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Contents

Figures

Tables

Abstract

Very little is known about remnant lake sturgeon stocks and the consequences of fishery management options on those stocks. Since 1997, assessment surveys by the Michigan Department of Natural Resources (MDNR) have characterized the St. Clair River (SCR) lake sturgeon population as relatively young with steady or increasing recruitment. However, researchers have not been able to determine effectively the sex or maturity for most of the sturgeon they capture because few fish caught during surveys were releasing gametes. Consequently, data are sparse or lacking for sex ratio, maturity schedule, and sex-specific growth rates and age composition. As a result, MDNR sport fishing regulations designed to protect reproductively mature females are based on minimal data and may not provide adequate protection of mature females from harvest. Sex ratio, age at first maturity, and spawning frequency data are needed to assist in managing the population for sustainable sport and commercial harvest. Available techniques for sexing sturgeon range from very invasive surgery to a simple blood draw and laboratory assays. This study focused on identifying the sex of mature and immature lake sturgeon by measuring the sex hormones estradiol (E_2) and testosterone (T) and the phosphoprotein vitellogenin (Vtg) in blood plasma by radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA), and evaluating this technique as a tool in lake sturgeon population management. A total of 115 lake sturgeon were sampled from May through June in 2004 and 2005 from the SCR, Michigan, USA. Twelve fish were releasing gametes at the time of capture. Due to the reproductive biology of lake sturgeon, we were able to assign a sex designation to 56% of the captured sturgeon based on hormone levels, resulting in a sex ratio of 1 female to 2.7 males. Considering that lake sturgeon are threatened with extinction in Michigan, advantages to using blood plasma assays were that fish were not harmed, and sample collection was quick, simple, and inexpensive. On the other hand, turnaround time, cost, and specialized equipment and training for the laboratory hormone assays make the technique far less than ideal for MDNR needs. Alternative options may include using other non-destructive field methods presented in the literature (e.g., ultrasound and fiber-optic endoscope) in conjunction with plasma hormone assays both to provide immediate sex identification and to support research and development of plasma hormone assays designed for the field. Determining the sex ratio of the SCR lake sturgeon population is an important component of a management program so that sturgeon restoration measures can be taken. This research supports the 'Restoration ecology of native species and their habitats' theme of the Huron-Erie Corridor Initiative (www.huron-erie.org).

Introduction

Lake sturgeon (*Acipenser fulvescens*), the largest and most long-lived native fish species in the Great Lakes, are late-maturing and intermittent spawners. In some parts of their geographic range, first reproduction in males occurs at 8-12 years old, they spawn every 2-7 years, and they have a potential lifespan of 55 years, whereas females first reproduce at 14 to 33 years old, reproduce every 4-9 years, and have a potential lifespan of 80-150 years (Bruch et al., 2001). Lake sturgeon spawn in the spring over rocky substrate in rivers and streams with a swift current when the water temperature reaches 8.8-21.1°C (Bruch and Binkowski, 2002; Nichols et al., 2003). Lake sturgeon are listed by the state of Michigan as threatened with extinction (MDNR, 1999).

Unlike many populations of lake sturgeon, the population sampled for this study reside in the Great Lakes proper and spawn in a large, deep, connecting channel, as opposed to reproducing in a shallow tributary of the Great Lakes, or smaller inland lakes and tributary systems within the Great Lakes basin (Manny and Kennedy, 2002; Thomas & Haas, 2002; Nichols et al., 2003; Caswell et al., 2004). Also, the Huron-Erie Corridor (HEC, Fig. 1) is unobstructed by dams, allowing for both immigration and emigration of sturgeon. Tag returns (Thomas and Haas, 2002) and telemetry data (Caswell et al., 2004) have shown that lake sturgeon move freely within the HEC but do not travel very far outside the corridor.

Fig. 1. Map showing the boundaries of the Huron-Erie Corridor (www.huron-erie.org).

Until the 1800s, lake sturgeon were very abundant in the HEC but have been reduced to <1% of their historical abundance by spawning and nursery habitat destruction, overfishing, and pollution (Tody, 1974). Recently in the St. Clair River (SCR), lake sturgeon recruitment has been relatively consistent, possibly due to the implementation of the Clean Water Act in 1972 and conservative sport fishing regulations beginning with a spawning season closure in 1983 (Thomas & Haas, 2002). Since 1999, sport angling for lake sturgeon in Michigan waters of the SCR is open from 16 July to 30 September and the harvest limit is one fish per year per person, within the slot limit of 1067-1270 mm (42-50 inches). The objective of the slot limit is to minimize harvest of mature females. A fish that is harvested must be tagged and registered at a MDNR registration station within 24 hours of capture. Recreational angling in Ontario waters of the SCR is open year round, with a harvest limit of one fish per day per person and no size limit. Commercial fishing for lake sturgeon is not allowed in the SCR by either Michigan or Ontario; however, there is an active commercial fishery in Ontario waters of southern Lake Huron.

