# WORKSHOP PROCEEDINGS

# GREAT LAKES LAKE STURGEON GENETICS:
## STATUS, NEEDS, and STANDARDIZATION

**HOLIDAY INN – CHICAGO CITY CENTER**
**CHICAGO, ILLINOIS**
**December 8-9, 1999**

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Edited by:

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Lower Great Lakes Fishery Resources Office
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## INTRODUCTION

Interest in the restoration of lake sturgeon, as part of ecosystem rehabilitation, has become more clearly defined and continues to expand among natural resource agencies. Sound stewardship of fishery resources requires a fundamental understanding of how populations are structured genetically and the effects of anthropogenic forces on partitioning of genetic diversity. Quantifying genetic diversity and understanding how this diversity is partitioned (i.e., among lake basins and spawning populations within each basin) can provide managers and biologists with critical information to address important management questions. For example, the extent to which populations are genetically differentiated (and inferentially reproductively isolated) can be of importance in defining evolutionary significant units or management units. To date, traditional fisheries assessment methods (i.e., mark-recapture techniques) have failed to provide needed data. Therefore, genetics issues are at the forefront of lake sturgeon enhancement efforts. For example, hatchery broodstocks and stocking efforts are utilized as the primary management tool for restoring or enhancing populations. Broodstock production includes maintaining a high level of genetic diversity for environmental adaptability and fitness. Identifying the genetic makeup of lake sturgeon populations is crucial to help maximize genetic diversity among broodstocks and to ensure their integrity is maintained and not contaminated by stocking or transfer events.

To better guide lake sturgeon restoration and enhancement efforts in the Great Lakes, resource managers need a better understanding of the genetic structure among populations. Natural resource personnel from state, federal, and provincial agencies are all collecting tissue samples for genetic analysis; however, there is currently limited applicable genetic information to support lake sturgeon recovery efforts within the Great Lakes Basin. Preliminary findings suggest separate strains (stocks) exist between Lake Erie and Lake St. Claire (Porter *et al.* 1995), which have no physical barriers limiting movement between these systems. These conclusions require more detailed, standardized genetic analyses to determine the extent of separation/mixing within the populations.

The Lake Sturgeon Rehabilitation Strategy (Strategy) developed by the Michigan Department of Natural Resources supports the need for genetic research (Whelan and Hay-Chmielewski 1998). The Strategy emphasizes the importance of investigating the population structure of lake sturgeon and understanding the genetic composition of native populations. Every attempt should be made to assure the genetic integrity of existing populations is maintained, particularly where supplementation or reintroduction is necessary. Identifying genetically suitable sources of broodfish is also critical for stocking events.

Due to inconclusive results and a continued need for lake sturgeon genetic information, the Lake Sturgeon Committee of the U.S. Fish and Wildlife Service - Great Lakes Basin Ecosystem Team identified genetic standardization as a priority action item. Initially, to investigate issues related to standardization, the USFWS - Lake Sturgeon Committee prepared the report, entitled: Great Lakes Lake Sturgeon Genetics Status Assessment: An Analysis of Samples, Methods, and Standardization (Lowie 1999), which identified several discrepancies associated with standardized collection and analysis of genetic materials. For example, separate agencies collect different tissue samples and use different preservation techniques in the field.

Although a number of different analysis techniques are being used in lake sturgeon genetics research, mitochondrial DNA and microsatellite are most common. The results of these separate analyses are not comparable. Furthermore, if geneticists are using the same method, incompatibility can still occur when different markers, which are developed by the individual geneticist, are used. The report also highlighted the need for fishery managers to identify their needs and present these needs to geneticists in order to focus future research. A genetics workshop was recommended to discuss the issues presented in his report and formalize the resulting standardization methods.

Purpose

As the first step in a process for more definitive research, the purpose of the workshop was to initiate coordination and standardization of lake sturgeon genetic work in the Great Lakes. The workshop was formulated with the concerns of biologists and managers in mind to address two basic questions: (1) what do biologists and managers want to learn from genetic information and, (2) what do geneticists need to provide that information. Specific workshop objectives included: (1) share current genetic capabilities/technology with all participants, (2) identify information and research needs, (3) identify the best collection and analysis methods, (4) establish a network of communication between management agencies and research geneticists, and (5) identify funding sources to conduct the necessary research. The workshop started with a session examining the level of inconsistency in genetic research and how genetic research can be applied to lake sturgeon management. Geneticists presented the current state of knowledge regarding the genetics of Great Lakes lake sturgeon stocks. Finally, a working session was held to achieve overall workshop objectives. Workshop attendees included biologists, managers, researchers, and geneticists from several state, federal, provincial, and academic entities (Appendix A).

The purpose of this proceedings document is to summarize the presentations and discussions that occurred at the workshop as well as provide final decisions, where consensus was obtained, to standardize future lake sturgeon genetic work.

## ABSTRACTS

Below, in program order, is a brief summary of each presentation, discussion that followed, and the abstract as submitted by the author. Where multiple authors are listed, the presenter is identified with an asterisk. Submitted slide presentations are available in Appendix B. For more information on an individual research project, refer to Appendix A for contact information.

