# Gonadal development and sex-specific demographics of the shovelnose sturgeon in the Middle Mississippi River

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## Summary

Harvest of the shovelnose sturgeon, *Scaphirhynchus platorynchus* for caviar has increased. To determine whether populations can withstand increased harvest, detailed information regarding sexual demographics is needed. We describe gender and reproductive development of 306 shovelnose sturgeon from the Middle Mississippi River (River km 0–322) during September 2001 through December 2003. Using dissection and histology, we identified three of the four gonadal stages described previously for male lake sturgeon and all seven stages for females. Males reached maturity at a smaller size than did females. Gonads can be rapidly inspected for sex and stage of development for the shovelnose sturgeon. The sex ratio was not different from 1 : 1. Seven intersexual fish occurred. Female fecundity was positively related to body weight (number of eggs = 30.24 × body weight − 8392; P = 0.013; $r^2$ = 0.45) and weakly related to fork length (number of eggs = 146.37 × fork length − 66 176, P = 0.053, $r^2$ = 0.23).

## Introduction

The collapse of the historic caviar fisheries in the Volga River and Caspian Sea has increased demand for domestically produced caviar in the USA. However, reduced sturgeon stocks in North America have left few commercially viable populations. Only the shovelnose sturgeon *Scaphirhynchus platorynchus* is currently viable for commercial harvest in the US, leading to its increased harvest in the Mississippi River. In Illinois, commercial harvest of shovelnose sturgeon caviar has increased from 82.5 kg in 1997 (Williamson, 1998) to 2388.6 kg in 2001 (Maher, 2002). The shovelnose sturgeon is small-bodied and matures early, likely allowing populations to sustain higher levels of harvest than most other chondrostean species (Morrow et al., 1998). With increased pressure on this fishery, information on life history, population dynamics, and sexual demographics is critical for effective fisheries management.

Little information is currently available on sexual demographics and fecundity, and thus the population-level reproductive potential (Begon et al., 1996) of this species. Because shovelnose sturgeon females are preferentially harvested for caviar, proper management also requires information regarding the population sex ratio. Shovelnose sturgeon in the Middle Mississippi River (MMR, River km 0–322) may have a skewed sex ratio with four males for every one female (Jackson, 2004). However, this information was likely inaccurate because all specimens were obtained from a commercial egg fisherman at a time near spring spawning; sturgeon collected on a spawning run have been shown to have skewed sex ratios (Van Eenennaam et al., 1996). More reliable estimates using unbiased gear during a season when sturgeon are not staging to spawn is needed to determine the actual sex ratio in the MMR.

Gonads of immature female and male shovelnose sturgeon are difficult to distinguish. Moos (1978) described the reproductive development of the shovelnose sturgeon in the Missouri River using dissection and histology; however, many of these stages were based on sampling time during the year rather than the standard structural characteristics used by other studies. Carlson and Pflieger (1981) assigned stages of gonadal development to Missouri River shovelnose sturgeon using the color of the gonad with no accompanying histology. This approach does not provide insight into the development of either the spermatozoa or the oocytes in the gonads, which is critical to the understanding of reproductive development.

Both dissection and histology must be used when characterizing the stages of gonadal development for a species (e.g. lake sturgeon; Bruch et al., 2001). For lake sturgeon, seven developmental stages of female development and four for male development were identified (Bruch et al., 2001) that were directly related to previous structural assignments for other species of sturgeon (Conte et al., 1988; Dettlaff et al., 1993; Amiri et al., 1996a,b; Van Eenennaam et al., 1996). These stages provide an adequate framework to apply to the gonadal development of the shovelnose sturgeon. We described the stages of gonadal development for the shovelnose sturgeon and the reproductive demographics of the population in the MMR. As females are preferentially harvested this guide would enable managers to assess quickly the reproductive demographics of shovelnose sturgeon stocks.

## Methods

During September 2001 through December 2003, we sampled shovelnose sturgeon behind wing dikes with gill nets seasonally (based on meteorologic season) at various locations on the MMR for all gonadal stages. Sturgeon sampled were euthanized with a lethal dose of MS-222 (tricane methonsolfate), measured to the nearest millimeter fork length (FL), weighed to the nearest gram, and then dissected for gonadal evaluation. Stages of gonadal development were determined visually and a digital image was then taken. Females were assigned to one of seven stages and males to one of four stages using the criteria developed for lake sturgeon (Bruch et al., 2001). The gonad and associated fat were removed for determination of the gonadal somatic index (GSI), which was the weight of the

gonad and fat divided by the total body weight multiplied by 100.

Gonads from a subsample of each developmental stage were fixed in 10% neutral buffered formalin for histologic analysis. Five 5-micron sections taken through the middle of each gonad were processed using standard histologic techniques (i.e. stained with hematoxylin and counterstained with eosin). Histologic samples were viewed under a compound microscope (40–630×) with a top-mounted digital camera; digital images of each gonad section were captured.

To provide a non-lethal technique for staging, we compared the images captured during ultrasound sexing (Colombo et al., 2004) with the stages determined by dissection. Additionally, as other authors have staged shovelnose sturgeon gonads, results from this study were compared with those of Moos (1978) and Carlson and Pflieger (1981) (Table 1).

