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Original Article

The relationship between morphological and reproductive status in suitable Russian sturgeon, *Acipenser gueldenstaedtii* (Brandt and Ratzeburg, 1833) broodstocks

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Abstract: Owing to reduction of sturgeon stocks in various water bodies, artificial propagation has become significantly important for stock restoration and appropriate broodstock selection is vital in this process. Selection of suitable broodstocks may influence the quality and quantity of obtained eggs and larva. The present study aimed to examine correlation between some morphometric and reproductive parameters to find suitable brood fish for artificial reproduction in Russian sturgeon, *Acipenser gueldenstaedtii*. Forty fish free of any external disease symptom and abnormality were selected for the study. Following biometric measurements including weight, total length, abdominal girth, PV (distance between pectoral and pelvic fins), LX (distance between anal and caudal fins), polarization index (PI), gonadosomatic index, absolute fecundity, and fertilization rate, correlations between these morphometric and biological characteristics with emphasis on breeding were calculated. There was higher correlation between weight-absolute fecundity and length-PV in fish responded to artificial breeding, while correlation between age-PV, length-PV and weight-abdominal girth were higher in those fish not responded to artificial breeding. The results suggests that it is quite possible to select suitable Russian sturgeon spawners for artificial propagation based on combination of body weight, LX, PV, age, abdominal girth and total length, however the most useful criteria for the selection seems to be precise measurement of the polarization index.

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Introduction

The Caspian Sea is the most important habitat and home for five sturgeon species including Beluga (*Huso huso*), Ship (*Acipenser nudiventris*), Stellate (*A. stellatus*), Persian (*A. persicus*) and Russian sturgeon (*A. gueldenstaedtii*). Over-fishing, habitat and spawning ground degradation along with environmental pollution have caused these species to be included in the IUCN Red List of endangered and consequently in CITES appendices (IUCN, 2012). Several governments and organizations have considered conservation and rehabilitation of sturgeon stocks and various restoration programs, e.g., allocation of catch quota on the base of stock assessment programs, restoration of natural habitats and spawning grounds and developing artificial breeding strategies. Artificial propagation is a regular practice to produce and release sturgeon fingerlings into the Caspian Sea (Falahatkar et al., 2011).

Although Russian sturgeon is important for meat and caviar production, nevertheless its artificial propagation in the southern Caspian Sea basin has dramatically been hampered by lack of suitable spawners. In addition, large numbers of spawners do not respond efficiently to artificial induction which leads to failure of propagation processes. Ovulation in fish sometimes is unpredictable and failed because of state of gonad development and handling stress during transportation and maintenance in hatchery (Schreck, 2010; Williot et al., 2011). Due to high fecundity and survival rate of the obtained larvae in

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Russian sturgeon, precise determination of gonad developmental stage will lead to an increase in induced ovulation and ultimate enhancement in hatchery production (Hurvitz et al., 2008). Several studies have revealed that sex steroids are appropriate components for determination of maturity stages of brood fish (Ceapa et al., 2002; Webb et al., 2002; Alberto et al., 2008; Davail-Cuisset et al., 2008; Petochi et al., 2011). Since blood sampling is considered as an invasive technique to determine sex and maturity in sturgeon (Wildhaber et al., 2007), introduction of a method to enables us effectively monitor gonad development in spawners is crucial for field and hatchery practices (Falahatkar et al., 2011).

Morphometric parameters of ovarian follicles have been used for selection of suitable broodstock in artificial breeding (See Goncharov et al., 1999). Although this method is not as precise as physiological methods, however it might be very useful where laboratory facilities are not available. The most potent way to assess the reproductive potential of female sturgeon prior to hormonal injection is determining the polarization index and in vitro maturation competence of ovarian follicles (Lutes et al., 1987; Williot et al., 1991). In present study, several morphometrical and sexual indexes were examined to highlight correlations between those parameters. This helps us to determine the reliable parameters to predict ovulation to select suitable spawners in final stage of maturation.

