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Determination of Sex and Maturity in *Acipenser stellatus* by Using Ultrasonography

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Abstract: Knowledge of both sex and maturity stage without killing fish is important from many aspects. The establishment of accurate sex and maturity determination without external sexual dimorphism or when external characters are not developed has usually involved the use of either hormone radioimmunoassay or biopsy techniques. These methods are time and labor consuming as well as harmful to fish. Therefore, it would be economically important for caviar producing countries to develop accurate, effective and non-invasive method for determination of sex and maturity in sturgeons.Ultrasonography examination was performed to determine sex and differences between matured and immatured *Acipenser stellatus*. The 249 captured stellate sturgeons larger than 95 cm in length were examined with a linear transducer. Sex determination was carried out based on the differences in echogenicity and morphology of the ovaries and testes. Results of ultrasonography were confirmed at necropsy: 199 fish were female (63 matured and 136 immatured) and 50 were male (29 matured and 21 immatured). Ultrasonographic examination resulted in sex determination with 97.2% accuracy. Gonads of immature males may be difficult to discern. The speed of examination was around 30 seconds or less per fish depending on the stage of maturation of fish.

Key words: Stellate sturgeon, *Acipenser stellatus*, Ultrasonography, Sex determination, Immature gonads, Mature gonads

Introduction

Sexual dimorphism is uncommon in many important cultured species; therefore it is difficult to determine sex and predict maturation of wild and captive stocks until a short time prior to spawning. The development and evaluation of practical, non-lethal techniques for determination of sex and maturational status of important broodfish is critical to the advancement of aquaculture. Current techniques for determination of sex and characterizing maturation in fish include urogenital catherization (Lewis, 1962, Rees & Harrell, 1990), plasma lipophosphoprotein analysis (Thurston, 1967, Craik & Harvey, 1984), plasma vitellogenin concentrations (Mommsen and Walsh 1988; Sullivan *et al,.* 1991; Tao *et al,.* 1992), immunoagglutination methods (LeBail & Breton, 1981) and radioimmunoassay of blood steroid levels (Berlinsky & Specker, 1991 ; Woods & Sullivan, 1993).

Most of the currently used techniques are time and labor consuming, invasive and threatening on fish health and reproductive success. They frequently require blood and oocyte collection which can stress mature broodfish, introduce pathogens, and delay or prevent ovulation. Stress associated with catherization and blood sampling can result in direct mortalities. Moreover, these methods are not conclusive in determining sex or reproduction status throughout most of the year, particularly when the fish is not in spawning condition (Blythe *et al.,* 1994).
In contrast, ultrasonic imaging has proven useful as a non-invasive technique to determine the sex and maturity of many commercially and ecologically valuable species for example: coho salmon *"Oncorhynchus kisutch"* Walbaum (Martin *et al.*, 1983), Rainbow trout *"O. mykiss"* (Reimers *et al.*, 1987), Atlantic salmon *"Salmo salar"* L. (Reimers *et al.*, 1987, Mattson 1991), Pacific herring *"Clupea harengus pallasi"* Val. (Bonar *et al.*, 1989), cod *"Cadus morhua"* (Karlsen & Holm, 1994), Striped bass *"Morone saxatilis"* (Blythe *et al.*, 1994), Atlantic halibut *"Hippoglossus hippoglossusll"* (Shields *et al.*, 1993), Snakehead fish *"Channa striatus"* (Montgomery *et al.*, 1997) and Paddle fish *"Polyodon spathula"* (O'Brien *et al.*, 1997).

In this detailed study we found ultrasonic imaging as a useful tools for sex determination in sturgeon.

Materials and Methods

Five testes and ovaries of the necropsied fishes were scanned by ultrasonography in a water tank to gain enough knowledge of their appearance before scanning live fish. A Pie Medical 200 VET (B. mode – Real time), 5 and 7.5 MHz Linear waterproof transducer was used in this study. The scan-head of transducer was covered by a condom to protect it against rows of scutes and star-shaped plates of the skin. Stellate sturgeon (n=249) were captured randomly from the Caspian Sea by gillnet with a mesh size of 100 mm during March, April and May of 1999 and scanned. Before scanning, the fish were anesthetized and submerged in a seawater tank. The necessary acoustic coupling was provided by the water itself, through which attenuation of ultrasound was relatively low. Scanning was performed transducer by close contact of 1cm apart from the skin surface. Frontal views of right or left lateral side of the fish between pectoral fin and anal fin were obtained and wherever needed transverse view was also obtained. Echogenicity (the rate of echo from tissues to transducer which is shown as bright spots on monitor) and morphology of gonads by ultrasonographic findings were studied for evaluating the differences between testes and ovaries and their status of maturation. All images were recorded on videotape for subsequent analysis. During the scanning, constant focus, brightness, and constant setting were used and the gain settings were fixed.

Results obtained from ultrasonography were confirmed immediately by necropsy of the each fish.

Fork length and weight measurements were carried out for each fish specimen, and material for aging (fin ray section) was also collected.

