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# Gonadosomatic index and fecundity of Lower Missouri and Middle Mississippi River endangered pallid sturgeon estimated using minimally invasive techniques

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## Summary

Minimally invasive, non-lethal methods of ultrasonography were used to assess sex, egg diameter, fecundity, gonad volume, and gonadosomatic index, as well as endoscopy to visually assess the reproductive stage of *Scaphirhynchus albus*. Estimated mean egg diameters of 2.202 ± 0.187 mm and mean fecundity of 44 531 ± 23 940 eggs were similar to previous studies using invasive techniques. Mean *S. albus* gonadosomatic indices (GSI) for reproductive and non-reproductive females were 16.16 and 1.26%, respectively, while reproductive and non-reproductive male GSI were 2.00 and 0.43%, respectively. There was no relationship between hybrid status or capture location and GSI. Mean fecundity was 48.5% higher than hatchery spawn estimates. Fecundity increased as fork length increased but did so more dramatically in the upper river kilometers of the Missouri River. By examining multiple fish over multiple years, the reproductive cycle periodicity for hatchery female *S. albus* was found to be 2–4 years and river dwelling males 1–4 years. The use of ultrasonic and endoscopic methods in combination was shown to be helpful in tracking individual gonad characteristics over multi-year reproductive cycles.

## Introduction

Pallid sturgeon *Scaphirhynchus albus* Forbes & Richardson 1905 are endemic to the Missouri, and Middle and Lower Mississippi rivers, USA (Mayden and Kuhajda, 1997), and listed as a federally endangered species (Dryer and Sandovol, 1993). As with most sturgeons, this species is long-lived and requires access to large river systems to complete its life cycle (Pflieger, 1997). To understand the dynamics of *S. albus* populations throughout their range, knowledge of their reproductive biology and the factors affecting reproductive potential and spawning success are necessary (Wildhaber et al., 2011). Unfortunately, due to the rarity of reproductive adult *S. albus*, relationships among the various factors that influence the fish's reproductive characteristics (e.g. sex, size, age, geographic location, genetics, or hybridization) are difficult to collect. Development of this fundamental understanding requires accurate individual assessments of the sex and reproductive status and potential. This is difficult because sturgeon lack secondary sexual characteristics and have multi-year reproductive cycles (Scott and Crossman, 1973; Doroshov et al., 1997; Keenlyne, 1997). It is therefore necessary to develop and apply field assessment techniques that can quickly and reliably determine the sturgeon reproductive status and measure reproductive potential without injury to the fish.

Minimally invasive techniques such as ultrasonography and endoscopy have been used effectively to identify sex in fishes (see Bryan et al., 2007 for summary). Reproductive organs of sturgeon have been examined and described in numerous studies using an ultrasound (Moghim et al., 2002; Colombo et al., 2004; Wildhaber et al., 2005, 2007) and endoscope (Kynard and Kieffer, 2002; Wildhaber et al., 2005, 2007; Hurvitz et al., 2007; Divers et al., 2009; Matsche et al., 2011). Studies with sturgeon species have expanded these techniques to include measurements of internal fish reproductive structures such as egg diameter, gonad volume, fecundity, and reproductive stage (Kynard and Kieffer, 2002; Bryan et al., 2007; Wildhaber et al., 2007). In a previous study (Bryan et al., 2007), we compared these minimally invasive methods with more invasive methods (egg biopsy and necropsy) of estimating egg diameter, gonad volume and fecundity in shovelnose sturgeon *S. platorynchus* Rafinesque, 1820, a species closely related to *S. albus*. The objective of this study is to apply previous *S. platorynchus* developed methodology to the closely related but endangered *S. albus* and compare the minimally invasive ultrasonic and endoscopic techniques to the invasive techniques of estimating *S. albus* egg diameter, gonad volume, fecundity, and gonadosomatic index (GSI). Additional to the comparison of methods, we also determined the relationship between *S. albus* GSI and fecundity, and fish origin, hybrid status, and capture location. Due to the restricted abilities to perform invasive procedures on endangered species, this study has limited data to confirm the non-invasive estimates of internal reproductive structures.

## Materials and methods

### Fish source

*Scaphirhynchus albus* were collected by numerous State and Federal entities during the annual broodstock collection from 2006 to 2011 in the Interior Highlands [Mississippi River river kilometer (rkm) 1534.5 to Missouri River rkm 402.3 and the Osage River tributary] and the Central Lowlands Management Units (Missouri River rkm 402.3–1416.2 and the Platte and Kansas river tributaries; USFWS, 2008) (Fig. 1, Table 1). *S. albus* 750 mm or larger were sent to hatcheries as potential broodstock. Fish were not sent if they were previously determined to not qualify [e.g. hatchery or

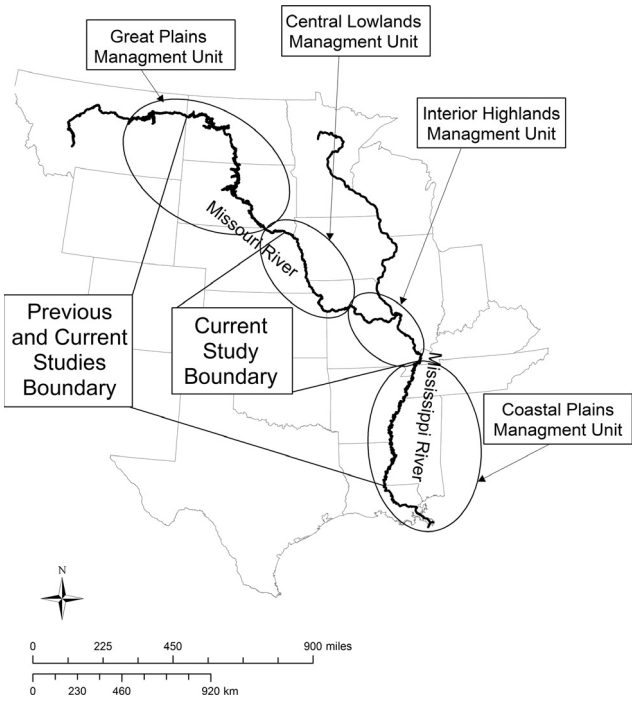


Fig. 1. Map of current study boundary extending from Lower Mississippi River, USA river kilometer (rkm) 1535.0 to Missouri River rkm 1416.2 encompassed by Central Lowlands and Interior Highlands management units (USFWS, 2008). Including previous *S. albus* studies, the boundary expands to Mississippi River rkm 506.0 upstream to the Missouri River rkm 2546.0 encompassed by all four management units

hybrid origin, intersex, or successfully spawned previously as broodstock (Kirk Steffensen, Nebraska Game and Parks Commission, personal communication)]. This includes three fish caught during 2006 propagation efforts from the Mississippi River below the Ohio River confluence, which is designated as the Coastal Plain Management Unit (Mississippi River rkm 0–1534.5). We also examined fish collected for telemetry tracking efforts of spawning sturgeon from 2003 to 2011 by U.S. Geological Survey (USGS) from the Lower Missouri River (rkm 0–1300; DeLonay et al., 2009) and which were at least 2 kg in weight. Additionally, we examined captive *S. albus* F1 broodstock, originating from spawned wild-caught individuals that were held at hatcheries their entire lives.

