COMPARING ULTRASONOGRAPHY AND ENDOSCOPY FOR THE IDENTIFICATION OF GENDER IN JUVENILE SIBERIAN STURGEON (*ACIPENSER BAERII*)

by

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(Under the Direction of Stephen J. Divers)

ABSTRACT

Early gender identification is essential in order to preferentially raise greater numbers of female sturgeon (Acipenseridae) for caviar production in aquaculture facilities. As many free-ranging species of sturgeon are threatened or endangered, the ability to confirm gender ante-mortem can be critically important for conservation and recovery projects. The goal of this study was to compare the accuracy of non-invasive ultrasonography and minimally-invasive endoscopy for the identification in 140, three and four-year-old Siberian sturgeon (*Acipenser baerii*), against the gold standard of gonadal biopsy and histology. Histology confirmed the gender for 128/140 (91.4%) fish. Histology and endoscopy agreed on the gender of 124/128 (96.9%) fish. Histology and ultrasonography agreed on 113/128 (88.3%) fish. Ultrasonography was significantly faster than endoscopy (11.8s versus 21.9s respectively). Endoscopy was more invasive but more accurate. The minor reduction in accuracy associated with non-invasive ultrasonography may be an acceptable trade-off in aquaculture and free-ranging applications.

INDEX WORDS: Sturgeon, *Acipenser baerii*, Ultrasonography, Endoscopy, Histology, Gender

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DEDICATION

This thesis is dedicated to all the individuals--family, friends, coaches, and professors--that have supported me throughout my education and career pursuits. I am humbled by the sincere love and care freely given by my peers and will cherish this chapter of life as a Master's student. This project confirms that I can do all things through Christ, my Savior and Redeemer, who gives me strength.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Introduction

Over the past few decades, there has been an increasing demand for minimally-invasive, rapid, non-lethal techniques to identify gender in fish. Sturgeon, in particular, are most notable for the harvest of their precious roe or caviar (Dumont et al., 2001). Over the past century, anthropogenic factors have caused a severe global decline of many sturgeon species, including most North American populations (Peterson et al., 2007). Recovery plans aimed to support sturgeon growth have not been effective due to minimal knowledge of basic life history information (Tripp et al., 2009).

To better protect these fish, extensive life history data, including gender identification, is key to help determine habitats critical for spawning, growth, and maturation. Due to the decline of free-ranging populations, aquaculture serves an ever-increasing role for caviar production. Gender identification is essential for the caviar industry, and early gender identification is economically desirable in order to preferentially raise greater numbers of females with limited aquaculture resources.

Sturgeon are sexually monomorphic, therefore, gender identification relies upon confirming the presence of either testes or ovaries by a variety of methods, including sex steroids, endoscopy, and ultrasonography (Wildhaber et al., 2005; Bryan et al., 2007; Hurvitz et al., 2007; Divers et al., 2009; Petochi et al., 2011). The diagnostic tool with the most accurate and efficient manner of gender identification in juveniles involves gonadal imaging. In

aquaculture facilities, gender is identified generally around 5 years of age. Identifying gender earlier than age 5 provides substantial financial incentives. Although their meat can be marketed, until gender is identified, males are costly in terms of consuming feed and occupy valuable space. Removing males early allows aquaculture resources to be dedicated to rearing a larger number of females and production of their more valuable roe. The benefits of early gender identification of free-ranging sturgeon, include identification of habitats essential for juvenile growth and maturation, and permit evaluation of population demographics to ensure sound management practices and recovery plans.

Endoscopy is a minimally-invasive, technique that permits direct, often magnified observation with low incidents of morbidity and mortality (Divers et al., 2009; Trested et al., 2010). Endoscopy is a commonly used diagnostic tool in fish medicine and is particularly valuable for the evaluation of internal viscera referred to as laparoscopy or coelioscopy (Boone et al., 2008). Endoscopy, including coelioscopy, has applications in commercial aquaculture, research, and aquarium fish. In sturgeon, two endoscopic techniques have been reported; (i) coelioscopy, in which the endoscope is inserted through the body wall to view the gonads and (ii) Mullerian duct endoscopy (gonoductoscopy), in which the endoscope is inserted into the urogenital duct to examine the gonad (Wildhaber et al., 2005). Both techniques have demonstrated high accuracy rates for identifying gender and determining reproductive stage (Wildhaber et al., 2005; Divers et al., 2009).

Ultrasonography is a well-established and non-ionizing form of diagnostic imaging. Ultrasonography is based on sound waves and is usually displayed as a two-dimensional tomographic image in gray-scale, which requires careful interpretation by a trained operator. Gender identification using ultrasonography in adult shovelnose (*Scaphirhynchus platorynchus*)

and the endangered pallid sturgeon (*Scaphirhynchus albus*), has been reported as 68-70%, less accurate compared to endoscopy (Wildhaber et al., 2005). As a result, more studies in sturgeon have focused on endoscopy due to its better accuracy and more intuitive image interpretation. Endoscopy is regarded as minimally-invasive, requiring a time-consuming surgical entry, although speed can develop with practice.

Several studies have confirmed the better accuracy of endoscopy (Hurvitz et al., 2007; Boone et al., 2008; Divers et al., 2009) over ultrasonography for identifying gender and determining reproductive status (Wildhaber et al., 2005). Although several studies have focused on comparing the effectiveness of ultrasonography to endoscopy, none have shown that ultrasonography is a more accurate, cost effective, and faster diagnostic imaging device (Wildhaber et al., 2005; Bryan et al., 2007).

There are few publications describing the use of ultrasonography to identify gender in immature sturgeon. This may, in part, be due to the subtle sonographic differences between the gonads of immature fish, and the greater technical skills required to interpret sonograms, compared to the color, topographic imaging of endoscopy. To the authors' knowledge, no studies have evaluated and compared ultrasonography and endoscopy to the gold-standard of gonadal histology in juvenile sturgeon. Furthermore, veterinary specialists in ultrasonography and endoscopy have rarely been involved in fish gender identification studies, which may have resulted in variability regarding technique and outcome.

The ability to view gonads can reveal pertinent biological information that can lead to better management and recovery plans for endangered sturgeon. In particular, ultrasonographic identification of gender in adults can be quickly performed, with minimal stress, and without the

need for anesthesia (Wildhaber et al., 2005). Wildlife agencies could feasibly utilize ultrasonography for non-invasive gender identification in free-ranging animals.

This study aims to directly compare ultrasonography and endoscopy to gonadal histology, using best practice standards. It is hoped that the collaboration between veterinary medicine, aquaculture and conservation organizations will improve the abilities of all parties to achieve higher accuracy rates, and implement sound aquaculture and conservation plans.

Literature Review

Taxonomy and Life History

Sturgeon (Acipenseridae) are one of the most ancient of vertebrates, with fossil records dating back more than 150 million years (Mims et al., 2002). The ranges of the 27 extant species extend from subtropical to subarctic waters in North America, Europe, and Asia. There are four genera including: *Acipenser, Huso, Scaphirhynchus,* and *Pseudoscarphirhynchus*. There are nine species of sturgeon in North American, most of which are threatened or endangered (Limburg and Waldman, 2009). Not only are sturgeon some of the oldest living vertebrates, they are some of the most long-lived. Atlantic, lake, and white sturgeon can live close to 80 years and pallid sturgeon can live for more than 40 years (Mims et al., 2002). Sexual maturity in sturgeon can exceed a decade, with intermittent spawning every 2-5 years. Typically, males mature a few years earlier than females. Like many late-maturing, long-lived vertebrates that are commercially harvested, many sturgeon populations are in serious decline (Braaten et al., 2009). Through increased management efforts and aquaculture, sturgeon conservation has gained momentum over the past decade (Rosenthal et al., 2009).

Similar to many salmonids, sturgeon are anadromous or potamodromous. Anadromous sturgeon, like the Russian sturgeon (*Acipenser gueldenstaedtii*) and Atlantic sturgeon (*Acipenser*

oxyrinchus oxyrinchus) live in oceanic or brackish water and migrate to freshwater rivers to spawn. Lake sturgeon (*Acipenser fulvescens*), shovelnose sturgeon (*Scaphirhynchus platorynchus*), and Siberian sturgeon (*Acipenser baerii*) are potamodromous and live in freshwater their entire lives, but do migrate within their habitats (Mims et al., 2002; Hamlin et al., 2011).

Reproductive Biology

Anatomically, the gonads are positioned longitudinally along the dorsal coelom on either side of the dorsal midline, just ventral to the kidneys. In adult sturgeon, the ovaries can account for 15-25% of a female's body weight (Mims et al., 2002). Age at sexual maturity ranges according to species, with hybrids (*A. naccarri* x *A. baerii*) as early as 6 years and Siberian sturgeon at 10 years (Petochi et al., 2011). When spawning commences, sturgeon broadcast their sperm and eggs. Following external fertilization, eggs become sticky and adhere to benthic rocks. Fry hatch 5-10 days after fertilization, depending on water temperature, and initial nourishment is derived from the yolk sac. Foraging for food begins when the yolk plug is excreted from the specialized intestine, called a spiral valve (Mims et al., 2002).

As they mature, fry consume zooplankton, annelids, and later crustaceans and mollusks. Sturgeon are bottom feeders that possess a protrusible, sucker-like mouth and taste-sensitive barbels. The unique and flexible mouth morphology enables them to grasp prey and exclude inorganic material before ingestion (Mims et al., 2002).

Caviar Industry

For centuries, sturgeon have been valued for their roe. As a result of the endangered status of many species, aquaculture serves as the major producer of sturgeon for the caviar industry. Although numerous aquaculture facilities exist in the US, most sturgeon and their

products originate from the Caspian Sea in countries like Russia, Kazakhstan, Azerbaijan, and Iran (Mims et al., 2002). The species most popular for caviar include beluga (*Huso huso*), Russian, Siberian, and stellate sturgeon (*Acipenser stellatus*).

High demand for caviar and excessive harvesting, coupled with pollution and habitat degradation, have resulted in many species becoming threatened or endangered as listed by the United Nation's Convention on the International Trade of Endangered Species of Wild Fauna and Flora or CITES (Mims et al., 2002). CITES restricts USA importation and exportation of listed species products, unless accompanied by a CITES permit issued through the US Fish and Wildlife Service.

Production of farm-raised sturgeon caviar has increased over the last few decades (Takahashi and Officer, 2010). Although facilities are intensive, require major capital investment, and necessitate skilled and constant monitoring, highly prized sturgeon can be raised in hatcheries and quality caviar and meat harvested for human consumption. As a result, aquaculture has benefited free-ranging sturgeon by decreasing commercial pressures on their natural populations.

However, during the hatchery and rearing process, juvenile sturgeon are vulnerable and require a complete nutritional program including a strict feeding schedule. Sturgeon can have one of the fastest growth rates of all freshwater fish, but like many long-lived vertebrates, reproductive maturity may take more than a decade before spawning can occur (Mims et al., 2002). Most male sturgeon are harvested for meat earlier, between ages 6-9 at approximately 9 kg (20 lbs) bodyweight. As a result, hatcheries aim to determine the gender of sturgeon as early as possible in order to remove males and focus resources on females for caviar production.

Gender Determination

Methods for gender identification and reproductive status have evolved as technology has advanced over the last decade. While mature gonads (able to spawn) can be differentiated based on gross visual inspection alone, immature gonads (not ready to spawn) must often be viewed using magnification or ultrasonographic imaging. Current methods are judged against endoscopic gonadal biopsy and microscopic examination of routinely prepared histologic sections as the definitive gold standard (Wildhaber et al., 2005; Hurvitz et al., 2007; Petochi et al., 2011).

Endoscopic Gender Identification

Endoscopy, a minimally-invasive surgical technique for direct observation, also permits the collection of biopsies for histologic confirmation of gender. Many studies that have utilized endoscopy have also demonstrated its accuracy in combination with low morbidity. An endoscopy study using both juvenile and adult shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*) concluded that endoscopy was 100% effective for gender identification when compared to histological findings (Matsche et al., 2011).

Although endoscopy in sturgeon is known to be accurate with high survival rates, endosurgical equipment and techniques have continued to evolve. In 2007, endoscopic evaluation of brook trout indicated a 96% accuracy for the correct identification of gender and reproductive status, with a 3.3% mortality rate limited to individuals less than 70 mm in snout-fork length (Swenson et al., 2007). The equipment used was a rigid 25º endoscope (Panoview Plus, Richard Wolf Medical Instruments, Vernon Hills, Illinois) equipped with a battery powered fiber optic light source (Welch Allyn, Model Solarc LB-21). The authors reported difficulties in viewing internal organs including gonads, especially in immature fish. Extended procedural

times probably accounted for the higher mortality of the smaller individuals (Swenson et al., 2007).

Most fish endoscopy studies have concentrated on mature individuals rather than juveniles (Swenson et al., 2007; Matsche et al., 2011). However, Hurvitz et al. (2007) performed a study that could identify gender with 100% accuracy, confirmed through histology, in juvenile Russian sturgeon. Their system consisted of a 4 mm, 14 cm long cytoscope with sheath that was employed to view the coelomic organs including the gonads. Using this system that relied on saline for insufflation to view the gonads more clearly, gender could be identified as early as 3.5 years of age (Hurvitz et al., 2007). The study was conducted using 10,563 farm raised Russian sturgeon, with gender confirmed by histological analysis from an average of 270 mg sample of gonadal tissue.

Divers et al. (2009) described a single-entry endoscopic technique that used a 2.7 mm rod-lens telescope (Karl Storz Veterinary Endoscopy America Inc [KSVEA], Goleta, CA), a 4.8 mm operating sheath (KSVEA), 1.7 mm biopsy forceps (KSVEA), telecam camera, 24 watt xenon-hybrid light source, 36 cm LCD monitor, and computer memory card slot for digital image capture and storage (Tele Pack, KSVEA). Gravity-fed sterile saline infusion was used to accurately identify gender and determine reproductive stage in mature free-ranging shovelnose and pallid sturgeon (*Scaphirhynchus albus*) (Divers et al., 2009).

In another study published in 2010, shovelnose sturgeon underwent a modified endoscopic technique. Instead of placing the endoscope directly into the body cavity or through the urogenital ducts, a threaded trocar was inserted through a ventral incision to allow a low-pressure air supply (Model AH801 aquarium air supply pump; Aquatic Ecosystems, Tampa, Florida) to insufflate the body cavity. The authors concluded that endoscopic evaluation using air

insufflation was safe and effective for identifying the gonads of adult shovelnose sturgeon (Trested et al., 2010).