Materials and Methods

Fish: Forty Russian sturgeons (27 females and 13 males) were caught at sturgeon fishing stations (Turkmen and Miankaleh Bay) by gill-net in the southern Caspian Sea basin during the winter season and transported (1.5 h) by a truck equipped with aeration to the Shahid Mardjani Sturgeon Hatchery Center in Golestan Province, Iran. The weight, length and age range of females were 10-39 kg, 112.5-167 cm, and 11-18 years old, respectively and were in range of 10-22 kg, 122-153.5 cm, and 10-15 years old, respectively, for males.

Maintenance and artificial breeding: Fish were kept under natural fluctuations over winter temperature and photoperiod. The collected fish were kept in broodstocks maintenance facilities in concrete ponds prior to oocyte examination from 2-6 days according to their fishing date. During the appropriate water temperature (March) for artificial breeding of Russian sturgeon, oocyte samples were taken by a special probe (Chebanov and Galich, 2011) to determine polarity index. Approximately, 10-15 eggs were sampled and put into 4% formaldehyde solution for later examination. For this purpose, eggs were put in boiled water for 2-3 minutes. Delicate sections were made across the animal to vegetal poles and polarization index (PI) was determined according to Dettlaff et al. (1993). The core idea is to estimate the stage of germinal vesicle migration under a dissecting microscope.

Based on the obtained PI, 10 broodstocks were suitable for hormonal induction and underwent hormonal treatment using common carp pituitary extract (males 40-60 mg; females 50-70 mg). Females were received intramuscular injection of two separate doses (10% as priming and 90% as resolving doses) in 12 hours interval (Pourasadi et al., 2009; Falahatkar and Efatpanah, 2011) at 14.5-15.5°C. Ovulation occurred within 22-26 h of resolving injection. All 13 caught males were received a single injection and spermiation occurred within 18-20 h. Sperm from 5 males were used for fertilization. Oocytes were collected traditionally by opening the abdomen (Pourasadi et al., 2008). Eggs were fertilized using 10 ml of milt diluted to 1:100 with addition of hatchery water. Following 5 min of fertilization, eggs were mixed with 1:10 clay suspension and softly stirred for 40 min until they were no longer sticky (Pourasadi et al., 2008). Eggs were then washed and transferred to Youshchenko incubator.

Morphometric and sex parameters: Fish weight (kg ± 50 g), total length (cm ± 1 mm), condition factor (CF = weight/total length^3 × 100), abdominal girth (cm ± 1 mm), PV (the distance between pectoral fin to pelvic fin; cm ± 1 mm), LX (the distance between

Table 1. Correlation (r) of measured parameters in Russian sturgeon broodstocks responded to hormonal injection (n = 10).

	Fertilization rate	PI	Absolute fecundity	GSI	Abdominal girth	LX	PV	Age	CF	Total length
Weight	-0.188	0.409	0.874**	-0.205	0.223	0.656*	0.619	0.415	0.623	0.647*
Total length	0.536	0.740*	0.555	-0.477	-0.123	0.830**	0.907**	0.803**	-0.186	
CF	0.168	-0.160	0.584	0.279	0.442	-0.018	-0.119	-0.288		
Age	-0.222	0.634*	0.229	-0.612	-0.364	0.680*	0.721*			
PV	-0.187	0.605	0.441	-0.447	0.112	0.840**				
LX	-0.249	0.511	0.497	-0.503	-0.129					
Abdominal girth	-0.170	-0.135	0.313	0.546						
GSI	0.007	0.024	0.200							
Absolute fecundity	-0.375	0.573								
PI	-0.299									

PI: polarization index; GSI: gonadosomatic index; LX: the distance anal fin to caudal fin; PV: the distance between pectoral fin up to the pelvic fin; CF: condition factor. * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

Table 2. Correlation (r) of measured parameters of Russian sturgeon broodstocks not responded to hormonal injection (n = 17).

