Source Population Assignment of Lake Superior Lake Sturgeon 2011 and 2016 samples

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Introduction

Lake sturgeon (*Acipenser fulvescens*) are of management concern throughout the Great Lakes basin. Knowledge regarding lake sturgeon ecology is primarily based on studies conducted during brief spawning periods in river habitats. Many of the identified threats to lake sturgeon recovery, such as habitat changes and the effects of dams, have occurred in tributary habitats (Auer 1999*a*, Houston 1987, Harkness and Dymond 1961). However, little is known about lake sturgeon ecology during the extended periods they spend in the open waters and bays of the Great Lakes. Lake sturgeon do not begin spawning until 15-20 years of age and once maturity is reached, an individual may only spawn every seven years (Harkness and Dymond 1961). Management cannot rely solely on data collected during spawning periods for critical information on lake sturgeon recruitment and ecology. Managers currently lack data on critical life stages (i.e., pre-adult) and the lack of movement data during these life stages diminishes the effectiveness of any implemented management actions.

Knowledge of lake sturgeon movements and habitat use during non-spawning times is limited. Available information suggests that lake sturgeon are capable of long-distance migrations (e.g., Auer 1999*b*) and tend to densely aggregate in relatively small areas (e.g., Smith and King 2005, Thomas and Haas 2002). Movement patterns can vary depending on life-stage (Peterson et al. 2007). Traditional mark-recapture methods or radio telemetry studies of tagged fish provide limited data about lake sturgeon movement because relatively few individuals from any breeding population are tagged or marked. Genetic techniques can overcome these obstacles and can identify which spawning populations are represented in non-spawning groups of lake sturgeon.

Breeding populations of lake sturgeon, especially in Lake Superior, exhibit a high degree of spatial genetic structure (Welsh et al. 2008). Spawning populations within Lake Superior had the highest level of genetic differentiation relative to the rest of the Great Lakes. These genetic differences allow for the identification of spawning population of origin in non-spawning lake sturgeon within Lake Superior. Lake sturgeon from Lake Winnebago (Wolf and Fox Rivers) have been stocked into Lake Superior (Schram et al. 1999) and this strain is also genetically distinguishable from native Lake Superior strains (DeHaan et al. 2006, Welsh et al. 2008). Genetic data have been successfully used to determine whether stocked Lake Winnebago sturgeon have migrated into the Sturgeon River and to assess natural levels of migration between the Sturgeon River and Bad River (Homola et al. 2010).

Project Objective:

- Determine the source spawning population of lake sturgeon collected throughout Lake Superior as part of the 2011 and 2016 lakewide index surveys and through other agency assessments.

Methods

Fin clips from lake sturgeon individuals collected from 2004-2011 and 2016 at sampling locations designated as high or medium priority by the USFWS Ashland FWCO were sent to our laboratory for processing.

DNA was extracted from fin clip samples using the Wizard SV® 96 Genomic DNA Purification System (Promega) or the GeneJET Genomic DNA Purification Kit (Thermo Scientific). Twelve polymorphic microsatellite loci (AfuG9, AfuG56, AfuG63, AfuG74, AfuG112, AfuG160, AfuG195, AfuG204 [Welsh et al 2003]; Afu68 [May et al 1997]; Afu68b [McQuown et al 2002]; Aox27 [King et al 2001]; Spl120 [McQuown et al 2000]) were organized into four multiplex groups and amplified using the Qiagen Multiplex Polymerase Chain Reaction (PCR) kit. Multiplex Group 1 included AfuG56, AfuG195, and Spl120; Multiplex Group 2 included AfuG9, AfuG74, and AfuG204; Multiplex Group 3 included AfuG112, AfuG160, and Aox27; and Multiplex Group 4 included Afu68, Afu68b, and AfuG63.

