

THE EFFECT OF CATIONS ON SPERM MOTILITY PERFORMANCE AND FERTILIZING ABILITY OF SILVER CARP *Hypophthalmichthys molitrix*

KHARA H*, BARADARAN SHAHROOZ N**, HADISEH DADRAS*, RAHBAR MINA***, AHMADNEJAD MOHADESEH**** KHODADOOST A***

*Islamic Azad University – Lahijan Branch, Faculty of Natural Resource, Lahijan, Iran

**International Sturgeon Research Institute, Rasht, Iran

***Islamic Azad University, Young Researcher Club, Lahijan Branch, Iran

****Inland Water Aquaculture Research Center, Bandar Anzali, Iran

(Received 14th March 2012)

The objective of the study was to investigate the effect of saline solution containing cations (Na^+ , K^+ , Ca^{+2} , Mg^{+2}) on sperm motility performance (duration of sperm motility and percentage of motile spermatozoa) and fertilizing capacity of sperm (fertilization rate, hatching rate, larvae length during hatching, larvae length during active feeding and survival rate) in silver carp. The results suggested that solutions containing ions did not improve the duration of sperm motility. The same was observed for the percentage of motile spermatozoa. Fertilization rate influenced by solutions containing Ca^{+2} , and other ions could not affect this parameter. The results showed that hatching rate was higher in solutions containing 99 mEq/L NaCl, 2 mEq/L MgCl_2 and 2, 4 mEq/L CaCl_2 respectively. Also, survival rate was higher in the solution containing 2 mEq/L MgCl_2 and 36 mg/dL KCl respectively. With regard to the obtained results, it was concluded that using appropriate activation medium can improve quality of fish sperm and subsequently increases artificial reproduction performance.

Key words: cations, fertilization capacity, saline solution, seminal plasma, silver carp, spermatozoa motility

INTRODUCTION

One of the most important factors responsible for the success of reproduction is the quality of male gametes. This implies not only the quality of the genetic material that is introduced into the egg during fertilization, but also the ability of sperm to move toward the female gametes, i.e. sperm motility. Sperm motility is a prerequisite factor determining sperm quality and fertilizing capacity (Alavi *et al.*, 2004). The quality of sperm is usually defined as motility, is a prerequisite factor which determines the semen fertilizing ability (Lahnsteiner *et al.*, 1997). Several factors influence sperm motility, such as pH (Alavi and Cosson, 2005 a; b), cations (Cosson, 2004; Alavi *et al.*, 2007), osmolality (Cosson, 2004; Alavi and Cosson, 2006; Alavi *et al.*, 2007) and dilution ratio (Alavi *et al.*, 2004) in

either aqueous environment or diluent. Motility of freshwater fish spermatozoa is triggered by a hypoosmotic medium (Cosson *et al.*, 1999). The duration of sperm motility is brief in most fish species (only lasts for 30 sec to few minutes) and varies between species: it depends on many factors affecting biochemical, physiological and metabolic characteristics of the broodstock and spermatozoa (Alavi *et al.*, 2008a). In industrial fish farming, one way to successful fertilization is to prolonge sperm motility with a modified activation medium (Alavi *et al.*, 2008a). The determining parameters that influence sperm motility will provide us with applied approaches to improve methods for artificial reproduction by developing immobilizing or activating media for fertilization (Billard *et al.*, 1995; Rodina *et al.*, 2004). To increase the efficiency in artificial fertilization trials, the composition of diluents is very important and the components of activating solutions must be adjusted according to species characteristics (Billard *et al.*, 1995; Alavi *et al.*, 2005b). Sperm quality is a key factor that determines the ability of sperm to successfully fertilise an egg. Also, fertilization success is depended on sperm motility, sperm/egg ratio and egg quality (Billard *et al.*, 1995). Previous studies have confirmed sperm motility parameters, especially the percentage of motile spermatozoa can be used to evaluate fertilization and hatching rate (Lahnsteiner *et al.*, 1998; Mansour *et al.*, 2005). The role of ions on sperm motility and fertilizing ability of spermatozoa has been reviewed by researchers (Stoss, 1983; Billard *et al.*, 1995; Cosson *et al.*, 1991; Cosson, 2004; Linhart *et al.*, 2008). The major ions involved in improving motility characteristics include sodium (Na^+), potassium (K^+), calcium (Ca^{+2}) and magnesium (Mg^{+2}). Silver carp, *Hypophthalmichthys molitrix* belongs to cyprinids, which globally produced 3.78 million tonnes in the world (FAO, 2008). Silver carp spawn in late spring and summer (April-September) when water temperature is relatively high. Studies on silver carp sperm are limited to cryopreservation. However, changes in silver carp sperm quality and fertilizability during the spawning seasons were reported by Mofizur Rahman *et al.*, (2011). To date, there is no information available about the influence of activation media on sperm motility of silver carp. Therefore, the objectives of the present study were to investigate the effects of cations (Na^+ , K^+ , Ca^{+2} , Mg^{+2}) on the motility performance of spermatozoa and their fertilizing ability in silver carp. The ionic composition of seminal plasma was also measured during the reproductive season.

