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# Laparoscopy, a minimally-invasive technique for sex identification in cultured great sturgeon *Huso huso*

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ABSTRACT

Laparoscopy is a fast, effective, and minimally invasive method for determining the sex and reproductive stage of great sturgeon (*Huso huso*) via a small (1 cm) incision in the abdominal wall. The system consists of a 7.5 mm, 22 cm long cystoscope sheath, fiber-optic light, halogen cold light source, video camera with a control unit, and a color monitor, and allows direct viewing of the abdominal organs of a fish. In this study, it was used on 120 farm raised great sturgeon ranging in age from 3 to 16 years. Sex identification was successfully performed in all fish which 95.8% sexed with laparoscopy alone. Five of 120 sturgeon required gonadal biopsy and microscopic examination to confirm sex. The unidentified fish were smaller and thinner than others. Results showed that both sexes could be identified with this system as early as 3 years of age and the sex ratio under culture conditions of females, males and unidentified sex were 56.6, 39.2 and 4.2%, respectively. While most reproductive applications of laparoscopy in great sturgeon related to the visual identification of gender and reproductive stage, the ability to biopsy the gonad enabled these identifications to be objectively assessed using histology. Our results suggest that laparoscopy is an efficient technique for sex identification at different ages and determination of gonadal development stages in great sturgeon. The ability to safely perform minimally invasive reproductive surgery in this species may have important management and conservation benefits during the culture period or wild population assays.



## 1. Introduction

Great sturgeon or beluga *Huso huso* is native to the Caspian Sea, the Black Sea and the Azov Sea. The high growth rate of great sturgeon makes it a very suitable candidate for commercial aquaculture. Rearing of sturgeon has only recently begun, prompted by their declining populations in natural habitats and the rise in meat and caviar prices (Birstein et al., 1997). However, little information is available on broodstock management, gonadal development, and puberty under culture conditions for great sturgeon compared to other cultured sturgeon species (Amiri et al., 1996; Doroshov et al., 1997; Van Eenennaam and Doroshov, 1998; Linares-Casenave et al., 2003; Hurvitz et al., 2007).

Sturgeons differ from most teleosts by their greater age at puberty and longer ovarian cycles. Sexual development in wild great sturgeon is completed under natural conditions after 12 to 14 years in males and 16 to 18 years in females (Holčik, 1989). In general, under aquaculture conditions sturgeon maturity is usually reached at an earlier age than is seen under natural conditions (e.g., Doroshov et al., 1997).

The sex and stage of maturity of sturgeon must be known for reproductive behavior and spawning activity and as means of ensuring a broodstock selection with a known sex ratio. Unfortunately, sturgeons have no secondary sexual characteristics. Moreover, sturgeons have multi-year reproductive cycles further complicating the ability to distinguish between sexes and determine maturation (Moos, 1978; Doroshov et al., 1997). The ability to assess sturgeon reproductive status in the field quickly, reliably, and without injury to the fish has proven difficult because current methodology requires physical examination of gonad tissue. The absence of external markers for sexing has motivated search for a practical technique for internal examination of the gonads for sex identification (Hurvitz et al., 2007; Falahatkar et al., 2008; Keyvanshokooh and Gharaei, 2010). Caviar production is the main purpose of sturgeon aquaculture and a reliable method is needed to separate the fish according to sex (Hurvitz et al., 2007). While females remain in culture ponds or tanks for many more years under conditions of optimal growth and development, males are destined for meat production. Furthermore, sexing of great sturgeon cannot be carried out with non invasive methods before the age of 3 (Falahatkar et al., 2008), while sex differentiation in other

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sturgeons is completed at as early as 1 to 2 years of age (Doroshov et al., 1997; Hurvitz et al., 2005). In older females approaching puberty, information on ovarian developmental stage is critical for the quality of caviar production. Vitellogenesis in cultured sturgeons can last up to 3 or 4 years (Doroshov et al., 1997) with large variations among individual fish of the same age (Amiri et al., 1996). Therefore, selection of females for estimating the optimal time for caviar harvest requires several examinations of each fish.

Knowledge of both sex and stage of maturity is important from many aspects especially in valuable fish like sturgeons. Since there is no clear external sexual dimorphism in sturgeons, either among maturing or non-maturing individuals, development of sex identification methods is needed. Consequently, determining sex and stage of maturity in fish without external sexual dimorphism, or when external characters are not developed, has usually involved either the use of invasive techniques such as biopsy or intensive techniques such as hormone radioimmunoassay (Wildhaber et al., 2005, 2007). These methods are time and labor consuming, because the fish must be examined individually to minimizing the handling and physical harms, as previously indicated by a positive correlation between time of exposure to stressors and plasma levels of cortisol (Thomas and Robertson, 1991; Falahatkar, 2010). Noninvasive methods for sexing live fish using ultrasonography and endoscopy have been recently developed for shovelnose sturgeon *Scaphirhynchus platorynchus* (Wildhaber et al., 2005, 2007). Although these new techniques need to examine the fish individually, but less time is needed to identifying the fish. The main objective of this study was to test the criteria used for determining the sex of shovelnose sturgeon, on great sturgeon. Following Wildhaber et al. (2005), we quantified and established a reliable minimally invasive technique and the ability to use laparoscopy as a technique for sexing and assessing stage of maturity in great sturgeon grown under aquaculture conditions at different age groups.