***Great Lakes Lake Sturgeon Genetics Status Assessment***
Chris Lowie - USFWS, LGLFRO (Slides on pages 22-24)

Summary
Chris provided an overview of how this workshop came to inception. A document titled, *Great Lakes Lake Sturgeon Genetics Status Assessment: An Analysis of Samples, Methods, and Standardization (Lowie 1999),* was presented. Based on the information reported by participating agencies, it is apparent that enhanced interagency coordination is necessary to guide future research on this topic.

Abstract
The U.S. Fish and Wildlife Service's (Service), Region 3 and Region 5 Great Lakes Basin Ecosystem Team (Ecoteam) identified lake sturgeon restoration and passage as a basin-wide issue on which to focus its efforts during the next fiscal years. A cross-regional and cross-program committee was established to identify and address priority action items, one of which includes standardizing genetic data collection and analysis. Multiple agencies are collecting different tissue samples from several waterbodies to accommodate various analysis methodologies. We, as resource managers and researchers, need to address the compatibility of the techniques and sampling regimes (i.e. cooperation) currently being used by various agencies. The objective of this paper is to compile information on existing genetic samples and programs conducted by various natural resource agencies to determine compatibility and identify potential standardization methods. Based on the information reported by participating agencies, it is apparent that enhanced interagency coordination is necessary to guide future research on this topic. The best collection, fixing, and preserving method should be identified and standardized among agencies to obtain clean and functional samples. The results of mitochondrial DNA (mtDNA) and microsatellite analyses are not directly comparable; however used together, the two results can be combined rather than compared. Allelic identification among analyses must be reviewed for consistency and standardized, if necessary. A sturgeon genetic marker library is being developed, which could be a positive step toward standardizing analyses. Finally, a coordinated effort to identify lake sturgeon genetic needs must occur among state, federal, and provincial resource managers and biologists in the Great Lakes Basin. These needs must be articulated to research geneticists, who in turn can provide practical information needed to manage lake sturgeon populations.

***Why Genetic Information is Important and How it Can be Used: General Background with Relevance to Sturgeon Conservation and Management***
Kim Scribner - Michigan State University

Summary
Kim gave a brief course in population genetics and how molecular techniques can be used to assist traditional fish management. Examples included defining Evolutionary Significant Units or Management Units, estimating effective population size, evaluating broodstock management, determining breeding sites of origin, and for forensics purposes. Follow-up discussions focussed on mating schemes when in a field situation. For example, when a biologist obtains only one

ripe female and one male, what should be done? Will one pair maintain, increase, or diminish the genetic diversity? Understanding the population size and genetic variation among the adults will help biologists make the decision.

Abstract
Recent advances in molecular biology have led to the development of numerous novel genetic markers which vary in modes of inheritance and in rates of evolutionary change. These markers have created many new opportunities for the study of fish ecology, behavior, and evolutionary history. This presentation will attempt to capture the diversity of issues, which can be addressed with molecular genetic markers by examining a number of case studies involving species from our laboratory and from the literature. Sound stewardship of fisheries resources necessitates a fundamental understanding of how populations are structured genetically and of the effects of anthropogenic forces on levels and partitioning of genetic diversity. The extent to which populations are isolated can be of importance in defining Evolutionary Significant Units (ESU's) or Management Units (MU's), and the extent of contemporary and/or historical gene flow among spatially segregated populations. Time-series data of per generation changes in gene frequency can be used to estimate effective population size in a variety of contexts, including threatened or exploited populations and to document the efficacy of broodstock management practices within hatcheries. Genetic markers are increasingly used in estimates of harvest derivation, or to place individuals with some probability to breeding sites of origin. Genetic markers when used in a forensics context are increasingly employed in studies of recruitment and survival and to examine interesting features pertaining to species behavioral ecology. Genetic markers have become a principle means for elucidating mating systems, reproductive strategies, and in estimation of male and female reproductive success. Species identification in contexts of food habits analyses or assessments of species assignment for larval fishes are also routinely practiced. Increasingly, hybridization either through purposeful introductions of non-indigenous species or via natural processes is becoming a leading area of conservation concern. Genetic markers can be used to examine the incidence and geographic context of inter-specific hybridization, identify causal factors, and document directionality of matings.

***Genetic Considerations for Captive Breeding Programs to Preserve Genetic Variability***

Harold Kincaid - USGS-BRD, Wellsboro (Slides on pages 25-30)

Summary
The previous discussion led directly to Harold's presentation, summarizing a breeding plan utilized with white sturgeon populations (Kincaid 1995). The plan focussed on establishing a self-sustaining population by restoring the natural age structure and preserving genetic variability. Harold insured that actual procedures will differ among restoration programs; however, the general principles presented can be applied to most programs. There was some contradiction between Scribner and Kincaid with regard to the best mating scheme. As stated above, knowing the population size and genetic variation among the population will help determine the best scheme.

