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Milt quality determination of a critically endangered fish, Olive barb (*Puntius sarana*, Ham.) in Bangladesh

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Abstract
The present study was aimed to evaluate the characteristics of the olive barb sperm. Milt was collected fortnightly from 49 male fish (mean weight 90.8 g and length 18.64 cm) from April to July in 2008. In the olive barb ejaculated milt, volume (μl/g), motility (%), duration of motility (s), concentration ($\times 10^{10}$/ml) and pH values were found to be 6.06±0.32, 88.27±0.71, 171.41± 7.41, 5.16±0.05 and 7.75±0.04, respectively. Milt volume was significantly ($P<0.05$) correlated with sperm concentration. Milt volume, sperm concentration, motility and duration of motility significantly varied ($P<0.05$) during spawning season.

Key words: *Puntius sarana*, Endangered, Milt quality

Introduction

Olive barb, *Puntius sarana* (Hamilton 1822), locally known as sarpunti is a very popular barb in Bangladesh and now critically endangered. It is the largest barb available in the Indian sub-continent. Though the fish was abundantly available in our open water system in the past, due to over exploitation and various ecological changes in its natural habitats, it is now critically endangered in Bangladesh (IUCN 2000). This important food fish will disappear from Bangladesh unless proper steps are urgently taken for development of appropriate aquaculture techniques along with proper conservation strategies.

Aquaculture farming requires good quality seed and for that proper management of male brood stock is a prerequisite .Management of broodstock is highly species-specific and its success depends upon many factors. (Billard *et al.* 1995). Determination of sperm density has been used to evaluate the sperm quantity (Rurangwa *et al.* 2004) that may influence the fertilization (Piros *et al.* 2002). For successful breeding programme it is essential to know the sperm characteristics. Various factors affect the sperm quality of fish including season, photoperiod, collection technique, temperature, time of collection, age and disturbances in spermatogenesis (Baynes and Scott 1989, Cabrita *et*

al. 2001, Asturiano *et al.* 2005). Sperm volume, spermatozoa concentration, percentages of motile sperm, sperm pH are usually considered for evaluating sperm quality (Bloom and Ottobre 2001; Tekin *et al.* 2003). No information is available pertinent to sperm quality of olive barb *Puntius sarana* in Bangladesh. Hence, the objective of the study was to evaluate the sperm quality of olive barb *Puntius sarana*.

Materials and methods

In the present study, *Puntius sarana* was collected from two natural depressions (Chalan *beel*, Natore district and Tola *haor*, Netrokona) during the month of January and February, 2008. The average size of male fish was 70g and 110g in Chalan *beel* and the Tola *haor*, respectively. The collected fish were reared in the ponds of Fisheries Field Complex, Bangladesh Agricultural University, Mymensingh. Fish were collected from the brood rearing ponds and kept in the tank of the hatchery of Faculty of Fisheries. Then collected broods were injected with PG at the rate of 2 mg PG/kg body weight for easy collection of milt samples. For sperm collection at first males were fished out from the tank using scoop net. Then fish was laid on the foam fixing in dorsal position and urogenital pore was wiped. Gentle pressure was applieed through the abdomen to remove urine, water, gut exudates and mucus and these were removed with tissue as much as possible for avoiding contamination. A 3 ml plastic syringe was used to collect the sperm. When the milt seemed concentrated, the mouth of the syringe was inserted into the urogenital pore to fill with sperm and then sperm was immediately transferred to the icebox. After collection, sperm samples were transported to the laboratory under cold conditions (7–10 °C). Ejaculated sperm volume was determined by the measuring pipette and expressed as μl. Milt pH were determined with a pH indicator strips (pH: 0–14; Merck, Germany). Sperm motility was evaluated visually for the percent motility (%) after activation in table salt (NaCl). The duration of motility (sec) was also recorded by stopwatch from the initial contact between the activation solution and milt until almost all of the spermatozoa (up to 20%) were immotile. One or two drop 0.9% NaCl was placed on a glass slide and then a drop of 1-2 µl fresh milt was poured to induce the initiation of motility. A light microscope (Novex K-range, Holland) was used at 400 magnifications to determine the percent motility. Sperm concentration was determined using haemacytometer (Germany) and expressed as number of cells$\times 10^{10}$/ml. Milt was diluted 4,000 times in a 0.9% NaCl solution. For preparing 4,000 times dilution, at first microtube containing 990 μl of the 0.9 % NaCl solution was taken and then 10 μl of milt was carefully added to the tube and the content was mixed carefully. From this microtube, 10 μl of milt suspension was taken out and transferred to another microtube containing 390 μl of 0.9 % NaCl solution and finally 4,000 times diluted milt was prepared. A droplet of the diluted milt was placed on a haemocytometer (depth 0.1 mm) with cover slip. The slide was left undisturbed for approximately 5 min to allow the milt cells to settle on focal plane. The number of milt in 5 large squares of the counting chamber was counted under the microscope at 40 times magnification.