MATERIAL AND METHODS

Brood fish, egg and sperm collection

The experiments were carried out in June 2010 at the Kasmahi Company, Rasht, Iran. Broodstocks (15 mature males and 15 mature females) were captured from the hatchery pools, during the spawning season. Fish were transferred to the site of the experiment, and acclimated for 2 weeks in 4000 L tanks. Fish used in the experiment ranged from 3.2 to 3.8 and 6.2 to 7.9 kg total weight and 63 to 82 and 71 to 86 cm total length for males and females, respectively. Silver carp males were injected intramuscularly with Carp Pituitary Gland Hormone (cPG) at a dose of 0.5 mg kg^{-1} . In addition, females were injected intramuscularly with a double

injection of 2 mg kg⁻¹ cPG. The first injection, 10% (0.2 mg/kg) cPG, was given 12h before the second injection (1.8 mg/kg). Semen was collected after spermiation, approximately 12 h after spawning induction. Semen of each male was collected and sperm batches transported to the laboratory under cold conditions (4°C) until used for analysis and fertilization. Care was taken to avoid contamination of the semen with water, mucus, blood, faeces or urine. Stripping of females was carried out 12 h after the second injection.

Sperm motility assessment

To examine the effects of cations on sperm motility performance, Na⁺ (NaCl) (85, 92 and 99 mEq/L), K⁺ (KCl) (36, 42 and 48 mg/dL), Mg²⁺ (MgCl₂) and Ca²⁺ (CaCl₂) (2, 4 and 6 mEq/L) were used. All solutions were buffered with 30-40 mM Tris-HCl, adjusted to pH 8.5 ± 0.2. Distilled water was used as the control. Sperm motility was evaluated visually for the percentage of motile spermatozoa after activation and total duration of motility (in seconds). To induce the initiation of motility, sperm was triggered directly in activation solutions at a ratio of 1: 2000 and immediately recorded with a 3 CCD video camera (Panasonic 240 Japan) mounted on a dark-field microscope (Leica USA). The duration of sperm motility was measured immediately after initiation of sperm activation until 100% spermatozoa were immotile. Percentage of motility was defined as the percentage of progressively motile spermatozoa within each activated sample. Progressively motile spermatozoa were defined as actively swimming in a forward motion. Only forward moving sperm was judged as motile and sperm cells that vibrated in place were not considered motile (Aas *et al.*, 1991). Analysis of sperm motility was carried out in triplicate for each sample at room temperature (20-22°C), using light microscopy under 400 × magnification.

Evaluation fertilizing ability

Fresh eggs were obtained from females and pooled just prior to assay. To control variation among the qualities of egg pools, the eggs were obtained from same-age females cultivated under the same conditions. Fertilization was performed in dry plastic dishes and 50 g of eggs (approximately 50000 eggs) was placed into each dish. The fertilization solution (3 g of urea, 4 g of NaCl in 1 L distilled water) was used according to the dry fertilization method. Batches of eggs were inseminated with solutions containing 85, 92 and 99 mEq/L NaCl, 36, 42 and 48 mg/dL KCl, 30, 32, 35 2, 4 and 6 mEq/L CaCl₂ and MgCl₂ respectively. Distilled water was used as the control. Following fertilization, the eggs were stirred for 1 h and then rinsed with hatchery water and placed into the incubator. Fertilization rate was determined as the percent of eyed eggs about 6 h after fertilization. For incubation, the so-called "Vase" device was used. The success of fertilization was evaluated by the percentage of eggs reaching the morula stage, 4h after fertilization. Hatching occurred between 1-2 days after fertilization.