Abstract
Restoration of depressed sturgeon populations may require development of captive breeding programs in situations where natural reproduction or recruitment is inadequate to sustain the population. When required, the captive breeding program needs to provide for systematically population expansion to a self-sustaining level that restores the natural age structure and preserves the underlying genetic variability. Genetic considerations include knowledge of population size and structure, generation interval, spawning intervals, and life history characteristics. In addition, the program must insure that the effective population size (Ne) is as large as possible and the genetic contribution of all parents is approximately equalized throughout the program. Actual procedures in each restoration program will differ, however, the genetic principles applied in the Kootenai River white sturgeon case study can be applied in most sturgeon restoration programs. The Kootenai River white sturgeon is a closed population that has not produced a successful year class since 1974. A captive breeding plan was developed in 1994 to begin the restoration process. Objectives were to preserve the remaining genetic variability, to restore the natural age class structure and to gradually expand the population. Assuming a 20-year generation interval and the ability of fisheries agencies to capture 5 mature males and 5 mature females per year, the captive breeding program would achieve a generation effective population size (Ne) of 200. Brood fish are paired randomly to spawn in pairs or in diallel mating designs to produce individual families. Families are reared separately to maintain family identity until the fish can be marked. Fish are stocked at age 1 or 2 so that all fish can be PIT tagged to assure positive identification of stocked fish by year class and family after return to the river. Stocking fish as fall fingerlings would reduce the potential for adaptation to the hatchery environment. Number of fish stocked per family is equalized based on expected rate of survival to 20 years-of-age. Stocking rates were selected to yield an average (estimated) of 4 breeding pairs per family at age 20. Natural selection during the 19+ years in the river environment before maturity will introduce variability in the actual genetic contribution of families to the next generation. Stocking numbers should be adjusted in future years as the realized survival rate become known. Brood fish are tagged at capture to identify those individuals that have produced families in the captive culture program. When sufficient brood fish are available, these fish will be returned to the river to minimize multiple spawning of individual fish. Because the captive breeding program is designed to produce limited numbers of breeding adults per family, a slowly expanding natural population should result that does not exaggerate the contribution of a few parents as occurs in typical hatchery supplementation programs. The captive breeding plan could be discontinued once spawning habitat is re-established and successful natural spawning and recruitment are demonstrated.

***Conservation Genetics of Lake Sturgeon in Central Canada***

Moira Ferguson – University of Guelph (Slides on pages 31-34)

Summary
Moira presented information on the appropriate choice of genetic markers for lake sturgeon research. Mitochondrial DNA analysis did not provide the resolution to identify management units. Based on research, it appears microsatellite markers meet the necessary criteria to answer research questions. One limitation is that microsatellite markers require a high number of

samples, typically 50 per population. Biologists collecting samples should not however be fixed on this number, as information can be gained from fewer samples.

Abstract
I will use completed and planned research at the University of Guelph to foster a discussion on the appropriate choice of genetic markers for lake sturgeon genetic research. The development of novel marker systems for fisheries applications has been fueled by the need to detect genetic variation. Although some applications will place greater emphasis on genetic differences among groups (stock structure) and some will focus on differences among individuals within populations (pedigree analysis), the detection of polymorphism remains the key. We have used mitochondrial DNA (restriction fragment length polymorphisms and direct sequencing of the control region) to determine the genetic population structure of lake sturgeon from the northern part of their range. Despite an extensive search for polymorphism, most of the lake sturgeon analyzed belonged to either one of two haplotypes. Although our analysis provided important information on the glacial history it did not have the resolution to identify management units. Ongoing research by Michael Robinson (Ph.D. student) will provide genetic information that will be used for the wise management and conservation of lake sturgeon in the Rainy River. We are comparing the genetic diversity of lake sturgeon in the Rainy River to those being cultured in a hatchery as well as archived samples (to be collected from aboriginal historical sites). Pedigree analysis of the cultured fish will be used to determine the relative success of individual males and females. Finally, we are comparing the Rainy River data to that of sturgeon collected from throughout the range. Successful achievement of our objectives requires that we use a genetic marker system that is hypervariable, unambiguous (i.e. single locus, codominant expression of alleles) and can be analyzed with minute quantities of genetic material. We believe that microsatellite loci are the only markers available that meet these criteria.

***Analysis of Lake Sturgeon Population Structure with Microsatellite Data***

*Eve McQuown[1], Charles C. Krueger[2], Harold L. Kincaid[3], Graham A.E. Gall[1], and Bernie May[1,2] (Slides on pages 35-36)

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Summary
Eve presented data on the genetic variation of six lake sturgeon populations throughout the Great Lakes. She used seven microsatellite markers; two derived from lake sturgeon, one from Atlantic sturgeon, and four from shovelnose sturgeon. Four of the markers were disomic in nature while the other three were tetrasomic. Preliminary data was presented showing discrimination among the populations, based on genetic variation. Further analysis showed where the allelic differences occurred. The extent of differentiation among the populations and the inheritance of the markers will be further analyzed.