Results

Evaluation of milt quality of olive barb

For evaluating the milt quality of olive barb, milt from forty nine fish of two stocks namely the Chalan *beel* and the Tola *haor* were evaluated. The size of *haor* olive barb ranged between 70 and 178 g (mean 111.69±5.73) in body weight and 18 and 24 cm (mean 20.23±0.35) in total length. On the other hand, size of Chalan *beel* olive barb ranged from 35 to 98 g (mean 67.17±2. 90) in body weight and 15 to 20 cm (mean 16.85 ±0.25) in total length. The mean (±S.E.) of percentage of motility, sperm concentration ($x10^{10}$/ml), ejaculated milt volume (μL/g) and milt pH of *haor* olive barb were found 87.12±0.97, 5.18x10^{10}±0.06, 5.27±0.36, 7.79±0.05, respectively (Table 1). In Chalan *beel* olive barb the mean of percent motility of fresh milt, sperm concentration (×1010/ml), ejaculated milt volume (μL/g) and milt pH were found 89.57±0.99, 5.15×10^{10}±0.07, 6.97±0.49, 7.70±0.05, respectively.

Table 1. Milt characteristics of olive barb milt (mean ± standard error)

Items	Stock			
	Haor	Range	Chalan *beel*	Range
Number of fish	26		23	
Weight (g)	111.69±5.73	70-178	67.17±2. 90	35-98
Length (cm)	20.23±0.35	18-24	16.85±0.25	15-20
Milt volume (μL/g)	5.27±0.36	3.11-9.73	6.97±0.49	2.04-13.0
Milt pH	7.79±0.05	7.5-8.0	7.70±0.05	7.5-8.0
Sperm concentration	5.18 x10^{10}±0.06	4.3-5.6 x10^{10}	5.15 x10^{10}±0.07	4.3-5.7 x10^{10}
Fresh motility (%)	87.12±0.97	80-95	89.57±0.99	80-95
Duration of motility (s)	177.96±10.13	110-290	164.0±10.88	70-244

It was found that weight of fish was significantly correlated ($P<0.01$, $r = 0.68$) with milt volume. There was no significant correlation of pH with other parameters (Table 2). Length of fish was also correlated ($P<0.05$, $r = 0.62$) with milt volume. Fresh milt motility and milt volume were significantly correlated with duration of milt motility ($P<0.05$, $r = 0.3$) and sperm concentration ($P<0.05$, $r = 0.36$), respectively. There were no strong correlation found among length, weight, milt pH, fresh motility and duration of motility (Table 2).

