

**Genetic Guidelines for the Stocking of Lake Sturgeon (*Acipenser fulvescens*)  
in the Great Lakes Basin**



**Miscellaneous Publication 2010-01**

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**April 2010**

# Genetic Guidelines for the Stocking of Lake Sturgeon (*Acipenser fulvescens*) in the Great Lakes Basin

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Citation: Welsh, A.B., Elliott, R.F., Scribner, K.T., Quinlan, H.R., Baker, E.A., Eggold, B.T., Holtgren, J.M., Krueger, C.C., May, B. Genetic guidelines for the stocking of lake sturgeon (*Acipenser fulvescens*) in the Great Lakes basin. Great Lakes Fish. Comm. Misc. Publ. 2010-01.

Great Lakes Fishery Commission  
2100 Commonwealth Blvd., Suite 100  
Ann Arbor, MI 48105-1563

**April 2010**

ISSN: 1090-106x (print)  
1553-8087 (online)

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Printed on recycled paper.  
Miscellaneous Publication 2010-01

## Table of Contents

<b>Abstract</b> .....	<b>1</b>
<b>Introduction</b> .....	<b>1</b>
<b>Background</b> .....	<b>2</b>
Genetic Risks of Stocking .....	4
<i>Outbreeding Depression</i> .....	4
<i>Loss of Within-Population Genetic Variation</i> .....	6
<i>Artificial Selection</i> .....	8
Conservation Stocking Principles .....	9
Recommendations .....	10
<b>Genetic Stocking Guidelines</b> .....	<b>10</b>
<b>Step 1: Identification of Genetic Stocking Units</b> .....	<b>12</b>
Purpose .....	12
Methods.....	12
Results .....	17
Application.....	21
<b>Step 2: Identification of Priority Conservation populations</b> .....	<b>24</b>
Purpose .....	24
Background and Rationale .....	25
<b>Step 3: A Stocking Decision Tree</b> .....	<b>27</b>
Purpose .....	27
Stocking Decision Tree .....	27
<i>Decision 1</i> .....	27
<i>Decision 2</i> .....	28
<i>Decision 3</i> .....	28
<i>Decision 4</i> .....	29
<i>Decision 5</i> .....	30
<i>Decision 6</i> .....	30
<b>Step 4. Stocking Program Design and Implementation</b> .....	<b>31</b>
Purpose .....	31
Section 1. Collection Targets and Mating Techniques.....	32
<i>Gamete Collection</i> .....	32
<i>Fertilized Egg or Larvae Collection</i> .....	34
<i>Individual Transfers</i> .....	34
<i>Rationale for Collection Targets and Mating Techniques</i> .....	35
Section 2. Rearing Techniques .....	37
<i>Rationale</i> .....	37
Section 3. Stocking Numbers .....	39
<i>Rationale</i> .....	41
Section 4. Release Techniques .....	42
<i>Rationale</i> .....	43
Section 5. Evaluation.....	44
<i>Rationale</i> .....	44
<b>Acknowledgements</b> .....	<b>45</b>
<b>References</b> .....	<b>46</b>



# **Genetic Guidelines for the Stocking of Lake Sturgeon (*Acipenser fulvescens*) in the Great Lakes Basin**

## **ABSTRACT**

Many lake sturgeon populations (*Acipenser fulvescens*) in the Great Lakes basin are far below historic population sizes, and several fishery management agencies are interested in promoting species recovery. Due to low adult abundance and poor levels of natural recruitment in many populations, stocking has been advocated as an important management tool. With increased interest in lake sturgeon stocking, genetic data and theories can provide valuable information to guide stocking decisions. Genetic data were analyzed to examine genetic relationships between different spawning locations. Six genetic stocking units were defined across the Great Lakes basin, and criteria for the identification of priority conservation populations were established. Input was received from managers and biologists from throughout the basin regarding management and stocking issues concerning lake sturgeon. A decision tree was created, based on genetic principles and foreseeable management scenarios, to assist managers in selecting appropriate donor populations for stocking sites. Recommendations for the design and implementation of a stocking program were included in the guidelines along with a general overview of literature and principles on which the guidelines were developed. The development of these guidelines can provide a model for the incorporation of genetic data into management decisions targeting species conservation.

## **INTRODUCTION**

These guidelines use existing genetic data and population genetics theory to assist decision making regarding the stocking of lake sturgeon (*Acipenser fulvescens*). The guidelines apply to areas within the Great Lakes basin as well as to inland lakes connected to the Great Lakes (including those lakes separated from the Great Lakes basin by dams). They are intended to guide the development of regional stocking strategies and to be incorporated into lake sturgeon management plans. The guidelines are not prescriptive for every management situation because all possible circumstances cannot be anticipated. Principles are described and recommended rules for their application are offered in the form of a decision tree that addresses different management situations.

Comprehensive lake sturgeon management plans should be developed that address issues such as evaluation of impediments to population recovery and definition of management goals (Krueger and Decker 1999). Stocking should be incorporated into lake sturgeon management plans when it addresses and helps to solve impediments. These guidelines address genetic concerns that arise when stocking is used as a management action. For stocking to be a priority management tool, it is imperative that non-genetic factors be considered as well. A thorough evaluation of habitat, existing demographic data, and other relevant data should be completed prior to the integration of these genetic guidelines into the stocking portion of a lake sturgeon management plan.

Portions of this document were developed from information presented and discussed by a workgroup of the Lake Michigan Lake Sturgeon Task Group. Participants in this workgroup, in addition to the authors, are listed in the Acknowledgements of this document and include representatives from state, federal, and tribal natural-resource agencies having expertise with lake sturgeon biology and culture and experts from various universities with experience in sturgeon genetics.

The guidelines do not represent a policy statement from federal, provincial, state, or tribal natural-resource agencies nor from the Great Lakes Fishery Commission. Instead, they represent scientific advice on the part of the authors for the management of lake sturgeon in the Great Lakes.

## **BACKGROUND**

Many lake sturgeon populations throughout the Great Lakes basin are remnants of their historical numbers. Overfishing, habitat deterioration (including the construction and operation of dams), and poor water quality contributed to lake sturgeon extirpation in many Great Lakes locations and drastically reduced the size of the remaining populations (Smith 1972). Lake sturgeon may also have suffered some level of mortality from sea lamprey (*Petromyzon marinus*) predation (Patrick 2007). Although conditions for lake sturgeon have improved in many ways, recovery has been slow or absent, and few efforts have been directed specifically towards sturgeon recovery. Lake sturgeon do not reach sexual maturity until 14-33 years of age (Harkness and Dymond 1961), and evidence exists for spawning-site fidelity (Auer 1999; DeHaan et al. 2006). Therefore, because lake sturgeon take a long time to mature, recovery in population size and natural recolonization of vacant spawning sites by lake sturgeon often may not meet the recovery time frame desired by the public, fisheries stakeholders, and management agencies.



Fish-management agencies use stocking (placement of artificially propagated fish or naturally developing eggs or the translocation of post-larval fish into water bodies) to accomplish a variety of purposes. Stocked fish or fertilized eggs can be used to supplement and rehabilitate existing marginal populations, develop new populations, or reintroduce fish to a location where they have been extirpated. Many naturally reproducing populations of vertebrates have been re-established within their natural ranges by the use of stocking as a management action, often by adult translocation (e.g., greater prairie chicken (Westemeier et al. 1998; Bouzat et al. 2009). Successful reintroductions of fish, however, seem to be less common than for other groups of animals or, at least, less reported. Examples of successful reintroductions of fish include flannelmouth sucker (*Catostomus latipinnis*) (Mueller and Wydoski 2004), Gila topminnow (*Poeciliopsis occidentalis occidentalis*) (Simons et al. 1989, greenback cutthroat trout (*Oncorhynchus clarki*) (Harig et al. 2000), brown trout (*Salmo trutta*) (Caudron et al. 2006), and lake trout (*Salvelinus namaycush*) (Hansen et al. 1995). Translocation stocking was used for the successful flannelmouth sucker and greenback cutthroat trout reintroductions. Stocking of artificially propagated (hatchery-origin) fish were used for Gila topminnow, brown trout, and lake trout reintroductions.

Stocking can potentially speed the recovery of lake sturgeon populations by reducing the dependency on the slow process of natural recolonization. For example, in the state of New York, lake sturgeon are listed as a threatened species. The New York State Department of Environmental Conservation requires the establishment of self-sustaining lake sturgeon populations in at least eight separate locations within their historic range for delisting (Carlson et al. 2002). To accomplish this objective, New York is actively pursuing a lake sturgeon stocking program to reintroduce those extirpated. The states of Michigan and Wisconsin have reintroduced lake sturgeon to waters where they have been extirpated through stocking of eggs, larvae, fingerlings, and occasionally adults (translocation). Hatchery-reared fish also have been used by state natural-resource agencies to supplement naturally reproducing lake sturgeon populations in Lake Winnebago (Wisconsin) and Black Lake (Michigan). Rearing facilities also have been employed to improve survival by bringing larval fish collected from the wild into a culture facility for several months before releasing them back into the wild. The Little River Band of Ottawa Indians has employed this technique on the Big Manistee River using a facility located streamside (Holtgren et al. 2007), and the Michigan Department of Natural Resources and Environment and Michigan State University have employed this technique on the Black River using a streamside facility and a traditional off-site hatchery (Crossman 2008).

The goal of management can also include providing for the customs of Native Americans, who may desire lake sturgeon for cultural or ceremonial purposes in addition to wanting them restored to native waters. This interest stimulated the reintroduction of lake sturgeon to the upper St. Louis River (Minnesota) by the Fond du Lac Band of Lake Superior Chippewa and the upper Wolf River (Wisconsin) by the Menominee Tribe of Wisconsin (Runstrom et al. 2002). Streamside rearing on the Big Manistee River (Michigan) by the Little River Band of Ottawa Indians (Holtgren et al. 2007) has been implemented to protect wild-captured sturgeon larvae for release as juveniles. Although stocking and artificial rearing can be an important part of sturgeon management, caution must always be exercised to avoid potential negative genetic consequences on both reintroduced and persisting lake sturgeon populations.

### **Genetic Risks of Stocking**

The genetic risks from stocking include outbreeding depression, an inadequate representation of genetic diversity in the captive population or the stocked progeny, and/or artificial selection. The genetic consequences of stocking may not be observed readily because of the lake sturgeon's life history and its relative inaccessibility during non-spawning periods. Therefore, a risk-adverse management approach should be adapted. This section provides brief information on the theories behind these genetic risks because these theories have been detailed thoroughly elsewhere (e.g., Miller and Kapuscinski 2003; Reisenbichler et al. 2003; Araki et al. 2007).

#### **Outbreeding Depression**

Outbreeding depression is reduced fitness, measured as reproductive success, resulting from the interbreeding between genetically distinct populations or species (Hallerman 2003; Edmands 2007). Reduction in fitness is due to a loss of genetic adaptations or a disruption of co-adapted gene complexes (several genes working together, resulting in the expression of a certain trait (Dobzhansky 1941)) that were present in the original population (Lynch 1991). For example, outbreeding can result in the loss of genetic adaptations for migrations (Altukhov and Salmenkova 1987). The loss of adaptation may occur in either the first or second generation of offspring after the interbreeding between distinct populations, but the reduction in fitness will not be observed until the adaptation that was lost is needed for survival or reproduction. Reductions in fitness take many forms, and there appear to be no reliable indicators for the effect that outbreeding will have on fitness (McClelland and Naish 2007).