Abstract
Overharvest by commercial fisheries and habitat alteration, such as construction of dams which impede migration to spawning sites, has led to the decline, and in some cases extinction, of lake sturgeon populations. Management programs to restore sturgeon have been implemented, but life history characteristics (reach sexual maturity at a late age and long periods of time between spawning) and the lack of information about the population structure of lake sturgeon has hindered decision-making. In order to gain a better understanding of lake sturgeon population structure, we examined variation at seven microsatellite loci within and among seven populations: Lake Erie, Lake Matagami, Menominee River, Wolf River, St. Lawrence River, and Des Prairies River. Two of these loci were derived from a lake sturgeon genomic library (May et al.1997), one from an Atlantic sturgeon library (King et al., unpub. data), and four from a shovelnose sturgeon library (McQuown et al., unpub. data). Four of these loci appear disomic, displaying either a single band or two bands of equal intensity. Three loci display banding patterns characteristic of four gene doses with four banded patterns of equal intensity, three bands with one darker than the other two,and two bands in an apparent three to one intensity. These loci have been used to genetically characterize lake sturgeon population structure. The results we report will be beneficial in identifying management units and designing programs to aid in the restoration of naturally-sustaining Great Lakes lake sturgeon populations.

***Molecular Genetic Characterization of Spatial Population Structure of Remnant Lake Sturgeon Populations in the Upper Great Lakes Basin***

Kim Scribner - Michigan State University

Summary
Kim stressed that the critical information needed to restore or rehabilitate lake sturgeon populations is the degree of population structuring within and among spawning areas, drainages, and lake basins. Kim is in the initial stages of lake sturgeon research, focussing on reproductive isolation among populations. Distribution information indicates lake sturgeon in the Great Lakes move large distances; between lake basins. Therefore, tissue samples for genetic analysis should be collected primarily from spawning populations to determine stock differentiation (or reproductive isolation). Tissue samples from individuals in open lake systems can be used to track them back to breeding sites of origin. Participants stated that limiting tissue collection to breeding individuals makes it even more difficult for biologists to obtain a sufficient number of samples. Breeding individuals are best case scenario; however, field personnel should not limit collections. Any samples are useful.

Abstract
Since the mid-1800's lake sturgeon (*Acipenser fulvescens*) have suffered dramatic declines coincident with over harvest and loss and blockage of spawning habitat. Range-wide declines in number and distribution have resulted in assignment of 'Vulnerable' and 'Threatened' status throughout the species entire North American range. Rehabilitation strategies in the upper Great Lakes Basin are designed to conserve and rehabilitate self-sustaining populations. Specifically, management efforts should be directed towards conservation and rehabilitation of populations which are currently still viable, as the diversity represented in these remnant populations constitute the remaining raw material to use for recovery across the upper Great Lakes basin. Further, in situations where populations have been

extirpated from historical habitats, management should strive to re-establish self-sustaining populations. One fundamental piece of information of critical importance for species restoration is the degree of population structuring within and among spawning areas, drainages, and lake basins. We will highlight details regarding specific questions our laboratory is addressing using molecular genetics data. Molecular genetics data are being used to infer degree of reproductive isolation among extant remnant populations and of the degree of drift in gene frequency over time. In addition, information on degree of genetic differentiation among breeding populations can be used to assign individuals or groups of individuals in open lake systems to breeding sites of origin. Genotypic data from spawning adults and out-migrating juveniles can be used to determine reproductive success and recruitment.

### ***Preliminary Results from Genetic Studies of Population Structure in the Lake Sturgeon of the Great Lakes***

*P.A. Fuerst[1,2], T.M. Cavender[2], B. Porter[2,3], J. Krieger[1], and J. Maybruck[1]

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Summary
Paul presented data from his research, which includes using several molecular techniques to determine genetic differences among sturgeon species and Great Lakes lake sturgeon populations. All techniques showed similar results among the populations. In addition, Paul's findings were very similar with Eve's, on populations examined by both researchers. Different microsatellite markers were used by each researcher.

Abstract
A variety of molecular techniques have been applied to study the structure of lake sturgeon populations. Mitochondrial DNA sequences have been collected to relate the lake sturgeon to the other four *Acipenser* and three *Scaphirhychus* species in North America. These data clearly show that the seuqence from *Acipenser fulvescens* forms a clade with the shortnose sturgeon (*A. brevirostrum*) as a sister species. No differences were noted between the mitochondrial genome sequences of the three *Scaphirhynchus* taxa. Genetic differences between localities within the lake sturegon have been examined using VNTR probes, RAPD analysis and microsatellite locus comparison. These studies each suggest that there may be differences between localities, and that an east-west component of differences may exist. Using a set of three VNTR probes, a set of fish obtained from Lake Erie and Lake St. Clair were compared with progeny of fish collected from the Wolf River of Wisconsin. Fish from the two eastern localities were significantly more similar to each other than they were to fish from Wisconsin, suggesting that population difference may exist. Further preliminary studies were pursued using RAPDs (Randomly Amplified Polymorphic DNA). A set of fish obtained at four localities were analyzed. These localities were the Wolf River and Menominee River from Wisconsin and the St. Clair River and Lake Erie. UPGMA analysis of the RAPD distances between populations indicate that the two Wisconsin and the two eastern populations form two clusters, again suggesting that east-west population differences exist. The analysis of this same data by cladistic methods also suggests that population differences exist, including an east-west component. A preliminary analysis of

these same localities using a microsatellite locus shows the same clustering of populations. An extension of the RAPD analysis to include some additional populations (Sturgeon River and Black River) continues to suggest local differentiation. Finally, investigations have been undertaken to examine the potential for obtaining historical information on sturgeon populations utilizing museum specimens which have been preserved by formalin treatment or other preservation methods. Comparisons of duplicate tissue samples which have been preserved using formalin or alcohol indicate that sequence information may not be reliably preserved in formalin fixed material, but that microsatellite allele sizes may be unaffected. (We thank the Wisconsin, Michigan and Ohio DNRs for assistance in collecting material and the USFWS, Ohio Sea Grant and the National Science Foundation for partial support of the work reported here).