## 2. Materials and methods

### 2.1. Fish and rearing conditions

Great sturgeon broodstocks were caught in the Caspian Sea and transported to the Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Complex, located in Rasht, Guilan, Iran. Fish were injected with common carp pituitary extract according to Conte et al. (1988) and eggs were collected traditionally by killing the fish and fertilized with appropriate sperm (ratio of 1 L egg with 10 mL of sperm) and washed with silt solution for removing the adhesion after the fertilization (Conte et al., 1988; Pourasadi et al., 2008). Then eggs were incubated in special trays egg incubator (29.3 × 39 × 12.8 cm depth) in which water comes from the below of tray and follows across the single layer of eggs and finally exit from the above tray with a design known as a Youshchenko that was originally developed in Russia. The resulting fry were then cultured in circular concrete tanks (2.5 m internal diameter and 17 cm depth) that were based on the design developed by the Russian Federal Institute of Fishery and Oceanography (VNIRO). Finally, juveniles were transferred to earthen ponds for grow out. Fish not used as part of the Caspian Sea stocking program were adapted to artificial diet in the concrete tanks. During the first year, these fish were maintained under routine hatchery conditions, in circular fiberglass 1000-L flow-through tanks (200 cm diameter and 32 cm depth). After reaching an average weight of 300 g, they were transferred to 2-ha rectangular earthen ponds and held under natural photoperiod with water temperatures between 3 and 16 °C in winter (November–February) and 20 to 29 °C during the rest of the year. They were fed twice a day with supplementary trout pellet feed (containing 38% protein and 14% fat), at 0.5 to 2% of their biomass daily, depending on the season and the fish size. These fish represent groups 2–4 in Table 1.

At another farm (Morvarid Ghorogh Sturgeon Farm, Talesh, Guilan, Iran), 100 g or greater fish were acquired from the Shahid Dr. Beheshti Sturgeon Fish Propagation and Rearing Complex. They were fed 3–4 times daily with a commercial pellet (Biomar®, 50% protein, 18% fat) at 0.5 to 3% of biomass until they reached 3 years of age. This group represents group 1 in Table 1.

### 2.2. Laparoscopy

Experiments were conducted using uniquely tagged, farmed great sturgeon of known age (Table 1). Fish were starved for at least 2 days prior to examination. During identification of sex with laparoscopy, length, and wet weight were also measured.

For gonadal examination, a laparoscope system (#21.00a, LUT GmbH, Denzlingen, Germany) was constructed (described by Hurvitz et al., 2007 with some modifications). Briefly, it consisted of a 7.5 mm cystoscope sheath 30°, 22 cm long and 7.5 mm in diameter, incorporating an optic light transmission fiber, connected to a halogen cold light source (150 W, #30801, Gima, Gessate, MI, Italy) and a miniature videocamera connected to a 14-in color monitor (Shahab Co, Tehran, Iran) (Fig. 1A). With this system, the internal organs of the fish could be viewed along the entire body cavity and the pictures of selected points could be monitored and saved digitally as an individual record (Fig. 2).

Fish were held in a 36 $m^3$ Kouranski holding tank (which is special rectangular concrete tank for brood fish with internal slopes; 14.30 × 14 × 1.5 m) at Shahid Dr. Beheshti Sturgeon Farm and 1250 L concrete tanks (40 cm depth and 200 cm diameter) at Morvarid Ghorogh Sturgeon Farm before the examination. Individual fish was put into the rectangular plastic container (400-L volume) filled with fresh 15–19 °C flowing water and 150 mg $L^{-1}$ clove powder for sedating the fish. The fish were then placed on a table for examination. To view gonads through the body wall, following Wildhaber et al. (2005), an 8 to 10 mm anterior to posterior incision was made with a sterile scalpel in the ventral wall of the body cavity toward the posterior end of the fish through which the cystoscope sheath was inserted (Fig. 1B). The incision was opened with a trocar (Unimax, Hsin Tien, Taipei) for better access to the abdominal space. The scope was equipped with a 1-L sterile saline solution and an internal canal through which the organs could be rinsed for a clear view. Sex identification with laparoscopy was usually performed in less than 3 min. After the examination, the incision was closed with a simple interrupted stitch using a silk suture with a thickness of 0.9 mm and a surgery needle (no. G 414/4, ACUFIRM, Dreieich, Germany). The incision was disinfected with Povidone–iodine solution 10% (Kishmedifarm, Kish, Iran) and Chloramphenicol spray (Afagh, Tehran, Iran) and the fish was injected with 1 mL of oxytetracycline

