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MANAGEMENT BRIEF

Assigning Sex and Reproductive Stage to Adult Lake Sturgeon using Ultrasonography and Common Morphological Measurements

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Abstract

Sex determination of fish species is difficult to assess when sexual dimorphism and gametes are not apparent. For threatened and endangered fish species, noninvasive techniques are needed when determining sex to minimize stress and the potential for mortality. We evaluated the use of a portable ultrasound unit to determine sex of Lake Sturgeon *Acipenser fulvescens* in the field. Ultrasound images were collected from 9 yellow-egg (F2, F3), 32 black-egg (F4, F5), and 107 fully developed male (M2) Lake Sturgeon. Two readers accurately assigned sex to 88–96% of fish, but accuracy varied in relation to maturity stage. Black-egg females and fully developed males were correctly identified for 89–100% of the fish sampled, while these two readers identified yellow-egg females only 33% and 67% of the time. Time spent collecting images ranged between 2 and 3 min once the user was comfortable with operating procedures. Discriminant analysis revealed the total length : girth ratio was a strong predictor of sex and maturity, correctly classifying 81% of black-egg females and 97% of the fully developed males. However, yellow-egg females were incorrectly classified on all occasions. This study shows the utility of using ultrasonography and a total length : girth ratio for sex determination of Lake Sturgeon in later reproductive stages around the spawning season.

Fish biologists use sex and maturity information to determine population demographics, assess factors that may be limiting the reproductive capacity of a population, select fish for propagation programs, regulate harvest, and assess life history strategies (Beamesderfer et al. 1995; Williot et al. 1997; Bruch 1999; Caron et al. 2002). However, sex determination of fish species that are not sexually dimorphic can be difficult. The sturgeons (family Acipenseridae) are an example of a fish family in which phenotypic sexual dimorphic traits are not easily apparent. Yet, the ability to determine sex of individual sturgeon is important for conservation as 19 of the 27 sturgeon species are considered endangered in their native range (IUCN 2015).

A number of different techniques that also minimize stress and the potential for mortality have been developed to determine the sex and maturity of sturgeons. Endoscopy, gonadal tissue biopsy, sex steroid profiles, laparotomy, morphology, and ultrasonography all have been used to obtain sex and maturity information from sturgeons (Moghim et al. 2002; Webb et al. 2002; Vescei et al. 2003; Colombo et al. 2004; Wildhaber et al. 2007; Lallaman et al. 2008; Chebanov and Galich 2009; Craig et al. 2009; Petochi et al. 2011). No single technique has as yet gained dominance as the candidate methods vary in accuracy, cost, risk of harm to the subject, the skill level or amount of training required to collect the data, and the time required to collect and process data and samples.

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Ultrasonography has been used to study fish reproduction for 30 years on more than 21 different fish species (Novelo and Tiersch 2012). Ultrasonography has advantages over other methods in that acquisition of results is immediate, per-sample cost is low after the necessary technology has been obtained, and the procedure is nonintrusive, which greatly minimizes stress on the subject. Disadvantages of ultrasonography include the relatively high cost of ultrasound units (US$5,000–$45,000 for portable units), the amount of training required to interpret ultrasound images, and a lack of units with the durability to perform in a field setting. While ultrasonography is regularly applied as a tool to determine the sex and maturation of sturgeon (Chebanov and Galich 2009; Novelo and Tiersch 2012), it has not been evaluated for Lake Sturgeon *Acipenser fulvescens*, a species of conservation concern across the Laurentian Great Lakes and upper Mississippi River drainage basin. In addition, the efficacy of using ultrasonography to identify the sex and maturation of Lake Sturgeon in the field has yet to be determined.

Morphological and external characteristics have also been used to determine the sex of adult sturgeon (Billard 2002; Vescei et al. 2003; Maltsev and Merkulov 2006; Podushka 2008; Falahatkar and Poursaeid 2013). Maltsev and Merkulov (2006) used craniological measurements to determine the sex of adult Russian Sturgeon *A. gueldenstaedti*. Using discriminant analysis, a model was developed that correctly classified sex for 90% of the individuals sampled. Falahatkar and Poursaeid (2013) used a suite of commonly collected morphological measurements to describe the sex of Beluga *Huso huso*, (also known as Great Sturgeon) and determined that the sex of the fish could not be correctly identified using only external characteristics. Vescei et al. (2003) accurately sexed 82% of live sturgeon using the shape of the urogenital opening; however, accuracy varied with species. Live White Sturgeon *A. transmontanus* were accurately sexed on all occasions, while the sex of Shortnose Sturgeon *A. brevirostrum* and Atlantic Sturgeon *A. oxyrinchus* was determined 71% and 82% of the time, respectively. This same technique has been applied to Beluga and Lake Sturgeon with no success (Vescei et al. 2003; Falahatkar and Poursaeid 2013). Body color and the shape and structure of fins have also been evaluated (Billard 2002; Podushka 2008); however, due to accuracy and other shortcomings, none of these methods are commonly used to determine the sex of adult sturgeon. Morphological measurements that can be used to describe the sex of adult Lake Sturgeon would be helpful to managers when other methods of sex determination are not possible. Therefore, the objective of the current study was to determine the accuracy of ultrasonography and common morphological measurements at assigning sex and maturity to Lake Sturgeon during the spring spawning season.