We used the 6 stage scale of gonad maturity (Bagenal, 1978). In this study, the "mature female" refers to female stage IV (with granules of caviar), the "immature female" to stages II and III, the "mature male" to stages III and IV (with lobulated testes) and the "immature male" to stage II (with narrow strip testes) (Plates 1-4).

Plate 1: A schematic figure of mature female gonad of Stellate sturgeon

Plate 2: A schematic figure of immature female gonad of Stellate sturgeon

Plate 3: A schematic figure of mature male gonad of Stellate sturgeon

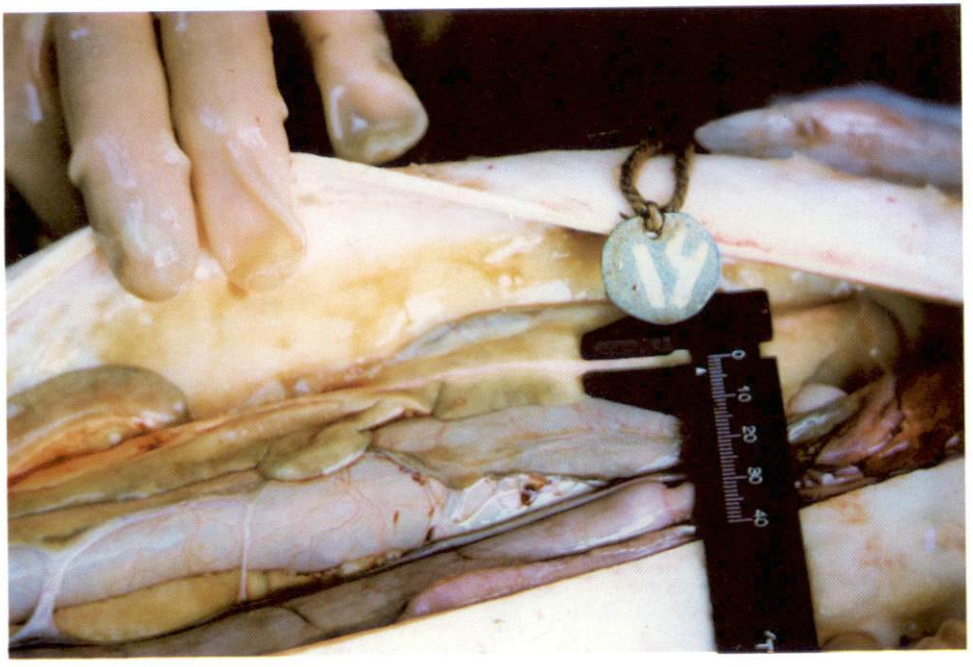

Plate 4: A schematic figure of immature male gonad of Stellate sturgeon

Results

The ultrasonographic findings of gonad appearance were : The gonad images of matured females were light, granulated and echogenic without any actual shape due to the size of ovaries (Fig.1), the gonads of immature females appeared more hyperechoic, heterogenic and lamellated in shape with indistinct margin and usually more hyperechoic than abdominal muscles (Fig.2). Matured testes images were homogenic, dark, lobulated and smooth with a distinct margin and usually more hypoechoic than abdominal muscles (Fig.3). Immature male testes were narrow with string-like appearance and were embedded in hypoechoic gonad fat and often were difficult to discern due to their small size (Fig.4).

From 249 examined fish, 199 (80%) fish were female and 50 (20%) were male. Table 1 shows the range of fork length, weight and age of the fish and table 2 shows the accuracy of ultrasound sex and maturity determination.

Table 1: Range of fork length, weight and age of the scanned stellate sturgeon.

Sex	No.	Fork length (cm)	Weight (kg)	Age (year)
Female	199	95-150	4.5-16	6-16
Male	50	100-141	4.5-13	6-15

Ultrasonographic accuracy for sex and maturity determination was 97.2%. The speed of the procedure was around 30 seconds or less per fish and depended on the sex and maturity of fish. Diagnosis of immatured males took more time than others.

Table 2: Accuracy of sex and maturity determination of stellate sturgeon using Ultrasound

Sex	Maturity Status	Ultrasonographic Diagnosis (No)	Necropsy Diagnosis (No)	Accuracy (%)
Female	Mature	63	63	100
	Immature	135	136	99.3
	Total	198	199	99.5
Male	Mature	28	29	96.5
	Immature	16	21	76.2
	Total	44	50	88
Total		242	249	97.2