Fecundity for mature females was quantified by removing five 1-g samples from each ovary and counting the eggs in each subsample. Mean number was calculated for each ovary present (left and right) and these two means were used to calculate number of eggs per ovary and per gram of fish (Keenlyne et al., 1992). To determine if fecundity was related to body size, total number of eggs for both ovaries was regressed against weight and FL of the fish.

### Statistical analysis

To determine if the ratio of males to females was different from one to one analysis of proportions was used (Sokal and Rohlf, 2000). In addition to sex ratio, it is important to determine if the length frequency is similar between sexes. Differences in length-frequency histograms were analyzed using a Kolomogorov–Smirnov test. Differences in the mean GSI among stages were analyzed using one-way ANOVA and Tukey's HSD test.

### Results

#### Sex ratios

A total of 308 shovelnose sturgeon were sampled for sex identification. Of these, 150 were identified as males and 149 as females, with the remaining nine fish being indeterminate or intersexual (n = 7). The ratio of males to females was not different from one to one ($\chi^2 = 0.000$, P = 1.0). Length frequency distributions did not differ between sexes (ks = 0.0859, P = 0.57) (Fig. 1).
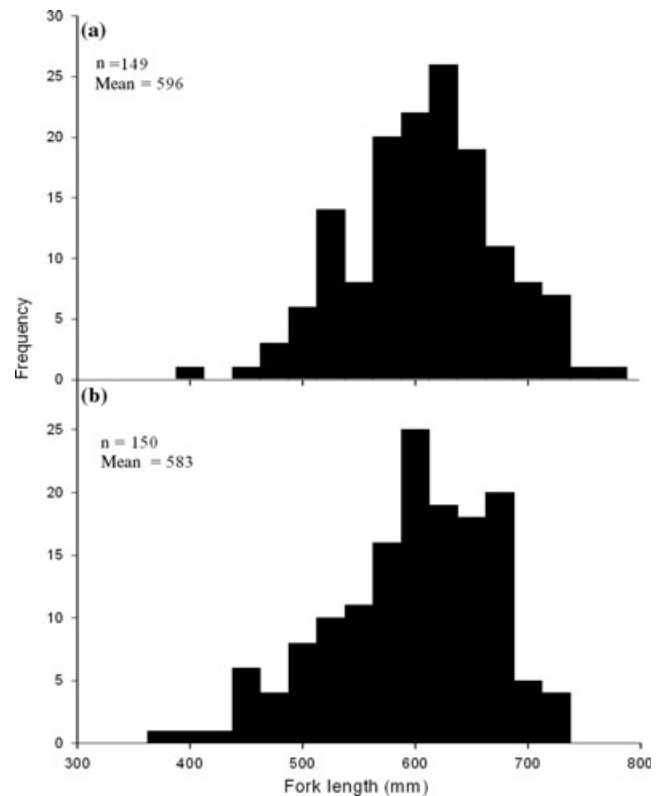


Fig. 1. Length-frequency histogram of (a) female and (b) male shovelnose sturgeon sampled in the Middle Mississippi River (River km 0–322)

### Male development

Virgin males (Mv; 12%, n = 19) were identified as having a small, ribbon-like testis embedded in a relatively small amount of testicular fat. Mean FL of Mv was 453 ± 8 mm (n = 19) and ranged from 369 to 500 mm, suggesting that male shovelnose sturgeon do not become reproductive until they reach approximately 500-mm FL. Mean GSI of Mv males (0.83 ± 0.10%) was lower than the other stages of male development (ANOVA, P < 0.05). Testes of Mv males were pink, with white to yellow testicular fat (Fig. 3a). Ultrasound images of the Mv males exhibited a small testicular material that was lightly colored (Fig. 3c). Histologic evaluation of stage Mv male testes displayed seminiferous tubules devoid of mature spermatozoa (Fig. 3b). Spermatogonia were embedded within the seminiferous tubules (Fig. 3b).

Table 1

Comparison of stages of gonadal development for the shovelnose sturgeon among these studies, Carlson and Pflieger (1981) and Moos (1978). Stages from the current study follow those determined for lake sturgeon (Bruch et al., 2001)

| Description | Study from Bruch et al. (2001) | Carlson and Pflieger (1981) | Moos (1978) |
|---|---|---|---|
| Small pink testis with small amount of testicular fat | Mv | N/A | I |
| Yellow testis large amount of testicular fat | MI | 1 | II and III |
| Large pink testis | MII | 2 and 3 | IV and V |
| Well ordered ovarian folds small amount of ovarian fat | Fv | N/A | I |
| Ovarian folds large amount of ovarian fat | FI | 1 | II |
| Clear to yellow small oocytes | FII | 2 | III |
| Yellow and light green oocytes | FIII | 3 | III |
| Large black oocytes | FIV | 4 and 5 | IV |
| Spawning female | FV | 5 | V |
| Translucent ovary | FVI | 6 | VI |
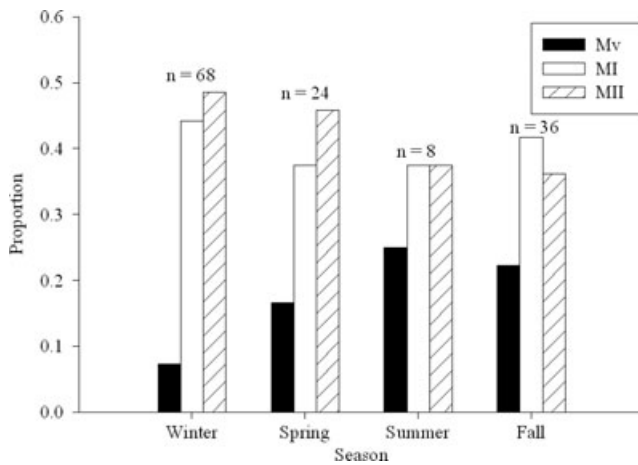
Fig. 2. Proportion of occurrence of stages of male shovelnose sturgeon development by season sampled from the Middle Mississippi River (River km 0–322) during September 2001 through December 2003