The sex ratio of lake sturgeon in the SCR is not known. Fishery managers need to know the status of the female sturgeon for accurate population evaluations because they mature later and spawn less frequently than males (McLeod et al., 1999). Lake sturgeon are not externally dimorphic (Vecsei et al., 2003). Currently, biologists determine sex only if a fish is releasing gametes or by making an incision to visually inspect the gonads. However, due to the protected status of the species in the state of Michigan, surgery is rarely performed. Knowing the sex ratio of lake sturgeon in the SCR is important because harvest regulations are based on sex-specific length, weight, and age data. Currently, this dataset is limited to those few individuals that when collected are freely releasing gametes; 14% of lake sturgeon sampled by the Michigan Department of Natural Resources (MDNR) from 1997-2007 were releasing gametes. Methods used to identify the sex of North American sturgeons include gross visual and histological studies (Bruch et al., 2001), ultrasound and endoscope (Kynard and Kieffer, 2002; Colombo et al., 2004; Wildhaber et al., 2005), and analyses of sex steroids in blood plasma (Webb et al., 2002; Feist et al., 2004). Measuring the sex steroids estradiol (E_2) and testosterone (T) and the phosphoprotein vitellogenin (Vtg) is less invasive than surgery and analysis of relative concentrations of these indicators can be used to assign sex because of sex-specific profiles of reproductively cycling fish (Rosenblum et al., 1987; Cornish, 1998; Barannikova et al., 2004). Given the limitations on using surgical procedures with the lake sturgeon, we chose to assign sex using blood sex hormones. For this study, we followed the methods presented by Webb et al. (2002) because the technique was minimally invasive, non-destructive, and had an accuracy rate of 85% for females and 79% for males. We also evaluated the potential utility of plasma vitellogenin protein, an egg-yolk precursor, as a female-specific indicator.

In sturgeon, as in most teleosts, plasma sex steroid concentrations are low or undetectable until gametogenesis, when E_2 generally predominates in females and T generally predominates in males. Seasonal changes in male and female lake sturgeon hormone levels are such that T and E_2 are elevated in the spring prior to spawning, drop drastically post-spawn, and are elevated again in the fall (McKinley et al. 1998). Vtg is the primary precursor of egg yolk proteins and may be used to determine female gender under natural conditions (Heppell et al. 1999). Therefore, our objective in this study was to determine the sex of sexually mature and immature

lake sturgeon by measuring the concentrations of sex hormomes E_2 and T and the phosphoprotein Vtg in blood plasma by radioimmunoassay (RIA) and an enzyme-linked immunosorbent assay (ELISA), and to evaluate this technique as a tool for lake sturgeon population management. RIA and ELISA are the accepted methods used for analyzing steroid hormones and Vtg in fish plasma, respectively. There are many papers in the literature that discuss these assays and their use with a wide variety of fish species. For simplicity, an overview of reproductive steroids in fish and their analysis by RIA has been discussed by Kime (1993). Information on Vtg and its analysis by ELISA was presented by Heppell et al. (1999).

Methods

Study area

The HEC (Fig. 1) is 160 km long from southern Lake Huron to the western basin of Lake Erie. It includes southern Lake Huron, the St. Clair River, Lake St. Clair, the Detroit River, and the western basin of Lake Erie. The HEC separates Michigan and Ohio, USA from Ontario, Canada. The Michigan shoreline is highly developed for industry, navigation, and residences, and the waterway is heavily used for intra- and international shipping, sport and commercial fishing, and recreational boating. The SCR flows from north to south between lakes Huron and St. Clair in the northern portion of the Huron-Erie Corridor.

Detailed descriptions of the SCR can be found in other publications (e.g. Edsall et al., 1988; Edsall and Gannon, 1991; Bolsenga and Herdendorf, 1993; Thomas and Haas, 1999). Lake sturgeon for the present study were collected near known spawning grounds at the headwaters of the SCR near Lake Huron and in the North Channel of the deltaic mouth, upstream of Lake St. Clair (Manny and Kennedy, 2002; Fig. 1).

Sample collection

Lake sturgeon were sampled from the SCR during May and June in 2004 and 2005 following the setline procedures described in Thomas and Haas (1999). Data were collected for morphometrics, relative condition factor, and age for each lake sturgeon as described in Craig et al. (2005). Those fish releasing gametes were identified as male or female in the field. Approximately 2 mL of blood was drawn from the caudal vein of 115 fish (Fig. 2), dispensed into a vaccutainer coated with sodium heparin, and stored on wet ice until returning to the USGS-Great Lakes Science Center, at which time the blood samples were spun at 3500 rpm for 10 min at room temperature. Plasma was transferred to cryovials and stored at -80ºC until they were shipped overnight on dry ice to the USGS-Columbia Environmental Research Center for radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) analyses.

Fig. 2. Collecting blood from the caudal vein, immediately posterior to the anal fin.

Steroid extraction and analysis

Plasma samples for steroid analyses were extracted using diethyl ether to separate steroids bound to proteins. The steroid-containing ether phase was quick-frozen on dry ice, and the resulting supernatant was placed in a 30°C water bath and evaporated under nitrogen. The steroid residues were then reconstituted in phosphate buffered saline with 1% gelatin (PBSG, pH 7.0) and stored at -20°C until assayed.

Estradiol and T were assayed using the RIA method. Steroid concentrations were determined through competition of the standard or extracted sample and a constant amount of radiolabeled steroid using a fixed antibody titer. Radiolabeled E_2 and T were obtained from Perkin Elmer (Wellesley, MA). The antibodies against E_2 and T were purchased from Sigma-Aldrich (St. Louis, MO) and MP Biomedicals (Solon, OH), respectively. Extracted samples were incubated with the antibodies and radiolabeled steroid overnight at 4°C. The following day, a chilled solution of dextran-coated activated charcoal in PBSG (0.4% dextran, 0.625% charcoal) was added and allowed to incubate before centrifugation at 0°C for 20 min at 2800 rpm. A portion of the resulting supernatant was added to a scintillation vial containing 5 mL of scintillation cocktail (EcoLume®, Fisher Scientific). A scintillation count was performed, with the resulting values showing the amount of antibody bound to labeled steroid, which is inversely proportional to the amount of free steroid in the sample. A standard curve of a known serial dilution of each hormone was used to allow calculation of steroid concentrations in the unknown

samples. Lake sturgeon samples were analyzed in duplicate, and results are expressed as picograms (pg) of steroid/mL of blood plasma.