	PI	Abdominal girth	LX	PV	Age	CF	Total length
Weight	0.298	0.933**	0.697**	0.885**	0.905**	0.501*	0.846**
Total length	0.111	0.665**	0.774**	0.953**	0.918**	-0.013	
CF	0.539	0.696**	0.023	0.114	0.190		
Age	0.121	0.783**	0.780**	0.939**			
PV	0.124	0.732**	0.726**				
LX	0.029	0.542*					
Abdominal girth	0.473						

PI: polarization index; LX: the distance between the anal fin the caudal fin; PV: the distance the pectoral fin to pelvic fin; CF: condition factor. * Correlation is significant at the 0.05 level; ** Correlation is significant at the 0.01 level.

anal fin to caudal fin; cm ± 1 mm), ovary weight (kg ± 10 g), gonadosomatic index (GSI = gonad weight/body weight × 100) and age were determined for all broodstocks. Weight of 100 oocytes and absolute fecundity (number of oocyte/fish) were determined in broodstocks responded to hormonal injection. For estimation of fish age, the first pectoral fin ray was sampled. A thin slide of 0.5-1 mm thickness was prepared and after putting into the glycerin solution for 24 h, the cross-section detail was studied using stereoscope (Hughes et al., 2005). Absolute fecundity was determined by below formula:

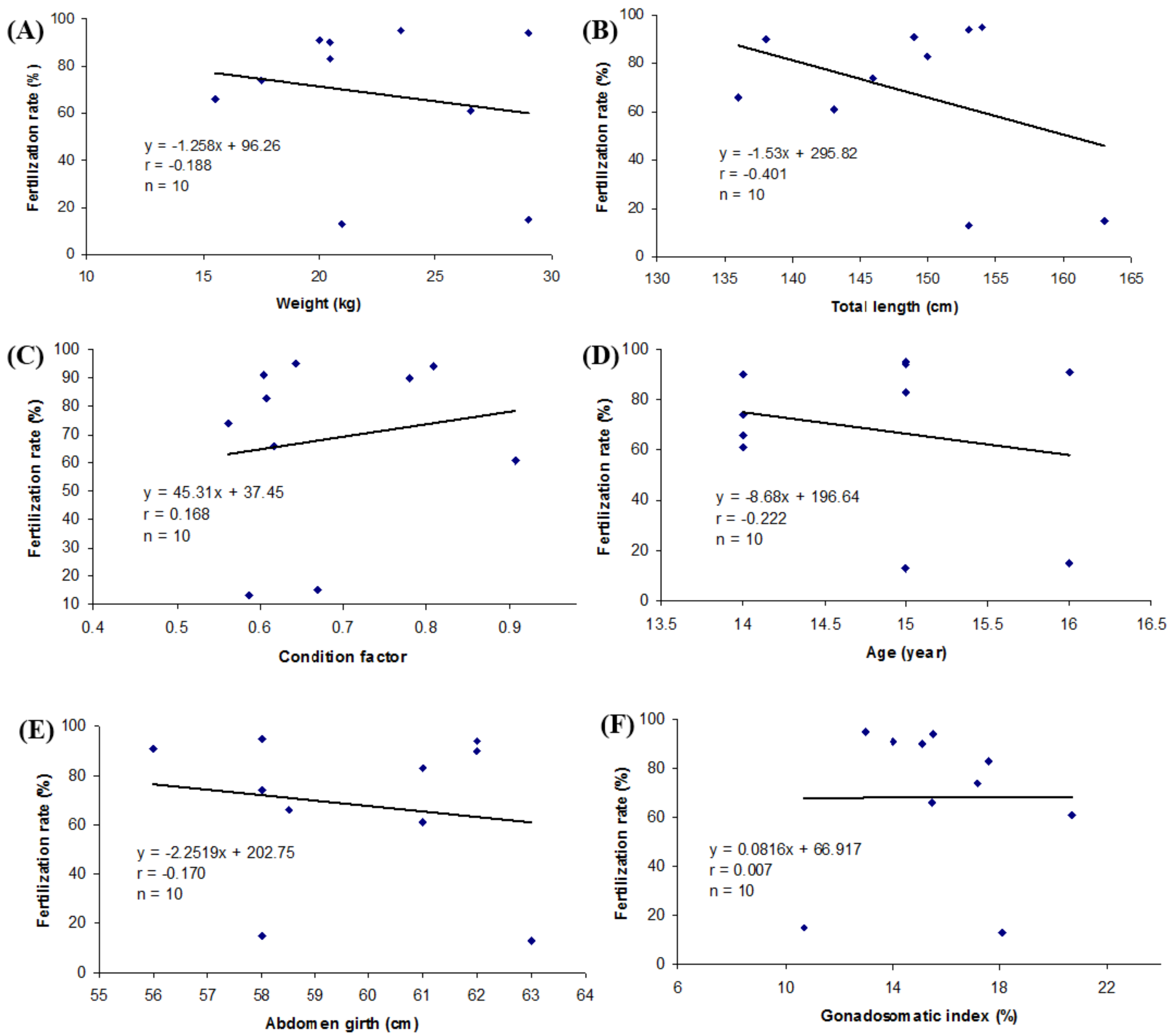
Absolute fecundity = Number of eggs per gram × total weight (g) of ovaries (eggs)