Males of stage one (MI; 44%, n = 65) were present in every season (Fig. 2) and similar in appearance to males of stage Mv, with the chief difference being the amount of testicular fat. Mean GSI of stage MI males (2.5 ± 0.25%) was greater than that of Mv males (ANOVA, P < 0.05). Stage MI males were 593 ± 7-mm FL and ranged between 493 and 715 mm. Testes of stage MI males were yellow and larger, with more testicular fat than Mv males (Fig. 3d). Individuals of this stage were easily sexed using ultrasound. Testes of stage MI males appeared smooth and light-gray in the ultrasound images (Fig. 3e). Histologic analysis of MI males appeared similar to the virgin males. The seminiferous tubules were devoid of developed sperm, with numerous spermatogonia dispersed in a homogenous fashion (Fig. 3f).

Stage two (MII; 44%, n = 65) males were present in all seasons (Fig. 2) and were characterized as fully developed releasing sperm during the spawning season (April through June). Mean size of MII shovelnose was 614 ± 6-mm FL and ranged from 499 to 719 mm. The primary difference between MII and MI males was the amount of testicular fat. MII males had reduced testicular fat surrounding the testis (Fig. 3g). The mean GSI of MII males (3.5 ± 0.22%) was greater than that of stage Mv and MI (ANOVA, P < 0.05) males due to an increase in the size of the testis from MI to MII. The testes of dissected MII males were white with a pink undertone (Fig. 3g). Ultrasound imaging effectively identified the sex of stage MII males. The testes of MII males appeared as smooth dark gray structures in the ultrasound images (Fig. 3i). Histologic analysis of this stage displayed the presence of mature spermatozoa packed into the seminiferous tubules (Fig. 3h). The spermatozoon of the shovelnose sturgeon was similar to that of other sturgeon, having an elongated head (Fig. 3h).

### Female development

Virgin females (Fv) had a mean length of 508 ± 8-mm FL (range 400–600 mm) and were present in all seasons (Fig. 4), comprising 18% (n = 25) of the females. Mean GSI of stage Fv (1.95 ± 0.26%) shovelnose sturgeon was low due to scarce ovarian fat (Fig. 5a). Fv females were grossly characterized as having a yellow gonad with orderly, well-formed ovarian folds with no grossly discernable oocytes; further, no atretic oocytes from previous spawnings were present (Fig. 6a). Fv females were difficult to distinguish from males using ultrasound due to the small size of the ovary. However, we were able to identify ovarian folds in three of four virgin females (Fig. 6a). Histologically, Fv female ovaries contained nests of pre-meiotic oogonia that stained lightly acidophilic (Fig. 7a). Some post-meiotic or primary oocytes could be seen in the follicular epithelium of virgin female. These early oocytes contained a large central nucleus (germinal vesicle) and stained strongly basophilic (Fig. 6a).

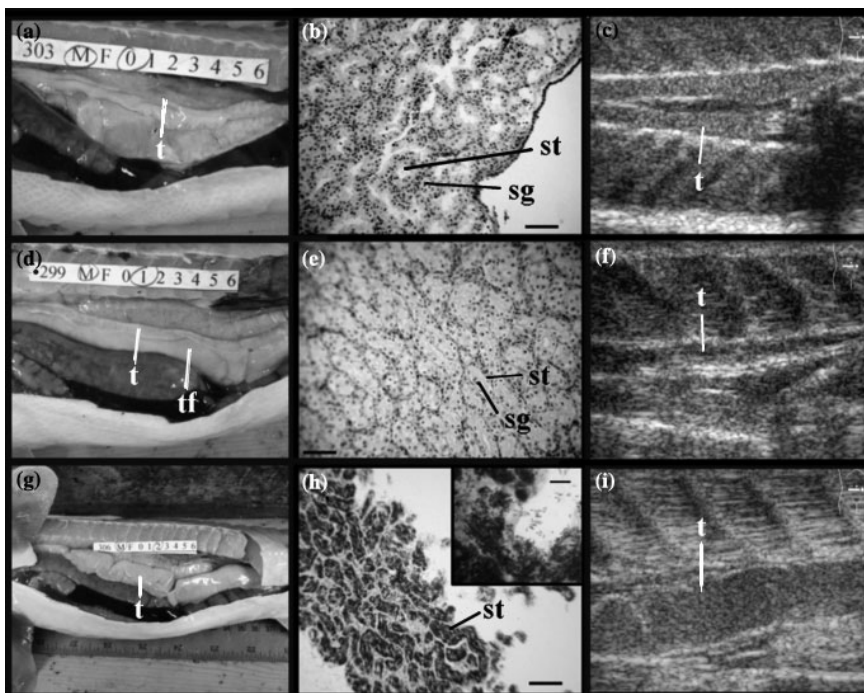Stage one (FI; 40%; n = 61) females were present in all seasons (Fig. 4) and were 604 ± 7 mm, ranging between 511