Estradiol assay sensitivity was 665 pg/mL at 20% binding and 9 pg/mL at 80% binding. Testosterone assay sensitivity at 80% binding was 6 pg/mL and 299 pg/mL at 20% binding. Cross-reactivities of the antibodies used in these assays with other similar steroids are reportedly less than 10% according to the vendors (for E_2 and T). Estradiol intra-assay variation was 12%, and interassay variation was 3 percent. Testosterone intra-assay variation was 1%, and interassay variation was 16 percent. The assay was validated for measurement of E_2 and T by verifying that serial dilutions of sample were parallel to a standard curve. The slopes were 0.99 and 0.91 for E_2 and T, respectively. These values were obtained from the regression of hormone measured and concentration of hormone added to a plasma sample.

Vitellogenin analysis

Analysis of vitellogenin was conducted by using ELISA [adapted from Folmar et al. (1996)]. Antibodies specifically against lake sturgeon Vtg are not available, however antibodies to both shovelnose sturgeon (*Scaphirhynchus platoyrnchus*) and white sturgeon (*A. transmontanus*) Vtg were available. Cross-reactivity between the shovelnose sturgeon antibody and lake sturgeon Vtg antigen was very poor. In preliminary trials using the shovelnose sturgeon antibody and lake sturgeon plasma of females with vitellogenic eggs, serially diluted samples showed no variation in absorbance values indicating no cross-reactivity of the shovelnose sturgeon Vtg antibody with lake sturgeon vitellogenin. This suggests cross-reactivity was very limited between these species. However, when plasma from vitellogenic lake sturgeon was serially diluted and white sturgeon antibody was applied, a predictable trend was seen, with diluted samples having high absorbance values and less diluted samples having lower values. This suggests white sturgeon antibody and lake sturgeon antigen exhibit some cross-reactivity, and was therefore used in analyses for the present study.

A direct ELISA was performed by coating a 96-well microtiter plate with purified shovelnose Vtg (prepared by Kevin Kroll, Senior Chemist, University of Florida, Center for Environmental and Human Toxicology, FL, USA) and incubating overnight at room temperature. Additionally, samples and standards (again, purified shovelnose sturgeon vitellogenin used) were pre-incubated with the primary (rabbit anti-white sturgeon) antibody (a gift of Javier Linares-Casenave, University of California, Davis, CA, USA) overnight at room temperature. The following day, the coated plate was washed with wash buffer (phosphate buffered saline, pH 7.35 with 0.1% Tween 20) and blocked for non-specific binding by incubating with bovine serum albumin. The pre-incubation samples were added in duplicate to the plate and incubated for 2 hrs at room temperature. The plate was then washed with the buffer before the addition of the secondary antibody [goat anti-rabbit (Sigma, St. Louis, MO)] with an alkaline phosphate conjugant. It was necessary to incubate this mixture overnight at room temperature in order to have sufficient binding. After 24 hours, the plate was washed as before, and the substrate developer, 4-nitrophenyl phosphate (pNPP) (Sigma, St. Louis, MO) was added and allowed to incubate to create a yellow precipitate. Plates were read in an absorbance plate reader (Spectra MAX 190, Molecular Devices, Sunnyvale, CA) at 405 nm. In a direct ELISA,

Vtg concentrations are usually determined by quantifying the absorbance values in relation to the known values of the standard curve. However, there was no purified lake sturgeon protein with which to develop a standard curve and there was only limited cross-reactivity of the white sturgeon antibody with both the shovelnose and lake sturgeon vitellogenin. Therefore, Vtg concentrations from lake sturgeon samples are reported as absorbance units.

Intra-assay and interassay variation was 4 percent. The assay was tested to determine if Vtg measurement was accurate by creating a serial dilution of a plasma sample to determine if it was parallel to the standard curve. A slope of 0.72 was obtained from the regression of Vtg measured and concentration of Vtg added to a plasma sample.

Assignment of sex

A fish was assigned a sex of male or female based on concentrations of plasma E_2 and T, in combination with Vtg and the E_2:T ratio. Data for shovelnose sturgeon (Wildhaber et al., 2007) were used as reference values for establishing assignment criteria. The ratio of estradiol to androgen is commonly used to assess sex steroid data (Folmar et al., 1996; Goodbred et al., 1997; Ceapa et al., 2002) because females typically have more estradiol than testosterone (i.e., $E_2/T>1.0$) and males typically have more testosterone than estradiol (i.e., $E_2/T<1.0$), but the range of this value for an individual can vary widely depending on reproductive condition. We identified females as having E_2 >100 pg/mL and/or E_2:T ≥1 and males as having T ≥500 pg/mL and E_2:T <1. In cases where the E_2:T ratio was <1 but Vtg was high (absorbance <0.15) the fish was identified as female. Plasma hormone data from the 12 lake sturgeon for which sex was confirmed, based on exuded gametes, were used in the model equations for predicting sex of white sturgeon (Webb et al., 2002) to determine how well measurement of lake sturgeon E_2 and T predicted phenotypic sex.

Statistical analyses

Sex-specific descriptive statistics are provided as means ± standard deviations. Webb et al. (2002) developed sex classification models using stepwise discriminant function analysis (DFA) to choose the best variables for predicting sex and stage of sexual maturity, and quadratic DFA to classify white sturgeon into groups of sex or sex and stage of maturity. For this study we focused only on assigning a sex to each lake sturgeon and therefore used the two equations for sex only. Sex was assigned by the greater of the two values. Analysis of variance and pairwise comparisons (Tukey) were used to test for differences in measured parameters (alpha=0.05) among the different sex assignments.

