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FACTORS AFFECTING MOTILITY, VIABILITY, SHORT AND LONG TERM PRESERVATION OF SPERMATOZOA OF FOUR SPECIES OF INDIGENOUS ORNAMENTAL FISHES

ANI JOSEPH, BOBAN, V. L & JAYAPRAKAS V

Department of Aquatic Biology & Fisheries, University of Kerala, Trivandrum, Kerala, India

ABSTRACT

The ornamental fishes are important not only because of their aesthetic value but also due to their immense commercial value in export trade. Biologists all over the world fear that the genetic strains and variability of wild fish stocks are being depleted at a rapid pace. So they 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 and long terms. The fishes used for the study were *Rasbora daniconius*, *Puntius filamentosus*, *Parambassis dayi* and *Hyporhamphus xanthopterus*, collected from Vellayani Lake in Trivandrum district of Kerala, India. High percentage of spermatozoan viability and sperm cell concentration was observed in four species of fishes. Longest duration of spermatozoan motility was observed in fertilizing solution. The spermatozoa showed longer duration of motility at lower temperature in the four species. Longer duration of spermatozoon motility can be found in alkaline pH. Motility of spermatozoa in dilute salt solutions maintained longer than in freshwater. In the short term preservation, spermatozoa of oxygenated samples were motile up to 72 hours. In the unoxygenated samples the spermatozoa were motile only up to 24 hours. Three different extenders (Extender I - Fish Ringer solution, Extender II - 7% Glucose solution, Extender III – 7% Sucrose solution) with different concentrations of two cryoprotectants, DMSO and Glycerol were used for the cryopreservation of spermatozoa. In the cryopreserved milt of *P. dayi*, the highest motility was recorded in extender I and 7.5% DMSO combination. In *H. xanthopterus*, the maximum duration of motility was exhibited by 7.5% DMSO in combination with extender III.

KEYWORDS: Extender, Motility, Preservation, Spermatozoa, Viability

INTRODUCTION

The increasing development of aquaculture industry projects the demand for developing suitable, economically viable repeatable methods for both short term and long term preservation of spermatozoa of fishes. 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 and long durations.

In the majority of freshwater fishes, the fertilisation 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 factors such as ions, pH, salinity, temperature, affect the duration of motility of

spermatozoa.

Good management of broodstock requires clear knowledge of the quality of gametes produced. The quality of spermatozoa is usually assessed by the intensity of motility (Goryezko and Tomasik, 1975). The sperm cell concentration is also related to the fertility of the spermatozoa. Viability of spermatozoa also determines the quality of spermatozoa. Assessing the percent of viable sperm is essential for storage of fish sperms in banks.

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. Air and preferably pure oxygen are most suitable for maintaining cell viability (Withler and Morley, 1968). The cryopreserved gametes can be stored in liquid nitrogen (-196^0 C) for a long period of time with out any deleterous effect. Cryopreservation makes possible the almost indefinite storage of desirable genes to save the unique characteristics. 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). Preservation of fish spermatozoa is particularly useful in families with asynchronous maturation habits, allows selective breeding and stock improvements and enables the conservation of genome (Harvey et al., 1982).

The development of reliable techniques in gamete preservation would offer both practical and economic advantages to aquaculture. 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 preservation of spermatozoa of selected freshwater fishes. The fishes used for the study are *Rasbora daniconius, Puntius filamentosus, Parambassis dayi* and *Hyporhamphus xanthopterus* (Figs. 1, 2, 3 & 4). Fishes were collected from Vellayani Lake in Trivandrum district of Kerala.

Figure 1: Rasbora Daniconius

Figure 2: Puntius Filamentosus

Figure 3: Parambassis Dayi

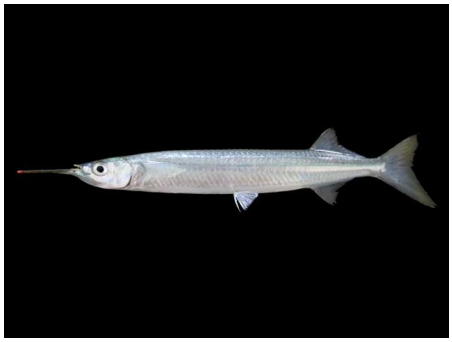

Figure 4 Hyporhamphus Xanthopterus

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.

