Genetic Analyses

DNA was extracted from samples using QIAGEN DNeasy® kits (QIAGEN Inc.) according to manufacturers' protocols. All samples were diluted to a DNA concentration of 20 ng/ul for use in PCR reactions.

Individuals were genotyped at 8 microsatellite loci including *LS-68* (May et al. 1997), *Afu68b* (McQuown et al. 2002), *Spl120* (McQuown et al. 2000), *Aox27* (King et al. 2001), *AfuG9, AfuG63, AfuG74, AfuG112* (Welsh et al. 2003). PCR reactions were conducted in 25 µl volumes containing 100ng DNA, 10X PCR Buffer (1M Tris-HCl, 1M MgCl2, 1M KCl, 10% gelatin, 10% NP-40, 10% Triton-X), additional MgCl2 as determined by optimizations (1.0ul: *LS-68, Afu68B, Spl120, AfuG112,* 1.5ul: *AfuG9*), 2mM of each dNTP, 10 pmol of forward and reverse primer and 0.5 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 (*AfuG9, AfuG63, AfuG112:* 48C; *AfuG74*: 50C; *Aox27:* 53C; *LS68, Afu68B:* 56C; *Spl120: 62C)* and 72° C for 1 minute. PCR products were run on 6% denaturing polyacrylamide gels and visualized on a Hitachi FMBIOII scanner. Allele sizes were determined using commercially available size standards (MapMarkerTM, BioVentures Inc.) and based on standard samples of known genotype. To minimize error, all genotypes were independently scored by two experienced lab personnel and verified again after data were entered into electronic databases.

Additionally, the mtDNA size polymorphisms used by DeHaan (2003) were examined for all individuals. Reactions were carried out using the primer tPrto123 (Brown et al. 1996) and SturgD1R1 (DeHaan 2003), which was developed to flank a repeated region (~82 bp) in the D-loop element. This region was identified by Buroker et al. (1990) and also used by Brown et al. (1992). Reactions were carried out in 50µL reaction volumes, under conditions as listed in DeHaan (2003). PCR reactions for both mtDNA and microsatellites were carried out in Robocycler96 thermocyclers (Stratagene, Inc.). PCR products were analyzed on 1% agarose gels with 5μL of ladder, stained with ethidium bromide, and visualized using a UV lightbox. The number of tandemly repeated motifs (1,2,3,4) was recorded for each individual.

Statistical Analyses

Background

Mixed stock analysis (MSA) uses differences in allelic and genotypic frequencies among baseline (breeding) populations to estimate proportional contributions of baseline populations to a given mixture sample (Pella and Masuda 2001). When dealing with multiple populations that co-occur in a region and in the absence of large numbers of tagged individuals or physical criteria to discriminate populations based on phenotypes (Langerhans et al. 2003) or parasites (Criscione et al 2006), genetic methods offer an important tool to estimate proportional contribution of spawning populations to mixtures. In general, the accuracy of MSA methods increase as a function of the number of polymorphic markers used, assuming that there are differences in allelic frequencies among baseline populations (Kalinowski 2004**)**, and when allele frequencies are estimated with minimal error (Guinand et al. 2004).

Mixture analysis was conducted using the Statistical Program for the Analysis of Mixtures, version 3.7b (SPAM, Debevec et al. 2000, ADFG 2003). SPAM uses a Bayesian approach, incorporating information on allele and expected genotype frequency from baseline populations as priors to inform population allocation of mixtures to putative populations of origin. In contrast to other (frequentist) approaches, the Bayesian method incorporates information about the genetic similarities across stocks in allocating mixtures to populations of origin, which increases both accuracy and precision of estimates (Pella and Masuda 2001). Additionally, this method is accurate even in the presence of rare alleles (in mixtures or baselines) and when composition estimates begin to differ greatly across baselines (Pella and Masuda 2001). This study examined individuals from a harvest sample (population of origin is not known) that were assigned to populations of most probable origin by repeated resampling (over a series of MCMC iterations) of both baselines (individuals of known origin) and mixtures (harvested individuals of unknown origin).

The first step of analysis in SPAM is to treat each baseline as a set of unknown individuals and proportionally assign each baseline to possible populations of origin. In this study, baseline populations are remnant spawning populations of lake sturgeon across the Lake Michigan basin. The purpose of these simulations was to determine accuracy, precision, and allocation bias when assigning individuals from a mixture sample (admixed population) to source population. In a dataset with completely accurate population allocation, 100% of each mixture would be assigned to the same baseline population and proportion allocations to the other baseline populations would be 0%. Deviations from 100% allocation indicate both the magnitude and direction in which misassignments are likely to occur in empirical mixtures of unknown composition. Misallocation may be due to two different factors: populations that are not sufficiently characterized (e.g., low sample size and imprecision in estimates of allele frequency; Guinand et al. 2004), or as a result of genetic similarities across populations (Kalinowski 2004). In the second case, the magnitude of misallocation will reflect degrees of genetic differentiation among baseline populations (Kalinowski 2004). Baseline populations characterized by higher misallocation proportions are likely to be genetically similar to other baseline populations. Given a sufficient degree of accuracy is realized for baseline assignments, based on simulation analysis, empirical mixtures are subsequently analyzed.

Analyses of baseline populations

Using data from known spawning individuals from four Lake Michigan tributaries, including the Fox River, "Michigan" (represented by the Manistee River), Menominee River, and Oconto River/Peshtigo River, 100% simulations were conducted using SPAM 3.7b (ADFG 2003). Individuals from the Peshtigo and Oconto Rivers were combined into a single composite baseline population because of similarities in allelic frequency (DeHaan et al. 2006). Simulation analyses were based on 1000 MCMC replicates, with both the mixtures and the baselines re-sampled at every iteration. Each iteration was checked for sufficient convergence (defined as 95% or more) to ensure that simulations were converging on the true global maximum.

Analyses of mixture composition

Using multilocus genotypes of individuals from samples collected from the 2001 harvest on the lowest section on the Menominee River, analyses were conducted to estimate the contribution of baseline populations to the harvest sample. These analyses are based on 1000 MCMC replicates, with both the mixtures and the baselines re-sampled at every iteration. Again, iterations were checked for convergence (see above).

Results

Mixture analysis: simulations

The results from simulation analyses support a fairly high level of accuracy and precision in assignment to breeding populations, with assignments ranging from 0.880-

0.953 and 90% confidence intervals which include 1 (Table 5), even though the majority of the streams supporting spawning populations are in very close proximity. Significant misassignments between baseline populations are rare, as can be seen in the number (zero) of off-diagonal assignments that have confidence intervals that do not include zero. Misassignments reflect population genetic structure. For example, there is more misassignment from the Fox River to the Oconto/Peshtigo Rivers than from the Fox River to the Menominee River, and the Fox and Oconto/Peshtigo Rivers are more genetically similar. Directionality of assignment error may reflect population-level phenomena, such as unidirectional migration between populations, as observed in this study between the Oconto and Peshtigo Rivers when they are examined as separate populations.