**Table 1**
Sex ratio and mortality post examination using laparoscopy for different groups of great sturgeon.

| Group (total length; cm) | Farm | n | Age | Female | Male | Sex ratio | Mortalities |
|---|---|---|---|---|---|---|---|
| 1 (116.7 ± 11.3) | Morvarid Ghorogh | 29 | 3 | 20 | 9 | 1:0.45 | 0 |
| 2 (118.2 ± 10.5) | Shahid Beheshti | 59 | 6 | 33 | 26 | 1:0.79 | 0 |
| 3 (136.6 ± 8.4) | Shahid Beheshti | 31 | 9 | 16 | 15 | 1:0.94 | 0 |
| 4 (210) | Shahid Beheshti | 1 | 16 | 1 | – | – | 0 |
| All classes | – | 120 | 3–16 | 70 | 50 | 1:0.71 | 0 |

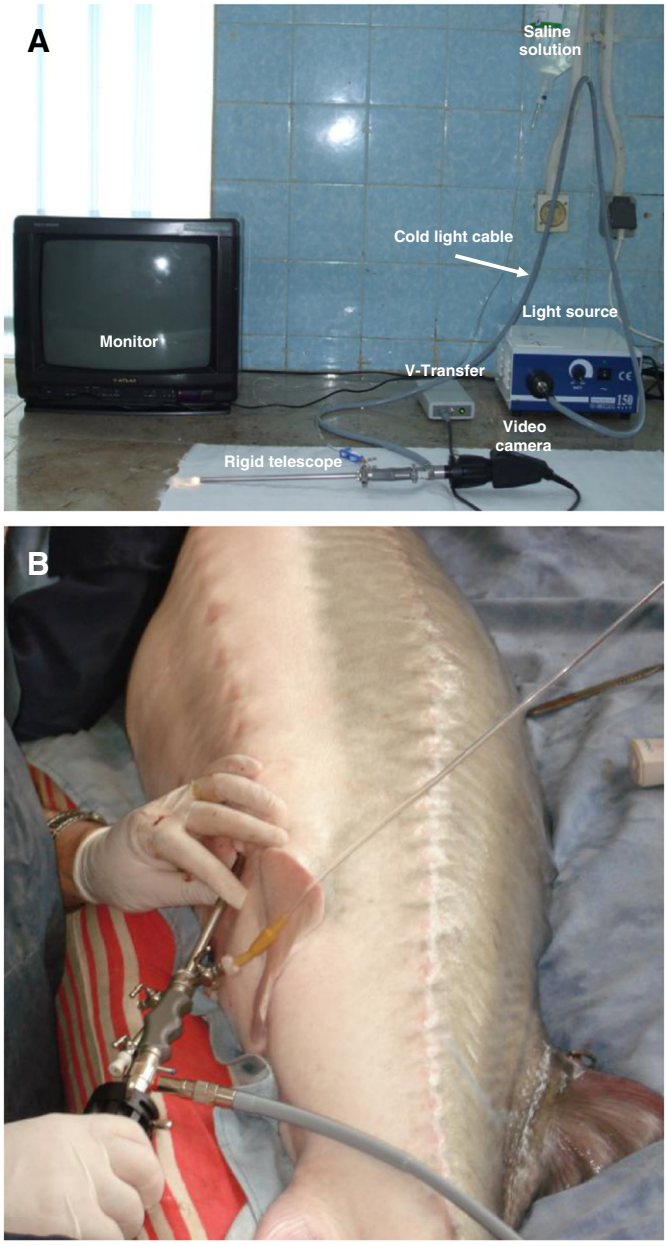


**Fig. 1.** Laparoscopy arrangement: A. System components: a 7.5 mm rigid telescope, with a video camera, connected to a halogen cold light source with fiber-optic light transmission cable, attached to a camera control, and displayed on a color monitor. B. Great sturgeon under laparoscopic examination through an abdominal incision.

10% (Razak, Tehran, Iran) as an antibiotic. Finally, the fish was placed in fresh clean water for recovery. The laparoscopic method was also used to assess gonadal development in both males and females. We used gonadal stages of shovelnose described by Moos (1978) and Wildhaber et al. (2007) with some modification according to our experience with great sturgeon (Table 2).

### 2.3. Sex identification

A total of 120, 3- to 16-year-old great sturgeon in four different groups were examined using laparoscopy. Sex identification was done using laparoscopic inspection of gonads for the presence of the same structures described by Wildhaber et al. (2007) such as oocytes, ovarian folds, and testicular lobes. For fish that could not be sexed based on laparoscopy, gonadal biopsy and microscopic examination were used to identify sex (Fig. 3). The biopsy required a 4-cm incision in the abdomen to allow for direct inspection and sampling of the gonads (Conte et al., 1988), but we made an incision with 2.5–3 cm for direct observation and biopsy of gonads. After that, incision was closed with one or two simple suture as described above. Samples were prepared according to Amiri et al. (1996) for gonad histology examination.