At capture, fish weight (nearest 5 g), fork length (FL; nearest mm), and capture rkm were recorded. A genetic sample determined whether the fish was a *S. albus* or a hybrid from a *S. platorynchus* × *S. albus* spawn (further referenced as hybrid status) and whether it was identified as a progeny from previous hatchery propagation efforts or assumed progeny of natural spawn when genetic information could not determine otherwise (further referenced as origin).

### Invasive methods

If the fish was a reproductive female, an incision was made for a telemetry surgery or for spawning preparations and an egg sample taken to determine mean diameter using microscopy. Prespawn egg samples were preserved in 10% neutral buffered formalin, and if collected within 7 days of the minimally invasive exam (removed 14 out of 31 samples), were used to measure the maximum diameter of 10 individual ripe eggs using a dissecting microscope (accuracy of $\pm$ 0.01 mm; see Bryan et al., 2007 for details). If the fish gonad was small (e.g. male or a non-reproductive female), gonad samples were not taken due to the potential of wounding internal organs or adversely affecting the function of the gonad; rather, the gonads were visually examined to determine sex through the incision. If no incision was made and the fish spawned in the hatchery, gametes were collected after hatch-

Table 1
Number and source of *Scaphirhynchus albus* examined during this study (FL = fork length, GSI = gonadosomatic index). Numbers in parentheses indicate gender confirmation using an invasive exam and when two numbers are present, a microscopic egg diameter measurement

| | | | | | | Missing data | | Fish in analyses | |
|---|---|---|---|---|---|---|---|---|---|
| Targeted fish | Sex | Initial fish exams | Incorrect gender | Multiple annual exams | Total fish exams | Weight not within 31 days | Factor in GSI analysis | GSI | Fecundity |
| Wild broodstock collection[a] | | | | | | | | | |
| Central Lowlands Management Unit from 2006 to 2011 | | | | | | | | | |
| $\geq$ 750 mm FL | F | 103 (42) | 1 | 9 | 93 (37–12) | 16 | 1 | 76 (31) | 28 (26) |
| | M | 174 (69) | 1 | 15 | 158 (56) | 15 | 14 | 129 (50) | 0 |
| Interior Highlands Management Unit from 2006 to 2011 | | | | | | | | | |
| $\geq$ 750 mm FL | F | 40 (16) | 1 | 3 | 36 (14–1) | 4 | 5 | 27 (10) | 9 (8) |
| | M | 21 (8) | 1 | 0 | 20 (7) | 2 | 5 | 13 (4) | 0 |
| Costal Plain Management Unit in 2006 | | | | | | | | | |
| $\geq$ 750 mm FL | F | 2 (0) | 0 | 0 | 2 (0) | 0 | 0 | 2 (0) | 2 (0) |
| | M | 1 (0) | 0 | 0 | 1 (0) | 0 | 0 | 1 (0) | 0 |
| Captive Broodstock in 1992, 1997, and 2002 | | | | | | | | | |
| Ready for spawning | F | 43 (22) | 0 | 0 | 43 (22) | 0 | 43 | 0 | 0 |
| Tracking | M | 34 (6) | 0 | 0 | 34 (6) | 0 | 34 | 0 | 0 |
| Lower Missouri River from 2003 to 2011 | | | | | | | | | |
| $\geq$ 2 kg[b] | F | 41 (25) | 7 | 0 | 40 (24–4) | 0 | 24 | 16 (11) | 8 (8) |
| | M | 49 (29) | 1 | 2 | 40 (20) | 0 | 23 | 17 (9) | 0 |
| Total | F | 229 (105) | 9 | 12 | 214 (97–17) | 20 | 73 | 121 (52) | 47 (42) |
| | M | 279 (112) | 3 | 17 | 253 (89) | 17 | 76 | 160 (63) | 0 |

[a]Fish were not examined if they were previously determined to not qualify for spawning [e.g. hatchery or hybrid origin, intersex, or successfully spawned previously as broodstock (Kirk Steffensen, Nebraska Game and Parks Commission, personal communications)].
[b]All fish were >670 mm FL.

ery spawning for sex determination and fecundity. Using all of these sources, sex was confirmed in 186 of 467 fish (Table 1). Gonads were assigned a stage of maturity based on *S. platorynchus* studies (Wildhaber et al., 2007) and either non-reproductive or reproductive (further referenced as reproductive status), where reproductive fish contained gonads that had the potential for maturing during the upcoming spawning season. For both sexes, non-reproductive fish were in stages I, II, III, and VI (immature, developing, vitellogenic/spermatogenic, and post-spawning, respectively); reproductive fish were in stages IV and V (pre-spawning and spawning, respectively). Total spawned egg count at the hatchery was collected using the von Bayer method (i.e. volumetric; von Bayer, 1908) and used as a fecundity estimate. Relative fecundity was calculated by dividing fecundity by fish wet weight in grams.

#### Minimally invasive methods

Because of the physical similarity between *S. platorynchus* and *S. albus*, the same ultrasonography and endoscopy techniques used on *S. platorynchus* in Bryan et al. (2007) were applied to *S. albus*. What follows is a brief summary of the methods and any modifications. Fish were examined during early spring and summer (March to May) to determine the gonad reproductive stage and consequently whether spawning would likely occur during the current spawning season. As the ultrasonic and endoscopic techniques are most accurate in assessing whether the closely-related *S. platorynchus* are in a reproductive (stages IV and V) or non-reproductive state (stages I, II, III and VI; Wildhaber et al., 2005; Bryan et al., 2007), we used both in combination to determine the sex and reproductive state (combined technique further referenced as UE) of the fish.

An ultrasound exam was performed by scanning each live submerged fish's body cavity in the transverse and frontal planes using either the Sonosite 180 Plus® with a L38/10–5 MHz multiple frequency, linear probe or Sonosite MicroMaxx® with a L38e/10–5 MHz multiple frequency, linear probe covered with a plastic sleeve and gel (Sonosite, Inc., Bothell, WA). Scans were conducted using the settings: 2D mode, general image optimization, small parts exam type, and depth and gain were adjusted as needed. The gonad characteristics (gonad volume, egg diameter, fecundity and GSI) were calculated from transverse gonad cross-sectional bitmap images from the ultrasound. The gonad cross-sectional area and diameters of all clearly defined eggs were measured from each cross-sectional image using IMAGE-PRO PLUS® version 6.3.0.512 software. An area-weighted number of random subsample of egg diameters was taken from each cross-section (mean number of eggs measured per fish and standard deviation was $28 \pm 9$). The total gonad volume was estimated using the three cross-sectional areas and an external gonad length measurement using gonad end points determined by the ultrasound. Fecundity was calculated using egg volume and gonad volume estimated from the ultrasound [ellipsoid volume with an axis ratio of 0.8781 (Bryan et al., 2007)]. Gonadosomatic index was calculated by dividing gonad weight by fish weight. Gonad weight was calculated using the gonad density:stage relationship from Bryan et al. (2007) with stage V for reproductive and stage II for non-reproductive fish. Fecundity was only calculated on reproductive female *S. albus*.

A Zibra Milliscope® flexible endoscope (Zibra Corp., Westport, MA) was used for endoscopic exams. Fish were examined by inserting the probe (<4.0 mm in diameter) into the urogenital duct, viewing the gonad structures through the urogenital duct wall. We documented the presence and color of eggs in females or presence of lobed testis or milt in the urogenital duct in males (Wildhaber et al., 2007).