***Identification and Conservation of Genetic Diversity in Atlantic Sturgeon* (Acipenser oxyrinchus)**

*Adrian Spidle and Tim King - USGS-BRD, Leetown (Slides on pages 37-41)

Summary

Adrian presented work conducted at the Leetown Science Center on population structuring of Atlantic and Gulf Sturgeon. Microsatellite variation in these species did allow precise discrimination of stock structure in Atlantic and Gulf sturgeon. This work demonstrated a success story in differentiating sturgeon populations over a large geographic area and provided an example of how we could proceed in the Great Lakes. It is likely that discrete stocks of lake sturgeon are present in the Great Lakes.

Abstract

The Atlantic sturgeon (*Acipenser oxyrinchus*) and the gulf sturgeon (*A. o. desotoi*) are under consideration for listing under the US ESA, and listed as threatened under the US ESA, respectively. In order to assess levels of genetic variation remaining in these species, and identify population structure for specific management goals, we have developed and screened a number of microsatellite loci. Atlantic sturgeon are differentiated at microsatellite loci among the St Lawrence River, St John River, Hudson River, Delaware River, Chesapeake Bay, and Altamaha River. Gulf sturgeon sampled from the Pearl, Yellow, and Apalachicola Rivers were correctly assigned to their drainage using multilocus microsatellite genotypes. These preliminary results indicate that microsatellite variation can allow precise discrimination of stock structure in Atlantic and gulf sturgeon.

***Microinjection as a Means of Developing a Molecularly Tagged Stock of Lake Sturgeon,* Acipenser fulvescens (Rafinesque)[§]**

Z.M.G. Sarwar Jahangir[1,3] and Ronald A. Eckhardt[2] (Slides on page 42)
(Slides on pages ; also see their web page at )

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Summary
Sarwar presented information on the use of a molecular tag (gene) to develop an identifiable stock of lake sturgeon. They developed a stock of transgenic lake sturgeon that expressed the gene by producing an enzyme called beta galactosidase. As a result, tissues from a transgenic individual turned blue in color when treated with a particular reagent, X-gal. This method may be applied to develop a stock of fish and make them identifiable through successive generations. Furthermore, using the same technique, other genes essential to lake sturgeon survival may be incorporated into a stock. Several questions arose regarding the legality and effects of stocking these fish in nature. Sarwar indicated this method may be used experimentally to develop a stock of lake sturgeon for a fully depleted and confined small water body. The legality to release them in a larger water system would depend on local environmental laws. Furthermore, the effects of these transgenic individuals on the natural environment is not known and should be further studied.

Abstract
Lake sturgeon is an endangered/threatened species in all states where it occurs. The ability to identify beheaded, skinned, filleted or otherwise altered lake sturgeon in the field is essential for effective conservation measures. In order to evaluate a possible method to provide such an ability, we developed a molecularly tagged stock of lake sturgeon carrying and expressing bacterial beta-galactosidase as a molecular tag. Over 5 days, spawning pairs of lake sturgeon were captured from the Wolf River, New London, WI, from which milt and ova were collected by stripping. The ova were fertilized by mixing with milt, dejellied with bentonite and microinjected with bacterial beta-galactosidase genes in a mobile laboratory van. The injected embryos were hatched and the sac fries reared at the Center for Great Lake Studies, Milwaukee, WI, for up to six months under the same conditions as control fish. Samples of developing embryos and sac fries from the microinjected ova were tested for the expression of beta-galactosidase by reaction with X-gal (5-bromo-4-chloro-3-indolyl-B-galactoside). Approximately 39 % were found to be expressing bacterial beta-galactosidase as indicated by the development of a blue color in the X-gal solution. None of the controls showed this reaction. When six-month old fingerlings obtained from the microinjected ova were tested with X-gal, their entire skin, brain, nerve cord, liver, kidney, fins, olfactory bulb and nasal epithelium developed an intense blue color while the controls showed no reaction. These observations

support the prediction that lake sturgeon, molecularly tagged with bacterial beta-galactosidase DNA, may be easily distinguished from non-tagged fish using a relatively simple color test. Hence, they could potentially be used to determine the size of lake sturgeon populations following standard release/recapture methods. Or, molecularly tagged fish could be released into nature and could easily be identified after capture (regardless of alterations), thus, improving conservation enforcement.

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The authors gratefully acknowledge the participation and support of Professor Fred P. Binkowski, Center for Great Lake Studies, University of Wisconsin - Milwaukee, 600 E. Greenfield Avenue, Milwaukee, WI 53204, and Ronald M. Bruch, Department of Natural Resources, State of Wisconsin, 905 Bay Shore Drive, P.O. Box 2565, Oshkosh, WI 54903 in the above research.