Mixture analysis: estimations

Results from a mixture analysis of the 2001 Menominee harvest fishery (Table 6) shows that the majority of the fish present in the lower section of the Menominee River (n=104) are of Menominee River origin (estimate=0.810, CI = 0.749-0.899). However, the results of mixed-stock analysis also show a non-zero contribution from another baseline population grouping, the neighboring Peshtigo and Oconto populations (estimate=0.1901, CI=0.111-0.269). As the 90% confidence interval on this estimate does not include zero, the results are statistically significant.

Discussion

If harvest occurs at a single location, patterns of movement of potentially harvested individuals significantly affects their probability of being harvested. For example, if individuals tend to stay closer to natal tributaries, and harvest occurs in river mouths, those individuals will have an increased probability of being harvested. If only one population, or a specific group of populations is being targeted, given non-random movement, incidental harvest may be decreased. If individual movement is random, however, the location of harvest may not affect rates of incidental harvest. Studies across a number of species have found support for both random (e.g., Watts et al. 2004) and non-random dispersal of individuals across available habitat (e.g. Potvin and Bernatchez 2001, Frazer et al. 2004, Frazer et al. 2005).

Results from MSA of lake sturgeon sampled from open-water habitats (chapter one) have shown that habitat occupancy is non-random with respect to natal stream and that natal philopatry may extend beyond the spawning period. These results may support the management assumption that all individuals present in the lower Menominee River in the fall are likely of Menominee origin. However, while individuals in the lower river may be more likely to originate from that population, proximity to a breeding population does not indicate population of origin with great certainty, as individuals from breeding populations around Green Bay are found throughout multiple areas of open-water habitat throughout the bay (chapter one). MSA results show that the majority of fish present in the lower section of the river are of Menominee River origin (n=104) (estimate=0.810, 90%CI=0.729-0.891), or approximately 84 individuals. However, MSA results also show a non-zero contribution from another baseline population grouping, the neighboring Peshtigo and Oconto populations (estimate=0.1901, 90%CI=0.111-0.269), or approximately 20 individuals, which have a combined adult spawning population size estimated to be less than 250 individuals (Elliott 2003).

While individuals are only targeted once a year at a single location, given the lack of information about adult habitat use and movement, the potential impact of this harvest on other lake sturgeon populations is not insignificant. Given the longevity of this species (Baker 1980), relatively low natural levels of adult mortality (Houston 1987), and current low numbers of all lake sturgeon populations (Holey et al. 2000), MSA results (~17%, or approximately 20, of the 2001 harvest were not of Menominee River origin) suggest that the harvest of non-targeted individuals has had significant impacts on neighboring populations of lake sturgeon. Given the length of time that harvest has been occurring on the lowest section of the Menominee, it is possible that, over multiple years, non-targeted individuals have been harvested at similar rates annually, which would represent a significant impact on neighboring populations.

Over the past 10 years (1996-2006), there have been a total of 628 individuals harvested on the downstream section (section 4) of the Menominee. Since harvest pressure on Section 4 has increased only in recent years (e.g. harvest in 1996= 32 individuals, harvest in 2005= 136 individuals, M. Donofrio, personal communication), and lake sturgeon are long-lived and exhibit a relatively delayed sexual maturation, the effects of this fishery on recruitment for populations throughout Green Bay may not be observed at this time (see chapter two). However, such a result does not necessarily indicate that the Menominee River harvest has no impact on recruitment in remnant populations of lake sturgeon.

The potential negative impact of harvest on lake sturgeon populations has not gone unnoticed by managers. In an effort to reduce fishing pressure on lake sturgeon in the Menominee River, different size limits were implemented in 2000, alternating between a 50” and a 70” minimum length for fish harvested. However, since the new limits were put in place, fishing effort has decreased in the even-numbered years (70” limit) and increased in alternate years (50” limit), effectively maintaining a constant average pressure, rather than decreasing the fishing pressure, on the Menominee River population (M. Donofrio, personal communication). In 2002 and 2000, the larger size limit resulted in no harvest (Kornely and Meronek 2004). A recent report offered that harvests of large female lake sturgeon should be reduced (Kornely and Meronek 2004). The importance of retaining large individuals in the population can be seen in a recent review which (Birkeland and Dayton 2005) highlights the danger of unequally targeting age/size classes, particularly because larger (older) individuals may be disproportionately contributing to recruitment. Because of the potentially detrimental effects on the Menominee River population, Kornely and Meronek (2004) suggest that “on the whole, the harvest on the Menominee River is too high.” Harvest on all sections of the river continues to be closely monitored by managers, with shorter harvest seasons and increased size limits implemented in 2006. Additionally, in 2006, Section 4 was changed to a catch-and-release fishery, so while anglers are still allowed to catch sturgeon, direct take is not allowed (M. Donofrio, personal communication).

In order to examine the management assumption that individuals in the lower river are staging for spring spawning, as well as assess the likely impact of harvest on the Menominee River population, all harvested fish in the Menominee River have been examined for stage of gonadal development since the 1998 harvest year. Gonadal developmental stage is determined based on a field guide for identifying sex and gonadal development of lake sturgeon (Kornely and Meronek 2004). The downriver section (below Menominee Dam) exhibited a sex-biased harvest (57% female, 43% male). However, the proportion of harvested individuals that were likely to spawn the following spring is high, with 62% of females and 80% of males classifying as individuals that will spawn the following spring. These observations provide support for the hypothesis that a large proportion of the fish gathering in the river, and therefore potentially subject to harvest, may be staging for spring spawning (Kornely and Meronek 2004).

MSA analysis of individuals harvested in additional years would allow managers to better gauge how frequently different populations have been affected by this harvest and therefore the extent to which the management assumptions have been violated. As changes have been seen over time in some aspects of the harvested individuals, such as size and stage of sexual maturity (Kornely and Meronek 2004), it is also possible that the degree to which different populations are impacted by this harvest has changed over time. Future research should examine multiple years of harvest, as tissue samples (fin clips or pectoral fin rays) have been taken from harvested individuals for many years (M. Donofrio, personal communication). Understanding the effect that harvest may have on other remnant populations of lake sturgeon will help managers interpret current and past (e.g., retrospective recruitment, chapter two) patterns in other lake sturgeon populations.

APPENDIX A : TABLES AND FIGURES

Table 1: Results from simulation analysis of baseline populations using program SPAM and data from eight microsatellite loci. Values shown are averages of posterior probabilities of assignment, over 1000 MCMC replicates, across all individuals sampled from four baseline population groups, and the associated 90% symmetric confidence intervals. Fox = Fox River (n=71), MI = Manistee River (n=80), Men=Menominee River (n=41), Oc/Pe=Oconto/Peshtigo Rivers (n=91)

		TO						
	Fox	**CI (Fox)**	**MI**	**CI (MI)**	**Men**	**CI (Men)**	**Oc/Pe**	**CI (Oc / Pe)**
FROM Fox	0.894	0.800-0.967	0.013	0-0.039	0.009	0-0.029	0.055	0-0.131
MI	0.013	0-0.045	0.969	0.911-0.999	0.001	0-0.009	0.007	0-0.026
Men	0.026	0-0.066	0.020	0-0.046	0.874	0.764-0.964	0.058	0.001-0.125
Oc/Pe	0.054	0.003-0.116	0.006	0-0.026	0.006	0-0.022	0.928	0.856-0.987

Table 2: Results of estimation analyses of open-water mixture collections using program SPAM and data from eight microsatellite loci. Values shown are averages of posterior probabilities of assignment, over 1000 MCMC replicates, across all individuals sampled from four baseline population groups, and the associated 90% symmetric confidence intervals.