#### Gonadosomatic index and fecundity analysis

We were interested in the relationships between GSI or fecundity, and the capture rkm, FL, sex, hybrid status, origin, and reproductive status. Fish were excluded from this analysis if sex was not correctly identified during the UE exam (Table 1). Twenty-five fish had multiple within-year exams, with 10 females and 12 males having two exams; one female and one male with three exams; one female with four exams. To maintain independence for statistical tests, only the first exam of each fish was used (29 exams excluded). This resulted in 467 usable fish exams of which 186 fish had sex confirmation. For the GSI and fecundity analysis, external and internal physical measurements of the fish had to be collected within 31 days from the UE exam (Table 1) so that the recorded physical measures would be similar to those during the UE exam. None of the *S. albus* examined living in the hatchery had physical measurements taken within 31 days of our UE exam, consequently all hatchery-origin *S. albus* included in the analysis lived in the river. Lastly, any fish with missing data in any factor of the analysis was removed (Table 1). This resulted in a total of 281 fish exams in the GSI and 47 fish in the fecundity analysis (Tables 1 and 2).

Capture location (measured as rkm) was used to determine latitudinal patterns. Tributary captured fish had a capture rkm set to the confluence mainstem rkm (four fish). For the GSI analysis, the most upstream fish capture rkm was 1283.5 (22 rkms below Gavins Point Dam, Yankton, South Dakota), and rkm 1243.5 for the fecundity analysis. Lowest rkm for both the GSI and fecundity analysis was Mississippi River rkm 1498.6. Capture rkm was adjusted to put it in the context of a single river length based on the spatial extent of the study (i.e. rkm 1498.6 on the Lower Mississippi River, set as 0, to its confluence with the Missouri River and upstream through the Missouri River to where the most-upstream fish was caught at rkm 1283.5 set as rkm 1634.5).

*Scaphirhynchus albus* GSI and fecundity were $\text{Log}_{10}$ transformed to meet normality assumptions. A generalized linear Multiple Regression model with two-way interactions was used to assess the relationship between GSI and capture rkm, FL, sex, hybrid status, origin, and reproductive status. The hybrid status*origin interaction was not included in the model since no hybrid sturgeon were produced in the hatchery. Reproductive status, sex, hybrid status and origin were considered qualitative variables; FL and rkm were continuous quantitative variables. Simple linear regression and the Tukey–Kramer multiple comparison test were used to further assess the significant relationships within the multiple regression model. Additionally, we used a generalized linear Mutiple Regression model with two-way interactions for each subgroup (reproductive or non-reproductive males or females) to assess the relationship between GSI and capture rkm, FL, hybrid status, and origin. For all tests, we used SAS 9.2 TS Level 2M0 software

Table 2
Statistics of *Scaphirhynchus albus* used in the multiple regression analysis examining how ultrasound estimated gonadosomatic index (GSI) or fecundity were affected by the fish's capture river kilometer, fork length (fl), sex, hybrid status, origin, and reproductive status. Variance reported as one standard deviation and the back transformed means reported in parentheses

| | Pallid sturgeon | | | | | |
|---|---|---|---|---|---|---|
| | Hatchery | | Wild | | Wild hybrid sturgeon | |
| | Non-reproductive | Reproductive | Non-reproductive | Reproductive | Non-reproductive | Reproductive |
| Female | | | | | | |
| Number of fish | 24 | 5 | 45 | 33 | 5 | 9 |
| Mean weight (g) | 1988 ± 513 | 3467 ± 714 | 2866 ± 1086 | 3606 ± 1131 | 2694 ± 1169 | 2976 ± 1181 |
| Mean fl (mm) | 814 ± 59 | 925 ± 53 | 899 ± 101 | 943 ± 90 | 850 ± 111 | 827 ± 73 |
| Mean $Log_{10}$ GSI | −0.08 ± 0.37 (0.84) | 1.2 ± 0.12 (17) | 0.04 ± 0.28 (1.1) | 1.1 ± 0.12 (12) | 0.03 ± 0.35 (1.1) | 1.2 ± 0.06 (16) |
| Mean egg diameter (mm) | | 3.12 ± 0.335 | | 3.00 ± 0.282 | | 2.98 ± 0.273 |
| Mean $Log_{10}$ fecundity | | 4.6576 ± 0.11 (45 452) | | 4.5764 ± 0.15 (37 707) | | 4.5976 ± 0.09 (39 594) |
| Mean eggs per g of wet fish | | 13 ± 1.4 | | 12 ± 4.5 | | 15 ± 5.5 |
| Male | | | | | | |
| Number of fish | 22 | 4 | 47 | 73 | 7 | 7 |
| Mean weight (g) | 1872 ± 432 | 2911 ± 701 | 2477 ± 1086 | 2844 ± 752 | 2125 ± 498 | 3035 ± 826 |
| Mean fl (mm) | 801 ± 46 | 892 ± 78 | 875 ± 89 | 909 ± 62 | 827 ± 52 | 904 ± 96 |
| Mean $Log_{10}$ GSI | −0.47 ± 0.23 (0.34) | 0.25 ± 0.03 (1.8) | −0.49 ± 0.27 (0.32) | 0.16 ± 0.12 (1.5) | −0.48 ± 0.36 (0.33) | 0.29 ± 0.09 (2.0) |

(SAS Institute Inc., 2002–2003), considered a P-value $\leq 0.05$ as significant, reported means from back-transformed means, all variance estimates as one standard deviation, and all factor level comparisons using model parameter estimates.

#### Comparison of GSI and fecundity results with previous studies

*Scaphirhynchus albus* can grow larger than those in our GSI analysis (Brown, 1955), consequently, we were interested in whether our estimated FL:GSI and FL:fecundity relationships would change if data from previously reported measures, some from *S. albus* much larger than those in this study, were included. There are 38 previous reports of *S. albus* GSI, 23 using the invasive weight-based methodology (Henry and Ruelle, 1992; Keenlyne et al., 1992; Keenlyne and Jenkins, 1993; George et al., 2012; Table 3) and 15 using minimally invasive ultrasonography (Bryan et al., 2007; some captured in the Great Plains Management Unit, rkm 1416.2–3394.4). Using GSI and fecundity estimates from previous studies (Table 3), we tested whether the linear regression generated with and without any previous study data were different using an *F*-test.

## Results

A total of 467 *S. albus* were examined for this study, of which 214 were females, making the male to female ratio 1.2:1 (69 reproductive females and 115 reproductive males). Thirteen *S. albus* were reproductive multiple times during this study. Three female fish that lived at the hatchery since birth were reproductive at 2-, 3-, and 4-year intervals, but this could have been shorter since the fish were not examined every year. Two males at the hatchery since birth were examined more than once and both were reproductive in consecutive years 2006 and 2007. Eight wild-origin males living in the river were reproductive multiple years during the study, two in consecutive years. Four of the eight males were reproductive every other year, however, only one of those fish was examined during the intervening year. The final two males were reproductive at a maximum of 3- and 4-year intervals. We did not examine more than once any wild-origin female *S. albus* living in the river in reproductive condition.

#### Egg diameter

In this study, 17 reproductive female *S. albus* had a mean egg diameter. The ultrasonic egg diameters were lower than the actual diameters by 0.504 ± 0.216 mm or 18.7% (ultrasound = 2.202 ± 0.187 mm; actual = 2.707 ± 0.124 mm). To adjust our egg diameter estimates for this error and the resulting fecundity calculation, we added the average difference between the methods to the ultrasound mean egg diameter of each fish.

