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Some Factors Affecting Spermatozoan Motility of Two Freshwater Fishes, *Puntius filamentosis* and *Amblypharyngodon mola*

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The effect of certain factors influencing the motility of spermatozoa of two species of fresh water fishes, *Puntius filamentosis* and *Amblypharyngodon mola* was evaluated. The biological properties and quality of semen were examined. The biological properties and quality of spermatozoan motility was estimated by activating in fresh water, distilled water, 1% glucose and fertilizing solution. The spermatozoa showed maximum duration of motility in fertilizing solution in both fishes. In *A. mola* the spermatozoan motility gradually increased from 1% seawater reaching the longest duration of motility of 1611.2 sec at 15% seawater and thereafter declined to 181 sec at 20% seawater. In *P. filamentosis* maximum duration of spermatozoan motility was observed at 5% seawater and then declined showing no motility at 20% seawater. The spermatozoa of *P. filamentosis* exhibited maximum duration of motility at 10°C and in *A. mola* at 20°C. Duration of motility increased with increase in the pH showing the maximum motility at pH 8.0 in both fishes. The spermatozoa of both fishes showed high percentage viability. The sperm cell concentration of *P. filamentosis* (20.56×10^9 sperms ml^{-1}) was less than that of *A. mola* (23.71×10^9 sperms ml^{-1}).

Key words : Spermatozoan motility, *Puntius filamentosis, Amblypharyngodon mola*.

Spermatozoa in majority of fishes are immotile within the testis as well as in the undiluted milt. The spermatozoan motility is a useful parameter to assess the quality of sperm cell. Sperm motility is initiated when semen is diluted with an activating medium such as freshwater, saline or ovarian fluid (Scott & Baynes, 1980). In the majority of freshwater fishes, the fertilization is external and spermatozoan motility may be influenced by various parameters of the external environment. Several environmental factors such as ions, pH, osmolality, salinity and temperature of the external medium affect the motility of the spermatozoa of fishes. The percentage of motile sperm has been used as a criteria for assessing potential fertility of semen (Terner, 1986). The success of fertilization depends upon the duration of motility of spermatozoa (Billard *et al.* 1993). The pH of the medium is one of the major factors regulating the sperm motility. In the experiments with different species of tilapia sperm, Chao *et al.* (1987) found that pH 7, 8, or 9 had the least adverse effect on sperm motility. Billard & Cosson (1988) indicated that in media of pH 7.5 trout sperm motility is totally inhibited but Ca^{2+} and Mg^{2+} ions can overcome this problem. In general, ions like Na^+, Ca^{2+}, Mg^{2+} and K^+ play important roles in the motility of spermatozoa. Almost all fishes show prolonged duration of spermatozoan motility in alkaline pH. Duration of sperm motility is reported to increase from acid to alkaline medium (Thorogood & Blackshaw, 1992). Duration of sperm motility is also dependent on temperature of the medium.

In the present study an attempt was made to develop a suitable activating

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medium for artificial fertilization and to evaluate the influence of salinity, temperature and pH on the duration of sperm motility of two species of fresh water fishes, *P. filamentosis* and *A. Mola.*

Materials and Methods

Male fishes of *A. mola* (Mean weight 6 g, Mean length 7.3 cm) and *P. filamentosis* (Mean weight 108 g, Mean length 9.8 cm) were used for the study. The fishes used for milt collection were starved for one day to avoid mixing of milt with the excreta. Anal region was cleaned using towel and blotting paper. Milt was collected into 1.5 ml Eppendorf tubes by applying gentle abdominal pressure. Extreme care was taken to avoid contamination of milt with blood, urine or mucus.

The duration of motility was estimated in different activating media such as fresh water (Na 16 ppm, K 16 ppm, Ca 10 ppm, Mg 0.5 ppm, Cl 19 ppm, SO_4 7 ppm and CO_2 12 ppm) 1% glucose, distilled water and fertilizing solution (3 g urea and 4 g NaCl l^{-1}, Woynarovich & Horvath, 1980). The milt and one of the above media were mixed at a ratio of 1:20 on a clean glass slide, a cover glass applied and the active forward movement of the spermatozoa were observed under a prefocussed microscope. The time at which 80% of spermatozoa in the field became immotile was recorded. Method used by Terner (1986) was followed for evaluating the duration of motility. The duration of motility of spermatozoa was noted in three fields each for five fishes and the mean motility calculated.