	Fox	CI (Fox)	MI	CI (MI)	Men	CI (Men)	Oc/Pe	CI (Oc/Pe)
SGB/ DYK/ PEN/ ORA	0.287	0.149-0.445	0.023	0-0.099	0.007	0-0.115	0.683	0.487-0.788
PRA/ YRA	0.244	0.158-0.370	0	0-0.065	0.176	0.104-0.255	0.58	0.429-0.659
PRF/ MAR	0.327	0.092-0.440	0.221	0.176-0.5	0.332	0.0670-0.351	0.12	0.034-0.461
LSS/ LSN	0.268	0.170-0.384	0.037	0.017-0.121	0.165	0.081-0.218	0.531	0.391-0.634
CRA	0.207	0.072-0.363	0.102	0.027-0.185	0.477	0.319-0.634	0.214	0.064-0.374
WAI/ DCO	0.315	0.135-0.519	0.194	0.064-0.333	0.165	0.031-0.308	0.326	0.116-0.539
LTB	<0.001	-	0.998	0.941-1.0	0.002	0-0.004	0	-

Table 3: Evidence for lack of genetic homogeneity across pooled mixtures based on likelihood ratio test implemented in program SPAM 3.7b as described in text. Tests were carried out over 5,000 MCMC replicates. “North” = WAI/DCO/CRA, “South” SGB/PEN/DYK/ORA, “Middle A” = LSS/LSN/YRA/ORA, “Middle B” = PRA/MAR.

Comparison	**p^a=**	**Conclusion**
across all sample groups	0.0002	significant heterogeneity
North vs. South	0.0002	significant heterogeneity
North vs. Middle A	0.0646	homogeneous mixture
North vs. Middle B	0.4235	homogeneous mixture
Middle A vs. Middle B	0.0002	significant heterogeneity
South vs. Middle A	0.2580	homogeneous mixture
South vs. Middle B	0.0002	significant heterogeneity

[a] *= Monte Carlo P-values are approximated using the method of Davison and Hinkely (1997), where p=(1+Q)/(1+R) with Q=the number of log likelihood ratios greater than or equal to the observed log likelihood ratio, and R= the number of simulation replicates.*

Table 4: Posterior probabilities of population assignment across known spawning individuals for four baseline breeding population groups. Posterior probabilities were derived using program STRUCTURE (Pritchard et al. 2000). Fox = Fox River (n=71), MI = Manistee River (n=80), Men=Menominee River (n=41), Oc/Pe=Oconto/Peshtigo Rivers (n=91)

		TO			
		Fox	**MI**	**Men**	**Oc/Pe**
FROM	**Fox**	0.543	0.155	0.169	0.132
	MI	0.106	0.722	0.085	0.087
	Men	0.098	0.111	0.630	0.161
	Oc/Pe	0.305	0.127	0.154	0.414

Table 5: Results from simulation analysis of baseline populations using program SPAM and data from eight microsatellite loci and a mtDNA size variant. Values shown are averages of posterior probabilities of assignment, over 1000 MCMC replicates, across all individuals sampled from four baseline population groups, and the associated 90% symmetric confidence intervals. Fox = Fox River (n=71), MI = Manistee River (n=80), Men=Menominee River (n=41), Oc/Pe=Oconto/Peshtigo Rivers (n=91)

	To Fox	**CI (Fox)**	**MI**	**CI (MI)**	**Men**	**CI (Men)**	**Oc/Pe**	**CI (Oc/Pe)**
FROM Fox	0.880	0.790-0.952	0.015	0-0.0411	0.002	0-0.009	0.068	0.016-0.138
MI	0.016	0-0.053	0.945	0.865-0.998	0.001	0-0.008	0.020	0-0.056
Men	0.190	0-0.052	0.211	0-0.051	0.887	0.775-0.973	0.051	0-0.123
Oc/Pe	0.045	0.001-0.101	0.009	0-0.028	0.003	0-0.012	0.933	0.862-0.990

Table 6: Results of estimation analyses of individuals harvested in the 2001 Menominee Hook-and-Line fishery (section of river between dam and Lake Michigan), using program SPAM and data from eight microsatellite loci and a mtDNA size variant. Values shown are averages of posterior probabilities of assignment, over 1000 MCMC replicates.

	Fox	**CI (Fox)**	**MI**	**CI (MI)**	**Men**	**CI (Men)**	**Oc/Pe**	**CI (Oc/Pe)**
MHL	0	-	0	-	0.824	0.749-0.899	0.176	0.104-0.248