### 2.4. Statistical analysis

Deviation from the expected 1:1 sex ratio for each sturgeon group was tested using the Chi-square test at 95% significance level, using SPSS 13.0 software (Chicago, IL). Independent samples *t*-test was performed to compare mean weight of females and males in each group. Data are presented as mean ± standard deviation.

## 3. Results

We found laparoscopy to be a highly effective method for identifying sex and determining maturity of immature and mature great sturgeon. A total of 120 fish were examined: 68 (56.7%) were found to be females, 47 (39.2%) males, and 5 (4.1%) could not be sexed through laparoscopy and thus, common biopsy methods were used for sex identification. For the 5 fish that could not be sexed by laparoscopy, high fat stores obscured the gonads, making determination between fat and gonad difficult. These fish were in pre-vitellogenic stage (Fig. 3A). Consequently, 68 of 70 (97.1%), 47 of 50 (94%) and 115 of 120 (95.8%), as female, male and for both sexes were correctly identified, respectively. Visual examination immediately and 1 month after surgery indicated no significant hemorrhage or surgical trauma associated with any procedure in any fish. Also no mortality was observed in any age class after sex identification by laparoscopy.

According to monitoring the gonads of fish by laparoscopy and confirmed by histology, different stages of ovarian and testis development were identified (Table 3). Also, the color and descriptive morphology for further examination by laparoscopy in great sturgeon was made in both sexes.

Overall effectiveness for sex identification and maturity assessment were highest for groups 2 and 3 (Table 4). The female to male ratio was not significantly different from the expected 1:1, according to Chi-square test ($p>0.05$) for groups 2 and 3 but it was significantly different for group 1 and all fish combined ($p<0.05$; Table 1).

There were no significant differences between weight of males and females in any group ($p>0.05$). The unidentified fish were smaller in condition factor and thinner than the others with an average weight of 7.2 ± 2.9 kg.

The ability to identify sex changed with age (Table 4). The greatest effectiveness (100%) was achieved in periods where gonads were largest at age 9 and 16. The ability to identify sex was higher than 93% when age of fish was over 3 years. The mean effectiveness for all fish combined was 95.8% (97.4 in females and 94% in males) with sex and gonad stage identifiable throughout the study.

Three-year-old fish from Morvarid Ghorogh Sturgeon Farm had an average weight of 7.6 ± 2 and 7.9 ± 2.1 kg for females and males, respectively. They were examined by laparoscopy for gonadal development and all females had ovaries at the pre-vitellogenic stage and males had testes at early spermatogenesis.

In 6-year-old fish from Shahid Dr. Beheshti Sturgeon Farm average weight for females and males were 9.9 ± 1.7 and 10.6 ± 3.2 kg, respectively. All females had ovaries at the pre-vitellogenic stage and males had testes at mid spermatogenesis. Nine-year-old fish from the same hatchery had an average weight of 11.9 ± 3.6 kg for females and 12.5 ± 3.7 kg for males, the maturity stages were vitellogenic and spermatogenesis, respectively (Fig. 3B and D). Only one 16-year-old female with 52 kg body weight was examined and it was at the post-vitellogenic and migratory nucleus stage (Table 4).