Fig. 3. Stages of male gonadal development of the shovelnose sturgeon: (a) Mv dissected, (b) Mv 100× magnification (scale bar = 200 μm), (c) Mv ultrasound, (d) MI dissected, (e) MI 250× magnification (scale bar = 80 μm), (f) MI ultrasound, (g) MII dissected, (h) MII 100× magnification (scale bar = 200 μm) insert 250× (scale bar = 80 μm), and (i) MII ultrasound. t, testis; tf, testicular fat; st, seminiferous tubule; sg, spermatogonia
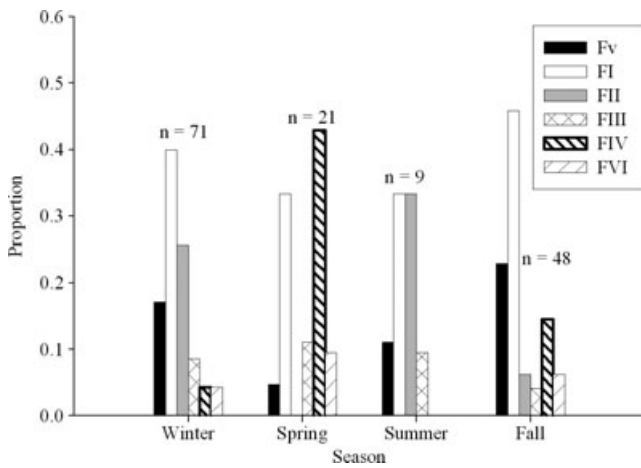
Fig. 4. Proportion of occurrence of stages of female shovelnose sturgeon development by season sampled from the Middle Mississippi River (River km 0–322) during September 2001 through December 2003

and 734 mm. Mean GSI remained low in the FI (4.01 ± 0.26%) sturgeon but varied due to large differences in the amount of ovarian fat (Fig. 5b). Although similar in color to the ovaries of the virgin females, a large amount of ovarian fat and atretic oocytes were present and ovarian folds were ragged (Fig. 5b). Oocytes were not discernable in FI females. Ultrasound imaging easily identified prominent ovarian folds (Fig. 6b).

Histologically, the ovaries of FI females contained many small oocytes that stained strongly basophilic (Fig. 7b). These primary oocytes had migrated to the walls of the ovigerous lamellae. The nuclei (germinal vesicle) of these previtellogenic oocytes were centrally located and contained numerous provitelline nucleoli (Fig. 7b). The oogonial nests present in Fv females were no longer present, because meiosis I and formation of the primary oocytes were completed (Fig. 7b).

Stage two (FII) females (16%; n = 24) were present in all seasons except in the spring (Fig. 4). The mean length (608 ± 8-mm FL) and range (535–695 mm FL) of FII females was similar to FI females. Stage FII females differed grossly from FI females due to visually identifiable oocytes. Also, mean GSI of FII females (7.03 ± 0.33%) was greater (ANOVA, P < 0.001). Clear to yellow oocytes were distributed throughout the ovary of FII females (Fig. 5c). A large amount of ovarian fat with small un-pigmented oocytes gave the ovary a salt-and-pepper-like appearance. Although ultrasound was efficient at distinguishing the females of stage FII from males due to the clearly identifiable ovarian mass, oocytes were not clearly identifiable in the ultrasound image, making distinction between FI and FII females difficult (Fig. 6c).
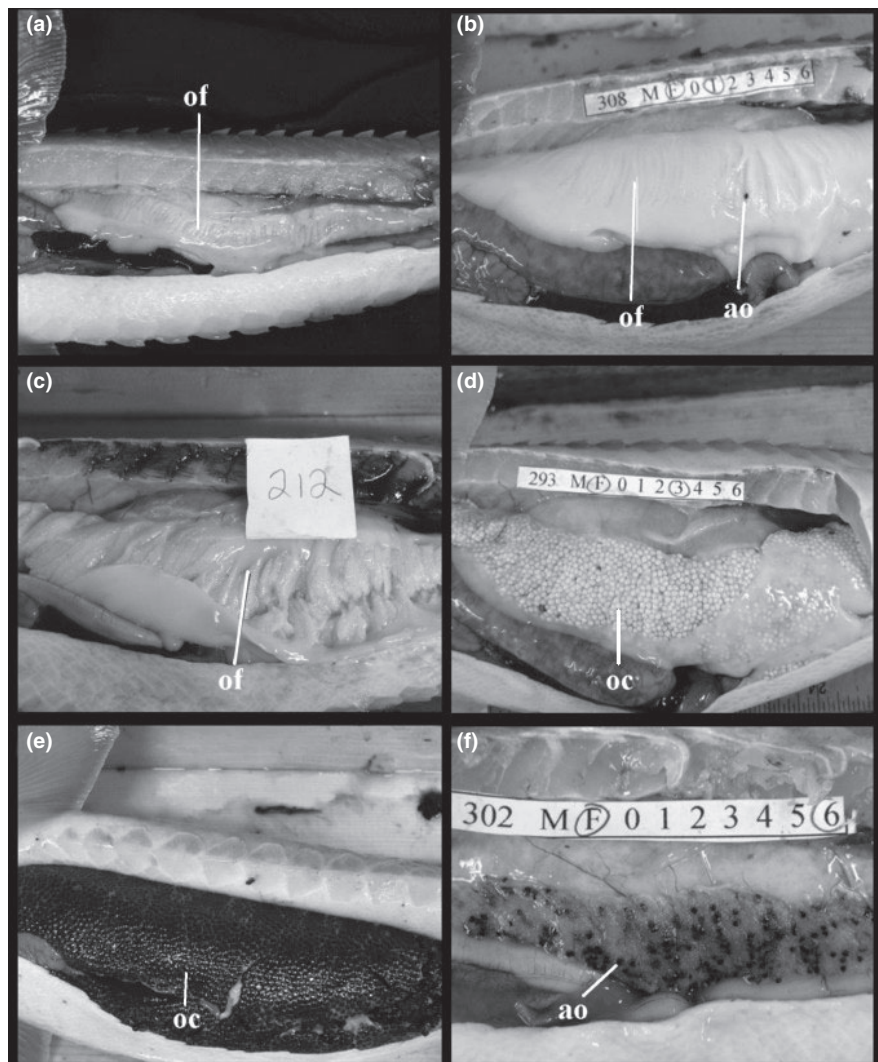