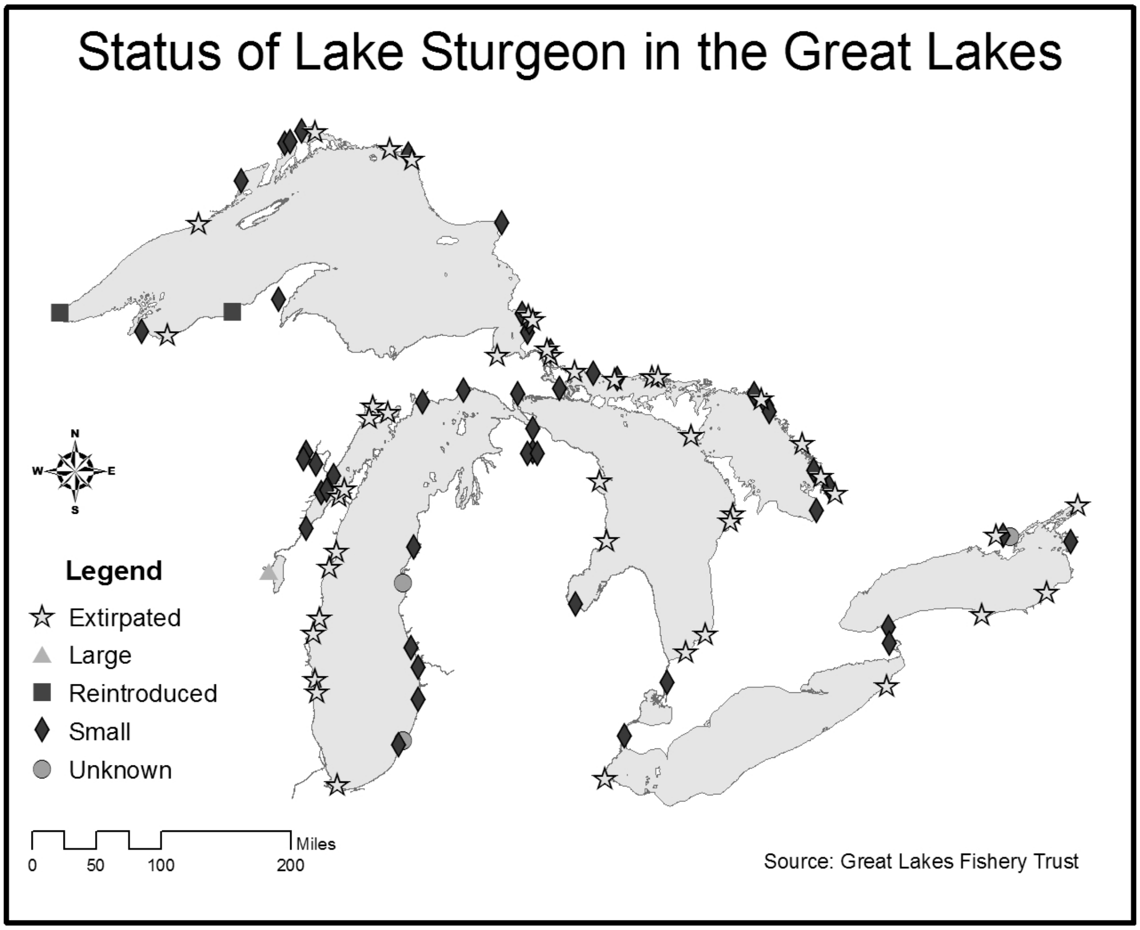

Figure 1: Status of historically-present populations of lake sturgeon, *Acipenser fulvescens*, across the Great Lakes region. Symbols indicate population status (extant or extirpated) and size if estimates are available (“large” ≥1000 spawning individuals, “small”<1000 spawning individuals). (Adapted from Holey et al. 2000)

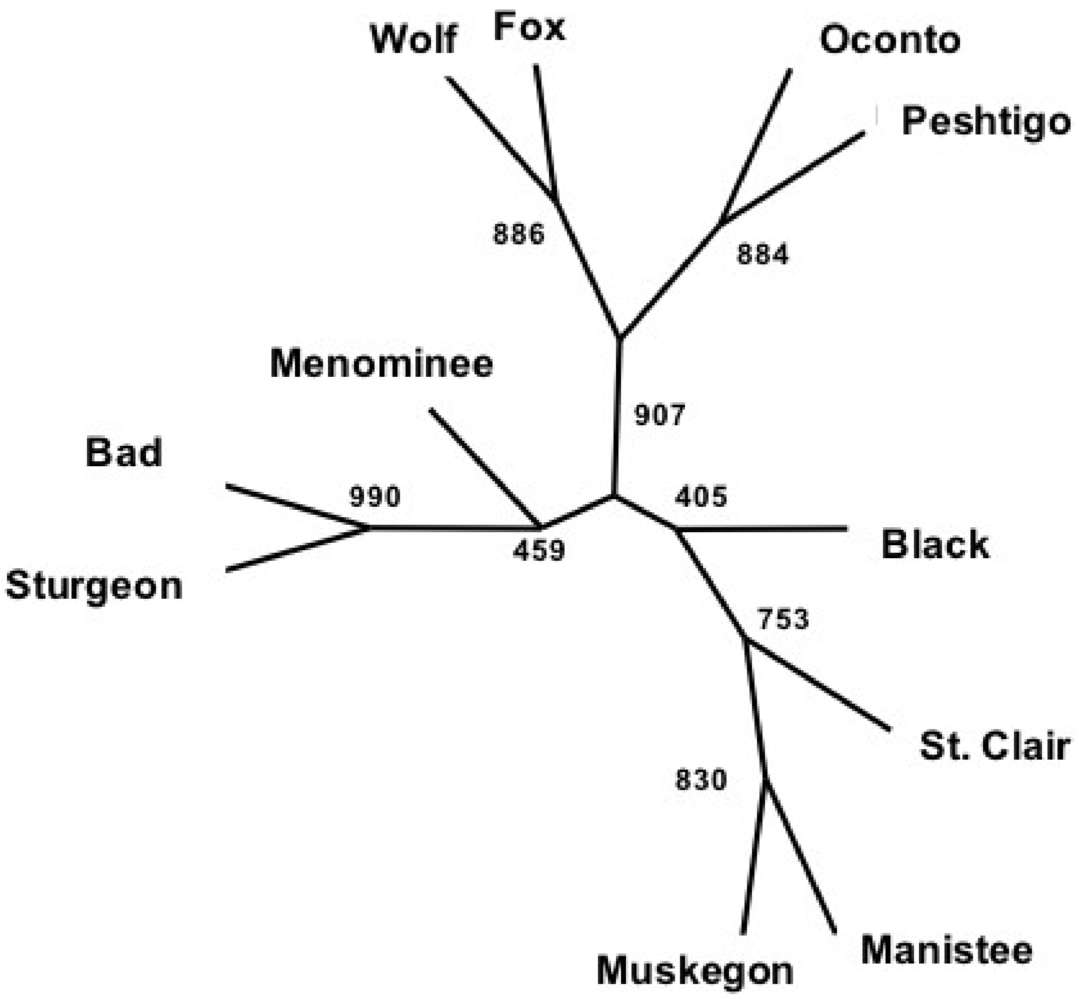

Figure 2: Neighbor-joining tree of Cavalli-Sforza and Edward's chord distances based on 8 microsatellite loci for 11 riverine populations of lake sturgeon throughout the Great Lakes, showing spatial genetic population structure. Numbers represent bootstrap support for each branch out of 1000 replicates. (From DeHaan et al. 2006)