After transferring the fertilized eggs to the incubators, the overall fertilization rate at blastula stage was recorded with collecting 200-300 eggs and counting live and dead eggs.

Statistical analyses: Data were analyzed using SPSS (version 12; Chicago, IL) to examine correlation between parameters and success of artificial reproduction. Normality of data and homogeneity of variances were tested by Kolmogorov-Smirnov and Levene's tests, respectively. The independent samples t-test was used to compare fish responded to hormonal treatment and those indifferent to hormonal treatment at 95% confidence level.

Results

Correlations of measured parameters in responsive and non-responsive broodstocks to hormonal treatment are presented in Tables 1 and 2. There were no significant correlation between fertilization rate and any measured indexes in responsive females. Regarding PI, a significant correlation was found only with age ($r = 0.634$, $P = 0.049$). Significant correlation was also observed between weight and absolute fecundity ($r = 0.874$, $P = 0.001$), length and PI ($r = 0.740$, $P = 0.014$), egg weight and fish length ($r = -0.782$, $P = 0.07$), egg weight and age ($r = -0.685$, $P = 0.029$), PV and LX with age ($r = 0.721$, $P = 0.019$; $r = 0.680$, $P = 0.031$, respectively) in broodstocks responded to artificial breeding.



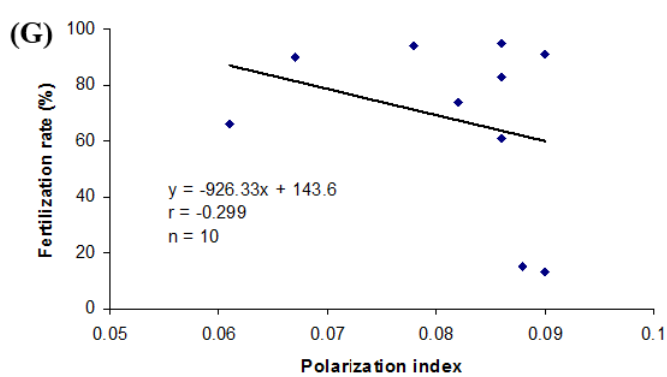


Figure 1. Regression of fertilization rate with weight (A), total length (B), condition factor (C), age (D), abdomen girth (E), gonadosomatic index (F) and polarization index (G) of Russian sturgeon broodstocks (n = 10) in southern basin of the Caspian Sea which responded to hormone injection.

In non-responsive females to hormonal treatment no correlation was observed between PI and any measured parameters, while significant correlations were observed between age and PV (r = 0.939, P<0.000), length and PV (r = 0.953, P<0.000), age and LX (r = 0.780, P<0.000) and weight and abdomen girth (r = 0.933, P<0.000).

Mean weight of responsive spawners (10 specimens) to artificial breeding was 22 ± 4.6 kg and those non-responsive (17 specimens) was 22.1 ± 7.5 kg. There were no significant differences between these two groups (P>0.05). No significant difference was

Table 3. Some measured indices (mean ± SD) in broodstocks responded (n = 10) and not responded (n = 17) to hormonal injection. Asterisks show significant differences between the two means (P<0.05).

