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# FACTORS AFFECTING THE MOTILITY AND SHORT TERM PRESERVATION OF SPERMATOZOA OF TWO SPECIES OF INDIGENOUS ORNAMENTAL FISHES, *RASBORA DANICONIUS* AND *PUNTIUS FILAMENTOSUS*

**Ani Joseph and Jayaprakas, V.***

*Dept. of Aquatic Biology and Fisheries, University of Kerala, Thiruvananthapuram, Kerala.*
***Corresponding author: jayaprakasvasu@yahoo.in***

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**Abstract:** To combat the serious threat of biodiversity, biologists, aquaculturists and specialists are trying to preserve germplasm of various fishes through gene banks. Various parameters that play major roles in the viability of spermatozoa such as, duration of sperm motility in different media, at different temperatures, in different salinities and at various pH levels were evaluated along with the influence of various factors on the duration of motility and viability of spermatozoa when preserved for short duration. High percentage of spermatozoan viability of fresh semen was observed in two species of fishes, *R. daniconius* was 92% and *P. filamentosus* was 93.6%. The sperm cell concentration of *R. daniconius* and *P. filamentosus* was 14.32 x $10^9$ and 20.56 x $10^9$ spermatozoa per ml respectively. Longest duration of spermatozoan motility was observed in fertilizing solution (25.8 and 97.6 seconds) and the spermatozoa showed least duration of motility in distilled water (11.2 and 47.2 seconds) in *R. daniconius* and *P. filamentosus* respectively. The spermatozoa showed longer duration of motility at lower temperature. The spermatozoa of *R. daniconius* showed maximum duration of motility (33.4 seconds) at 18$^0$C and the least duration of motility was observed at 30$^0$C (12 seconds). In the case of *P. filamentosus* mean duration of motility was high at 18$^0$C (94.2 seconds) and the least duration of motility was recorded at 30$^0$C. In *R. daniconius* maximum duration of spermatozoan motility was shown at pH 8.5 (14.6 seconds). The least duration of motility was observed at pH 6 (10.6 seconds). In *P. filamentosus* longest duration of motility was found in the alkaline pH 8 (38.8 seconds). In *R. daniconius* the maximum duration of motility was noticed at 1% salinity (13.6 seconds) and least duration of motility at 10% salinity (7.8 seconds). Maximum duration of motility was observed at 10% salinity (47 seconds) in *P. filamentosus* and the least duration of motility at 3% salinity (40.2 seconds). In the case of *R. daniconius* and *P. filamentosus* the spermatozoa were motile only up to 24 hours in the unoxygenated samples. In the oxygenated samples, spermatozoa of *R. daniconius* were motile only up to 25.6 seconds in the fresh milt, which was reduced to 8.2 seconds after preservation for 72 hours and in the *P. filamentosus*, the spermatozoa were motile up to 95.2 seconds in the fresh milt, which was reduced to 16.8 seconds after preservation for 72 hours. The unoxygenated sample of *R. daniconius* showed considerable reduction in the duration of sperm motility from 26 seconds to 8.4 seconds after storage for 24 hours and in the *P. filamentosus* reduction in the duration of motility was found from 95.8 seconds to 16.2 seconds.

***Key words:*** Motility, viability, spermatozoa, preservation.

## INTRODUCTION

Biologists all over the world fear that the genetic strains and variability of wild fish stocks are being depleted or drastically diminishing at a rapid pace due to overfishing, indiscriminate use of pesticides, siltation and habitat destruction. Many species of fishes are at the brim of extinction. Biologists, aquaculturists and specialists are trying to preserve germplasm of various fishes through gene banks to combat the serious threat to biodiversity.

Clear knowledge of the factors which influence, initiate and prolong motility will help to standardise a medium which yield maximum period of motility and minimum damage to spermatozoa. This would also enhance the fertilization rate. In the majority of freshwater fishes, the fertilization is external and the motility of spermatozoa is subjected to wide fluctuations in the various physico-chemical and biological parameters of the aquatic environment (Chambeyron and Zohar, 1990). Several environmental parameters such as, ions, pH, salinity, temperature affect the duration of motility of spermatozoa.