Alternatively, outbreeding can result in hybrid vigor or increased fitness in hybrids, likely due to the masking of deleterious alleles (Remington and O'Malley 2000). In populations that are highly inbred and experiencing detrimental fitness consequences, very low levels of immigration can improve the fitness of the population (Tallmon et al. 2004). Straying of even a few stocked individuals, over time, could exceed the low levels of immigration required to improve fitness without diluting locally adaptive alleles.

Lake sturgeon populations in the Great Lakes basin harbor substantial genetic diversity (DeHaan et al. 2006; Welsh et al. 2008), and mixing lake sturgeon populations genetically could disrupt their co-adapted gene complexes. Data show that the majority of lake sturgeon populations are genetically distinct from each other (McQuown et al. 2003; DeHaan et al. 2006; Welsh et al. 2008). If reintroduced lake sturgeon stray to nearby natural populations for spawning, outbreeding depression may occur and disrupt genetically based adaptive differences. In salmonids, straying appears to be more frequent among hatchery-produced fish than wild fish (Quinn 1993; Schroeder et al. 2001). In the absence of imprinting, stocked sturgeon may also be more likely to stray. Therefore, outbreeding depression should be a major concern when implementing a stocking strategy.

Lake sturgeon, because of their long generation time and life spans, have not been studied for evidence of outbreeding depression. However, several examples exist in the literature that have demonstrated the occurrence of outbreeding depression in populations of other fish species. Offspring from wild-bred populations of rainbow trout (*Oncorhynchus mykiss*) had higher survival in Lake Superior tributary streams in Minnesota than offspring resulting from crosses between wild-bred and hatchery strains (Miller et al. 2004). Hatchery-hybrid offspring had a reduced chance of surviving the first winter. In this case, the effects of outbreeding may have been amplified when the offspring were faced with harsh environmental challenges. Similarly, reduced fitness, measured by changes in embryo development time and survival, was observed in progeny from crosses of three geographically separate populations of southeast Alaska coho salmon (*Oncorhynchus kisutch*) relative to control lines (Granath et al. 2004). In pink salmon (*Oncorhynchus gorbuscha*), decreased survival in the F<sub>2</sub> generation of crosses between genetically distinct populations provided evidence for outbreeding depression due to the breakdown of co-adapted gene complexes (Gilk et al. 2004). Outbreeding between largemouth bass (*Micropterus salmoides*) populations with genetic differentiation (i.e.,  $F_{ST}$ ) values of 0.05 (comparable to values observed in lake sturgeon studies in the Great Lakes) resulted in increased infectious disease susceptibility (Goldberg et al. 2005). Therefore, intentional mixing of genetically distinct populations should be used only in limited circumstances when populations are suffering from inbreeding depression (Edmands 2007).

## **Loss of Within-Population Genetic Variation**

Within-population genetic variation may be reduced when a representative sample is not obtained from the donor population during collection of the brood stock and/or propagation of gametes collected from adults. Whether gametes, larvae, or adults are collected, only a small portion of the population is represented, and, therefore, a representative sample of the genetic diversity may not be present. The allele frequencies in wild and captive populations can be different, and some low-frequency alleles may be absent in captive populations (Allendorf and Ryman 1987). When collecting individuals from donor populations, an adequate number of individuals should be sampled to ensure that genetic diversity is fully represented. Collecting individuals across the spatial and temporal variation shown by a spawning population should be considered when making collections. Crossman (2008) provided evidence for genetic differences between adults spawning early and late in the season as well as adaptations of early-larval life-history traits to environmental conditions at the time of spawning.

Inbreeding, or the mating of close relatives, is expected to decrease a population's viability (Mills and Smouse 1994) due to inbreeding depression, i.e., reduced fitness measured by survival and reproductive success. Inbreeding depression can be caused by recessive deleterious genes that are expressed because of increased homozygosity, or by a decrease in heterozygotes, where heterozygotes have a fitness advantage (Charlesworth and Charlesworth 1987). The resultant decrease in heterozygosity may not be observed for several generations, and numerically depressed populations may have large numbers of related individuals (i.e., high coancestry) without an apparent decrease in heterozygosity. Currently, no evidence exists for inbreeding depression in remnant lake sturgeon populations. DeHaan et al. (2006) did not observe a correlation between genetic diversity and population size. Species with long generation times may be buffered from the effects of inbreeding (Lippe et al. 2006). Polyploidy may also protect lake sturgeon from inbreeding depression. Multiple chromosomal copies can delay homozygosity (Allendorf and Waples 1996). However, if evidence of inbreeding depression is found in the future, then management efforts may focus on enhancing the genetic diversity of inbred populations. Modeling efforts may be able to provide insight into the risk of inbreeding depression for remnant lake sturgeon populations (Schueller and Hayes, unpublished data).

Inbreeding may increase under artificial production through the mating of related adults and/or the release of large numbers of related offspring, possibly leading to inbreeding depression. Ryman (1970) observed decreased survival in inbred families of hatchery-released Atlantic salmon (*Salmo salar*) compared to non-inbred families also released from the hatchery. Reduced survival and growth was observed also in hatchery-reared inbred Pacific salmon (*Oncorhynchus* spp.) (Kincaid 1983). Additionally, if adults from populations characterized by high coancestry are used as a donor stock for stocking, the resulting progeny will be inbred and likely will be less fit than progeny from matings between unrelated adults.

The effective population size,  $N_e$ , is relevant to managers engaged in lake sturgeon rehabilitation because  $N_e$  determines the rate of inbreeding accrual, loss of genetic diversity, or change in allele frequencies.  $N_e$  is the size of a hypothetical ideal population that would experience the same amount of genetic change as the population under consideration (Wright 1931, 1938) and is often smaller than census population numbers ( $N$ ) due to unequal sex ratios, variance in family sizes, and changes in population size over generations (Kimura and Crow 1963). In hatchery settings, the large number of eggs from female sturgeon and the difficulty in handling adult fish can encourage the use of small numbers of parents. However, these practices can decrease  $N_e$ . Crossman (2008) reared eggs from different females separately in hatcheries and documented large variance among females in egg and larval survival, which dramatically reduced  $N_e$ .

A trade-off exists, known as the Ryman-Laikre effect (Ryman and Laikre 1991), in which a gain in the total production of offspring through propagation is accompanied by a reduction in  $N_e$  and a loss of genetic diversity in the population as a whole. Supplementation with offspring from comparatively few adults, but with higher survival relative to wild progeny, will increase variance in reproductive success in the entire population (including both wild and hatchery-produced fish), resulting in a decrease in  $N_e$ . The effect is magnified as the proportion of hatchery progeny increases. This trade-off can be avoided when the ratio of  $N_e$  to the census population size ( $N_e:N$ ) of the captive population is greater than the corresponding ratio in the wild population (Waples and Do 1994). Natural populations often have an  $N_e:N$  ratio less than one. Therefore, the corresponding ratio in the captive population should be close to one to avoid the Ryman-Laikre effect. If a wild population is chosen to receive stocked fish (supplementation), then it is likely that the natural population in the area has a small population size. The hatchery contribution could swamp the genetic contribution made by the natural population, resulting in a decreased effective population size. This result was realized in simulations of proposed stocking of Gulf sturgeon (*Acipenser oxyrinchus desotoi*) into the Suwanee River, Florida (Tringali and Bert 1998).

## **Artificial Selection**

Hatchery rearing conditions can create a different selection regime for sturgeon relative to environmental conditions experienced by individuals in natural populations. This shift in selection pressure can occur during the collection of source individuals, the rearing of offspring, and/or the release of offspring (Busack and Currens 1995; Campton 1995). Changes in selection pressure can result in a different genetic makeup for the hatchery fish in comparison to wild fish and may favor genotypes that are not well suited to life under natural conditions or to a changing environment (Araki et al. 2007; Hutchings and Fraser 2008). The decrement in fitness of hatchery progeny is a function of the time their genes experience hatchery selection (Lynch and O'Hely 2001; Ford 2002). These principles have been used to establish regional policies governing the use of hatchery-reared fish (e.g., Pacific salmon (Moberg et al. 2005)).

When collecting source individuals, separate collections should be made both temporally and spatially at the spawning site. Collecting at a single time during the spawning run may inadvertently select for early or late spawning times (Crossman 2008). Collecting at a single location within the spawning site may inadvertently select for certain habitat preferences that, if genetically based, could result in the lack of representation of potential ecotypes in the resulting offspring.

During rearing, selection under hatchery conditions can result in the survival of sturgeon adapted to the hatchery environment and in the loss of traits required for long-term survival and reproductive success in the wild. For example, sturgeon stocking within the Azov Sea basin (Russia) has been occurring since the late 1950s. Within this basin, the Kuban River has experienced a loss of ecotypes, demonstrated by a shortened spawning run and breeding season (Chebanov et al. 2002). The loss of diversity has been attributed to the rearing conditions and the selective breeding of productive females used within the stocking program, resulting in artificial selection that diminishes the diversity of the sturgeon being released.

Timing of the release of hatchery offspring also can result in artificial selection through lack of exposure to the selective pressures faced by certain life stages in the wild. If the heaviest natural selective pressure and resulting mortality are experienced when sturgeon are young and the sturgeon are released into the wild at a later life stage, the released sturgeon are protected from the beneficial effects of natural selection. This effect is the conundrum of hatchery propagation because hatcheries purposefully protect fish past critical life stages that come before they are stocked. Hatchery winnowing can result in a much higher effective population size for the hatchery populations compared to natural populations, leading to the Ryman-Laikre effect described in an earlier section (Loss of Within-Population Genetic Variation).

## **Conservation Stocking Principles**

For the reasons stated above, stocking may result in negative consequences to remnant lake sturgeon populations and to the populations being supplemented or reintroduced through stocking. Therefore, stocking plans should be discussed and coordinated with other agencies that manage lake sturgeon populations within the area where released fish are expected to occur. Stocking should be implemented only when other actions will not accomplish management goals or objectives within a reasonable time. When stocking is deemed necessary, the following principles should be adopted to minimize negative genetic consequences. These principles are discussed in more detail later.

- The genetic structure and diversity of existing healthy natural populations should be preserved.
- Selection of appropriate donor stocks for reintroduction should be based on their likely genetic similarity to neighboring populations.
- The effective population size of lake sturgeon populations, both wild and hatchery produced, should be maximized. Practices such as maximizing the number of parents used for each generation and equalizing family contributions help maximize the effective population size.
- Mating practices should preserve the genetic diversity of the donor population so as to reduce the potential for inbreeding.
- Rearing techniques for propagated fish should promote homing so as to minimize the likelihood of straying by hatchery fish.
- Sturgeon that are released into the wild should represent the natural genetic diversity of the donor population.