Each 10 µl Multiplex PCR reaction contained 1x QIAGEN Multiplex PCR Master Mix, 0.2 µM of each fluorescently labeled forward primer (0.1 µM for Afu68), 0.2 µM of each reverse primer (0.1 µM for Afu68), RNase-free water, and 20 ng extracted template DNA. Thermal cycling for Multiplex Group 1 consisted of an initial denaturation at 95°C for 15 minutes followed by 15 cycles consisting of a denaturation period of 30 seconds at 94°C, annealing period of 1 minute 30 seconds at 60°C with a 0.5°C decrease per cycle, and an extension period of 1 minute at 72°C. This was followed by 15 additional cycles consisting of a denaturation period of 30 seconds at 94°C, an annealing period of 1 minute 30 seconds at 52°C, and an extension period of 1 minute at 72°C. A final extension period of 30 minutes at 60°C and a 10°C hold ended the reaction. Thermal cycling for Multiplex Group 2, Multiplex Group 3, and Multiplex Group 4 consisted of an initial denaturation at 95°C for 15 minutes followed by 35 cycles consisting of a denaturation period of 30 seconds at 94°C, annealing period of 1 minute 30 seconds at 57°C, and an extension period of 1 minute at 72°C. This was followed by a final extension period of 30 minutes at 60°C. A 10°C hold ended the reaction. Thermal cycling was completed using Eppendorf Mastercycler Nexus and BioRad C1000 Touch thermal cyclers.

Due to the presence of non-specific amplification in the multiplex PCR, Spl120 was amplified in a separate PCR reaction. This 10 µl reaction contained 1x Colorless Go Taq Flexi Buffer (Promega), 1.5mM $MgCl_2$, 0.2mM deoxynucleotide triphosphate (dNTP) mix (Promega), 0.2 µM fluorescently labeled forward primer, 0.2 µM reverse primer, 0.05 units Go Taq Flexi DNA polymerase (Promega), and 40 ng extracted template DNA. Thermal cycling consisted of an initial denaturation at 95°C for 2 minutes followed by 20 cycles consisting of 30 seconds at 92°C and 40 seconds at 70°C with a 0.5°C decrease of the second step per cycle. This was followed by 20 cycles consisting of 30 seconds at 92°C and 40 seconds at 60°C with a 1 second increase of the second step per cycle. A 10°C hold ended the reaction.

The amplified products were analyzed using a Beckman Coulter GenomeLab™ GeXP Genetic Analysis System. Allele peaks were identified using the GenomeLab™ Fragment Analysis Software v3.2.42 by Beckman Coulter.

In order to determine the origin of non-spawning lake sturgeon, a baseline of spawning populations/regions from various locations throughout the Great Lakes was used (Table 1). Spawning populations below Niagara Falls were excluded due to their low likelihood of moving into Lake Superior. Some spawning populations had to be combined into regions due to low genetic differentiation among populations, resulting in low assignment accuracy. The Michipicoten River spawning population was equally similar to the Goulais River and Black Sturgeon/Pic/White, decreasing assignment accuracy; therefore, the Michipicoten was eliminated from the baseline. Most baseline spawning populations were previously analyzed using the 12 standardized microsatellite loci (Welsh et al. 2008; Scribner, unpublished data). Additional spawning populations that were included in the baseline but not previously published include the White River (Ontario), the Garden River, and the Serpent River. The Garden River and Serpent River were genetically indistinguishable each other and from the Mississaugi River; therefore, they were combined (along with the Spanish River) into a Lake Huron group for the baseline.

Genetic assignment testing was done using a conditional maximum likelihood approach, as implemented in the software ONCOR (Kalinowski et al. 2008). Simulations were first done to test the assignment power of the microsatellite loci and the baseline. Baseline genotypes were used to simulate mixture genotypes where each baseline population represented 100% of the mixture sample, with sample sizes equal to the baseline sample size (Anderson et al. 2008).

Assignment testing was then conducted to determine the most likely spawning site of origin of non-spawning lake sturgeon individuals. For sample groups with sufficient sample sizes ($N > 50$), a mixed stock analysis was conducted based on all sampled individuals, with 95% confidence intervals calculated based on 1000 bootstrap replicates. When the lower confidence interval includes 0, then the possibility exists there are truly no migrants from that spawning population. However, the bootstrapping method removes individuals from the mixture; in cases where there are few migrants from that spawning population (especially in mixtures with smaller sample sizes), some bootstrap replicates may result in an estimated proportion of 0 in the mixture. Therefore, individual assignment testing may have improved performance in detecting rare migrants. Individual assignment based on genotype frequencies and mixture proportions was used on all individuals to determine the probability of each individual belonging to its most likely spawning site of origin, using the method of Rannala and Mountain (1997), as implemented in the software ONCOR. The probability associated with each assignment was calculated, with individuals with probabilities <0.80 being considered as "unassigned". Reasons for this include stringency of the assignment criterion, power of the microsatellite loci, genetic differentiation among