Sperm Cell Concentration

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

Percentage Viability of Spermatozoa

The percentage sperm viability is an index of fertility. Viability was determined using eosin-nigrosin dye exclusion method (Chao *et al.*, 1975). Five samples of each species of fish were used to record the percentage viability and the mean values were worked out. A drop of milt was placed on a glass slide. It was mixed with one drop of 5% eosin and two drops of 10% nigrosin. A relatively thin spermatozoa smear was prepared on glass slide. Then it was air dried and focussed 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

$$\text{Percentage viability} = \frac{\text{Number of live spermatozoa}}{\text{Total number of spermatzoa}} \times 100$$

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 (3 g urea and 4 g NaCl in one litre distilled water) was estimated. The duration of motility of spermatozoa of five specimens from each species was examined. 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 prefocused microscope. A stop watch was used to measure the duration of motility.

Effect of Temperature on Duration of Motility of Spermatozoa

Duration of motility of spermatozoa was estimated in different temperatures from 18^0 C to 30^0 C with an interval of 3^0 C in fertilizing solution. 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 Spermatozoan Motility

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). Five replications in each species of fish and mean motility was worked out.

Effect of Salinity on Duration of Spermatozoan Motility

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. Five 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 4^0C (non frozen) on Duration of Spermatozoan Motility

Freshly collected milt was divided into equal volumes (0.5ml) and placed inside 8 polythene bags (12×15 cm). Four bags were filled with oxygen gas and the other four with out oxygen and were kept 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, fertilizing solution. The duration of motility was assessed until no motile spermatozoa were found. Five replications of each treatment was maintained.

Cryopreservation

Stripped milt was collected in 1.5 ml polypropylene storage vials, capped tightly and stored in crushed ice until processed. Three different constituents of extenders were used for the study (Extender I - Fish Ringer solution, Extender II - 7% Glucose solution, Extender III – 7% Sucrose solution). Two widely accepted cryoprotectants, DMSO and glycerol were used for the study. The concentrations used in the study were 5%, 7% and 10%. All the three extenders and cryoprotectants were added in the ratio 1:3 (one part of semen to three parts of the extender/cryoprotectant). Samples after 30 days were taken out and analysed for the spermatozoan motility.

Statistical Analysis

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

RESULTS

The spermatozoa of four 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 all the four species was milky white in appearance. The semen was found to be viscous in all the three species except *P. dayi*. The semen of *P. dayi* was found to be watery.

Viability of Spermatozoa

High percentage of spermatozoan viability of fresh semen was observed in four species of fishes. Spermatozoan viability of *Rasbora daniconius* was 92%, *Puntius filamentosus* was 93.6%, *Parambassis dayi* was 95.9% and *Hyporhamphus xanthopterus* was 93.3%.

Sperm Cell Concentration

The spermatozoa concentration showed significant difference among four species of fishes. The sperm cell concentration of *R.daniconius, P.filamentosus, P. dayi* and *H. xanthopterus* were 14.32×10^9, 20.56×10^9, 15.08×10^9 and 12.55×10^9 spermatozoa per ml respectively.

Effect of Activating Media on Spermatozoan Motility

Longest duration of spermatozoan motility was observed in fertilizing solution (25.8, 97.6, 74.4, 53.8 seconds) in *R.daniconius, P.filamentosus, P. dayi* and *H. xanthopterus* respectively (Table 1). The result of analysis of variance showed that the mean duration of spermatozoan motility differed significantly in various activating media. F-ratio - Highly significant ($P<0.01$).

Table 1: Effect of Various Activating Media on Duration of Spermatozoan Motility

Activating Media	Mean Duration of Motility (sec)			
	R. daniconius	***P. filamentosus***	***P. dayi***	***H. xanthopterus***
Fertilizing Solution	25.8 ± 1.393	97.6 ± 2.502	74.4 ± 5.354	53.8 ± 0.374
1% Glucose solution	16.2 ± 0.200	57.2 ± 0.583	59.4 ± 0.510	29.4 ± 0.510
0.35% Saline Solution	14.8 ± 0.374	50.2 ± 0.374	54.4 ± 0.600	30.2 ± 0.374
Fresh water	12.4 ± 0.245	47.2 ± 0.374	24.8 ± 0.374	21.4 ± 0.510
Distilled Water	11.2 ± 0.374	44.4 ± 0.245	27..8 ± 0.374	18.4 ± 0.510

3.4 Effect of Temperature on Spermatozoan Motility

The spermatozoa showed longer duration of motility at lower temperature. The spermatozoa showed maximum duration of motility at 18^0C (33.4, 94.2, 42.8, 36.2 seconds) in *R.daniconius, P.filamentosus, P. dayi* and *H. xanthopterus* respectively (Table 2). ANOVA showed that the mean duration of spermatozoan motility at different temperatures differ significantly in four species of fishes. F-ratio - Highly significant ($P<0.01$).