The gender and reproductive status of Atlantic and shortnose sturgeon have also been determined using endoscopy (Matsche et al., 2011). A pair of Ternamian EndoTip cannulae were inserted through the ventral body wall of fish to provide access for a 5 mm rigid laparoscope and biopsy forceps. All sturgeon survived and scars from incisions were no longer evident 9-12 months post-surgery. The authors also reported that sex identification was accurate in all but immature fish.

Ultrasonographic Gender Identification

The advancement of diagnostic imaging, including ultrasonography, in fish has been rapid over the past decade (Masoudifard et al., 2011; Petochi et al., 2011). Ultrasonography offers a completely non-invasive, safe, reliable, and effective means of gonadal imaging (Wildhaber et al., 2005; Bryan et al., 2007; Masoudifard et al., 2011). Most ultrasonographic studies performed for gonadal interpretation have also reported some limitations, particularly regarding accuracy when compared to endoscopy (Wildhaber et al., 2005; Bryan et al., 2007).

a) Application and Limitations

There are various factors limiting the use of ultrasonography in the practice of gender identification in sturgeon, namely: (i) fish morphology, (ii) characteristics of the ultrasonography equipment, and (iii) ultrasonographer's ability to locate and identify gonads of immature sturgeon.

(i) Fish Morphology

The accessibility of acceptable acoustic windows and ability to locate and evaluate internal organs is often related to the size of the fish. The gonads of smaller and generally more immature

fish are more difficult to image (Wildhaber et al., 2005). A second morphological impediment are the ossified and modified dermal scutes, which create a barrier to sound waves and hinders ultrasonographic evaluation of structures deep within the body. To avoid these physical barriers, the ultrasound transducer, or probe, must be positioned in such a way that allows ultrasound waves to pass between scutes to the internal organs. As a result, the probe is positioned off of the scutes and is often placed along the ventrum.

(ii) Characteristics of Ultrasonography Equipment

There is a large amount of variability between available ultrasound machines, with some having preferred features such as faster processing speeds, image optimization, spatial compounding, tissue harmonic imaging, Doppler imaging, and DICOM compatible digital image and video capture for later review. Microconvex and linear array high frequency transducers are desired for high-resolution images of small or superficial body parts.

The earliest ultrasonographic studies were performed using large machines and lower frequency transducers that could not function in the field. Some studies reported using a non-portable Shimadzu SDU-400 Plus, with a 7.5 MHz linear transducer (Wildhaber et al., 2005; Bryan et al., 2007), while more recent investigations have utilized portable units, such as the Sonosite 180-Plus with a 6-13 MHz linear transducer (Colombo et al., 2004; Masoudifard et al., 2011). These portable units, especially when battery-powered, have obvious logistical advantages when working in the field, but have previously suffered from inferior image quality or functionality when compared to their non-portable counterparts (Wildhaber et al., 2005). Recent advances have improved the image quality of many portable machines to make them much more comparable to the larger stand-alone units (Masoudifard et al., 2011).

(iii) Ultrasonographer's Ability to Locate and Identify Gonads of Immature Sturgeon

The difficulty associated with identifying gender, especially in juveniles, is a combination of the intimate relationship between the gonads and surrounding fat, coupled with the similar acoustic properties of immature gonads and fat. In addition to differentiating different tissues (fat and kidneys), understanding tomographic anatomy, the ability to optimize image quality, and accurately measure and document, and an overall working knowledge of sturgeon anatomy is essential (Colombo et al., 2004).

b) Accuracy

In 2002, a study reported high accuracy for gender identification using ultrasonography, and confirmed by necropsy, in stellate sturgeon (Moghim et al., 2002). All sturgeon sampled were less than 95 cm (snout to fork length) and gonads were more easily identified due to the large size of each fish. This study, using a non-portable machine, reported an accuracy of 97% for both mature (spawning) and immature (not yet spawning) sturgeon, although gender identification for immature males was more difficult to discern. This study also reported the speed of the ultrasonographic examination to be about 30 seconds per fish (Moghim et al., 2002).

Another study that performed ultrasonography on euthanized fish reported 86% accuracy for identifying gender in mature and immature male and female shovelnose sturgeon (Colombo et al., 2004). The researchers examined gonads using a portable Sonosite 180-Plus ultrasound with a 5 MHz linear probe. Four unspecified observers determined gender based on a consensus from the ultrasound viewing. Gender was confirmed at necropsy, but without histology.

Wildhaber et al. (2005) reported limitations in distinguishing between males and immature or spent (post spawning) female sturgeon, and indicated that the default gender identification was often male if the gonads were not distinguishable from other body tissues or

there was an absence of any tissue remnants suggestive of a female (Wildhaber et al., 2005). This approach to gender identification may not be the most correct, but it is an appropriate method to determine one gender over another in a non-lethal manner.

Ultrasonographic examination was performed in 80, 3-year-old juvenile beluga sturgeon using a Sonosite portable ultrasound unit with a linear 6-13 MHz transducer (MicroMaxx-USA) (Masoudifard et al., 2011). The authors suggested that a high frequency transducer and transverse scanning contributed to a 97% accuracy rate for gender identification in which gonadal shape and echogenicity were determining factors (Masoudifard et al., 2011).

Overall, ultrasonography offers a non-invasive, safe, reliable, and rapid method for identifying gender in sturgeon. Higher frequency transducers, which provide greater detail and permit an evaluation of gonadal shape and echnogenicity, tend to be most accurate (Masoudifard et al., 2011). The effectiveness of ultrasonography depends largely on the capabilities of the ultrasonographer and the equipment, coupled with the maturity and size of the fish. The larger more mature the fish, the easier and more accurate the ultrasonographic evaluation. Despite some interpretational difficulties, ultrasonography is credited as being the least invasive and most rapid methodology available. Even though difficulties relating to the interpretation of ultrasonographic images do exist, most studies have not utilized specialty-trained ultrasonographers (e.g. Diplomates of the American College of Veterinary Radiology). Any initial ultrasound feasibility study involving immature fish is ideally conducted using high-end equipment and a highly trained ultrasonographer to interpret images and validate a given technique prior to its attempted use by less extensively trained personnel, because if such a specialist cannot identify gender then there is no merit to the technique in lay-person hands.

Histology

Gonadal biopsies are often collected following the endoscopic examination of gonads to definitively determine gender and confirm the accuracy of other evaluation methods (Wildhaber et al., 2005; Hurvitz et al., 2007; Divers et al., 2009; Matsche et al., 2011; Petochi et al., 2011). Gonadal samples are obtained using small biopsy forceps inserted either through an endoscopy sheath or triangulated separately to the endoscopy telescope and camera (Boone et al., 2008; Divers et al., 2009). Biopsy samples are placed in tissue cassettes and fixed in 10% neutral buffered formalin. Tissues are embedded in paraffin wax, cut in 4-5 µm sections, mounted on glass slides and stained with hematoxylin and eosin (H&E) prior to microscopic evaluation (Allen, 1992).

Physical Restraint, Anesthesia, Recovery, and Post-Operative Care

Tricaine methanesulfonate is the only FDA approved fish anesthetic agent in the USA, and is therefore preferred for any invasive and potentially painful procedures, including endoscopy (Boone et al., 2008; Divers et al., 2009; Trested et al., 2010; Matsche et al., 2011). Sturgeon are typically induced in a bath containing 150 mg/L MS222 and 300 mg/L of sodium bicarbonate buffer. Sturgeon are then translocated to a surgery station with a water recirculation system to provide a constant flow of aerated water over the gills and maintained with 80 mg/L of MS222 and 160 mg/L of sodium bicarbonate (Divers et al., 2009).

Fish have been reported to recover well following anesthesia, with very low morbidity or mortality (Hernandez-Divers et al., 2004; Boone et al., 2008; Divers et al., 2009; Matsche et al., 2011). In laboratory settings, fish are placed in recovery tanks containing vigorously aerated water. In the field, fish may recover along the stream bank in live nets, suspended in water.

MS222 has a 30 day withdrawal period, and therefore no fish by-product can be used for human consumption within the 30 days after administration.

Analgesics are typically not used in studies involving gender identification because the techniques are minimally invasive and excessive discomfort is not anticipated. In addition, no analgesics have been evaluated regarding tissue residuals in fish and fish products for human consumption.

Literature Cited

Allen, T. 1992. Hematoxylin and eosin. Laboratory Methods in Histotechnology. Armed Forces Institute of Pathology, Washington, DC: 53-58.

Boone, S.S., S.J. Hernandez-Divers, M.G. Radlinsky, K.S. Latimer, and J.L. Shelton. 2008. Comparison between coelioscopy and coeliotomy for liver biopsy in channel catfish. Journal of the American Veterinary Medical Association, 233(6): 960-967.

Braaten, P.J., D.B. Fuller, R.D. Lott, and G.R. Jordan. 2009. An estimate of the historic population size of adult pallid sturgeon in the upper missouri river basin, montana and north dakota. Journal of Applied Ichthyology, 25(0): 2-7.

Bryan, B.J.L., M.L. Wildhaber, D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, and M.L. Annis. 2007. Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon. Journal of Applied Ichthyology, 23(4): 411-419.

Colombo, R.E., P.S. Wills, and J.E. Garvey. 2004. Use of ultrasound imaging to determine sex of shovelnose sturgeon. North American Journal of Fisheries Management, 24(1): 322-326.

Divers, S.J., S.S. Boone, J.J. Hoover, K.A. Boysen, K.J. Killgore, C.E. Murphy, S.G. George, and A.C. Camus. 2009. Field endoscopy for identifying gender, reproductive stage and gonadal anomalies in free-ranging sturgeon (*scaphirhynchus*) from the lower mississippi river. Journal of Applied Ichthyology, 25(s2): 68-74.

Dumont, H., A. Werth, D. Aubrey, R. Jenkins, J. Switzer, M. Farvar, K. Razavi, R. Shapour, H. Govan, and M. Pourkazemi. 2001. The sturgeon. The IUCN Commission of Environmental, Economic, and Social Policy(8).

Hamlin, H.J., M.R. Milnes, C.M. Beaulaton, L.C. Albergotti, and L.J. Guillette Jr. 2011. Gonadal stage and sex steroid correlations in siberian sturgeon, acipenser baeri, habituated to a semitropical environment. Journal of the World Aquaculture Society, 42(3): 313-320.

Hernandez-Divers, S., R. Bakal, B. Hickson, C. Rawlings, H. Wilson, M. Radlinsky, S. Hernandez-Divers, and S. Dover. 2004. Endoscopic sex determination and gonadal manipulation in gulf of mexico sturgeon (acipenser oxyrinchus desotoi). Journal of Zoo and Wildlife Medicine, 35(4): 459-470.

Hurvitz, A., K. Jackson, G. Degani, and B. Levavi-Sivan. 2007. Use of endoscopy for gender and ovarian stage determinations in russian sturgeon (acipenser gueldenstaedtii) grown in aquaculture. Aquaculture, 270(1-4): 158-166.

Limburg, K.E., and J.R. Waldman. 2009. Dramatic declines in north atlantic diadromous fishes. BioScience, 59(11): 955-965.

Masoudifard, M., A.R. Vajhi, M. Moghim, R.M. Nazari, A.R. Naghavi, and M. Sohrabnejad. 2011. High validity sex determination of three years old cultured beluga sturgeon (huso huso) using ultrasonography. Journal of Applied Ichthyology, 27(2): 643-647.

Matsche, M.A., R.S. Bakal, and K.M. Rosemary. 2011. Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (acipenser brevirostrum) and atlantic sturgeon (acipenser oxyrinchus oxyrinchus). Journal of Applied Ichthyology, 27(2): 627-636.

Mims, S., A. Lazur, W. Shelton, B. Gomelsky, and F. Chapman. 2002. Species profile: Production of sturgeon. Southern Regional Aquaculture Center, 7200.

Moghim, M., A. Vajhi, A. Veshkini, and M. Masoudifard. 2002. Determination of sex and maturity in acipenser stellatus by using ultrasonography. Journal of Applied Ichthyology, 18(4,Äê6): 325-328.

Peterson, D., P. Vecsei, and C. Jennings. 2007. Ecology and biology of the lake sturgeon: A synthesis of current knowledge of a threatened north american acipenseridae. Reviews in Fish Biology and Fisheries, 17(1): 59-76.

Petochi, B.T., P. Di Marco, V. Donadelli, A. Longobardi, I. Corsalini, D. Bertotto, M.G. Finoia, and G. Marino. 2011. Sex and reproductive stage identification of sturgeon hybrids (acipenser naccarii × acipenser baerii) using different tools: Ultrasounds, histology and sex steroids. Journal of Applied Ichthyology, 27(2): 637-642.

Rosenthal, H., R. Bruch, T. Haxton, and P. Allen. 2009. Proceedings of the inaugural meeting of the north american chapter of the world sturgeon conservation society, august 19,äê21, 2008. Journal of Applied Ichthyology, 25: 1-1.

Swenson, E.A., A.E. Rosenberger, and P.J. Howell. 2007. Validation of endoscopy for determination of maturity in small salmonids and sex of mature individuals. Transactions of the American Fisheries Society, 136(4): 994-998.

Takahashi, S., and F. Officer. 2010. Sturgeon conservation and the role of japan. The State of Wildlife Trade in Japan, 10: 42.

Trested, D.G., R. Goforth, J.P. Kirk, and J.J. Isely. 2010. Survival of shovelnose sturgeon after abdominally invasive endoscopic evaluation. North American Journal of Fisheries Management, 30(1): 121-125.

Tripp, S., Q. Phelps, R. Colombo, J. Garvey, B. Burr, D. Herzog, and R. Hrabik. 2009. Maturation and reproduction of shovelnose sturgeon in the middle mississippi river. North American Journal of Fisheries Management, 29: 730-738.

Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, M.L. Annis, and J.A. Allert. 2005. Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. Journal of Fish Biology, 67(1): 114-132.

