

# Changes in Sperm Quality of Silver (*Hypophthalmichthys molitrix*) and Bighead Carps (*Hypophthalmichthys nobilis*) during the Spawning Season

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## Abstract

This study investigated the changes in spermatological parameters and biochemical composition of the seminal plasma of silver carp *Hypophthalmichthys molitrix* and bighead carp *Hypophthalmichthys nobilis* across three spawning seasons (early, mid and late). While silver carp had similar levels of motile sperm in early and mid seasons (early: 96±1%; mid: 95±1%), highest motile sperm in bighead carp was found only in the early season (95±1%). Sperm concentration in the three spawning seasons were  $3.15 \pm 0.02 \times 10^9 \text{ mL}^{-1}$  (early);  $2.91 \pm 0.04 \times 10^9 \text{ mL}^{-1}$  (mid);  $2.60 \pm 0.14 \times 10^9 \text{ mL}^{-1}$  (late) in silver carp and  $2.96 \pm 0.03 \times 10^9 \text{ mL}^{-1}$  (early);  $2.79 \pm 0.02 \times 10^9 \text{ mL}^{-1}$  (mid);  $2.65 \pm 0.12 \times 10^9 \text{ mL}^{-1}$  (late) in bighead carp. In silver carp, fertilization rate observed across spawning seasons were 75±3% (early); 61±1% (mid); 61±2% (late). However, drastic changes in fertilizability was observed in bighead carp (early: 79±3%; mid: 60±3% and late: 25±2%). Of the seminal plasma components, chloride and total protein levels were significantly changed in both fishes over seasons. The results of this study have implications in improving the quality of fish seed by improving the quality of fish sperm.

## Introduction

Silver carp *Hypophthalmichthys molitrix* and bighead carp *Hypophthalmichthys nobilis*, respectively, are top one and six of all cultured fin fishes globally producing 3.78 and 2.32 million tonnes annually (FAO, 2008). Both are alien species in Bangladesh and contributed nearly 12% of the country's total fish yield (FRSS, 2008). However, nearly 97% of the total hatchlings (350.03 tonnes spawn) are being produced in 756 hatcheries (DoF, 2007). But production and supply of fish spawn from these hatcheries are not reliable in terms of quality and quantity in relation to the demand particularly in the late spawning season. Poor fry/fingerling production performance between spawning seasons results in higher production cost, lower quantity of seed and lesser access by the end users.

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Silver and bighead carps spawn in late spring and summer (April-September) when water temperature is relatively high. But they do not spawn equally between early and late seasons of reproductive cycles. The rate of fertilization has been observed only 10-15% in August-September (late season) compared to over 80% in April-May-June (early season) period (Abdul Halim, Hatchery Manager, Jagorany Chakra Fish Hatchery, Jessore, Bangladesh; pers. comm. 2006). While milt from a single male has been found to be enough to fertilize the eggs of two or three females in April-June period, milt from four or five males has resulted in even lower than 10% fertilizability in August-September period. This lower fertilizability in the late spawning season could have resulted from poor quality of sperms. As a result, hatchery owners cannot produce the required quantity of fish seed in the latter season, which would otherwise be over-wintered, and used for stocking into pond, by taking the advantage of earlier beginning of the culture season (February-March). Early beginning of stocking expands the culture duration of warmer season, which will in turn increase fish yield.

Sperm quality plays an important role by influencing the fertilization of eggs. However, fish farming industries have focused mostly on the quality of eggs and larvae rather than that of sperm (Rurangwa et al. 2004). However, production of healthy larvae does not depend only on the quality of eggs but also on the quality of sperm. Good quality sperm can ensure the production of valuable offspring for aquaculture (Bromage and Roberts, 1995).

Sperm qualities have been well studied in salmonids and in some fresh water fishes (Morisawa et al. 1983b; Billard et al. 1995; Alavi et al. 2008). While Morisawa et al. (1983a) has demonstrated the effect of potassium and osmolality on the sperm motility of common carp *Cyprinus carpio*; crucian carp *Carassius carassius* and gold fish *C. auratus*, sperm biology of common carp has been reported by Billard et al. (1995). Bozkurt et al. (2008) has reported the spermatological parameters (sperm volume, motility, density and pH) and their relationships with seminal plasma composition in grass carps *Ctenopharyngodon idella* emphasizing on the sperm motility. Alavi et al. (2008) have also demonstrated a seasonal change in sperm morphology, density, motility and seminal plasma composition in the barbell, *Barbus barbus*.