Results

One-hundred fifteen lake sturgeon were sampled. Insufficient blood sample volume resulted in no analysis of four samples. Low blood sample volume resulted in partial analysis of 43 fish (steroids but not Vtg were analyzed for 39 samples, Vtg but not steroids were analyzed for four samples). The remaining 68 fish samples were fully analyzed as described above. Sex assignments based on plasma indicator criteria were made for 61 of the 111 individual plasma

samples assayed. The remaining 50 fish could not be confidently sexed based on the plasma criteria.

The accuracy of laboratory-assigned sex identification was not calculated because gonads could not be inspected. However, 12 of the tested fish were releasing gametes at the time of capture and provided an opportunity to validate a subset of the sex assignments. Sex determination by lab analyses and field examination for the12 fish agreed in all but two cases (Fig. 3). A female that was releasing eggs had hormone levels that classified it as a male (low E_2 and high T) and Vtg was also low. A male releasing milt was classified as undetermined because it had hormone levels below criteria thresholds (low E_2 and low T) despite an E_2:T <1; no Vtg data were available.

Using the hormone data from these same 12 lake sturgeon, the white sturgeon predictive models for sex (Webb et al., 2002) accurately identified 3 of the 4 field-assigned females and 7 of the 8 field-assigned males. The same 2 individuals that were misclassified by the model were also misclassified by the assay. All laboratory-assigned females were also predicted to be female using the model. However, the model results only matched 27 of the 45 laboratory-assigned males. All of the samples not classified by the laboratory assay were classified as female by the model.

Hormone values did not differ between the field-identified and laboratory-identified groups of males or females (Table 1). Estradiol measurements were significantly greater for all females than all males regardless of method of identification but the sexes had equivalent testosterone levels (Table 1). The fish for which sex identification could not be made had estradiol values comparable to males and lower than females but testosterone values were significantly lower than both males and females (Table 1).

The white sturgeon Vtg antibody showed some limited cross-reactivity with lake sturgeon Vtg antigen. However, lack of a purified lake sturgeon Vtg protein for a standard did not allow direct quantification of the amount of Vtg in each sample. Nevertheless, there was sufficient competition for white sturgeon antibody between the lake sturgeon (sample) and shovelnose sturgeon Vtg antigens (plate coating) to observe a relative difference in assay absorbance values for field-validated males and females. Although Vtg could not be accurately quantified in this assay, relative absorbance values were used to differentiate high (low absorbance values) from low (high absorbance values) Vtg concentrations. Means did not significantly differ between field-identified males and females because of fish to fish variation. Mean Vtg units for laboratory-identified males and females were significantly different (Table 1). Vitellogenin values did not differ for a given sex irrespective of method of identification (Table 1). Vitellogenin measured in unknowns was more like that measured in males than females (Table 1). Fifty percent of the field-identified females and 83% of the lab-identified females fell below 0.15 absorbance value threshold (Fig. 4). All males and unknowns had absorbance values greater than 0.15.

Fish of the same sex did not differ in age or weight irrespective of method of identification (Table 1). Laboratory males were younger and smaller than both sets of females (Table 1) but field males were similar in age and weight to females (Table 1). Fish of unknown sex were younger and smaller than females (Table 1) and field-identified males (Table 1) but not

laboratory-identified males (Table 1). The total length among field males, females and lab females did not differ, these fish were larger than lab males and the unknowns (Table 1). There were no differences in condition among any of the groups we identified (Table 1).

The sex ratio (M:F) of the 12 fish identified in the field was 2:1, whereas the sex ratio of the 50 fish identified by plasma alone was 2.8:1. When all 62 fish (lab plus field: 45 males, 17 females) were considered, the sex ratio was 2.7:1.

Table 1
Mean, standard deviation (SD), range, and sample size for morphometrics, blood plasma sex steroids, and vitellogenin for lake sturgeon assigned to a sex, based on field criteria (field) or laboratory criteria (lab). Those fish that could not be assigned a sex were identified as unknown. Means across columns with no or the same superscripts are not significantly different at $p>0.05$.

		field males	field females	lab males	lab females	unknowns
Total length	mean	1373[a]	1516[a]	1373[b]	1494[a]	1040[b]
(mm)	SD	90	111	32	85	239
	range	1275-1505	1412-1654	632-1505	1321-1613	654-1702
	N	8	4	37	13	49
Weight	mean	16[ab]	25[a]	10[bc]	25[a]	8[c]
(kg)	SD	4	4	6	4	6
	range	10-21	22-30	2-27	15-31	2-29
	N	8	4	37	13	49
Age	mean	22[ab]	30[a]	15[bc]	29[a]	13[c]
(years)	SD	5	8	7	6	7
	range	16-29	24-39	5-28	22-40	4-38
	N	7	3	35	11	39
Condition (*Kn*)	mean	0.948	1.014	0.988	1.038	0.998
(sex-specific)	SD	0.148	0.151	0.127	0.089	0.102
	range	0.778-1.173	0.881-1.188	0.700-1.280	0.876-1.221	0.777-1.235
	N	8	4	37	13	49
E_2	mean	26 [a]	3903 [b]	26[a]	2471[b]	29 [a]
(pg/mL)	SD	10	4704	7	3773	7
	range	14-40	25-9536	15-47	26-12468	12-43
	N	8	4	36	13	46
T	mean	3468 [a]	4607 [a]	2543 [a]	2673 [a]	174 [b]
(pg/mL)	SD	1554	2714	1873	2098	122
	range	71-5023	648-6835	457-6472	220-5513	22-470
	N	8	4	36	13	46
Vtg	mean	0.29 [a b]	0.25 [a b]	0.32 [a b]	0.11 [b]	0.27 [a]
(relative absorbance	SD	0.07	0.26	0.15	0.09	0.11
units)	range	0.18-0.34	0.01-0.60	0.17-0.69	0.01-0.32	0.17-0.62
	N	4	4	21	12	31