METHODS

Lake Sturgeon collection.—Fish collection took place in the connecting channel between Lakes Huron and Erie known as the St. Clair–Detroit River system (SCDRS). Lake Sturgeon were collected using baited setlines deployed just prior to or during the spring spawning season in April–June, 2012–2014. Setlines consisted of a mother line with 25 or 50 hooks (1/0 and 9/0 sizes) baited with Round Gobies *Neogobius melanostomus* (Thomas and Haas 1999). Setlines were fished overnight and occasionally over two nights when inclement weather prevented retrieval. Lake Sturgeon captured were held in a 378-L holding tank with recirculating water on a research vessel. Total length, FL, weight, and girth (largest diameter posterior to the pectoral fins) were collected from each fish.

The sex of individual Lake Sturgeon was assessed in two ways. An initial determination of sex was made by applying hand pressure to the abdomen just posterior to the pectoral fins and moving towards the urogenital opening. If eggs or milt were observed to exit the urogenital opening, sex was assigned as a black-egg spawning female (F5) or a fully developed spawning male (M2), respectively (Bruch et al. 2001). If eggs or milt were not present fish sex was denoted as unknown. Second, visual inspection of the gonads following a laparotomy was performed on a subsample of Lake Sturgeon (>1,200 mm TL) that were to receive an acoustic transmitter as part of a companion study evaluating the movements of adult Lake Sturgeon throughout the SCDRS. Sex and maturity were assigned according to Bruch et al. (2001) (Table 1), and the incision was closed with sutures immediately after tag implantation.

Ultrasound data collection.—A portable SonoSite MicroMaxx Ultrasound System (SonoSite, Bothell, Washington) with a L38e/10-5 MHz transducer was used to collect images from each Lake Sturgeon that received an acoustic transmitter. The cost was $18,390, which included the SonoSite MicroMaxx unit, the L38e/10-5 MHz linear transducer (scan depth, 3.8-9.0-cm), SiteLink Image Manager 3.4.5 software (required to export images from the unit), a case for the unit, and a sun shade for scanning under direct sunlight. A small amount of ultrasound gel was placed in a Ziploc bag to decrease air interference with the Lake Sturgeon and transducer probe. The bag was placed over the transducer probe and held in place using a rubber band. To optimize image quality, the gain, display brightness, and depth settings were adjusted with the probe on the Lake Sturgeon. The depth setting was adjusted depending on the size of the fish and ranged from 3.8 to 9.0 cm. The exam type was constant and set to vascular. Images were collected in two-dimensional (2D) mode, and the best image was captured by adjusting the optimization setting: Gen (balance between resolution and penetration), Res (best possible resolution), and Pen (best possible penetration). The optimization setting adjusts the focal zone, aperture size, frequency (center and bandwidth), and waveform to enhance image quality (MicroMaxx 2008).

During ultrasound image collection, each Lake Sturgeon was restrained by two people and either placed on a measuring board

TABLE 1. Sex and maturity assigned to Lake Sturgeon as defined by Bruch et al. (2001).

Sex	Maturity	Classification
Female	Female virgin (Fv)	Small feathery looking, beige colored ovarian tissue.
	Early developing female (F1)	Pinkish–beige ovarian tissue with "brain-like" folds; visible whitish eggs < 0.5 mm in diameter.
	Early yellow-egg female (F2)	Yellowish–beige ovarian tissue with deep "brain-like" folds; eggs 1–2 mm in diameter.
	Late yellow-egg female (F3)	Large yellowish ovaries with deep lateral folds; yellow–greenish eggs 2.5 mm in diameter.
	Black-egg female (F4)	Large dark ovaries with deep lateral folds; shiny dark gray to black eggs 3 mm in diameter.
	Black-egg spawning female (F5)	Spawning female; eggs loose in body cavity; gray to black eggs 3.5 mm in diameter.
	Spent or recovering female (F6)	Ovaries are folded; ovarian tissue appears mushy, pinkish, and flaccid.
Male	Male virgin (Mv)	Testes ribbon-like to lumpy with lateral creases or folds; dark gray to cream color.
	Developing male (M1)	Testes tubular or lobed; light to dark gray color; substantial amounts of fat.
	Fully developed male (M2)	Testes large; cream to whitish color; deeply lobed filling the abdominal cavity.
	Spent or recovering male (M3)	Testes are much reduced; distinct lobes and whitish to cream color; little fat.