Changes in sperm quality over seasons have also been reported in some fishes other than carps. For example, in rainbow trout *Oncorhynchus mykiss*, sperm motility and seminal sodium, potassium, calcium, magnesium, chloride and osmolality have been reported by Morisawa et al. (1983b). In jundia, *Rhamdia quelen*, Borges et al. (2005) have found annual variations in semen characteristics and seminal plasma biochemical composition. Rouxel et al. (2008) have observed maximum sperm concentration and seminal fluid components (pH, protein, Na<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>) in the middle period of spawning season in Atlantic cod, *Gadus morhua*. However, changes in sperm quality across spawning seasons in silver and bighead carps have never been reported. The current study was, therefore, designed to investigate the changes of sperm quality, and physical and biochemical characteristics, of silver and bighead carps' semen across three spawning seasons in Bangladesh.

## Materials and Methods

### *Experimental Fishes*

Silver ( $57.9 \pm 2.2$  cm;  $2.2 \pm 0.2$  kg; mean  $\pm$  SEM) and bighead carps ( $61.9 \pm 0.6$  cm;  $2.8 \pm 0.2$  kg; mean  $\pm$  SEM) broods of Bangladesh Rural Advance Committee (BRAC, an NGO ) Fish and Prawn Hatchery, Rajendrapur, Gazipur, Bangladesh were used as the experimental fishes. A total of 50 (25 male and 25 female) fishes were used as the stock animal. This study was undertaken during the commercial operation of this hatchery between April and September 2009 dividing the spawning season of silver and bighead carps into three seasons as early (April to May), middle (June to July ) and late (August to September).

### *Conditioning and hormonal induction of brood fishes*

Selected brood fishes were conditioned by holding them in 1,600 L flow-through-tank system for a period of 6 hr prior to induction by hormone. Male and female broods were held in different holding tanks and given mechanical aeration. Hormonal solution was prepared and applied to the brood fishes following standard protocols (Chondar, 1994). Human chorionic gonadotropin (HCG; Fuda Hormone Factory, Xiamen, China) and the extract of the commercially available pituitary gland (PG; Ducamar Company, Cantabria, Spain) were used as stimulating agents. Female fishes were given a single dose of  $200 \text{ IU HCG} \cdot \text{kg}^{-1}$  body weight (BW) as stimulating dose and  $500 \text{ IU HCG} + 3 \text{ mg PG} \cdot \text{kg}^{-1}$  BW as the resolving dose. In males,  $2 \text{ mg PG} \cdot \text{kg}^{-1}$  BW was applied.

### *Milt and egg collection*

Milt samples were collected from sexually matured males in sterilized plastic vials by pressing gently on the abdomen. Samples were drawn every week during the entire study period with three replicates. Males were used repeatedly across all three seasons. However, eggs were collected from matured female broods into a plastic bowl by stripping immediately after ovulation and mixed with milt for fertilization. Rate of fertilization was measured 8 hr after fertilization by examining a minimum of 150 eggs per replicate following the technique described by Butts et al. (2009).

### *Detection of sperm motility (%)*

Sperm motility was determined immediately after collection of fresh milt sample. Freshly collected milt sample was diluted with 0.3% NaCl (activating solution) at 10:1 ratio (10 mL activation solution and 1 mL milt sample). Diluted samples (4-5  $\mu\text{L}$ ) was placed on a slide covered by a cover slip (22 $\times$ 22 mm) and sperm motility was determined using a microscope (biomicroscope, XSZ21-5DN, China) connected with a laptop (DELL, Germany) at 1600x magnification and expressed as percentage.

### ***Determination of sperm concentration ( $\times 10^9 \text{ mL}^{-1}$ )***

Sperm concentration was determined using improved Neubauer counting chamber (area 1 mm<sup>2</sup> and depth 0.1 mm) (Germany). Milt sample was diluted with 0.3% NaCl solution at 1000:1 ratio. The diluted sample was placed on the counting cells of a Neubauer chamber with a cover slip and was left for approximately 10 min to allow the sperm cells to be settled on the counting chamber. The sperm cells were counted by a compound light microscope (LABOMED CXRII, USA) at 160x magnification connected to a CCD camera (Unican, HV-2616, Japan) and picture was displayed on a monitor (Samsung 17 inch CRT monitor, Japan) through a TV card (PERPECT Smart Power, China). Sperm concentration was calculated following Alavi et al. (2006).