Spermatozoan motility was scored after activating with freshwater following an arbitrary scale (Chambeyron & Zohar, 1990), as; 0 = 100% immotile; I = <30% motile; II = 30-50% motile; III = 50-70% motile and V = >80% show active motility. Viability which is the number of live or viable spermatozoan in the milt was estimated by the standard eosin-nigrosin dye exclusion method of Chao *et al.* (1975), and expressed as a percentage of the total number of spermatozoa.

The concentration of spermatozoa was determined using improved Neubar counting chamber (Buyukhatipoglu & Holtz, 1978).

Duration of spermatozoan motility was observed in different pH levels from 5 to 9.5 with an interval of 0.5 in distilled water. pH was adjusted with 0.05 M NaH_2PO_4 from pH 5 to 7 and with 0.05 M Na_2HPO_4 from pH 7.5 to 9.5. A digital pH meter with combined glass electrode (Systronics, Model 335) was used. Effect of salinity on the duration of spermatozoan motility was determined in 1, 3, 5, 10, 15, 20 and 25% sea water in distilled water. The effect of temperature on the duration of sperm motility was estimated by activating the spermatozoa in freshwater at temperatures from 5° to 35°C at an interval of 5°C. The milt and activating media were acclimatised to the respective temperatures by storing at that temperature for one hour prior to the experiment. The temperatures from 5 to 25°C were maintained by adjusting the air conditioner, 30°C was the ambient temperature and 35°C was maintained in a water bath.

Results and Discussion

A high percentage of spermatozoan viability was observed in *P. filamentosis* (93.56%) and *A. mola* (89.01%) (Table 1). Viability is the most reliable index of the physiological condition of spermatozoa and correlates well with fertility. Several investigators have followed the assumption that the most commonly used laboratory assays for semen quality are motility and viability of the spermatozoa (Erdahl,

Table 1. Biological parameters of spermatozoa

Parameters	*Puntius filamentosis*		*Amblypharyngodon mola*	
	Mean	SD	Mean	SD
Viability, %	93.56	1.688	89.01	1.412
Spermatozoan concentration (sperms ml^{-1})	$20.56x10^9$	2.604	$23.71x10^9$	5.672
Motility Score	V		V	
Nature of milt	Viscous		Viscous	
Colour of milt	White		White	

1986; Terner, 1986). The spermatozoan concentration varies between species; some of the values reported : $16.5x10^9$ ml^{-1} in bluegill sunfish, $39.6x10^9$ ml^{-1} in carp and $45.1x10^9$ ml^{-1} in perch (Erdahl, 1986). It is reported to reach highest values during breeding season (Suquet *et al.*, 1992). The present results confirm the species-wise variation in concentration.

In both species longest duration of spermatozoan motility was observed in fertilizing solution while the shortest duration was noticed in distilled water (Fig. 1).

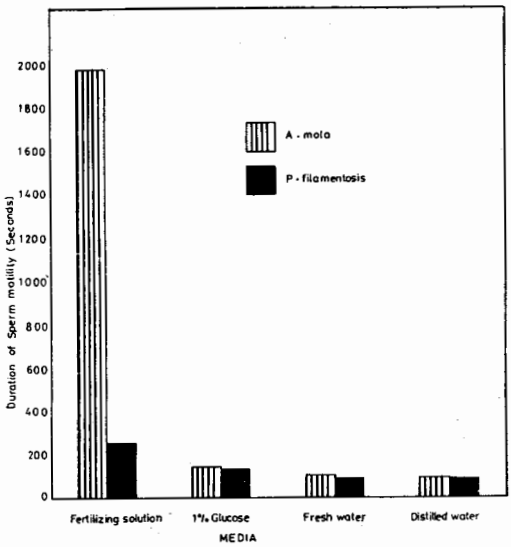

Fig. 1. Spermatozoan motility in various activating media.

The spermatozoan motility in fertilizing solution was significantly higher than ($P<0.01$) that in other media. Motility score is an important measure of milt quality. Spermatozoan motility in *Poecilia reticulata* was 60 min in ringer solution (Billard, 1978), and 1304 sec in fertilizing solution in *Anabas testudineus* (Sheeja, 1994) showing that the duration of sperm motility vary in different activating media and between fishes. In the present study also the duration of motility between *A. mola* and *P. filamentosis* varied considerably. Fertilizing medium was the best activating medium for effecting maximum fertilization of the two species studied.

