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The effects of different times of reproductive migration on biochemical compounds of ovarian fluid and on fertilization rate of Persian sturgeon (*Acipenser persicus*) brood stocks

Milad Kabir, Mohamad R. Imanpoor, and Masoud Asghari

Department of Fisheries, Gorgan University of Agriculture Sciences and Natural Resources, Gorgan, Iran. Corresponding author: M. Kabir, mld.kabir.17@gmail.com

Abstract. In this study we investigated the effects of different times of reproductive migration on biochemical compounds fluctuation (calcium, magnesium, glucose, cholesterol, total protein, sodium and potassium) and on the percentage of haematocrit of coelomic fluid and on the fertilization rate of 14 Persian sturgeon brood stocks, during the spawning season (in March of 2008 until May of 2008). For this purpose, the period of sampling was divided to three groups, beginning (March), middle (April) and end (May) of spawning season of these fishes. The average of fork length and total weight of fishes before extraction of eggs of Persian sturgeon brood stocks in treatments 1, 2 and 3 were 166.5 ± 6.36, 156.71 ± 8.69 and 174.5 ± 2.12 cm, also 33.0 ± 1.41, 30.1 ± 7.19 and 34.5 ± 0.70 kg, respectively. On the other hand, the average of percentage of fertilization rate in treatments 1, 2 and 3 were respectively 166.5 ± 6.36, 156.71 ± 8.69 and 174.5 ± 2.12 cm, 33.0 ± 1.41, 30.1 ± 7.19 and 34.5 ± 0.70 kg , 67.5 ± 6.36, 68.3 ± 17.86 and 49.5 ± 3.53 percent. Our results showed that there were significant differences in calcium (5.41±0.51 - 1.65±0.03 and 11.99±1.41 mg dL^{-1}), glucose (37.99±15.10 - 23.61±6.45 and 11.3±3.26 mg dL^{-1}), cholesterol (15.55±3.79 - 8.26±2.25 and 10.33±1.88 mg dL^{-1}) and sodium (185.94±38.86 - 103.98±31.78 and 180.75±6.47 mM L^{-1}) between these treatments ($P<0.05$) but in the percentage of fertilization rate, haematocrit and other calculated biochemical compounds, there were no significant differences ($P>0.05$). Overall, significant and insignificant fluctuations in coelomic fluid in spawning season of these fishes demonstrated that ovarian fluid in treatments number 1 (March) and 3 (May) had better characteristics for sperm mobility compared to treatments 2 (April).

Key Words: Different times of reproductive migration, biochemical compounds, coelomic fluid, Persian sturgeon.

خلاصه. در طی این تحقیق به بررسی اثرات زمان مهاجرت مولدین قره برون روی تغییرات بیوشیمیایی (کلسیم، منیزیم، گلوکز، کلسترول، پروتئین کل، سدیم و پتاسیم) و درصد لقاح مایع تخمدانی 14 مولد قره برون در طول فصل تکثیر (اسفند 1387 الی خرداد 1388) پرداخته شد. برای این منظور این بازه زمانی به 3 تیمار شامل ابتدای دوره (اسفند)، میانه دوره (فروردین) و انتهای دوره (اردیبهشت) تقسیم شد. میانگین طول چنگالی و وزن کل قبل از استحصال تخم قبل از استحصال تخم در مولدین مادهٔ قره برون در تیمارهای 1، 2 و 3 به ترتیب 166/5±6/36، 156/71±8/69 و 174/5±2/12 سانتی متر، 33±1/41، 30/14±7/19 و 34/5±0/70 کیلوگرم بود. از طرف دیگر، میانگین درصد لقاح در تیمارهای 1، 2 و 3 67/5±6/36، 68/28±17/86 و 49/5±3/53 درصد بود.
نتایج تحقیقات ما نشان داد که اختلاف معنی داری مابین کلسیم (5/41±0/51 – 1/65±0/03 و 11/99±1/41 mg dl^{-1})، گلوکز (37/99±15/10 – 23/61±6/45 و 11/3±3/26 mg dl^{-1})، کلسترول (15/55±3/79 – 8/26±2/25 و 10/33±1/88 mg dl^{-1}) و سدیم (185/94±38/86 – 103/98±31/78 و 180/75±6/47 mM Lit^{-1}) در تیمارهای ما وجود دارد ($P<0.05$) اما مابین درصد لقاح، هماتوکریت و سایر ترکیبات شیمیایی محاسبه شده اختلاف معنی داری مشاهده نشد ($P>0.05$). روی هم رفته، نوسانات معنی دار و غیر معنی دار مایع سلومیک در طول فصل تکثیر این ماهیان نشان داد که مایع تخمدانی در تیمارهای شماره 1(اسفند) و 3 (اردیبهشت) ویژگی های بهتری را برای تحرک اسپرم در مقایسه با تیمار 2 (فروردین) دارا می باشند.
کلمات کلیدی: زمانهای مختلف مهاجرت تولید مثلی، ترکیبات بویشیمیایی، مایع سلومیک، قره برون