CHAPTER 2

COMPARING ULTRASONOGRAPHY AND ENDOSCOPY FOR THE IDENTIFICATION OF GENDER IN JUVENILE SIBERIAN STURGEON (*ACIPENSER BAERII*)[1]

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Abstract

The present study compares the effectiveness of ultrasonography and endoscopy to identify gender in juvenile Siberian sturgeon (*Acipenser baerii)* using gonadal histology as the gold standard. The goal of this study is to accurately identify gender in 3 and 4-year-old, juvenile Siberian sturgeon using non-invasive ultrasonography and minimally-invasive rigid coelioscopy. A total of 140, 3 and 4-year-old Siberian sturgeon were anesthetized and evaluated using ultrasonography and endoscopy, with each fish having a 2.5mm^3 gonadal biopsy taken to confirm gender histologically. Histology confirmed the gender for 128/140 (91.4%) fish. Histology and endoscopy agreed on the gender of 124/128 (96.9%) fish. Histology and ultrasonography agreed on 113/128 (88.3%) fish. Ultrasonography was significantly faster than endoscopy (11.2s versus 22.3s respectively), while endoscopy was more invasive but more accurate. Diagnostic accuracy, efficiency to complete the procedure, and invasiveness are factors to consider in commercial and free-range applications. Early gender identification in aquaculture is economically advantageous, such that food and spatial resources are targeted to select for greater numbers of females. Since many species of free-ranging sturgeon are threatened or critically endangered, the ability to view gonads in fish species can reveal pertinent biological information that can lead to better management and recovery plans.

Introduction

Diagnostic imaging of fish and wildlife is common in clinical practice and veterinary research, as well as in many aquacultured species. In sturgeon aquaculture, the ability to determine gender is essential for caviar production. Because sturgeon are sexually monomorphic, or lack external sexual characteristics, the most accurate method to determine gender is through examination of the gonads (Hurvitz et al., 2007).

All *Acipenseriformes* are long-lived vertebrates and typically do not reach sexual maturity until they are at least 8-10 years of age (Mims et al., 2002; Hurvitz et al., 2007; Stahl et al., 2009). The ability to view internal viscera in the most efficient and safe manner is critical to the utility of any diagnostic device. Historically, gender cannot be truly identified until after age 5, and this is most often performed using minimally-invasive endoscopy.

The harvest of only mature females is required for caviar production; therefore, it is economically advantageous for hatcheries to determine gender as early as possible to cull males (Chebanov and Galich, 2009). In most commercial sturgeon farms, male fish are typically harvested before maturity, because sturgeon meat has little economic value compared to the caviar produced by females. Coincidentally, hatcheries must identify the gender of vast numbers of fish, sometimes thousands, ideally in a relatively short time period. Until gender is identified, the prolonged culture of male sturgeon is relatively inefficient on a cost-revenue basis, because they occupy valuable culture space that could otherwise be used to raise additional females. Consequently, early gender identification provides substantial financial benefits by allowing commercial farms to maintain a higher proportion of female fish. Unfortunately, current methods have demonstrated gender identification is not effective for fish younger than age 4 or 5 and few studies have focused on gender determination in immature sturgeon.

For aquaculture purposes, ultrasonography and endoscopy are the primary methodologies used to identify gender and reproductive condition of sturgeon and other fishes. Endoscopy has become a vital diagnostic tool in veterinary medicine, while ultrasonography has pioneered the advancement of non-invasive imaging. However, more gender identification studies have favored endoscopy due to its reported higher accuracy (Hurvitz et al., 2007; Boone et al., 2008; Divers et al., 2009; Matsche et al., 2011).

Endoscopy, although regarded as the most accurate imaging technique, is minimally-invasive and may be more time consuming than ultrasonography. Recent studies comparing ultrasonography and endoscopy have shown that ultrasonography is not as accurate in identifying sturgeon gender. Wildhaber et al. (2005) compared the effectiveness of ultrasonographic and endoscopic techniques to identify gender in adult male and female shovelnose sturgeon (*Scaphirhynchus platorhynchus*) and found that ultrasonography, the least invasive technique, correctly identified gender only 68-70% of the time when confirmed by gonadal histology. Colombo et al. (2004) reported 86% accuracy in gender identification in male and female, juvenile and adult free-ranging shovelnose sturgeon confirmed using visual inspection following euthanasia.

A lack of information exists on early gender identification and distinguishing juvenile from adult sturgeon. In addition, no study has thoroughly evaluated the accuracy of ultrasonographic gender assessments using specialty trained ultrasonographers (e.g. Diplomates of the American College of Veterinary Radiology). Therefore, the goal of this study is to evaluate ultrasonography and endoscopy using board certified veterinarians, to accurately identify gender in juvenile Siberian sturgeon (*Acipenser baerii*). The null hypothesis being tested states that there will be no difference in accuracy and speed in ultrasonography and endoscopy

compared to gonadal histology in gender identification of juvenile Siberian sturgeon. The alternative hypothesis states that a difference will exist in accuracy and speed of ultrasonography and endoscopy compared to gonadal histology in gender identification in juvenile Siberian sturgeon.

Methods

Fish Culture

All procedures were approved by the University of Georgia's Institutional Animal Care and Use Committee (AUP # A2011 05-008-Y1-A0). Siberian sturgeon eggs were originally purchased overseas, imported to Georgia, and reared at the Cohutta Fisheries Center of the University of Georgia's Warnell School of Forestry and Natural Resources. One-hundred forty sturgeon were held in indoor 46,370 L, below ground, concrete runs inside a Cohutta Fisheries building. The sturgeons sampled had not previously been identified as male or female. The sturgeon raceways were equipped with biological and mechanical filters, providing a 50-75% recirculation on a daily basis. Makeup water was provided ad libitum from a nearby spring that was plumbed to the head of the raceways. Sturgeon were fed a slow sinking extruded trout 40% protein pellet diet (Silver Cup, Murray, Utah) twice per day for a total daily feed rate of 0.75% body weight per day. Dissolved oxygen, pH, temperature and ammonia were maintained well within the recommended range for commercial sturgeon culture (Hochleithner and Gessner, 1999).

Anesthesia and Surgical Procedures

All anesthesia, surgical and non-surgical procedures were performed on August 1 and August 2, 2011. The sturgeon were fasted for 48-72 hours before surgery to decrease any potential stress associated with feeding as well as to minimize regurgitation. One-hundred and

ten, 3-year-old sturgeon (mean fork length = 71.2 cm [range 57-89.5]; mean wet weight = 1970 g [range 816-4066 g]) and 30, 4-year-old sturgeon (mean fork length = 69.5 cm [range 58.4-80]; mean wet weight = 1990 g [range 1250-3030 g]) were randomly chosen from the same hatchery cohort.

Anesthesia was induced by placing each fish in an aerated 132.5 L plastic container that contained a 150 mg/L solution of tricaine methanesulfonate (MS-222) (Finquel, Argent Chemical Laboratories, Redmond, WA) buffered with 300 mg/L sodium bicarbonate. After each fish lost righting reflex (known as stage II of anesthesia; (Stoskopf, 1993)), it was weighed and immediately transferred to a surgical table, and placed in right dorsolateral recumbency. The surgery table was constructed from a large utility cart with a fenestrated surgery platform and gravity-fed return to a reservoir tank situated underneath (Figure 2.1). A submersible (or external) water pump provided a continuously re-circulating supply of maintenance anesthesia (80mg/L of MS-222 buffered) from the reservoir to the top of the surgical table where rubber tubes delivered water into the buccal cavity of the fish through the gender assessment examinations. The re-circulating anesthesia system delivered 80mg/L of MS-222 buffered with 160 mg/L of sodium bicarbonate. The setup was designed to split the water supply such that two fish could be maintained simultaneously before used water drained passively back into the reservoir tank. Anesthesia solution water recirculated through a number of fish for up to 4 hours until it was replaced with fresh solution.

Surgical instruments, including endoscopy equipment (telescopes, sheaths, and biopsy forceps), were sterilized by immersion in 0.55% ortho-phthalaldehyde (CIDEX OPA) solution (Advanced Sterlization Products, Irvine, CA) for 20 min, then rinsed in sterile water before each use. One-liter bags of sterile saline were suspended approximately 1 m above the surgery

platform and connected to one of the ports on the operating sheath using an intravenous fluid administration set (Primary I.V. Set, 185 cm, 15 drops/sec, Hospira Inc, Lake Forest, IL). This gravity-fed saline-infusion provided mild distension during coelioscopic evaluation.

Ultrasonographic Evaluation

Ultrasound evaluations were conducted using a portable Philips CX50 ultrasound device with a 7-15 MHz transducer (Core Ultrasound, Grand Rapids, MI). Ultrasound evaluations were performed on anesthetized fish using the basic ultrasonographic procedures previously reported (Zwiebel and Sohaey, 1998). Once the fish was placed in right dorsal lateral recumbency, fork length for left and right sides was measured using a flexible, waterproof measuring tape.

Due to the presence of ossified scutes within the skin, which interfere with transduction of ultrasonic wave transmission, the transducer was positioned in between the rows of lateral and abdominal scutes (Figure 2.2). The transducer equipped with a 1.5 cm standoff pad, was placed directly on the left side dorsal to the abdominal row of scutes (Figure 2.2). Because sturgeon gonads are positioned laterally on either side of the dorsal midline and occupy most of the length of the coelom, the ultrasonographer scanned along the length of the left gonad beginning at the most cranial aspect of the coelom and continued to the most caudal end of the gonad in both dorsal and transverse planes. Gonadal length was measured manually with ultrasonographic guidance. After the length of the left gonad was measured, the widest portion of the gonad was measured with digital calipers in the transverse plane. For evaluation of the right gonad, the sturgeon was repositioned in left dorsal lateral recumbency and the procedure was repeated.

The ultrasonographer interpreted the appearance of the gonads, and gender was identified according to the echogenicity, echotexture, and uniformity of the gonad. Hyperechoic, or gonads that appeared brighter relative to adjacent muscle, and fine echotexure with an overall uniform

composition were identified as male. Female fish were identified by the presence of tortuous hyperechoic bands, with coarse echotexure and heterogeneous composition.

The amount of time elapsed to ultrasonographically determine gender in 17 of the 3-year-old Siberian sturgeon was measured using a stopwatch. Times were recorded beginning with initial contact of the transducer to the fish, to the time that the ultrasonographer could identify gender, which was blinded to the endoscopist.

Endoscopic Evaluation and Biopsy

Following completion of the ultrasound evaluation, fish were transferred to the opposite side of the surgery platform and re-positioned in right lateral recumbency for endoscopic evaluation. Endoscopic evaluations were conducted in a field situation as aseptically as possible using sterilized equipment. Endoscopic evaluations used a rigid telescope system comprised of a 2.7 mm x 18 cm 30 ^{o} telescope (63017BA, Karl Storz Veterinary Endoscopy America Inc [KSVEA], Goleta, CA), 4.8 mm (14.5 Fr) operating sheath (67065CC, KSVEA), 1.7 mm (5 Fr) biopsy forceps (67161Z, KSVEA), 5 mm telescope, optical biopsy forceps (1 gm biopsy cup), telecam camera, 24 watt xenon-hybrid light source, 36 cm (14") LCD monitor, and computer memory card slot (PCMCIA) for capture and storage of digital images (20043120-20 Tele Pack, KSVEA).

The incision site for the telescope was located one third of the distance from the pelvic to pectoral fins, between the two abdominal scutes (Figure 2.3). A 1 cm area was wiped with a sterile cotton-tipped applicator. A 4-5 mm incision was made using a number 11 scalpel blade. Small, straight hemostats were used to puncture and enter into the coelom. The hemostats were then replaced by the telescope and operating sheath. Sterile saline infusion was initiated and controlled by the port lever on the operating sheath. Once infusion was sufficient, a detailed

examination of the left and right gonads was performed using a previously described technique (Divers et al., 2009).

The gender of the animal was visually identified and recorded digitally. Gender was identified endoscopically based on the presence of follicular structures within ovaries and tubular structures within testes. Digital images of gonads were then recorded and a small biopsy of the gonad was collected using the 5 Fr (1.7 mm) biopsy forceps inserted through the instrument channel of the operating sheath. The biopsy tissue was then gently shaken into sterile saline within a 5 ml sterile tube. The biopsy sample was placed between sponge pads and positioned inside a histology cassette and labeled with the identification of each fish (M490 Histosette, Biomedical Marketing Associates, 2472 Citation Ct, Wexford, PA 15090). Within a minute, the cassette was placed into a 1 L plastic container with 10% neutral buffered formalin. Following telescope removal from the coelom, the infused saline was permitted to passively escape from the fish, and the incision was closed with a single suture of 3-0 antibiotic-impregnated poliglecaprone 25 (Monocryl-Plus, Ethicon Inc., Somerville, NJ) (Figure 2.3). The duration of the endoscopic procedure, from scalpel incision entry to gender identification, was then recorded.

Recovery

Following completion of both the ultrasonographic and endoscopic evaluations, each fish was transferred to an 1892 L plastic holding tub containing aerated tank water for recovery. Recovery was defined as the return of righting reflexes and normal swimming behavior. After recovery, fish were returned to their original raceway.

Histology

Sponge pads containing the biopsy specimens were transferred to a second tissue cassette pre-labeled with a unique University of Georgia Department of Pathology accession number, corresponding to the animal's identification number, and returned to 100% non-buffered formalin. The tissues were processed routinely by dehydration in a graded series of ethanol solutions of increasing strength, followed by clearing in a series of xylenes, embedding in paraffin, and sectioning at 5 μm. Prepared slides were stained with hematoxylin and eosin (H&E) (Allen, 1992).

Statistical Analysis

a) Method analysis

Comparison of correctness for gender identification by ultrasonography and endoscopy (compared to histology) within each age group was conducted using a McNemar test of symmetry (Everitt, 1992). This analysis was conducted to compare the correctness of the ultrasonography and endoscopy methods within each age group. To learn if differences between the two methods were significant, two indicator variables were created to indicate whether each method correctly detected the gender for each observation. The variables were assigned a value of 1 if the respective method was correct and 0 otherwise. A McNemar test of symmetry was then conducted to see whether one method outperformed the other. The McNemar test results indicated whether the off-diagonals of a table comparing the two methods were symmetric or not. In this case, the diagonal from upper left to lower right represented fish for which both tests were correct, or both tests were incorrect. If the off-diagonals were "symmetric", or contained approximately the same number of entries in each cell, it would indicate which test was correct and which test was incorrect when only one of the tests was incorrect. If the *P*-value was

significant (<0.05), then the tests were not random. This test was conducted for both 3-year-old fish and 4-year-old fish.

b) Gender analysis

A Fisher's exact test was conducted to determine whether true gender influenced correctness of each method for each age group (Everitt, 1992). This test was conducted separately for endoscopy and ultrasonography for 3-year-old fish.

c) Age analysis

The Fisher's exact test was also conducted to determine whether age influenced gender identification correctness for ultrasonography and endoscopy. In this test, both age cohorts were combined to determine whether correctness of identification is independent of age for ultrasonography and endoscopy.