baseline spawning populations, and the potential presence of unsampled spawning populations. There is a balance between assignment accuracy and the ability to assign individuals (Berry et al. 2004). With a higher assignment threshold (e.g., ≥90%), accuracy would increase but more individuals would be unassigned. In contrast, more individuals could be assigned if a lower assignment threshold was selected but accuracy would decrease.

Results and Discussion

Based on the simulations, there was high assignment accuracy among the spawning populations/regions (Table 1), indicating sufficient genetic differentiation for accurate assignment of non-spawning individuals. All assignment accuracies (i.e., percent correctly assigned) for the spawning baseline were ≥ 90%.

In general, most of the non-spawning lake sturgeon stay geographically close to their spawning site of origin (Tables 2, 3). For example, most of the sturgeon (75.1%) collected at the mouth of the Bad River originated from the Bad River spawning population. However, there were also several Sturgeon River individuals (11.7%) captured at that location. Another example is at Chequamegon Bay, where most individuals originated from the nearby spawning populations of the Bad/White Rivers and Sturgeon River (51.2% and 18.4%, respectively). Some locations, like Batchawana and Goulais Bays, have a large mixture of individuals from multiple spawning populations, including individuals from the Bad/White Rivers, Sturgeon River, Goulais River, Black Sturgeon/Pic/White Rivers, and Lake Huron.

Some long-distance migrants were also observed at many sampled locations. Individuals from the north shore of Lake Superior (Black Sturgeon/Pic/White Rivers) were detected in more distant locations such as the Bad River, Chequamegon Bay, Keweenaw Bay, the Ontonagon River, and the Sturgeon River (Table 3). In contrast, the only long-distance migrants observed along the north shore of Lake Superior were some Sturgeon River individuals in the Pic River area (Table 3). Individuals from Lake Huron were detected in distant locations, such as the Bad River, Chequamegon Bay, Keweenaw Bay, the Ontonagon River, the Pic River, the St. Louis River, the Sturgeon River, and western Lake Superior (Table 3). The Lake Huron group in the spawning population baseline includes the Garden, Serpent, Mississaugi, and Spanish Rivers. The most likely populations contributing these migrants are the Garden and Serpent Rivers because of their proximity to Lake Superior.

Individuals from the Wolf/Fox Rivers were identified at the Apostle Islands, Bad River, Batchawana Bay, Goulais Bay, Grand Portage, Keewenaw Bay, Lake George, Montreal River, St. Louis River, and western Lake Superior. These individuals are likely from the stocking efforts that occurred in the St. Louis River. Lake sturgeon were thought to have been extirpated from the St. Louis River and stocking occurred in this river beginning in 1983. From 1983-1994, the source population for stocking

was from the Lake Winnebago system (Wolf/Fox rivers) in western Lake Michigan. From 1998-2000, the Sturgeon River was used as a source population for fish stocked in the St. Louis River. Sturgeon River fish have also been stocked in the Ontonagon River in twelve years from 1998-present. The Menominee River was purportedly used as a source during some years of stocking; however, no individuals with Menominee River origins were detected at any sampled locations. In the St. Louis River, 75.8% of the captured sturgeon originated from the Wolf/Fox Rivers, while 10.9% originated from the Sturgeon River (Table 2). Natural migrants from Lake Huron (most likely from the Garden or Serpent Rivers) were also detected at the St. Louis River.

Some spawning populations appear to stray more than other spawning populations. Sturgeon River individuals were detected at 20 out of 25 sampled locations, being only absent from Lake Huron locations and the White River in Wisconsin. Lake Huron individuals were also detected at 20 out of 25 sampled locations. The higher rate of straying of Sturgeon River individuals could be due to lack of homing in stocked Sturgeon River individuals. However, straying of the stocked Wolf/Fox individuals was less, with Wolf/Fox individuals being detected at 10 locations. The level of straying observed in Wolf/Fox individuals is comparable to the levels observed in other natural populations, such as the Bad and Goulais Rivers (11 and 12 out of 25 locations, respectively). In contrast, the Kaministiquia River has a very low rate of straying, with individuals only being captured in 3 out of the 25 locations, with the largest percentage at Chequamegon Bay (4.3%). This likely supports the idea that the Kaministiquia River is a river-resident population.

