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The effects of time of reproductive migration on the biochemical composition and mobility trait of sperm in Persian sturgeon (*Acipenser persicus*) brood stocks

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Abstract. In this study the effects of reproductive migration of Persian sturgeon on some mobility traits of sperm (duration of sperm movement and the percentage of motile sperms) and on biochemical compounds (e.g. calcium, magnesium, glucose, total protein, sodium and potassium) of semen was investigated. For this purpose, time of reproductive migration in these brood stocks were divided to three periods (i.e. treatment 1 = March, treatment 2 = April and treatment 3 = May) and in each period we sampled from those male fishes which had approximately the same sizes. The results showed that the duration of sperm movement (110.00±28.28, 327.85±85.38 and 55.00±7.07), the percentage of motile sperms (70.50±2.12, 88.60±8.38 and 66.00±0.00) and amounts of glucose (6.25±2.78, 1.18±0.44 and 7.78±1.25), total protein (0.19±0.05, 0.41±0.10 and 0.07±0.00), potassium (3.57±1.31, 2.57±0.77 and 4.46±0.14) and calcium (2.19±0.31, 2.59±0.81 and 11.99±6.35) of these fishes were significantly different at different times of the reproductive migration ($P<0.05$). But no significant difference was observed between these treatments for the percentage of spermatocrit, cholesterol and sodium and magnesium ions ($P>0.05$). Generally speaking, male brood stocks of Persian sturgeons in terms of mentioned parameters are more appropriate in April compare to March and May months.

Key Words: Time of reproductive migration, biochemical compounds, sperm mobility traits, Persian sturgeon.

خلاصه.در اين مطالعه اثر زمان مهاجرت توليد مثلي مولدين قره برون روي برخي پارامترهاي اسپرم شناختي (طول دوره تحرك اسپرم و درصد اسپرم هاي متحرک) و ترکيبات بيوشيميايي (يون هاي کلسيم، منيزيم، گلوکز، پروتئين کل، سديم و پتاسيم) سمن بررسي شد. به اين منظور زمان مهاجرت توليد مثلي مولدين به سه دوره زماني (تيمار 1= اسفند ، تيمار 2= فروردين و تيمار 3= ارديبهشت)تقسيم و در هر دوره زماني از ماهي نر حدوداً هم اندازه نمونه برداري شد. نتايج تحقيق نشان داد که طول دوره تحرك اسپرم (28/28±110/00، 85/38±327/85 و 0/07±55/00)، درصد اسپرم های متحرک (2/12±70/50، 8/38±88/60 و 0/00±66/00) و مقادير يون هاي گلوکز (2/78±6/25، 0/44±1/18 و 1/25±7/78)، پروتئين کل (0/05±0/19، 0/10±0/41 و 0/00±0/07)، پتاسيم (1.31±3/57، 0/77±2/57 و 0/14±4/46) و کلسيم (0/31±2/19، 0/81±2/59 و 6/35±11/99) در زمانهاي مختلف مهاجرت توليد مثلي مولدين ماهي قره برون اختلاف معني داري (**P<0/05**) داشتند. اما درصد اسپرماتوکريت، کلسترول و يون هاي منيزيم و سديم در زمانهاي مختلف مهاجرت توليد مثلي مولدين ماهي قره برون اختلاف معني داري (**P>0/05**) وجود نداشت. در مجموع مي توان گفت نمونه هاي ماهي قره برون در فروردين ماه به لحاظ پارامترهاي حرکتي و ترکيبات بيوشيميايي نسبت به ماههاي اسفند و ارديبهشت مناسب تر هستند.

کلمات کليدی. زمان مهاجرت توليد مثلي، ترکيبات بيوشيميايي، پارامترهاي حرکتي اسپرم، قره برون.

Introduction. Sturgeons are among the most valuable seafood species and they are known since antiquity and due to this long historical record these species are called living fossils. 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 the Acipenseridae family that widely spread in the southern part of Iranian coastal waters of Caspian Sea. In 1997, this species for the first time was introduced as an independent species (Birstein et al 1997). After 1971, artificial reproduction and rearing of sturgeon were conducted to the general tendency to develop the breeding of this fish in the southern Caspian Sea (Azari Takami 1992). Sturgeons are reared to produce fingerlings, to reconstruct natural supplies and improve its population or to produce market size fishes (Chebanov & Billard 2001).