Spermatozoa concentration gives the number of spermatozoa in unit volume of milt and hence the total number of spermatozoa a fish yield can be ascertained. The rate of fertility may be highly influenced by sperm cell concentration of milt, chances of fertilization increases with high spermatozoan count. Viability of spermatozoa also determines the quality of spermatozoa. Viability depicts the live or biologically functional spermatozoa. Assessing the percent of viable sperm is essential for storage of fish sperms in banks. Sperm motility and viability are well correlated.

The success of any economically productive artificial insemination programme depends up on maximal utilizsation of available gametes. Spermatozoa may preserve for either short term or long term. The period of storage may range from a few hours to a few days in short term preservation. The preserved gametes can be in course of time used for constituting the species, if they happen to get extinct in nature (Das and Pandey, 1998). Short term preservation of fish spermatozoa is particularly useful in families with asynchronous maturation habits. Under these conditions the gametes can be collected and kept under suitable condition for later use. Air and preferably pure oxygen are most suitable for maintaining cell viability (Withler and Morley, 1968). The development of reliable techniques in gamete preservation would offer both practical and economic advantages to aquaculture.

The ornamental fishes are important not only because of their aesthetic value but also due to their immense commercial value in export trade. More than 200 species of freshwater ornamental fishes are bred in the country. The immense potential of ornamental fishes for large scale employment generation as well as increase in export earnings highlight the need for the preservation of gametes and embryos which facilitates to preserve the germplasm of endangered species of fishes.

## MATERIALS AND METHODS

### The fishes used for the study

A series of experiments were conducted to assess the effect of factors influencing the motility, viability and short term preservation of spermatozoa of selected freshwater fishes. The fishes used for the study are *Rasbora daniconius, Puntius filamentosus* (Figs. 1 & 2). Fishes were collected from Vellayani Lake in Trivandrum district of Kerala.

### Collection of milt

Milt was collected from live fishes by applying gentle pressure on the abdominal region. Care was taken to ensure that blood, faeces, urine, water or mucus did not contaminate the milt.

**Fig1.** *Rasbora daniconius*

**Fig2**. *Puntius filamentosus*

**Motility of spermatozoa**

An attempt was made to find out a suitable medium in which the duration of motility could be prolonged. Various activating media like freshwater, distilled water, 1% glucose solution, 0.35% saline and fertilizing solution (3 g urea and 4 g NaCl in one litre distilled water) were used to activate the spermatozoa. The milt and fertilizing solution were mixed together in 1:20 ratio on a clean glass slide covered with a cover glass and observed the active forward movement of spermatozoa under pre-focused microscope (Figs. 3 & 4). Motility of the spermatozoa was estimated within 30 seconds using a microscope (45x magnification). A stop watch was used to measure the duration of motility. For all motility trials six samples for each species were used. Each sample was collected from a single fish.

**Sperm cell concentration**

The spermatozoa concentration of the milt was assessed using Neubar counting chamber. Six samples were used for each species of fish to work out the mean sperm cell count.

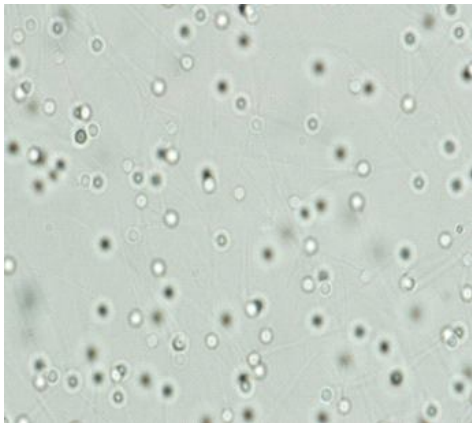

**Fig. 3**. Micrograph of spermatozoa of *Rasbora daniconius*

**Percentage viability of spermatozoa**

The percentage sperm viability is an index of fertility. Viability is expressed in percentage. Viability was determined using eosin-nigrosin dye exclusion method (Chao *et al.*, 1975). Six samples of each species of fish were used to record the percentage viability and the mean values were worked out. Each sample was collected from a single fish for each species.