Introduction. Most of sturgeons of the world (92-90%) are extracted from Caspian Sea (Deetlaff et al 1993). Persian sturgeons (*Acipenser persicus* Borodin, 1897) are anadromous fishes, belonging to Acipenseridae family that spread widely in the southern part of Iranian coastal waters of Caspian Sea. During spawning season, males and females are extracted from the river (Kohneshahri & Azari Takami 1974). Breeding and growth of Sturgeon in Iran are in research and development phases (Alavi et al 2002).

The gametes of external fertilized fishes, such as sturgeons, are released simultaneously into fresh or salt water, during spawning. This potential hostile aquatic environment induces physiological changes which result in the activation of both sperm mobility and unfertilized ovum (Jamieson 1991). Due to limited time of sperm mobility and also due to closing micro pill, fertilization ability of ovums decreased to less than a minute (Billard 1986). Hence, ovums were maintained in a cavity named coelomic cavity and were washed with a liquid with the same viscosity which is called ovarian, coelomic or peritoneal fluid, to prevent the activation of ovums in the natural environment. The osmolality of the coelomic fluid may prevent ovum activation while ovums are stored in the peritoneum (Billard & Jalabert 1974, Billard 1988). Coelomic fluid may also help ovums of sturgeons to be fully matured before going out of the mother's body (Ginzburg 1968). For example, in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792), fertilization rate increased from 88% to 100% immediately following ovulation, in other word after ovums had been bathed in coelomic fluid in the peritoneal cavity for 4–6 days (Springate et al 1984). Ovarian fluid is a maternally derived liquid that surrounds the egg mass inside the female fish and is expelled during spawning (Turner & Montgomerie 2002). Ovarian fluids can keep the eggs fertilized for more than 10 minutes (Lahnsteiner 2002). The ovarian fluid which surrounds the eggs during spawning may influence different fertilization aspects, such as sperm mobility characteristics (Lahnsteiner 2002) or sperm-egg recognition (Amanze & Iyengar 1990). This important fact that brood stocks can successfully fertilize all of eggs will cause consuming a lot of energy by them to produce eggs and ovarian fluids.

Several studies have been done on the diversity of compounds of ovarian fluid and these differences are associated with physiological conditions of female brood stocks, quality and maturity of eggs (Lahnsteiner et al 1999, Lahnsteiner 2000).

There are numerous studies which observed chemical composition of seminal fluid during spawning season (Rideout et al 2004; Cruz-Casallas et al 2007; Rosengrave et al 2009) but information on the composition of ovarian fluid is limited. Lahnsteiner et al (1995) and Wojtczak et al (2007) reported that ovarian fluid compositions during spawning season are different between brood stocks. Aegerter & Jalabert (2004) have studied the changes in osmolality, pH and protein concentration of ovarian fluid in spawning season of rainbow trout; such variations may cause changes in physiological conditions during the spawning season. On the other hand, changes in the maturity and quality of eggs can affect on ovarian fluid composition (Lahnsteiner et al 1999; Lahnsteiner 2000; Aegerter & Jalabert 2004). Such changes in the quality of ovarian fluid may influence the sperm mobility, fertilization success and perhaps the survival and growth of larva.

Some factors in the ovarian fluid protect the spermatozoa (Billard 1983), such as the presence of proteins or carbohydrates in the ovarian fluid (Yoshida & Nomura 1972; Lahnsteiner 2002). Turner & Montgomerie (2002) suggested that some components of ovarian fluid can influence ATP metabolism and, hence, increase sperm longevity and swimming speed. The enhancement of sperm mobility and its viability in ovarian fluid has been mainly attributed to the coelomic fluid ionic balance (Scott & Baynes 1980). Ions of ovarian fluid, especially sodium can significantly reduce osmotic shock to spermatozoa (Wojtczak et al 2007).