	Weight (kg)	Total length (cm)	Condition factor	PV (cm)	LX (cm)	Age (year)	PI	GSI (%)	Abdomen girth (cm)
Responsive	22.3 ± 4.6	148.5 ± 8.1	0.7 ± 0.1	58.5 ± 3.2	28.2 ± 2.2	14.8 ± 0.8	8.1 ± 1.0*	15.7 ± 2.8*	59.3 ± 2.9
Non-responsive	22.1 ± 7.5	144.3 ± 14.3	0.7 ± 0.1	56.4 ± 6.4	27.5 ± 3.0	14.5 ± 1.7	12.5 ± 6.2	22.1 ± 6.5	60.9 ± 8.0

PV: the distance between pectoral fin to pelvic fin; LX: the distance between the anal fin up to the caudal fin; PI: polarization index; GSI: gonadosomatic index.

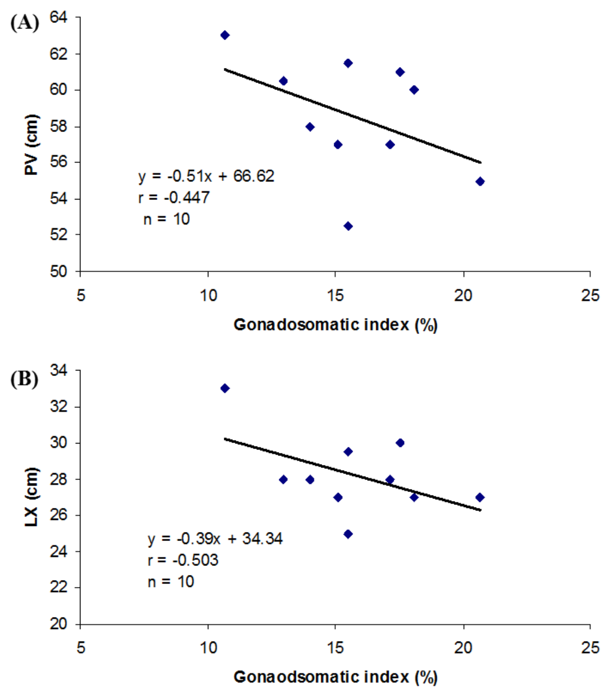


Figure 2. Regression of PV (A) and LX (B) with gonadosomatic index (%) of Russian sturgeon broodstocks (n = 10) in southern basin of the Caspian Sea which responded to hormone injection.

noticed between total length, CF, PV, LX, abdomen girth and age (P>0.05), while PI and GSI were significantly different in two groups (P<0.05; Table 3).

Figure 1 (A-G) shows correlation of some parameters in fish responded to artificial breeding with fertilization rate. There was no positive correlation in any measured parameters except for fertilization rate and CF (Fig. 1A). Negative correlation also was found between PV (Fig. 2A; r = -0.447) and LX (Fig. 2B; r = -0.503) with GSI.

Discussion

This is important to find a simple approach to select sturgeon breeders for artificial propagation and present study showed some relations but not powerful to examine the suitable broodstocks. It is clear that the age and body size of fish are closely related and when the effect of size is eliminated, the effect of age on fecundity may be small or inconsistent (Wootton, 1991), which is in correlation with our findings. Colombo et al. (2007) and Tripp (2007) reported a significant positive relationship between fecundity and weight of the shovelnose sturgeon (*Scaphirhynchus platorynchus*) harvested from middle of Mississippi River. Fecundity and both fork length and wet weight in upper Wabash River female shovelnose sturgeon (Kennedy et al., 2006) demonstrated positive relationships > 69%. Positive relationships between fecundity and fork length were observed in Atlantic sturgeon (*A. oxyrinchus*) in more than 70% and in the lower Mississippi River over 40% of female sturgeons (Van Eenennaam et al., 1996; Van Eenennaam and Doroshov, 1998; Wildhaber et al., 2006). Also, some positive relationships were observed in Siberian sturgeon (*A. baeri*) breeders for selection of suitable fish (Williot et al., 1991). Žikov and Petrova (1993) reported a significant relationship between absolute fecundity with body length, age and weight in pikeperch (*Stizostedion lucioperca*). Similar results were observed on absolute fecundity and body weight of Russian sturgeon in present study. These results show that fecundity is influenced by the egg size, species, season and environmental conditions. Fecundity varies among populations of the same species and also differs from one year to another. A key feature with fecundity is that it increases (with certain exceptions) in accordance with fish growth. A large fish lays more eggs than a small one and the correlation between fecundity and weight is higher than the correlation between length and age

(Wootton, 1991).