## **Recommendations**

The following recommendations implement the above principles and provide a foundation for the subsequent guidelines:

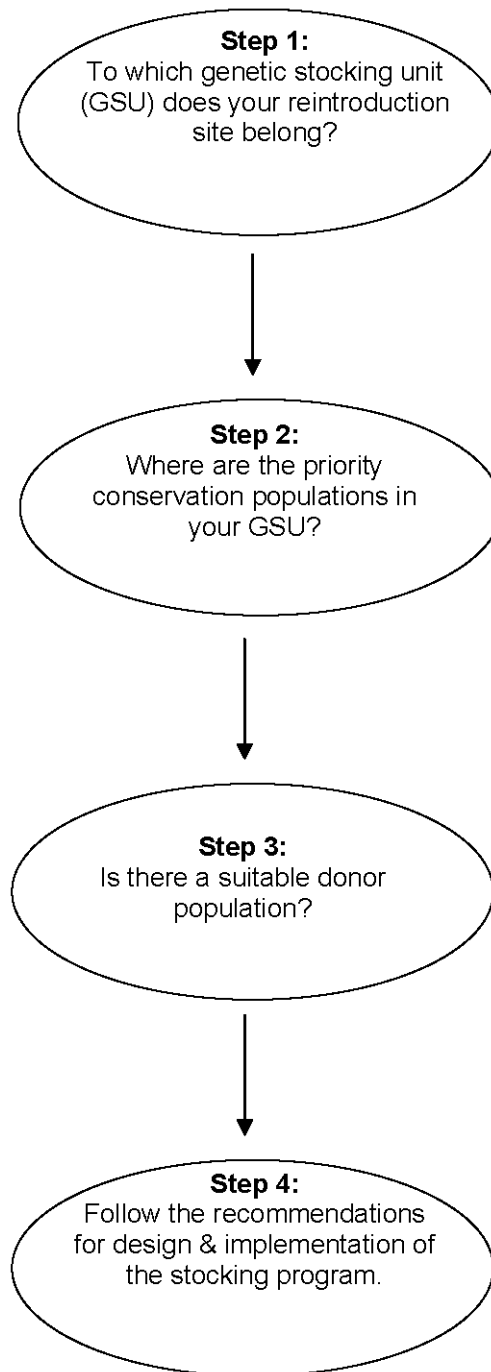
- Assess current population status and trends at the target river as well as suitability of existing habitat for maintaining a self-sustaining population.
- Evaluate potential for existing populations to colonize the target river.
- Use appropriate donor stock.
- Maximize the number of parents used each year.
- Equalize family contributions.
- Continue stocking over a sufficient period to generate a genetically diverse population with multiple age-classes.
- Monitor stocked population and neighboring populations (including the donor population).

## **GENETIC STOCKING GUIDELINES**

Genetic stocking guidelines, divided into four steps, were developed using the above conservation stocking principles and recommendations, and they are intended to help evaluate the appropriateness of stocking for different management situations and to guide decision making and implementation (Fig. 1). The guidelines recommend methods for implementing a stocking program that reduces genetic risks to lake sturgeon populations. The first step in the guidelines identifies populations or groups of populations in the Great Lakes to consider as potential donors and those populations likely to be affected by stocking. The second step identifies populations that should be given a high priority for preservation. These populations have characteristics that are deemed important to preserve, and management actions that affect them should be conservative. The third step uses a decision tree that incorporates the information from the previous two steps so as to evaluate whether a suitable donor population exists. The fourth step recommends procedures for implementing a stocking program. The first three steps begin with a section describing purpose and rationale, followed by a section providing relevant background information, and ending with suggested management actions. The fourth step is divided into sections describing the aspects of a stocking program and methodological suggestions, including a detailed rationale.



Fig. 1. Progression of steps in the genetic stocking guidelines.



## **STEP 1: IDENTIFICATION OF GENETIC STOCKING UNITS**

*Action Item: Identify the genetic stocking unit(s) (GSU) or unassigned population geographically closest to the proposed stocking site.*

### **Purpose**

The purpose of this section is to help identify the gamete source population best suited for the site being considered for stocking. A genetic stocking unit is a population or group of populations that may be used as a donor source for stocking within that GSU. GSUs will also be used to assess which spawning populations are most likely to be affected by stocking. Conservation units have been identified based on both genetic and ecological data (e.g., Crandall et al. 2000; Fraser and Bernatchez 2001; Palsbøll et al. 2006). However, the GSUs described in these guidelines were defined solely by genetic data and are not intended for management purposes beyond decisions regarding stocking. This step will identify GSUs that contain populations that are likely to be genetically similar. The use of such a population as a donor at other sites within that GSU can reduce the likelihood of outbreeding depression.

### **Methods**

To define GSUs, data for 30 lake sturgeon populations, based on standardized microsatellite markers, were compiled from the University of California-Davis (Welsh et al. 2008) and Michigan State University (DeHaan et al. 2006) (Fig. 2, Table 1). These markers have been standardized among different laboratories to facilitate data synthesis. All samples were from spawning adults unless otherwise noted. Data were compiled from eight microsatellite loci used in common by the two laboratories. Pairwise  $F_{ST}$  values were calculated for each pair of spawning populations that had more than 20 samples to determine if the spawning populations were genetically distinct.  $F_{ST}$  values can range from 0 to 1, with increasing values corresponding to increasing genetic differentiation. Populations with less than 20 samples lacked statistical power to detect significant genetic differences. Genetic differences between spawning populations may be a result of reproductive isolation due to natal homing or genetic drift.

Fig. 2. Map of sample locations. Circles indicate sampling location. Numbers correspond to the locations in Table 1.

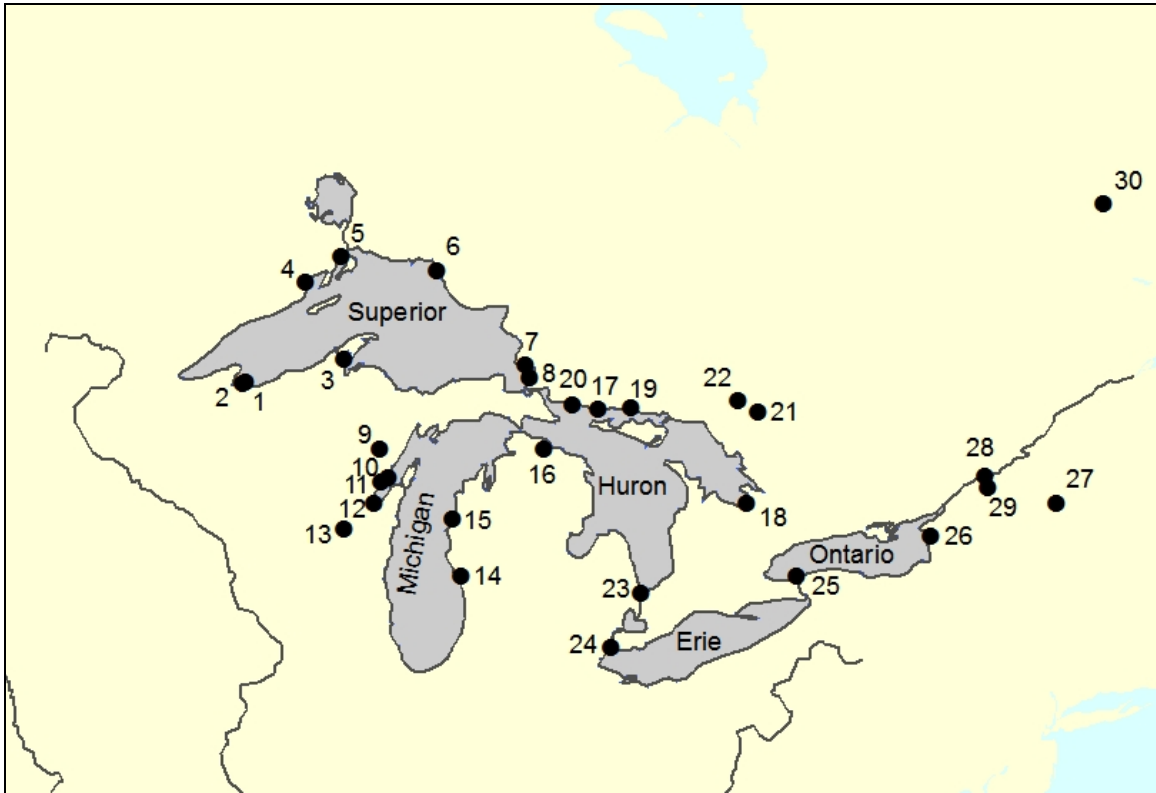


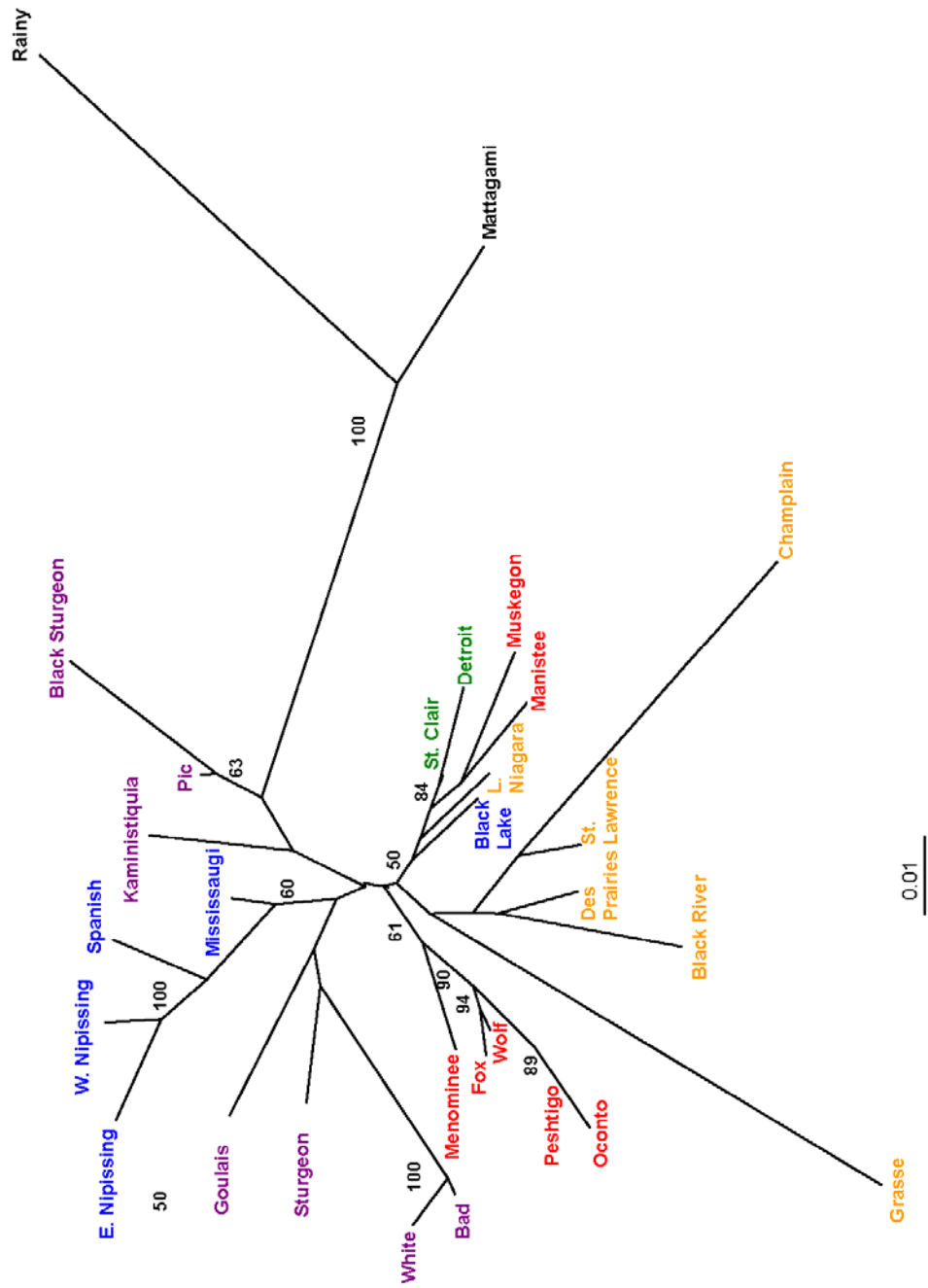
Table 1. Spawning locations included in the designation of GSUs. Location on Fig. 2 and sample sizes are noted. Samples from non-spawning individuals are denoted with an asterisk.