(28 cm width × 200 cm length) out of the water or submerged in the holding tank. Irrespective of the holding technique, the transducer probe was not submerged in the water. Six images were collected from each fish along the frontal and transverse planes as described by Chebanov and Galich (2009). While along the transverse plane, the ventral abdominal surface of the fish was scanned and images were collected from three different locations: ventral transverse (within 25 cm of the urogenital opening), middle transverse (between ventral transverse and anterior transverse images), and anterior transverse (within 25 cm of the insertion points of the pectoral fins). Along the transverse plane, the notochord was included in each image when possible and used as reference when interpreting internal structures (Figure 1a). Along the frontal plane, the mid-lateral region between the side and abdominal scutes was scanned. Images were collected from three different locations: ventral frontal (within 25 cm of the urogenital opening), middle frontal (between ventral transverse and anterior transverse images), and anterior frontal (within 25 cm of the insertion points of the pectoral fins). The transducer orientation remained constant for all images collected along each plane. Since each image was stamped for date and time, collection time for all six images was recorded. Bitmap images were stored on a compact flash card within the SonoSite MicroMaxx unit and exported to a computer using SiteLink Image Manager 3.4.5 software. Sex and maturity was assigned by analyzing images on the computer, independent of morphological data.

Ultrasound image analysis.—Due to difficulties distinguishing between the F2 and F3 as well as the F4 and F5 female reproductive stages in the field, reproductive stages of females were grouped together for analysis. All F2 and F3 females were grouped and considered as yellow-egg females and all F4 and F5 females were grouped and considered as black-egg females. Only one early developing (F1) and one spent or recovering (F6) female were captured along with five developing males (M1) and one spent or recovering male (M3) based on field verification through the incision. As a result of small sample sizes, these reproductive stages were not included in the analysis, and the echogenicity (ability of tissue to reflect sound waves) was only characterized for yellow-egg and black-egg females, along with fully developed (M2) males (Bruch et al. 2001; Moghim et al. 2002; Colombo et al. 2004; Wildhaber et al. 2007; Chebanov and Galich 2009). The definitions and characteristics described below were taken from Chebanov and Galich (2009). Yellow-egg females were characterized as having heterogeneous gonadal echostructures (organs containing different echogenicity or intensities), in which the gonads lacked a clear boundary with no bright hyperechoic (bright-white echoes of higher amplitude or density than the surrounding medium) lines, were not bound to muscle tissue, and seemed to resemble an overlapping cloud-like structure (Figure 1a, b). Yellow-egg female echostructures were also characterized as grainy, possibly containing ovigerous folds. Black-egg female gonads were characterized by the presence of single large eggs within the image (Figure 1c, d). The eggs absorb the echosignal well; therefore eggs were only present a few centimeters below the muscle tissue. Fully developed male gonads were characterized as