Results

Gender Identification

All surgical and anesthetic procedures were performed without complications and all fish recovered without evidence of morbidity. Morphometric data and results of gender determination in the 3 and 4-year-old groups of fish are summarized (Tables 2.1 and 2.2). Histological analyses of gonadal biopsies confirmed gender in 128 out of 140 individual fish. These analyses also revealed 3 individuals were intersex (Figure 2.4) while the remaining 9 individuals had undifferentiated gonads. Gonad samples confirmed as intersex or undifferentiated were removed from subsequent statistical analyses. Overall, out of the 128 confirmed genders, ultrasonography correctly identified 88.3% for all fish (85.2% 4-year-old, 89.1% 3-year-old) while endoscopy correctly identified 96.9% of all fish (100% 4-year-old, 96.9% 3-year-old) (Table 2.3).

Ultrasonographic Evaluation

Fish gender was initially identified using ultrasonography. Less developed gonads were similar in echogenicity to adjacent muscle (Figure 2.5). Gonads that were more developed were increased in echogenicity when compared to adjacent tissues, namely overlying muscle (Figure 2.6). Gonads with tortuous hyperechoic bands were identified as female (Figure 2.7). Gonads that lacked the bands with a more uniform hyperechoic composition were considered males (Figure 2.6). The gonads of intersex individuals were confirmed through histology, but were often not identified at the time of the evaluation (Figure 2.8). Of the 17, 3-year-old fish examined, the mean time of ultrasonographic gender identification was 11.2 seconds per fish (Table 2.4).

Endoscopic Evaluation

Following ultrasonography, fish gender was identified using endoscopy. Gonads that showed any evidence of oocytes, whether white or yellow in color, were indicative of a female (Figure 2.9). Gonads that appeared smooth with a sinusoidal pattern were identified as male (Figure 2.10). Of the 17, 3-year-old fish examined, the endoscopic method mean was 22.3 seconds per fish (Table 2.4).

Histological Evaluation

Histology confirmed the gender for 128/140 (91.4%) fish. Twelve gonadal samples were largely composed of undifferentiated cells with tubular organization and gender could not be identified (Figure 2.11). Many of the confirmed male samples showed early signs of spermatogenesis (Figure 2.12 and 2.13). Females with early follicular development were also distinct with multiple primary growth oocytes (Figure 2.14).

Statistical Analysis

a) Method Analysis

Results of the McNemar test of symmetry showed that for the age 3 cohort, ultrasonography misidentified gender in 11 out of 101 fish, or 10.9%; endoscopy misidentified gender in 4 out of 101 fish, or 4.0% (Table 2.5) The gender of 89 individuals was correctly identified and three individuals were misidentified simultaneously from both methods. There were eight cases that endoscopy gave correct results and ultrasonography gave incorrect results. Conversely, there was only one case where ultrasonography was correct and endoscopy was incorrect. As a result, the McNemar test was statistically significant at the 0.05 level (P=0.0391).

Results of the McNemar test of symmetry showed that for the age 4 cohort, ultrasonography misidentified gender in 4 out of the 27 fish, or 14.81%; endoscopy misidentified gender in zero out of the 27 fish, or 0%. There were 23 observations that correctly identified gender by both methods. There were four cases that endoscopy gave correct results and ultrasonography gave incorrect results, and no cases where endoscopy gave incorrect results. The McNemar test was not statistically significant at the 0.05 level (P=0.1250). However, since endoscopy performed perfectly for 4-year-old fish, the data suggests that a larger sample size may indicate a significant difference between instruments for 4-year-old fish.

b) Gender analysis

Results of Fisher's exact test showed that for the age 3 cohort, the correctness of ultrasonography was related to true gender (Table 2.6), but this did not appear for endoscopy (Table 2.7). The P-value of 0.0065 was significant for ultrasonography, but was not significant for endoscopy (P=0.3009). Tables 2.6 and 2.7 show both methods were more accurate in this sample for males than females (21.95% vs. 3.33% and 7.32% vs. 1.67%), but this difference is much larger for

ultrasonography because endoscopy is overall more accurate and, therefore, true gender was not a significant influence.

For 4-year-old fish, this test was conducted only for ultrasonography, since endoscopy results were all correct. The Fisher's exact test *P*-value was not significant for ultrasonography of four-year-old fish (P=0.5956) (Table 2.8).

c) Age analysis

The *P*-value for Fisher's exact test was not significant for either ultrasonography (P=0.5195) or endoscopy (P=0.5782).

Discussion

This study presents the first direct comparison of accuracy between ultrasonography versus endoscopy for gender identification in juvenile Siberian sturgeon. Both methods were interpreted as successful for gender identification. Ultrasonography, the non-invasive method, proved less accurate, but faster, with regards to the speed of gender identification. Endoscopy was more invasive and required more time, but was the more accurate method for gender identification. Ultrasonography was significantly male-biased, in that female fish were more likely to be misidentified than male fish. In contrast, endoscopic gender identification was unbiased.

The most immature, or least developed fish were more difficult to identify with either method, especially using ultrasonography. Interestingly, age was not a significant factor in gender identification for either method in the study populations of 3 and 4-year-old fish. However, age is a significant factor in gender identification as an overall practice. As fish age, their gonads develop and increase proportionally in size becoming easier to identify using either ultrasonography or endoscopy. Age may not have been significant in this study because the two

cohorts were not only similar in age but also in morphometrics, which influences method accuracy.

The effectiveness of ultrasonography for gender identification in this study (88%) was similar to previously published studies (Colombo et al., 2004; Wildhaber et al., 2005; Masoudifard et al., 2011). Colombo et al. (2004) demonstrated 86% overall accuracy, while others have reported a range of 68-97.5% using ultrasonography (Wildhaber et al., 2005; Masoudifard et al., 2011). The ultrasonographer in our study was a diagnostic imaging specialist using established protocols and techniques that should result in less variability and greater consistency of observations. However, there were limitations to the effectiveness of ultrasonography. Most notably, identifying gender in small, less developed fish was difficult. In the age 3 cohort, out of the 11 fish with incorrectly identified genders, 10 were lower in both weight and fork length to the overall mean of that age cohort. In the age 4 cohort, out of the 4 fish with incorrectly identified genders, two were lower in both weight and fork length to the overall mean of the age cohort. If the gonads showed no evidence of female gonadal tissue, they were classified as male. This is the same classification procedure reported previously (Wildhaber et al., 2005).

There are numerous factors that support ultrasonography as a useful and accurate tool. First, the portability of ultrasound machines makes this an ideal method for both the laboratory and the field. In this study, the Philips ultrasound machine was lightweight (6.2 kg) and could be powered by an AC battery. Second, this machine had broadband transducer with a wide range of available frequencies. The ideal transducer for a given fish is dependent on its size and shape (Wildhaber et al., 2005). Optimal image acquisition is then dependent on the depth of the region of interest. The higher the frequency, the shallower the depth to which the ultrasound can

penetrate the tissue, but the greater the detail (Wildhaber et al., 2005). The unit used in this study recorded fish gender at 13 MHz. Third, the speed of interpretation of gender was fast and non-invasive. The primary anatomical characteristic that distinguished gender was the tortuous hyperechoic bands in females, while male gonads lacked hyperechoic bands and had a more uniform hyperechoic composition.

Endoscopy is also a valuable method for gender identification. It, too, can be operated in the field, and enables a direct observation of gonads and, therefore, a more accurate assessment of gender due to the magnification of the observed organs. Endoscopy exhibited limitations as well, primarily the limited ability to distinguish between gonad and fat. Similar to ultrasonography, if obvious ovarian tissue could not be visualized, the animal was recorded as a male. These observations were also similar to those reported by Wildhaber et al. (2005).

The results of this study show that ultrasonography and endoscopy are both accurate methods for early gender identification in juvenile Siberian sturgeon. Although endoscopy was more accurate, ultrasonography has the advantage of being rapid, non-invasive, and does not require anesthesia (Wildhaber et al., 2005). Aquaculturists and wildlife biologists would benefit from the use of ultrasonography and endoscopy for early identification of gender in order to rear greater numbers of female sturgeon for caviar production, and to reveal pertinent biological information that can lead to better management of habitats critical to the life history of these nomadic fish.

The tradeoffs between accuracy and precision of the two methods must be carefully considered based on the particular situation where gender identification is needed. As such, the high accuracy of endoscopy may be of paramount importance for field biologists studying wild populations, whereas aquaculturists may conclude that the efficiency of gender identification is

more important when large numbers of fish must be examined quickly. Regardless of which method is used, this study demonstrates that histological examination is critical in establishing baseline conditions regarding age and or size of juvenile sturgeon assessed.

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Literature Cited

Allen, T. 1992. Hematoxylin and eosin. Laboratory Methods in Histotechnology. Armed Forces Institute of Pathology, Washington, DC: 53-58.

Boone, S.S., S.J. Hernandez-Divers, M.G. Radlinsky, K.S. Latimer, and J.L. Shelton. 2008. Comparison between coelioscopy and coeliotomy for liver biopsy in channel catfish. Journal of the American Veterinary Medical Association, 233(6): 960-967.

Chebanov, M., and E. Galich. 2009. Ultrasound diagnostics for sturgeon broodstock management. FSGTSR. Education-South. Publ.

Colombo, R.E., P.S. Wills, and J.E. Garvey. 2004. Use of ultrasound imaging to determine sex of shovelnose sturgeon. North American Journal of Fisheries Management, 24(1): 322-326.

Divers, S.J., S.S. Boone, J.J. Hoover, K.A. Boysen, K.J. Killgore, C.E. Murphy, S.G. George, and A.C. Camus. 2009. Field endoscopy for identifying gender, reproductive stage and gonadal anomalies in free-ranging sturgeon (*scaphirhynchus*) from the lower mississippi river. Journal of Applied Ichthyology, 25(s2): 68-74.

Everitt, B., 1992. The analysis of contingency tables. Chapman & Hall/CRC.

Hochleithner, M., and J. Gessner, 1999. The sturgeons and paddlefishes. Acipenseriformes.

Hurvitz, A., K. Jackson, G. Degani, and B. Levavi-Sivan. 2007. Use of endoscopy for gender and ovarian stage determinations in russian sturgeon (acipenser gueldenstaedtii) grown in aquaculture. Aquaculture, 270(1-4): 158-166.

Masoudifard, M., A.R. Vajhi, M. Moghim, R.M. Nazari, A.R. Naghavi, and M. Sohrabnejad. 2011. High validity sex determination of three years old cultured beluga sturgeon (huso huso) using ultrasonography. Journal of Applied Ichthyology, 27(2): 643-647.

Matsche, M.A., R.S. Bakal, and K.M. Rosemary. 2011. Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (acipenser brevirostrum) and atlantic sturgeon (acipenser oxyrinchus oxyrinchus). Journal of Applied Ichthyology, 27(2): 627-636.

Mims, S., A. Lazur, W. Shelton, B. Gomelsky, and F. Chapman. 2002. Species profile: Production of sturgeon. Southern Regional Aquaculture Center, 7200.

Stahl, M.T., G.W. Whitledge, and A.M. Kelly. 2009. Reproductive biology of middle mississippi river shovelnose sturgeon: Insights from seasonal and age variation in plasma sex steroid and calcium concentrations. Journal of Applied Ichthyology, 25(0): 75-82.

Stoskopf, M.K., 1993. Fish medicine. WB Saunders Co.

Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, M.L. Annis, and J.A. Allert. 2005. Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. Journal of Fish Biology, 67(1): 114-132.

Zwiebel, W.J., and R. Sohaey, 1998. Introduction to ultrasound. WB Saunders.

Table 2.1 Minimum and maximum fork length, weight, and gonadal length, for 3 and 4-year-old Siberian sturgeon.

	Weight (kg)		Fork Length (cm)		Gonad Length (cm)	
	3 yr	4 yr	3 yr	4 yr	3 yr	4 yr
Minimum	0.82	1.25	57	58.42	10	13.97
Maximum	4.1	3.03	89.5	80	28	23
Mean	1.97	1.99	71.23	69.5	20.24	18.90
Standard Deviation	±0.56	±0.42	±6.34	±5.18	±3.08	±2.21

Table 2.2 Distributions of fish gender in 3 and 4-year-old Siberian sturgeon as determined by ultrasonography, endoscopy, and histopathology (true gender).

Gender	Ultrasonography	Endoscopy	Histopathology
Female	46	55	55
Male	94	85	73
Other	-	-	12
			Total = 140

Other – includes undifferentiated and intersex samples

Table 2.3 Summary of identification of gender for ultrasonography and endoscopy confirmed histologically in 3 and 4-year-old Siberian sturgeon.

	Ultrasonography		Endoscopy	
Age class	3	4	3	4
# Sampled and confirmed histologically	101	27	101	27
# Correct	90	2	97	27
% Correct	89%	85%	97%	100%

Table 2.4 Recorded time for gender identification using ultrasonography and endoscopy in 3-year-old juvenile Siberian sturgeon.

Fish #	Ultrasonograhy (sec)	Endoscopy (sec)	Histologic Gender (confirmed)
1	7.32	17	Male
2	9.41	30.27	Male
3	4.9	24.6	Female
4	9.86	18.23	Male
5	7.21	12.29	Female
6	26.56	21.8	Male
7	8.85	18.5	Male
8	10.21	20	Male
9	7.33	38.6	Male
10	3.76	15.8	Male
11	4.66	35.58	Female
12	10.67	17.8	Male
13	9.32	24.3	Male
14	9.31	22	Male
15	26.93	24.3	Female
16	19.63	24.1	Male
17	14.62	14	Female
Average:	11.21	22.30	

Table 2.5 Correctness for ultrasonography vs. endoscopy for 3-year-old Siberian sturgeon.

Correctness for Ultrasonography vs. Endoscopy			
3 Year Old	**Endoscopy**		
Ultrasonography	**1**	**0**	**Total**
1	89	1	90
0	8	3	11
Total	97	4	101

McNemar's Test	
Statistic (S)	5.4
DF	1
Exact P-Value	0.0391

Table 2.6 Correctness for ultrasonography vs. gender for 3-year-old Siberian sturgeon.