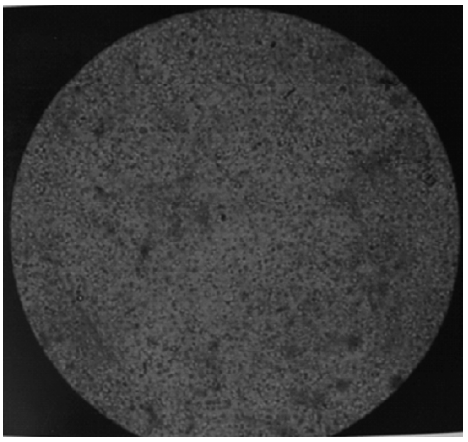

**Fig4.** Micrograph of spermatozoa of *Puntius filamentosus*

A drop of milt was placed on a glass slide. One drop of 5% eosin was added to the milt and mixed thoroughly. Then two drops of 10% nigrosin were added to it and mixed well. A relatively thin spermatozoa smear was prepared on glass slide. Then it was air dried and focused under a compound microscope (45 x magnifications). The live spermatozoa were observed as blue or ash colour and dead as pink or red. The percentage viability was calculated as follows:

Percentage viability = (Number of live spermatozoa/Total number of spermatoozoa )× 100

**Effect of activating media on spermatozoan motility**

An attempt was made to find out a suitable medium in which the duration of motility can be prolonged for longer periods. Motility is considered as the most reliable index of fertility. The sperm motility in freshwater, distilled water, 1% glucose solution, 0.35% saline solution and fertilizing solution was estimated.

**Effect of temperature on duration of motility of spermatozoa**

A series of experiments were conducted to evaluate the effect of temperature on duration of motility of spermatozoa. Duration was

estimated in different temperatures from 18$^0$ C to 30$^0$ C with an interval of 3$^0$ C in fertilizing solution. It was examined by adjusting the temperature of A/C in an air conditioned room. Five replicated observations in each temperature for each species of fish were recorded and the mean values were worked out.

**Effect of pH on duration of spermatozoon motility**

An experiment was conducted to demonstrate the effect of pH on the duration of sperm motility. Media of different pH were prepared by adding disodium hydrogen phosphate and sodium dihydrogen phosphate to distilled water. The solutions of various pH ie., 6, 6.5, 7, 7.5, 8, 8.5, 9 were prepared and duration of motility at each of these pH levels was evaluated. (Terner, 1986; Goodall *et al*., 1989). Four replications in each species of fish and mean motility was worked out.

**Effect of salinity on duration of Spermatozoon motility**

An experiment was conducted to assess the effect of different grades of salinity on the duration of motility of spermatozoa. Media of different grades of salinity such as 1%, 3%, 5%, 10%, 20% were prepared and the duration of motility at each of these salinities was evaluated. Four replications in each salinity for each species were maintained and the mean motility was recorded.

**Short term preservation**

Experiments were conducted to preserve the spermataozoa for short duration (non frozen) at 4$^0$ C in the refrigerator. The period of storage ranged from a few hours to a few days.

**Effect of short term storage at 4$^0$C (non frozen) on duration of spermatozoon motility**

Freshly collected milt was divided into equal volumes (0.5ml) and placed inside polythene bags (12×15 cm) and filled the bags with oxygen gas from a cylinder and tied tightly. Similarly milt was placed inside polythene bags (12×15cm) filled with air and tied tightly. Five replications of each treatment were maintained. These samples were stored at 4$^0$ C in the refrigerator. The oxygenated samples were filled with fresh oxygen twice daily. After every 24 hours the samples were taken out and the observations on duration of motility was recorded after activating with the activating media, such as, fertilizing solution. The duration of motility was assessed until no motile spermatozoa were found.

**Statistical analysis**

Analysis of variance method (ANOVA) was employed to determine the statistical significance of various treatments.

**RESULTS**

The spermatozoa of two species of fishes used for the study were immotile in the semen and were found to be motile only by activating it with suitable media.The milt of both the species was milky white in appearance and the semen was found to be viscous.

**Viability of spermatozoa**

High percentage of spermatozoan viability of fresh semen was observed in two species of fishes. Spermatozoan viability of *R. daniconius* was 92% and *P. filamentosus* was 93.56%.