## WORKING SESSION

To conclude the workshop, an open discussion session was held to achieve the overall meeting objectives of identifying information and research needs, identifying the best collection and analysis methods, and establishing collaboration and funding sources to conduct future research. The session was facilitated by Gary Whelan (Michigan Department of Natural Resources) and Chris Lowie scribed.

The following bullets provide a summary of the main issues discussed. Where consensus was obtained, decisions are documented to standardize and guide future lake sturgeon genetics work.

♦ ***Information Needs***

The following needs were identified by participants:

- Stock identification
- Mixed stock identification
- Stock variation
- Characterize successful reproductive populations
- Identify best sources for stocking
- Identify best stocking/mating strategies
- Identify genes responsible for survival
- Identify fitness and domestic selection
- Genetic tagging
- Individual identification
- Impact of stocking on natural populations

Grouping and prioritizing these needs were beyond the scope of this workshop due to time constraints. Therefore, we identified the highest priority to focus further discussion. The group selected the need to identify stocks as the top priority.

- ***Collection, fixing, and preservation methods***

  Samples should be collected primarily from spawning populations; however, samples from open water fish are valuable. Fin tissue is the most effective, yet least invasive tissue type and should be provided in most circumstances. Soft tissue inadvertently gathered from collecting age structures is satisfactory. Use your best professional judgement as to the quantity of tissue collected. A minimum of one-half square inch is required; however, up to 1 square inch from adults is preferred. Simply water rinse and wipe hands and utensils to avoid contamination between subsequent samples. Air-drying was determined by geneticists to be the best method for fixing and preserving samples. Placing the sample in a paper envelope is satisfactory. This method also alleviates the difficulties associated with shipping alcohol or other preservatives. However, air-drying is not best for archiving samples due to possible bug infestation. Alternatively, preserving the sample in 95% alcohol (not denatured alcohol) is appropriate, particularly if archiving the sample. It is best to change the alcohol after the initial fixation for long term storage. Ensure the sample is submerged. If samples are frozen, keep them frozen until ready for analysis.

- ***Centralized depository for samples***

  Establishing a central depository for samples or data was discussed. Participants were reluctant to relinquish samples to one location, not knowing the final fate of them. However, a database of population genetic information would be acceptable. Participants agreed to include genetic information as part of a database being developed by the USFWS Great Lakes Basin Ecosystem Team, Lake Sturgeon Committee. Many partners will be involved in developing this database and concerns regarding availability of data (on the internet) are being addressed.

- ***Analysis methods***

  Microsatellite analysis is fastest for stock identification; however, experimentation with other methods will be left open for the future. It was stated that tetrasomic markers are more challenging to standardize due to the complexity of determining gene dosage. Therefore, there is some reluctance to working with tetrasomic markers. A library of disomic microsatellite markers is being established. More disomic microsatellite markers are needed to provide an adequate suite for addressing information needs. Existing and additional markers should be standardized and available for data comparisons between multiple labs. To enhance collaboration between labs and minimize competition for funds, one financial source is necessary. Contracts between agencies and genetic labs should specify how samples will be used and data ownership.

- ***Funding***

  Several granting agencies were identified who could potentially provide funding for a Great Lakes-wide study; including, the Great Lakes Fishery Trust, the Great Lakes Fishery Commission via appropriations from the Great Lakes Fish and Wildlife Restoration Act, or the Environmental Protection Agency's Great Lakes National

Program Office (GLNPO). Regardless of funding sources, this needs to be a collaborative effort among and between natural resource agencies and geneticists. The group agreed that Chris Lowie would develop two proposals in cooperation with participants. The first would focus on standardizing markers and the second on collecting samples from spawning populations throughout the basin.

## SUMMARY

Standardizing genetic data collected from existing populations will assist state, provincial, and federal biologists and managers throughout the Great Lakes determine if separate strains/stocks exist within their geographic area. This workshop catalyzed the process for obtaining valid and conclusive genetic analyses of Great Lakes lake sturgeon populations. Specifically, the workshop addressed several issues related to lake sturgeon genetics including identifying information needs, standardizing collection and analysis methods, and identifying the next step to determine the extent of genetic separation/mixing within populations. Participating biologists and managers determined that obtaining additional information on stock variation and identification was the highest priority. However, geneticists specified that additional markers need to be developed to meet this request, and to support other lake sturgeon genetic research (e.g. mating schemes/broodstock management). All participants agreed a large source of funding was necessary to accommodate such a research project.

Genetic issues are at the forefront of lake sturgeon enhancement efforts because of potential stocking or transfer events. This workshop demonstrated new and additional management options available when the genetic make-up of existing populations is known. The workshop and this document present the most current information available regarding collection, preservation, and analysis techniques. Certainly, advanced technologies in all aspects of genetics will arise in the future; however, the information presented here is expected to initiate a communication network between and among management agencies and geneticists for cooperative and standardized work in the future. With shrinking budgets, limited staff, and often inadequate equipment, partnerships are essential. For more current information on standardization techniques, please contact the editor or local natural resource agency.