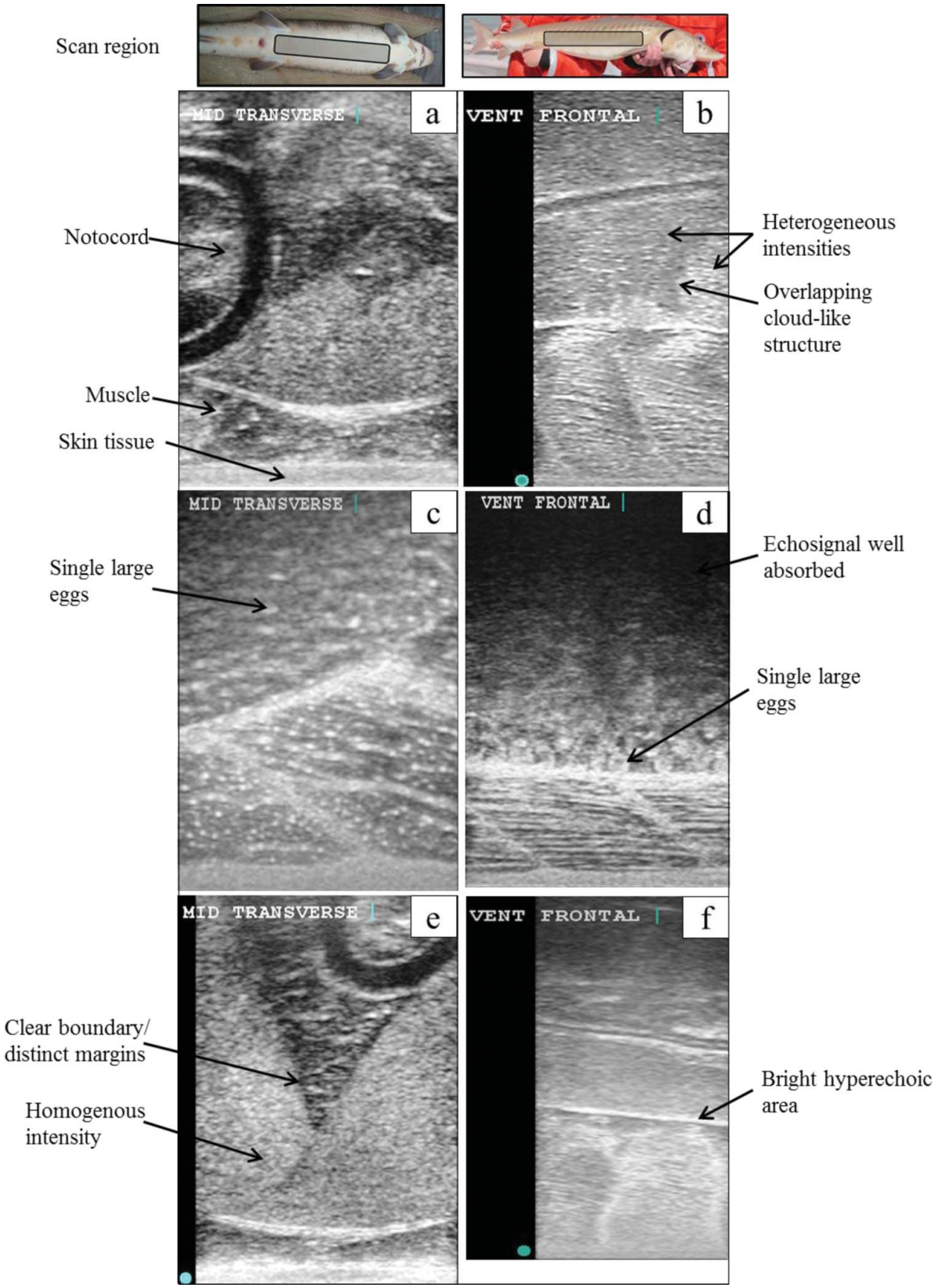

FIGURE 1. Scan region (transverse and frontal) and ultrasound images collected from (**a**, **b**) yellow-egg female, (**c**, **d**) black-egg female, and (**e**, **f**) fully developed male Lake Sturgeon. The notochord, muscle, and skin tissue features in (a) provide the reader a reference point when viewing images along the transverse plane. The arrows in (b) highlight the heterogeneous echosignal intensity and overlapping cloud-like structure characteristics used to assign sex and maturity to yellow-egg females. The arrows in (c) and (d) highlight the single large eggs and absorption of the echosignal apparent in black-egg females. The arrows in (e) and (f) highlight the distinct margins, homogenous echosignal intensity, and bright hyperechoic characteristics used to assign fully developed males.

having homogenous echostructures (uniform distribution of the echosignal; same intensity over the entire organ), in which the testes possibly were enclosed within bright hyperechoic lines, were fine grained with a clear boundary, and lacked ovigerous folds (Figure 1e, f). Based on these characteristics, two independent readers assigned sex and maturity. All six images were interpreted for each fish and the image or images containing the characteristics described above (e.g., bright hyperechoic area, clear boundaries) were noted. The best location of gonadal echostructure was recorded for each fish between the six locations where images were collected. The best location was defined as the location (i.e., ventral transverse, middle transverse, anterior transverse, ventral frontal, middle frontal, or anterior frontal) in which the characteristics of the gonadal echosignal described above could be most clearly discerned.