The start of sperm mobility, its swimming speed and its swimming duration are directly affected by concentrations of ions (K^+, Na^+, Ca^{2+}, Mg^{2+}), the osmotic rate, pH

(Stoss 1983; Wojtczak et al 2007), viscosity (Lauga 2007), dilution rate and other components of external medium (Yoshida & Nomura 1972; Stoss 1983; Billard et al 1995b; Cosson et al 1999; Ingermann et al 2003).

Since good quality of sperm influences on the survival of produced larvae and on improving the generation, quick assessment of sperm quality can be useful to select the appropriate brood stocks which have sperms with the betst quality (Rurangwa et al 2004). Sperm quality is a criterion for measuring the sperm ability in fertilizing the ovum. Therefore, parameters which influence on the fertilization of ovum are sperm quality parameters. The most important parameters which deal with the sperm quality are spermatocrit, sperm density, seminal plasma composition, duration of sperm movement and percentage of motile sperms (Rurangwa et al 2004).

A few researches were done on the physiology and biochemistry of sperm structure, sperm mobility and changes in characteristics of milts. Munkittrick & Moccia (1987) reported that rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792)), spermatocrit, sperm mobility and seminal plasma ions decreased during the reproduction season. Rurangwa et al (2004) reported that concentrations of spermatozoa in the rainbow trout and common carp (*Cyprinus carpio* Linnaeus, 1758) decreased at the end of spawning season.

Seminal plasma includes inorganic compounds (ions), organic compounds and enzymes. Inorganic compounds, including sodium, potassium, calcium and magnesium, play the role of preventers or stimulators in spermatozoa (Morisawa 1985) and organic compounds are important for metabolic activity (Lahnsteiner et al 1996).

Ionic compositions of the sperm may change during reproduction seasons (Alavi & Cosson 2006). Ions of seminal plasma may polarize the cell membrane and can stimulate sperm mobility (Morisawa et al 1999). Cations (often bivalent, such as calcium) have antagonistic effects to avoid effects of potassium on sperm mobility (Alavi & Cosson 2006). K^+ plays an essential role in sperm mobility in sturgeons (Alavi & Cosson 2005) . Over-increase of sodium will decrease the duration of sperm movement and the percentaga of motile sperms (Morisawa 1985; Alavi & Cosson 2006).

The purpose of this study was to determine the effects of different reproductive migration period on some mobility traits of sperm and on biochemical compounds of semen of Persian Sturgeon. Therefore, according to the information about compounds of sperm we can understand that in which month of reproduction season we can have sperms with better characteristics.

Material and Method. 7 milts (2 fish in first treatment, 3 fish in second treatment and 2 fish in third treatment) were sampled from Persian sturgeon's brood stocks during March until May, 2010, in Shahid Marjani center of sturgeon breeding and growth, Gorgan, Iran. Samples were experimented in the three treatments, i.e. beginning (March), middle (April) and end of reproduction season (May). Brood stocks were kept apart from each other in Kourenski pounds 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), and according to Van Eenennam et al (2001) method, depending on water temperature and fish weight (Billard 2000). Approximately 12 hours after injection, spermatogenesis took place. Milts were extracted with special syringes which were inserted into gonopore of alive males. After that, milts were put into 1.5 ml micro tube 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 University of Gorgan for future analysis. However, during analysis temperature of milt was 4°C to save their mobility.

In the laboratory, to measure the duration of sperm movement and the percentage of motile sperms (in less than 7 seconds), after adding distilled water (50 times of sperm volume, to stimulate sperm mobility), samples were put under the stereomicroscope (a microscope equipped with a CCD camera attached to a computer, Panasonic WV-CP240, Japan) (Cosson et al 2000) with magnification number 10 which recorded the sperm movement with a digital camera with high resolution. survival life of sperm for each sample was measured by stopwatch. The stopwatch started to work at