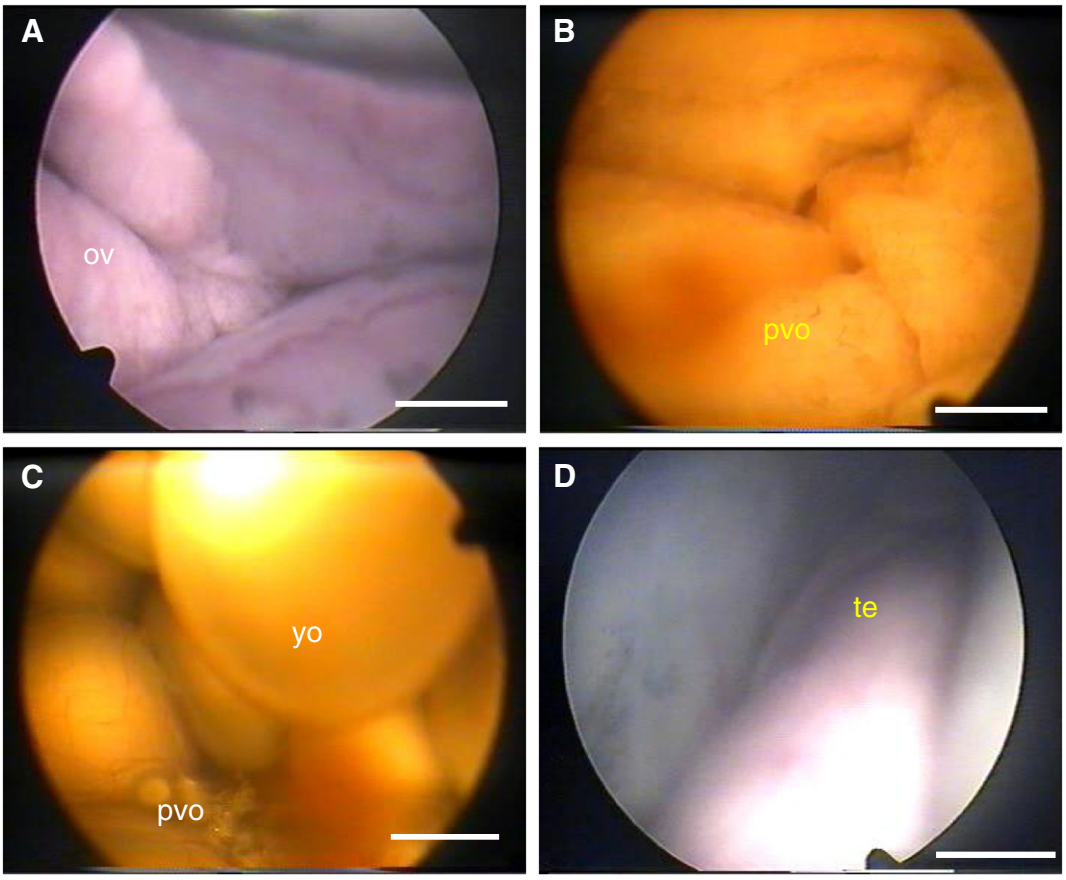


**Fig. 2.** Laparoscopic view of great sturgeon ovaries and testes: A and B: Ovaries (ov) in stage II. Oocytes are in pre-vitellogenic oocyte stage (pvo). C: Oocytes in stage IV (vitellogenic stage, yellow oocyte stage; yo) with some oocyte in stage II. D: Testes (te) in stage II (early spermatogenesis). Scale bar: 1 mm.

## 4. Discussion

Present study showed that the laparoscope was effective in visualizing the gonads and for sexing great sturgeon through an incision in the abdomen. Of the 120 fish examined using laparoscopy, 95.8% (n = 115) were correctly sexed. All attempted endosurgical procedures were successfully completed without causing significant hemorrhage or surgical trauma. Overall, the effectiveness of laparoscopy for sex identification of cultured great sturgeon observed in this study was similar to that seen by other studies (e.g., Wildhaber et al., 2005) and more effective than had been found in previous work on other fish species (e.g., Ortenburger et al., 1996).

Although some fish exhibit external morphological differences between sexes, there is relatively limited information available to determine the sex of sturgeons by dimorphic differences alone. Urogenital shape (Vecsei et al., 2003) and biometric head differences (Mal'tsev and Merkulov, 2006), while used to sex other sturgeon species, cannot effectively be used in great sturgeon (Falahatkar et al., 2008). This method has less than 70% accuracy in some North American sturgeons (Vecsei et al., 2003).

Several methods have been used for the examination of sex and gonad developmental stage of fish. Gonadal structures of sturgeons have also been examined using ultrasound (Moghim et al., 2002; Colombo et al., 2004; Wildhaber et al., 2005, 2007) and endoscopy (Kynard and Kieffer, 2002; Hernandez-Divers et al., 2004; Bryan et al., 2005; Wildhaber et al., 2005, 2007; Hurvitz et al., 2007; Divers et al., 2009). Each method for the examination of sex and gonad developmental stage in fish has advantages and disadvantages. The usual and most common technique is a 3- to 4-cm incision in the abdomen, through which the gonads can be visually examined. This method, known as "biopsy" with histological examination, is more stressful and results in an elevated cortisol response in inspected fish because of larger incision and sampling the gonad compare to laparoscopy (Falahatkar, 2010). For ripe females, a lesser incision of 1 cm is enough to perform a gonadal biopsy with 4-mm tygon tubing (Conte et al., 1988; Hochleithner and Gessner, 2001). Other non- or minimally invasive techniques like sonography and endoscopy are effective at identifying sex and stage and are seemingly harmless to the reproductive organs of the fish. In our study, no comparison was performed between laparoscopy and any of the above mentioned

**Table 2**
Descriptions of the physical characteristics of shovelnose sturgeon reproductive stages from Moos (1978) and Wildhaber et al. (2007) modified for great sturgeon.

| Stage | Females | Males |
|---|---|---|
| I | Few oogonia at gonad periphery | Lobules with a few primary spermatogonia |
| II | More and larger oogonia | Predominantly spermatogonia with occasional spermatocytes and spermatids |
| III | Yolk deposition | Spermatogenesis occurring with equal occurrence of spermatogonia, spermatocytes, spermatids, and spermatozoa |
| IV | Large full yolked oocytes with central nucleus | Predominately spermatids and spermatozoa with few spermatogonia |
| V | Nucleus at animal pole with micropyles in oocyte membrane | Predominately spermatozoa |
| VI | Large empty follicles, some with dark pigment | Unspent spermatozoa with spermatogonia |