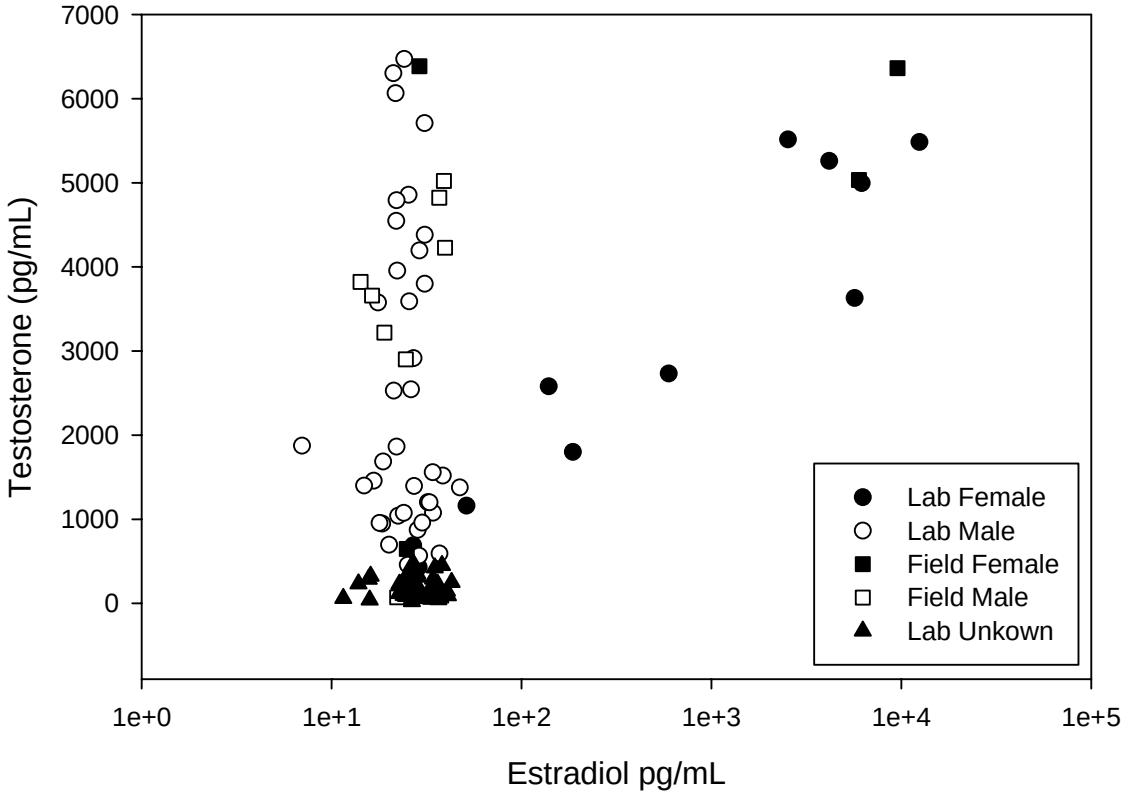

Fig. 3. Estradiol (E_2) and testosterone (T) values for female (closed symbol), male (open symbols), and unassigned (triangles) lake sturgeon. Arrows point to individual lake sturgeon whose field-identified sex did not match sex assigned through hormone and vitellogenin analysis.