the same time that milt was activated by water and stopped when sperm movement stopped (Leach & Montgomery 2000; Turner & Montgomery 2002). Then Adobe premier software (Version 6) was used to take pictures every 10, 20, 30, 40 seconds, after sperms activations (Fig. 1). This pictures were changed into 30 slides and we randomly selected four forms of them (i.e. form 1, 4, 7 and 10). The positions of 10 spermatozoa in these randomly selected pictures were observed and the percentage of motile sperms was calculated. It should be noted that the dilution ratio of milt to water was 1:50 so that under the microscope, about 50 sperms were viewed in each point (although this ratio was suggested by the Gallis et al (1991), Alavi et al (2002), Alavi & Cosson (2005), we also determined it by trial and error). All treatments were done in three replications and to avoid experimental error, all measurements were observed by a viewer (Cosson et al 2000).

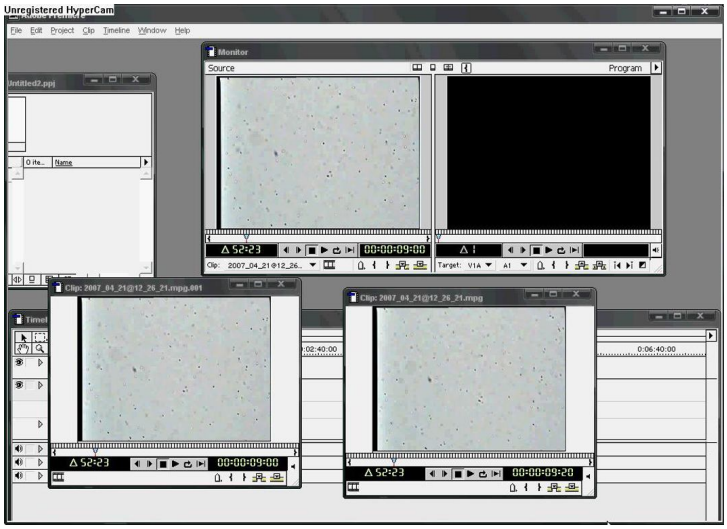

Figure 1. Use of Adobe premier software (Version 6) to calculate of the percentage of motile sperms.

Spermatocrit of milt is very different among males and between species and in different seasons (Patkin 1999). For measuring the spermatocrit, the tubes containing semen were centrifuged in the 3000 round of centrifugal machine for 8 minutes (Eppendorf AG 22311 Hamburg, centrifuge 5415D); then hematocrit reader was used (WPA LightWave - Diode - array S2000 UV/Vis) to determine the percentage of sperm to seminal fluid (percentage of white space to the total milt volume) (Fitzpatrick et al 2005).

In order to study the biochemical index of sperm, the samples were put into 1.5 ml micro tubes, were centrifuged in the 13,000 round rpm for 5 minutes and were transferred to new vials. The vials were stored at -20°C for future studies on biochemical compounds. Since the ions in seminal fluid may polarize cell membrane and stimulate sperm mobility (Morisawa et al 1999), we measured sodium and potassium ions concentrations by flame photometer (Jenway pfp England) and the concentrations of calcium, magnesium ions, glucose, total protein and cholesterol by the spectrophotometer (S200-UV.VIS England) in seminal fluid, using quantitative biochemical kits of serum or plasma parameters (Turker et al 2004). All tests took place at room temperature (20-22°C).

Our data were analysized statistically using One-Way ANOVA with Duncan test at the level of 95 % and also using SPSS, version 16. Statistically significant differences were set at the level of $P<0.05$ with ± standard deviation (SD).

Results and Discussion. The duration of sperm movement and the percentage of motile sperms had significant difference in the different reproductive migration periods of Persian Sturgeons ($P<0.05$). Furthermore, among the biochemical compositions of seminal fluid, the amounts of glucose, total protein, calcium and potassium were significantly different in these treatments ($P<0.05$). On the other hand, regarding the

percentages of spermatocrit, cholesterol, sodium and magnesium of seminal fluid, there was no significant difference between these periods (P>0.05).

Analysis of some biochemical parameters of semen at the initial, middle and final periods of migration in Persian sturgeons has been expressed in Table 1.

Furthermore, some movement parameters of sperm during spawning season of these fishes have been shown in Table 2.