#### Gonadosomatic index

When all 281 fish were included in the analysis (Table 2), there was a significant effect of sex*reproductive status, origin*FL and reproductive status*FL interactions on *S. albus* GSI (Table 4). Reproductive females had the highest mean GSI at 16.16% (Tukey–Kramer test, T-statistic = 13.75, $r = 47$, $v = 84$, P-value < 0.0001). Non-reproductive female GSI (mean = 1.26%) and reproductive male GSI (mean = 2.00%) were not significantly different from one another (Tukey–Kramer test, T-statistic = −2.30, $r = 74$, $v = 84$, P-value = 0.1014). Non-reproductive males had the lowest GSI (mean = 0.43%) of all groups (Tukey–Kramer test, T-statistic = 7.69, $r = 76$, $v = 74$, P-value < 0.0001). The slope of the relationship between the GSI and FL was greater for hatchery-origin fish FL than for wild-origin fish. When examining each individual group of fish, the FL*rkm interaction was significant in predicting the reproductive female *S. albus* GSI (Table 4). We did not find any differences in GSI between hybrid status groups of fish.

Table 3
List of *Scaphirhynchus albus* data fromprevious studies used as a comparison for gonadosomatic indexand fecundity estimates generated in this study fomultrasonography ('nr' means the general location or specific river kilometer for individual fish was not reported in article)

| Reference | River (State abbreviation) | River kilometer | Weight (grams) | Fork length (mm) | Estimation method | GSI | Fecundity | |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | Total egg count | Eggs per gram |
| Non-reproductive female | | | | | | | | |
| Bryan et al. (2007) | Missouri (ND) | 2546 | 18 600 | 1495 | Ultrasound | 0.8 | | |
| | Missouri (ND) | 2546 | 18 100 | 1565 | Ultrasound | 0.7 | | |
| Henry and Ruelle (1992)[a] | Mississippi (LA) | 506[b] | 3864 | 853 | Weight | 7.2 | | |
| | Mississippi (LA) | 506[b] | 4200 | 865 | Weight | 9.0 | | |
| | Mississippi (LA) | 506[b] | 4000 | 910 | Weight | 10.5 | | |
| | Mississippi (IL/LA) | nr | nr | 705 | Weight | 6.6 | | |
| | Mississippi (IL/LA) | nr | nr | 589 | Weight | 1.5 | | |
| | Mississippi (IL/LA) | nr | nr | 601 | Weight | 1.3 | | |
| | Mississippi (IL/LA) | nr | nr | 657 | Weight | 0.5 | | |
| Keenlyne and Jenkins (1993) | Mississippi (LA) | nr | 800 | 601 | Weight | 1.0 | | |
| | Mississippi (LA) | nr | 1200 | 657 | Weight | 1.0 | | |
| | Mississippi (LA) | nr | 2000 | 705 | Weight | 7.0 | | |
| | Mississippi (LA) | nr | 3900 | 853 | Weight | 7.0 | | |
| | Missouri (NE) | nr | 2200 | 864 | Weight | 3.0 | | |
| | Mississippi (LA) | nr | 4200 | 865 | Weight | 9.0 | | |
| | Mississippi (LA) | nr | 4000 | 910 | Weight | 11.0 | | |
| Reproductive female | | | | | | | | |
| Bryan et al. (2007) | Missouri (ND) | 2546 | 19 100 | 1434 | Ultrasound | 7.9 | 50 739 | 2.7 |
| | Missouri (ND) | 2546 | 16 800 | 1435 | Ultrasound | 9.7 | 94 279 | 5.6 |
| Henry and Ruelle (1992)[c] | Mississippi (LA) | 506[b] | 3050 | 800 | Weight | 23.9 | 61 992 | 20.3 |
| Keenlyne et al. (1992) | Missouri (ND) | 2173.4[d] | 17 110 | 1404 | Weight | 11.4 | 170 000 | 9.9 |
| George et al. (2012)[e] | Mississippi (LA) | 507[b] | 2850 | 827 | Weight | 13.8 | 50 759 | |
| | Mississippi (LA) | 507[b] | 3200 | 886 | Weight | 20.0 | 51 959 | |
| Non-reproductive Male | | | | | | | | |
| Bryan et al. (2007) | Missouri (ND) | 2546 | 12 200 | 1360 | Ultrasound | 0.6 | | |
| | Missouri (ND) | 2546 | 17 700 | 1379 | Ultrasound | 0.3 | | |
| | Missouri (ND) | 2546 | 16 300 | 1410 | Ultrasound | 0.7 | | |
| Keenlyne and Jenkins (1993) | Mississippi (MO) | nr | 1200 | 640 | Weight | 6.0 | | |
| Reproductive Male | | | | | | | | |
| Bryan et al. (2007) | Missouri (ND) | 2546 | 12 700 | 1348 | Ultrasound | 0.5 | | |
| | Missouri (ND) | 2546 | 20 900 | 1376 | Ultrasound | 0.3 | | |
| | Missouri (ND) | 2546 | 15 400 | 1400 | Ultrasound | 0.4 | | |
| | Missouri (ND) | 2546 | 13 600 | 1405 | Ultrasound | 0.6 | | |
| | Missouri (ND) | 2546 | 24 000 | 1450 | Ultrasound | 0.4 | | |
| | Missouri (ND) | 2546 | 21 800 | 1460 | Ultrasound | 0.9 | | |
| | Missouri (ND) | 2546 | 22 700 | 1550 | Ultrasound | 0.6 | | |
| | Missouri (ND) | 2546 | 24 900 | 1594 | Ultrasound | 0.9 | | |
| Keenlyne and Jenkins (1993) | Mississippi (LA) | nr | 1700 | 710 | Weight | 7.0 | | |
| | Mississippi (MO) | nr | 1600 | 724 | Weight | 4.0 | | |
| | Mississippi (LA) | nr | 1900 | 738 | Weight | 8.0 | | |
| | Missouri (ND) | nr | 10 400 | 1244 | Weight | 4.0 | | |

[a]The four males caught by Henry and Ruelle (1992) were not included because their reproductive state was not reported.
[b]Lower Missouri River riverkilometer.
[c]Possible hybrid between pallid and shovelnose sturgeon.
[d]Estimated using the middle riverkilometer for the free flowing section of the Missouri river in Morton county, ND.
[e]Physical measurements were from a frozen specimen that were at stage III in October. Used mean estimates.

**Fecundity**

Nineteen *S. albus* were spawned in the hatchery and total egg counts were recorded. Ultrasound estimates of fecundity were almost two times the final spawned egg total at the hatchery, with an average overestimate of 22 894 ± 23 025 eggs, or 48.5% (ultrasound = 44 531 ± 23 940 eggs and hatchery spawn = 22 940 ± 12 188 eggs, n = 47). The mean relative fecundity estimated using the ultrasound was 10.7 ± 4.9 eggs per one gram of total fish weight and 6.0 ± 3.0 for hatchery spawns. For all analyses, relative fecundity trends mimicked fecundity results presented below.

Fecundity did not differ significantly by hybrid status or origin of fish (hybrid status *F*-test, test statistic = 0.76, ndf = 1, ddf = 37, P-value = 0.3900; origin *F*-test, test statistic = 1.63, ndf = 1, ddf = 37, P-value = 0.2101), however, there was a significant interaction between rkm and FL (*F*-test, test statistic = 9.46, ndf = 1, ddf = 37, P-value = 0.0039). Ultrasound estimated fecundity of *S. albus* had a positive trend with FL as rkm increased but this trend was more pronounced in the higher rkms. We calculated the linear relationship between fecundity and FL with and without rkm (Table 5). These relationships were significant and the addition of rkm increased the amount of variation explained by the model by 24%.