Seminal plasma characteristics

Seminal plasma was separated from the semen by centrifugation (Eppendorf AG, Hamburg, Germany). Plasma was centrifuged twice to avoid

possible contamination with spermatozoa. Plasma samples were frozen at -20°C until analysis. The chemical composition of seminal plasma was measured by the colorimetric method using an Autoanalyser Technican (Caretium-XI-921, Germany) for Ca^{+2} and Mg^{+2} measurement and with a flamephotometer (Jenway PFP, England, Standard kits from Parsazmoon, Tehran, Iran) for Na^{+} and K^{+} in the Dr Fadaiees' Medicine Laboratory, Rasht, Iran.

Data analysis

The normal distribution of data was tested using the Shapiro-Wilk's test. Data were analysed using one-way ANOVA (SPSS 16). Duncan test was used for post hoc comparisons. Results are presented as mean \pm SE. Differences with a probability value of 0.05 ($p < 0.05$) were considered significant.

RESULTS

Sperm motility performance

Sperm characteristics and ionic composition of seminal plasma of silver carp are given in Table 1. Maximum (40.6 ± 8.5 s) duration of sperm motility was observed in solutions containing 99 mEq/l NaCl (Fig. 1a). The higher percentage of motile spermatozoa was obtained after triggering the motility in distilled water ($55 \pm 5\%$) compared to other concentrations of NaCl (Fig. 1b). A similar pattern was detected in terms of percentage of motile spermatozoa when semen was incubated with KCl solutions (Fig. 2b), but duration of sperm motility was higher in the solution containing 36 mg/dL KCl (Fig. 2a). In the case of MgCl_2 and CaCl_2 , duration of sperm motility was not influenced by activation solutions and higher values were observed in distilled water (Fig. 3 and 4a). A decreasing trend was observed in the percentage of motile spermatozoa when the dose of the solutions containing MgCl_2 and CaCl_2 gradually increased (Fig. 3 and 4b).

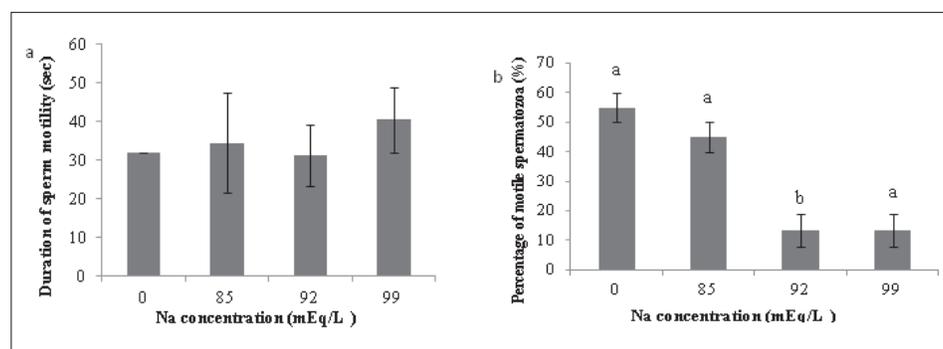


Figure 1. Effect of different concentrations of NaCl on (a) duration of sperm motility and (b) percentage of motile spermatozoa in silver carp. Values with different alphabetic letters are significantly different

Table 1. Sperm characteristics of silver carp

Variables	Minimum	Maximum	Mean \pm SE
Duration of sperm motility (sec)	45	27	36 \pm 9
Percentage of motile spermatozoa (%)	94	97	95 \pm 6
Sodium (mmol ⁻¹)	99	82	90 \pm 4.90
Potassium (mmol ⁻¹)	48	43	45 \pm 10.17
Magnesium (mmol ⁻¹)	14.6	10.1	7.3 \pm 2.44
Calcium (mmol ⁻¹)	2.5	1.2	1.5 \pm 0.47