**Sperm cell concentration**

The spermatozoa concentration showed significant difference between the two species of fishes. The sperm cell concentration of *R. daniconius* and *P. filamentosus* was 14.32 x $10^9$ and 20.56 x $10^9$ spermatozoa per ml respectively.

**Motility of spermatozoa**

The spermatozoa of the two species of fishes were immotile in the milt and found to be motile only by activating them using suitable media.

**Effect of activating media on spermatozoon motility**

The spermatozoa of *R. daniconius* were motile for 25.8 seconds in fertilizing solution. Longest duration of motility was observed in fertilizing solution. The spermatozoa showed least duration of motility in distilled water (11.2 seconds) (Table 1). In the case of *P. filamentosus* also the

spermatozoa showed longest duration of motility in fertilizing solution (97.6 seconds) and showed least duration of motility in distilled water (47.2 seconds) (Table 1). The result of analysis of variance showed that the mean duration of spermatozoan motility differed significantly in various activating media. F-ratio in *R. daniconius* was found to be 71.922 and in *P. filamentosus* it was 346.277 (P<0.01).

**Table 1.** Effect of various activating media on duration of spermatozoan motility of *R. daniconius* and *P. filamentosus*

| Activating Media | Mean Duration of motility (sec) | |
|---|---|---|
| | *R. daniconius* | *P. filamentosus* |
| Fertilizing Solution | 25.8 ± 1.393 | 97.6 ± 2.502 |
| 1% Glucose solution | 16.2 ± 0.200 | 57.2 ± 0.583 |
| 0.35% Saline Solution | 14.8 ± 0.374 | 50.2 ± 0.374 |
| Fresh water | 12.4 ± 0.245 | 47.2 ± 0.374 |
| Distilled Water | 11.2 ± 0.374 | 44.4 ± 0.245 |

**Effect of temperature on spermatozoon motility**

The spermatozoa showed longer duration of motility at lower temperature. The spermatozoa of *R.daniconius* showed maximum duration of motility (33.4 seconds) at 18$^0$C and the least duration of motility was observed at 30$^0$C (12 seconds) (Table 2). In the case of *P. filamentosus* mean duration of motility was high at 18$^0$C (94.2 seconds) and the least duration of motility was recorded at 30$^0$C (Table 2). The result of analysis of variance showed that the mean duration of spermatozoon motility at different temperatures differ significantly in both species of fishes. F-ratio (*R. daniconius)* was observed to be 395.744 and F-ratio (*P. filamentosus)* was 2272.219 (P<0.01).

**Table 2.** Effect of temperature on duration of spermatozoan motility of *R. daniconius* and *P. filamentosus*

| Temperature of media ($^0$C) | Mean Duration of motility (sec) | |
|---|---|---|
| | *R. daniconius* | *P. filamentosus* |
| 18 | 33.4 ± 0.510 | 94.2 ± 0.374 |
| 21 | 22 ± 0.316 | 78.8 ± 0.860 |
| 24 | 18.6 ± 0.510 | 56.4 ± 0.510 |
| 27 | 15.2 ± 0.374 | 47.4 ± 0.400 |
| 30 | 12 ± 0.316 | 28.6 ± 0.400 |

**Effect of pH on spermatozoon motility**

In *R. daniconius* maximum duration of spermatozoon motility was shown at pH 8.5 (14.6 seconds). The least duration of motility was observed at pH 6 (10.6 seconds) (Table 3). In *P. filamentosus* longest duration of motility was found in the alkaline pH 8 (38.8 seconds). The least duration of motility was found at pH 6 (29.6 seconds) (Table 3). As pH increased from 6 there was an increase in the duration of the spermatozoon motility up to pH 8 and then showed a decrease. The analysis of variance showed that the variation in spermatozoon motility is significantly different at various pH levels. In *R. daniconius*, F-ratio was observed as 36.741 and in *P. filamentosus*, F-ratio was 137.651 (P<0.01).