**Comparison of GSI and fecundity results with previous studies**

Three out of four relationships between GSI and FL differed significantly after data from previous studies were added to the models (non-reproductive female: *F*-test, test

Table 4
Results of the multiple regression analysis examining the influence of capture river kilometer (Rkm), fork length (FL), sex, hybrid status, origin, and reproductive status (RS) on *Scaphirhynchus albus* gonadosomatic index

| | | | Females | | | | Males | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | All fish combined | | Reproductive | | Non-reproductive | | Reproductive | | Non-reproductive | |
| Variation source | F statistic | P-value | F statistic | P-value | F statistic | P-value | F statistic | P-value | F statistic | P-value |
| Model | 89.75 | <0.0001* | 3.54 | 0.0030* | 3.64 | 0.0010* | 1.57 | 0.141 | 1.20 | 0.307 |
| Sex | 0.07 | 0.7885 | – | – | – | – | – | – | – | – |
| Hybrid status | <0.01 | 0.9644 | 0.69 | 0.41 | 0.80 | 0.373 | 0.86 | 0.358 | <0.01 | 0.965 |
| Origin | 3.93 | 0.0485* | 6.67 | 0.0139* | 4.63 | 0.0351* | 0.01 | 0.904 | 0.02 | 0.881 |
| RS | 38.57 | <0.0001* | – | – | – | – | – | – | – | – |
| FL | 2.37 | 0.1252 | 0.02 | 0.892 | 5.78 | 0.0192* | 0.68 | 0.412 | 0.18 | 0.672 |
| Rkm | 0.02 | 0.9017 | 8.43 | 0.0062* | 0.02 | 0.885 | 1.83 | 0.18 | 0.20 | 0.658 |
| Sex*Hybrid status | 0.26 | 0.6088 | – | – | – | – | – | – | – | – |
| Sex*Origin | 0.58 | 0.4470 | – | – | – | – | – | – | – | – |
| Sex*RS | 56.71 | <0.0001* | – | – | – | – | – | – | – | – |
| Sex*FL | 3.31 | 0.0698 | – | – | – | – | – | – | – | – |
| Sex*Rkm | 0.52 | 0.4716 | – | – | – | – | – | – | – | – |
| Hybrid status*RS | 1.19 | 0.2764 | – | – | – | – | – | – | – | – |
| Hybrid status*FL | 0.08 | 0.7783 | 0.79 | 0.381 | 0.48 | 0.489 | 0.44 | 0.509 | 0.10 | 0.756 |
| Hybrid status*Rkm | 3.46 | 0.0641 | 0.67 | 0.418 | 0.89 | 0.35 | <0.01 | 0.949 | 1.86 | 0.177 |
| Origin*RS | 0.42 | 0.5184 | – | – | – | – | – | – | – | – |
| Origin*FL | 4.35 | 0.0381* | 8.68 | 0.0055* | 4.85 | 0.0312* | 0.04 | 0.847 | 0.18 | 0.669 |
| Origin*Rkm | 0.18 | 0.6680 | 2.21 | 0.145 | 0.45 | 0.503 | 0.08 | 0.773 | 0.93 | 0.339 |
| RS*FL | 13.64 | 0.0003* | – | – | – | – | – | – | – | – |
| RS*Rkm | 1.01 | 0.3149 | – | – | – | – | – | – | – | – |
| FL*Rkm | 0.02 | 0.8905 | 7.52 | 0.0093* | 0.06 | 0.809 | 2.82 | 0.097 | 0.21 | 0.651 |
| Model degrees of freedom | 20 | | 9 | | 9 | | 9 | | 9 | |
| Error degrees of freedom | 260 | | 37 | | 64 | | 74 | | 66 | |
| Corrected Total degrees of freedom | 280 | | 46 | | 73 | | 83 | | 75 | |

*Indicates statistical significance.

statistic = 24.21, ndf = 1, ddf = 86, P-value < 0.0001; non-reproductive male: *F*-test, test statistic = 20.75, ndf = 1, ddf = 76, P-value < 0.0001; reproductive male: *F*-test, test statistic = 25.60, ndf = 1, ddf = 92, P-value < 0.0001). The relationship between *S. albus* GSI and fecundity, and FL for reproductive females did not change when the data from previous studies were added (GSI *F*-test, test statistic = 0.41, ndf = 1, ddf = 49, P-value = 0.5265; Fecundity *F*-test, test statistic = 0.78, ndf = 1, ddf = 49, P-value = 0.3827). Since three out of the six relationships changed when data from previous studies were added, we recalculated the FL relationships and statistics, combining both the previously reported *S. albus* data and current data from this study (Table 5).

The non-reproductive female and male GSI:FL relationship with all reported observations became nonsignificant and stayed nonsignificant, respectively, compared to the relationship that included only those fish in this study (Table 5). The reproductive female and male GSI:FL relationship stayed negative for both genders and stayed and became significant, respectively. The fecundity:FL relationship became more positive and significant when previous fish were included. The interaction between fecundity, rkm, and FL was still significant after the addition of the six previous reports of *S. albus* fecundity (Table 5; *F*-test, test statistic = 4.45, ndf = 1, ddf = 49, P-value < 0.001; Fig. 2).

## Discussion

The take of endangered *S. albus* is restricted and consequently the number of fish in this study using invasive methodology was limited. Even with limited invasive exams to measure the egg diameter (8% of fish) and fecundity (40% of fish), the error with the non-invasive methods was similar to the previously observed error with the closely related *S. platorynchus* (Bryan et al., 2007). In addition, our results are similar to those found for 22 previously dissected *S. albus* (Table 3). This would indicate that the estimates from the non-invasive and invasive methods would be comparable for fish with only non-invasive estimates. The non-invasive methods used in this study do have sources of error including gender misdiagnoses (Wildhaber et al., 2005), lag time between UE exam and physical measurements, opaque oviducts [which rarely occurred in this study even though it was problematic in Bryan et al. (2007)], and limited ultrasound measurement accuracy on objects <10 mm (corrected using an egg diameter factor to adjust for this inaccuracy). In addition, due to the limited size range of *S. albus* in this study combined with high variability in the non-reproductive female GSI and fecundity relationships, our conclusions about trends are limited. Until there is an increase in the take of *S. albus* for invasive examinations, the non-invasive methods of estimating GSI and fecundity are the most accurate measure for these internal parameters.