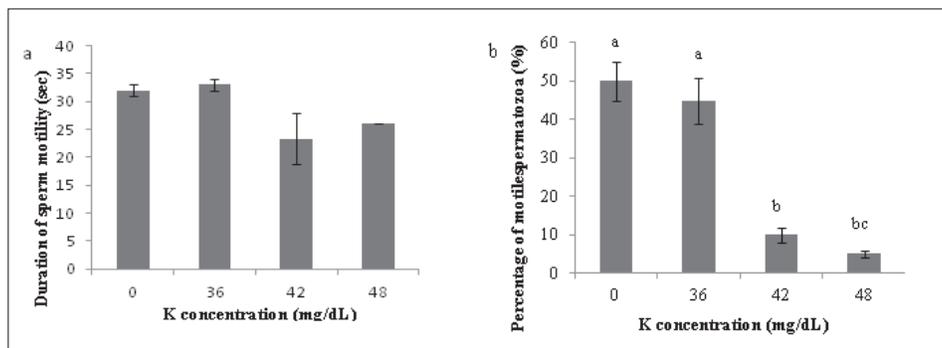


Figure 2. Effect of different concentrations of KCl on (a) duration of sperm motility and (b) percentage of motile spermatozoa in silver carp. Values with different alphabetic letters are significantly different

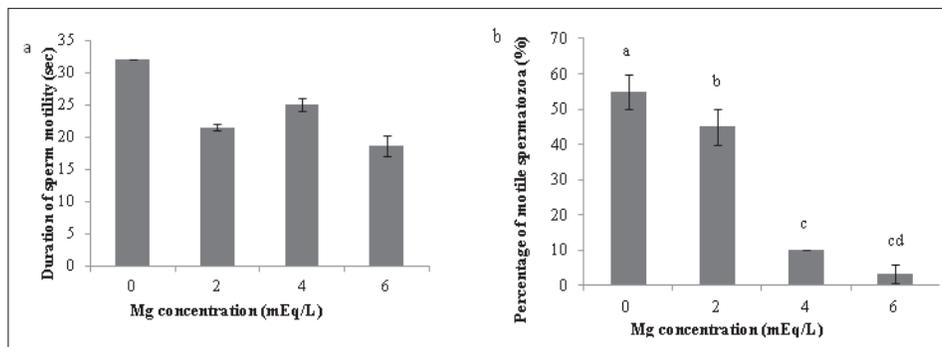


Figure 3. Effect of different concentrations of MgCl₂ on (a) duration of sperm motility and (b) percentage of motile spermatozoa in silver carp. Values with different alphabetic letters are significantly different

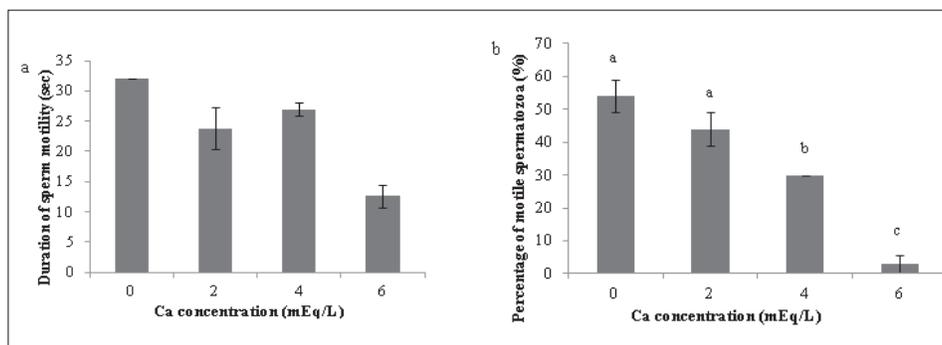


Figure 4. Effect of different concentrations of CaCl_2 on (a) duration of sperm motility and (b) percentage of motile spermatozoa in silver carp. Values with different alphabetic letters are significantly different

Fertilization capacity

Effects of cations (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) on fertilizing capacity of silver carp sperm are presented in Table 2-5, respectively. The solution containing cations (Na^+ , K^+ , Ca^{2+} and Mg^{2+}) did not affect fertilization rate and the highest values were observed in distilled water (Table 2-5). The maximum hatching rate was recorded when solutions containing 99 mEq/l NaCl, 2 mEq/L MgCl_2 and 4 mEq/L CaCl_2 were used respectively (Table 2, 4 and 5). Larvae length during hatching and active feeding was not changed by activation mediums. Survival rate was changed by solutions containing 99 mEq/l NaCl, 36 mg/dL KCl_2 and 2 mEq/L MgCl_2 and CaCl_2 , but their values were not significantly different (Table 2-5).