### ***Seminal plasma assay***

Milt samples were centrifuged (REMI RM12C, Laboratory Centrifuge, Germany) for 10 min at room temperature at 8,000 g to separate the seminal plasma and preserved at -20 °C until analysis. While seminal pH was measured using standard pH electrodes after 30 min of sampling, mineral and organic components (Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, Ca<sup>2+</sup>, total protein, albumin and cholesterol) were measured following the method described by Hatef et al. (2007).

### ***Data Analysis***

All percent data were transformed into square root before statistical analysis, while sperm concentration data were transformed into natural log. Data were analyzed using ANOVA followed by Tukey's HSD post hoc for multiple comparisons. Data have been presented as mean  $\pm$  SEM and analyzed by using the statistical software SPSS version 10.0 with the level of significance at  $p < 0.05$ .

## **Results**

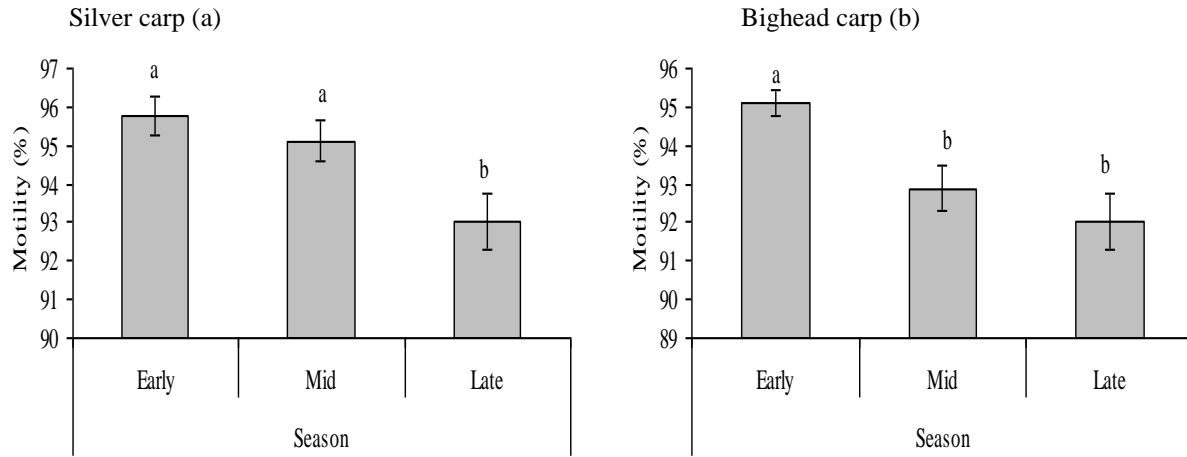
### ***Sperm motility (%)***

In silver carp, early (95.78 $\pm$ 0.49%) and mid seasons (95.11 $\pm$ 0.54%) had similar but significantly higher level of motile sperm than did the late season (93 $\pm$ 0.73%; Fig. 1a). Unlike silver carp, bighead carp had significantly higher sperm motility (95.11 $\pm$ 0.35 %) only in the early season than in the other two seasons (mid: 92.89 $\pm$ 0.59 %; late: 92 $\pm$ 0.73%; Fig. 1b).