Data analysis.—Principal component analysis (PCA) was used to identify the set of morphometric variables most closely associated with sex and reproductive stage. Linear discriminant analysis (LDA) was then used to predict sex and reproductive stage based on the variables identified in the PCA. Total length, weight, girth, TL : girth ratio, and TL : weight ratio were the morphological variables used in the analysis. Variables were standardized so they all had a variance value of 1 and mean value of 0 to eliminate bias by those that contained the most variance in the original data. Normality of variables was assessed using skewness. When the absolute value of skewness was >1, variables were transformed (McCune and Grace 2002). As a result, weight data were $\log_{10}(x)$ transformed to meet the normality assumption. Weight data were again $\log_{10}(x)$ transformed to meet the multivariate normality assumption and homogeneity of within-group variances were tested using Bartlett's test. Prior probabilities were assigned based on the number of Lake Sturgeon in each group and leave-one-out cross validation was performed to determine the accuracy of the discriminant analysis. All analysis was conducted in program R (R Core Team 2013).

Pearson's chi-square test (χ^2) of independence was performed to determine whether sex and reproductive stage assigned by reader 1 and reader 2 differed from the sex and reproductive stage determined through visual examination of the gonads. To evaluate reader bias, the sex and reproductive stage assigned by each reader was compared with the known sex and reproductive stage using an age-bias plot (Campana et al. 1995). In an age-bias plot (hereafter referred to as "sex and stage identification plot"), the mean age (with 95% CI) from the reader is plotted for each distinct age. In our case the assigned sex and reproductive stage provided by each reader was plotted against the known sex and reproductive stage based on visual examination of the gonads. If the 95% CI did not contain the 1:1 agreement line then the plot suggests a systematic difference between the reader and the actual sex and reproductive stage. Data analyses were performed using the FSA package in program R (Ogle 2014). An alpha value of 0.05 was used to determine statistically significant differences for all tests.

RESULTS

Over the 3 years of this study of Lake Sturgeon, the sex and maturity of 9 yellow-egg and 32 black-egg females and 107 fully developed males were determined through visual examination of the gonads. Total length, weight, girth, TL : girth ratio, and TL : weight ratio varied among Lake Sturgeon sex and reproductive stage (Table 2). The first two principal components in the PCA analysis accounted for 98% of the variation in sex and reproductive stage (Figure 2). The first principal component (PC1) explained 85% of the variance and represented a contrast between black-egg females and fully developed males. The factor loadings were of similar magnitude suggesting all variables were influential on the principal component (Table 3). The second principal component (PC2) explained an additional 13% of the variation and, based on factor loadings, the most influential variables were length and the TL : girth ratio.

Since all of the morphometric variables were influential on PC1, all were included in the LDA (Table 4). The first axis (LD1) accounted for 96.4% of the between-group variability, while LD2 explained 3.6%. The cross-validated model correctly predicted the sex of Lake Sturgeon in 87% of the occasions, but accuracy was highly dependent on sex and reproductive stage. The overall misclassification rate was 12.8%. Yellow-egg females were misclassified on all occasions, and the model classified them as either black-egg females 22% of the time or fully developed males 78% of the time. Of all individuals sampled 78% were correctly classified

TABLE 2. Sex and reproductive stage, along with mean and range (in parentheses) TL (mm), weight (kg), girth (mm), TL : girth ratio, and TL : weight ratio of Lake Sturgeon collected in the St. Clair–Detroit River system, 2012–2014.

Sex and reproductive stage	TL	Weight	Girth	TL : girth ratio	TL : weight ratio
Yellow-egg female	1,577 (1,432–1,740)	26.6 (20.2–37.5)	649 (572–758)	2.44 (2.19–2.67)	60.8 (46.4–73.8)
Black-egg female	1,629 (1,462–1,800)	34.9 (22.0–51.0)	734 (597–892)	2.27 (1.90–2.47)	48.8 (34.6–67.3)
Fully developed male	1,426 (1,207–1,699)	19.2 (11.4–34.0)	566 (473–730)	2.53 (2.13–2.94)	77.0 (48.2–105.8)