Ovarian fluid can have a selective effect on sperm if its effects on sperm behavior differ among competing males (Turner & Montgomerie 2002; Urbach et al 2005). However, comprehensive information on many aspects of ovarian fluid quality in sturgeons is limited. In this study, we determined ionic (calcium, magnesium, sodium and potassium) and biochemical (total protein, glucose and cholesterol) composition of ovarian fluid during different times of reproductive migration in Persian sturgeon brood stocks.

Material and Method. 7 Samples of coelomic fluid (2 fishes in the first treatment, 3 fishes in the second treatment and 2 fishes in the third treatment) were collected from Persian sturgeon brood stocks from March to May, 2008, in Shahid Marjani Center of

Breeding and Growth of Sturgeon, Gorgan, Iran. Samples were experimented in three treatments: beginning (March), middle (April) and end (May) of reproduction season. Brood stocks were kept in Kourenski ponds with 14-17°C and 8.2 mg O_2. The time of hormone injection was calculated on the basis of germinal vesicle (GV) and sexual maturity index (Deetlaff et al 1993), according to the method of Van Eenennam et al (2001), depending on water temperature and the fish weight (Billard 2000).

Approximately 24 hours after the first injection, spawning of female brood stocks has started. Then they were killed by striking their heads. After that brood stocks were dried and cleaned with a towel and blood samples were taken with cutting gills, the abdomens area were incised, the eggs were extracted and then separated from ovarian fluid by a net with tiny meshes and this fluid was purified and collected.

After that, coelomic fluids were put into 1.5 mL micro tubes, with the least upper atmosphere air space to minimize their air exchanges. Then a flask containing ice and samples was transferred to the Central Laboratory of Agricultural Sciences and Natural Resources at University of Gorgan for future analysis.

It should be noted that the percentage of fertilization rate as well as the proliferation of brood stocks were measured in this stage.

To evaluate the percentage of haematocrit, after centrifuging the tubes containing blood of fishes in 3000 rpm for eight minutes, haematocrit reader was used (WPA Light Wave-Diode-array S2000 UV/Vis) to determine the percentage of haematocrit (Fitzpatrick et al 2005). In order to determine the biochemical index of coelomic fluid, bloods of brood stocks were located into 1.5 ml micro tubes and tubes were centrifuged in the 13.000 rounds for 5 minutes and then they were transferred to new vials. Vials of coelomic fluid were stored at -20°C for future study on biochemical compounds. Since cell membrane can polarize the ions in seminal fluid and stimulate sperm mobility (Morisawa et al 1999), we measured sodium and potassium ions concentrations by flam photometer (Jenway pfp England) and also the concentrations of calcium, magnesium ions, glucose, total protein and cholesterol by the spectrophotometer (S200-UV.VIS England) in seminal and coelomic fluid, using quantitative biochemical kits of serum or plasma parameters (Turker et al 2004).

Statistical analysis of data was done in triplicate, by One-Way ANOVA with Duncan test at the level of 95%, using SPSS 16. Statistical significance was set at the level of $P<0.05$ with ± standard deviation (SD).

Results and Discussion. Average of fork length and total weight of the female brood stocks before eggs extraction in treatments 1, 2 and 3 were respectively 166.5 ± 6.36, 156.71 ± 8.69 and 174.5 ± 2.12 cm, 33.0 ± 1.41, 30.1 ± 7.19 and 34.5 ± 0.70 kg. Averages of fertilization rate were respectively 67.5 ± 6.36, 68.28 ± 17.86 and 49.5 ± 3.53 (Table 1).

Table 1

The average of fork length, total weight and fertilization rate of Persian sturgeon brood stocks among different reproductive migration (means ± S.D)

Experimental groups	*Time of migration (month)*	*Total weight (kg)*	*Fork length (cm)*	*Fertilization rate (%)*
1	March	33.00±1.41	166.5±3.36	67.50±7.09
2	April	30.14±7.19	156.7±5.69	68.28±17.86
3	May	34.5±3.53	174.5±2.12	49.5±3.53

Results of Table 1 show that there were no significant differences between different reproductive migrations in fork length, total weight and fertilization rates of *A. persicus* brood stocks ($P>0.05$).

The averages of the percentage of haematocrite in these brood stocks in treatments 1, 2 and 3 were respectively 29.22 ± 4.14, 24.15 ± 5.87 and 21.56 ± 3.47.

These differences were not significant (P>0.05). Also, the averages of fertilization rates of brood stocks through the spawning season were not significantly different (P>0.05).

