AACL BIOFLUX
Aquaculture, Aquarium, Conservation & Legislation
International Journal of the Bioflux Society

Investigation of chemical composition of ceolomic fluid and its effect on sperm motility traits in Prussian carp, *Carassius gibelio,* during spawning season

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Abstract. The effects of the composition of ceolomic fluid and sperm function are not well understood in teleostean fish species. The aim of the present study was to determine the concentration of the major inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-), organic composition (glucose and total protein) and pH of ovarian fluid in Prussian carp *Carassius gibelio* (Bloch, 1782), and to determine if the composition of these fluids influences sperm motility trait (percentage and duration of motility). In this purpose, sperm motility traits in 3 medium (ovarian fluid, fresh water + ovarian fluid and fresh water alone) were tested. The ovarian fluid was composed of sodium 133.1 ± 3.8 mM L^{-1}, potassium 2.4 ± 0.28 mM L^{-1}, calcium 0.56 ± 0.28 mM L^{-1}, magnesium 0.65 ± 0.23 mM L^{-1}, chloride 135.4 ± 3.92 mM L^{-1}, total protein 3.3 ± 0.46 gdL^{-1}, glucose 3.57 ± 0.48 mM L^{-1} and pH 8.22 ± 2.9. The results suggested that sperm movement duration was significantly higher in ovarian fluid than fresh water and fresh water + ovarian fluid during spawning season. The percentage of motile spermatozoa did not differ significantly among ovarian fluid, fresh water + ovarian fluid and fresh water in spawning periods. As a conclusion, the results of this study recommend the use of ovarian fluid, it is useful as an activation medium to improving sperm motility parameters of Prussian carp.

Key Words: Ceolomic fluid, sperm motility, Prussian carp, season spawning.

چکیده: اثر ترکیبات مایع سلومیک و عملکرد اسپرم در گونه های ماهیان (به خصوص ماهیان زینتی) به خوبی درک نشده است. هدف از مطالعه حاضر، تعیین غلظت یونهای اصلی غیر آلی (سدیم، پتاسیم، کلسیم، منیزیم و کلر)، ترکیبات آلی (گلوکز و توتال پروتئین) و pH مایع تخمدان در ماهی قرمز *Carassius gibelio* (*Bloch*,1782) و نیز بررسی تاثیر ترکیبات آن بر روی صفات حرکتی اسپرم (درصد و مدت زمان تحرک) بود. بدین منظور، صفات حرکتی اسپرم در 3 رقیق کننده (مایع تخمدان، آب شیرین + مایع تخمدان و آب شیرین به تنهایی) مورد آزمایش قرار گرفت. مایع تخمدانی متشکل از سدیم 3/8 ± 133/1 میلی مول در لیتر، پتاسیم 0/28 ± 2/4 میلی مول در لیتر، کلسیم 0/28 ± 0/56 میلی مول در لیتر، منیزیم 0/23 ± 0/65 میلی مول در لیتر، کلر 3/92 ± 135/4 میلی مول در لیتر، توتال پروتئین 0/46 ± 3/3 گرم در دسی لیتر، قند خون 3/57 ± 0/48 میلی مول در لیتر و pH= 8/22 + 0/29 بود. نتایج نشان داد که مدت زمان حرکت اسپرم در مایع تخمدان به طور قابل توجهی بالاتر از آب شیرین و آب شیرین + مایع تخمدان در طول فصل تخم ریزی بود. درصد تحرک اسپرم در میان مایع تخمدان، آب شیرین + مایع تخمدان و آب شیرین در دوره تخم ریزی تفاوت معنی داری نداشت. به عنوان نتیجه گیری، نتایج حاصل از این مطالعه نشان می دهد که استفاده از مایع تخمدانی به عنوان رقیق کننده، در جهت فعال‌سازی و بهبود پارامترهای حرکتی اسپرم ماهی قرمز مفید است.

کلمات کلیدی: مایع سلومیک، تحرک اسپرم، ماهی قرمز، فصل تخمریزی

Introduction. The Prussian carp (*C. gibelio*) is a fresh water that belongs to Cyprinidae. Most Prussian carp breed in captivity, particularly in pond settings. Breeding usually happens after a significant temperature change, often in spring. Males chase females, prompting them to release their eggs by bumping and nudging them. The reproductive biology of *Carassius* species is complicated by the occurrence of gynogenesis.