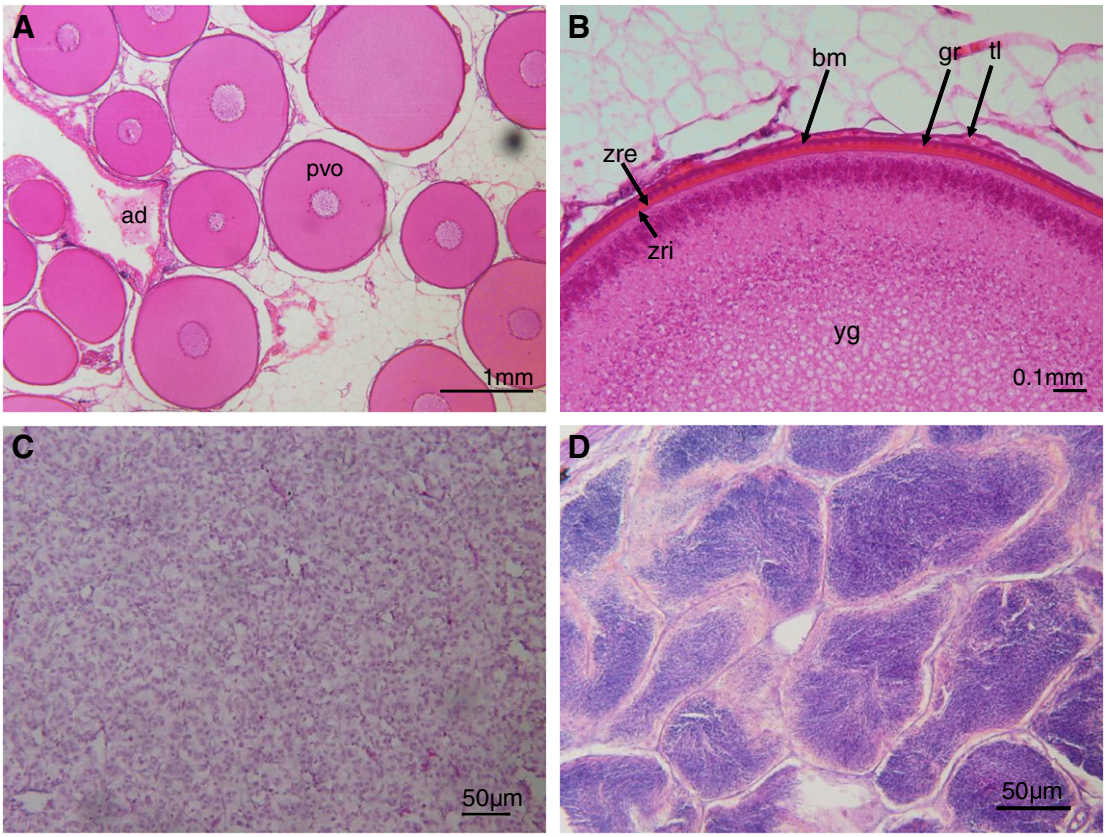


**Fig. 3.** Light microscopy showing maturity stages of great sturgeon (paraffin sections, hematoxylin-eosin stain). A: female. Pre-vitellogenic stage (pvo) and adipose tissue (ad). B: female. Magnification of oocyte layer in late pre-vitellogenic (zri: zona radiata interna, zre: zona radiate externa, gr: granulosa cells, tl: thecal layer, bm: basement membrance, yg: yolk globules). C: male with spermatogonia (early spermatogenesis). D: male with spermiation stage.

methods. However, it was found that laparoscopy could be used in both the field and the laboratory, similar to the results obtained by Wildhaber et al. (2005) and Hurvitz et al. (2007).

Using ultrasound for sex identification and reproductive staging of sturgeons has the advantage of being a non-invasive method. Accuracy rates are 86% (Colombo et al., 2004) to 97% (Moghim et al., 2002) in different species. Nevertheless, the use of ultrasound requires a high degree of expertise in order to analyze the images and the most difficulty to analyze the images are related to younger fish. Unlike ultrasound, laparoscopy enables a direct observation of oocyte color, size and distribution (Bryan et al., 2007; Wildhaber et al., 2005, 2007) and thus, allows a potentially better evaluation of developmental stage. This system provides magnification of the observed organs. This is an important advantage, especially for sex identification in 3-year-old fish when oocytes are as small as 0.12 to 0.18 mm, as noted by Hurvitz et al. (2005) for Russian sturgeon.