Ultrasound egg diameter estimates were closer to the actual egg diameter for *S. albus* than *S. platorynchus* using the same techniques (Bryan et al., 2007). The better egg diameter measures are likely due to the advancement in ultrasonic technology within the past 10 years. Additionally, Bryan et al. (2007) included eggs from stage III *S. platorynchus*, which tend to be smaller and more difficult to measure than eggs in this study. The ultrasound egg size with the adjustment factor for *S. albus* were within the range of previous reports (reproductive female mean egg diameter

Table 5
Results of the simple linear regression analysis on gonadosomatic index or fecundity of *Scaphirhynchus albus* with and without data from previous studies (fl = fork length and rkm = riverkilometer; Henry and Ruelle, 1992; Keenlyne et al., 1992; Keenlyne and Jenkins, 1993; Bryan et al., 2007)

| Fish group | Fork length range (mm) | Relationship | n | $r^2$ or $R^2$ | F Statistic | P-value | 95% Confidence interval for intercept | 95% Confidence interval for slope |
|---|---|---|---|---|---|---|---|---|
| Pallid sturgeon relationships from current study | | | | | | | | |
| Female | | | | | | | | |
| Non-reproductive | 692–1094 | $Log_{10}$ GSI = −1.4220 + 0.0016*fl | 74 | 0.2542 | 24.54 | <0.0001* | (−1.998, −0.8463) | (0.0010, 0.0023) |
| Reproductive | 740–1116 | $Log_{10}$ GSI = 1.5376 − 0.0004*fl | 47 | 0.1222 | 6.26 | 0.0160* | (1.207, 1.868) | (−0.0008, $-8.68 \times 10^{-5}$) |
| | 740–1116 | $Log_{10}$ fecundity = 4.1724 + 0.0005*fl | 47 | 0.0994 | 4.97 | 0.0309* | (3.794, 4.551) | ($4.37 \times 10^{-5}$, 0.0009) |
| | 740–1116 | $Log_{10}$ fecundity = 5.1013 − 0.0005*fl −0.0017*rkm +$1.76 \times 10^{-6}$ *rkm*fl[a] | 47 | 0.3372 | 7.29<br>95% C.I. for rkm<br>95% C.I. for fl*rkm | 0.0005* | (4.212, 5.991) | (−0.0015, 0.0005)<br>(−0.0030, −0.0005)<br>($3.17 \times 10^{-7}$,$3.20 \times 10^{-6}$) |
| Male | | | | | | | | |
| Non-reproductive | 726–1054 | $Log_{10}$ GSI = −0.9901 + 0.0006*fl | 76 | 0.0344 | 2.64 | 0.1086 | (−1.615, −0.3650) | ($-1.36 \times 10^{4}$, 0.0013) |
| Reproductive | 765–1057 | $Log_{10}$ GSI = 0.2740 − 0.0001*fl | 84 | 0.0033 | 0.27 | 0.6020 | (−0.0977, 0.6457) | (−0.0005, 0.0003) |
| Pallid sturgeon relationships combining current and previous studies | | | | | | | | |
| Female | | | | | | | | |
| Non-reproductive | 589–1565 | $Log_{10}$ GSI = −0.2504 + 0.0004*fl | 90 | 0.0209 | 1.87 | 0.1745 | (−0.7426, 0.2418) | (−0.0002, 0.0009) |
| Reproductive | 740–1435 | $Log_{10}$ GSI = 1.4879 − 0.0004*fl | 53 | 0.2040 | 13.07 | 0.0007* | (1.286, 1.6896) | (−0.0006, −0.0002) |
| | 740–1435 | $Log_{10}$ fecundity = 4.0720 + 0.0006*fl | 53 | 0.2730 | 19.16 | <0.0001* | (3.818, 4.326) | (0.0003, 0.0008) |
| | 740–1435 | $Log_{10}$ fecundity = 4.3272 + 0.0004*fl −0.0005*rkm +$4.15 \times 10^{-7}$ *rkm*fl[b] | 53 | 0.4598 | 13.9<br>95% C.I. for rkm<br>95% C.I. for fl*rkm | <0.0001* | (3.794, 4.860) | (−0.0002, 0.0010)<br>(−0.0009, −0.0002)<br>($1.9 \times 10^{-8}$,$8.10 \times 10^{-7}$) |
| Male | | | | | | | | |
| Non-reproductive | 640–1410 | $Log_{10}$ GSI = −0.6352 + 0.0002*fl | 80 | 0.0080 | 0.63 | 0.4306 | (−1.079, −0.1923) | (−0.0003, 0.0007) |
| Reproductive | 710–1594 | $Log_{10}$ GSI = 0.8422 − 0.0007*fl | 96 | 0.3329 | 46.91 | <0.0001* | (0.6424, 1.042) | (−0.0009, −0.0005) |

*Indicates statistical significance.
[a]Riverkilometer ranged from Mississippi river 1498.6 upstream to Missouri river 1243.5, a total of 1594.5 km.
[b]Riverkilometer ranged from Mississippi river 506 upstream to Missouri river 2173.4, a total of 3516.5 km.

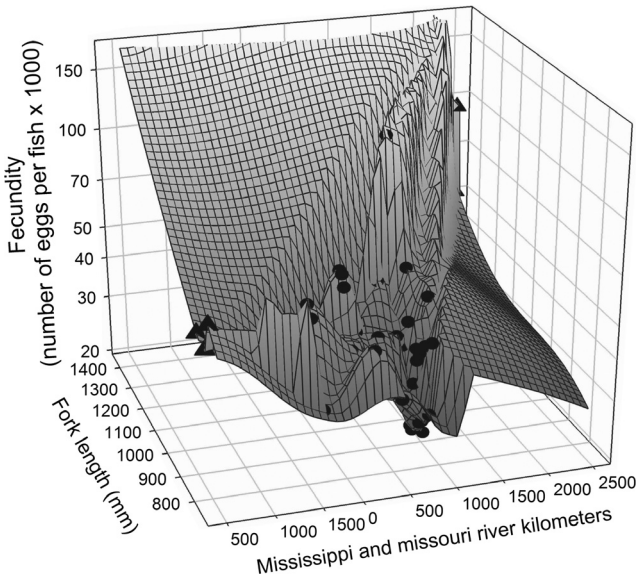


Fig. 2. Significant interaction between *Scaphirhynchus albus* fecundity, fork length, and river kilometer. Fecundity estimates are from the non-invasive ultrasound technique (circles) combined with previous studies (triangles) on pallid sturgeon using either the ultrasound or invasive weight method (note that not all data points are above the surface; Henry and Ruelle, 1992; Keenlyne et al., 1992; Bryan et al., 2007; George et al., 2012)

= 2.81 mm, std = 0.13, Henry and Ruelle, 1992; long axis egg length range from 2.5 to 3.0 mm (Keenlyne et al., 1992).

Using ultrasonography, male *S. albus* GSI is smaller but female *S. albus* GSI is similar to previous reports using invasive estimates (Table 3) and other sturgeon species (Billard and Lecointre, 2001; Wildhaber et al., 2007). The difference in males may be an artifact of the ultrasound technique or an actual decrease in GSI, but more estimates are required. GSI trends (Table 5) were consistent with *S. platorynchus* (Wildhaber et al., 2007) with reproductive female GSI the highest, non-reproductive females and reproductive males having similar GSI, and non-reproductive males having the lowest. The significant interactions between FL and origin of fish in the GSI multiple regression model was due to the limited size range and GSI of hatchery origin fish relative to the wild population (Table 2; only intensively stocked since 2001). As all hatchery *S. albus* have GSI measurements within the range of the wild *S. albus* GSI, we do not think this interaction will continue once all the stocked fish attain the same age and size as the wild population.

Ultrasound estimates of *S. albus* fecundity were almost two times the final spawned egg totals reported at the hatchery, although error in the ultrasound measurement should have been less (~5% based on Bryan et al., 2007). In the hatchery, female *S. albus* are hormonally induced to ovulate and the eggs are hand-stripped by repeatedly applying gentle pressure along the length of the abdominal wall. Eggs are stripped from females periodically during ovulation, which may last for several hours to more than a day. Eggs are not completely removed from most hatchery spawns of *S. albus*, unless surgical techniques are used [not performed in this study, but see Doroshov et al. (1983) for the technique]. Consequently, the ultrasound fecundity estimates are likely a more accurate reflection of true fecundity. Although the fecundity estimates from this study were lower than previously published measures of *S. albus* fecundity based on necropsy (Table 3), relative fecundity was within the range of *S. albus* and other species of sturgeon (Krykhtin and Gorbach, 1996; Billard and Lecointre, 2001).