The use of more powerful genetic markers (e.g., SNPs) would increase the power of assignment and allow for increased detection of genetic differentiation among spawning populations, likely resulting in more individuals being assigned to a source population. Efforts are currently underway to develop and use SNPs for the baseline population analysis, making this tool available in the future for assignment testing. Finally, low assignment probability could result from the individual originating from a spawning population that is not represented in the baseline. For example, Batchawana Bay has the highest percentage of individuals that could not be assigned (61.1%), indicating the potential presence of an unsampled spawning population in the Batchawana River. The absence of baseline data for current Lake Superior spawning populations is a limitation for this analysis/project.

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Project Deliverables

In addition to this final report, the following deliverables were also produced:

- Master spreadsheet with individual assignments for all samples and associated probabilities
- Oral presentation at the International Sturgeon Symposium in Vienna, Austria (September 2017)
- Poster presentation at the Great Lakes Lake Sturgeon Coordination Meeting (February 2018)
- Submitted manuscript to the Journal of Applied Ichthyology ("A reintroduced lake sturgeon population comes of age: a genetic evaluation of stocking success in the St. Louis River").

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Table 1. Accuracy of the spawning population/region baseline, with sample size, percent correctly assigned (accuracy), and 95% confidence intervals.

Spawning Population/Region	N	Accuracy (95% CI)
Bad/White	179	99.8% (98.9-100)
Sturgeon	48	94.3% (89.6-98.2)
Kaministiquia	85	98.9% (96.3-100)
Goulais	43	95.2% (89.3-98.8)
Black Sturgeon/Pic/White	178	98.5% (95.8-100)
Menominee	91	98.9% (96.6-100)
Wolf/Fox	103	98.0% (96.6-100)
Huron*	186	98.4% (96.2-100)

* Huron includes the Mississaugi, Spanish, Garden, and Serpent Rivers.

Table 2. Mixed stock analysis showing percentage of each spawning population/region in the sampled mixture, with associated 95% confidence intervals. Percentages highlighted in red are estimates where the lower 95% confidence interval does not include 0.

	N	Bad/White	Sturgeon	Kaministiquia	Goulais	Black Sturgeon/Pic/White	Menominee	Wolf/Fox	Huron
Bad	311	75.07	11.73	1.55	0	5.92	0	3.25	2.47
		(68.7-81.6)	(7.3-17.2)	(0-4.3)	(0-3.4)	(0.2-11.6)	(0-0.8)	(0.9-5.7)	(0-6.1)
Chequamegon	32	51.21	18.36	3.12	0	15.25	0	0.02	12.04
		(32.1-70.1)	(3.7-39.4)	(0-12.5)	(0-0)	(0-30.3)	(0-0)	(0-14.8)	(0-24.6)
Goulais	451	0.21	2.82	0.78	54.05	7.16	0.24	0	34.74
		(0-3.3)	(0-10.4)	(0-2.7)	(36.9-66.1)	(2.9-13.5)	(0-2.7)	(0-1.6)	(21.8-46.5)
Keweenaw	231	0.92	91.17	1.02	3.1	1.63	0.06	1.34	0.76
		(0-7.1)	(72.1-93.7)	(0-5.1)	(0.3-9.9)	(0-10.4)	(0-2.6)	(0-3.0)	(0-8.2)
Lake Huron	31	0	0	0	23.81	0.01	0	3.23	72.95
		(0-0)	(0-14.9)	(0-0.1)	(0-56)	(0-15.3)	(0-8.5)	(0-13.9)	(36.5-91.3)
Michipicoten	27	0	0.1	0	15.69	35.42	0	0	48.79
		(0-0)	(0-26.3)	(0-8.7)	(0-47.8)	(8.2-65.4)	(0-10.3)	(0-2.4)	(6.3-71.5)
Ontonagon	263	0.33	91.79	0	1.71	1.17	0	0	4.99
		(0-3.4)	(73.3-95.7)	(0-0.9)	(0-13.6)	(0-14.3)	(0-0.5)	(0-0)	(0-10.8)
Pic	108	0	1.73	0	0	93.99	0	0	4.28
		(0-0)	(0-6.6)	(0-2.7)	(0-6.7)	(80.3-99.0)	(0-0)	(0-0)	(0-15.2)
St. Louis	394	0	10.96	0	0	0.01	0	75.84	13.19
		(0-0.8)	(6.6-15.7)	(0-0.3)	(0-3.2)	(0-2.4)	(0-6.7)	(53.6-84.1)	(4.3-31.4)
Sturgeon	61	2.8	82.53	0	0	13.92	0	0	0.74
		(0-11.7)	(56.5-93.1)	(0-2.8)	(0-12.5)	(1.3-34.0)	(0-0)	(0-0)	(0-12.3)
Whitefish Bay	20	0	23.49	3.36	49.18	0	5.8	0	18.17
		(0-0.4)	(0-48.9)	(0-20.5)	(19.9-80.7)	(0-11.2)	(0-25.7)	(0-0.3)	(0-43.2)