Fig. 5. Dissected views of gonadal development stages in female shovelnose sturgeon: (a) Fv, (b) FI, (c) FII, (d) FIII, (e) FIV, and (f) FVI. of, ovarian fold; oc, ooctyte; ao, atretic oocyte
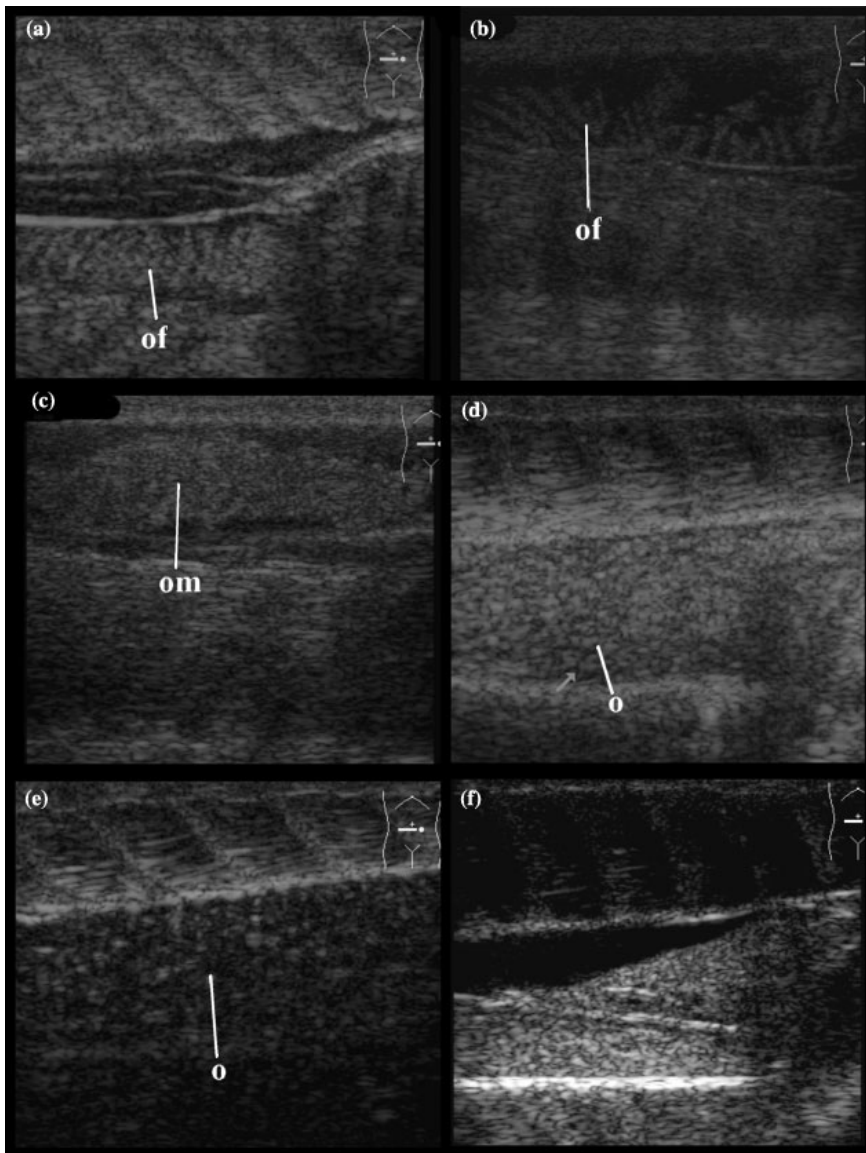
Fig. 6. Ultrasound images of gonadal development stages in female shovelnose sturgeon: (a) Fv, (b) FI, (c) FII, (d) FIII, (e) FIV, and (f) FVI. of, ovarian fold; om, ovarian mass; o, oocyte

Histologic examination revealed the beginning of yolk formation (vitellogeneses) in FII females, with the appearance of two distinct zones of yolk formation in the cytoplasm (Fig. 7c). The lightly acidophilic internal zone contained large yolk vacuoles (macroplatelets) (Fig. 7c) and the strongly acidophilic external zone appeared smooth microscopically with small yolk vacuoles (Fig. 7c). The germinal vesicles of these oocytes contained abundant euvitelline nucleoli that stained strongly basophilic (Fig. 7c). The cell wall showed the primordium of the zona radiata as a slightly acidophilic band inside the follicular envelope (Fig. 7c).