Table 2. Correlations between spermatological parameters and body traits in olive barb

	Length (cm)	Milt volume (μL/g)	Milt pH	Sperm concentration	Fresh motility	Duration of motility
Weight (g)	0.92**	0.68**	0.19	0.05	- 0.04	0.14
Length (cm)		0.62**	0.16	0.05	- 0.11	0.1
Milt volume (μL/g)			- 0.11	0.36*	0.12	- 0.09
Milt pH				- 0.05	- 0.07	- 0.03
Sperm concentration					0.28	0.25
Fresh motility						0.3*

**P<0.01, *P<0.05

At the beginning of spawning season milt volume of olive barb was lower and it gradually increased with the time (Fig. 1). During the experimental period highest milt volume of olive barb was observed in 5th fortnight (7.42±1.61) and lowest value observed in 2nd fortnight (3.94±0.54). However, milt volume was significantly varied (P<0.05) among the sampling months during the spawning season.

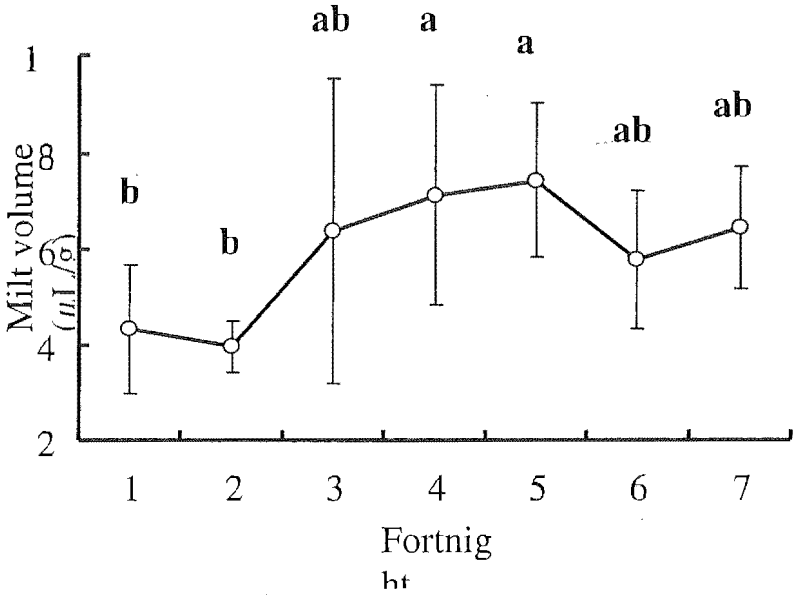

Fig. 1. Milt volume (μl/g) of olive barb at different fortnights during spawning season. Values superscripted by the same letter are not significantly different (P > 0.05). 1-2, 3-4, 5-6 and 7 fortnights represent April, May, June and July, respectively

Sperm concentration of fresh milt of olive barb was highest in 4th fortnight (5.40±0.16) of the sampling month. Like milt volume, sperm concentration was lower at the beginning of the spawning season and it gradually increased with time (Fig. 2). However, lowest concentration was found in the 7th fortnight (4.83±0.49) of the sampling months.

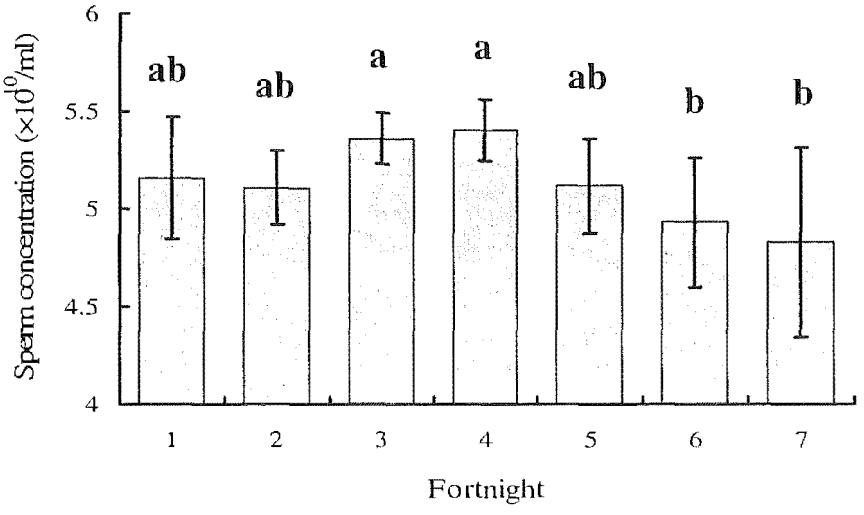

Fig. 2. Sperm concentration (x10^{10}/ml) of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significant different (P>0.05).
Data represents mean (columns) and standard deviation (bars)