Information on gonadal development in relation to fish weight and age is essential tool to manage sturgeon culture activities and caviar production (Hurvitz et al., 2008). Van Eenennaam and Doroshov (1998) demonstrated a relationship between individual fecundity with fork length and age in mature females of Atlantic sturgeon (*A. oxyrinchus*). In present study, these correlations were r = 0.555 and r = 0.229, respectively.

The results showed a very high correlation between absolute fecundity and fish weight (r = 0.874). Smith and Baker (2005) also demonstrated a relationship between weight and length for female Lake sturgeon (*A. fulvescens*) spawners (r = 0.776). There was no significant relationship between body size of white sturgeon (*Acipenser transmontanus*) broodstock and ovulation success in study conducted by Lutes et al. (1987). We observed a positive correlation between weight and total length and also between condition factor and fish age. This could be interpreted that increase in length does not necessarily coincide with increase in weight (Fawole, 2002).

In this study, we found a small negative regression of polarization index with fertilization rate, while the value was significantly different between responded and not-responded females. It seems that germinal vesicle (GV) position is one of the best parameters for selection of brood fish in spawning season and even transferring fish from wild to hatcheries. Former studies showed that GV position is a key morphological parameter for general discrimination of potentially responsive females as suggested by Lutes et al. (1987) and Williot et al. (1991). In white sturgeon, GV position and in vitro oocyte maturation showed high relationships with ovulation. There were also significant correlations between ovulation success and germinal vesicle breakdown, oocyte diameter and plasma concentration of 17α, 20β-dihydoxy progesterone (Lutes et al., 1987). It seems that the best way to test the ovarian follicles near to spawning is in vitro study (Mojazi Amiri et al., 2001; Williot et al., 2009). Also, a study on Persian sturgeon (*A. persicus*) revealed that the calcium content of the oocytes are positively correlated with the polarization index (Rafiee and Hajrezaee, 2011). Therefore, it is appropriate that to identify the possibility of some methods for pre-selection or for first selection of genitors for the reproduction based on morphometrical parameters; final selection (fish with hormonal treatment) being based on microscopical observation of oocytes (PI, oocyte size) and bio-tests.

Owing to deterioration of suitable spawning grounds for sturgeons in the rivers of the southern Caspian Sea, most of the broodstocks used for artificial breeding are caught at the Sea. Since these fish are at earlier stages of maturity, or even between 2 spawning seasons, they do not respond duly to hormonal treatments. Some 33-55% of Persian sturgeons caught and transported to the hatcheries does not respond efficiently to hormone injection and discarded from artificial breeding cycle (Azari Takami et al., 1997).

A major problem with artificial breeding of sturgeons in comparison to bony fishes is that the bony fishes have annual reproduction cycle, while this cycle in sturgeons depending upon the slow gonads development, environmental conditions, feed availability and some other parameters occurs in 2-5 year intervals. Therefore, one may not guarantee the success of artificial breeding based on age and other parameters since fish may appear mature with appropriate length and weight in fact in the period between two consecutive spawning. Consequently, majority of the results obtained in teleost species are not compatible with sturgeons.

In conclusion, considering the facts mentioned above, selection of spawners based on morphometric parameters e.g., tracking the position of nucleus and other measurements and indices are practical methods to select suitable broodstocks for artificial propagation and discard unsuitable ones for caviar production. Our study demonstrated that it is quite possible to select suitable broodstocks for artificial propagation based on combination of weight, LX, PV, age, abdomen girth and length, but the most practical tools for this selection which can be consider is polarization index. Undoubtedly physiological parameters like sex steroids and corticosteroids measurements should also be given special priority for selection of sturgeon spawners with regards to their fragile stock condition which leaves no place for try and error. Based on previous findings, our results confirmed that examination of GV position and some in vitro studies on oocyte maturation response may be adequate tools for selection of Russian sturgeon females in artificial propagation. Detailed studies are required to combine practical approaches and sophisticated physiological and biochemical methods for better selection of broodstock to secure successful breeding programs.

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