Identifying number	River	Sample size
Lake Superior:		
1	Bad	136
2	White	43
3	Sturgeon	78
4	Kaministiquia*	85
5	Black Sturgeon*	57
6	Pic	33
7	Batchawana	6
8	Goulais	23
Lake Michigan:		
9	Menominee	68
10	Peshtigo	54
11	Oconto	18
12	Fox	46
13	Wolf	111
14	Muskegon	17
15	Manistee	89
Lake Huron:		
16	Black Lake	114
17	Mississaugi	52
18	Nottawasaga	8
19	Spanish	47
20	Thessalon	3
21	East Lake Nipissing	35
22	West Lake Nipissing	40
Lake Erie:		
23	St. Clair	100
24	Detroit*	33
Lake Ontario:		
25	Lower Niagara*	50
26	Black	11
St. Lawrence:		
27	Lake Champlain	26
28	St. Lawrence	54
29	Grasse	28
30	Des Prairies	14

Genetic distances (Cavalli-Sforza and Edwards' chord distance (Cavalli-Sforza and Edwards 1967)) between populations with greater than ten samples were also calculated to generate trees describing the relationships among the different populations (Fig. 3). Two locations in the Hudson Bay drainage, Mattagami River ( $n = 40$ ) and Rainy River/Lake of the Woods ( $n = 27$ ), were included in the analysis to offer a perspective on out-of-basin differences.

A Bayesian approach for identifying population clusters was implemented using the software STRUCTURE (Pritchard et al. 2000). Without using prior information about sampling location, STRUCTURE tests the likelihood of various numbers of population clusters ( $K$ ), given the genetic data and based on Hardy-Weinberg equilibrium and linkage disequilibrium. Populations with fewer than ten samples were therefore included in the analysis. The natural-log likelihood values were plotted and consistent genetic discontinuities were identified among the most likely values of  $K$ . Clusters were determined by identifying those populations that consistently grouped apart for several likely values of  $K$ . Membership coefficients to the different clusters were calculated for each population.

Fig. 3. Neighbor-joining tree (based on Cavalli-Sforza and Edwards' chord distance) with sampled populations with 10+ samples, based on genetic distance calculated using eight microsatellite loci. Numbers correspond to bootstrap values > 50% (out of 10,000 replicates). Purple text is Lake Superior, red text is Lake Michigan, blue text is Lake Huron, green text is Lake Erie, orange text is Lake Ontario/St. Lawrence, and black text is Hudson Bay.



## Results

After correcting for multiple comparisons, the majority of populations were statistically distinguishable from each other based on genetic data ( $p < 0.01$ , Table 2). Comparisons that were not genetically distinct include the following pairs: Bad vs. White, Fox vs. Wolf, Black (tributary to Black Lake) vs. Detroit vs. lower Niagara, East Nipissing vs. West Nipissing, Detroit vs. St. Clair vs. lower Niagara, and lower Niagara vs. St. Lawrence.

Our GSU designations correspond to consistencies among the results of the neighbor-joining tree (Fig. 3), the STRUCTURE analysis (Table 3, Fig. 4), and the pairwise  $F_{ST}$  values (Table 2). In general, the genetic data indicated that populations within Lake Superior are highly differentiated, populations on the eastern shore of Lake Michigan are more closely related to Lake Huron populations than to populations from Green Bay (Lake Michigan), and populations in Lakes Huron, Erie, and Ontario are less genetically differentiated.

Table 2. Pairwise  $F_{ST}$  values between all spawning locations with more than 20 samples. Numbers correspond to locations in Fig. 2 and Table 1. Bold values shaded gray are not significant (after correction for multiple comparisons,  $p > 0.01$ ).



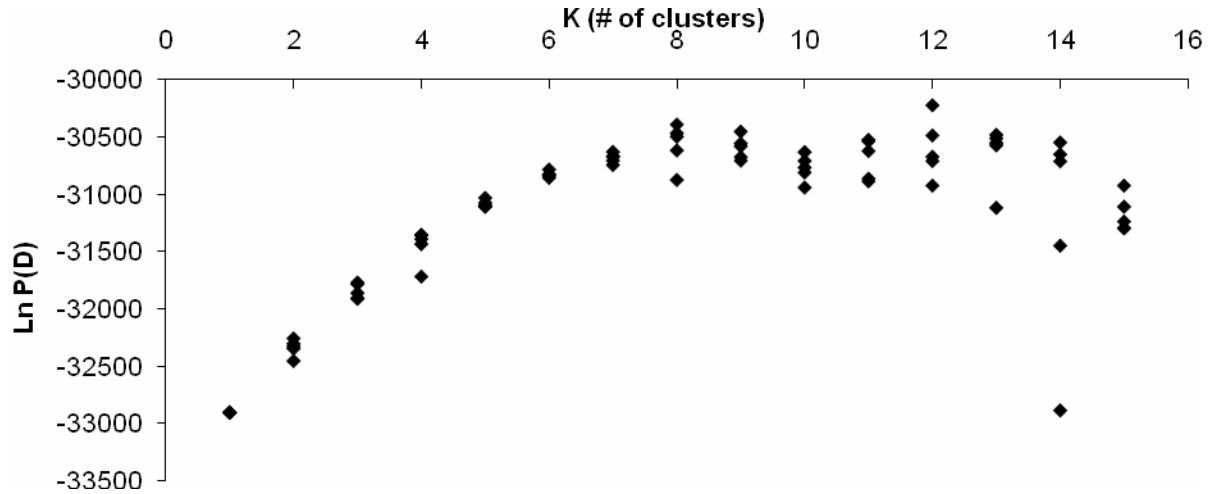


Table 3. Membership coefficients for each spawning location to the clusters identified by STRUCTURE ( $K = 7$ ), with highest cluster membership in bold (see Table 1 and Fig. 2 for site locations).

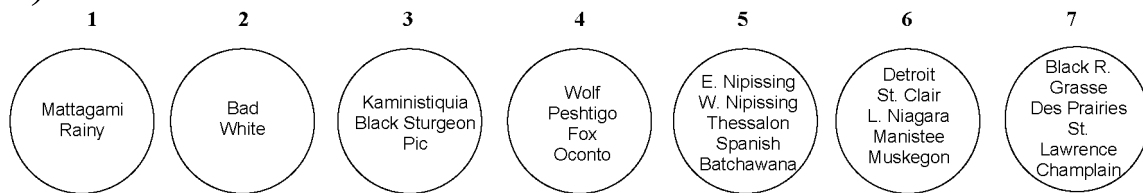
Spawning site	Cluster						
	1	2	3	4	5	6	7
Mattagami R.	0.02	<b>0.91</b>	0.01	0.01	0.02	0.02	0.02
Rainy R.	0.02	<b>0.92</b>	0.01	0.01	0.01	0.01	0.01
Bad R.	0.04	0.05	0.05	0.05	0.02	0.06	<b>0.72</b>
White R.	0.04	0.03	0.08	0.05	0.03	0.08	<b>0.69</b>
Sturgeon R.	0.18	0.12	0.11	0.09	0.07	0.16	<b>0.27</b>
Kaministiquia R.	0.11	0.10	0.11	0.09	0.07	<b>0.47</b>	0.06
Goulais R.	0.15	0.03	<b>0.26</b>	0.12	0.08	0.18	0.18
Batchawana R.	0.23	0.02	<b>0.54</b>	0.07	0.03	0.05	0.07
Black Sturgeon R.	0.05	0.13	0.06	0.03	0.08	<b>0.60</b>	0.06
Pic R.	0.14	0.07	0.10	0.07	0.09	<b>0.47</b>	0.06
Menominee R.	0.21	0.06	0.08	0.08	<b>0.25</b>	0.24	0.09
Wolf R.	0.13	0.04	0.08	0.09	<b>0.54</b>	0.10	0.02
Manistee R.	0.06	0.02	0.09	<b>0.50</b>	0.11	0.14	0.08
Muskegon R.	0.09	0.03	0.07	<b>0.41</b>	0.15	0.16	0.11
Peshtigo R.	0.07	0.03	0.14	0.11	<b>0.47</b>	0.15	0.04
Fox R.	0.09	0.04	0.04	0.15	<b>0.54</b>	0.10	0.03
Oconto R.	0.08	0.02	0.06	0.06	<b>0.58</b>	0.14	0.06
Black Lake	<b>0.24</b>	0.03	0.16	0.23	0.14	0.12	0.07
Mississaugi R.	0.11	0.04	<b>0.26</b>	0.18	0.15	0.21	0.07
E. Lake Nipissing	0.06	0.02	<b>0.73</b>	0.09	0.05	0.04	0.02
W. Lake Nipissing	0.06	0.04	<b>0.67</b>	0.07	0.05	0.08	0.03
Thessalon R.	0.03	0.02	<b>0.70</b>	0.02	0.02	0.03	0.19
Nottawasaga R.	0.14	0.03	0.20	<b>0.33</b>	0.13	0.10	0.09
Spanish R.	0.05	0.03	<b>0.55</b>	0.17	0.06	0.12	0.02
Detroit R.	0.16	0.04	0.19	<b>0.40</b>	0.09	0.08	0.04
St. Clair R.	0.18	0.03	0.13	<b>0.36</b>	0.11	0.08	0.11
L. Niagara R.	0.21	0.07	0.10	<b>0.31</b>	0.11	0.14	0.06
Black R.	<b>0.30</b>	0.22	0.06	0.21	0.11	0.09	0.02
Grasse R.	<b>0.64</b>	0.05	0.08	0.03	0.04	0.07	0.11
Des Prairies R.	<b>0.41</b>	0.08	0.10	0.12	0.09	0.11	0.10
St. Lawrence R.	<b>0.49</b>	0.07	0.09	0.13	0.12	0.07	0.03
Lake Champlain	<b>0.51</b>	0.07	0.05	0.05	0.26	0.03	0.03

Fig. 4. Results from STRUCTURE analyses: a) natural-log likelihood values for cluster numbers ( $K$ ) 1-15, with 5 replicates for each cluster number; b) diagram depicting cluster groupings. Populations with poor assignment to a cluster include: Sturgeon, Goulais, Menominee, Black Lake, Mississauga, and Nottawasaga.

a)



b)



## Application

Substantial literature exists on the use of genetic data to delineate evolutionarily significant units (ESUs) and management units (MUs). The ESU concept was used by Waples (1991, 1995) to identify salmon populations for conservation. His ESU definition was incorporated into the National Marine Fisheries Service's listing criteria, under the Endangered Species Act (ESA), for distinct population segments. Under this definition, an ESU is a population that "can be shown to be reproductively separate from other populations and have unique or different adaptations" (Waples 1991). Genetic data can be used to demonstrate reproductive isolation and the genetic uniqueness of populations (e.g., Hedrick et al. 2001). This definition of ESUs has been criticized as being too narrow and not fulfilling the purposes of the ESA (Pennock and Dimmick 1997). Moritz (1994) narrowed the ESU definition even further, stating that an ESU is a population that is "reciprocally monophyletic for mitochondrial DNA alleles and show[s] significant divergence of allele frequencies at nuclear loci." Moritz also introduced the definition of MUs as "populations with significant divergence of allele frequencies at nuclear or mitochondrial loci, regardless of the phylogenetic distinctiveness of the alleles" (Moritz 1994). Subsequently, other authors have proposed ESU and MU definitions that encompass criteria beyond genetic considerations. Crandall et al. (2000) raised important concerns about the focus on reciprocal monophyly, which can exclude adaptive genetic differences between populations. These authors also stressed the importance of the incorporation of ecological data in ESU designations. Fraser and Bernatchez (2001) proposed an ESU definition that acknowledges the defining criteria will often depend on the context. Palsbøll et al. (2006) proposed combining demographic and genetic parameters to generate biologically meaningful MUs.