Correctness for Ultrasonography vs. Gender			
3 Year Old	**True Gender**		
Ultrasonography	**f**	**m**	**Total**
1	32 78.05	58 96.67	90
0	9 21.95	2 3.33	11
Total	41	60	101

Fisher's Exact Test Ultrasonography	
P-Value	0.0065

Table 2.7 Correctness for endoscopy vs. histologic gender for 3-year-old Siberian sturgeon.

Correctness for Endoscopy vs. Gender			
3 Year Old	**True Gender**		
Endoscopy	**f**	**m**	**Total**
1	38 92.68	59 98.33	97
0	3 7.32	1 1.67	4
Total	41	60	101

Fisher's Exact Test Endoscopy	
P-Value	0.3009

Table. 2.8 Correctness for ultrasonography vs. histologic gender in 4-year-old Siberian sturgeon.

Correctness for Ultrasonography vs. Gender			
4 Year Old	**True Gender**		
Ultrasonography	**f**	**m**	**Total**
1	11 78.57	12 92.31	23
0	3 21.43	1 7.69	4
Total	14	13	27

Fisher's Exact Test Ultrasonography	
P-Value	0.5956

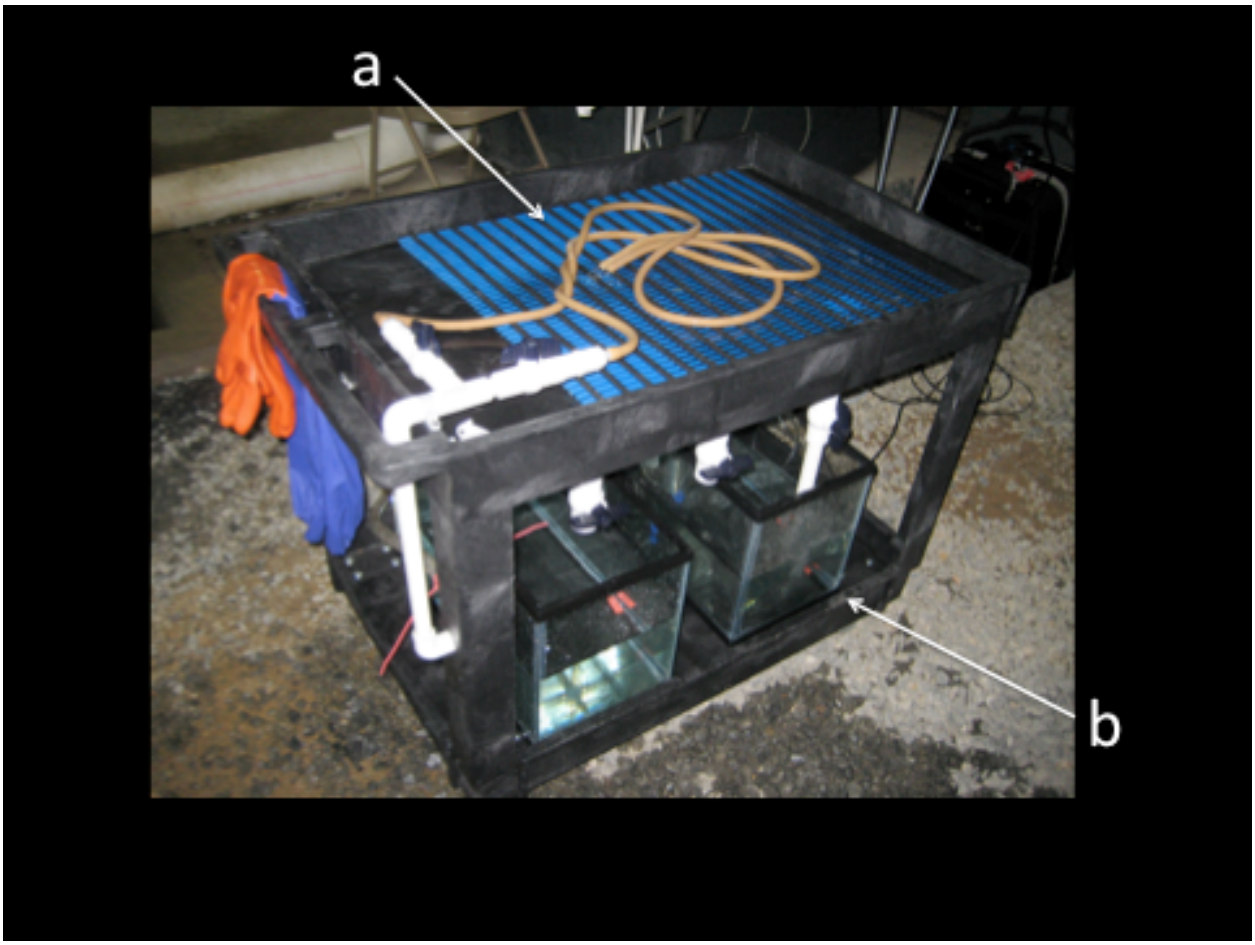

Figure 2.1 Sturgeon surgery station including split rubber tubes above (a) and two reservoir tanks for maintenance anesthesia below (b).

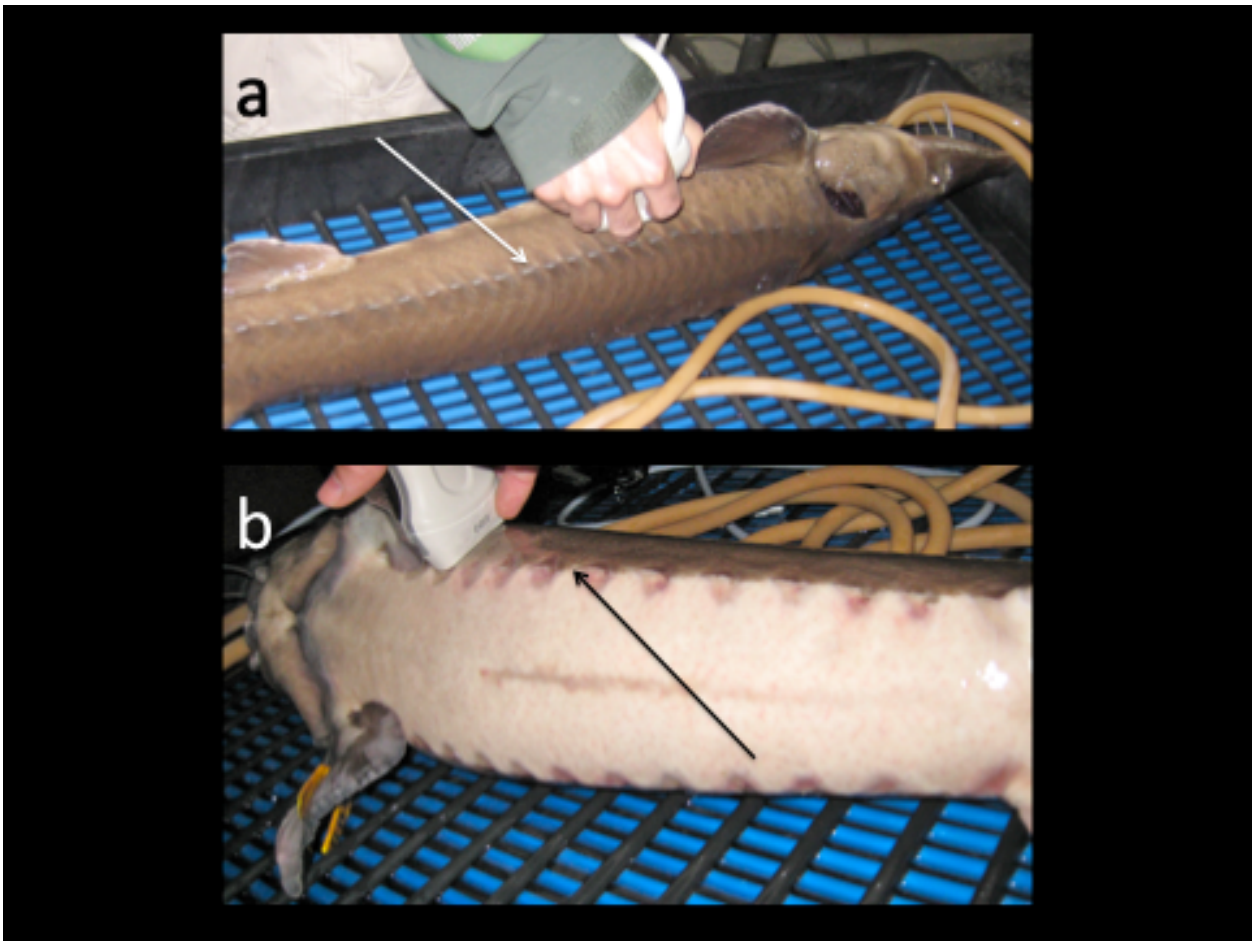

Figure 2.2 (a) Sturgeon in right lateral recumbency with ultrasonographic transducer positioned ventral to the lateral scutes (white arrow). (b) Sturgeon in right lateral recumbency with ultrasonographic transducer positioned dorsal to the abdominal scutes (black arrow).

Figure 2.3 (a) Sturgeon in right lateral recumbency prior to incision between abdominal scutes (arrow). (b) Sturgeon in right lateral recumbency following suture closure (arrow).

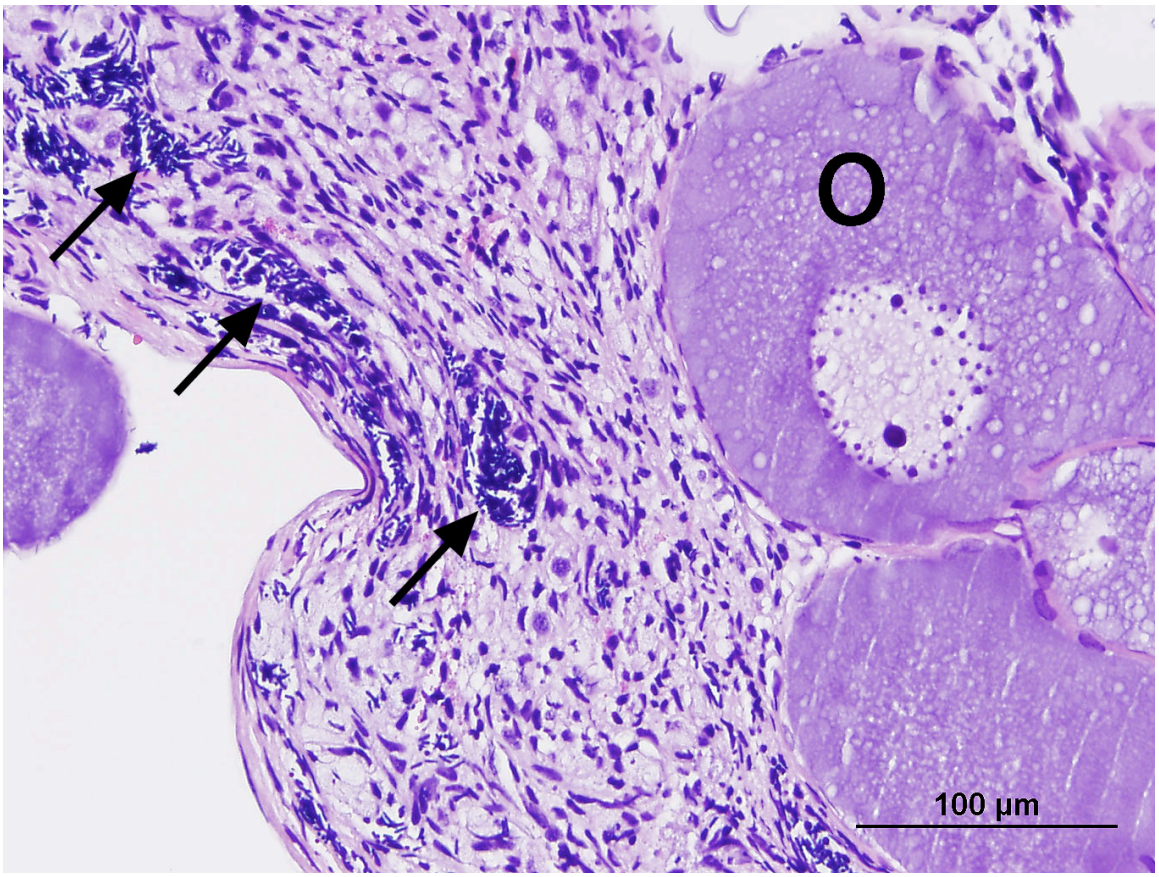

Figure 2.4 Histological section of a gonadal biopsy from a 3-year-old intersex Siberian sturgeon. Narrow testicular tubules (black arrows) are filled by elongate intensely basophilic heads of mature sperm. There is a primary growth oocyte (O) with multiple nucleoli distributed around the nuclear periphery. (H&E)