High quality gametes are necessary for successful aquaculture programs. In species with external fertilization, sperm typically remain inactive until they are released by the male, but may show enhanced motility in the presence of the ovarian fluid (also called ceolomic fluid) that is typically released with eggs by the female during reproductive season (Scott & Baynes 1980). It has been shown that ceolomic fluid influences sperm motility in fish (Turner & Montgomerie 2002; Dietrich et al 2008). Fish ovarian fluid has a unique composition regarding the presence of the organic and inorganic components which support the viability of spermatozoa (Morisawa et al 1983; Piironen & Hyvarinen 1983; Stoss 1983; Lahnsteiner et al 1993). Ovarian fluid also contains various nutrients, metabolites and hormones (Hirano et al 1978; Lahnsteiner et al 1995; Ingermann et al 2001). Some author have been shown that protein and carbohydrate fractions of ovarian

fluid prolonged the motility of *Oncorhynchus mykiss*) sperm (Yoshida & Nomura 1972) but, Na^+ and K^+ levels have statistically significant positive and negative relations, respectively, with the percentage of motile cells (Lahnsteiner et al 1996). Koya et al (1993), found that Elkhorn sculpin *Alcichthys alcicornis* (Herzenstein) sperm stayed active for >60 min in ovarian fluid, much longer than in other media tested, including sea water. Atlantic cod *Gadus morhua* sperm activated in ovarian fluid swam significantly faster (mean and maximum speeds) than sperm activated in sea water alone (Litvak & Trippel 1998). They also observed that sperm swam faster in the vicinity of eggs and suggested that this may have been due to ovarian fluid on the egg surface. It has been already claimed that the motility parameters of the spermatozoa (such as the duration, intensity and the percentage of motility) are higher when the motility of spermatozoa were triggered in the ovarian fluid (Billard 1983, 1992; Turner & Montgomerie 2002). Though the mechanism by which ovarian fluid enhances sperm motility remain unknown (Morisawa 1994), it is clear that ovarian fluid generally increases the sperm movement duration in externally fertilizing fish. Little is known about the quantitative effects of ovarian fluid on other aspects of sperm movement (Turner & Montgomery 2002). The ovarian fluid surrounding the eggs during spawning may influence various aspects of fertilization, such as sperm motility characteristics (Lahnsteiner et al 2001). Increasing of the sperm motility by ceolomic fluid has been attributed mainly to the ions present in ceolomic fluid (Scott & Baynes 1980). There have been few investigations of the relation between composition of ovarian fluid and sperm motility such as Chinook salmon, *Oncorhynchus tshawytscha* (Rosengrave et al 2009) and Brown trout, *Salmo trutta caspius* (Hatef et al 2009; Hajirezaee et al 2010). In some teleost species, the percent of motile cells and duration of sperm motility altered subject to seminal fluid (Billard & Cosson 1992; Lahnsteiner et al 1996). The objective of the present study was to examine effects of the presence of ceolomic fluid of Prussian carp (*C. gibelio*) with respect to the concentrations of the major inorganic ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-), organic composition (protein and glucose) and pH. We investigated whether composition of ovarian fluid influenced sperm motility characteristic (sperm movement duration and percentage of motile spermatozoa).

Material and Method. Female and male Prussian carp were obtained from a reared hatchery at Nahar Khoran, Gorgan, Iran. To stimulate fish for spawning injected intraperitoneally: 0.5 ml kg^{-1} Ovaprim (sGnRHa+Dompridon). Milt samples were collected during the 2010 spawning season (February, March, April and May) from 31 sexually mature two-year-old male Prussian carp (TL: 17.6 ± 1.85 cm, TW: 60.43 ± 8.48 g). Fish were dried to avoid activation of sperm by water, urine and blood, and then milt was collected by applying gentle bilateral abdominal pressure. Ovarian fluid was also collected from mature two-year-old female Prussian carp during the 2010 spawning season (n= 12 February, n= 12 March, n= 12 April and n= 12 May). The ovarian fluid was then pipetted gently out of the egg batch and into screw–cap tubes with minimal head space to minimize air equilibration. Ovarian fluids were centrifuged at 3000 rpm for 8 min. The pH of ovarian fluids was immediately determined using a laboratory pH meter (pH meter, Iran T.S. co 462) and samples were frozen at -20^{0}C until the análisis moment. Two mineral (Ca^{+2} and Mg^{+2}) and two biochemical parameters (total protein and Glucose) of the ovarian fluid were measured spectrophotometric method (WPA-S2000-UV/VIS Cambridge - UK). The concentration of Na^+ and K^+ were determined with flame photometer (Jenway PFP 7, England) (standard kits from Parsazmoon, Tehran, Iran).