Evaluations

A workshop evaluation form was sent to all participants to determine if overall and individual objectives were met at the meeting. Also, the evaluation solicited suggestions to improve and expand future lake sturgeon workshops and reveal any unfulfilled expectations of participants. Of the 43 people who attended the workshop, 16 evaluations were received. Persons indicated that the two most important objectives were: the desire to enhance their knowledge of genetics issues and research results (n=12), and establish collaboration, partnerships, and interaction with other lake sturgeon researchers (n=10). All 16 persons indicated their objectives were achieved. Specific aspects of the workshop contributing to the achievement of objectives were: the presentations of research results (n=7), and the open discussion session (n=6). Other positive aspects commonly noted included, the diverse agency representation and overall potential for future cooperation and standardization.

Suggestions to improve the workshop included: use a facilitator to keep on schedule, increase Canadian and Tribal representation, increase the scope to include the entire range of lake sturgeon, focus on the immediate application of current information, and include a poster session to create more interaction. These suggested improvements were helpful; however, some were not original objectives of this workshop and others were controlled by the participants and the direction and tempo of the discussions.

Future workshop topics that were suggested (in majority order) included, rehabilitation planning and stocking, continued genetics standardization, and other collaborative techniques. Additional topics noted were status and assessment techniques, standardized database (status), hatchery techniques and research, and contaminants.

Progress

Since the workshop, additional questions have been raised and addressed, and progress has been made on several action items that were discussed in the Working Session. The following paragraphs describe recent activities conducted as a result of the workshop.

- ***Sample collection***

  A list was developed identifying candidate rivers potentially hosting a spawning population of lake sturgeon (Appendix C). These rivers reflect high priority areas from which genetic samples are needed. If anyone has the ability to survey these rivers for lake sturgeon and collect genetic samples, please utilize the information in this document and contact the editor or local natural resource agency.

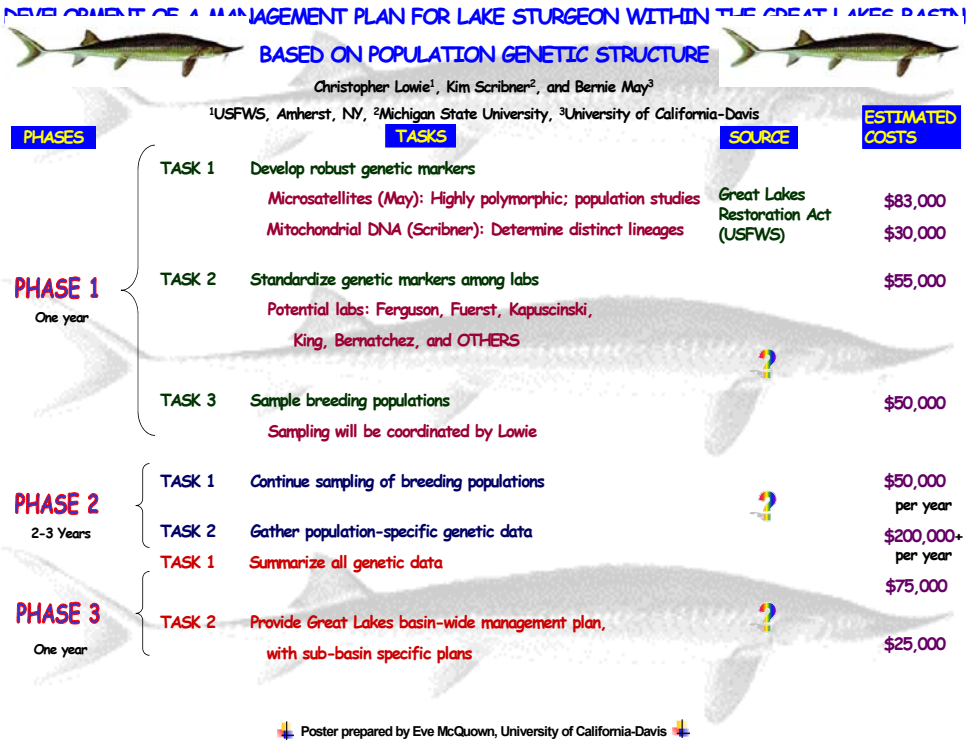
  Biologists questioned what to do with samples while markers are being developed and standardized or until a larger study can take place. I recommended holding all samples until a basin-wide study occurs. If immediate analysis is required, identify the fate of your samples and data results in a written agreement with the geneticist.

- ***Centralized database***

  The USFWS Lake Sturgeon Committee requested funds to compile a comprehensive database of lake sturgeon tributaries in the Great Lakes. Information on genetic samples will be included in this project.

- ***Funding***

  A proposal was developed by C. Lowie, K. Scribner, and B. May, and submitted to several funding agencies. The project proposal addresses needs under a large, comprehensive and cohesive framework to develop a management plan for lake sturgeon in the Great Lakes based on population genetics. The proposal outlines a series of tasks to be completed in three phases over a five-year period. To date, funding has been secured for a portion of Phase I, which includes developing a set of functional microsatellite and mitochondrial DNA (mtDNA) markers. These markers will provide the necessary information for determining the extent of genetic separation/mixing within Great Lakes populations and standardizing the markers for future research. Additional funds are needed for sample collection, stock structure analysis, and preparing a basin-wide management plan. The comprehensive project proposal represents a collaborative effort among and between natural resource agencies and geneticists.