Due to the high value of persian sturgeon brood stocks, experts took a great effort (using a syringe) to extract their reproductive substance without killing them. therefore we were not able to biometry them. Since these factors are the quality parameters of sperm, it can be said that in the second treatment (middle period of migration), compared to other periods, the quality of sperm was more suitable.

Sperms of different fish species are different regarding the start of sperm mobility (Cosson et el 1999), duration of sperm movement, the percentage of motile sperms (Billard & Cosson 1992) and the pattern of sperm movement (Ravinder et al 1997). In other words, these parameters in the middle period of reproductive migration in *A. persicus* (treatment 2) were more compared to the initial period (treatment 1) and final period (treatment 3) of reproductive migration (Table 2). In a study on cod (*Gadus morhua* Linnaeus, 1758) by Suquet et al (1992) and Rouxel et al (2008), the percentage of motile sperms in the beginning, middle and end of migration of this species were respectively 43±15, 52±25, 41±15; so, similar of our research, there was not significant difference between them (P>0.05). At the middle of reproduction season, immigration is more compared to the beginning and the end of the reproductive migration. Spermatocrit and sperm densities in seminal fluid are generally used to evaluate the quality of fish sperm (Rurangwa et al 2004). In the present study there were not significant differences between percentages of spermatocrit during the spawning season (P>0.05) (Table 2). Similar to the results of our research, Suquet et al (1992) and Rouxel et al (2008) showed that also in cod the percentages of spermatocrit were not significantly different in different periods of season of reproductive migration (P>0.05).

Compounds of seminal plasma have important effects on those fishes which have external fertilization (Morisawa & Suzuki 1980). Ionic compounds may also change during spawning season (Alavi & Cosson 2006). Sodium is one of the dominant ions in seminal plasma (Alavi & Cosson 2006) and excessive increase in sodium concentration will cause decreases in duration of sperm movement and the percentage of motile sperms (Morisawa et al 1983). Our results show that there was no significant difference in sodium concentrations in plasma between our treatments in Persian sturgeons (P>0.05) (Table 1).

Potassium is involved in the storage of spermatozoa in the static state (Bayanes et al 1981). Morisawa & Suzuki (1980) reported that a potassium ion is a suitable inhibitory factor which prevent sperm movement in seminal plasma. Recent studies have shown that the ranges of inhibitory effects of potassium on the stimulation of the sperm mobility chang during reproductive season. High percentage of sperms will be motile even in high concentrations of potassium (40 and 80 mmol) (Billard & Cosson 1992). Difference in potassium concentrations between water and seminal plasma caused the start of sperm movement (Morisawa et al 1983). Our study shows that the concentration of K^+ at the beginning of the reproductive season increased (3.57±1.31) and then gradually decreased in the middle of the season (2.57±0.77), but at the end of the spawning season it reached to its maximum level (4.46±0.14) (P<0.05). Besides, since at the middle of the spawning season we observed the reduction of potassium concentration (2.57±0.77) and increases in the duration of sperm movement (327.85±85.38) and in the percentage of motile sperms (88.60±8.38), we can conclude that the increase in potassium ions will decrease sperm movement parameters (Tables 1 and 2). In other words, it can be said that potassium ion concentrations are different between different seasonal reproductive migrations of *A. persicus* (similar to the results of a research by Alavi & Cosson (2006)).

Table 1

Some biochemical parameters of semen in Persian sturgeon brood stocks among different reproductive migration (means ± S.D)

Experimental groups	*Glucose mg/dl*	*Cholesterol mg/dl*	*Total protein g/dl*	*Magnesium mg/dl*	*Calcium mg/dl*	*Sodium mM/Lit*	*Potassium mM/Lit*
1	6.25±2.78[a]	5.28±2.62	0.19±0.05[b]	1.14±0.53	2.19±0.31[b]	50.44±30.83	3.57±1.31[ab]
2	1.18±0.44[b]	8.18±3.34	0.41±0.10[a]	1.03±0.54	2.59±0.81[b]	61.04±31.87	2.57±0.77[b]
3	7.78±1.25[a]	4.92±2.11	0.07±0.00[b]	0.40±0.11	11.99±6.35[a]	40.59±5.29	4.46±0.14[a]

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

Table 2

Percents of spermatocrite and duration of sperm movement and percent of motile sperms in Persian sturgeon brood stocks among different reproductive migration (means ± S.D)