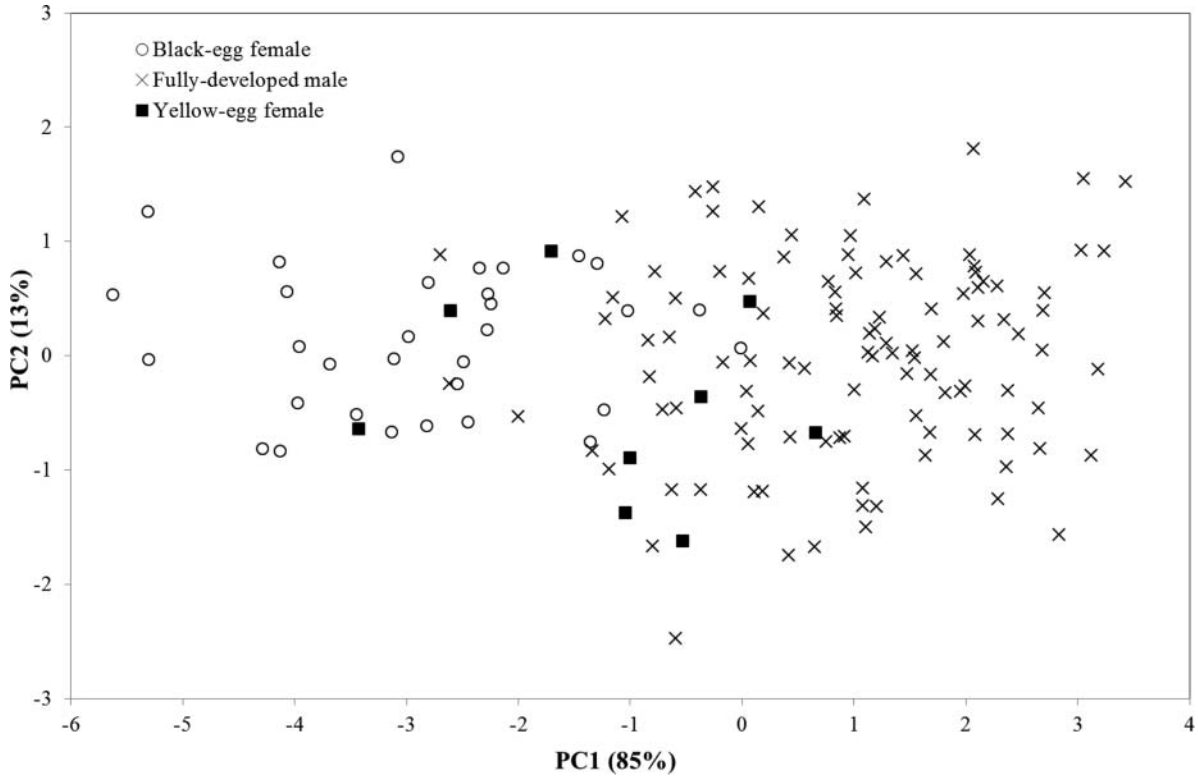

FIGURE 2. A plot of principal components (PC1 and PC2) from a PCA examining the sex and reproductive stage of Lake Sturgeon using morphological variables.

as black-egg females, 19% were misclassified as fully developed males, and 3% were misclassified as yellow-egg females. Of the individuals sampled and classified as fully developed males 97% were classified correctly, 2% were misclassified as black-egg females, and <1% were classified as yellow-egg females. Closer examination of the TL : girth ratio variable indicated that the largest TL : girth ratio for black-egg females was 2.47. Sixty-five percent of the fully developed males and 56% of the yellow-egg females had a TL : girth ratio > 2.47.

Correct assignment of sex and maturation stage of Lake Sturgeon from ultrasound images was best for black-egg females and fully developed males (Table 5). Reader 1 correctly assigned sex to 95% of females and 89% of males. The reproductive stage was correctly assigned to 67% of yellow-egg females, 100% of black-egg females, and 89% of fully developed males. When discrepancies were observed, they were primarily between yellow-egg females and fully developed males. Reader 1 assigned yellow-egg females as fully developed males in 22% of the individuals sampled and fully developed males as yellow-egg females in 11%. Reader 2 correctly assigned sex to 88% of the females and 96% of the males. The reproductive stage was correctly assigned to 33%

TABLE 3. Component loadings for the first two principal component axes (PC1 and PC2) from the PCA examining the sex and reproductive stage of Lake Sturgeon using morphological variables. The percent variance explained by each axis is denoted at the bottom of the table.

Morphological variable	PC1	PC2
Length	−0.43	−0.55
Girth	−0.48	0.11
Weight	−0.48	−0.17
TL : weight ratio	0.48	0.06
TL : girth ratio	0.36	−0.81
Percent of variance explained	85.2	13.5

TABLE 4. Linear discriminants for the morphological variables used to determine sex and reproductive stages of black-egg female, fully developed male, and yellow-egg female Lake Sturgeon; LD1 and LD2 are the two interpreted axes.

Morphological variable	LD1	LD2
Length	0.003	0.034
Girth	−0.008	−0.018
Weight	−23.362	−51.604
Length : weight ratio	−0.145	−0.245
Length : girth ratio	1.861	−6.452

TABLE 5. Field-verified sex and reproductive stage of Lake Sturgeon (based on visual examination of gonads) and the sex and reproductive stage assigned by each reader based on ultrasound images. The number and percentage (in parentheses) correctly assigned are reported.