Table 2: Effect of Temperature on Duration of Spermatozoan Motility

Temperature of Media (^{0} C)	Mean Duration of Motility (sec)			
	R. daniconius	*P. filamentosus*	*P. dayi*	*H. xanthopterus*
18	33.4 ± 0.510	94.2 ± 0.374	42.8 ± 0.374	36.2 ± 0.374
21	22.0 ± 0.316	78.8 ± 0.860	36.4 ± 0.245	32.6 ± 0.510
24	18.6 ± 0.510	56.4 ± 0.510	31.8 ± 0.374	30.0 ± 0.316
27	15.2 ± 0.374	47.4 ± 0.400	29.0 ± 0.447	28.0 ± 0.316
30	12.0 ± 0.316	28.6 ± 0.400	24.8 ± 0.583	25.4 ± 0.678

3.5 Effect of pH on Spermatozoan Motility

In *R. daniconius* maximum duration of spermatozoan motility was shown at pH 8.5 (14.6 seconds). The longest duration of motility was found in the pH 8 (38.8, 59.2, 53.4 seconds) in *P.filamentosus*, *P. dayi* and *H. xanthopterus* respectively (Table 3). ANOVA showed that the variation in spermatozoan motility is significantly different at various pH levels. F-ratio - Highly significant (P<0.01).

Table 3: Effect of pH on Duration of Spermatozoan Motility

pH	Mean Duration of Motility (sec)			
	R. daniconius	*P. filamentosus*	*P. dayi*	*H. xanthopterus*
6	10.6 ± 0.245	29.6 ± 0.245	33.2 ± 0.663	19.0 ± 0.447
6.5	11.6 ± 0.245	32.4 ± 0.245	40.0 ± 0.447	21.4 ± 0.510
7	12.0 ± 0.200	33.6 ± 0.245	46.0 ± 2.025	32.0 ± 0.837
7.5	12.8 ± 0.200	34.6 ± 0.245	52.6 ± 0.748	40.2 ± 0.663
8	13.4 ± 0.245	38.8 ± 0.200	59.2 ± 1.281	53.4 ± 0.510
8.5	14.6 ± 0.245	35.8 ± 0.200	52.2 ± 0.374	34.6 ± 0.812
9	11.2 ± 0.200	35.0 ± 0.316	30.0 ± 0.707	24.6 ± 0.748

3.6 Effect of Salinity on Duration of Spermatozoan Motility

In *R. daniconius* the maximum duration of motility was noticed at 1% salinity (13.6 seconds) and in *P. filamentosus* maximum duration noticed at 10% salinity (47 seconds). The maximum duration of motility was noticed at 3% salinity (55.8, 48.6 seconds) in *P. dayi* and *H. xanthopterus* respectively (Table 4). ANOVA showed that there is significant difference in the duration of motility with salinity. F-ratio - Highly significant (P<0.01).

Table 4: Effect of salinity on duration of spermatozoan motility

Salinity (%)	Mean Duration of Motility (sec)			
	R. daniconius	*P. filamentosus*	*P. dayi*	*H. xanthopterus*
1	13.6 ± 0.245	44.2 ± 0.735	32.4 ± 1.208	24.4 ± 0.510
3	12.8 ± 0.374	40.2 ± 0.374	55.8 ± 0.663	48.6 ± 0.812
5	12.4 ± 0.510	44.6 ± 0.400	42.6 ± 1.077	33.8 ± 0.663
10	8.2 ± 0.374	47.0 ± 0.316	28.6 ± 0.510	27.0 ± 0.949
20	Immotile	Immotile	17.2 ± 0.735	16.2 ± 0.374

3.7 Effect of Short Term Storage at 4^0 C on Duration of Speramatozoan Motility

A decrease in the sperm motility was observed after short term preservation. In the oxygenated samples, spermatozoa were motile up to 72 hours. In the unoxygenated samples the spermatozoa were motile only up to 24 hours (Tables 5 & 6). ANOVA showed that the decrease in duration of spermatozoan motility was found to be statistically

significant. F-ratio - Highly significant (P<0.01)].