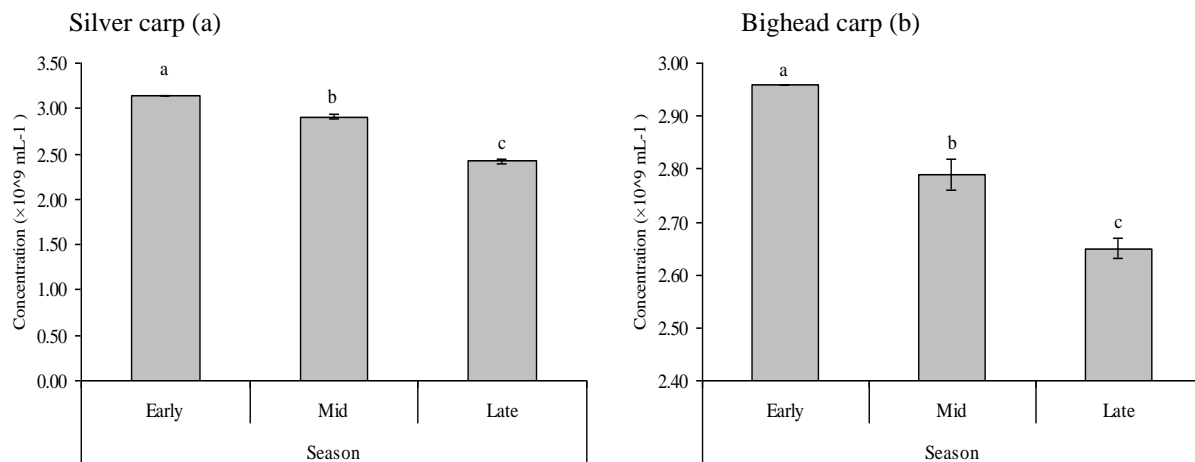
### ***Sperm concentration ( $\times 10^9 \text{ mL}^{-1}$ )***

Sperm concentration declined in each sequential reproductive season both in silver and bighead carps as the spawning season progressed. But the most remarkable difference was observed in bighead carp. In both fishes, sperm concentration was significantly changed over seasons. While the highest sperm concentration was found in the early season (silver carp: 3.15 $\pm$ 0.02 mL<sup>-1</sup>; bighead

carp:  $2.96 \pm 0.03 \text{ mL}^{-1}$ ), the late season had the lowest (silver carp:  $2.42 \pm 0.14 \text{ mL}^{-1}$ ; bighead carp:  $2.65 \pm 0.12 \text{ mL}^{-1}$ ; Fig. 2a, b).



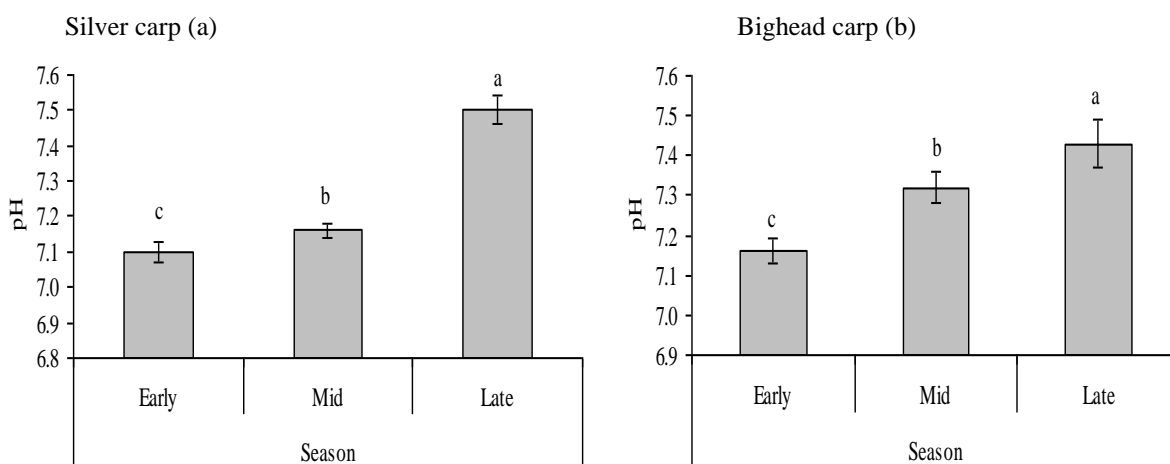
**Fig. 1 (a, b).** Sperm motility rate (%) in silver carp and bighead carp found during the regular commercial operation in Rajendrapur BRAC hatchery, Bangladesh in three spawning seasons. Milt samples were taken every week in triplicate. Bars (mean  $\pm$  SEM) with different letters denote significant differences (ANOVA, HSD;  $p < 0.05$ ).



**Fig. 2 (a, b).** Sperm concentration ( $\times 10^9 \text{ mL}^{-1}$ ) in silver carp and bighead carp found during the regular commercial operation in Rajendrapur BRAC hatchery, Bangladesh in three spawning seasons. Milt samples were taken every week in triplicate. Bars (mean  $\pm$  SEM) with different letters indicate significant differences (ANOVA, HSD;  $p < 0.05$ ).