Analysis of some biochemical parameters of coelomic fluid at the beginning, middle and final periods of migration in Persian sturgeons is expressed in Table 2.

Table 2

Concentrations principal inorganic ions and biochemical compositions of the ovarian fluids of Persian sturgeon brood stocks among different reproductive migration (means ± S.D)

Experimental groups	*Glucose mg dL^{-1}*	*Cholesterol mg dL^{-1}*	*Total protein g dL^{-1}*	*Magnesium mg dL^{-1}*	*Calcium mg dL^{-1}*	*Sodium mM L^{-1}*	*Potassium mM L^{-1}*
1	37.99± 15.10^{a}	15.55± 3.79^{a}	0.50± 0.25	1.47± 0.83	5.41± 0.51^{b}	185.94± 38.86^{a}	3.52± 1.61
2	23.61± 6.45^{ab}	8.26± 2.25^{b}	0.41± 0.15	0.85± 0.11	1.65± 0.03^{c}	103.98± 31.78^{b}	5.35± 0.05
3	11.3± 3.26^{b}	10.33± 1.88^{ab}	0.64± 0.00	1.03± 0.59	11.99± .41^{a}	180.75± 6.47^{a}	4.28± 0.10

Different letters (a, b and c) indicate significant differences between groups (P<0.05).

Results of Table 2 indicated that during spawning season there were significant differences between coelomic fluids of female Persian sturgeons in calcium, glucose, cholesterol and sodium (P<0.05).

Since in most fish species the ovarian cavity is placed after the oviduct, mature oocytes that have been released out of the follicles discharge into the ovarian cavity (Nagahama 1983). Ovarian fluid is formed by filtered blood plasma and secreted actively from ovary epithelia (Hirano et al 1978). The pH of the fluid ranged from 8.4 to 8.8 and it contains sodium, potassium and calcium ions, glucose, fructose, cholesterol, phospholipids, proteins and free amino acids (Lahnsteiner et al 1995). The origin of this fluid and its components is quite unknown. However, some components of the fluid are believed to be derived from the blood (Matsubara et al 1985) and therefore these components could be produced and secreted into the blood by the ovary as well as by other tissues.

In those fishes were activated in coelomic fluide swimming speed of the motile sperm and long-time direct movement of spermatozoa increased, compared to the other groups activated in fresh water, salt water or buffer solution [(arctic charr (*Salvelinus alpinus alpinus* (Linnaeus, 1758)) - Turner & Montgomery (2002), Urbach et al (2005); Atlantic cod (*Gadus morhua* Linnaeus, 1758) - Litvak & Trippel (1988); rainbow trout - Rosengrave et al (2008)]. Continuity of sperms lives and the increase in sperm mobility by ovarian fluid is attributed to ionic balance of ovarian fluid (Scott & Baynes 1980) and according to researches by Rime et al (2004), long term remaining of eggs in the body are effects on ovarian fluid composition. Unlike above results, Elofsson et al (2003) have investigated some effects of coelomic fluid on spermatozoa motion of *Spinachia spinachia* (Linnaeus, 1758). In this species, coelomic fluid in saline solution had no effect on the spermatozoa motion.

According to the research by Scott & Vermeirssen (1994), ovarian fluid has pheromone activity which causes interactions to stimulate oviparous fishes. Rosengrave et al (2008) suggest that one or more components of a female's ovarian fluid differentlly influence the males' sperm behavior.

Carbonate and bicarbonate were the principal buffer ions in ovarian fluid. Viscosity of ovarian fluid is more than water viscosity (Rosengrave et al 2009). High viscosity would maintain high ionic concentrations close to the egg surface. Additionally, it would provide low shear, laminar flow conditions adjacent to the eggs, which have been found to be necessary for successful fertilization in another external fertilizer, the Red Abalone (*Haliotis rufescens* Swainson, 1822) (Riffell & Zimmer 2007).

Many studies have been made on compounds of seminal fluid in several species (Jamieson 1991; Alavi & Cosson 2006) but information about composition of ovarian fluid is limited (Hirano et al 1978; Lahnsteiner et al 1995; Wojtchzak et al 2007). There is no basic analysis of the ovarian fluid composition and the possible inter specific differences. Van Heerde et al (1993) have analyzed the organic and inorganic compounds of ovarian fluid in *O. mykiss*. On the other hand, postovulatory keeping of the eggs in the body cavity can affect on the composition of the ovarian fluid (Rime et al 2004). Data on ovarian fluid composition could provide the basis for further study on the development of artificial extending for storage and conservation of eggs or for quality control of ovum.