**Table 3.** Effect of pH on duration of spermatozoan motility of *R. daniconius* and *P. filamentosus*

| pH | Mean Duration of motility (sec) | |
|---|---|---|
| | *R. daniconius* | *P. filamentosus* |
| 6 | 10.6 ± 0.245 | 29.6 ± 0.245 |
| 6.5 | 11.6 ± 0.245 | 32.4 ± 0.245 |
| 7 | 12 ± 0.200 | 33.6 ± 0.245 |
| 7.5 | 12.8 ± 0.200 | 34.6 ± 0.245 |
| 8 | 14.6 ± 0.245 | 38.8 ± 0.200 |
| 8.5 | 13.4 ± 0.245 | 35.8 ± 0.200 |
| 9 | 11.2 ± 0.200 | 35 ± 0.316 |

**Effect of salinity on duration of spermatozoon motility**

In *R. daniconius* the maximum duration of motility was noticed at 1% salinity (13.6 seconds) and least duration of motility at 10% salinity (7.8 seconds) (Table 4). Maximum duration of motility was observed at 10% salinity (47 seconds) in *P. filamentosus*. The spermatozoa showed least duration of motility at 3% salinity (40.2 seconds) (Table 4). No motility was found in 20% salinity in both species of fishes. The analysis of variance showed that there is significant difference in the duration of motility with salinity. F-ratio (*R. daniconius)* was 39.000 and F-ratio (*P. filamentosus)* was 33.816 ($P<0.01$).

**Table 4.** Effect of salinity on duration of spermatozoon motility of *R. daniconius* and *P. filamentosus*

| Salinity (%) | Mean Duration of motility (sec) | |
|---|---|---|
| | *R. daniconius* | *P. filamentosus* |
| 1 | 13.6 ± 0.245 | 44.2 ± 0.735 |
| 3 | 12.8 ± 0.374 | 40.2 ± 0.374 |
| 5 | 12.4 ± 0.510 | 44.6 ± 0.400 |
| 10 | 8.2 ± 0.374 | 47 ± 0.316 |
| 20 | Immotile | Immotile |

**Effect of short term storage at $4^0$ C on duration of speramatozoan motility**

A decrease in the sperm motility was observed after preservation. In the case of *R. daniconius* and *P. filamentosus* the spermatozoa were motile only up to 24 hours in the un oxygenated samples (Table 5). In the oxygenated samples, spermatozoa of *R. daniconius* were motile only up to 25.6 seconds in the fresh milt, which was reduced to 8.2 seconds after preservation for 72 hours (Table 5) and in the *P. filamentosus*, the spermatozoa were motile up to 95.2 seconds in the fresh milt, which was reduced to 16.8 seconds after preservation for 72 hours (Table 5). The un-oxygenated sample of *R. daniconius* showed considerable reduction in the duration of sperm motility from 26 seconds to 8.4 seconds after storage for 24 hours and in the *P. filamentosus* reduction in the duration of motility was found from 95.8 seconds to 16.2 seconds. The result of analysis of variance showed that the decrease in duration of spermatozoan motility

was found to be statistically significant. F-ratio (*R. daniconius)* preserved in oxygen atmosphere was 308.281 and F-ratio (*P. filamentosus)* preserved in oxygen atmosphere was 9130.864. F-ratio *(R. daniconius)* preserved under non-oxygenated atmosphere, 860.444 and F-ratio *(P. filamentosus)* preserved under non-oxygenated atmosphere, 15681.60 (P<0.01).

**Table 5.** Effect of short term storage at $4^0$ C on duration of spermatozoon motility of *R. daniconius* and *P. filamentosus*

| Period of storage (hours) | Mean Duration of motility (sec) | | | |
|---|---|---|---|---|
| | *R. daniconius* | | *P. filamentosus* | |
| | Oxygenated | Unoxygenated | Oxygenated | Unoxygenated |
| 0 | 25.6 ± 0.510 | 26 ± 0.447 | 95.2 ± 0.374 | 95.8 ± 0.374 |
| 24 | 18.6 ± 0.510 | 8.4 ± 0.400 | 45 ± 0.316 | 16.6 ± 0.510 |
| 48 | 12 ± 0.316 | - | 25.4 ± 0.400 | - |
| 72 | 8.2 ± 0.374 | - | 16.8 ± 0.374 | - |
| 96 | - | - | - | - |