In the context of lake sturgeon genetic management, several of these proposed definitions for ESUs and MUs do not meet the purposes of these guidelines. Prior to designating any unit for conservation, the goals of management should be defined and the units should facilitate achievement of that management goal (Taylor and Dizon 1999). One of the goals of these guidelines is to assist with the selection of appropriate donor populations that would reduce the risk of outbreeding depression from stocked fish that stray. Therefore, donor populations should be selected that are likely to be the least genetically differentiated from the natural remnant populations most likely to absorb stocked strays. Because the population at the reintroduction site may have been extirpated, it is impossible to accurately determine which donor population is most genetically similar.

Therefore, the GSU designations need to incorporate this uncertainty and make assumptions (using genetic and non-genetic data) about which population is likely to be the most appropriate donor. Fig. 5 depicts the current GSU designations based on existing data, and Fig. 6 shows the location of the GSUs.

Fig. 5. GSU designations based on a combination of pairwise  $F_{ST}$  values, neighbor-joining tree, and STRUCTURE results (colors relate to map in Fig. 6).

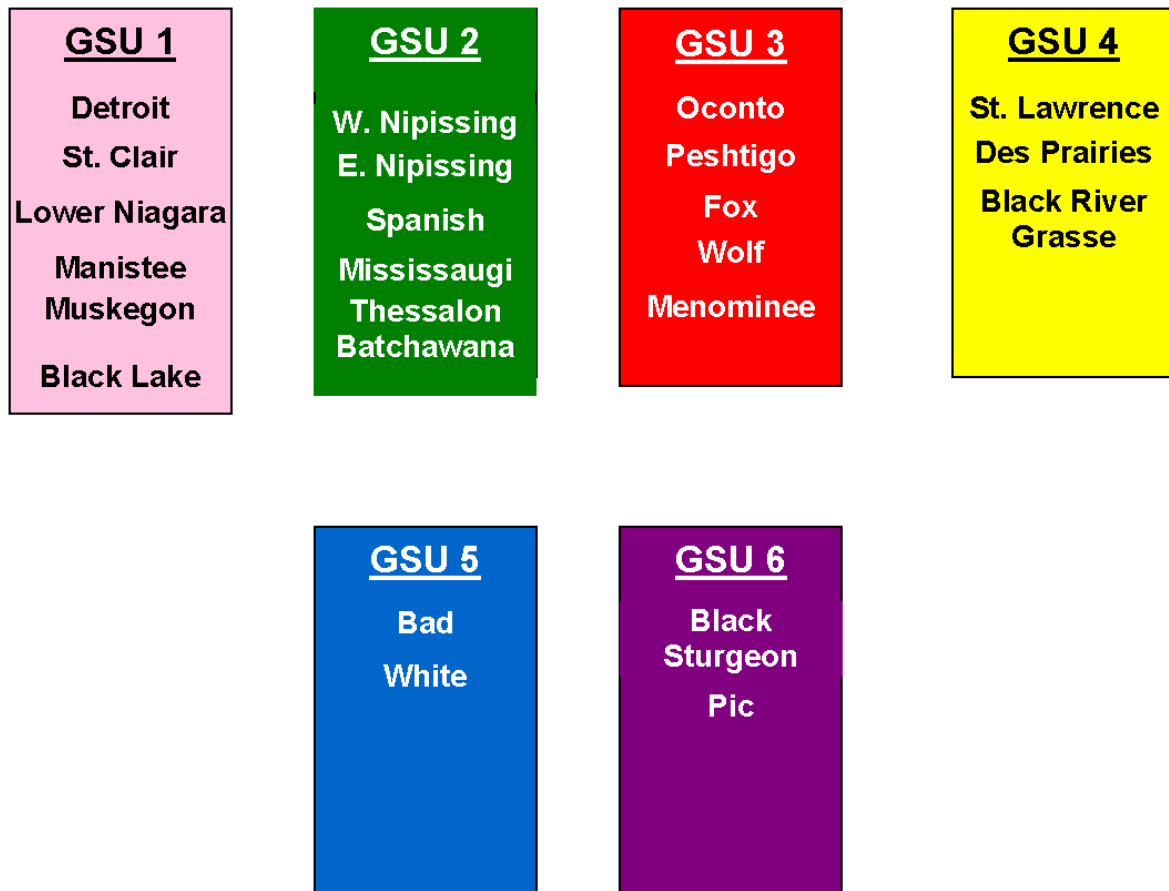
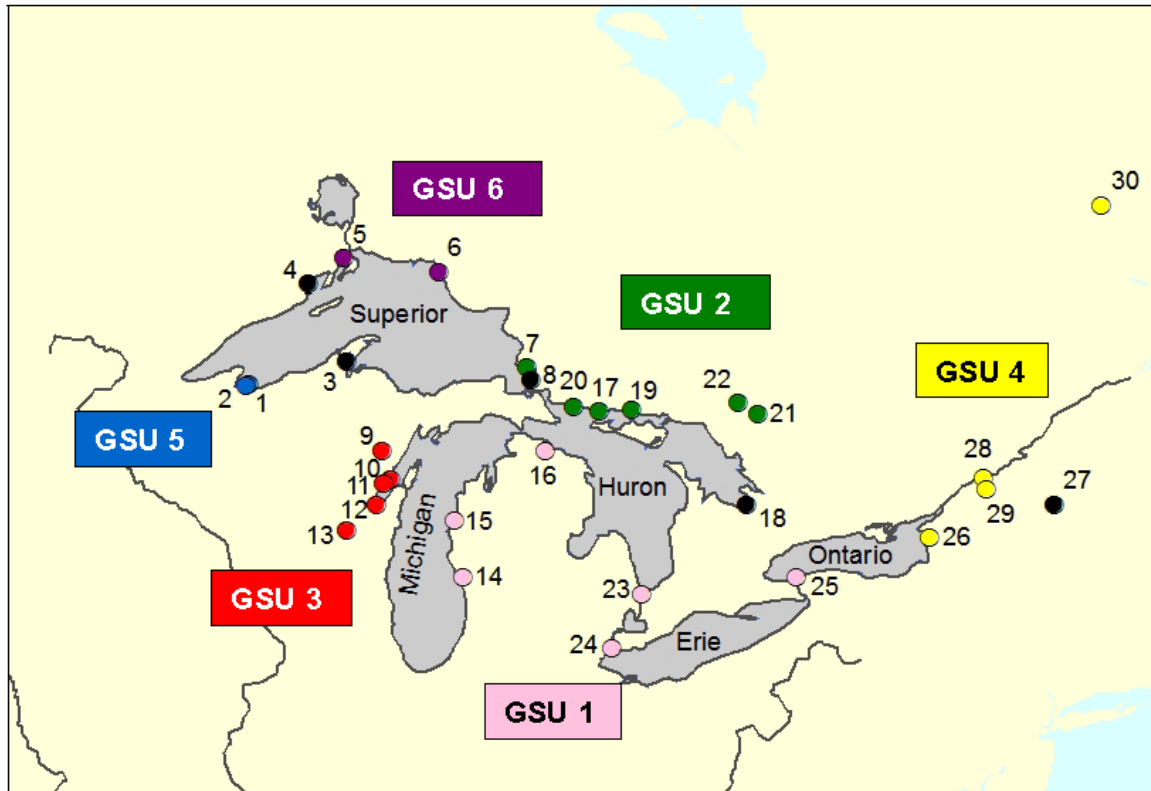


Fig. 6. Sampled Great Lakes spawning locations color-coded to its corresponding GSU (see Fig. 5). Numbers correspond to sample locations described in Fig. 2. Black dots correspond to populations that do not consistently group with other sampled populations.



In summary, based on the genetic data analyses, many distinct regions where natural populations remain (northern Lake Superior, southern Lake Superior, northern Lake Huron, Green Bay, and the St. Lawrence area) are strongly differentiated from each other and have been separated into six separate GSUs (Figs. 5, 6). The populations from locations distributed across the central region of the Great Lakes from the lower state of Michigan, Lake St. Clair, and the Niagara region appear less differentiated (despite their more dispersed distribution) and are grouped into a single GSU. Some populations (Sturgeon River, Kaministiquia River, Goulais River, Nottawasaga River, and Lake Champlain) could not be assigned to a GSU and are represented by black dots in Fig. 6.

Three guidelines for the identification of appropriate donor populations for stocking follow from these data:

- Because most populations are genetically distinct from each other, stocking programs should exercise caution to avoid outbreeding and the disruption of remnant population structure.
- Management actions within a lake basin (even within a GSU contained in a single lake basin) should not be considered in isolation from surrounding lake basins and surrounding GSUs.
- Uncertainty remains about GSU designations. Not all populations and stocking sites are grouped within a GSU. When GSU designations remain uncertain, populations geographically closest to the stocking site can be a potential donor population. GSU designations should be updated when additional genetic data become available.

## **STEP 2: IDENTIFICATION OF PRIORITY CONSERVATION POPULATIONS**

*Action Item: Identify any priority conservation populations that are located within the relevant genetic stocking units.*

### **Purpose**

The purpose of this section is to identify those populations that should be given high priority for conservation; such populations should reproduce at sites where human activities are expected to have a limited effect upon the population. A population can be a priority conservation population if it meets any of the three criteria described in this section. Each GSU should contain at least one priority conservation population to ensure that its genetic diversity will likely be represented in the future. Some spawning populations may be a high priority for conservation from a genetic perspective because they contribute substantially to the overall genetic variability of lake sturgeon in the Great Lakes and represent populations whose genetic makeup may be relatively unaltered by human activities. A priority conservation population can be identified based on variables such as high genetic divergence, unique life-history characteristics, or being a natural, self-sustaining population. At locations where such populations exist, extra caution in identifying donor populations for stocking must be exercised, and culture and stocking methods that promote the imprinting of cultured fish should be used.

## Background and Rationale

Different methods that use both genetic and ecological data for prioritizing populations have been developed for species other than sturgeon. For example, Allendorf et al. (1997) prioritized Pacific salmon populations by first determining the extinction probability for a population. If a population had a relatively high extinction probability, the population was then ranked with a scoring system according to the genetic and ecological consequences of extinction using criteria such as genetic divergence and the status of nearby populations. However, while the objectivity of a scoring system is laudable, the ability to identify appropriate scores is questionable (Wainwright and Waples 1998). Perkins et al. (1993) used levels of genetic differentiation for the identification of heritage brook trout (*Salvelinus fontinalis*) populations that should be conservation priorities. Petit et al. (1998) proposed a method of prioritization based solely on genetic markers. Those authors evaluated a population's contribution to the genetic variability of the species as a whole by examining among-population genetic divergence and within-population genetic diversity. Moritz (2002) stressed the importance of maintaining historically isolated lineages as well as varied landscapes with viable populations for preserving evolutionary potential.

The criteria used for prioritizing sturgeon populations in these guidelines include not only genetic variables, but also include biological variables that may account for adaptive differences among populations and that integrate the prioritization approaches described above. Sole reliance on the use of neutral genetic markers for prioritization may underestimate the degree of adaptive divergence between populations (McKay and Latta 2002). The criteria to be used for the identification of priority conservation populations are the amount of genetic differentiation, the presence of unusual life histories, and evidence of being close to a natural (unperturbed) state.