Table 2. Effect of different concentrations of NaCl on fertilization capacity of silver carp sperm

Na concentration (mEq/L)	Fertilization rate	Hatching rate	Larvae length during hatching	Larvae length during active feeding	Survival rate (%)
Distilled water	81.6±0.09 ^a	43.2±7.5 ^c	4.40±0.11	5.0±0.09	88.1±2.7
85	51.6±2.8 ^b	60.0±5.0 ^{ab}	4.07±0.12	0	0
92	20.0±5.0 ^d	16.0±1.9 ^d	3.20±0.12	5.5±0.08	84±1.7
99	35.0±5.0 ^c	70.0±5.0 ^a	4.00±0.15	4.8±0.13	92±0.61

Columns with different alphabetic letters are significantly different ($p < 0.05$)

Table 3. Effect of different concentrations of KCl on fertilization capacity of silver carp sperm

K concentration (mg/dL)	Fertilization rate	Hatching rate	Larvae length during hatching	Larvae length during active feeding	Survival rate (%)
Distilled water	81.6±10.5 ^a	43.2±7.5 ^a	4.1±0.11	5.0±0.09	88.1±2.7
36	51.0±2.08 ^b	41.6±2.8 ^b	4.4±0.2	4.3±0.05	91.6±0.58
42	2.0±0.46 ^d	1.3±0.31 ^d	0	0	0
48	11.6±2.8 ^c	6.6±1.8 ^c	0	0	0

Columns with different alphabetic letters are significantly different ($p < 0.05$)

Table 4. Effect of different concentrations of MgCl₂ on fertilization capacity of silver carp sperm

Mg concentration (mEq/L)	Fertilization rate	Hatching rate	Larvae length during hatching	Larvae length during active feeding	Survival rate (%)
Distilled water	81.6±9.5 ^a	43.20±7.5 ^b	4.1±0.1	5.0±0.09	88.1±2.7
2	60.0±5 ^b	80.00±5 ^a	3.7±0.25	4.4±0.27	90.6±0.58
4	3.6±1.5 ^c	2.00±0.65 ^c	0	0	0
6	0.67±0.01 ^d	0.33±0.05 ^d	0	0	0

Table 5. Effect of different concentrations of CaCl₂ on fertilization capacity of silver carp sperm

Ca concentration (mg/dL)	Fertilization rate	Hatching rate	Larvae length during hatching	Larvae length during active feeding	Survival rate (%)
Distilled water	80.6±8.4 ^a	43.2±7.2 ^c	4.1±0.11	5.0±0.39	88.1±2.7
2	60.0±5 ^b	70.0±5 ^{ab}	4.0±0.06	5.1±0.08	91.1±0.16
4	50.0±5 ^{bc}	75.0±5 ^a	4.4±0.13	5.0±0.08	90.0±0.03
6	0 ^d	0 ^d	0	0	0

Columns with the different alphabetic letters are significantly different ($p < 0.05$)

DISCUSSION

To our knowledge, this is the first observation about the effects of saline solution containing cations (Na⁺, K⁺, Ca⁺², Mg⁺²) on sperm motility performance and fertilizing capacity of sperm in silver carp. The duration of sperm motility is usually very short (a few minutes in freshwater species) and could be influenced by external environmental factors such as pH, temperature, ions, and osmolality