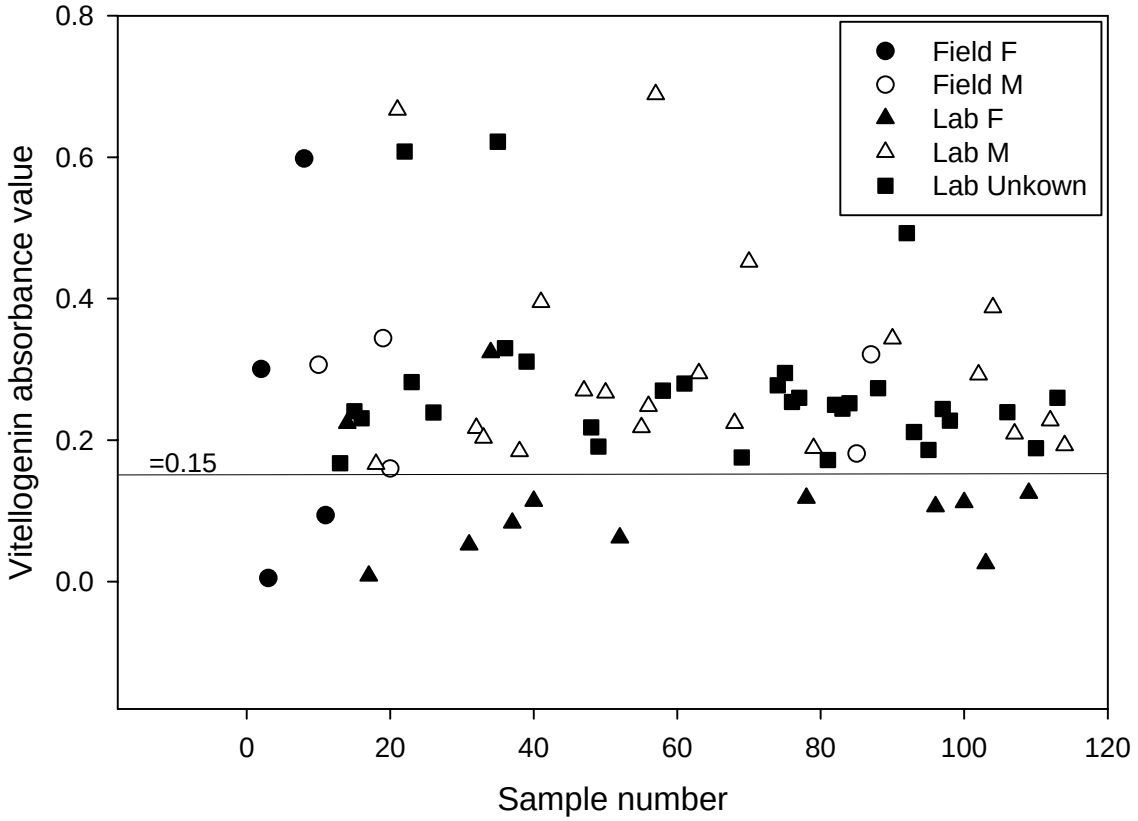

Figure 4. Scatter plot of absorbance values for an individual fish plasma sample resulting from analysis of lake sturgeon vitellogenin. Sex of field-identified fish was verified by observation of fish releasing gametes at time of capture. Sex of laboratory-identified fish was based on plasma criteria (see text). The horizontal line shows the absorbance value break-point that separates all males and fish that could not be assigned a sex from a majority of field and laboratory identified females.

Discussion

Sex ratio of SCR lake sturgeon

In fishes, plasma sex steroid concentrations are low or undetectable until puberty when gametogenesis begins (Okuzawa, 2002). During and after puberty, sex steroids will show seasonal and sex-specific patterns (Fitzpatrick et al., 1986; Rosenblum et al., 1987; Cornish 1998). In general, adult females with recrudescent ovaries or gravid females throughout vitellogenesis produce high levels of both E_2 and Vtg in contrast with males that are always low in E_2 but high in T during spermatogenesis (Redding and Patiño, 1993). Upon completion of gamete maturation proximal to spawning and after spawning, elevated sex steroids will decrease in both females and males (Rosenblum et al., 1987; King et al., 1994).

McKinley et al. (1998) showed that lake sturgeon steroid levels were high at pre-spawn, fell immediately after spawning, and then were elevated again in the fall. Similar results have been reported for several other sturgeon species for steroids and vitellogenin (Chapman et al., 1987; Amiri et al., 1996; Linares-Casenave et al., 2003; Barannikova et al., 2004). The wide range of E_2 and T values we observed for females and T values we observed in males suggests that the lake sturgeon in this study were collected during the spawning period. That these individuals were reproductively mature and not immatures is supported by their age and size. Also, given the age and size of some of the unknowns, we failed to assign sex to some individuals because post-spawn hormone levels had fallen below our criteria that could differentiate among males, post-spawn females, and immatures. Our ability to distinguish males from females could have been greater had the fish been collected in the very early spring or fall instead of late spring-early summer spawning period.

Confirmation of laboratory sex identification was limited to field observations. The field notations of male or female (based on releasing gametes from ripe and running individuals) provided valuable information that both validated assay results and supported inconsistencies. However, it was impossible to develop a quantitative model for predicting sex from hormones and vitellogenin because a limited number of lake sturgeon were releasing gametes at the time of capture. Our use of an existing model (Webb et al., 2002) developed for white sturgeon accurately identified 75% of the females and 88% of the males identified as such in the field. Model application and laboratory criteria yielded the same results and provide support for the criteria. Results were comparable to an 85% and 79% accuracy for female and male white sturgeon, respectively, modeled by Webb et al. (2002). The model seemed to overestimate the number of laboratory-identified female sturgeon as demonstrated by classification of all individuals below our established hormone criteria as females. Conversely, when Ceapa et al. (2002) applied the same model to predict sex of migrating *A. stellatus*, males were greatly overestimated. Our results may be expected from using this particular model given its greater accuracy for predicting sex of mature fish over immature fish and low accuracy (30%) in identifying immature males (Webb et al., 2002).

Despite the sex-specific differences in reproductive hormone concentrations between males and females, these measures alone are not completely reliable as a metric to assign sex because of their natural fluctuations during the reproductive cycle. This is particularly true post-spawn when both estrogens and androgens in males and females are at their lowest concentrations. Nevertheless, our results suggest that the majority of the

fish we could not assign sex to were immature or post-spawn males but not post-spawn females. Estradiol, Vtg, age, weight and length for the unassigned fish were most like those of the laboratory identified males and uniquely, as a group these fish had lower testosterone levels than all females and all males that we identified.