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# APPENDIX A
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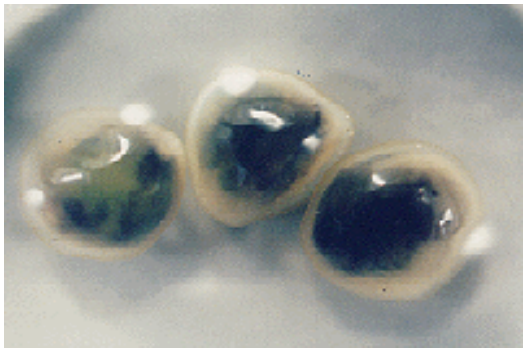
# APPENDIX B

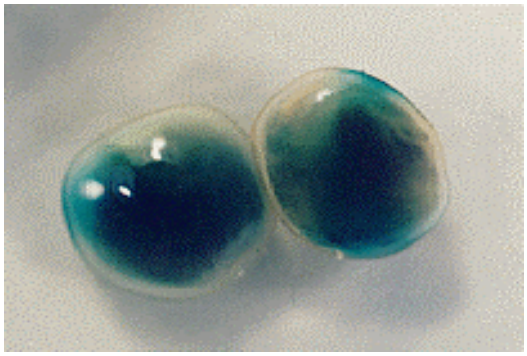
# SLIDE PRESENTATIONS

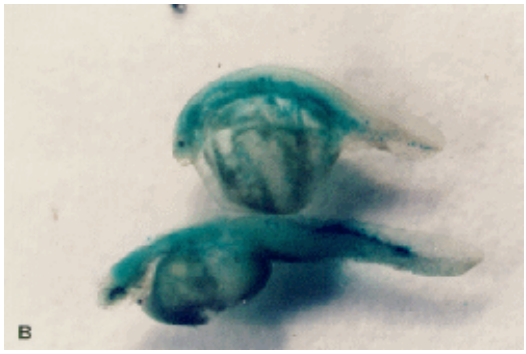
**Contact Chris Lowie at Chris_Lowie@fws.gov**

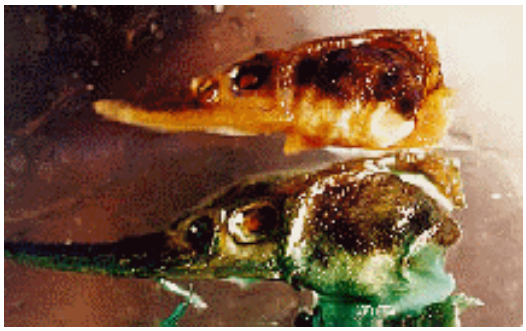
Sarwar Jahangir
Partial slides taken from associated web site:
http://nysedexperimental.brooklyn.cuny.edu/libc/WEBSHARE/WWWROOT/index.html

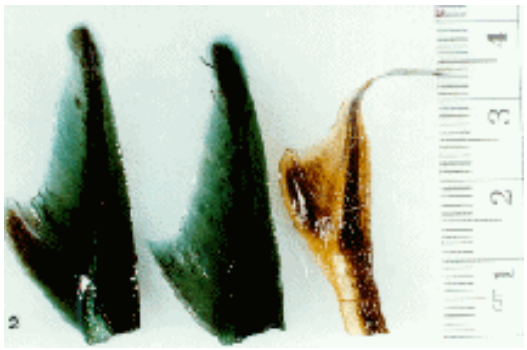
Below are visual examples of results of the experiment. You can see that the B-Galactosidase uptake and expression is present in all stages of development. The presence of the B-Galactosidase is evident by the blue color of the fish. Those that do not appear blue are the controls. This type of transgenic procedure, is a formidable weapon against the poaching of endangered fish species.









# APPENDIX C

## LIST OF CANDIDATE RIVERS FOR LAKE STURGEON FROM WHICH GENETIC SAMPLES ARE NEEDED FOR GENETIC ANALYSIS

***Lake Ontario – St. Lawrence River***

| | | |
|---|---|---|
| Black River | Upper St. Lawrence River | Raquette River |
| St. Regis River | Oswegatchie River | Oak Orchard Creek |
| Salmon River (Canada) | Trent River | Credit River |
| Lower Niagara River | | |

***Lake Erie – Lake St. Claire***

| | |
|---|---|
| Upper Niagara River | Maumee River |
| Grande River (Canada) | Thames River |

***Lake Huron***

| | | |
|---|---|---|
| Carp River | Au Sable River | Rifle River |
| Tittabawasee River | | |

***Lake Michigan***

| | | |
|---|---|---|
| Lower Menominee River | Peshtigo River | Oconto River |
| Lower Fox River | Indian Lake/Manistee River | |
| Muskegon River | | |

***Lake Superior***

| | | |
|---|---|---|
| Kaministiquia River | Michipicoten River | Big Pic River |
| Goulais River | Gravel River | Black Sturgeon |
| Nipigon River | Batchawana River | Tahquamenon River |
| Ontonogan River | Pigeon River | |