Almost similar milt pH was observed in spawning season and no significant different observed in different fortnight during the experimental period. Initial milt motility was rather low at the beginning of the season and a high variation (P<0.05) in milt motility of olive barb fresh milt was found among different fortnight of the sampling months. The milt motility was highest in the 4th fortnight (92.5±2.67) and lowest in the 1st fortnight (85±3.54) during the sampling month (Fig. 3).

Duration of motility (s) of olive barb was variable (P<0.05) among the fortnight during the experimental period. Duration of motility (up to 20% motility) gradually increased and observed highest in the 4th fortnight (222.38 ± 40.91) and then decreased (Fig. 4). However, lowest duration of motility was observed in the 1st fortnight (127± 34.02) of the sampling months.

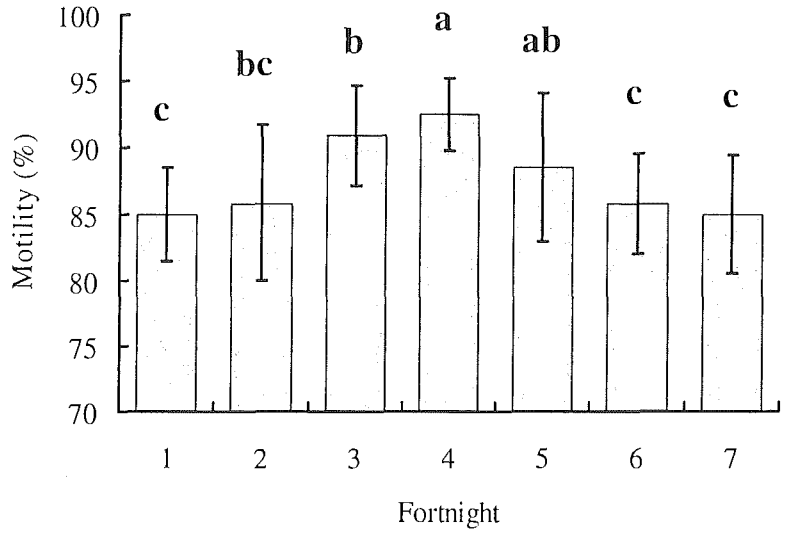

Fig. 3. Percentage of sperm motility of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significantly different (P > 0.05).
Data represents mean (columns) and standard deviation (bars)