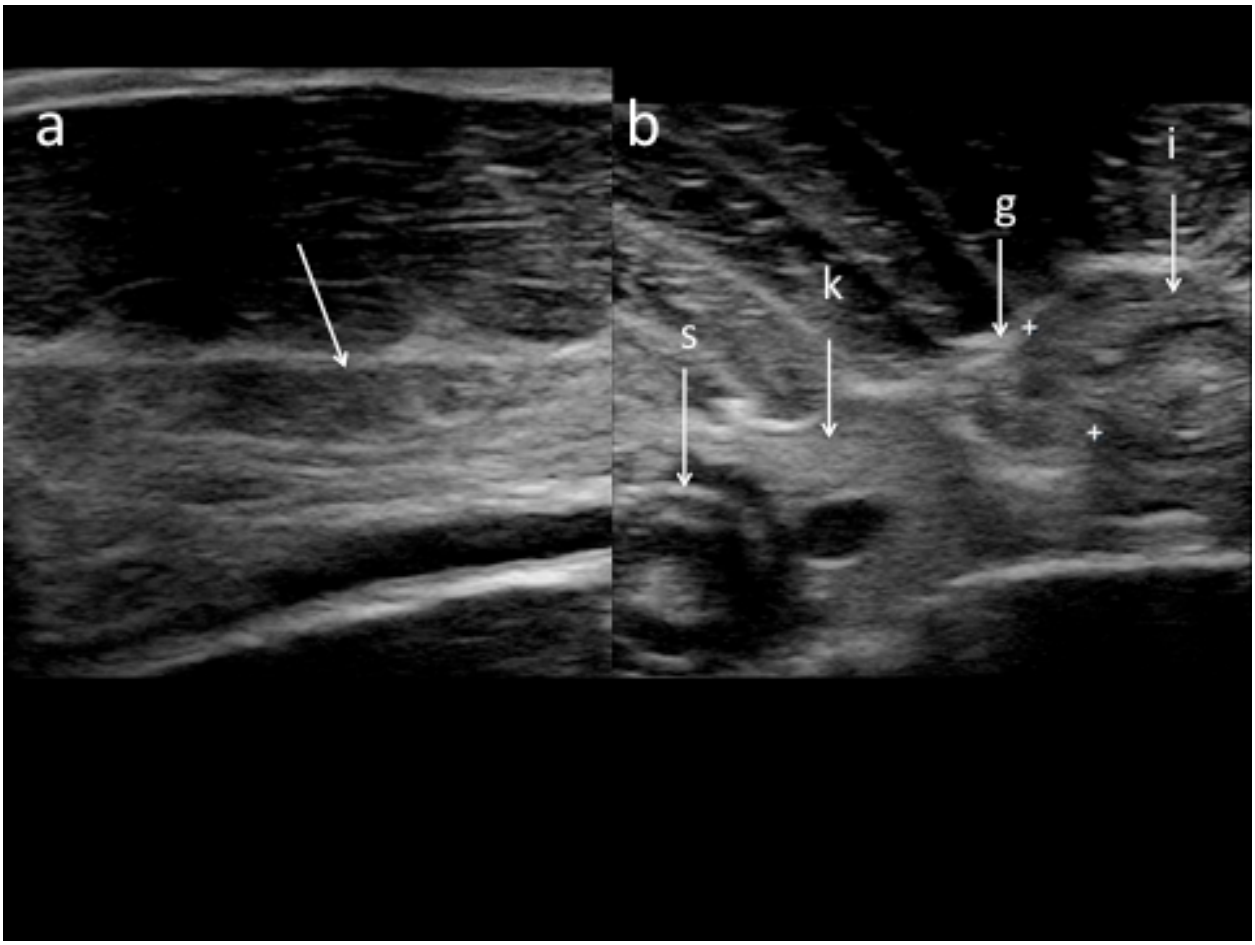

Figure 2.5 Dorsal (a) and transverse (b) plane ultrasound images of the right gonad (arrows) of a 4-year-old Siberian sturgeon with undetermined gender due to lack of gonadal development. The gonad is mildly, diffusely heterogeneous and similar in echogenicity to overlying muscle. Other organs include (s) spinal column, (k) kidney, (g) gonad, and (i) gastrointestine.

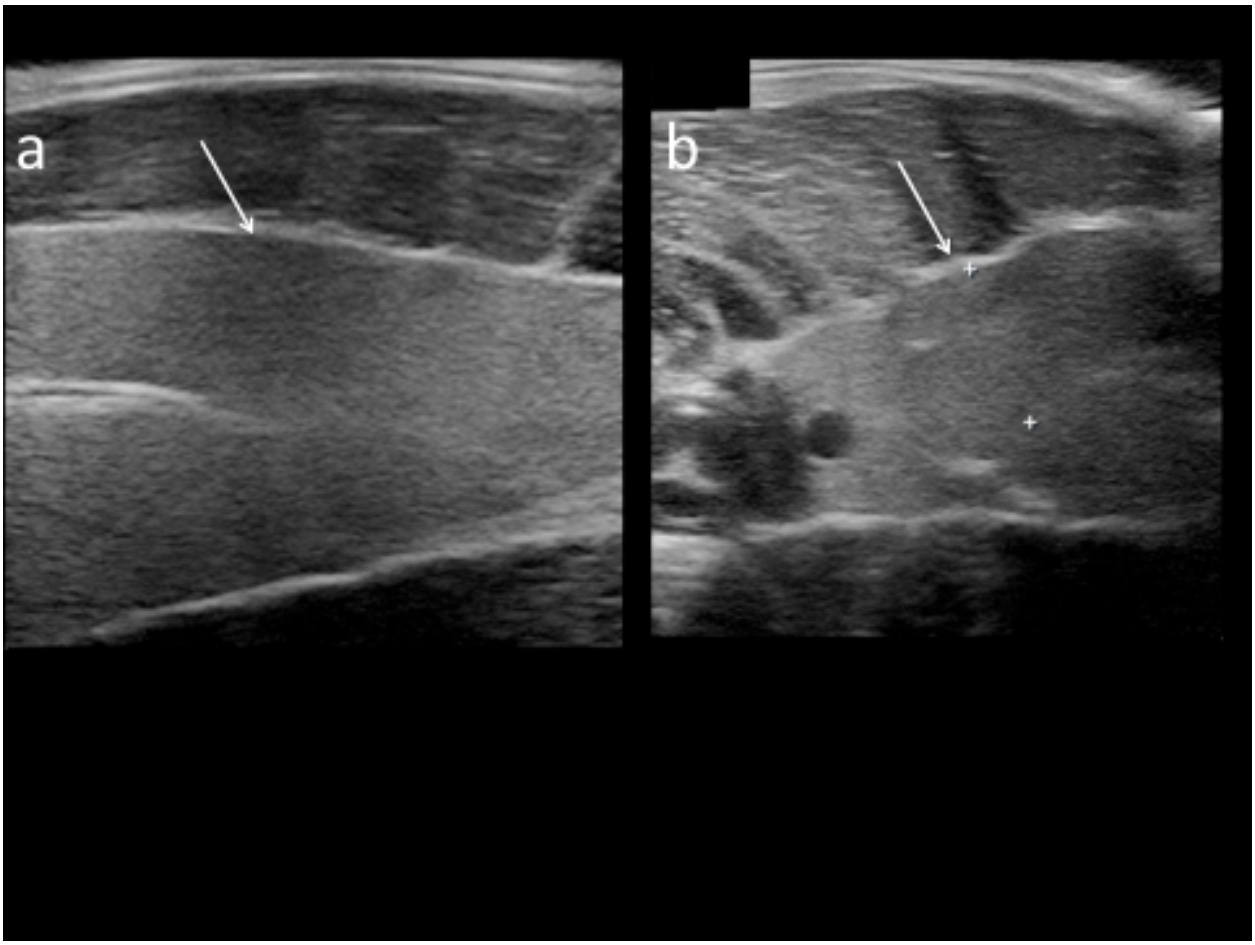

Figure 2.6 Dorsal (a) and transverse (b) plane ultrasound images of the right gonad (arrows) of a 4-year-old Siberian sturgeon ultrasonographically identified as male and confirmed through histology. The gonad was homogeneously hyperechoic with a fine echotexture.

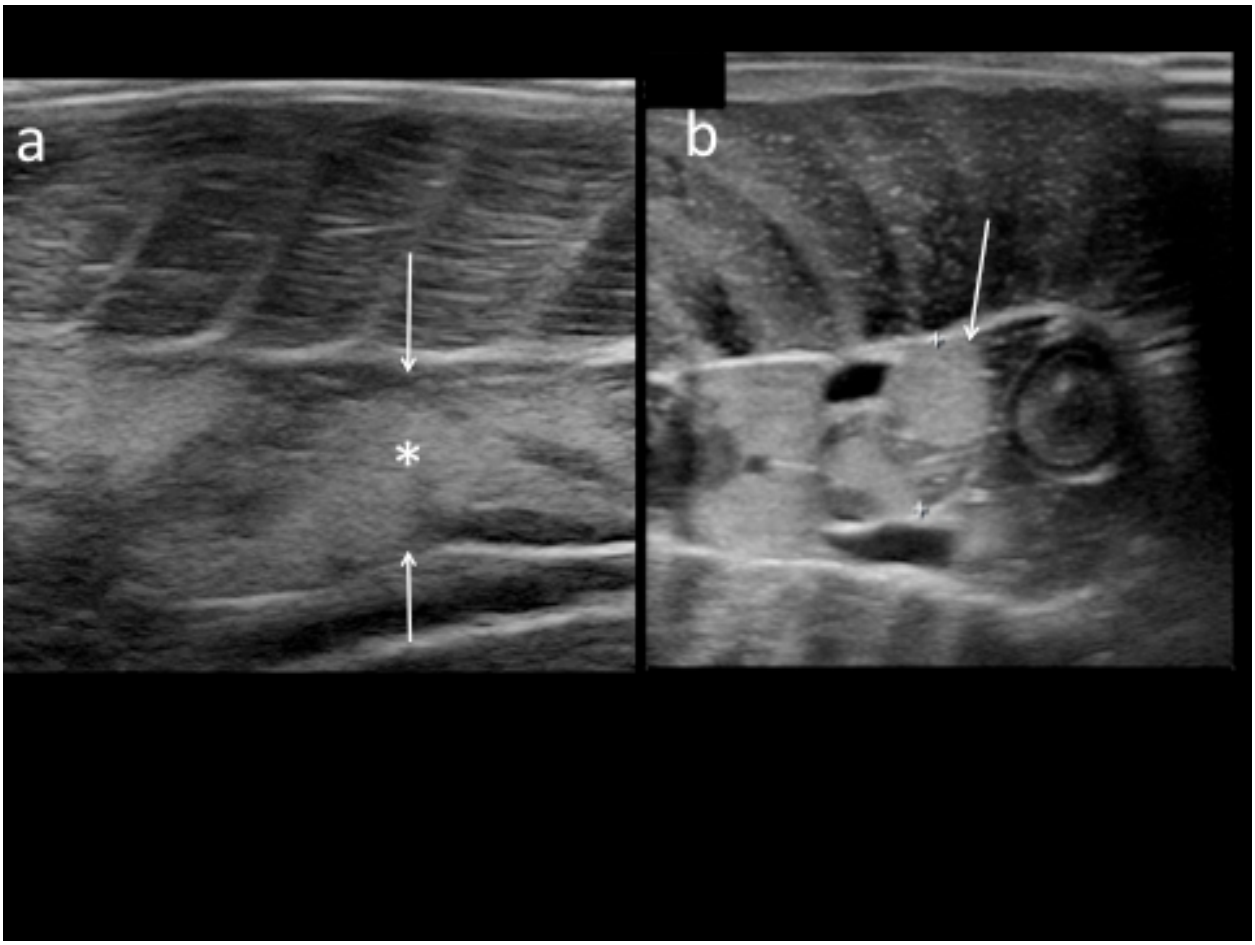

Figure 2.7 Dorsal (a) and transverse (b) plane ultrasound images of the right gonad (arrows) of a 3-year-old Siberian sturgeon ultrasonographically identified as female and confirmed through histology. Undulant hyperechoic tissue (asterisk) was identified throughout center of gonad.

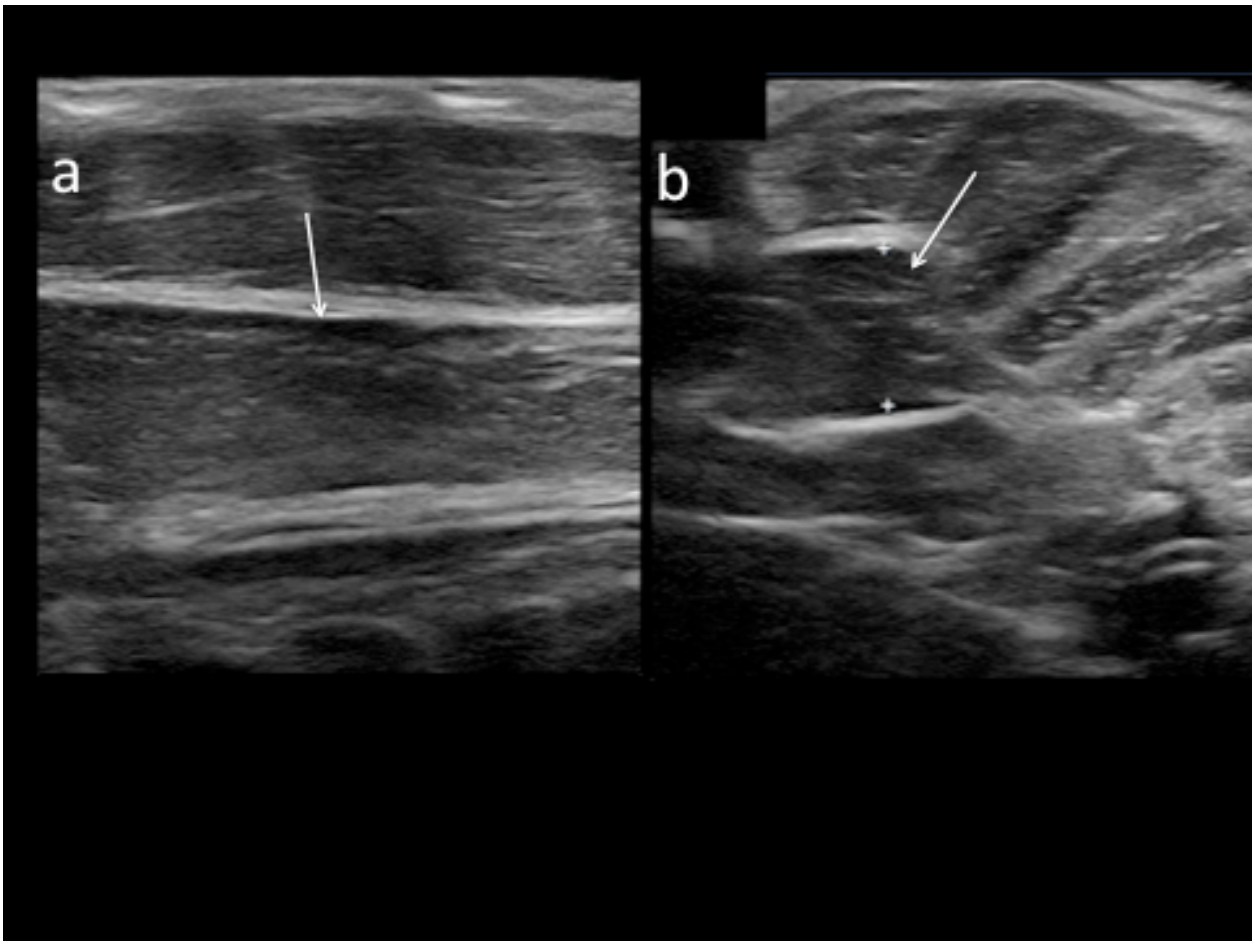

Figure 2.8 Dorsal (a) and transverse (b) plane ultrasound images of the left gonad (arrows) of a 3-year-old Siberian sturgeon identified histologically as intersex. The gonad had heterogeneous hyperechoic tissue throughout.