Sperm motility analysis. The effect of ovarian fluid on sperm motility trait was examined. Therefore, sperm motility trait in three solutions (fresh water from the reared hatchery, ovarian fluid + fresh water from the reared hatchery and ovarian fluid alone) was tested in each month during breeding season. One µl of milt was thoroughly mixed (for approximately 5 s) with 1000 µl of above solutions, and then pipetted onto a glass slide. Samples reviewed at 200× on a negative phase-contrast microscope (Leica USA) and the motility was presented as the percentage and duration of motility. The duration of sperm motility was measured immediately after initiation of sperm activation until 100 % spermatozoa were immotile and expressed as sperm movement duration. Only

forward moving sperm were judged motile, those simply vibrating or turning on their axes was considered immotile (Aas et al 1991).

Statistical analysis. Data analysis of variance (ANOVA) was done with Duncan test for the comparison of mean values resulting from the various treatments at a significance level of $P< 0.05$. Before analysis by ANOVA, data was used for normality of data distribution and homogeneity of variance. The data of sperm motility durations showed a normal distribution and were then analyzed with ANOVA with subsequent Duncan test for the comparison of mean values resulting from the various treatments at a significant level of $P< 0.05$. Results are presented as mean ± SD. Statistical analyses were performed with SPSS 16 for windows statistical package.

Results and Discussion. Ionic and biochemical compositions of the ovarian fluid of Prussian carp are presented in Table 1.

Table 1

The ionic and biochemical compositions of the ovarian fluid in Prussian carp

Parameters	*February*	*March*	*April*	*May*
Sodium (mM L^{-1})	133.4 ± 4.4[a]	134 ± 2.1[a]	129 ± 5.2[a]	136 ± 3.6[a]
Potassium (mM L^{-1})	2.3 ± 0.1[a]	2.7 ± 0.42[a]	2.1 ± 0.24[a]	2.5 ± 0.36[a]
Calcium (mM L^{-1})	0.51 ± 0.2[a]	0.49 ± 0.36[a]	0.54 ± 0.42[a]	0.52 ± 0.32[a]
Magnesium (mM L^{-1})	0.68 ± 0.20[a]	0.65 ± 0.26[a]	0.66 ± 0.15[a]	0.64 ± 0.32[a]
Chloride (mM L^{-1})	136.3 ± 4.2[a]	131.7 ± 5.4[a]	135.4 ± 3.6[a]	138.2 ± 2.4[a]
Glucose (mM L^{-1})	3.6 ± 0.42[a]	3.2 ± 0.76[a]	3.7 ± 3.6[a]	3.8 ± 0.23[a]
Total protein (gdL^{-1})	3.25 ± 0.86[a]	3.68 ± 0.41[a]	3.1 ± 0.23[a]	3.4 ± 0.36[a]
pH	8.2 ± 0.1[a]	8.3 ± 0.2[a]	8.2 ± 0.2[a]	8.2 ± 0.1[a]

Values with the same superscript are not significantly different.

A higher duration of sperm motility was observed after triggering the motility in ovarian fluid and mixture of ovarian fluid with fresh water compared to that observed in freshwater during spawning season (Fig. 1) ($P< 0.05$), but no significant difference was observed between ovarian fluid and the mixture of ovarian fluid and fresh water ($P> 0.05$). The maximum and minimum sperm movement duration (59.23 ± 10.17 and 27.31 ± 8.5) in ovarian fluid (February) and fresh water (March) were recorded respectively.

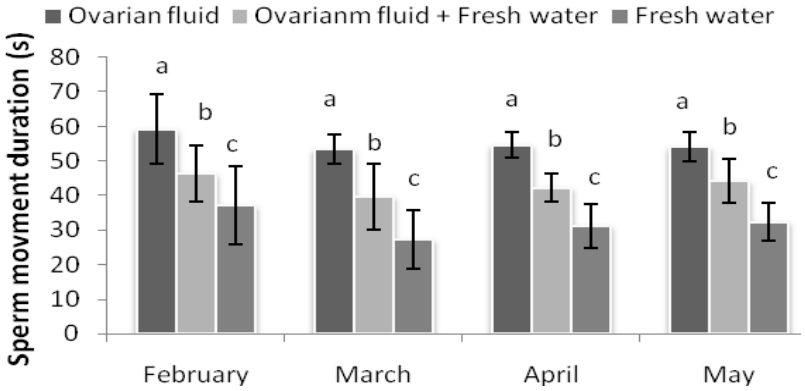

Figure 1. Duration of sperm motility in *Carassius gibelio* after triggering sperm motility in ovarian fluid, ovarian fluid+ fresh water and fresh water alone; significant differences are indicated with different superscripts.