The negative relationship found in this study between reproductive *S. albus* GSI and length was unexpected. However, the GSI:length relationship was negative in reproductive female lake sturgeon *A. fulvescens* (Rafinesque 1817) (Bruch et al., 2006) but also positive in reproductive female *S. platorynchus* (Kennedy et al., 2006) and Persian sturgeon *A. persicus* (Borodin, 1897) (Nazari et al., 2010). A negative GSI:length relationship would indicate the increase in gonad size relative to body size is not consistent during the life of the fish. This seems to indicate for certain sturgeon species that relative size change of the gonads when transitioning between reproductive stages is not as great in larger fish. However, due to the limited number of larger fish in our relationship and lack of age determination, further investigation will be needed to determine the cause of this negative relationship.

As the geographic range of *S. albus* contains large climatic variation from Montana to Louisiana (Fig. 1), the fecundity*FL*rkm interaction found in this study could imply a trend in temperature variation. The rkm factor was only significant in models that incorporated fish size, either in the response variable (i.e. GSI and relative fecundity) and/or predictive variable (i.e. FL), which could imply that this relationship is due to natural variation in *S. albus*. These results suggest a countergradient variation (Conover and Schultz, 1995) involving fecundity and temperature, similar to other physiological trends that vary over different temperatures found in fish species inhabiting large geographic areas that contain large climatic variation (e.g. silversides *Menidia* sp., Yamahira and Conover, 2002; *Pomacentrus coelestis*, Kokita, 2003; Arctic charr *Salvelinus alpinus*, Power et al., 2005; Atlantic cod, *Gadus morhua*, Thorsen et al., 2010). Other rkm trends have been found for *S. albus* including genetic variation (Schrey and Heist, 2007), morphometric trends (Keenlyne et al., 1994; Murphy et al., 2007), and variation in the growth rate of juveniles (Shuman et al., 2011). However, all rkm trends should be assessed with caution since they are confounded with many factors such as habitat, temperature, prey abundance, resource limitations, flow regime, and/or river size. Whether the fecundity*FL*rkm relationship is due to climate variation, resource limitations, fish age, or other longitudinal river trends will only be determined through further study of this species, involving more fecundity estimates from the extreme latitudes in the *S. albus* range, using reciprocal transplants, and/or controlled laboratory experiments (Conover and Schultz, 1995).

Using minimally invasive techniques repeatedly on the same individual sturgeon in the Lower Missouri River suggest a more variable reproductive cycle for *S. albus* than previously reported (Dryer and Sandovol, 1993; Keenlyne and Jenkins, 1993). Ten river-caught male *S. albus* found in our study had an estimated reproductive cycle of 1–4 years, whereas the previous anecdotal estimate (Dryer and Sandovol, 1993) was 2–3 years. Most wild male sturgeon species have a spawning periodicity of one to 5 years (Chapman et al., 1996; Billard and Lecointre, 2001; Erickson and Webb, 2007; Tripp et al., 2009). The three hatchery-maintained female *S. albus* of this study also had shorter reproductive cycles (2–4 years) than the previous estimate of 3–10 years for wild river-dwelling fish (Dryer and Sandovol, 1993;

Keenlyne and Jenkins, 1993). This result is expected to be due to the optimal growth conditions of a hatchery, whereby the culture environment usually decreases the sturgeon minimum cycle time by 2 years (Chapman et al., 1996; Doroshov et al., 1997; Williot and Brun, 1998; Billard and Lecointre, 2001; Williot et al., 2005). Most wild female sturgeon species have a spawning periodicity of 2–11 years (Billard and Lecointre, 2001; Erickson and Webb, 2007; Tripp et al., 2009). Based on the results of this study, spawning periodicity in *S. albus* ranges from 1 to 4 years for males and a minimum of 2 years for females.

In conclusion, the minimally invasive ultrasonic and endoscopic techniques were successful at estimating GSI, fecundity, and egg size of *S. albus* and their measures were similar to invasive biopsy methods. These techniques can be used in a laboratory setting or in the field and provide a minimally invasive assessment of sturgeon sex and reproductive condition. They are very useful in determining reproductive characteristics of populations of sturgeon at risk and crucial in assessing impacts from the environment or species restoration efforts.

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## References

von Bayer, H., 1908: A method of measuring fish eggs. Bull. U. S. Bureau Fish. **28**, 1011–1014.

Billard, R.; Lecointre, G., 2001: Biology and conservation of sturgeon and paddlefish. Rev. Fish Biol. Fish. **10**, 355–392.

Brown, C. J. D., 1955: A record-size pallid sturgeon, *Scaphirhynchus albus*, from Montana. Copeia **1955**, 55.

Bruch, R. M.; Miller, G.; Hansen, M. J., 2006: Fecundity of lake sturgeon (*Acipenser fulvescens*, Rafinesque) in Lake Winnebago, Wisconsin, USA. J. Appl. Ichthyol. **22**, 116–118.

Bryan, J. L.; Wildhaber, M. L.; Papoulias, D. M.; DeLonay, A. J.; Tillitt, D. E.; Annis, M. L., 2007: Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon. J. Appl. Ichthyol. **23**, 411–419.

Chapman, F. A.; van Eenennaam, J. P.; Doroshov, S. I., 1996: The reproductive condition of white sturgeon, *Acipenser transmontanus*, in San Francisco Bay California. Fish. Bull. **94**, 628–634.

Colombo, R. E.; Wills, P. S.; Garvey, J. E., 2004: Use of ultrasound imaging to determine sex of shovelnose sturgeon. N. Am. J. Fish. Manage. **24**, 322–326.

Conover, D. O.; Schultz, E. T., 1995: Phenotypic similarity and the evolutionary significance of countergradient variation. Trends Ecol. Evol. **10**, 248–252.

DeLonay, A. J.; Jacobson, R. B.; Papoulias, D. M.; Simpkins, D. G.; Wildhaber, M. L.; Reuter, J. M.; Bonnot, T. W.; Chojnacki, K. A.; Korschgen, C. E., 2009: Ecological requirements for pallid sturgeon reproduction and recruitment in the Lower Missouri River: a research synthesis 2005–08. U.S. Geological Survey, Scientific Investigations Report 2009–5201, Columbia, pp. 59.

Divers, S. J.; Boone, S. S.; Hoover, J. J.; Boysen, K. A.; Killgore, K. J.; Murphy, C. E.; George, S. G.; Camus, A. C., 2009: Field endoscopy for identifying gender, reproductive stage and gonadal anomalies in free-ranging sturgeon (*Scaphirhynchus*) from the lower Mississippi River. J. Appl. Ichthyol. **25**, 68–74.

Doroshov, S. I.; Clark, W. H.; Lutes, P. B.; Swallow, R. L.; Beer, K. E.; McGuire, A. B.; Cochran, M. D., 1983: Artificial propagation of the white sturgeon *Acipenser transmontanus* (Richardson). Aquaculture **32**, 93–104.

Doroshov, S. I.; Moberg, G. P.; Van Eenennaam, J. P., 1997: Observations on the reproductive cycle of cultured white sturgeon, *Acipenser transmontanus*. Environ. Biol. Fishes **48**, 265–278.