Reader	Assigned sex and reproductive stage	Field-verified sex and reproductive stage		
		Yellow-egg female	Black-egg female	Fully developed male
Reader 1	Yellow-egg female	6 (67)	0 (0)	12 (11)
	Black-egg female	1 (11)	32 (100)	0 (0)
	Fully developed male	2 (22)	0 (0)	95 (89)
Reader 2	Yellow-egg female	3 (33)	0 (0)	4 (4)
	Black-egg female	1 (11)	32 (100)	0 (0)
	Fully developed male	5 (56)	0 (0)	103 (96)

of the yellow-egg females, 100% of black-egg females, and 96% of fully developed males. Again, when discrepancies were observed, they were primarily between yellow-egg females and fully developed males. Reader 2 classified yellow-egg females as fully developed males in 56% of the individuals sampled and fully developed males as yellow-egg females in 4% of the individuals. Pearson's chi-square test showed no statistical difference between sex and reproductive stage based on visual inspection of the gonads and sex and reproductive stage assigned by reader 1 ($\chi^2 = 0.51$, df = 2, $P = 0.77$) or reader 2 ($\chi^2 = 2.73$, df = 2, $P = 0.26$). Reader 1 showed a systematic difference when assigning sex and reproductive stage to fully developed males, often assigning them as yellow-egg females. Reader 2 showed no systematic differences (Figure 3).

The best location to view gonadal echostructure characteristics of Lake Sturgeon depended on sex and reproductive stage (Table 6). Yellow-egg female gonad characteristics were most often present at the ventral transverse, middle transverse, and middle frontal image locations. The gonadal characteristics of black-egg females could be seen well at all image locations, while the fully developed male gonadal characteristics were best viewed at the middle transverse and middle frontal locations. Using the time stamps on the ultrasound images, the time to collect all six images ranged between 2 and 3 min, once the user was comfortable with the procedures.

DISCUSSION

The sex of Lake Sturgeon was accurately assigned to 88% and 96% of the individuals sampled by the two readers, respectively, using ultrasonography. Classification of Lake Sturgeon in later stages of development was highly accurate; 89% and 96% for fully developed males and 100% for black-egg females for the two readers. However, at earlier stages of development accuracy declined and only 33% and 67% of yellow-egg females were correctly classified by the two readers. The ability to accurately predict sex of sturgeons is dependent upon time of year and reproductive stage of the sturgeon species being studied. Moghim et al. (2002) evaluated the use of ultrasonography on Stellate Sturgeon *A. stellatus* and correctly classified 97–100% of females and mature males, while only 76% accuracy was obtained on immature males. Colombo et al. (2004) correctly determined the sex of Shovelnose Sturgeon *Scaphirhynchus platorynchus* in 86% of the individuals sampled; however, accuracy of assigning sex in virgin females