The present study analyzed ovarian fluid compositions in females of Persian sturgeon, because there is little information available about the ovarian fluid compositions in sturgeons (especially in Persian sturgeon) and most of studies have been done on females of salmonids family. We compared our results with other studies results on salmonids fish. According to our findings the ranges of concentration of ovarian fluid in Persian sturgeons during the spawning season were as following: sodium 72-224 mM L^{-1}, potassium 1.61-5.53 mM L^{-1}, calcium 1.62-12.7 mM L^{-1}, magnesium 0.64-2.31 mM L^{-1}, total protein 0.25-0.76 g/dL, cholesterol 6.01-19.35 mg dL^{-1}, and glucose 53.1-8.04 mM L^{-1}. Our results show that there are significant differences between coelomic fluids of females of Persian sturgeons, through these periods, in calcium, glucose, cholesterol and sodium ($P<0.05$). We found that that findings on the composition of the coelomic fluid of *A. persicus*, in terms of a number of compounds, is similar to findings of Hatef et al (2009) who investigated the composition of ovarian fluid in Caspian brown trout (*Salmo trutta caspius* Kessler, 1877). According to their findings the ovarian fluid has sodium 164.4 ± 4.4 mM L^{-1}, potassium 1.8 ± 0.1 mM L^{-1}, calcium 0.6 ± 0.1 mM L^{-1}, magnesium 0.4 ± 0.02 mM L^{-1}, chloride 127.4 ± 5.9 mM L^{-1}, total protein 389.5 ± 89.6 mg 100 mL^{-1}, cholesterol 9.3 ± 1.2 mg dL^{-1}, and glucose 3.3 ± 0.2 mM L^{-1}.

We noticed significant differences in the ionic composition of *A. persicus* ovarian fluid collected at different periods of the spawning season. Such seasonal variation could cause changes in a female's physiological status during the spawning season, along with changes in egg quality and maturity. All these will affect on the composition of the ovarian fluid (Lahnsteiner et al 1999; Lahnsteiner 2000; Aegerter & Jalabert 2004). But Taati et al (2010) observed that ionic (Na^+, K^+, Cl^-, Ca^{+2} and Mg^{+2}) and biochemical (total protein, glucose) compositions of ovarian fluid did not change during spawning period, whereas Lahnsteiner et al (1995) reported ovarian fluid compositions (Na^+, K^+, Ca^{+2} and glucose) of four species including rainbow trout, brook trout (*Salvelinus fontinalis* (Mitchill, 1814)), lake trout (*Salmo trutta lacustris* Linnaeus, 1758) and Danube salmon (*Hucho hucho* (Linnaeus, 1758)) compounds (Na^+, K^+, Cl^-, Ca^{2+} and Mg^{2+}) and low concentrations of organic substances such as sugars, cholesterol and protein (Rurangwa et al 2004).

Lahnsteiner et al (1995) also found considerable intraspecific variation in the composition of the ovarian fluid in four salmonid species (rainbow trout, brook trout, lake trout and Danube salmon). The intra specific variation in ovarian fluid composition could be resulted partly from variations in post-ovulation maturation within the coelomic cavity, in the physiological status of the female, and in egg quality (Lahnsteiner 2000; Lahnsteiner et al 1999).

Similar to our results, Rosengrave et al (2009) for all ion concentrations observed statistically significant differences in ovarian fluid compositions of Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum, 1792)) between sampling dates, during the spawning season. They found that the ionic composition of the ovarian fluid varied among individual females and the largest relative differences were observed in K^+, Ca^{2+} and Mg^{2+} due to the high concentration of carbonate and bicarbonate, respectively.

Our results show that there is no significant difference in concentrations of potassium between coelomic fluids of Persian sturgeons during different times of migration season ($p>0.05$) but maximum and minimum of this ion were respectively observed in April and March. The ovarian fluids with low K^+ concentrations or saline medium without K^+ ions can prolong sperm mobility and subsequently increase the

fertilizing ability. Several studies have shown that the extent of inhibitory of sperm mobility by K^+ ion change during spawning season (Alavi & Cosson 2006; Alavi et al 2008).

The duration of sperm movement and the percentage of motile sperms can decrease by an excessive increase of Na^+ (Morisawa et al 1983). Alavi & Cosson (2005) have demonstrated that sperm concentration shows more biological sensitivity to the concentration of sodium when it comes to 50 mM or more. Similar to our results, Suquet et al (2005) reported that during breeding season of Atlantic cod, Na^+ ion concentration significantly changed ($P<0.05$).