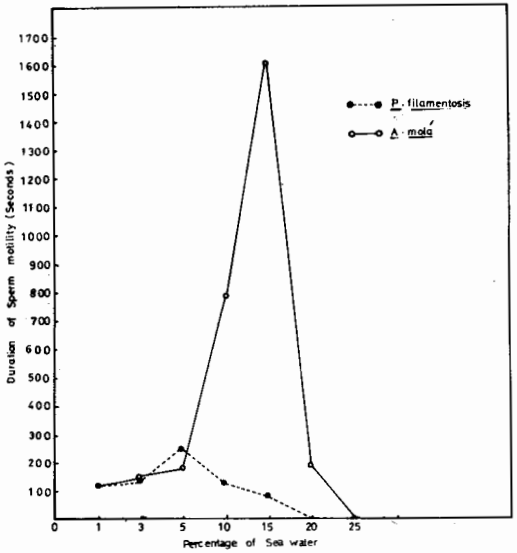

Fig. 2. Spermatozoan motility in varying concentrations of seawater.

The response in duration of sperm motility to salinity is shown in Fig. 2. The duration of motility increased at higher salinities in both fishes which may be attributed to similar osmotic concentration of the activating media (Morisawa and Suzuki, 1980) and seminal-plasma. The high osmolality of sea water may be a major factor influencing activation of spermatozoa. Solutions of NaCl and KCl between 50 and 300 mOsm initiate motility

Table 2. Duration of spermatozoan motility in distilled water at various pH

pH	*Puntius filamentosis*				*Amblypharyngodon mola*			
	Duration of sperm motility, sec		% of motile spermatozoa		Duration of sperm motility, sec		% of motile spermatozoa	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5.00	Immotile	-	-	-	-	-	-	-
5.50	66.08a	0.90	45	7.7	100.76a	7.87	45	10.6
6.00	162.08b	15.32	55	13.9	195.60b	18.90	55	11.7
6.50	332.20c	80.68	60	10.9	314.88c	45.63	65	8.9
7.00	413.80d	36.78	85	9.9	463.16d	93.60	85	6.8
7.50	498.00e	14.50	90	6.9	1375.20e	99.90	90	6.7
8.00	603.22f	21.60	95	4.4	1846.80f	53.40	95	4.9
8.50	518.20g	3.31	90	5.9	1423.20eh	57.92	85	4.8
9.00	386.48h	52.22	90	2.9	1528.00g	48.58	80	6.9
9.50	309.68i	43.20	85	2.7	1435.20h	73.29	80	5.6

Means followed by the same letter in each column do not differ significantly ($p>0.01$) (Duncan's multiple range test)

of sperm cells in *Gambusia affinis* and *P. reticulata* (Morisawa and Suzuki, 1980).

The pH of an activating medium also greatly influences the duration of motility. Duration of spermatozoan motility increased with increase in the pH showing the maximum motility at pH 8.0 for both *A. mola* and *P. filamentosis* (Table 2). The spermatozoan motility varied significantly with pH ($P<0.01$). Similarly the percentage motility of spermatozoa also varied with pH, being lesser at acidic pH and increasing as the pH increased. Experiments in tilapia have shown that pH 7, 8 and 9 had the least adverse effect on motility (Chao *et al.*, 1987). In most of the fishes the spermatozoa were immotile at pH 5. Alkaline pH was found to be more conducive to spermatozoan motility and fertilizability of carps (Jayaprakas, 1995).

Table 3. Duration of spermatozoan motility in fresh water at different temperatures

Temperature °C	*Puntius filamentosis*				*Amblypharyngodon mola*			
	Duration of sperm motility, sec		% of motile spermatozoa		Duration of sperm motility, sec		% of motile spermatozoa	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	77.36a	2.85	80	10.4	82.36a	0.26	75	8.6
10	100.92b	2.21	95	2.9	84.64b	6.26	80	10.1
15	93.56c	1.69	85	4.1	88.72c	6.35	90	4.2
20	87.64d	1.39	80	6.8	101.16d	3.34	95	3.9
25	74.00e	3.46	75	4.9	86.68e	2.48	85	5.8
30	89.32d	8.37	60	8.8	86.64e	5.75	75	5.6
35	60.40f	2.97	55	2.9	60.80f	2.75	70	6.9

Means followed by the same letter in a column do not differ significantly ($p>0.01$) (Duncan's multiple range test)

Spermatozoan motility lasted longer at cooler temperatures, viz., 10°C for *P. filamentosis* and 20°C for *A. mola* (Table 3). Longer durations of motility at 20°C have been recorded for *P. reticulata* (60 min, ringer solution) and *Sparus auratus* (6 min, seminal plasma) by Billard (1978) while even freshwater at 4°C gave a fairly long duration of 1224 sec for *A. testudineus* (Sheeja, 1994). The longer duration at sperm motility at cooler temperatures has also been confirmed by Bimal Lal (1993) for carp sperm at 10°C.

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