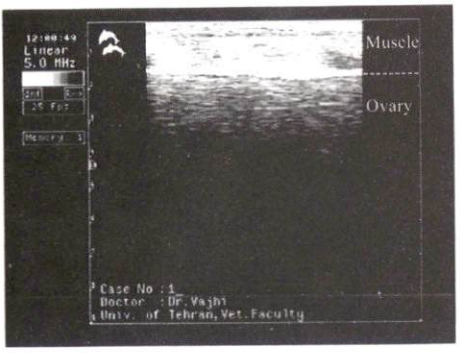

Fig.1: Sonographic image of mature female of stellate sturgeon

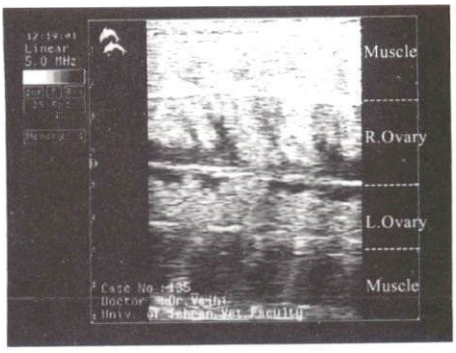

Fig. 2 : Sonographic image of immature female of stellate sturgeon

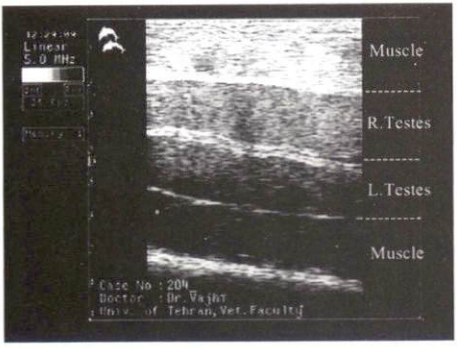

Fig.3: Sonographic image of mature male of stellate sturgeon

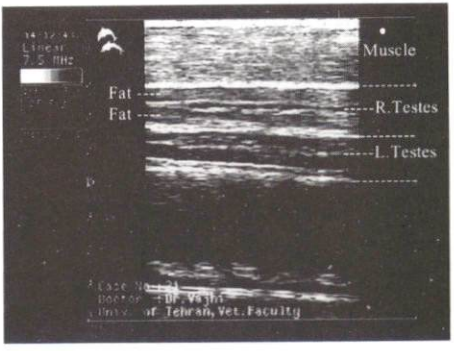

Fig. 4 : Sonographic image of immature male of stellate sturgeon

Discussion

As mentioned before, our samples were randomly chosen from fishes larger than 95 cm. In this range of size, it was never observed immature male and female in stage I at necropsy. Usually male and female in stages V and VI are found in the rivers during the spawning season and since only the sea fishing captures used in this study, these stages were not included in this experiment. Therefore this study was exclusively conducted on immature and mature male and female stages II, III and IV. Sex composition in our samples was %20 male and %80 female that is almost close to the sex composition of stellate sturgeon population in the southern part of the Caspian Sea (Moghim & Hassannia, 1997).

Although there are differences in the morphology and development of the gonads among species, the ultrasound images of the gonads in stellate sturgeon were almost similar to the earlier observations in salmonides (Mattson, 1991), herring (Bonar *et al,*. 1989), cod (Karlsen & Holm, 1994), snakehead fish (Montgomery *et al*., 1997) and paddle fish (O'Brien *et al.,* 1997). Ultrasonography images of matured female gonads were light and granulated, and oocytes could be visualized as whitish-grey dots at the top of the screen and a marked shadow was visible on the image, opposite to the transducer. Mature male gonads were seen as dark grey areas or hypoechoic with coiled and convoluted lobes. The gonads of immature males were difficult to identify due to small gonad size.

The gonads of immature female stellate sturgeon were discerned by ultrasonic morphology and echogenisity and they appeared hyperechoic, heterogenic and lamellated. Immature females of salmon were detected by small size of the ovary (Reimers *et al.,* 1987 ; Mattson, 1991) but for cod it is not possible to differentiate immature females (Karlsen & Holm, 1994).

Sinilar to other studies, the accuracy of sex determination of mature fishes was greater than immature fishes. But, sex determination of immature fishes in other studies was very difficult or impossible, while on stellate sturgeons the accuracy of sex determination was 99.3% and 76.2% for immature females and males, respectively. The acoustical properties and large gonad size of stellate sturgeon also helped in sex identification.

Previously published ultrasound studies have reported qualitative observations on fish scanned only at one time during the year, primarily in the spawning season when

physical expression of sperm and ovarian biopsy would be simple and more conclusive, however, potentially more disruptive than ultrasound examination. Martin *et al.*, (1983) found that mature coho salmon could be sexed accurately with ultrasound one month before spawning, but they examined only 5 adult fish (2 females). Reimers *et al.*, (1987) were able to distinguish the sex of rainbow trout and Atlantic salmon but not until 5 months before spawning. Karlsen & Holm (1994) reported sex determination of cod which accuracy depends on season. The highest accuracy was achieved before spawning when gonads were the largest. In these fishes, the interval between reproduction periods is one year, but this period for stellate sturgeon of the Caspian Sea population is about three to four years (Silov *et al.*, 1971; Holcik, 1989). It means that various stages of maturity can be found in the sea, during the year. Thus, although this study was conducted during the spring, but accuracy of sex and maturity determination by ultrasonography may be the same during other seasons, however, more investigation is needed.

This study can help determining immature females in the sea fishing because of its high speed and accuracy. Identifying immature females can help in releasing them in a harmless manner, to preserve this valuable caviar producing fish. Furthermore, the results can be used in selecting suitable fish for artificial propagation and mono sex culture purposes.

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