(Cosson 2004; Alavi and Cosson, 2006). Therefore, determination of optimum parameters for the activation medium is very important to increase the efficiency of artificial reproduction (Alavi *et al.*, 2004). It has been shown that the ionic composition of the activating solution influences the initiation and duration of sperm motility (Marian *et al.*, 1993). Sperm motility is affected also by the ionic composition of the diluent as previously reported (Billard and Cosson, 1992). Research has documented the effect of ions on the initiation of motility and the duration of sperm in an effort to determine the biosensitivity of sperm. The longer period of sperm motility in saline solution is related to the delayed energy loss of spermatozoa for movement (Alavi and Cosson, 2006). However, other factors such as ATP content in the sperm, the number of mitochondria, osmolality and pH are involved in this process. In contrast to other studies (Stoss, 1983; Billard *et al.*, 1995), the duration of sperm motility in *H. molitrix* was not prolonged in saline solutions compared with freshwater. Morisawa *et al.*, (1983) showed that K^+ increases sperm motility (duration and percentage of motility) in some cyprinids. Similar to other Cyprinidae, we found that Na^+ and K^+ were the most predominant ions in seminal plasma. Literature reveals wide variations in the ionic composition of seminal plasma. Na^+ and K^+ ions concentrations were reported to be in the 94-107 and 39-78 in cyprinids (Kruger *et al.*, 1984; Billard *et al.*, 1995), respectively. For example, K^+ concentration differs among cyprinids. Values have ranged from 1.93 MmL^{-1} in *Tinca tinca* (Linhart *et al.*, 2003) to 98 MmL^{-1} in *Barbus barbuis* (Alavi *et al.*, 2008 b). K^+ measured 28.8 MmL^{-1} in *Barbus sharpeyi* (Alavi *et al.*, 2010). This difference in seminal Na^+ and K^+ concentrations denotes species-specific characteristics (Ciereszko *et al.*, 2000). The differences between our results and other studies could be related to several parameters, including spawning time of the fish species (Suquet *et al.*, 1994), contamination of semen by urine during stripping (Suquet *et al.*, 1994), phagocytosis of sperm in the testis during the degeneration stage of spermatogenesis (Lahnsteiner *et al.*, 1993), thinning and hydration of semen during spermiation (Morisawa *et al.*, 1979). Previous studies have confirmed that sperm motility plays an important role to in the determination of the fertilizing ability of sperm (Billard, 1992; Billard *et al.*, 1995). Fertilizing ability of spermatozoa increases by using appropriate activation mediums that increase the duration of motility. In this study, the highest sperm motility was obtained in distilled water compared to saline solutions; consequently the maximum fertilization rate was observed after activation of sperm in distilled water. In our experiment, the highest hatching rate was recorded in solutions containing 99 mEq/l NaCl, 2 mEq/L $MgCl_2$ and 4 mEq/L $CaCl_2$ respectively (Table 2, 4 and 5). High sperm motility and fertilization rates were detected after activation of sperm in 45 mM NaCl, 5 mM KCl, 30 mM Tris-HCl, pH 8.0 (Saad and Billard, 1987; Billard *et al.*, 1995; Perchec *et al.*, 1995; Linhart *et al.*, 2003; Linhart *et al.*, 2008). Saad and Billard (1987) reported fertilization rates lower than 5% and 25% after activation of sperm in freshwater and carp activation solution (CAS, containing 45 mM NaCl, 5 mM KCl, 30 mM Tris-HCl adjusted to pH of 8.0). The spermatozoa incubated in K^+ rich medium showed an interestingly high fertilizing ability after second motility triggering in carp activation solution (CAS) (Linhart *et al.*, 2008). Further studies, reported that salmonid spermatozoa, when immersed in saline, ovarian fluid or

diluted seawater maintained motility and fertility for extended periods, i.e. hours or days (Ellis and Jones, 1939). The differences observed between this study and others might be related to the quality of eggs and the management conditions used during artificial insemination.

ACKNOWLEDGEMENTS:

The present work was conducted at Lahijan Islamic Azad University, Iran. The authors wish to thank the manager and staff of laboratory center for their valuable contribution.

Address for correspondence:

Khara Hossein
Islamic Azad University – Lahijan Branch
Faculty of Natural Resource
Department of Fishery and Aquaculture
Lahijan, Iran
P.O. Box: 1616
h_khara1974@yahoo.com