Table 5: Effect of Short Term Storage at 4^0 C on Duration of Spermatozoan 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	-	-	-	-

Table 6. ***Effect of short term storage at 4^0 C on duration of spermatozoan motility of P. dayi and H. xanthopterus***

Period of Storage (Hours)	Mean Duration of Motility (sec)			
	P. dayi		*H. xanthopterus*	
	Oxygenated	Unoxygenated	Oxygenated	Unoxygenated
0	72.4 ± 0. 872	63.0 ± 1.414	46.2 ± 0.663	38.4 ± 1.364
24	38.4 ± 1.364	22.2 ± 0.735	25.8 ± 0.663	-
48	27.4 ± 1.327	-	18.6 ± 0.678	-
72	16.0 ± 0.894	-	15.0 ± 0.837	-
96	-	-	-	-

3.8 Cryopreservation of Spermatozoa

Table 7: ***Effect of Cryopreservation on Duration of Spermatozoan Motility of P. dayi and H. xanthopterus Among Different Extenders and Different Concentrations of DMSO***

Combinations of Extender and Cryoprotectant Added Semen	Mean Duration of Motility (sec)	
	P. dayi	*H. xanthopterus*
Milt + Ex1 + 5% DMSO	26.8 ± 0.733	24.6 ± 0.926
Milt + Ex1 +7. 5% DMSO	63.4 ± 0.747	45.2 ± 1.592
Milt + Ex1 + 10% DMSO	52.2 ± 0.581	34.2 ± 1.655
Milt + Ex2 + 5% DMSO	33.4 ± 1.073	17.4 ± 0.814
Milt + Ex2 + 7.5% DMSO	54.2 ± 1.279	34.6 ± 1.029
Milt + Ex2 + 10% DMSO	44.4 ± 1.288	27.2 ± 0.966
Milt + Ex3 + 5% DMSO	27.0 ± 0.894	14.4 ± 0.926
Milt + Ex3 + 7.5% DMSO	54.4 ± 1.364	46.6 ± 1.029
Milt + Ex3 + 10% DMSO	41.4 ± 0.747	32.6 ± 1.073

Three different extenders (Extender I - Fish Ringer solution, Extender II - 7% Glucose solution, Extender III – 7% Sucrose solution) with different concentrations of two cryoprotectants, DMSO and glycerol were used for the cryopreservation of spermatozoa. In the cryopreserved milt of *P. dayi*, the highest motility was recorded (63.4 seconds) in extender I and 75% DMSO combination (Table 7). The extender I with 10% glycerol showed the motility of 53.8 seconds. In *H. xanthopterus*, the maximum duration of motility was exhibited by 75% DMSO in combination with extender III (46.6 seconds). The highest motility in glycerol was recorded (44.4 seconds) from extender I with 10% glycerol (Table 8).

Table 8. ***Effect of Cryopreservation on Duration of Spermatozoan Motility of P. dayi and H. xanthopterus Among Different Extenders and Different Concentrations of Glycerol***

Combinations of Extender and Cryoprotectant Added Semen	Mean Duration of Motility (sec)	
	P. dayi	***H. xanthopterus***
Milt + Ex1 + 5% glycerol	24.8 ± 0.966	16.8 ± 0.859
Milt + Ex1 +7. 5% glycerol	29.4 ± 1.073	30.4 ± 0.510
Milt + Ex1 + 10% glycerol	53.8 ± 0.662	44.4 ± 0.814
Milt + Ex2 + 5% glycerol	24.4 ± 0.747	15.2 ± 0.662
Milt + Ex2 + 7.5% glycerol	29.2 ± 1.592	32.0 ± 0.836
Milt + Ex2 + 10% glycerol	42.4 ± 1.431	39.2 ± 0.581
Milt + Ex3 + 5% glycerol	18.0 ± 0.546	15.6 ± 1.364
Milt + Ex3 + 7.5% glycerol	31.0 ± 1.673	26.4 ± 0.926
Milt + Ex3 + 10% glycerol	40.2 ± 1.114	32.6 ± 1.073

DISCUSSIONS

The quantitative measures such as milt-volume, spermatozoa concentration, viability and motility give better picture for the effective utilization in fertilization and also determine the success of fertilization. In the case of majority of freshwater fishes the sperms are typically immotile in the undiluted milt and are activated only in a medium. 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) and in yellowfin bream (Thorogood and Blackshaw, 1992). A high rate of sperm motility was reported in the yellowfin bream, *Acanthopagrus australis* (Thorogood and Blackshaw, 1992). In the present study fertilizing solution was found as the best activating medium for the four species of fishes investigated.

The sperm cell concentration of the semen is also related to the fertility of the spermatozoa. In the four species of fishes *R. daniconius, P. filamentosus, P. dayi* and *H. xanthopterus* investigated the sperm cell concentration is highest in *P. filamentosus*. The results clearly indicate that the sperm cell concentration in the milt differ in different species of fishes. The sperm cell concentration (53×10^9 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 (3.6967×10^{12} cells / ml) in *Chanos chanos*. According to Aas *et al*. (1991) spermatozoa concentration have direct effect on the rate of fertilization.