Differences in allele frequencies between healthy populations indicate that gene flow is limited and that a potential exists for unique genetic adaptations to accumulate over time. Various statistical analyses can be used to determine the degree of genetic divergence such as  $F_{ST}$ , genetic distance measures, and factorial correspondence analysis. Using eight microsatellite loci, the average  $F_{ST}$  across all spawning populations is 0.08. Populations having an average  $F_{ST}$  value greater than 0.08 can be considered as having relatively high levels of genetic differentiation. However, high levels of differentiation should not be a result of isolation by artificial barriers. Those populations that have relatively high levels of natural genetic differentiation from other populations should be identified as priority conservation populations. Examples of populations that meet this criterion would be the Bad River ( $F_{ST} = 0.09$ ), Kaministiquia River ( $F_{ST} = 0.09$ ), and Black Sturgeon River ( $F_{ST} = 0.09$ ), all of Lake Superior.

Life-history characteristics that relate to unusual migratory characteristics, spawning behavior, and age and growth patterns are examples of attributes that denote potential priority conservation populations. An example of an alternate life-history strategy is year-round river residency. Most populations within the basin show a migratory life history, with fish leaving rivers as juveniles to feed and grow in the Great Lakes and adults returning to natal rivers to spawn. Other examples include the existence of a shoal- or reef-spawning population that completed its life history in a lake or bay, or a population with multiple spawning runs (e.g., early and late). Whether these behavioral differences are associated with inherited adaptive differences is uncertain. A conservative approach should assume some genetic basis exists for these life histories. Life-history traits used for the identification of priority conservation populations should not be the result of human-altered environments or human activities. For example, river residency that is the result of the presence of dams is not a life-history trait that should be given priority. The Kaministiquia River (Lake Superior) and the St. Clair River would be examples of populations that may have unusual life-history traits. Telemetry and capture data from the Kaministiquia River population suggest that most fish reside in the river all year (M. Friday, Ontario Ministry of Natural Resources, personal communication, 2008). Sturgeon spawning in the St. Clair River do so in deep water (>10-m deep), and a year-round river-resident population exists at this location (Boase 2003; Thomas and Haas 2004).

Populations that are freely moving (without being blocked by artificial barriers from using critical portions of their historical home range), have not been impacted by stocking, have retained sufficient genetic diversity, and are recruiting naturally are closest to a natural (unperturbed) state and should be priorities for conservation. These populations most likely represent closely the natural genetic diversity of lake sturgeon populations unaltered by human activities. Rivers that fit this criterion exist in each GSU. The Sturgeon River (Lake Superior) and the St. Clair River are two examples.



## **STEP 3: A STOCKING DECISION TREE**

### **Purpose**

This step helps with decision making about whether to reintroduce or supplement a lake sturgeon population at a selected location. Reintroduction is defined as the establishment of lake sturgeon at a spawning location where it is absent. Supplementation is defined as stocking at a location where a sturgeon population currently exists but where the population is below a desired abundance. This step also guides the selection of donor stocks and the implementation of stocking or supplementation strategies. Donor stocks supply gametes for propagation of lake sturgeon in hatcheries. The decisions made at this step are guided by genetic considerations unique to the site targeted for reintroduction or to the population intended for supplementation. As the decision tree is being used, the reasons used to make decisions at each step should be documented to allow for internal and external review.

### **Stocking Decision Tree**

#### **Decision 1**

- A. If the goal of stocking is to have a self-sustaining population, were or are the impediments that contributed to the original population's extinction, decline, or lack of recovery remedied, or planned to be remedied, by the time the stocked fish return to spawn?

YES: Go to Decision 2.

NO: Stocking is not recommended. Research and management should focus on remedying the impediments limiting lake sturgeon sustainability prior to reintroduction. Management should develop a plan with measures that have the potential to remedy the impediments that prevent sustainability. At this point, Step 3 of the guidelines has been completed, and Step 4 should not be started until the impediments at the site are remedied or will be remedied by actions under way.

- B. If the goal of stocking is not to have a self-sustaining population, go to Decision 2.

The purpose of Decision 1 is to ensure that suitable conditions exist to support a self-sustaining population. Stocking will not result in lake sturgeon recovery if the fundamental reasons for the population's decline or extinction were or are not addressed. These impediments should be examined before stocking. This decision emphasizes the

importance of evaluating all biological factors important to lake sturgeon recovery, not just those associated with stocking. For supplementation, addressing Decision 1 may result in the natural recovery of the population without a need for stocking.

## **Decision 2**

- A. Is there a high likelihood that a lake sturgeon population exists at the selected site?
- YES: Go to Decision 3.
- NO: Go to Decision 5.
- B. If the existence of a population is unknown, Step 3 of the guidelines has been completed. Assessments should be conducted to determine the status of the population, including abundance, reproductive success, genetic diversity, and threats to viability, and then the previous decisions should be repeated.

The purpose of Decision 2 is to determine whether stocking will affect the population that remains at the proposed stocking site. Future decisions will aim at trying to minimize impacts to the existing population at the site.

## **Decision 3**

- A. Is a high risk of extinction predicted for this population? High risk of extinction is defined as a >50% reduction in population size has occurred over the last 75 years, even though the causes of reduction have ceased, or a >30% reduction in population size has occurred over the last 75 years, even though the causes of reduction have remained or are unknown.
- YES: Begin supplementation with individuals from this population using the recommendations in Step 4. Supplementation also may be conducted with individuals from populations within the GSU if the existing population is too small or has low genetic diversity. Step 3 of the guidelines has now been completed; go to Step 4.
- NO: Go to Decision 4. The data suggest the population likely will sustain itself, although perhaps at levels below management objectives.
- B. If data are insufficient to predict the risk of extinction, Step 3 of the guidelines has been completed. Conduct assessments to determine the status of the population including abundance, reproductive success, genetic diversity, and threats to viability and then repeat the previous decisions.

The purpose of Decision 3 is to determine the viability of the existing population in order to assess whether supplementation is critical to that population's sustainability. If a population is at a high risk of extinction, then supplementation may be implemented to prevent extirpation of the population. Ongoing research may provide insight into identifying populations that are at high risk of extinction (Schueller and Hayes, unpublished data). If a high risk of extinction does not exist, supplementation may meet other goals, such as restoring fish-community function, establishing a recreational fishery, providing for tribal ceremonies/subsistence, and/or controlling invasive species. Criteria for reductions in population size are based on the International Union for Conservation of Nature and Natural Resources criteria for vulnerable taxa (International Union for Conservation of Nature and Natural Resources 2001).

#### **Decision 4**

Was this population identified as a priority conservation population in Step 2?

YES: Supplementation should not be conducted at this site, as the genetic importance of this stock could be compromised. Step 3 of the guidelines has been completed; do not proceed to Step 4.

NO: Supplementation may be implemented as per Step 4. Supplementation should not use individuals from other populations. Step 3 has been completed; proceed to Step 4.

The purpose of Decision 4 is to preserve the genetic diversity of priority conservation populations. If the population has been identified as a priority conservation population, supplementation could compromise its genetic integrity by decreasing genetic diversity, increasing relatedness, selecting for traits that are advantageous in captivity, and decreasing effective population size. The risks of stocking outweigh the benefits because the population is not likely to become extinct. If the population is not a priority conservation population, supplementation may be used if the population is not meeting fish-community and/or management objectives.

## **Decision 5**

Does at least one population within the GSU identified in Step 1 have sufficient numbers (so that gamete collection will not affect recruitment and diversity in the donor population), sufficient genetic diversity (as measured by average number of alleles and average heterozygosity), and logistical feasibility to serve as a donor stock?

YES: Go to Decision 6.

NO: Reintroduction is not recommended at this site. An appropriate donor population is not available. Management should focus on conservation of existing remnant populations until a potential donor becomes available. Step 3 of the guidelines has been completed; do not proceed to Step 4.

The purpose of Decision 5 is to determine whether a suitable donor population exists. A donor population should be from the same GSU to reduce the risk of outbreeding depression. The donor population should also have sufficient numbers of females and males to provide an adequate representation of genetic diversity and to reduce the relatedness of offspring. Sufficient genetic diversity in the donor population is important. Use of a population that is inbred or has experienced a genetic bottleneck could result in an inbred reintroduced population that lacks evolutionary potential. Logistical feasibility takes into account factors such as ease of donor capture, accessibility of spawning site, and cost. Similar habitat characteristics at the reintroduction site and at the site of the donor population also may be beneficial. If no donor stock exists within the GSU identified in Step 1, reintroduction is not recommended at this site because the threat to natural populations within the GSU outweighs the benefits of reintroduction.

## **Decision 6**

Is the proposed stocking site geographically isolated from all priority conservation populations identified in Step 2 or has the risk of reintroduced individuals straying to these sites been removed?

YES: Stocking at the proposed site can occur using the selected donor stock(s). Step 3 of the guidelines has been completed; proceed to Step 4.

NO: If an appropriate donor stock(s) has been selected for the proposed site, stocking can proceed providing propagation techniques ensure long-term fidelity of the stocked fish to the proposed site (see rationale below). Only gametes or fertilized eggs from the donor population should be used; drifting larvae from another river should not be used (see rationale below). Step 3 of the guidelines has been completed; proceed to Step 4.

The purpose of Decision 6 is to evaluate the potential impact of stocking on priority conservation populations. If stocked individuals stray and reproduce at sites containing a priority conservation population, the genetic integrity of that population could be compromised. A very conservative approach in donor selection and breeding-plan implementation needs to be adopted. Some of the GSUs cover large geographic ranges and may have barriers that impede straying, particularly in an upstream direction. In these instances, the threat of individuals straying to priority conservation populations is reduced. Examples of locations with barriers include sites below Niagara Falls and sites below the Moses Saunders Dam on the St. Lawrence River (Fig. 2). Wherever a potential exists for stocked fish to stray into the spawning sites used by priority conservation populations, propagation techniques that promote imprinting should be used. A current example is streamside rearing using gametes or fertilized eggs from the donor population. Due to the uncertainty of the life stage at which imprinting occurs and to the potential for reduced genetic diversity due to relatedness of collected individuals, the collection and use of drifting larvae from the donor population is not recommended.

## **STEP 4. STOCKING PROGRAM DESIGN AND IMPLEMENTATION**

### **Purpose**

The purpose of this section is to describe breeding guidelines that will reduce the genetic risks associated with propagation after the use of Steps 1-3 has determined that stocking should proceed. Steps 1-3 evaluated the genetic risks of reintroduction, introduction, or supplementation at the selected site and identified an appropriate donor population that reduces the risk of outbreeding depression. Based upon the results of Steps 1-3, including the documentation developed for each decision and the recommendations derived in this step, a management agency should be able to write a proposal for the artificial propagation needed for reintroduction, introduction, or supplementation and seek review from the lake committee with responsibility for that Great Lake (see <http://www.glfc.org/lakecom/>). Sections of these guidelines may be used within management plans, as appropriate.