REFERENCES

1. Aas GH, Refstie T, Gjerde B, 1991, Evaluation of milt quality of Atlantic salmon, *Aquaculture*, 95, 125-32.
2. Alavi SMH, Cosson J, Karami M, Abdolhay H, Mojazi Amiri B, 2004, Chemical composition and osmolality of seminal fluid of *Acipenser persicus*; their physiological relationship with sperm motility, *Aqua Res*, 35, 1238-43.
3. Alavi SM, Cosson J, 2005a, Sperm motility and fertilizing ability in the Persian sturgeon *Acipenser persicus*, *Aqua Res*, 36, 841-50.
4. Alavi SMH, Cosson J, 2005b, Sperm motility in fishes: I. Effects of temperature and pH, *Cell Biol Inter*, 29, 101-10.
5. Alavi SMH, Cosson J, 2006, Sperm motility in fishes: (II) Effects of ions and osmolality, *Cell Biol Inter*, 30, 1-14.
6. Alavi SMH, Rodina M, Policar T, Kozak P, Psenicka M, Linhart O, 2007, Semen of *Perca fluviatilis* L.: Sperm volume and density, seminal plasma indices and effects of dilution ratio, ions and osmolality on sperm motility, *Theriogenology*, 68, 276-83.
7. Alavi SMH, Linhart O, Coward K, Rodina M, 2008 a, Fish spermatology: implication for aquaculture management. In: Alavi SM H, Cosson J, Coward K, Rafiee R, Editors, *Fish spermatology*, Oxford, Alpha Science Internationally Ltd, 39761.
8. Alavi SMH, Psenicka M, Rodina M, Policar T, Linhart O, 2008 a, Changes of sperm morphology, volume, density and motility and seminal plasma composition in *Barbus barbuis* (Teleostei: Cyprinidae) during the reproductive season, *Aqua Liv Res*, 21, 75-80.
9. Alavi, SMH, Jorfi E, Hatf A, Mortezaei S AS, 2010, Sperm motility and seminal plasma characteristics in *Barbus sharpeyi* (Gunther, 1874), *Aqua res*, 41, e688-e94.
10. Billard R, Cosson MP, 1992, Some problem related to the assessment of sperm motility in freshwater fish, *J Exp Zool*, 261, 122-31.
11. Billard R, 1992, Reproduction in rainbow trout: sex differentiation, dynamics of gametogenesis, biology and preservation of gametes, *Aquaculture*, 100, 263-98.
12. Billard R, Cosson J, Perche G, Linhart O, 1995, Biology of sperm and artificial reproduction in carp, *Aquaculture*, 124, 95-112.
13. Ciereszko A, Glogowski J, Dabrowski K, 2000, Biochemical characteristics of seminal plasma and spermatozoa of freshwater fishes. In: Tiersch TR, Mazik PM, editors, *Cryopreservation of Aquatic Species*, Baton Rouge: World Aquaculture Society 20-48.