A. persicus is sensitive to concentrations of Ca^{2+}; Ca^{2+} at 100 µM which can prevent the deterrent effects of K^+ (Cosson et al 1999, Alavi et al 2004). Data of Cosson et al (1991) strongly suggest that calcium ions can be of high importance in regulation of sperm mobility. The favorable effect of the coelomic fluid on washed gametes may also cause the presence of Ca^{2+} which is essential for fertilization (Ginsburg 1968). Calcium, like sodium, could reduce inhibitory effects of potassium on the activation of spermatozoa in sturgeons (Billard et al 1999; Alavi et al 2002). Ca^{2+} and Mg^{2+} ions in ovarian fluid were significantly correlated to each other, which may reflect variation in protein concentration.

A research on intracellular mechanisms of sperm mobility in teleost fishes has proved key role of magnesium in starting the activation of sperm mobility, especially in membrane of sperm (Cosson et al 1999). Alavi & Cosson (2005) reported that in *A. persicus* with 10 mmol magnesium, highest duration of sperm movement and percentage of motile sperms were observed. Rosengrave et al (2009) found that the mobility percentage increased after increasing concentration of Mg^{2+}. Our results showed no significant differences during spawning season in concentration of magnesium in coelomic fluid of *A. persicus* ($p>0.05$).

Cholesterol may also have protective effect against environmental changes (especially temperature). A research has shown that in trout there is a clear correlation between cholesterol and the duration of sperm movement (Secer et al 2004). In our experiment, the concentration of cholesterol was higher in the beginning and at the end of the spawning season ($P>0.05$).

Proteins may function as signaling molecules that have a chemo kinetic or chemo tactic effect on sperm. These peptide signaling molecules have been found on the surface of the unfertilized ovum of sea urchins (Echinoidea) (Neill & Vacquier 2004). Differences in the protein content of the coelomic fluid might explain the high percentage of success in artificial insemination when the eggs of different females are inseminated in water (Fredrich 1981). Moreover, Matsubara et al (1985) investigated the protein composition of the ovarian fluid of rainbow trout. Ginzburg (1968) has suggested that coelomic fluid proteins in fish play an important role in controlling sperm mobility. Protein changes are important because of the protective role they play for spermatozoa in many fish species (Rouxel et al 2008). In addition, White & Macleod (1963) indicated that protein has a protective role. Our results showed insignificant difference in concentration of total protein in coelomic fluid ($p>0.05$) but maximum and minimum of this ion were respectively observed in May and April.

Bahre Kazemi et al (2010) demonstrated that eyeing rate and the concentration of cholesterol, protein, and calcium of ovarian fluid of Caspian brown trout were negatively correlated. Furthermore, regarding ovarian fluid, hatching rate and the concentration of glucose, cholesterol, protein, and calcium were negatively correlated. Some of our data are similar to their findings. According of our data (Tables 1 and 2), wherever the concentrations of cholesterol, total protein and calcium of coelomic fluid decreased and the fertilization rate of eggs increased. However, these differences were not significant ($P>0.05$).

Conclusion. Our data provide evidence that the sturgeons have mechanisms to stabilize the concentrations of a number of hormones and metabolites in the coelomic fluid to the optimum level, which may be important for their direct effects on the gonads washed by

this fluid and the eggs which matured after ovulation. The present study also showed intra specific variations in the ionic composition of ovarian fluids through the spawning season which may cause better selection of sperms by the female fish. However, whether such selection operates during natural mating, still remains to be demonstrated.

According to the reasons which were mentioned in previous sections, the increase in the calcium ion and potassium ion will increases sperm mobility, and on the other hand magnesium, sodium, total protein and cholesterol have positive effects on sperm mobility. Since these ions in March and May, compared to April, have higher and suitable concentrations, it can be concluded that the chemical composition of ovarian fluid have better characteristics in beginning and end of the reproduction season, compared to the middle of the spawning season in Persian sturgeons (*A. persicus*).

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Authors:
Milad Kabir, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, e-mail: mld.kabir.17@gmail.com
Mohamad Reza Imanpoor, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, mr_imanpoor@yahoo.com
Masoud Asghari, Department of Fishery, Gorgan University of Agricultural Sciences and Natural Resources, Iran, Golestan, Gorgan, Shahid Beheshti Avenue, Postal code: 49138-15739, masoudasghari_14@yahoo.com
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