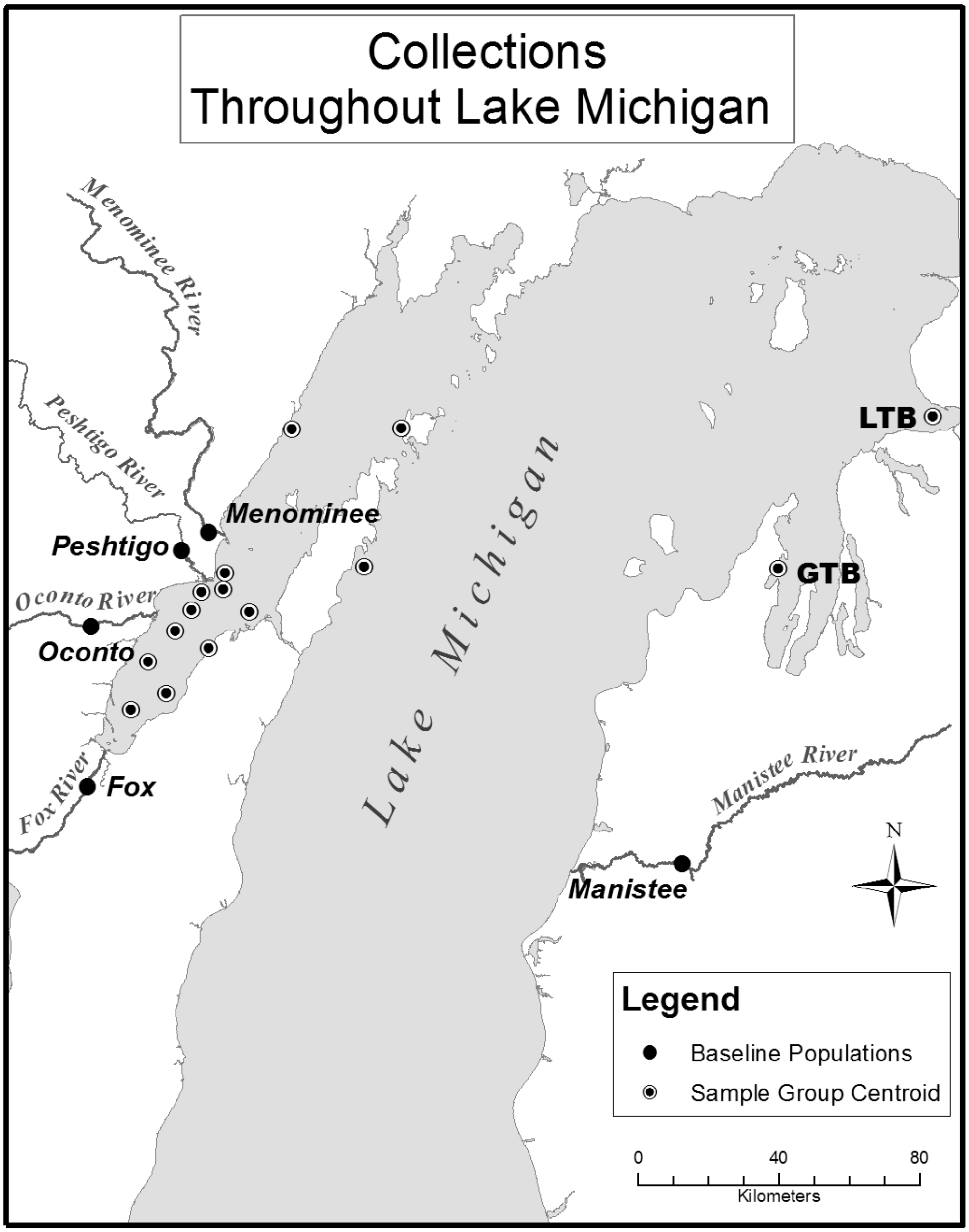

Figure 3: Map of (1) breeding populations, sampled from tributaries of Lake Michigan. Spawning individuals were sampled from the Fox (n=72), Menominee (n=42), Oconto/Peshtigo (n=91) and Manistee (n=80) Rivers. The Oconto and Peshtigo Rivers were combined for final analyses due to genetic similarity (DeHaan et al. 2006). (2) Sampling locations throughout Lake Michigan. Open-water habitats were sampled during 1999-2006. Points on the map represent the mean latitude/longitude for all samples collected in a region. Samples were pooled across all collection years for analysis purposes.

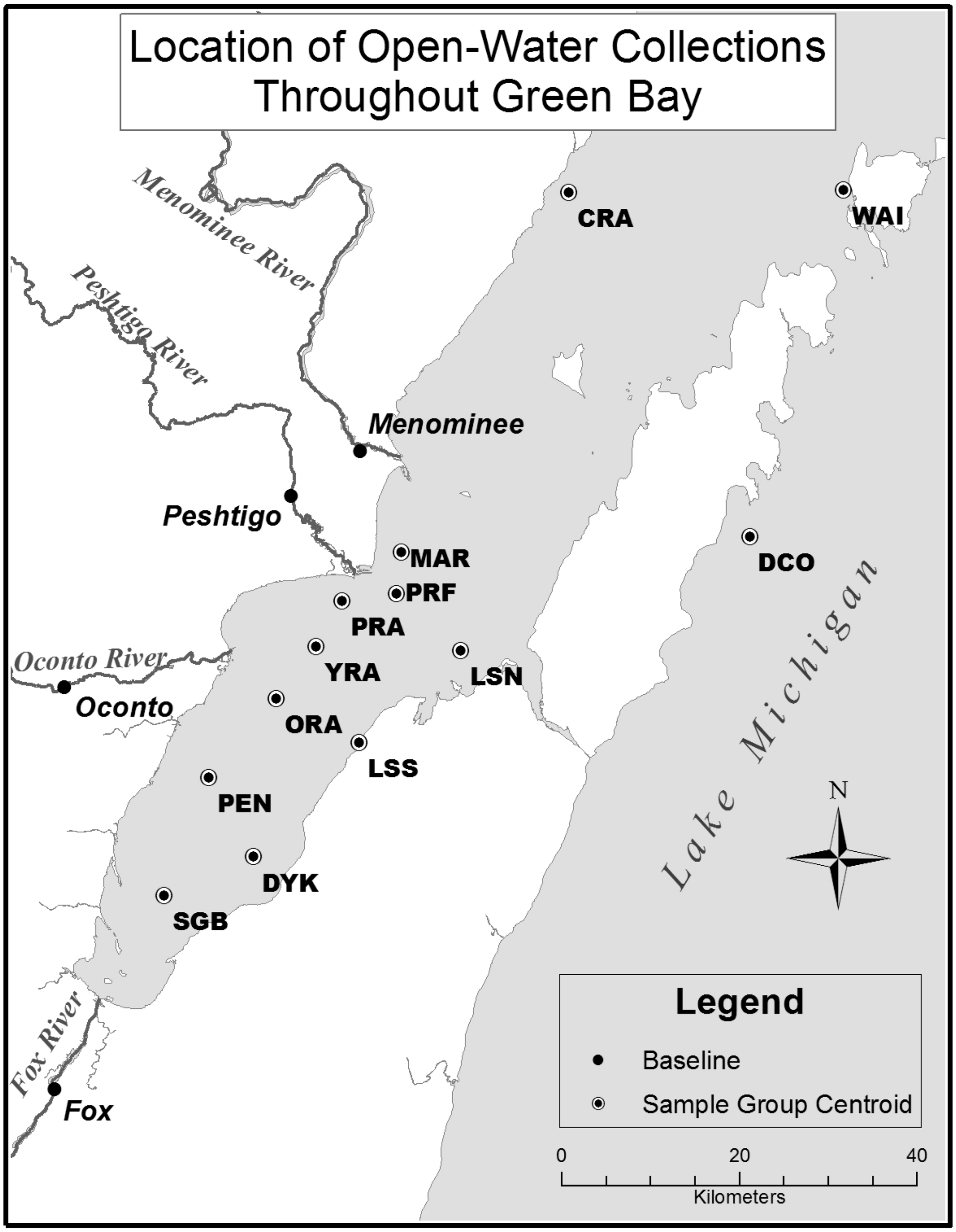

Figure 4: Sampling locations throughout Green Bay, sampled from 1999-2006. Points on the map represent the mean latitude/longitude for all samples collected in a given location. Samples were pooled across all years for analysis. WAI/DCO = Washington Island/Door County Area (n=47), CRA = Cedar River Area (n=75), MAR = Marinette trap net (n=23), PRF = Peshtigo Reef Area (n=57), PRA = Peshtigo River Area (n=93), YRA = Young's Reef Area (n=59), LSN = Little Sturgeon Area/north (n=24), LSS = Little Sturgeon Area/south (n=136), ORA = Oconto River Area (n=49), PEN= Pensaukee Area (n=17), DYK = Dykesville Area (n=27), SGB = Southern Green Bay (n=14).