## Section 1. Collection Targets and Mating Techniques

### Gamete Collection

Gamete collection is often the most appropriate strategy for reintroduction into waters where sturgeon are extirpated. Gamete collection may also be suitable when supplementing an existing population, assuming adequate numbers of adult donors are available. To proceed, use the following recommendations:

1. Gamete collection should target a total of 250 females and 250-1,250 males over the lifetime of the stocking (resulting in an  $N_e > 500$  and average yearly  $N_e \geq 20$  over a 25-year period). See Table 4 for the appropriate number of matings to achieve this yearly effective population size. In locations where this number is not feasible due to limited donor population size, minimum acceptable numbers as low as 100 females and 100 males summed over a period of at least 25 years can be used (resulting in an  $N_e = 200$  and average yearly  $N_e \geq 8$ ). An estimate of the number of eggs needed from each female can be determined a priori based on expected survival rates during incubation and rearing so that sufficient progeny are available to meet stocking targets (see Section 3) without encumbering propagation facilities with an unusable excess. Stocking will likely need to occur over many years to obtain the target number of parents and to achieve an adequate age structure in the established population.
2. Adults should be captured throughout the spawning season from the donor spawning population(s) and at several locations within the spawning site to maximize the diversity in the genetic and the reproductive-strategy characteristics represented in collected gametes.
3. When available, use up to five males per female. Divide the number of males by the number of females to determine the number of males to be mated with each female. Then divide the eggs from each female into equal allotments corresponding to the number of males with which the female will be mated. Gametes should be kept separate by family (male-female pair) so that the number of progeny from each family can be equalized during the rearing process (see Section 2). Do not pool sperm or reuse males.
4. Mark all parents (PIT tags suggested) so that their use in propagation will be known if they are recaptured in future collections, and collect tissue samples for genetic analysis to establish a baseline of parental genotypes. This record keeping is critical for long-term evaluation and success.

Table 4. The number of male and female parents required to achieve annual effective population sizes of at least 8.

	Number of parents	Female																				
		3	4	5	6	7	8	9	10	11	12											
Male	4		8.0																			
	5		8.9	10.0																		
	6	8.0	9.6	10.9	12.0																	
	7	8.4	10.2	11.7	12.9	14.0																
	8	8.7	10.7	12.3	13.7	14.9	16.0															
	9	9.0	11.1	12.9	14.4	15.7	16.9	18.0														
	10	9.2	11.4	13.3	15.0	16.5	17.8	19.0	20.0													
	11	9.4	11.7	13.8	15.5	17.1	18.5	19.8	20.6	22.0												
	12	9.5	12.0	14.1	16.0	17.7	19.1	20.6	21.8	23.0	24.0											
	13	9.8	12.2	14.4	16.4	18.2	19.8	21.3	22.6	23.8	25.0											
	14	9.9	12.4	14.7	16.8	18.7	20.4	21.9	23.3	24.6	25.8											
	15	10.0	12.6	15.0	17.1	19.1	20.9	22.5	24.0	25.4	26.7											
	16	10.1	12.8	15.2	17.5	19.5	21.3	23.0	24.6	26.1	27.4											
	17	10.2	13.0	15.5	17.7	19.8	21.8	23.5	25.2	26.7	28.1											
	18	10.3	13.1	15.7	18.0	20.2	22.2	24.0	25.7	27.3	28.8											
	19	10.4	13.2	15.8	18.2	20.5	22.5	24.4	26.2	27.9	29.4											
	20	10.4	13.3	16.0	18.5	20.7	22.9	24.8	26.7	28.4	30.0											

Note: Effective population size is calculated using the equation  $N_e = 4N_fN_m / (N_f + N_m)$ . This equation does not take into account variation in family size. Therefore, family sizes need to be relatively equal. Values inside the bold line correspond to recommended mating combinations that achieve the target annual  $N_e$  of 20. All other values shown correspond to mating combinations that achieve minimum annual  $N_e$  of 8 and should only be used for those donor populations of insufficient size to support target numbers. Unlisted values are below the minimum yearly number or require the reuse of males, which is not recommended. Table is modified from the Breeding and Stocking Protocol for Cultured Atlantic Sturgeon (Pierre et al. 1996).

## **Fertilized Egg or Larvae Collection**

For supplementation of an existing population, the collection of larvae or fertilized eggs may be very suitable because it has the potential to gather representative fish from more parents than may be feasible with direct collection of gametes (Crossman 2008). When access to, or availability of, adults of reproductive age from the donor population(s) is limited or difficult, collecting deposited eggs or drifting larvae may be the only option. Proceed using the following recommendations:

1. As with direct gamete collection, collected fertilized eggs or drifting larvae should come from the natural spawning of at least 250 females and 250-1,250 males over the lifetime of the stocking. This collection can be evaluated during the course of the supplementation effort through genetic analysis of reared fish.
2. Because fertilized eggs and drifting larvae collected from the wild are usually contributing to natural production, limit collections to 10% or less of available drifting larvae or fertilized eggs. Collect eggs and/or larvae from different locations within the spawning site and at different times throughout the spawning or larval drift period.
3. Larval collection for transfer to other river systems is not appropriate for reintroduction at sites not completely isolated from priority conservation populations (see Step 3). Due to uncertainty about the mechanism of imprinting in lake sturgeon, the risk of outbreeding depression resulting from straying to these populations is considered too great.

## **Individual Transfers**

Where donor populations exist upstream of a migration barrier within targeted rehabilitation waters (within a river system), downstream transfers of any life stage, including adults, is a viable and preferable alternative to stocking artificially reared fish. Such transferred fish at maturity should home to the target waters. The target number of transfers should be 250 females and 250-1,250 males of diverse ages. This number can be accumulated over an extended time period (e.g., over 25 years). If the donor population is limiting, a transfer of 4 females and at least 4 males each year would meet the minimum requirements. Adult transfers should not exceed 5% of the donor adult stock in any year, and between-river transfers should not be conducted (see below).



## Rationale for Collection Targets and Mating Techniques

Lake sturgeon gamete collection, propagation, and stocking require a sustained, long-term effort if a population is to be restored or enhanced in a genetically conservative way. The intermittent spawning and late sexual maturity of lake sturgeon require a long-term commitment. Due to the protracted maturity schedule for lake sturgeon, gametes can be collected over one generation (25 years) to meet the overall target number.

A re-established population with a minimum effective population size of 500 is recommended (Lande 1988). This number will ensure that the long-term evolutionary potential and a sufficient level of genetic diversity are maintained in the stocked population. In donor populations that have a population size that cannot support the recommended number, a minimum of 200 should be sufficient (Allendorf and Ryman 1987). This minimum number will increase the likelihood of sampling low-frequency alleles and will not disqualify potential donor populations that would be optimal in reducing the risk of outbreeding depression. Sampling donor populations at this scale has been demonstrated to maintain the allele frequencies observed in the donor population (Page et al. 2005).

An effective population size per year of 20 ( $N_e = 20$ ) is recommended for donor populations of large size (e.g., Wolf and St. Lawrence Rivers). This yearly target will ensure that the overall minimum number ( $N_e = 500$ ) will be met within a 25-year period. If the yearly effective population size exceeds 20, the overall target will be met sooner. If the donor population cannot support that number, a minimum effective population size per year equal to 8 ( $N_e = 8$ ) for 25 years is recommended. For the white sturgeon (*Acipenser transmontanus*), 500 parents over 50 years (10 parents each year; yearly  $N_e = 10$ ) were recommended (Pollard 2002); for the Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*), 100 parents over 10+ years (yearly  $N_e = 6$ ) (Pierre et al. 1996); and for the paddlefish (*Polyodon spathula*) 50 parents over 5 years (yearly  $N_e = 10$ ) (MICRA Paddlefish Sturgeon Committee 1998). The Kootenai River white sturgeon conservation aquaculture program recommends an annual minimum target of  $N_e = 10$ , which has been surpassed in most of the recent years the program has operated (Kootenai Tribe of Idaho 2007). The recommended mating scheme is a partial factorial design where females are mated with subsets of the available males. This mating scheme increases genetic diversity and increases  $N_e$  by reducing variance in family size (Fiumera et al. 2004). Simulation studies have demonstrated that a complete factorial design (a portion of each female's eggs are fertilized by each male) maximizes  $N_e$  compared with a monogamous mating design where the eggs from each female are mated with a single male (Fiumera et al. 2004). Implementation of a complete factorial design may be necessary when variance in family size is large. However, possible negative consequences of a complete factorial

design include an increase in relatedness of offspring (Miller and Kapuscinski 2003). The recommended mating scheme offers an increase in genetic diversity and  $N_e$  while decreasing the relatedness among offspring. Equalization of family sizes further increases the  $N_e$ , resulting in an  $N_e$  similar to the size achieved through a complete factorial design. Sperm should not be pooled; otherwise, sperm competition may result in the over-representation of a subset of males (Campton 2004).

When collecting fertilized eggs or drifting larvae from wild populations, the number collected should not exceed that required to meet the objectives of supplementation, and their removal should not compromise the productivity of the donor population. In most situations, the recommendation is to collect up to only 10% of the available production. The recommended numbers of transferred adults (<5% of adult donor stock) were based on mortality rates that could be sustained without having a detrimental effect upon the donor population.

## Section 2. Rearing Techniques

1. Rear fish in a manner that promotes the imprinting and adaptation of stocked fish to the receiving waters. The use of streamside rearing is currently being and is likely to meet this objective (Holtgren et al. 2007; Crossman 2008). Other techniques offering the same advantages should be considered as they are developed. Rearing techniques should also address the trade-offs between artificial-rearing time, domestication selection, release size, and survival.
2. Maintain equal familial representation (+5% of the established stocking number) during the rearing process, at release, and across the entire stocking period (25 years). Excess offspring should not be released into open waters but may be used for captive research or provided to private aquaculture, zoos, or aquaria.
3. Employ the best rearing practices, which seek to produce a good-quality fish reared under conditions that mimic, to the greatest degree possible, the average natural conditions at the site where sturgeon will be introduced. These practices will minimize the probability of domestication selection.
4. Monitor the health of propagated sturgeon as well as sturgeon in the donor population.

### Rationale

Rearing techniques will depend upon the intent of the stocking activity (e.g., to re-establish an extirpated population or to rehabilitate an existing but depressed population through supplementation). Efforts should be made to facilitate the imprinting of reared sturgeon to their receiving waters. Straying of only a few reared fish of one genetic source into waters containing a genetically different population could result, over time, in the loss of the genetic identity of that population. Streamside rearing is one method currently being tested that is expected to maximize the likelihood of imprinting and thus minimize the risk of straying of stocked fish (Holtgren et al. 2007). Other viable and existing techniques should be considered and investigated. Streamside rearing is defined here as a technique of rearing gametes and fish in ambient receiving waters appropriate for that life stage (eggs and/or larvae through stocked juveniles). This technique will likely require locating rearing facilities at remote field locations, and will add logistics and expense to artificial-production operations. Other techniques offering the same advantages should be considered as they are developed and recommended. Rearing techniques should also address the tradeoffs between artificial rearing time, domestication selection, release size, and survival. Directly stocking eggs or very early-stage larvae could also reduce the risk of straying.

Rearing practices should be adopted that will maintain equal family representation. Equal representation can occur through separate rearing or marking individuals with unique marks by family. If propagation logistics require combining families during the rearing process, this action should be delayed until after significant periods of mortality have passed. Families could be combined incrementally as space dictates. When combining families is deemed necessary, family contributions should be equalized. By equalizing family sizes, the ratio of the effective population size to the census population size is closer to one, thereby minimizing the loss of genetic diversity and avoiding the Ryman-Laikre effect.

The numbers of gametes to rear per family should be determined a priori based on expected survival rates during incubation and rearing so that the target stocking number is attained with all families contributing equally throughout the entire period of stocking (25 years). However, equalization of family sizes (+5%) at stocking does not necessitate reduction of all families to the size of the smallest annual production group. Doing so could unduly compromise the intended demographic benefits of the effort. Instead, offspring from those families that are below the target number will simply be under-represented and will likely necessitate the rearing of additional families in future years to meet propagation targets. Further, the numbers stocked from other families should not be increased to make up for this shortfall but should be kept as targeted originally.

During captive rearing, water-quality conditions including temperature, dissolved oxygen, dissolved nitrogen, turbidity, and other important parameters should mimic ambient conditions in the receiving waters and meet fish-culture and fish-health standards. Cultured fish should be monitored for health to keep them in good condition, free of debilitating and/or lethal diseases, and free of chronic stress symptoms to prevent introduction and dissemination of communicable diseases.