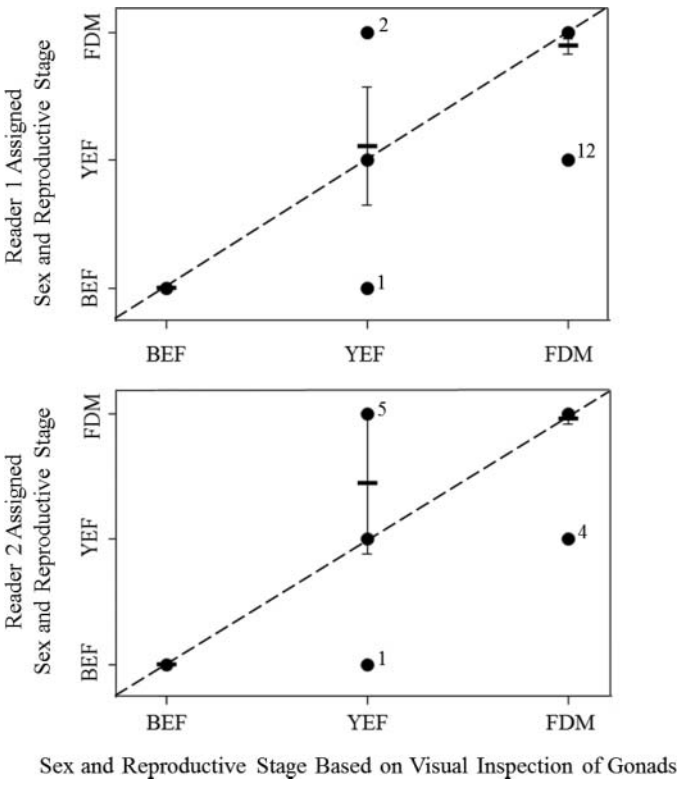

FIGURE 3. A sex and stage identification plot plotting the assigned sex, as determined by reader 1 and reader 2, against the known sex and reproductive stage of Lake Sturgeon based on visual examination of the gonads. The 95% CIs are shown as vertical bars. When the CI does not contain the dashed 1:1 line, differences exist. The number of fish incorrectly assigned can be seen next to the black points, which represent the actual sex and reproductive stage assigned by each reader. BEF = black-egg female, YEF = yellow-egg female, FDM = fully developed male.

TABLE 6. The percent of occasions in which reader 1 or reader 2 assigned ventral transverse, middle transverse, anterior transverse, ventral frontal, middle frontal, or anterior frontal as the best location in which the characteristics of the gonadal echostructure in Lake Sturgeon could be most clearly discerned.

Sex and reproductive stage	Image location					
	Ventral transverse	Middle transverse	Anterior transverse	Ventral frontal	Middle frontal	Anterior frontal
Yellow-egg female	22.6	38.7	6.5	6.5	22.6	3.2
Black-egg female	14.4	19.6	16.0	16.0	18.4	15.6
Fully developed male	12.7	41.7	7.4	6.8	28.1	3.3

(Fv) and F6 females decreased to 75% and 40%, respectively. Similar to the current study, Wildhaber et al. (2006) had difficulties distinguishing between males and early developing female Shovelnose Sturgeon. Further research is needed to assess the accuracy of ultrasonography to determine the sex and maturity of Lake Sturgeon during early developmental stages and at different times of the year.

Discrepancies in the current study were primarily between distinguishing fully developed males and yellow-egg females; however, several reproductive stages of Lake Sturgeon were not included in the analysis. Including these reproductive stages would likely decrease the accuracy of correctly assigning maturity to fully developed males and yellow-egg females. The accuracy of assigning sex and reproductive stage using ultrasound images may be improved as readers become more familiar with gonadal echostructure characteristics and the misclassification of yellow-egg females may decrease with sample size and experience. The gonadal echostructure characteristics of black-egg females are so distinct that accuracy would likely not decline at this reproductive stage. Since the SCDRS contains year-round resident Lake Sturgeon, all reproductive stages are available to be captured during the spring spawning season (Caswell et al. 2004). This increases the chances of incorrectly classifying sex and reproductive stage. Many populations of Lake Sturgeon are anadromous, migrating up tributaries to spawn in the spring (Harkness and Dymond 1961). In most cases, these populations will primarily consist of fully developed males and black-egg females, increasing the chances of correctly identifying sex and reproductive stage using ultrasonography.

The identification of a morphological characteristic allowing for the discrimination between male and female sturgeon would be of great value for sturgeon researchers. As a result, several morphological measurements and external traits have been evaluated (Billard 2002; Vescei et al. 2003; Maltsev and Merkulov 2006; Podushka 2008; Falahatkar and Poursaeid 2013). In the current study, discriminant analysis revealed that common morphological measurements may be used to predict sex and reproductive stage, correctly classifying 78% of black-egg Lake Sturgeon females and 97% of fully developed males. However, yellow-egg females were incorrectly classified on all occasions and were classified as fully developed males in 78% of the individuals sampled. This analysis suggests that these morphological variables can be used to separate black-egg females and fully developed males during the spring spawning season, and its utility should be tested on other populations of Lake Sturgeon where these reproductive stages primarily exist.

The correct classification in 88–96% of individuals sampled indicates that ultrasound is a useful noninvasive technique to determine the sex and reproductive stage of Lake Sturgeon in the field during the spawning season. In the current study, 70% of males but only 5% of the females expressed gametes when pressure was applied to the abdomen. Thus using gamete expression alone, the ratio of reproductively active females to males in the SCDRS was 1:37.5. The ratio reverted to 1:3.3 based on the sex assignment from the ultrasound images. This estimate indicates the operational sex ratio (Emlen and Oring 1977). Since only a proportion of Lake Sturgeon in a population spawn each year and males spawn more frequently than females (Harkness and Dymond 1961), the actual sex ratio of females to males may be closer to 1:1. Due to the small proportion of fish that expressed gametes in this study, collecting ultrasound images from Lake Sturgeon during spring spawning assessments will likely improve our sex ratio estimate in the SCDRS. This information can then be used to make informed management decisions regarding the Lake Sturgeon population in this system.

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