### Seminal pH

Level of seminal plasma pH had an increasing trend over the seasons in both species. In both fishes, pH level was found highest in the late season (silver carp:  $7.50 \pm 0.04$ ; bighead carp:  $7.43 \pm 0.06$ ), while the lowest level was observed in the early season (silver carp:  $7.10 \pm 0.03$ ; bighead carp:  $7.16 \pm 0.03$ ; Fig. 3a, b). Higher level of pH was also detected in the mid season (silver carp:  $7.16 \pm 0.02$ ; bighead carp:  $7.32 \pm 0.04$ ) than did the early.



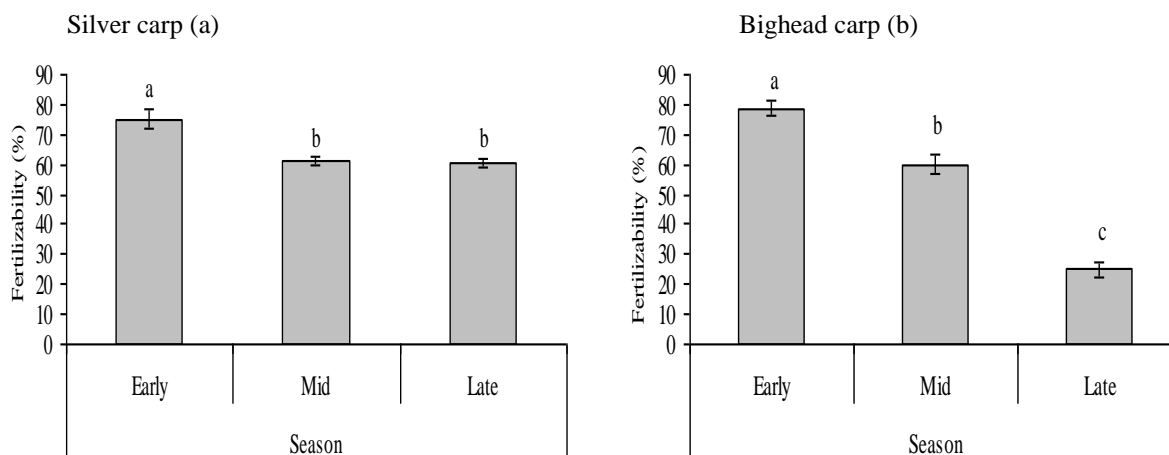
**Fig. 3 (a, b).** Seminal pH in silver carp and bighead carp found during the regular commercial operation in Rajendrapur BRAC hatchery, Bangladesh in three spawning seasons. Milt samples were taken every week in triplicate. Bars (mean  $\pm$  SEM) with different letters are significantly different (ANOVA, HSD;  $p < 0.05$ ).

### Fertilizability (%)

In both fishes, early season had significantly higher level of fertilizability (silver carp:  $75.10 \pm 3.25\%$ ; bighead carp:  $78.72 \pm 2.55\%$ ) than did the other two seasons (silver carp, mid:  $61.37 \pm 1.31\%$ ; late:  $60.73 \pm 1.41\%$ ) except bighead in which the lowest level was found in the late (mid:  $59.92 \pm 3.39\%$ ; late:  $24.85 \pm 2.33\%$ ; Fig. 4a, b).

### Sodium ( $\text{Na}^+$ mM L<sup>-1</sup>)

In silver carp, a significant difference in seminal plasma  $\text{Na}^+$  ion concentration was detected in all three spawning seasons. While the highest level of  $\text{Na}^+$  ion was found in the early season, late season had the lowest level. The level of seminal  $\text{Na}^+$  found in the mid season was higher than in the late but lower than did the early season (Table 1a). Unlike silver carp, bighead carp had similar levels of  $\text{Na}^+$  both in the early and mid seasons with the lowest level in the late season (Table 1b).



**Fig. 4 (a, b).** Fertilization rate (%) in silver carp and bighead carp found during the regular commercial operation in Rajendrapur BRAC hatchery, Bangladesh in three spawning seasons. Milt samples were taken every week in triplicate. Bars (mean  $\pm$  SEM) with different letters denote significant differences (ANOVA, HSD;  $p < 0.05$ ).