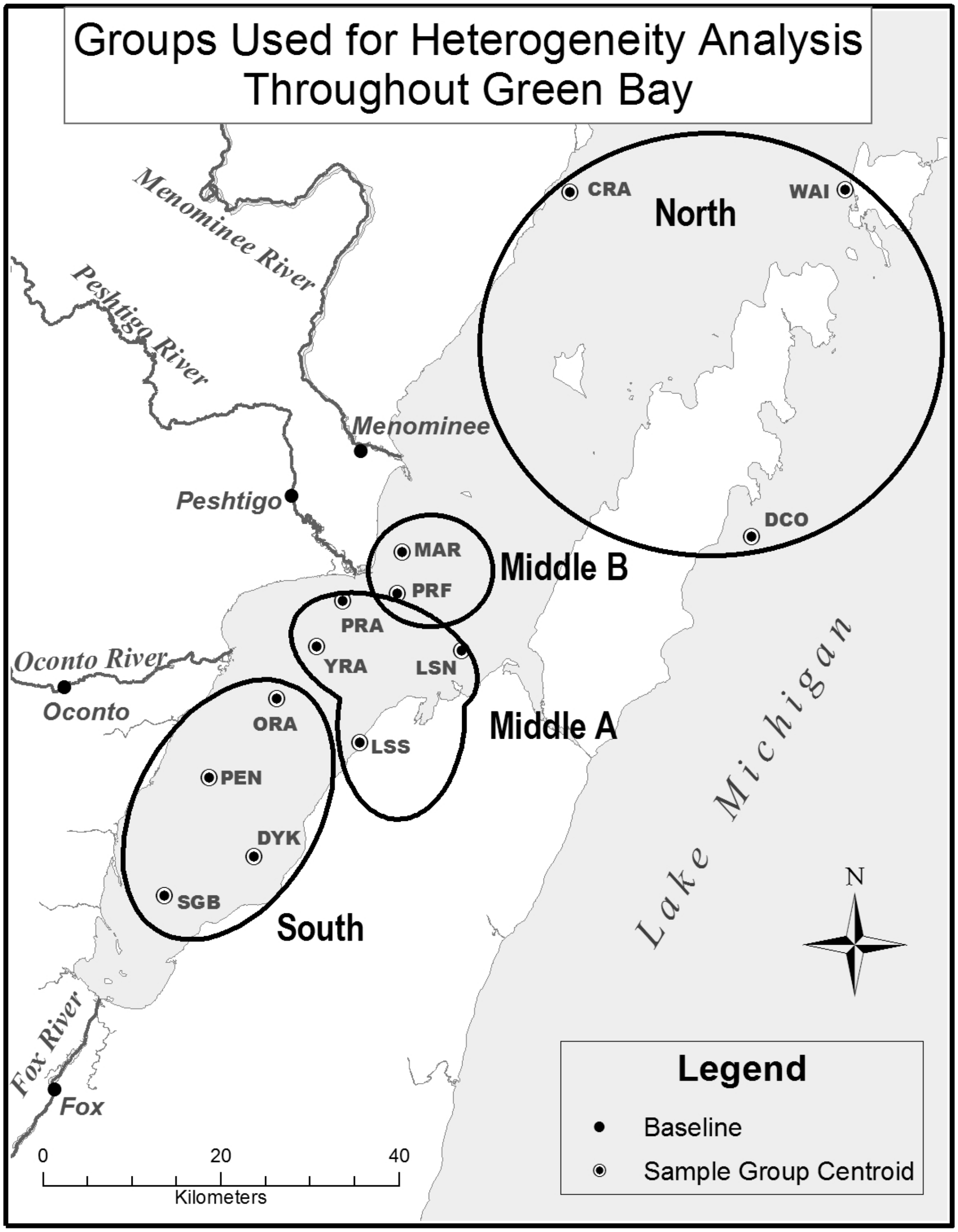

Figure 5: A map of groups used for heterogeneity analysis of mixtures across Green Bay (see text for details). “North” = WAI/DCO/CRA, “South” = SGB/PEN/DYK/ORA, “Middle A” = LSS/LSN/YRA/ORA, “Middle B” = PRA/MAR.