Accurate determination of Vtg could improve sex identification of lake sturgeon. Vitellogenin is only produced in females in response to increasing estradiol levels during growth of oocytes. Immature fish and males should have no or very low vitellogenin. Specker and Anderson (1994) developed one of the first enzyme-linked immunosorbent assays (ELISA) for Vtg to differentiate between striped bass lacking sexual dimorphism. However, routine extensive determination of Vtg requires species-specific reagents and this has limited the use of vitellogenin. In recent years, recognition that Vtg's responsiveness to estrogen makes it a useful bioindicator of a fish's exposure to estrogenic chemicals has led to the development of additional ELISAs (Jones et al., 2000). Direct measurement of Vtg and its use together with sex hormones to determine sex or reproductive stage of sturgeon is limited to only a few cases (Amiri et al., 1996; Ceapa et al., 2002; Wildhaber et al., 2007). Results from the present study, despite using a highly modified white sturgeon ELISA assay, showed that measurement of Vtg could be a useful means to determine lake sturgeon sex. A cut-off value was identified that effectively separated the males from females that we had identified with steroids. All of the unknowns separated with the males despite being identified as females in the model. This suggests the sensitivity of the modified assay was quite low. Using a Vtg assay specifically developed for shovelnose sturgeon, Wildhaber et al. (2007) showed that, regardless of reproductive stage, female Vtg levels were 100-fold greater than males. Purification of lake sturgeon Vtg and development of a specific lake sturgeon Vtg antibody would likely provide biologists with an ELISA assay adequate to determine sex of all but the youngest of the immature lake sturgeon.

Although lake sturgeon are listed as a threatened species in Michigan, data suggest that the SCR population is healthy and rebounding. Scuba divers have documented spawning activity and egg deposition (Nichols et al., 2003); juveniles have been caught, tagged, and recaptured (Thomas and Haas, 1999; Thomas and Haas, 2002); population estimates, although tenuous, indicate that the population is relatively large (Thomas and Haas, 2002); body condition is near the upper limits of ranges reported in the literature for other lake sturgeon populations at similar latitudes (Craig et al., 2005); and age composition suggests that recruitment since 1973 has been steady or increasing (Thomas and Haas, 2002; Craig et al., 2005). This study on sex ratio suggests that the slot limit for the Michigan sport fishery is effective. All 15 fish identified as female in this study were larger than the upper limit of harvestable size, thus supporting the objective of the slot limit regulation of protecting reproductively mature females from harvest. The implementation of slot limit size restrictions has also been effective in the recovery of white sturgeon populations in the Columbia River (Rienman and Beamesderfer, 1990).

Lake sturgeon sex ratios reported in the literature range from 1:1.1 to 1:9.6 female:male (Roussow, 1957; Haugen, 1969; Folz and Meyers, 1985; Lyons and Kempinger, 1992; Auer, 1999; Bruch and Binkowski, 2002; Smith and Baker, 2005). Method of sex determination, spatial and temporal proximity to spawning events during collection, and selective fishing practices all may contribute to the wide variation in

reported sex ratios for sturgeon. While a population sex ratio of 1:1 may be expected (Zubova, 1986), our conservative estimate indicates that the lake sturgeon operational sex ratio based on reproductively active individuals (Emlin and Oring, 1977) in the SCR is 1 female to 2.7 males. When Moos (1978) studied shovelnose sturgeon year-round in the Missouri River, he similarly reported an overall population sex ratio of 1:1.2, whereas the ratio of only those that were identified as spawners was 1:2.3. The Michigan Department of Natural Resources has sampled 1,111 lake sturgeon from 1997-2007, of which 14% of the fish were releasing gametes, resulting in a ratio of 1:6.6. Lake sturgeon, like shovelnose sturgeon, are intermittent spawners and only 45% of the adults within a population may be sexually active and spawn during a given season (Bruch, 1999; Bruch et al., 2001; Bruch and Binkowski, 2002). Because males mature at an earlier age and spawn more frequently than females, it is reasonable that there would be more spawning males per female during any given spawning period.

Genetic diversity within lake sturgeon populations is the cornerstone to successful natural reproduction (Drauch and Rhodes, 2007). Polyandrous and polygynous breeding by lake sturgeon maximizes the genetic diversity of offspring by maximizing the opportunity for each spawning adult to mate with numerous individuals (Bruch and Binkowski, 2002). When numbers of fish are low, diversity is increased when the ratio of spawning females to males favors females because eggs are more limited than sperm. In the present study, we have estimated the number of reproductively mature lake sturgeon females relative to males using non-invasive methods as 1 female to 2.7 males.

Current sport fishing regulations for lake sturgeon harvest in Michigan waters of the SCR and Lake St. Clair are based on a limited dataset of sex-specific length, weight, and age data collected from fish releasing gametes at the time of capture. Sex ratio information is needed by fishery managers to estimate spawning stock biomass because usually only female fish and their maturity and fecundity are considered in such analyses. The sex ratio of a fish population is most valuable when combined with length or age data or both, and these data are particularly important for species such as lake sturgeon, which show sexual dimorphism in growth, distribution, habitat use, vulnerability to capture, or other behaviors (Fabrizio and Richards, 1996). Researchers have been collecting both length and age data of lake sturgeon in the Great Lakes for well over a decade. The addition of sex ratio to that data would help refine sport fishing regulations for lake sturgeon in parts of their range, should that be necessary.

RIA and ELISA as tools in lake sturgeon management

Fishery managers need to understand the complex nature of the lake sturgeon reproductive cycles so that effective management and restoration procedures can be developed. Age at first maturity and duration between spawning events for both sexes within and among populations is highly variable (Peterson et al., 2007, and references within). Another obstacle to studying their reproduction is that lake sturgeon are not externally dimorphic and the temporal period when gametes are easily extruded by squeezing is short compared with many other freshwater fish species. Their threatened status in Michigan makes it undesirable to use invasive techniques such as surgery to determine sex, while the population-specific variation makes it difficult to directly apply what is known about other populations to the lake sturgeon in the SCR. Each population

needs to be thoroughly studied in its own right if managers are going to make science-based decisions about restoration and management efforts.