The yellow egg female (Stage FIII) was relatively rare (7.5%; n = 11) but was present in every season (Fig. 4). Mean length of FIII females was 606 ± 17-mm FL and ranged between 555 and 735 mm. Although the amount of ovarian fat was generally lower than that of the FII females, mean GSI (9.37 ± 0.88%) was relatively high. The ovary of FIII females was easily identified grossly by the presence of large yellow and light green oocytes (Fig. 5d). The ovaries of the FIII females were easily identified by ultrasound imagery (Fig. 6d). Although the ovarian folds were no longer distinguishable, the relatively large oocytes were distinguishable.

Ovaries of FIII females showed the presence of large oocytes with a large central nucleus (Fig. 7d); the cellular matrix was composed of both micro- and macroplatelets distributed in a two-layer fashion. The large macroplatelets were distributed adjacent to the nucleus and the microplatelets formed a region close to the cell membrane (Fig. 7d). The euvitelline nucleoli now stained slightly acidophilic (Fig. 7d). The cell membrane of the oocyte had become more complex with the presence of two distinct zona radiata that stained acidophilic (Fig. 7d). Many striations apparent in the zona radiata, producing a columnar appearance (Fig. 7d).

Black egg stage FIV females comprised 12.8% (n = 19) of females and were present in all seasons except for the summer (Fig. 4). Mean length of FIV females was 655 ± 9-mm FL and ranged from 569 to 713 mm FL. Grossly, the ovaries of FIV females were unmistakable due to the presence of large, black oocytes (Fig. 5e). The mean GSI of 18.9 ± 1.01% for FIV females was greater than that of any other stage (ANOVA; P < 0.001). Oocytes of the FIV females were easily identified with ultrasound (Fig. 6e).

Melanosomes were present inside the oocyte cell membranes of the FIV female (Fig. 7e), giving the eggs their characteristic
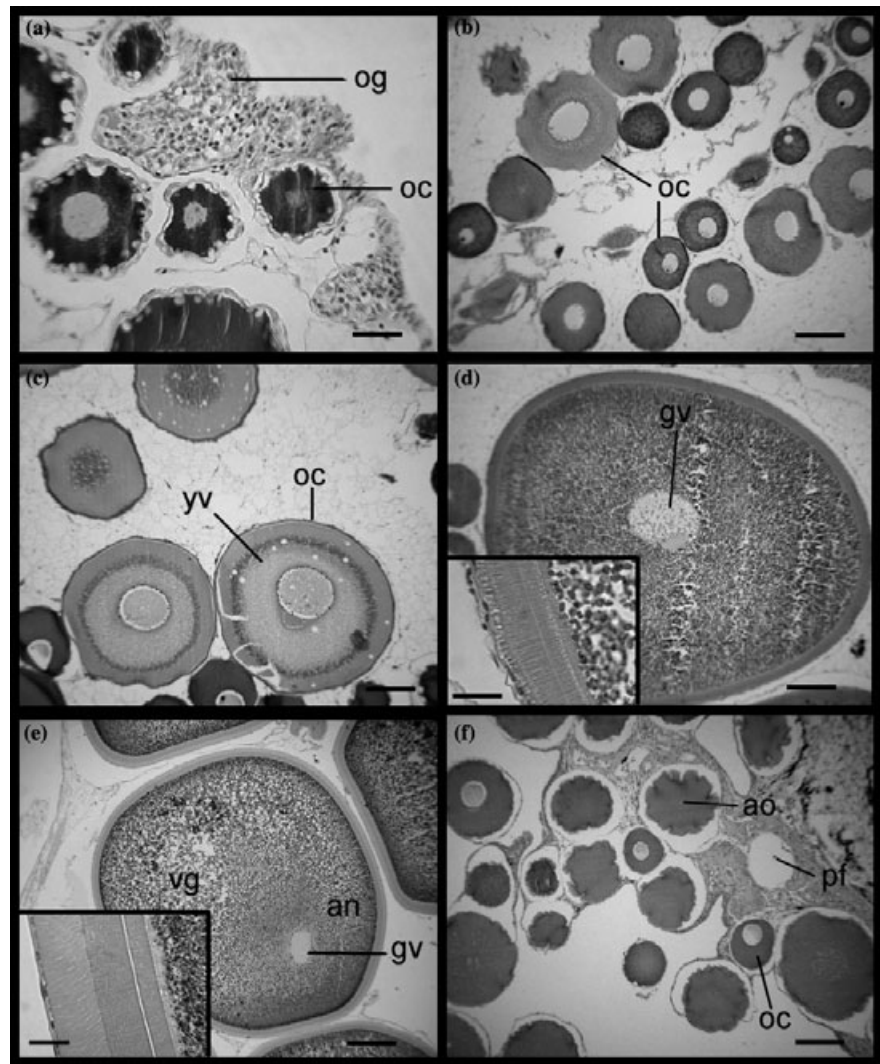
Fig. 7. Histologic micrographs of gonadal development stages in female shovelnose sturgeon: (a) Fv 250× magnification (scale bar = 80 μm), (b) FI 100× magnification (scale bar = 200 μm), (c) FII 100× magnification, (d) FIII 40× magnification (scale bar = 500 μm), insert 630× magnification (scale bar = 32 μm), (e) FIV 40× magnification, insert 630× magnification, and (f) FVI 100× magnification (200 mm). og, oogonia; oc, oocyte; gv, germinal vesicle; an, animal hemisphere; vg, vegetal hemisphere; ao, atretic oocyte; pf, post-ovulatory follicle