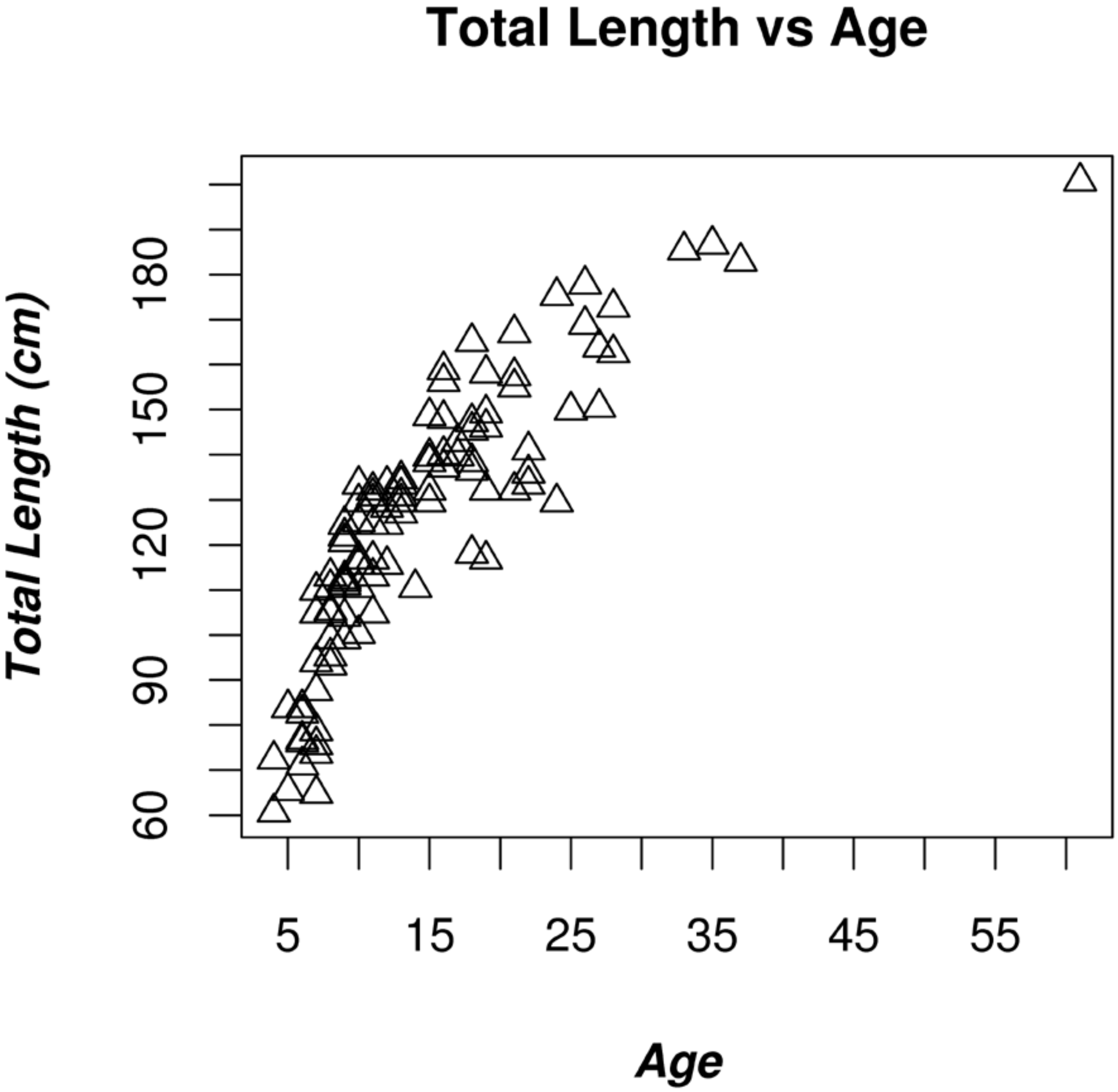

Figure 6: A scatter plot of length and age for 102 individuals of known age (r=0.91), determined by analysis of pectoral fin ray cross-section (see Gunderman and Elliott 2004), which are a representative subset of all individuals captured in the open-waters of Green Bay in 2002-2003. (Data from Gunderman and Elliott 2004.)

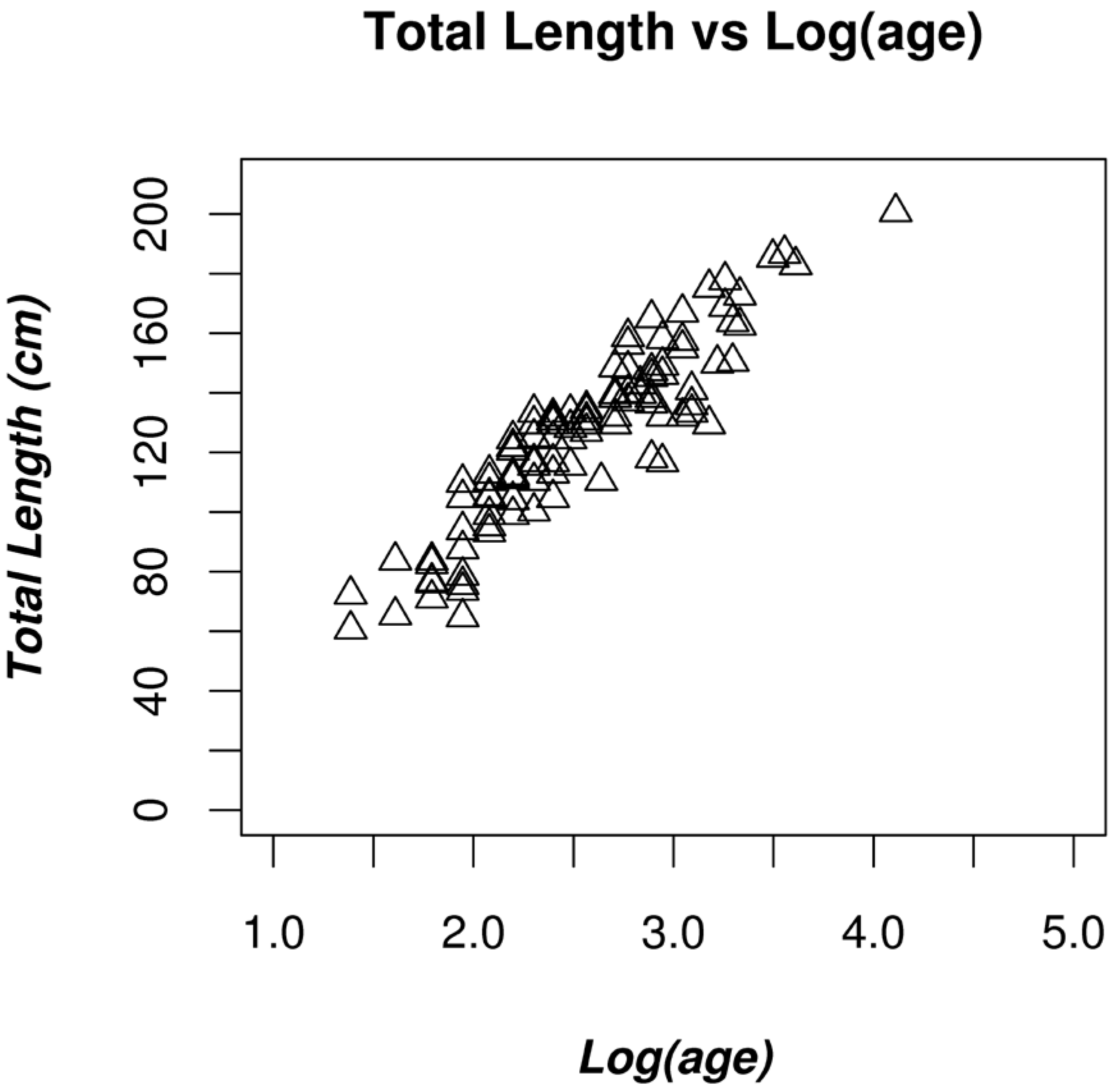

Figure 7: A joint density plot of log(age) and length for 102 individuals of known age determined by analysis of pectoral fin ray cross-section (see Gunderman and Elliott 2004), which are a representative subset of all individuals captured in the open-waters of Green Bay in 2002-2003. (Data from Gunderman and Elliott 2004)