White (Wisc.)	47	89.34	0	0	0	0.02	0	0	10.64
		(76.8-97.9)	(0-3.7)	(0-0)	(0-3.7)	(0-11.5)	(0-3.6)	(0-0)	(0-20.6)
W. Lake Sup.	74	1.92	5.46	0	1.67	0	0	85.81	5.14
		(0-6.7)	(0-21.7)	(0-5.3)	(0-7.6)	(0-3.6)	(0-6.3)	(67.5-94.7)	(0-21.7)

Table 3. Individual assignment results for each sampling location. Number of samples with assignment probabilities ≥ 80% (N), and percentage of assigned individuals representing identified spawning groups are included. Non-zero percentages are highlighted in red.

	N	Bad/White	Sturgeon	Kaministiquia	Goulais	Black Sturgeon/Pic/White	Menominee	Wolf/Fox	Huron
Apostle Islands	5	40.0	20.0	0.0	0.0	0.0	0.0	40.0	0.0
Bad	231	79.7	12.1	0.4	0.9	2.6	0.0	2.6	1.7
Bark Point	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Batchawana	33	3.0	3.0	0.0	30.3	12.1	0.0	3.0	48.5
Blind	6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
Chequamegon	23	60.9	17.4	4.3	0.0	8.7	0.0	0.0	8.7
Goulais	196	1.5	7.7	1.0	45.9	9.7	0.0	0.5	33.7
Grand Portage	6	0.0	16.7	0.0	0.0	0.0	0.0	83.3	0.0
Huron Bay	1	0.0	100.0	0.0	0.0	0.0	0.0	0.0	0.0
Keweenaw	158	3.2	88.0	0.0	1.9	2.5	0.0	1.9	2.5
Lake George	5	0.0	0.0	0.0	20.0	0.0	0.0	20.0	60.0
Michipicoten	12	0.0	8.3	0.0	16.7	8.3	0.0	0.0	66.7
Montreal	7	28.6	28.6	0.0	14.3	0.0	0.0	28.6	0.0
Ontonagon	176	1.1	91.5	0.0	1.7	0.6	0.0	0.0	5.1
Pic	55	0.0	5.5	0.0	1.8	81.8	0.0	0.0	10.9
Prairie Cove	4	0.0	25.0	0.0	0.0	50.0	0.0	0.0	25.0
Prairie River	4	0.0	25.0	0.0	0.0	50.0	0.0	0.0	25.0
Saxon Harbor	4	0.0	75.0	0.0	0.0	0.0	0.0	0.0	25.0
Spanish River	5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
St. Louis	226	0.0	15.9	0.0	0.4	0.0	0.0	67.3	16.4
Sturgeon	37	5.4	83.8	0.0	0.0	5.4	0.0	0.0	5.4
Tahquamegon	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	100.0
W. Lake Superior	49	2.0	6.1	0.0	2.0	0.0	0.0	81.6	8.2

White (Wisc.)	36	94.4	0.0	0.0	0.0	0.0	0.0	0.0	5.6
Whitefish Bay	11	0.0	36.4	0.0	54.5	0.0	0.0	0.0	9.1