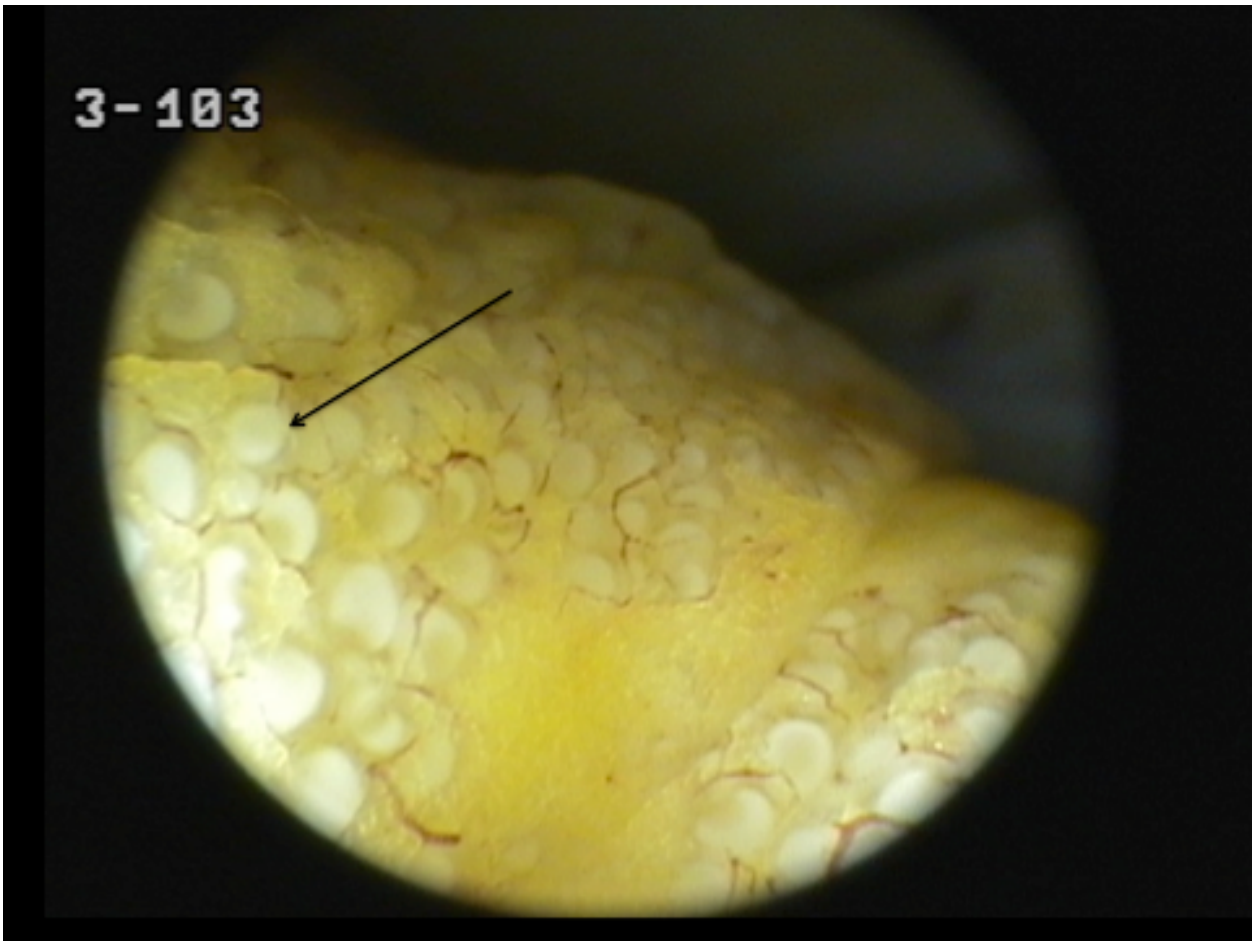

Figure 2.9 Endoscopic image of a 3-year-old female Siberian sturgeon gonad confirmed histologically. Tiny oocytes (arrow) invisible to naked eye but clearly identifiable and interspersed within gonadal fat.

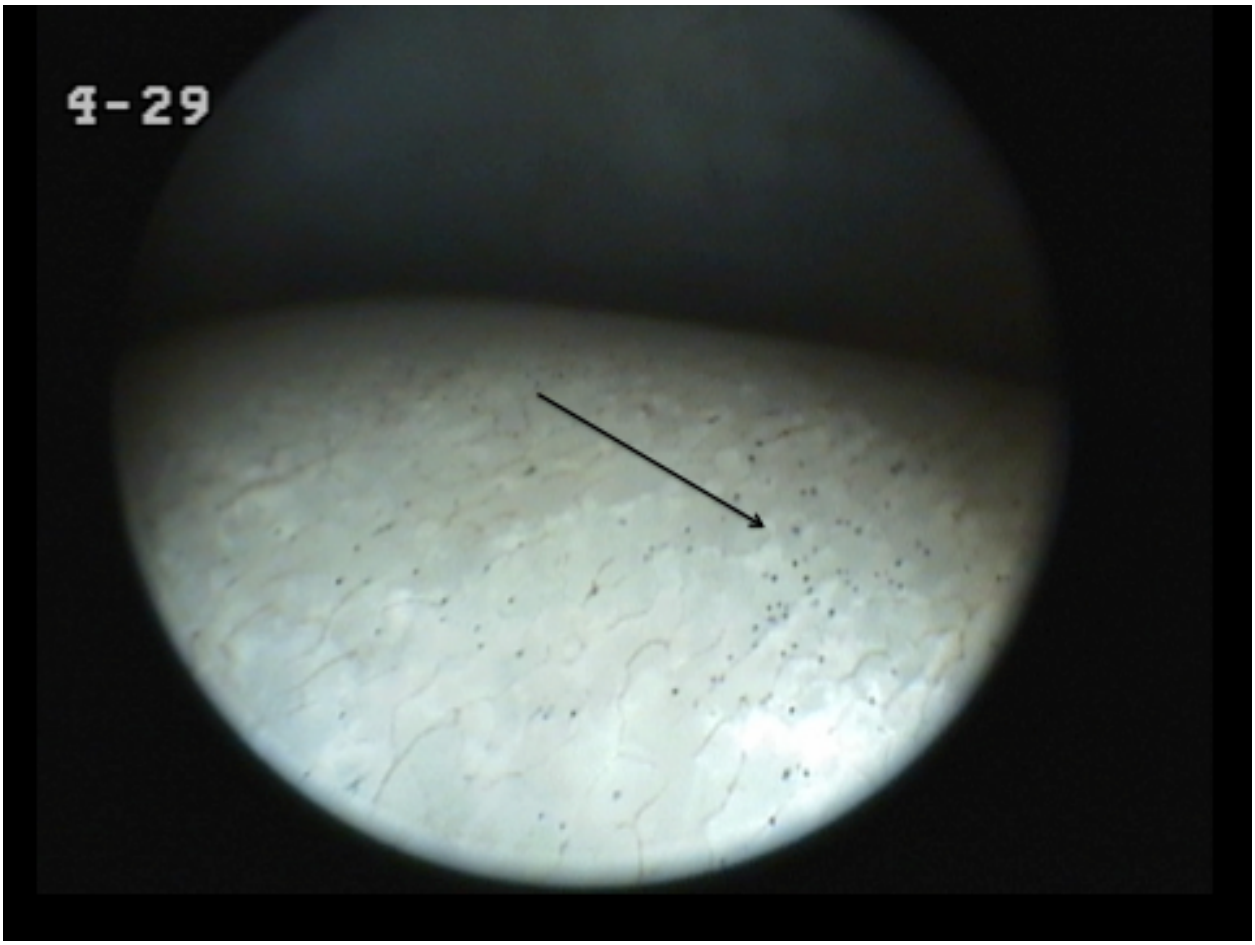

Figure 2.10 Endoscopic image of a 4-year-old male Siberian sturgeon gonad confirmed histologically. Gonads appeared smooth with a sinusoidal pattern (arrow).

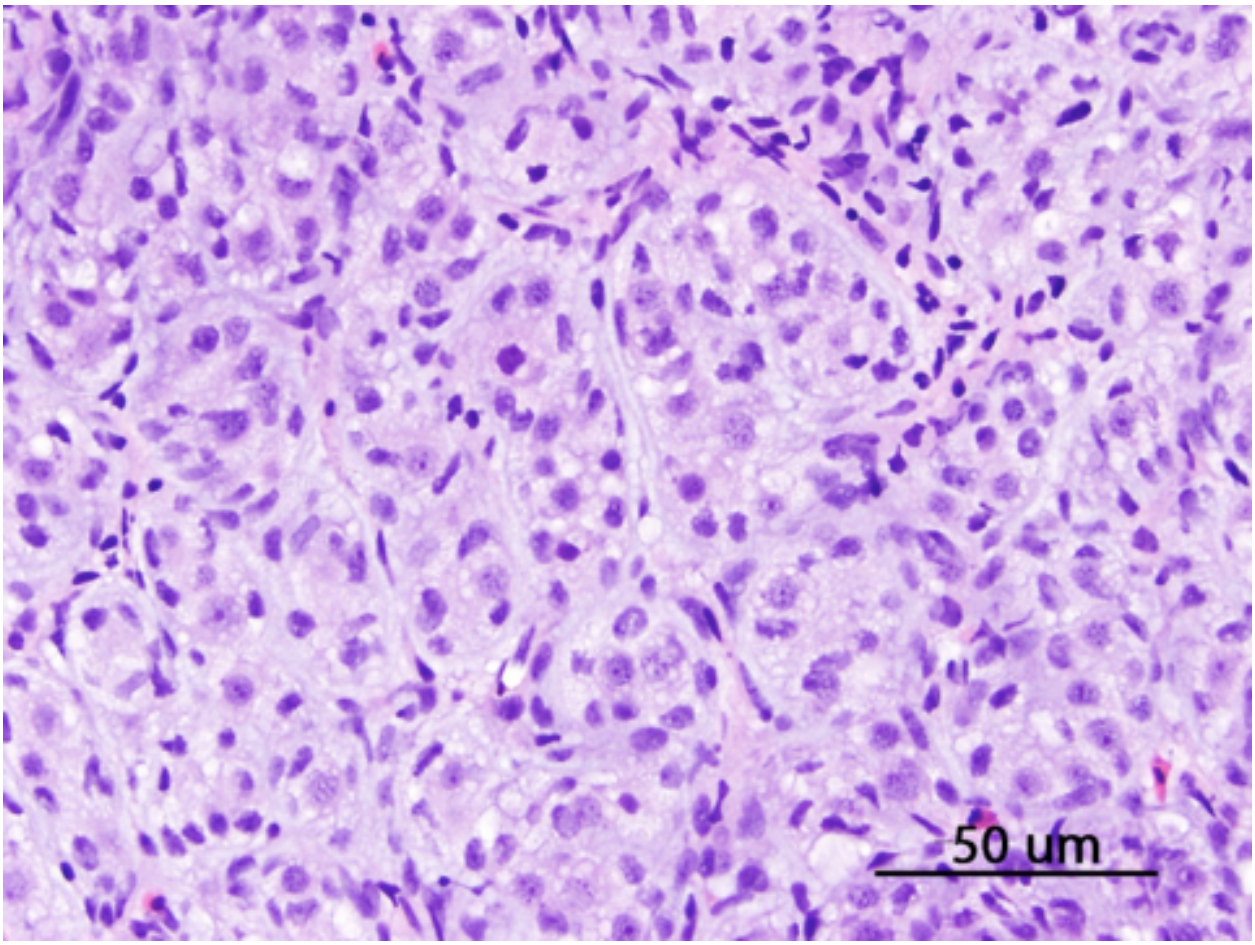

Figure 2.11 Histological section of an undifferentiated gonad biopsied from a 3-year-old Siberian sturgeon of unconfirmed gender. (H&E)

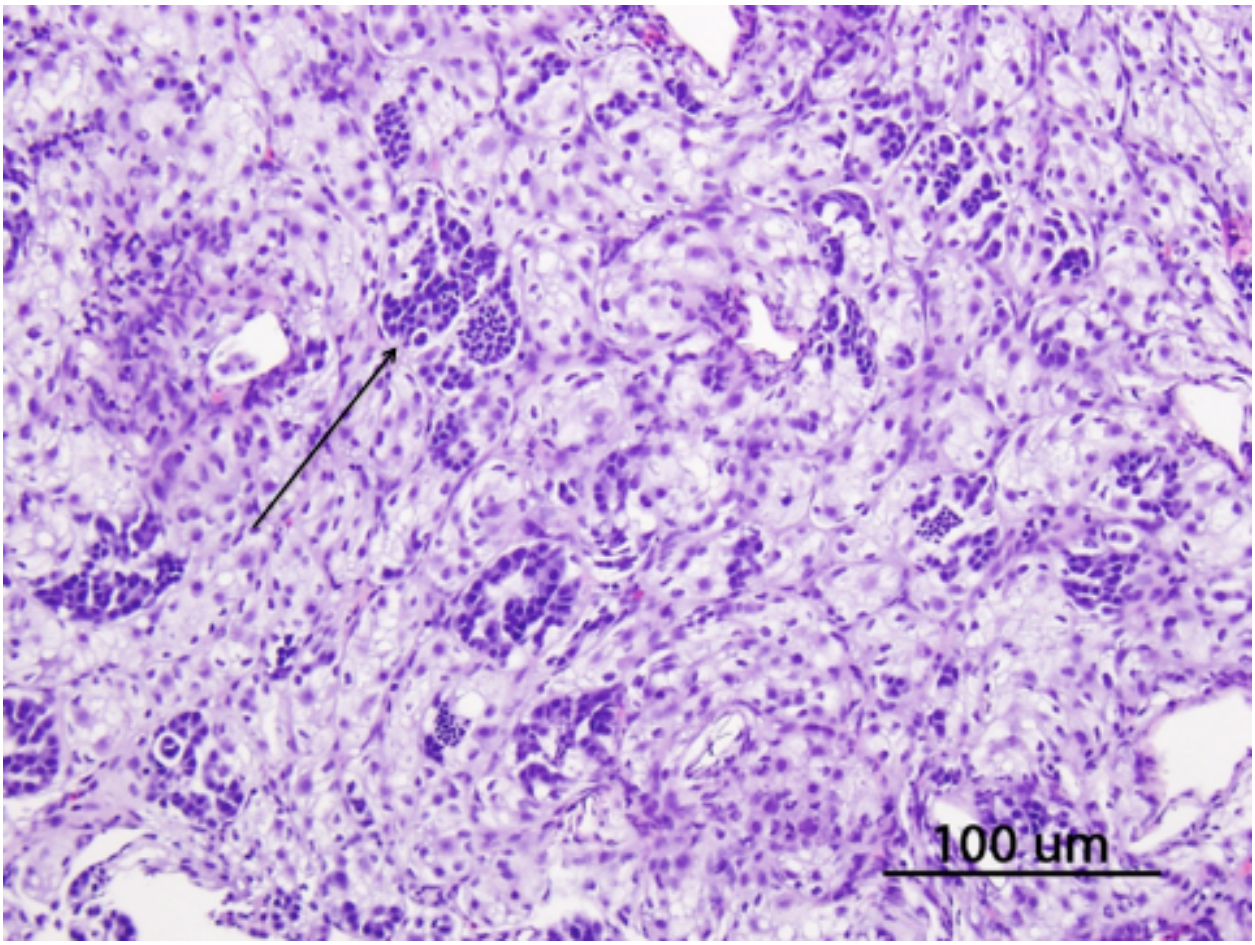

Figure 2.12 Histological section of a gonadal biopsy from a 3-year-old male Siberian sturgeon demonstrating progressive spermatogenesis in individual testicular lobules (arrow). (H&E)