**Table 1 (a & b).** Biochemical composition of seminal plasma of silver and bighead carps sampled during regular commercial operation of BRAC hatchery, Rajendrapur, Gazipur, Bangladesh in three spawning seasons. Milt samples were taken every week in triplicate. Means ( $\pm$  SEM) within row with different letters are significantly different (ANOVA, HSD;  $p < 0.05$ ).

#### 1a: Silver carp

Variables	Seasons		
	Early	Mid	Late
Sodium ( $\text{Na}^+$ mM L <sup>-1</sup> )	105.16 $\pm$ 3.48 <sup>a</sup>	97.33 $\pm$ 3.77 <sup>b</sup>	95.60 $\pm$ 4.33 <sup>c</sup>
Potassium ( $\text{K}^+$ mM L <sup>-1</sup> )	44.69 $\pm$ 2.52 <sup>b</sup>	48.20 $\pm$ 2.36 <sup>a</sup>	43.42 $\pm$ 0.34 <sup>b</sup>
Chloride ( $\text{Cl}^-$ mM L <sup>-1</sup> )	122.46 $\pm$ 5.66 <sup>a</sup>	117.35 $\pm$ 2.51 <sup>b</sup>	113.46 $\pm$ 4.50 <sup>c</sup>
Calcium ( $\text{Ca}^{2+}$ mM L <sup>-1</sup> )	2.98 $\pm$ 0.17 <sup>b</sup>	3.08 $\pm$ 0.12 <sup>a</sup>	2.91 $\pm$ 0.08 <sup>c</sup>
Total protein (mg dL <sup>-1</sup> )	213.88 $\pm$ 9.49 <sup>a</sup>	144.66 $\pm$ 6.12 <sup>b</sup>	136.00 $\pm$ 10.11 <sup>b</sup>
Albumin (mg dL <sup>-1</sup> )	63.00 $\pm$ 1.50 <sup>a</sup>	56.44 $\pm$ 1.66 <sup>b</sup>	53.66 $\pm$ 4.80 <sup>b</sup>
Cholesterol (mM L <sup>-1</sup> )	3.82 $\pm$ 0.17 <sup>a</sup>	3.18 $\pm$ 0.13 <sup>b</sup>	2.91 $\pm$ 0.15 <sup>b</sup>

#### 1b: Bighead carp

Sodium ( $\text{Na}^+$ mM L <sup>-1</sup> )	101.81 $\pm$ 4.98 <sup>a</sup>	97.52 $\pm$ 4.80 <sup>a</sup>	93.53 $\pm$ 5.08 <sup>b</sup>
Potassium ( $\text{K}^+$ mM L <sup>-1</sup> )	46.13 $\pm$ 3.24 <sup>b</sup>	48.24 $\pm$ 3.61 <sup>a</sup>	48.29 $\pm$ 2.71 <sup>a</sup>
Chloride ( $\text{Cl}^-$ mM L <sup>-1</sup> )	118.75 $\pm$ 6.38 <sup>a</sup>	114.66 $\pm$ 3.46 <sup>b</sup>	112.22 $\pm$ 2.48 <sup>c</sup>
Calcium ( $\text{Ca}^{2+}$ mM L <sup>-1</sup> )	3.57 $\pm$ 0.15 <sup>a</sup>	3.54 $\pm$ 0.09 <sup>a</sup>	3.24 $\pm$ 0.20 <sup>b</sup>
Total protein (mg dL <sup>-1</sup> )	175.00 $\pm$ 8.26 <sup>b</sup>	180.33 $\pm$ 4.84 <sup>a</sup>	176.00 $\pm$ 6.80 <sup>b</sup>
Albumin (mg dL <sup>-1</sup> )	53.88 $\pm$ 2.85 <sup>b</sup>	57.33 $\pm$ 1.95 <sup>a</sup>	53.00 $\pm$ 3.46 <sup>b</sup>
Cholesterol (mM L <sup>-1</sup> )	2.61 $\pm$ 0.11 <sup>a</sup>	2.31 $\pm$ 0.16 <sup>b</sup>	2.06 $\pm$ 0.21 <sup>c</sup>

***Potassium ( $K^+$  mM L<sup>-1</sup>)***

In silver carp, the highest level of  $K^+$  was measured in the mid season, while the late season resulted in the lowest (Table 1a). Bighead carp had similar but significantly higher concentration of  $K^+$  both in the mid and late seasons than did in the early (Table 1b).