Experimental groups	*Time of migration (month)*	*Spermatocrit (%)*	*Sperm movement (s)*	*Motile sperms (%)*
1	March	8.33±1.50	110.00±28.28[b]	70.50±2.12[b]
2	April	7.00±0.95	327.85±85.38[a]	88.60±8.38[a]
3	May	6.40±1.73	55.00±7.07[b]	66.00±0.00[b]

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

Studies by Cosson (2004) show that calcium has mmol (mM) role in increasing the sperm movement parameters. Other studies on common carp showed that preventing calcium ions to enter seminal fluid will prevent sperm motility (Krasznai et al 2000). This study shows that concentrations of calcium vary during reproductive migrations and have significant differences in Persian sturgeons ($P<0.05$). In this study maximum calcium concentration was at the end of spawning season (11.99±6.35) (Table 1). But a study conducted by Suquet et al (1992) showed that calcium concentration at the middle of cod migration was more compared to the other periods of reproductive migration.

There is little information about the effect of magnesium ion on the sperm mobility of bony and Acipenserid fishes. A research which was on intracellular mechanisms of sperm mobility in bony fishes showed important and key role of magnesium in starting of sperm mobility (Cosson et el 1999). This study showed (Table 2) that concentrations of magnesium (1.14±0.53, 1.03±0.54 and 0.40±0.11) in the different reproductive migration periods had no significant differences ($P>0.05$). Alavi & Cosson (2005) showed that in those *A. persicus* which had 10 mmol magnesium the highest duration of sperm movement and the highest percentage of motile sperms were observed. Scheuring (1925) reported that some ions such as sodium, calcium and magnesium can neutralize the inhibitory effect of potassium.

Glucose in seminal plasma prepares energy requirements for testis during spermatogenesis or for lipid synthesis in spermatozoa (Soengas et al 1993). Secer et al (2004) reported significant correlations ($P<0.05$) between glucose and duration of sperm mobility in rainbow trout. Present study showed significant difference in glucose between reproduction migration periods of different fishes ($P<0.05$). This factor was lower in treatment 2 (1.18±0.44) (Table 1).

White & Macleod (1963) mentioned the protective role of protein. Bozkurt et al (2006) showed that high protein content (9.42±3 g/dl) is necessary for semen of brown trout (*Salmo trutta trutta* Linnaeus, 1758). Secer et al (2004) also reported that there is a significant correlation ($P<0.05$) between protein amount and potassium and calcium amounts which have considerable effects on sperm mobility. In this study, there were significant differences between protein levels during reproduction seasons ($P<0.05$). Protein level in treatment 2 (April) was higher (0.41±0.10) compared to other treatments (0.19±0.05 and 0.07±0.00). Cholesterol exists in the seminal plasma of freshwater fishes (Billard et al 1995a). Cholesterol may have protection effect against environmental fluctuations (especially temperature) (Secer et al 2004). Scientists also showed that in trout there is a clear correlation between the duration of sperm mobility and cholesterol. In the present study, there was no significant difference in cholesterol content between the reproduction migration periods of male brood stocks ($P>0.05$). However the concentration of cholesterol increased in the middle of the spawning season (8.18±3.34) (Table 1).

Conclusion. This study was done on mobility traits (duration of sperm movement were 327.85-55.00 and the percentage of motile sperms were 88.60-66.00) and biochemical parameters of sperms (Glucose = 7.78-1.18, Cholesterol = 8.18-4.92, Total protein = 0.41-0.07, Magnesium = 1.14-0.40, Calcium = 11.99-2.19, Sodium = 61.04-40.59 and Potassium = 2.57-4.46) of Persian sturgeon in different reproductive migration periods. Our data indicates that sperms of this fish brood stocks in second treatment (duration of sperm movement = 327.85±85.38, percentage of motile sperms = 88.60±8.38, Glucose = 1.18±0.44, Cholesterol = 8.18±3.34, Total protein = 0.41±0.10, Magnesium = 1.03±0.54, Calcium = 2.59±0.81, Sodium = 61.04±31.87 and Potassium = 2.57±0.77) (April) have more suitable characteristics compare to March and May.

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