black color. The germinal vesicle and the microplatelets had migrated into the animal hemisphere of the oocytes (Fig. 7e). The macroplatelets coalesced in the vegetal hemisphere (Fig. 7e). The cell membrane had segregated into three distinct zona radiata (Fig. 7e). All three layers of the zona radiata contained striations and stained lightly acidophilic (Fig. 7e).

Only one spawning female was obtained during the study. This individual was caught by a commercial fisherman at a water temperature of 19°C. The spawning female freely expressed oocytes when the abdomen was stroked using the Bruch Stroke (Bruch et al., 2001).

Spent females (FVI) comprised 5% (n = 8) of the total catch of females (Fig. 4) and were 638 ± 11-mm FL, ranging between 597 and 705-mm FL. The FVI females were present during three of the four seasons, with no spent females caught in the summer (Fig. 4). This was due to the low catch rates of shovelnose sturgeon during summer of the year. Mean GSI of FIV (1.63 ± 0.43%) females was similar to that of Fv females. Most ovaries were pink to translucent, with few atretic follicles (n = 5) (Fig. 7f), while others had many atretic oocytes suggesting follicular atresia (n = 3). Ultrasound imaging was not reliable, with 75% (n = 4) of FVI females being identified as males (Fig. 7f).

Histologic examination of the stage FVI females revealed the presence of both atretic oocytes and the developing oogonia (Fig. 7f). At high magnification atretic oocytes stained lightly acidophilic, and were identified as having an irregular shape with degradation of the cellular wall (Fig. 7f). The developing primary oocytes for the next batch of eggs were similar to oocytes of stage FI (Fig. 6f).

**Fecundity**

The mean fecundity of FIV female shovelnose sturgeon was 30 767 ± 2143 eggs (n = 13), or 23.6 ± 1.26 eggs per gram of fish weight. Fecundity of females was positively related to the weight of the female with the number of eggs = 30.24 × body weight − 8392 (P = 0.013, $r^2$ = 0.45). Additionally, there was a weak, positive relationship between FL of the female and fecundity (number of eggs = 146.37 × FL − 66 176, P = 0.053, $r^2$ = 0.23).

**Intersexuality**

Seven intersexual sturgeon were sampled during this study. Some obscure hermaphrodites may have been missed because all of the identified intersexual fish were predominantly males (Fig. 8a). The intersex fish all had patches of oocytes embedded in the testicular tissue (Fig. 8a). Only one intersexual fish was scanned with ultrasound and was misidentified as a male with only testes evident (Fig. 8b). Histologic analysis of these fish confirmed the presence of both male and female tissues
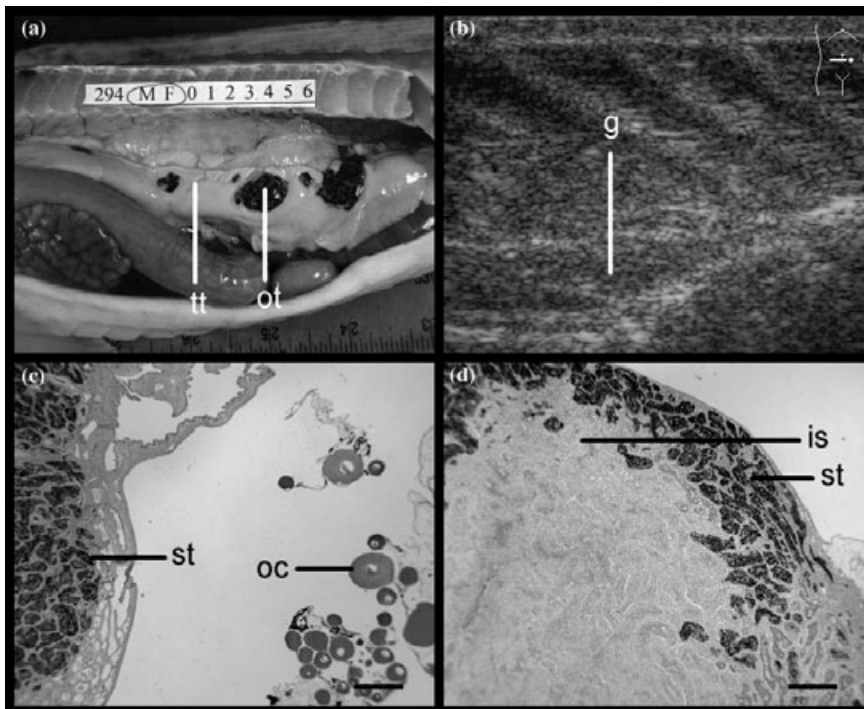
Fig. 8. Example images of intersexual shovelnose sturgeon sampled from Middle Mississippi River (River km 0–322): (a) dissected, (b) ultrasound, (c) 100× magnification (scale bar = 200 μm), and (d) 250× magnification (scale bar = 80 μm). oc, oocyte; ot, ovarian tissue; g, gonad; is, seminiferous tubules devoid of sperm; st, seminiferous tubules; tt, testicular tissue

(Fig. 8c). The male tissue had reduced reproductive output with most of the seminiferous tubules being devoid of spermatozoa (Fig. 8d). Only primary oocytes were visible similar to those of a FI female (Fig. 8c).