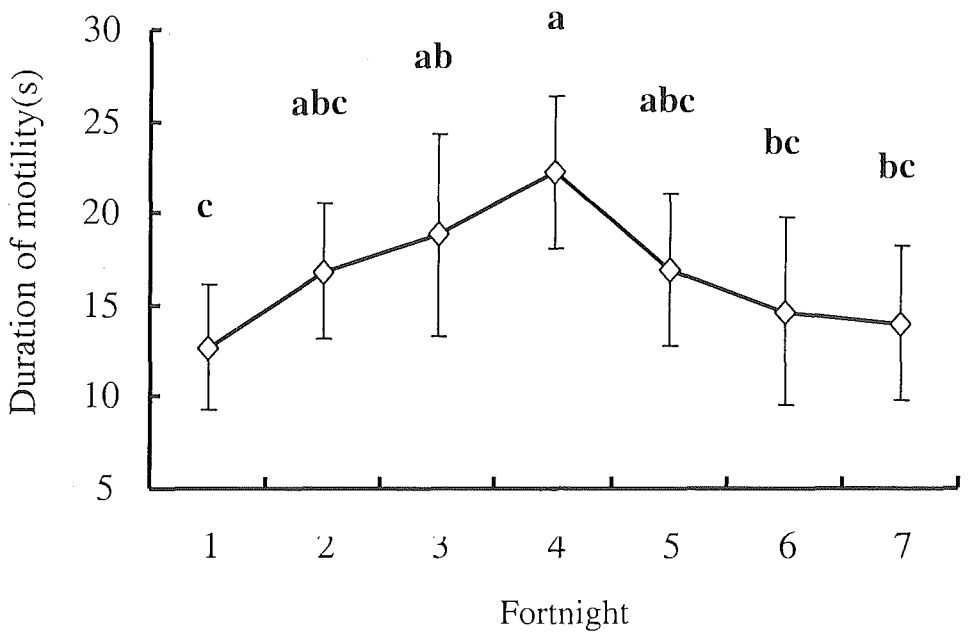

Fig. 4. Duration of sperm motility (s) of olive barb at different fortnights during spawning season.
Values superscripted by the same letter are not significant different (P > 0.05).
Data represent means (columns) and standard deviation (bars)

Discussion

Several factors contribute to variation in milt quality including biological characteristics of the brood stock (age, length and weight) (Trippel and Neilson 1992, Hoysak and Liley 2001), the rearing conditions for brood fish (Morisawa *et al.* 1979) and the methods of spawning induction (Caille *et al.* 2006). The milt quality of fish is changed with spawning, showing decreases in the duration of motility, percentage of motility and spermatozoa concentrations at the end of spawning (Lahnsteiner *et al.* 1998, 2005, Liley *et al.* 2002 and Aral *et al.* 2005). The present study confirms that the milt volume of olive barb was lower at the beginning of spawning season and it gradually increased with the time. During the experimental period highest ejaculated milt volume of olive barb was observed in 5th fortnight and lowest value observed in 2nd fortnight. These results suggest the milt volume changed during the spawning season. Sperm production increases from the beginning to middle of the spawning season and declines again at the end of the spawning season in many freshwater species (Lahnsteiner *et al.* 1998, Liley *et al.* 2002, Tekin *et al.* 2003 and Aral *et al.* 2005). Fish milt concentration has been assessed by three main techniques including haemocytometer counting, spermatocrit and spectrophotometry. Haemacytometer was used to determine sperm concentration in this experiment. Sperm concentration was linearly correlated with milt volume and sperm concentration was also varied between the sampling fortnights and highest in the middle of the spawning season. Fish sperm concentration is an important parameter in hatchery reproduction management and it is highly variable and depends on species, individuals, fish size, and season (Glogowski *et al.* 1999). In this study, the average milt motility of olive barb fluctuated in the sampling period. Lahnsteiner *et al.* (2005) reported that sperm motility pattern changed during spawning season. Milt motility in males could be due to either milt preparation procedures or the period of spawning season. The duration of milt motility was significantly varied in different fortnight. This result suggests that season also may impact on the duration of the motility. The milt pH in olive barb was found to be slightly alkaline in the present study. There was no significant difference observed in the milt pH among different fortnight in entire sampling months. The pH has been reported as one of the major sperm activating factors in fish species (Stoss 1983). The duration of sperm motility in *Petromyzon marinus* decreased with an increase in pH, but the percentage of motile cells did not change over the pH range 6.0–9.0 (Ciereszko *et al.* 2002). According to Ingermann *et al* (2002) on pH sensitivity of sperm motility in *Acipenser transmontanus* demonstrated that sperm maintained at high pH (more that 8.2) had appreciable motility when added to water but that the motility was inhibited when there was maintained at low pH (less than 7.5).

Conclusions

The olive barb, *Puntius sarana* farming should be expanded both for restocking in the natural habitat and aquaculture, there is an increasing need to improve the breeding process and for that largely standardized gamete management and handling. The study describes for the first time, milt characteristics of olive barb. Observation of milt characteristics represent valuable baseline information for establishing milt quality standard and provide background information that may be useful for breeding programs to save this species from extinction.

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