Dryer, M.; Sandovol, A., 1993: Recovery plan for the pallid sturgeon (*Scaphirhynchus albus*). U.S. Fish and Wildlife Service, Bismark, pp. 55.

Erickson, D. L.; Webb, M. A. H., 2007: Spawning periodicity, spawning migration, and size at maturity of green sturgeon, *Acipenser medirostris*, in the Rogue River, Oregon. Environ. Biol. Fishes **79**, 255–268.

George, S. G.; Slack, W. T.; Hoover, J. J., 2012: A note on the fecundity of pallid sturgeon. J. Appl. Ichthyol. **28**, 512–515.

Henry, C. J.; Ruelle, R., 1992: A study of pallid sturgeon and shovelnose sturgeon reproduction. U.S. Fish and Wildlife Service, Pierre, pp. 19.

Hurvitz, A.; Jackson, K.; Degani, G.; Levavi-Sivan, B., 2007: Use of endoscopy for gender and ovarian stage determinations in Russian sturgeon (*Acipenser gueldenstaedtii*) grown in aquaculture. Aquaculture **270**, 158–166.

Keenlyne, K. D., 1997: Life history and status of the shovelnose sturgeon, Scaphirhynchus platorynchus. Environ. Biol. Fishes **48**, 291–298.

Keenlyne, K. D.; Jenkins, L. G., 1993: Age at sexual maturity of the pallid sturgeon. Trans. Am. Fish. Soc. **122**, 393–396.

Keenlyne, K. D.; Grossman, E. M.; Jenkins, L. G., 1992: Fecundity of the pallid sturgeon. Trans. Am. Fish. Soc. **121**, 139–140.

Keenlyne, K. D.; Henry, C. J.; Tews, A.; Clancey, P., 1994: Morphometric comparisons of upper Missouri River sturgeons. Trans. Am. Fish. Soc. **123**, 779–785.

Kennedy, A. J.; Sutton, T. M.; Fisher, B. E., 2006: Reproductive biology of female shovelnose sturgeon in the upper Wabash River, Indiana. J. Appl. Ichthyol. **22**, 177–182.

Kokita, T., 2003: Potential latitudinal variation in egg size and number of a geographically widespread reef fish, revealed by common-environment experiments. Mar. Biol. **143**, 593–601.

Krykhtin, M. L.; Gorbach, E. I., 1996: Fecundity of the kaluga *Huso dauricus* and the Amur sturgeon *Acipenser schrencki*. J. Ichthyol. **36**, 56–50.

Kynard, B.; Kieffer, M. C., 2002: Use of a borescope to determine the sex and egg maturity stage of sturgeons and the effect of borescope use on reproductive structures. J. Appl. Ichthyol. **18**, 505–508.

Matsche, M. A.; Bakal, R. S.; Rosemary, K. M., 2011: Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*). J. Appl. Ichthyol. **27**, 627–636.

Mayden, R. L.; Kuhajda, B. R., 1997: Threatened fishes of the world: *Scaphirhynchus albus* (Forbes & Richardson, 1905) (Acipenseridae). Environ. Biol. Fishes **48**, 420–421.

Moghim, M.; Vajhi, R.; Veshkini, A.; Masoudifard, M., 2002: Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. J. Appl. Ichthyol. **18**, 325–328.

Murphy, C. E.; Hoover, J. J.; George, S. G.; Killgore, K. J., 2007: Morphometric variation among river sturgeons (*Scaphirhynchus* spp.) of the Middle and Lower Mississippi River. J. Appl. Ichthyol. **23**, 313–323.

Nazari, R. M.; Ghomi, M. R.; Aquiloni, L., 2010: Interrelationships among egg, larvae and maternal characteristics in Persian Sturgeon *Acipenser persicus*. Braz. Arch. Biol. Technol. **53**, 1093–1095.

Pflieger, W. F., 1997: Fishes of Missouri. Missouri Department of Conservation, Jefferson City.

Power, M.; Dempson, J. B.; Reist, J. D.; Schwarz, C. J.; Power, G., 2005: Latitudinal variation in fecundity among Arctic charr populations in eastern North America. J. Fish Biol. **67**, 255–273.

SAS Institute Inc., 2002–2003: SAS Software, Release 9.1. Cary, NC, USA.

Schrey, A. W.; Heist, E. J., 2007: Stock structure of pallid sturgeon analyzed with microsatellite loci. J. Appl. Ichthyol. **23**, 297–303.

Scott, W. B.; Crossman, E. J., 1973: The sturgeons and paddlefishes: order Acipenseriformes. Fish. Res. Board Can. **184**, 77–116.

Shuman, D. A.; Klumb, R. A.; Wilson, R. H.; Jaeger, M. E.; Haddix, T.; Gardner, W. M.; Doyle, W. J.; Horner, P. T.; Ruggles, M.; Steffensen, K. D.; Stukel, S.; Wanner, G. A., 2011: Pallid sturgeon size structure, condition, and growth in the Missouri River Basin. J. Appl. Ichthyol. **27**, 269–281.

Thorsen, A.; Witthames, P. R.; Marteinsdóttir, G.; Nash, R. D. M.; Kjesbu, O. S., 2010: Fecundity and growth of Atlantic cod (*Gadus morhua* L.) along a latitudinal gradient. Fish. Res. **104**, 45–55.

Tripp, S. J.; Phelps, Q. E.; Colombo, R. E.; Garvey, J. E.; Burr, B. M.; Herzog, D. P.; Hrabik, R. A., 2009: Maturation and reproduction of shovelnose sturgeon in the Middle Mississippi River. N. Am. J. Fish. Manage. **29**, 730–738.

U.S. Fish and Wildlife Service, 2008: Pallid sturgeon (*Scaphirhynchus albus*) range wide stocking and augmentation plan. U.S. Fish and Wildlife Service, Denver, pp. 55.

Wildhaber, M. L.; Papoulias, D. M.; DeLonay, A. J.; Tillitt, D. E.; Bryan, J. L.; Annis, M. L.; Allert, J. A., 2005: Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. J. Fish Biol. **67**, 114–132.

Wildhaber, M. L.; Papoulias, D. M.; DeLonay, A. J.; Tillitt, D. E.; Bryan, J. L.; Annis, M. L., 2007: Physical and hormonal examination of Missouri River shovelnose sturgeon reproductive stage: a reference guide. J. Appl. Ichthyol. **23**, 382–401.

Wildhaber, M. L.; DeLonay, A. J.; Papoulias, D. M.; Galat, D. L.; Jacobson, R. B.; Simpkins, D. G.; Braaten, P. J.; Korschgen, C. E.; Mac, M. J., 2011: Identifying structural elements needed for development of a predictive life-history model for pallid and shovelnose sturgeons. J. Appl. Ichthyol. **27**, 462–469.

Williot, P.; Brun, R., 1998: Ovarian development and cycles in cultured Siberian sturgeon, Acipenser baeri. Aquat. Living Resour. **11**, 111–118.

Williot, P.; Brun, R.; Rouault, T.; Pelard, M.; Mercier, D.; Ludwig, A., 2005: Artificial spawning in cultured sterlet sturgeon, *Acipenser ruthenus* L., with special emphasis on hermaphrodites. Aquaculture **246**, 263–273.

Yamahira, K.; Conover, D. O., 2002: Intra- vs. interspecific latitudinal variation in growth: adaptation to temperature or seasonality? Ecology **83**, 1252–1262.

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