## DISCUSSION

In the case of majority of freshwater fishes the sperms are typically immotile in the undiluted milt and are activated only in a medium. This is highly useful for sperm preservation, since in the undiluted state no energy is utilized by sperms. The spermatozoa of two species of fishes investigated in the present study are found to be immotile in the milt and they are activated in a medium, such as, freshwater, saline, fertilizing solution. The immotility of the spermatozoa in the milt was also reported in salmonids (Scott and Baynes, 1980; Terner, 1986) in tilapias (Chao *et al*., 1987) in yellow fin bream (Thorogood and Blackshaw, 1992) and in rainbow trout (Lahnsteiner *et al*., 1998). A high rate of sperm motility was reported in *Sillago ciliata* (Goodall *et al*., 1989) and in the yellow fin bream, *Acanthopagrus australis* (Thorogood and Blackshaw, 1992).

The sperm cell concentration of the semen is also related to the fertility of the spermatozoa. In the two species of fishes *R. daniconius* and *P. filamentosus* investigated the sperm cell concentration is highest in *P. filamentosus*. The sperm cell concentration *of P. filamentosus* is $20.56 \times 10^9$ cells / ml and in the *R. daniconius,* it is $14.32 \times 10^9$ cells / ml. The results clearly indicate that the sperm cell concentration in the milt differ in different species of fishes. The sperm cell concentration ($53 \times 10^9$ cells / ml) in the grey mullet was very high when compared with other fishes (Chao *et al*., 1975). Hara *et al*. (1982) reported an enormously high number of sperms ($3.6967 \times 10^{12}$ cells / ml) in *Chanos chanos*. According to Aas *et al*. (1991) spermatozoa concentration have direct effect on the rate of fertilization. The sperm cell concentration in the milt varies with species. It is very high in *Sillago sihama* ($74.44 \times 10^{-9}$ $ml^{-1}$) (Benno, 2000). The sperm cell concentration in common carp is $34.6 \times 10^{-9} ml^{-1}$ (Chao *et al*., 1987). The high sperm cell concentration is essential to ensure high rate of fertilization as it takes place in the aquatic environment which is subjected to the stresses of the environmental parameters, such as, water currents, waves, tides and turbulence. Sperm cell concentration is reported to show maximum values at the peak of breeding season. The presence of large number of spermatozoa is an adaptation to ensure enhanced fertility and high rate of recruitment of the young ones.

Spermatozoon motility and the duration of motility have been correlated with fertility in many species of fishes (Terner, 1986; Chao *et al*., 1987; Goodall *et al*., 1989). In the present study, maximum duration of sperm motility has been obtained in fertilizing solution for the two species of fishes. Spermatozoa exhibited short duration of motility in distilled water (Table 1). The trout spermatozoa are reported to be motile as briefly as 30 seconds (Terner, 1986). Different species of tilapia exhibited duration of motility of 4-5 minutes (Chao *et al*., 1987). The shorter duration of motility in fresh water was reported by previous investigators (Terner, 1986; Saad *et al*., 1988). Billard (1978) recorded the spermatozoon motility of 3600 seconds in *Poecilia reticulata* using ringer solution, the highest motility period ever reported in fresh water fishes. Total motility duration of 3-26 minutes has been reported in the turbot, *Scophthalmus maximus* (Suquet *et al*., 1992 b). Longer duration of motility in fertilizing solution was reported in carp (Jaechnichen, 1992) and *Etroplus suratensis* (Bindhu, 1999).

Knowledge of the factors influencing the activation and duration of motility of sperms have tremendous practical application in the short term and long term preservation of spermatozoa. The temperature of the medium has profound effect on the duration of sperm motility. In the two species of fishes, investigated in the present study, prolonged duration of motility is observed when the sperms are brought to lower temperatures. Maximum duration of motility was obtained at $18^0$C for *R. daniconius* and *P. filamentosus*. Duration of sperm motility gradually decreased as the temperature of the medium increased in the case of two species of fishes investigated. The results also compare with the observations of Thorogood and Blackshaw (1992) in yellow fin bream spermatozoa. The spermatozoa of Yellow fin bream (*A. australis*) were found to remain active for significantly longer duration at low temperature. Earlier works have also reported the longer duration of motility at lower temperature; $10^0$C for carp (Jayaprakas and BimalLal, 1996) and $5^0$C for *E. Suratensis* (Bindhu, 1999). The temperature of the activating media may change the physical and chemical composition of the seminal plasma. When the enzymatic system of the seminal plasma changes the whole function of spermatozoa may be affected which leads to changes in duration of motility.