The dynamics of the percentage of motile spermatozoa as a function of time post activation is shown in Figure 2. The percentage of motile sperm had not significant changes (P> 0.05) in ovarian fluid compared to those of ovarian fluid + fresh water and the fresh water alone.

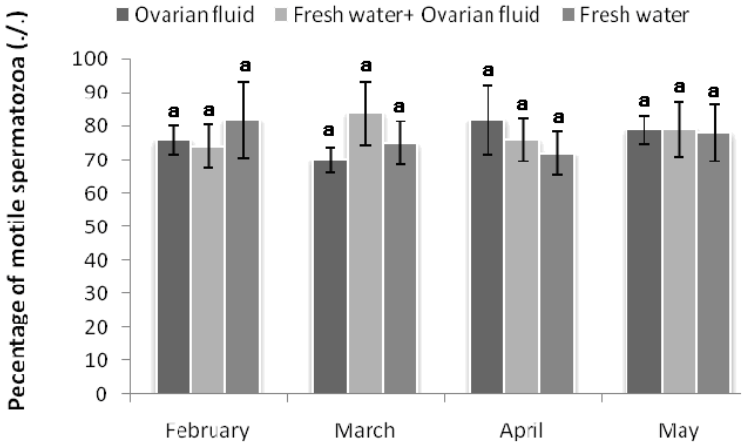

Figure 2. Percentage of motile spermatozoa in *Carassius gibelio* after triggering sperm motility in ovarian fluid, ovarian fluid + fresh water and freshwater alone. Values with the same superscript are not significantly different.

To our knowledge, this is the first study showing the composition of the ovarian fluid in Prussian carp. In teleost fish, sperm motility is one of the biomarkers used for assessment of sperm quality (Lahnsteiner et al 1998). The present results are showed that ovarian fluid enhances sperm movement duration (Fig. 1). It has already been demonstrated that sperm motility is a key factor determining the fertilizing ability of sperm (Billard 1992; Billard et al 1995). Turner & Montgomery (2002) found that effects on sperm movement were enhanced with increasing concentrations of ovarian fluid. It is not found which component(s) in ovarian fluid enhances sperm motility. Billard (1983) claimed that some substances in ovarian fluid or seminal plasma protect sperm. The protein and carbohydrate fractions of ovarian fluid (Yoshida & Nomura 1972; Lahnsteiner 2001) or pH enhance the motility (Wojtczak et al 2007). Litvak & Trippel (1998) observed that sperm of Atlantic cod swam 30% faster in ovarian fluid at 30 s post-activation than in salt water. In addition, some chemical constituents of ovarian fluid influence ATP metabolism such that the rate and duration of energy production are increased (Turner & Montgomerie 2002). In our recent work, we found that duration of sperm motility was higher in ovarian fluid than fresh water. This may be due to composition of female ovarian fluid (Rosengrave et al 2009), or some chemical constituents of ovarian fluid influence ATP metabolism such that the rate and duration of energy production is increased (Perchec et al 1995; Lahnsteiner et al 1997). More work is needed to identify the cellular processes involved (Ward & Kopf 1993; Morisawa 1994). In our study, although ovarian fluid enhanced sperm movement duration, there was no effect on percentage of motile spermatozoa. In contrast, Hatef et al (2009) reported that percentage of motile spermatozoa in *Salmo trutta caspius* were significantly higher in ovarian fluid than freshwater. Also Elofsson et al (2003) showed that percentage of motile spermatozoa in stickleback presented no difference between sperm diluted in sea water alone or sea water with addition of ovarian fluid in agreement with our results. Recent studies have shown that the ovarian fluids of Prussian carp females enhance the sperm motility characteristics that is similar to previous researches (Urbach et al 2005; Dietrich et al 2008; Rosengrave et al 2009). This study suggested that ovarian fluid or (ovarian fluid + fresh water) can be used for increasing sperm movement. It is also useful for the triggering of sperm motility in Prussian carp and enhances the fertilizing ability of sperm.

Conclusions. This study suggested that ovarian fluid (or ovarian fluid + fresh water) can be used for increasing sperm movement. It is also useful for the triggering of sperm motility in Prussian carp and enhances the fertilizing ability of sperm.

Acknowledgements. The authors would like to thanks the staff of the fish Aquaculture station, Gorgan, Iran. The authors wish to thank staff of central laboratory in Gorgan University.

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Received: 15 August 2010. Accepted: 06 September 2010. Published online: 07 September 2010.
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How to cite this article:
Taati M. M., Mehrad B., Shabani A., Golpour A., 2010 Investigation of chemical composition of ceolomic fluid and its effect on sperm motility traits in Prussian carp, *Carassius gibelio,* during spawning season. AACL Bioflux **3**(3):227-232.