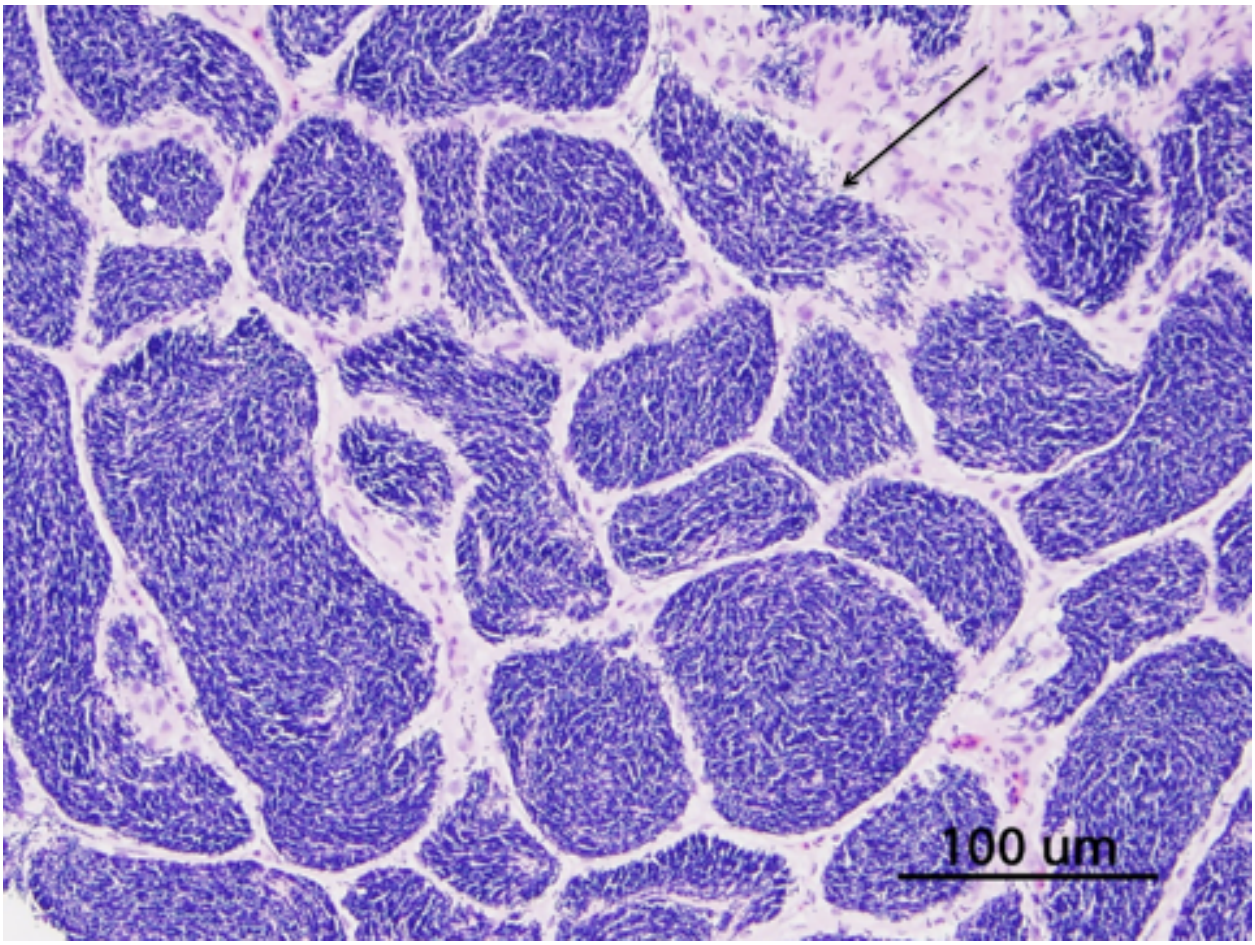

Figure 2.13 Histological section of a gonadal biopsy from a 3-year-old male Siberian sturgeon demonstrating large numbers of mature sperm distending from testicular tubules (arrow). (H&E)

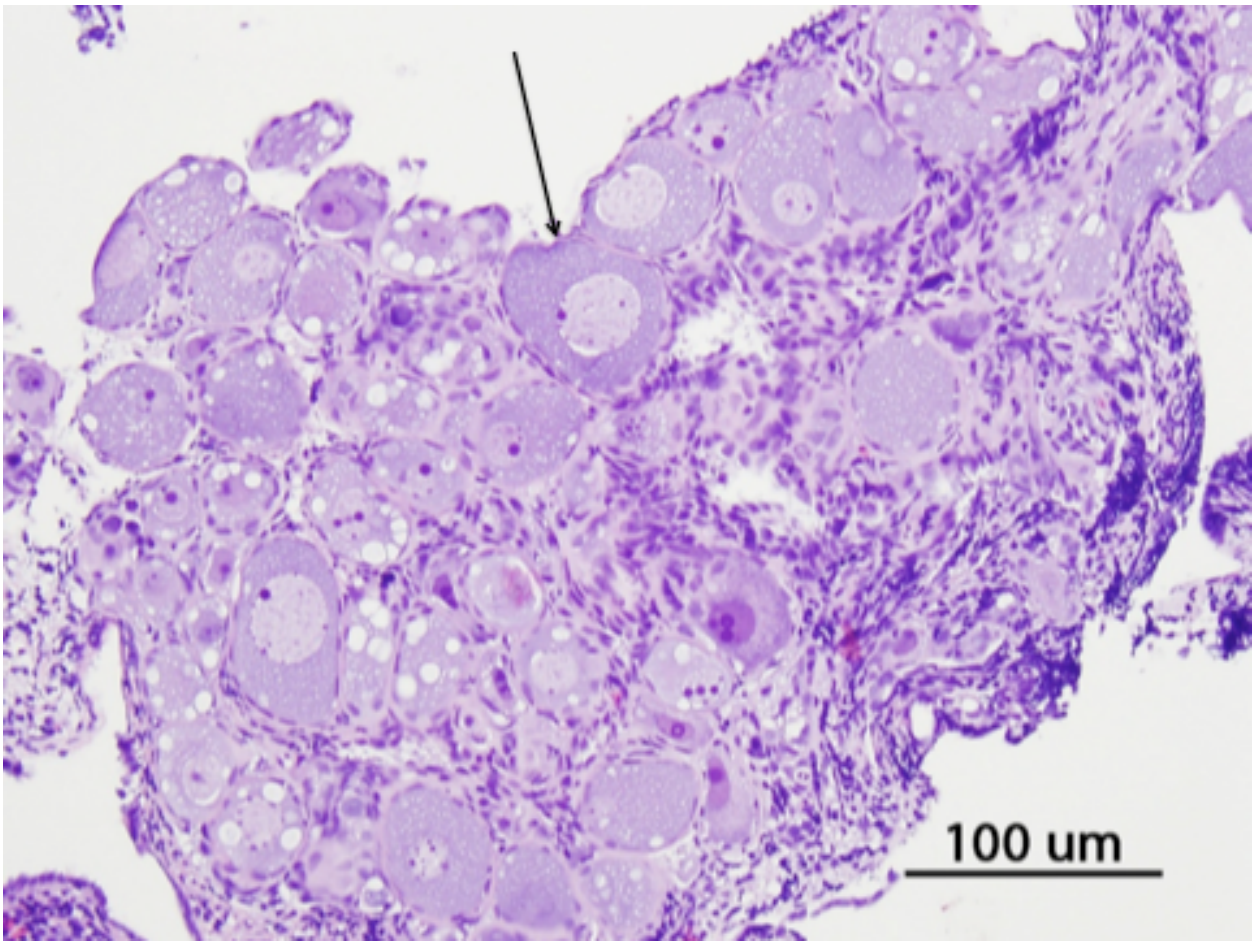

Figure 2.14 Histological section of a gonadal biopsy from a 3-year-old female Siberian sturgeon. The tissue is predominated by primary growth oocytes in various stages of development (arrow). (H&E)

CHAPTER 3

CONCLUSIONS

Diagnostic imaging is commonly used to identify gender in immature fish and reproductive stage in sexually mature individuals. Previously, most research has indicated that ultrasonography is not as accurate as endoscopy, and may not be the ideal method for gender identification for aquacultured or free-ranging sturgeon (Wildhaber et al., 2005; Bryan et al., 2007; Hurvitz et al., 2007; Divers et al., 2009; Matsche et al., 2011). In concurrence with other gender identification studies, the evaluation of ultrasonography and endoscopy was conducted and compared to gonadal histology, as well as the time required to identify gender. Most aquaculture facilities practice endoscopy because it provides direct observation of the gonads in a relatively short time frame. However, though endoscopy is minimally invasive, it still requires surgical entry into the coelomic cavity to view gonads and identify gender, unlike ultrasonography, which is completely non-invasive.

To address the controversy regarding the most accurate, efficient, and safe methodology to identify gender in fish, juvenile Siberian sturgeon (*Acipenser baerii*) were analyzed using a portable ultrasound machine and a rigid endoscope. The first objective was to evaluate the accuracy of gender identification using ultrasonography and endoscopy. The second objective was to measure the time elapsed to identify gender in both methods.

Ultrasound imaging showed distinct anatomical differences between males and females. Female gonads showed tortuous hyperechoic bands. Male gonads were uniformly hyperechoic relative to adjacent muscle when compared in immature fish. It was also noted the more

developed juvenile male gonads were increased in echogenecity on the ultrasound image, with strong contrast between gonad and adjacent tissue. Endoscopy identified gonads with evidence of oocytes as female. Male gonads were identified as smooth in appearance with a sinusoidal pattern.

The time recorded to identify gender was conducted in 17 fish. Ultrasonography correctly identified gender in a mean time of 11.2 seconds per fish. Endoscopy took twice as long, acquiring a mean of 22.3 seconds per fish to identify gender. Given the results, ultrasonography is regarded as the faster gender identification method.

Ultimately, the goal of this study was to present data to indicate which diagnostic imaging device is the more cost effective and accurate machine to identify gender in juvenile Siberian sturgeon. The results indicated that endoscopy was the more accurate, although invasive method, whereas ultrasonography was the faster, non-invasive method. Aquaculture thrives on the ability to identify gender at the earliest age as possible, in the fastest and most cost effective manner. The cost of a portable ultrasound machine with the minimum specifications required to identify gender in fish range from $20,000 to $50,000, which is comparable to the cost of endoscopic equipment, approximately $30,000.

Fish may be endoscoped numerous times throughout their life, beginning around age five and potentially extending through nine years. Ultrasonography provides the luxury of quickly identifying gender as early as three years without the need for anesthesia and surgery. This leads to a call for additional research to study different reproductive stages, especially when mature fish become ripe for harvesting caviar, using ultrasonography. Ideally, this may eliminate the need to repeatedly perform endoscopy throughout the lifetime of the fish.

Future gender and reproductive stage studies using ultrasonography should also be conducted in free-ranging sturgeon. With nearly all North American species of sturgeon protected under federal law, research would largely influence a better understanding of the life history of these migratory fish through gender and reproductive stage analyses. Identifying gender at prime spawning habitats and other aquatic areas critical to the growth and development of larval and juvenile sturgeon would influence future management decisions.

The current study indicates both methods are accurate to identify gender (vs. gonadal histology), with endoscopy being more accurate (96.9% vs. 88.3%) and ultrasonography as the faster method (time required to identify gender) in juvenile Siberian sturgeon. Furthermore, we believe ultrasonography would be valuable in the field in free-ranging sturgeon. With proper training, ultrasonography is an effective and practical method in identifying gender in sturgeon.

Literature Cited

Bryan, B.J.L., M.L. Wildhaber, D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, and M.L. Annis. 2007. Estimation of gonad volume, fecundity, and reproductive stage of shovelnose sturgeon using sonography and endoscopy with application to the endangered pallid sturgeon. Journal of Applied Ichthyology, 23(4): 411-419.

Divers, S.J., S.S. Boone, J.J. Hoover, K.A. Boysen, K.J. Killgore, C.E. Murphy, S.G. George, and A.C. Camus. 2009. Field endoscopy for identifying gender, reproductive stage and gonadal anomalies in free-ranging sturgeon (*scaphirhynchus*) from the lower mississippi river. Journal of Applied Ichthyology, 25(s2): 68-74.

Hurvitz, A., K. Jackson, G. Degani, and B. Levavi-Sivan. 2007. Use of endoscopy for gender and ovarian stage determinations in russian sturgeon (acipenser gueldenstaedtii) grown in aquaculture. Aquaculture, 270(1-4): 158-166.

Matsche, M.A., R.S. Bakal, and K.M. Rosemary. 2011. Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (acipenser brevirostrum) and atlantic sturgeon (acipenser oxyrinchus oxyrinchus). Journal of Applied Ichthyology, 27(2): 627-636.

Wildhaber, M.L., D.M. Papoulias, A.J. DeLonay, D.E. Tillitt, J.L. Bryan, M.L. Annis, and J.A. Allert. 2005. Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. Journal of Fish Biology, 67(1): 114-132.