14. Cosson J, Billard R, Gibert C, Dreanno C, Suquet M, 1999, Ionic factors regulating the motility of fish sperm In: Gagnon C, editor, The Male Gamete: From Basic to Clinical Applications, Illinois, Cache River Press, 161-86.
15. Cosson J, 2004, The ionic and osmotic factors controlling motility of fish spermatozoa, *Aqua Inter*, 12, 69-85.
16. Ellis WG, Jones JW, 1939, The activity of the spermatozoon of *Salmo salar* in relation to osmotic pressure, *J Exp Biol*, 16, 530-4.
18. FAO 2008, FAO Yearbook: Fishery and aquaculture statistics. Food and Agriculture Organization, Rome, Italy. 72.
19. Kruger JC, Smith GL, VanVuren JHJ, Ferreira JT, 1984, Some chemical and physical characteristics of the semen of *Cyprinus carpio* and *Oreochromis mossambicus*, *J Fish Biol*, 24, 263-72.
20. Lahnsteiner F, Patzner RA, Weismann T, 1993, The testicular main duct and the spermatic duct in some cyprinid fishes. II. Composition of seminal fluid, *J Fish Biol*, 44, 459-67.
21. Lahnsteiner F, Bergger B, Weismann T, Patzner RA, 1997, Sperm motility and seminal composition in the Turbot (*Lota lota*), *J Appl Ichthyol*, 13, 113-9.
22. Lahnsteiner F, Berger B, Weismann T, Patzner RA, 1998, Determination of semen quality of the rainbow trout by sperm motility, seminal plasma parameters and spermatozoa metabolism, *Aquaculture*, 163,163-81.
23. Linhart O, Rodina M, Bast J, Cosson J, 2003, Urinary bladder, ionic composition of seminal fluid and urine with characterization of sperm motility in tench (*Tinca tinca* L.), *J Appl Ichthyol*, 19, 177-81.
24. Linhart O, Alavi SMH, Rodina M, Gela D, Cosson J, 2008, Comparison of sperm velocity, motility and fertilizing ability between firstly and secondly activated spermatozoa of common carp (*Cyprinus carpio*), *J Appl Ichthyol*, 24, 386-92.
25. Marian T, Krasznai Z, Balkay L, Balazs M, Emri M, Bene L, 1993, Hypoosmotic shock inducing osmolality dependent permeabilization and structural changes in the membrane of carp sperm, *J Histochem Cytochem* 41, 291-98.
26. Mansour N, Ramoun A, Lahnsteiner F, 2005, Quality of testicular semen of the African catfish, *Clarias fipeneus* (Burchell 1982) and its relationship with fertilization and hatching success, *Aqua Res*, 36, 1422-8.
27. Mofizur Rahman MD, Rahman MS, Hasan M, 2011, Changes in Sperm Quality of Silver (*Hypophthalmichthys molitrix*) and Bighead Carps (*Hypophthalmichthys nobilis*) during the Spawning Season, *Asian Fish Sci*, 24, 413-25.
28. Morisawa M, Hirano T, Suzuki K, 1979, Changes in blood and seminal plasma composition of the mature salmon (*Oncorhynchus keta*) during adaptation to freshwater, *Comp Biochem Physiol*, 64, 325-9.
29. Morisawa M, Suzuki K, Shimizu H, Morisawa S, Yasuda K, 1983, Effect of osmolality and potassium on motility of spermatozoa from freshwater cyprinid fishes, *J Exp Zool*, 107, 95-103.
30. Perchec G, Jeulin C, Cosson J, Andre F, Billard R, 1995, Relationship between sperm ATP content and motility of carp spermatozoa, *J Cell Sci*, 108, 747-53.
31. Rodina M, Cosson J, Gela D, Linhart O, 2004, Kurokura solution as immobilizing medium for spermatozoa of tench (*Tinca tinca* L.), *Aqua Int*, 12:119 -31.
32. Saad A, Billard R, 1987, The composition and use of a sperm diluent in the carp, *Cyprinus carpio*, *Aquaculture*, 66, 329-45.
33. Stoss J, 1983, Fish gamete preservation and spermatozoan physiology. In: Hoar WS, Randall DJ, Donaldson EM, editors, *Fish Physiology*, NewYork, Academic Press, 305-50.
34. Suquet M, Billard R, Cosson J, Dorange G, Chauvaud L, Mugnier C, Fauvel C, 1994, Sperm features in turbot (*Scophthalmus maximus*): a comparison with other freshwater and marine fish species, *Aqu Liv Res*, 7, 283-94.

**UTICAJ KATJONA NA POKRETLJIVOST SPERMATOZOIDA I NA PLODNOST
SREBRNOG ŠARANA *Hypophthalmichthys molitrix***

KHARA H, BARADARAN SHAHROOZ N, HADISEH DADRAS,
RAHBAR MINA, AHMADNEJAD MOHADESEH I KHODADOOST A

SADRŽAJ

Cilj ovog istraživanja je bio da se ispita uticaj rastvora koji sadrže katjone (Na^+ , K^+ , Ca^{+2} , Mg^{+2}) na pokretljivost spermatozoida (trajanje pokretljivosti spermatozoida i procenat pokretljivih spermatozoa) i njihovu oplodnu sposobnost (stopa plodnosti, stopa izleganja, dužina larve tokom izleganja, dužina larve tokom aktivne ishrane i stopa preživljavanja) kod srebrnog šarana. Rastvori koji su sadržavali navedene jone nisu produžili trajanje pokretljivosti kao ni procenat pokretnih spermatozoida. Procenat oplodnje nije bio izmenjen rastvorima koji su sadržavali Ca^{+2} i druge jone. Rezultati su ukazali da je stopa izleganja bila viša u rastvorima koji su sadržali 99 mEq/L NaCl, 2 mEq/L MgCl_2 i 2,4 mEq/L CaCl_2 . Takođe je stopa preživljavanja bila veća u rastvoru koji je sadržavao 2 mEq/L MgCl_2 i 36 mg/dL KCl. Na osnovu dobijenih rezultata se može zaključiti, da odgovarajući medijum za spermatozoide može da poboljša kvalitet semena riba i posledično poveća uspeh veštačke oplodnje.