***Chloride ( $Cl^-$  mM L<sup>-1</sup>)***

In both fishes (silver and bighead carps), seminal  $Cl^-$  level continued to decline in the later seasons (mid and late) compared to the early season. While the highest level of seminal  $Cl^-$  ion was measured in the early season, the lowest level was measured in the late season. Mid season also had a higher level of  $Cl^-$  than in the late season (Table 1a, b).

***Calcium ( $Ca^{2+}$  mM L<sup>-1</sup>)***

In silver carp, while the highest level of  $Ca^{2+}$  was measured only in the mid season, both early and mid seasons resulted in higher level of  $Ca^{2+}$  in bighead carp than in the late (Table 1a, b). However, silver carp had significantly different levels of  $Ca^{2+}$  in the early and late seasons.

***Total protein (mg dL<sup>-1</sup>)***

While the highest level of total seminal plasma protein of silver carp was observed in the early season, significantly lower but similar level was detected in the latter two seasons (Table 1a). Unlike silver carp, bighead carp had the highest level of total seminal plasma protein in the mid season, while the other two seasons had similar but significantly lower levels (Table 1b).

***Albumin (mg dL<sup>-1</sup>)***

While silver carp had the highest level of seminal plasma albumin in the early season, bighead had in the mid. In silver carp, the latter two seasons had similar but significantly lower level of albumin. But in bighead carp, the early and late resulted in similar levels (Table 1a, b).

***Cholesterol (mM L<sup>-1</sup>)***

Both fishes had higher level of seminal plasma cholesterol in the early season compared to that of mid and late seasons except bighead had significantly lower level in the late season (Table 1a, b).



## Discussion

The observed similar sperm motility in both early and mid seasons in silver carp indicates equal responses of this fish in these seasons. Lower sperm motility in silver carp in the late season could have resulted from repeated use of the same male breeder for spawning. However, bighead carp did not respond similarly to silver carp. Single peak in early season in bighead carp indicates species difference. Bighead carp may not spawn well in excess of one time in a single season, which could be related to their breeding strategy. The first spawning had 7 months for maturation, while the latter two had shorter or even less than half the former duration for maturation. This variability in durations of maturation might have played a strong role in determining the quality of the sperm in terms of motility. The higher the duration available, the greater the chances of getting higher protein content which is the ultimate determinant of maturation and spermatogenesis. Seasonal changes in the sperm motility could also be related to fish age, nutrition and environmental conditions (Bozkurt et al. 2008). However, individual variation in sperm motility has also been demonstrated based on their ripeness (Tekin et al. 2003). The findings of this study are in agreement with the findings of Akcay et al. (2004) in mirror carp, *Cyprinus carpio* var. *specularis*.

Lower sperm concentration observed at the end of the spawning season indicates the lack of gonadal development in both fishes. The observed difference in the concentration of sperm across spawning seasons could have resulted from discontinuous spermatogenesis. Alavi et al. (2008) has demonstrated a similar seasonal change of sperm concentration ( $18.81 \times 10^9$  in March to  $12.45 \times 10^9$  mL<sup>-1</sup> in May) in barbel. Changes in sperm concentration might be related to gonadal development and maturation, which are also regulated by change in climate, day length and food supply. Seasonal changes in sperm concentration could also be related to differences in hormonal stimulation methods, environmental conditions, biological characters of the brood fish such as age and others (Piros et al. 2002).

The observed increasing trend of seminal pH over seasons in both silver and bighead carps indicates seasonal effect. This increasing trend in seminal pH in both fishes could be the result of environmental changes and complex chemistry among the seminal plasma components. No single constituent but the combined effect of all seminal plasma ingredients are responsible for this change in seminal pH throughout the spawning seasons. Lower seminal pH in the early and mid seasons indicates the best alkaline condition required for maintaining the viability of fish sperm. Similar findings have been confirmed by Emri et al. (1998) in common carp and Bozkurt et al. (2008) in grass carp.