Research on methods to identify the sex of sturgeon include gross visual and histological studies on lake sturgeon (Bruch et al., 2001), analyses of sex steroids in blood plasma of white sturgeon (Webb et al., 2002; Feist et al., 2004), and ultrasound and endoscope studies on shovelnose sturgeon (Colombo et al., 2004; Wildhaber et al., 2005), and shortnose sturgeon (*Acipenser brevirostrum*, Kynard and Kieffer, 2002). For this study, we chose to use the methods presented by Webb et al. (2002) because the technique was minimally invasive, non-destructive, and had an accuracy rate of 85% for females and 79% for males.

There were several advantages to field personnel in determining sex by RIA and ELISA. Whole blood samples were collected in the field while on a boat on the river; fish handling was relatively short and did not require administering antibiotics or anesthesia; and blood collection was fast and easy, required little training, and was inexpensive. Collecting and storing the plasma upon returning to the GLSC was also quick, easy, and inexpensive. However, there are several drawbacks to analyzing blood plasma hormones for lake sturgeon management. The assays cannot be done in the field, so further research on known sexes cannot be performed unless the fish is tagged and recaptured the next year, but recaptures are uncommon. Both assays require skilled personnel and equipment, and RIA also requires permits for working with radioactive reagents, thus requiring managers to outsource the laboratory processing. The vitellogenin assay uses an antibody to vitellogenin that can be species-specific, and only gulf sturgeon (*Acipenser oxyrhynchus desotoi)* vitellogenin antibodies are commercially available. The cost to run E_2, T, and Vtg ranges from $50 - $100 USD per fish, which is not included in the budget for most agencies, so must be obtained from outside funding sources through competitive grants.

The laboratory portion of the study produced mixed success attributable to two primary causes based on 1. the biology of the fish (as discussed above) and 2. the methods used in the field collection portion of the study. Procedurally, several modifications in the field collection could be made to ensure greater success by other researchers. At least 3 mL of whole blood could be collected from each fish so that a minimum of two 1-mL plasma samples could be available for assays. In addition to providing a backup in case something happens to one of the vials, 2 vials of plasma allow the laboratory to run steroids and Vtg independently, since Vtg should not be repeatedly frozen and thawed. This is not true for plasma steroids. It is highly desirable that a laboratory perform and provide the appropriate QA/QC to ensure data quality, and to do so, more plasma is needed either by bleeding more fish or collecting more blood per sturgeon. Both quantity and quality of plasma were issues in our analyses. Many of our vials contained red blood cells, indicating that plasma samples were handled improperly. Red blood cells may have contaminated the plasma during plasma transfer, or the red blood cells may have been lysed during bleeding or blood handling. Lysing can be prevented by removing the needle prior to slowly returning the blood to the heparinzed vaccutainer and being certain the blood remains on wet ice until it is centrifuged. Centrifugation should be done at 3500 rpm for no more than 10 min. If straw-colored plasma has not clearly separated, the sample can be centrifuged one more time, but no more, as the sample will become too warm. Care should be taken when transferring the

plasma from the vaccutainer to the cyrovial to ensure that no red blood cells are transferred.

There are other non-destructive techniques (e.g., endoscope and ultrasound) for sturgeon sex identification that may meet the immediate needs of managers. The fiber-optic endoscope (i.e., borescope) is a quick, cost-effective, easy-to-learn, low-tech sexing method, although is invasive because the endoscope is inserted into the urogenital opening. Ultrasound has many of the same advantages as the endoscope plus is non-invasive, but it tends to cost more and produce less accurate results. Depending on sturgeon species, spent, non-ripe, or immature females may be difficult to identify, and the technique has a limited ability to distinguish between fat stores and gonad (Wildhaber et al., 2005). Three studies on three species of sturgeon using these two techniques have produced very positive results. Kynard and Kieffer (2002) studied shortnose sturgeon and had 100% success identifying mature females and were able to stage eggs qualitatively, but they were not able to determine unripe or immature females from males. Overall, the success rate was 39% for shortnose sturgeon. Wildhaber et al. (2005) were able to identify immature or unripe females from males in shovelnose sturgeon. Overall, the success rate was 85%: 93% and 75% accuracy for males and females, respectively. Wildhaber et al. (2005) also looked at a limited number of the endangered pallid sturgeon (*Scaphirhynchus albus*) and had an overall success rate of 57%. This species tended to have a thick, opaque urogenital duct membrane, which prevented the researchers from viewing the gonads in some cases. The same authors also used a portable field ultrasound unit on both species, with an overall success rate of 68% for shovelnose sturgeon; 76% and 59% accuracy for males and females, respectively. For pallid sturgeon, the overall success rate was 86%. Colombo et al. (2004) also used a portable ultrasound on shovelnose sturgeon, with an overall success rate of 86%: 96% and 80% accuracy for males and females, respectively.

Conclusions

Final conclusions of this study are 1. Lake sturgeon can be sexed by RIA and ELISA, but higher rates of accuracy could be obtained from a species-specific predictive model based on seasonal hormone and vitellogenin profiles of all reproductive stages ; 2. sex assignment of lake sturgeon by RIA and ELISA does not meet the needs of managers in the HEC; 3. A quick, accurate, and reliable field assay for sex identification is needed; 4. Endoscopes and portable ultrasounds are non-invasive procedures that could be tested on lake sturgeon to determine their effectiveness in sexing lake sturgeon; and 5. Evidence in this study supports the Michigan slot limit of 1067-1270 mm as an effective tool in protecting large, reproductively mature females from harvest. Future studies should include the use of equipment such as endoscopes and ultrasounds for on-site sex identification and maturity classification while collecting blood plasma (following recommended methods modifications) and eggs (via biopsy) to support research aimed at designing a field hormone assay, staging maturity, and studying the reproductive cycle of this threatened population.

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