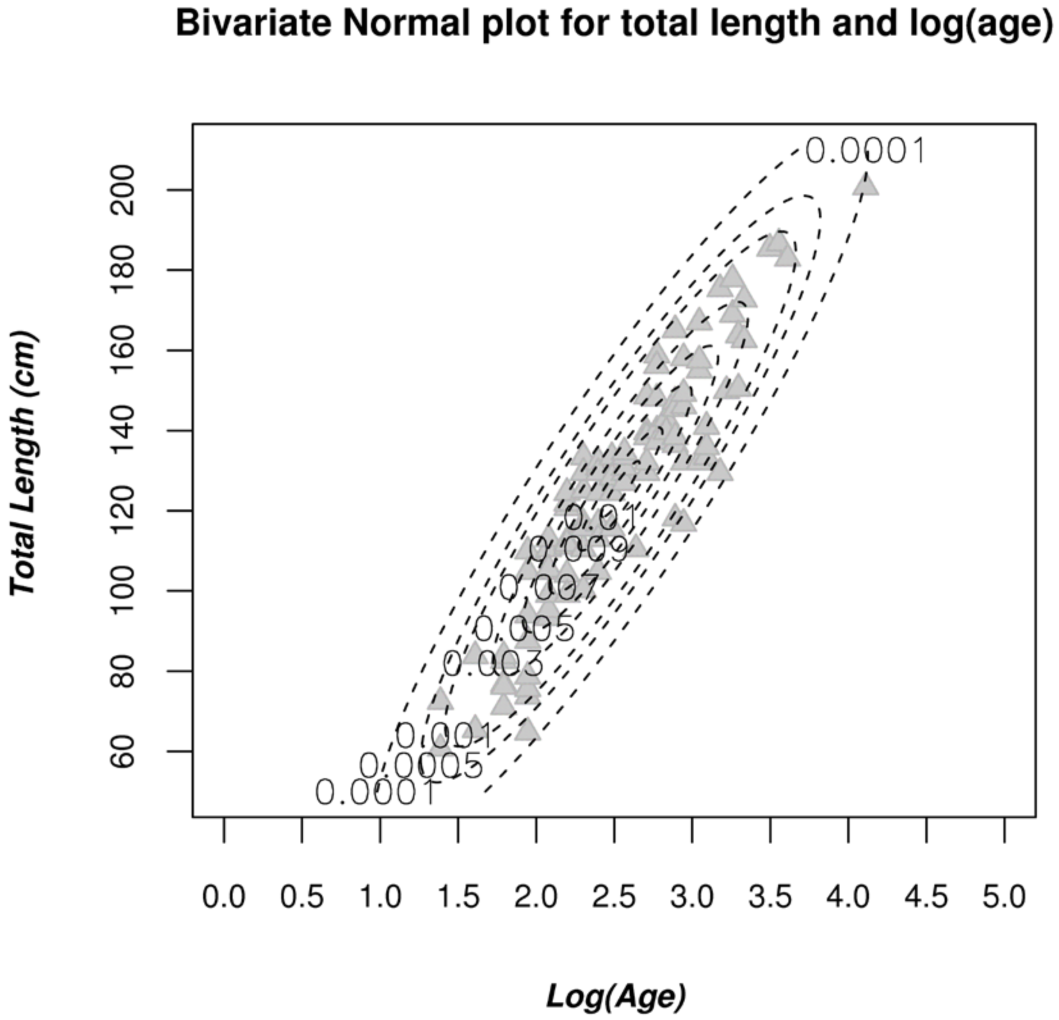

Figure 8: A joint density plot of log(age) and length for individuals of known age (aged by analysis of pectoral fin ray cross-section, see Gunderman and Elliott 2004). This yields a probability of log(age) given length for each defined age bin, allowing the incorporation of uncertainty in age assignment on an individual basis.

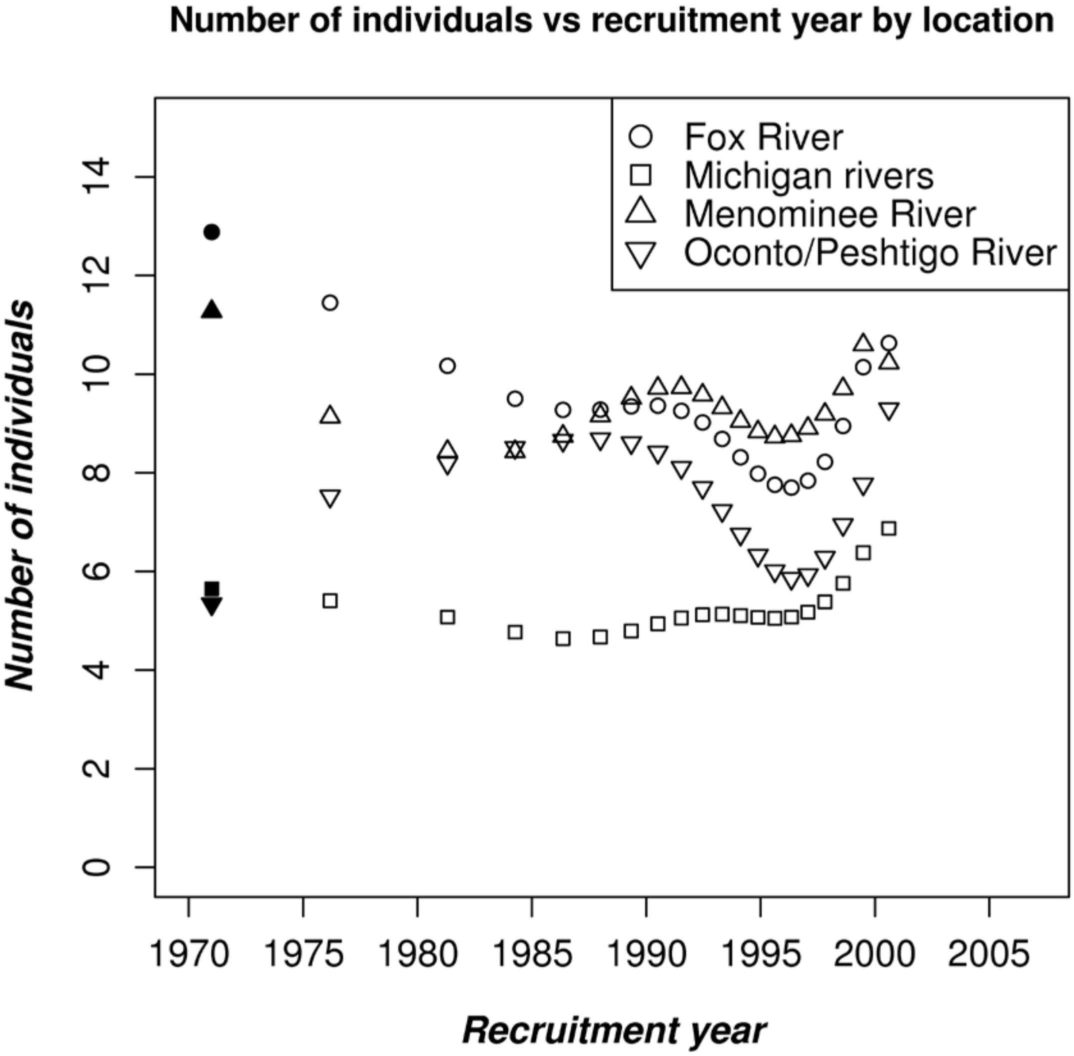

Figure 9: Results of location by recruitment year analysis (see text for details). Solid symbols on the left side of the graph indicate posterior probabilities of assignment to an age bin including year 1976 and all years previous. Analysis found that (1) irrespective of year, the effect of location is significant (populations likely had different sizes historically), (2) irrespective of location, the trend in recruitment is consistent, and (3) there is no evidence against non-independence across populations (precluding the investigation of a year*location effect).

APPENDIX B : BIBLIOGRAPHY

BIBLIOGRAPHY

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