Declining fertilization rate has been observed across spawning seasons in both silver and bighead carps. Significantly higher fertilizability observed in the first two seasons than in the late season in both fishes indicates their early responsiveness in spawning. Higher fertilizability in the early and mid seasons could have resulted because of higher sperm concentration. The lower fertilizability in the latter season perhaps could be related to less responsiveness due to

environmental requirements and gonadal maturation resulting from repeated use of the same breeder. Since silver and bighead carps begin to breed from March, and during the spawning season, same breeders are used for two or three times a year. This repeated use of the same breeder could be responsible for lower quality of sperm. Duration between spawning seasons may not be enough to get the gonads mature to produce good quality sex cells. Highest fertilizability in early season in both fishes indicates optimum response in spawning. However, higher fertilization rate than the current experimental fishes has been observed in catla, *Catla catla* (91-92%; Nandi et al. 2007).

The observed  $\text{Na}^+$  content in seminal plasma of silver and bighead carps is higher than that of common carp ( $75 \text{ mM L}^{-1}$ ; Morisawa et al. 1983a). However, in this study, seminal  $\text{K}^+$  level in both fishes are lower than in common carp ( $70 \text{ mM L}^{-1}$ ; Morisawa et al. 1983a). This difference in the seminal  $\text{Na}^+$  and  $\text{K}^+$  concentrations denotes species-specific characteristics (Ciereszko et al. 2000). The electrolytes ensure the viability of the sperm.  $\text{K}^+$  ion has a specific role in maintaining spermatozoa in quiescent state (Baynes et al. 1981). Low levels of  $\text{Na}^+$  and  $\text{K}^+$  ions are associated with low percentages of motile spermatozoa and such semen is considered to be of low quality. The observed low levels of  $\text{Na}^+$  and  $\text{K}^+$  may be related to the deficiency in the seminal plasma formation.  $\text{K}^+$  ion has an inhibitory effect on the initiation of sperm motility in salmonids (Lahnsteiner et al. 1998), which is associated with a reduction in sperm-fertilizing ability. In this study, percentage of motile sperm cells in the semen of silver and bighead carps has increased when electrolyte levels in the seminal plasma are increased.

Seminal  $\text{Cl}^-$  ion concentration of both fishes is higher than that of ocean pout ( $9.04\text{-}21.11 \text{ mM L}^{-1}$ ; Wang and Crim, 1997) but lower than in Caspian brown trout, *Salmo trutta caspius* ( $133.04 \pm 5.96 \text{ mM L}^{-1}$ ; Hatef et al. 2007). However, the  $\text{Cl}^-$  ion concentration has been reported to be in the range of  $96\text{-}110 \text{ mM L}^{-1}$  in cyprinids (Billard et al. 1995) which agrees with our findings. The observed  $\text{Ca}^{2+}$  level in the seminal plasma of silver and bighead carp was similar to brown trout, *Salmo trutta fario* ( $3.50 \pm 0.67 \text{ mM L}^{-1}$ ; Bozkurt et al. 2006). However, seminal plasma in both fishes had higher  $\text{Ca}^{2+}$  ion concentration compared to that of Caspian brown trout ( $1.68 \pm 0.20 \text{ mM L}^{-1}$ ; Hatef et al. 2007) and barbel ( $0.3\text{-}0.4 \text{ mM L}^{-1}$ ; Alavi et al. 2008). This variable concentration of seminal  $\text{Cl}^-$  and  $\text{Ca}^{2+}$  ion concentrations could be related to the secretion of seminal plasma from the spermatid duct epithelium (Lahnsteiner et al. 1993). The difference could also be related to several parameters including spawning season of the fish species and contamination of semen by urine during stripping (Suquet et al. 1994).

In the present study, the observed high concentration of total protein and albumin in the seminal plasma in both fishes indicates a high demand for protein. High level of protein might have a protective role in sperm motility. However, specific role of protein and albumin in fish semen is unknown. Seminal plasma protein prolongs the viability of spermatozoa as measured by sperm motility (Lahnsteiner et al. 2004). In this study, seminal cholesterol level of both fishes does not change much across spawning seasons. However, cholesterol could have a protective effect against environmental changes (especially on temperature) that might occur upon releasing fish semen into

water. The analysis of physical and bio-chemical components of semen revealed some species-specific characteristics, particularly  $K^+$  and protein levels.

## Conclusion

We found that the quality of sperm changes in both species as the spawning seasons progressed. No single factor is responsible for change in sperm quality. However, age and size of the breeders, repeated use of the same breeder over seasons, nutritional requirement, stocking density, hormonal manipulation and environmental changes together or individually may affect the sperm quality that changes between spawning seasons.

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