The pH of the activating medium has profound effect on the duration of motility of spermatozoa. In the present study, shortest duration of spermatozoon motility is recorded in the acidic pH and the longest in alkaline. In the present study duration of spermatozoon motility increased at higher pH levels showing maximum motility at pH 8.5 in *R. daniconius* while at pH 8 in the *P. filamentosus*. The results are in agreement with that of yellow fin bream in which a mildly alkaline medium is found more conducive to sperm activation and prolonged duration of motility than neutral or acidic environment (Thorogood and Blackshaw, 1992). The longer duration of spermatozoon motility in alkaline pH was also reported in perch (Lahnsteiner *et al*., 1995), *C. carpio* (BimalLal, 1993) and *A. mola* (Paul, 1998). The longer duration of spermatozoon motility in alkaline pH may be due to the change in concentration of the medium.

Salinity of the activating medium plays an active role on the duration of motility. Several studies have also indicated that the motility of spermatozoa in dilute salt solutions maintained longer than in freshwater (Buyukhatipoglu and Holtz, 1978; Goodall *et al*., 1989; Thorogood and Blackshaw, 1992). Longest duration of sperm motility was observed at 1% salinity in *R. daniconius* and in *P. filamentosus* it was at 10% salinity. Increased duration of sperm motility at higher salinity was reported by Palmer (1994) in pickey bream.

Viability and fertility are well correlated. High percentage of viable spermatozoa result in high rate of fertility. In the present experiment, the viability of spermatozoa of freshly collected semen of *P. filamentosus* is 93.56% and in the case of *R. daniconius*, it is 92%. This ideally shows that the spermatozoa of *P. filamentosus* exhibit high rate of fertility. The percentage viability of

*Ctenopharyngodon idella, Cirrhinus mrigala, Labeo rohita* and *Cyprinus carpio* was reported to be 94.45, 94.08, 93.08 and 93.93% respectively (Bimal Lal, 1993). Studies by Bindu (1999) revealed spermatozoan viability of *Osphronemus goramy* (94.04%), *Puntius sarana* (95.36%), *Labeo fimbriatus* (96.92%) and *Etroplus suratensis* (96.2%). Reports by Benno (2000) on the viability of spermatozoa of *Auxis rochei* (93.93%), *Trichurus savala* (86.06%), *Sillago sihama* (92%) and Mugil parsia (87.27%) are also comparable observations. Several investigators have followed the assumption that the most commonly used laboratory assay for semen quality is motility and viability of the spermatozoa. (Erdahl *et al*., 1984; Terner, 1986; Chao *et al*., 1992, Billard *et al*., 1992).

The preservation of oxygenated milt in the refrigerator for short duration is very useful for conducting artificial insemination programmes in hatcheries. It has been reported that the survival of fish sperm can be maintained at 0 to $4^0$C by providing adequate oxygen to the milt (Billard, 1981; Chao *et al*., 1992). The semen stored under oxygen atmosphere is found to be superior to sample stored in air. Survival of spermatozoa is prolonged under oxygen atmosphere (Billard, 1980). In the present study the motility of the spermatozoa are used to verify the effect of duration of storage on the spermatozoa at $4^0$C. Duration of motility was reduced significantly after storage for 24 hours in un-oxygenated milt and for 72 hours in oxygenated milt.

The decrease in the sperm motility after short term preservation has also been reported by several workers in different species of fishes (Chao *et al*., 1987, 1992). Buyukhatipoglu and Holtz (1978) were able to preserve the rainbow trout semen at $4^0$C under oxygen up to 15 days and under air up to 9 days. Previous studies showed that the fertilizing capacity which is normally maintained for only one day (Carpentier and Billard, 1978) may be prolonged to fifteen or even thirty days by addition of oxygen and antibiotics. The knowledge of factors that influence the duration of sperm motility can be applied to develop short term and long term preservation techniques. The results of the present study on activation and duration of sperm motility in various activating media and the effect of temperatures, pH and salinity levels on sperm motility are important in assessing the factors that contribute to the fertility of semen.

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