Except for the price (approximately 10,000 US dollars for whole instruments), there are some disadvantages to using laparoscopic techniques. Less successful in identifying sex of stages I–II (pre-vitellogenesis) great sturgeon using laparoscopy in our study was also performed by Wildhaber et al. (2005) in shovelnose sturgeon and Matsche et al. (2011) in shortnose and Atlantic sturgeons *Acipenser oxyrinchus oxyrinchus*. One of the errors in endoscopy is misidentification of fat stores as mature testes. Misclassification of immature fish as mature males accounted for error in maturity classifications, and one mature female was incorrectly classified as male by Wildhaber et al. (2005). This error is less of a problem in cultured fish because of known age and estimation of gonadal maturity stage. When sturgeon grows and gonads mature, relative fat content decreases allowing easier and faster identification of sex and maturity stage. In addition to fat stores, full stomachs occasionally impeded visibility of gonads. To minimize the latter problem, we allowed

**Table 3**
Laparoscopic view and gonadal morphology (Matsche et al., 2011) in great sturgeon with the diameter and color of oocyte and testis. Histological development stages follow those explained by Amiri et al. (1996) or Van Eenennaam and Doroshov (1998).

| Sex | Descriptive gonad morphology | Stage | Diameter (μm) | Color |
|---|---|---|---|---|
| Female | Ribbon-like gonad (smooth to rough surface) covered with pink/beige to orange fat | I and II | 100–200 | Semi-transparent |
| | Ovary pink with ovarian lamellae, brain like folds are well notable form lateral part | II | 200–800 | Pink to white |
| | Ovary with white to light-yellow oocytes and moderate to extensive fat | III | 800–2000 | White to yellow |
| | Ovary with yellow pigmented oocytes and little to moderate fat | III and IV | 2000–3000 | Yellow to gray |
| | Ovary large with gray oocytes and very little fat | IV | >3000 | Dark gray |
| | Numerous large gray or black eggs loose in coelom and no fat | V and VI | >3000 | Black |
| Male | Ribbon-like white testis covered with yellow to orange fat | I and II | – | Yellow to orange |
| | Tubular white testis with blood vessels and little to moderate fat | III | – | White |
| | Testis with small, white lobes and little to moderate fat and get brilliant | III and IV | – | White |
| | Moderate to large white testis | IV and V | – | White |

**Table 4**
Summary data on body weight and gonadal stage for great sturgeon age 3–16 years.

| Age | n | | Body weight (kg) | | Accuracy sexed (%) | Gonadal stage | |
|---|---|---|---|---|---|---|---|
| | F | M | Females | Males | | Females | Males |
| 3 | 20 | 9 | 7.6 ± 2 | 7.9 ± 2.1 | 93.1 | Pre-vitellogenesis | Early spermatogenesis |
| 6 | 33 | 26 | 9.9 ± 1.7 | 10.6 ± 3.2 | 94.9 | Pre-vitellogenesis | Mid-spermatogenesis |
| 9 | 16 | 15 | 11.9 ± 3.6 | 12.5 ± 3.7 | 100 | vitellogenesis | Spermatogenesis |
| 16 | 1 | | 52 | – | 100 | Post vitellogenesis; Migratory nucleus | – |

F: female, M: male.

2 days for evacuation of the intestinal tract before applying our method. Regardless, the effectiveness evident in our results support other studies that have found endoscopy to be a useful technique for determining maturity status and sex of mature fish (Moccia et al., 1984; Ortenburger et al., 1996; Bryan et al., 2007; Wildhaber et al., 2005, 2007; Hurvitz et al., 2007). Our results further indicate that use of endoscopy can be effective for sturgeon that mature at relatively small sizes and earlier ages which we had fish with 7 kg weight as the smallest fish in present study.

All 120 examined fish survived following the laparoscopic examination, delayed mortality was not observed and although growth rate was not affected (personal observation). Similar studies also showed 100% survival after endoscopy (Hernandez-Divers et al., 2004; Matsche et al., 2011). Therefore, it seems that this procedure has high quality and accuracy with these sizes of fish.

Sturgeon fishes are gonochoristic and verification for female heterogametic genetic sex identification has been shown for some species like white sturgeon *Acipenser transmontanus* (Van Eenennaam et al., 1999), bester *H. huso*×*Acipenser stellatus* (Omoto et al., 2005) and shortnose sturgeon *Acipenser brevirostrum* (Flynn et al., 2006). The sex identification system in sturgeons is classified as like as birds (ZZ male, WZ female; Devlin and Nagahama, 2002), in which the expected sex ratio of 1:1. In fact, Doroshov et al. (1997) found this ratio among hundreds of cultured white sturgeon. Nevertheless, Flynn et al. (2006) reported that normal, untreated populations of shortnose sturgeon reared in culture (Supreme Sturgeon and Caviar, Canada) typically have a sex ratio that is skewed relatively towards females (1:0.79 female:male). The results of Hurvitz et al. (2007) showed a significant deviation from the expected 1:1 sex ratio in favor of females (55% females, 40% males and 5% unidentified). Similarly, our results also present a sex ratio of 56.7% females, 39.2% males and 4.1% unidentified. It is possible that environmental factors related to intensive culture favor female survival. Sometimes sex ratio changes with environmental condition, fertilization process and because of the 3-year-old fish was reared in another farm, culture conditions may affected the sex ratio for the reason that of high mortality of males during the early stage of rearing or influence on fish during the sexual dimorphism. Different year classes of more than 100 or better, a few 1000 farmed fish needed to be sexed carefully to verify a real shift from 1:1, as our study of only 120 fish could be just a slight skew. Therefore, increasing number of examined fish will probably lead to the approximate sex ratio of 1:1. More study is needed to find why the sex ratio of sturgeon is changing during the culture period from the expected 1:1.