### Section 3. Stocking Numbers

1. Determine the numbers of each life stage to be stocked based on habitat availability and expected survival rates for the stocking site, accounting for the population size and recruitment from an existing remnant population (if present). Table 5 serves as a guide for the development of stocking targets based on the life stage to be stocked. Stocking numbers will vary, depending on the target adult population size.
2. Determine the number of fertilized eggs and larvae based on habitat availability and expected survival rates for the stocking site (Table 5). Assuming a 1.0% larval-to-fingerling-stage survival rate, stocking rates for larvae might typically range between 25,000 and 100,000 individuals. The number of eggs to be stocked will depend on the number that can be collected from female donors and fertilized by males. As many naturally fertilized eggs may be stocked as can be collected. The collections of wild fertilized eggs should not exceed a level that negatively affects recruitment to the donor population. Lacking site-specific data, 10% is a suggested maximum collection level.
3. Determine the number of juvenile or adult transfers based on habitat availability with a minimum equal to the number of donors specified in Section 1 (minimum of 250 females and 250-1,250 males over a period of at least 25 years, or if abundance in the donor population is limiting, then a yearly  $N_e > 8$ ).

Table 5. Sample annual stocking numbers of fingerling- and yearling-size fish necessary to achieve the recommended adult population based on a range of possible annual survival rates (assumes 25 years of stocking and a target adult population of 750 fish, ages 15-40).

Survival rate (%)			Number stocked	
Fingerling	Yearling	Adult	Fingerling	Yearling
10	50	90	7,000	700
		95	2,100	210
		98	975	98
10	75	90	4,700	470
		95	1,400	140
		98	650	65
10	90	90	3,900	390
		95	1,170	117
		98	540	54
25	50	90	2,800	700
		95	840	210
		98	390	98
25	75	90	1,880	470
		95	560	140
		98	260	65
25	90	90	1,560	390
		95	470	117
		98	220	54
50	50	90	1,400	700
		95	420	210
		98	195	98
50	75	90	940	470
		95	280	140
		98	130	65
50	90	90	780	390
		95	235	117
		98	110	54

## **Rationale**

The goal is to establish a founding population of at least 750 sexually mature lake sturgeon in each site targeted for rehabilitation. This number was selected because it represents the minimum number thought to be present in Great Lakes populations that are considered to be either stable or increasing in abundance (Sturgeon and Bad Rivers in Lake Superior and the Lower Menominee River in Green Bay). However, a target adult population size may vary depending on management goals and habitat availability. Stocking numbers will then need to be adjusted accordingly.

There are several ways to calculate the number of lake sturgeon to stock in a particular system to achieve this minimum population goal. The first way involves estimating the number of larvae, fingerlings, or yearlings that are produced by known natural populations having this abundance of mature fish. These numbers can then be used to set stocking targets in similar systems. Examples are limited, but for the Peshtigo River (Lake Michigan), larval production was estimated at approximately 6,200-23,000 and fingerling production at 108-1,260 in 2002-2007 (Benson 2004; Caroffino 2009).

The second way involves estimating and calculating survival rates at different life stages can be estimated and calculated. To meet the population abundance target of 750 sexually mature lake sturgeon per population after 25 years of stocking, the number of fish to stock at different life stages can be calculated using survival-rate estimates. However, survival rates may vary considerably among years and rivers or populations. For wild lake sturgeon in the Peshtigo River (Lake Michigan), survival from the larval to the fall-fingerling stage appeared to range from 1-10% (Benson 2004; Caroffino 2009). In Green Bay, total annual mortality for lake sturgeon ages 9-60 ranged from 5.1-7.0%, although this estimate was considered a maximum given an apparent increase in recruitment over the study (Elliott and Gunderman 2008). Total annual mortality for Manistee River fish ages 10-50 was estimated at 4.5% (Lallaman et al. 2008). Baker and Borgeson (1999) reported a 5% total annual mortality rate for adult lake sturgeon in Black Lake (Michigan), and Priegel and Wirth (1975) reported a total annual mortality of 5.4% for the Lake Winnebago population. Annual total mortality estimates for the Lake Winnebago population have ranged from 9.8-22.1% since 1953, with exploitation amounting to 1.0-11.5% (Bruch 1999). Total annual mortality for lake sturgeon in the St. Clair system was estimated at 9.0% (Thomas and Haas 2004). Crossman et al. (2009) estimated overwinter survival of age-0 lake sturgeon juveniles to be 40%. Survival of hatchery-reared white sturgeon in the Kootenai River was estimated at 64% during the year after release and at approximately 90% during all subsequent years (Kootenai Tribe of Idaho 2004). The amount of larval production for native populations and their life-stage survival rates should be priority research goals.

Survival-rate assumptions, particularly those for adult fish, greatly affect the number of fish needed for stocking. Table 5 provides examples of stocking rates to guide establishment of a population of 750 mature adults (ages 15-40) given three levels of assumed survival for three life stages. For example, assuming annual survival rates of 25% for fingerlings, 75% for yearlings, and 90% for adults, some 1,880 lake sturgeon fingerlings would need to be stocked per year for 25 years to attain an adult population of 750 fish. Increasing the assumed survival of adult fish by 5% (to 95%) reduces the number of fingerlings needed to 560 per year. Survival rates and habitat availability for all life stages will need to be determined and evaluated continually to maintain appropriate stocking rates.

#### **Section 4. Release Techniques**

1. Release propagated lake sturgeon at the earliest life stage possible, considering the trade-offs among survival, domestication or other culture effects, imprinting success, and genetic and demographic benefits and risks. Existing culture programs have found that rearing lake sturgeon to an age/size of 3-5 months and 4-8 inches is a reasonable approach for most target waters.
2. Establish a method to reliably mark or identify stocked fish after their release. If stocked fish are large enough, permanently mark all of them so that, at a minimum, stocking location and year-class can be determined for recaptured fish. Ideally, all stocked fish would be marked with PIT tags or other individual-specific internal tags. Using coded wire tags injected at specific locations with or without fin clips also may be useful. If size at stocking precludes reliable marking of individuals, consider the use of permanent mass-marking techniques and retain samples to genetically characterize stocked fish.
3. When families have been combined prior to stocking or when wild larvae or fertilized eggs have been collected and reared, collect tissue samples from all fish (or a sufficient sub-sample) to characterize their genetic lineage. If stocked fish are too small for non-destructive sampling, a subset should be sacrificed to provide the necessary tissue for genetic analysis.
4. Release lake sturgeon in locations where habitat is suitable and typical for the life stage(s) being stocked.
5. Use release techniques that increase chances for survival, such as acclimation pens, nighttime releases, and multiple releases over time.



## **Rationale**

Although releasing fish at the earliest possible life stage will maximize the likelihood of imprinting, fish may be held in streamside or hatchery facilities through much of the first growing season to reduce exposure to early mortality sources, such as predation, and to facilitate tagging or marking of individuals prior to release. To maximize survival and facilitate imprinting, fish should be released into receiving waters at locations where wild fish are known or would be expected to reside at that period in their life history.

Marking should identify individuals and/or individual families of origin, stocking location, experimental unit, date of release, and year-class. A marking technique should be used that can be interpreted universally. PIT tags afford this level of discrimination at a relatively small cost compared to the overall cost of implementing rehabilitation stocking. The potential benefits that will be afforded from evaluation justify the initial expense of tagging. Genetic analysis can also provide some of the needed information, but costs and logistics are likely to be greater. To evaluate final parental contribution and to facilitate future evaluation of the origin of returning adult fish, tissues should be collected for genetic analysis from representative samples of fingerlings and yearlings before they are stocked. Sampling is especially important for lots originating from wild eggs or drift larvae and for families that have been commingled prior to stocking.

## **Section 5. Evaluation**

1. Lake sturgeon management plans that involve stocking projects should contain detailed evaluation criteria that are explicitly linked to management objectives. Evaluation will provide the opportunity to learn over the considerable time periods of stocking and to adapt to unforeseen problems effectively. Benchmarks of stocking success should be developed for various life stages, behaviors, and time periods (e.g., every five years). Assessment and monitoring need to be carried out to gauge progress toward benchmarks. A commitment to evaluation is essential if a stocking program is to contribute new information and be adaptive.
2. The number and sex of donors should be recorded each year. If the number of donors falls short of the yearly target (see Section 1) for five consecutive years, the goal of an overall minimum contribution from at least 250 parents likely will not be met. A different donor population should be identified or rehabilitation efforts should be reevaluated.
3. Ten years after the first stocking event, rigorous evaluation of spawner returns should begin. This evaluation should include monitoring of neighboring rivers and other populations where stocked sturgeon might stray. If sexually mature strays are identified, reintroduction/supplementation should be modified to increase the likelihood of imprinting to the stocking site. If sufficient (target) numbers of ripe males are not detected in the stocked river from year 10 to year 20, the rationale for stocking should be reassessed, and adjustments should be made.
4. Twenty years after the first stocking event, rigorous evaluations of recruitment should begin (if natural recruitment was a stocking goal). If successful reproduction and recruitment are not detected in the target river from year 25 to year 30, or soon after mature females are detected on the spawning grounds, the reasons for failure should be identified, and adjustments should be made.

### **Rationale**

Due to the long-term commitment necessary for lake sturgeon stocking (up to one generation or 25 years), an opportunity exists to learn from stocking and to make modifications while the program is being implemented. The cost and effort required for effective stocking makes the creation of a thorough evaluation program a good investment. Defining clear objectives or criteria for the determination of success or failure reduces subjectivity in determining whether management actions need to be altered.

## **Acknowledgements**

Participants at the Lake Michigan Lake Sturgeon Task Group Genetic Conservation, Propagation, and Stocking Work Group Meeting held June 16-17, 2004, contributed to the development of these guidelines and included, in addition to the authors, Doug Aloisi, Paul Anders, Nancy Auer, Fred Binkowski, Dale Burkett, Tom Burzynski, Ron Bruch, Leonardo Congiu, Steve Fajfer, Brant Fisher, Mark Holey, Allison (Niggemyer) Evans, Don Reiter, Brian Sloss, Kregg Smith, Tom Thuemler, and Martha Wolgamood. Valuable comments, insight, and feedback were provided by Doug Aloisi, Paul Anders, Nancy Auer, Jim Boase, Dave Borgeson, Tom Burzynski, Doug Carlson, Bill Culligan, Dawn Dittman, Michael Donofrio, Rich Drouin, Brant Fisher, Brian Gunderman, Bob Haas, Liz Hay-Chmielewski, Daniel Hayes, Tracy Hill, Steve Hogler, Mark Holey, William Horns, Jim Johnson, Jerry McClain, Lloyd Mohr, Michael Morencie, Kurt Newman, Allison (Niggemyer) Evans, Holly Patrick, Amy Schueller, Brian Sloss, Mike Thomas, Gary Whelan, Emily Zollweg, and members of Kim Scribner's research team. The Great Lakes Fishery Commission hosted A. Welsh for three months as a visiting scientist to facilitate information gathering for this report. Funding was provided by the Great Lakes Fish and Wildlife Restoration Act and a University of California-Davis Dissertation Year Fellowship. The findings and conclusions in this document are those of the authors and do not necessarily represent the views of their associated agencies or institutions.

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## MISCELLANEOUS PUBLICATIONS

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- August 1993      A survey of fish-community and habitat goals/objectives/targets and status in Great Lakes areas of concern. J.H. Hartig. 95 p.
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