## Discussion

The patterns of male shovelnose sturgeon gonad development were similar to that of other sturgeon species. We identified three stages of male development coinciding with those identified for lake sturgeon (Bruch et al., 2001), white sturgeon (Conte et al., 1988), and the Russian sturgeon (Dettlaff et al., 1993). We failed to identify spent males, which also rarely occurred in lake sturgeon due to the relatively fast turnover of testicular tissue (Bruch et al., 2001). Bruch et al. (2001) found one spent male by chance after examining over 1000 specimens. In the MMR, all three stages of development were identified in all seasons, with running ripe, stage MII individuals being found only in the spring. Hence, the shovelnose sturgeon, like other North American sturgeon, likely spawns solely in the spring. The year-round presence of MII stage males suggests that energetic investment in gonadal tissue occurs considerably before spawning.

The female shovelnose sturgeon, like the males, developed similarly to other sturgeon species concerning gonad maturation. We identified all seven stages of female sturgeon gonadal development described for lake sturgeon (Bruch et al., 2001). Although the ovary structure of the acipenserids is different from teleosts, the development of the oocytes is similar (Groman, 1982; Yasutake and Wales, 1983). However, yolk deposition in the shovelnose sturgeon differed from other fish species in that the large yolk vacuoles formed a ring adjacent to the germinal vesicles. In fishes such as the striped bass, *Morone saxatilis*, the deposition of fat occurs in regions near the cell membrane (Groman, 1982). The mature oocytes of the shovelnose sturgeon had unequal distribution of yolk, characteristic of the acipenserids (Conte et al., 1988; Dettlaff and Vassetzky, 1991; Dettlaff et al., 1993; Bruch et al., 2001).

Gonadal designations asserted by other authors are comparable to those outlined here. Our staging designation agreed with those used by Moos (1978) and Carlson and Pflieger (1981) with few exceptions (Table 1) that arose from differences in the methods for stage designation. Unlike these previous studies, we used relatively objective, anatomical characteristics (i.e. independent of date or color) to provide a complete staging description with ultrasound or gross inspection. This guide mirrors those developed for other sturgeon taxa (Conte et al., 1988; Dettlaff and Vassetzky, 1991; Dettlaff et al., 1993; Bruch et al., 2001).

Length at maturity differed for male and female shovelnose sturgeon, with males becoming mature at 500-mm FL and females at approximately 570-mm FL. This suggests that males become mature at 4 years, whereas females do not reach maturity until age 6 (Jackson, 2004). There was considerable overlap in length between FI and Fv females attributable to differing reproductive cycles among individuals. Once length at maturity was reached, neither the male nor female shovelnose sturgeon appeared to spawn every year. This is supported by the presence of large stage MI (605 mm) males and stage FI (629 mm) females present in the spring. Intermittent spawning is common among the fishes in the order Chondrostei, with some species requiring as much as 7 years to complete a cycle of development (Bruch et al., 2001). Overlapping stages within seasons lends more support to intermittent spawning and differing reproductive cycles.

We have presented data from both dissection and histology, providing better insight into the development of oocytes and spermatocytes in the gonadal tissue, which was lacking in the staging designation developed by Carlson and Pflieger (1981). Ultrasound imaging was previously shown to be useful for distinguishing between the sexes of shovelnose sturgeon (Colombo et al., 2004; Wildhaber et al., 2005). Although we have not examined the fidelity of this equipment for staging, these preliminary results are promising. The only stage at which ultrasound was not effective was FVI. However, with further study this may be resolved by identifying those

structures that may be indicative of the stage. Previous studies have shown ultrasound sex-determination efficiency to be related to stage (Wildhaber et al., 2005). However, a cross-section image was used in determining sex and stage (Wildhaber et al., 2005); this approach may be less accurate, as those structural characteristics indicating stage are more prevalent in a transverse view (Colombo et al., 2004).

A total of seven intersex fish were identified in our sample, resulting in a 2% intersexuality rate in the population of shovelnose sturgeon in the MMR. This amount of intersexuality is consistent with the rate of 3% found by Carlson and Pflieger (1981) for shovelnose sturgeon and with 1% for Atlantic sturgeon found by Van Eenennaam et al. (1996). Recently completed research determined that some intersexuality can be attributed to increased estrogen-mimicking compounds found in the MMR shovelnose sturgeon (Koch, 2005).

The information presented here can be used as a guide for future shovelnose sturgeon studies, and allows for the comparison of reproductive development between sturgeon species. We have also provided a more complete guide by incorporating ultrasound images of each stage of gonadal development, heretofore lacking in previous studies of sturgeon gonadal development. Managers and biologists now have a quick and reliable method to assess the reproductive demographics of shovelnose sturgeon populations.

## Acknowledgements

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