Great sturgeon males were not the focus of this study except for sex identification. We found that the ovary of 3- to 6-year-old females were at the pre-vitellogenic stage. In most of the fish at this stage, oocytes at the oil droplet and cortical alveolar stages could be found, compared to the perinucleolus (60–200 μm) and oil droplet (200–400 μm) stage oocytes which is described by Amiri et al. (1996).

Egg color changes throughout the developmental stages from transparent to white in pre-vitellogenic, yellow in early vitellogenic, gray and black in post-vitellogenic which is showed melanin accumulation during these phases of oocytes growth. The term of 'Black oocyte' is described migratory nucleus stage with around 3 mm diameter. This size of great sturgeon oocyte can be used for caviar production or propagation after completion of final maturation of gonad.

Through the 9 years of rearing, both sexes of great sturgeon grew at the same rate demonstrating that there is no difference in energy consumption for gonadal development. As presented in Tables 1 and 3, growth rate of fish grown at the Shahid Dr. Beheshti Sturgeon Propagation and Rearing Complex was lower than at the Morvarid Ghorogh Sturgeon Farm. This was because of 2 years of growing in earthen ponds with poor feeding practices and using different feed. Fish body weight is a highly variable factor, depending, among the other parameters, on rearing conditions; consequently the data obtained in the present study does not necessarily represent the maximum growth rate of great sturgeon in captivity.

At the 9 years old, only a few of the 16 females were at the "black oocyte" stage. However, over 80% of the females were already at different stages of vitellogenesis. Previous studies showed that no apparent relationship was found between body size and oocyte maturity stages in cultured bester (Amiri et al., 1996), cultured Siberian sturgeon (Pelissero et al., 1991), or cultured Russian sturgeon (Hurvitz et al., 2007) and highly variable pubertal size was seen in white sturgeon in both raised and wild populations (Doroshov et al., 1997) as we observed in our study according to the body size and ovarian developmental stage. Laparoscopic view and gonadal morphology in great sturgeon oocytes showed the relative semi transparent color at stages I–II to pink-white at the end of stage II, but no different in weight of fish was found. It seems that this species could be stopped at the pre-vitellogenesis stage for many years.

High accuracy rate for sexing and maturity stage was found in larger fish and this indicates that when gonads grow to stage III or higher, is easier to be identified by laparoscope. Also, less time is needed to examine these fish because the gonad has higher volume and in both sexes it can be distinguished without any especial expertise which was required at early developmental stages.

Since the histological analysis could not be done at the field, and moreover it is not rapid a method for oocyte examination, our study showed that egg color and diameter (Table 3) could be a general indicator in female great sturgeon reproductive stage and can be determined using laparoscopic examination of the ovaries.

Although histological analysis coupled with blood chemistry will provide the most accurate assessment of sex and reproductive stage but those are needed more time for evaluation and expensive. Nevertheless, laparoscopy is a useful screening tool, especially for identifying sex at an early age and reproductive stage of great sturgeon. Because of this tool is compact and portable, make it practical for field applications, even in remote settings which can work with portable generator or even battery powers. However, laparoscopy requires knowledge of fish anatomy and experience in observing gonads at various stages of development by operator. Training and validation may minimize operator error as well as incidental injury or mortality to the fish. Also, laparoscopy can be useful for purposes other than observing sexual organs. The spleen, liver, visceral fat, and intestinal tract were also observed through the endoscope. The ability to collect diagnostic samples using nonlethal techniques such as endoscopy would be an important tool in valuable or endangered

species. Beside this, minimally invasive endoscopy permits the serial and repeated collection of samples from research fish without subjecting the animals to repeated, major laparotomy. Tissue collection for histology may require killing the fish or invasive procedures (e.g. abdominal surgery, catheterization through the gonoduct). In contrast to histology and blood chemistry, laparoscopy provides immediate information about sex and reproductive stage. The effective use of these minimally invasive instruments for fish species, make them suitable for use on threatened and endangered species. Using the laparoscope, which causes minimal damage or stress to the fish, is important to commercial farming of great sturgeon and other sturgeon species aimed at caviar production and propagation for wild stock management.

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