High percentage of viable spermatozoa result in high rate of fertility. High percentage of spermatozoan viability of fresh semen was observed in four species of fishes. 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 (BimalLal, 1993). Several investigators have followed the assumption that the most commonly used laboratory assay for semen quality is motility and viability of the spermatozoa. (Terner, 1986; Billard *et al*., 1992).

Spermatozoan motility and the duration of motility have been correlated with fertility in many species of fishes (Terner, 1986). In the present study, maximum duration of sperm motility has been obtained in fertilizing solution for the four species of fishes. The trout spermatozoa are reported to be motile as briefly as 30 seconds (Terner, 1986). Billard (1978) recorded the spermatozoan motility of 3600 seconds in *Poecilia reticulata* using ringer solution, the highest motility period ever reported in fresh water fishes. Longer duration of motility in fertilizing solution was reported in carp (Jaechnichen, 1992) and *Etroplus suratensis* (Bindu, 1999).

The temperature of the medium has profound effect on the duration of sperm motility. In the four 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 18^0C. The results also compare with the observations of Thorogood and Blackshaw (1992) in yellowfin bream spermatozoa. Earlier works have also reported the longer duration of motility at lower temperature; 10^0C for carp (Jayaprakas and BimalLal, 1996) and 5^0 C for *E. suratensis* (Bindu, 1999).

The pH of the activating medium has profound effect on the duration of motility of spermatozoa. In the present study, longest duration of spermatozoan motility is recorded in the alkaline pH. The results are in agreement with that of yellowfin bream in which a mildly alkaline 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 spermatozoan motility in alkaline pH was also reported in *C. carpio* (BimalLal, 1993).

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 (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. Longest duration of sperm motility was observed at 3% salinity in *P. dayi* and *H. xanthopterus*.

The preservation of oxygenated milt in ordinary 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 4^0C by providing adequate oxygen to the milt (Billard, 1981; Chao *et al*., 1992). Survival of spermatozoa is prolonged under oxygen atmosphere (Billard, 1980). In the present study, duration of motility was reduced significantly after storage for 24 hours in unoxygenated 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*., 1992). 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.

Selection of extenders, cryoprotectants and their composition play an important role for the cryopreservation of spermatozoa of fishes. Erdahl *et al*. (1984) obtained optimum fertility in the dilution ratio 1:3 for salmon spermatozoa. In the present study, the dilution ratio selected was 1:3 since many investigators (Legendre and Billard, 1980; Erdahl *et al*., 1984) obtained good results at this dilution. Regarding the selection of cryoprotectant also, the results show a species dependant variation. Glycerol was shown to have superior effect on cryopreservation of yellow fin bream *Acanthopagrus australis* (Thorogood and Blackshaw, 1992). The most effective DMSO concentration in *Sillago ciliata* was found to be between 7 and 10% of the medium (Kerby, 1983). The species dependent variation necessitates the development of species specific extenders, cryoprotectants and their percentage. In the present study, the concentration of DMSO to maintain the best result was found to be 7.5%. The concentration of glycerol in 10% was found to be most suitable than both 5% and 7.5%. In the fishes studied, DMSO proved its capability to protect the damages due to freezing than glycerol.

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.

CONCLUSIONS

Cryopreservation makes possible the almost indefinite storage of desirable genes to save the unique characteristics.

Preservation of fish spermatozoa is particularly useful in families with asynchronous maturation habits, allows selective breeding and stock improvements and enables the conservation of genome. The increasing development of aquaculture industry projects the demands for developing suitable, economically viable repeatable methods for both short and long term preservation of spermatozoa of fishes. The success of any economically productive artificial insemination programme depends up on maximal utilisation of available gametes. The development of reliable techniques in gamete preservation would offer both practical and economic advantages to aquaculture.

REFERENCES

1. **Aas, G. H., Refstie, T.** and **Gjerdde, B. 1991**. Evaluation of milt quality of Atlantic salmon. *Aquaculture*, **95**: 125-132.
2. **Billard, R. 1978**. Changes in structure and fertilizing ability of marine and freshwater fish spermatozoa diluted in media of various salinities. *Aquaculture*, **14**: 187-198.
3. **Billard, R. 1980**. Reproduction and artificial insemination in teleost fish. *Int. Congr. Anim. Reprod. Artif. Insemin. (proc.)* 9^{th}, 1980 RT-H-3, 327-337.
4. **Billard, R. 1981**. Short term preservation of of sperm under oxygen atmosphere in rainbow trout (*Salmo gairdneri*). *Aquaculture*, **23**: 287-293.
5. **Billard, R.** and **Cosson, M. P**. 1992. Some problems related to the assessment of sperm motility in freshwater fish. *J. Exptl. Zool*., **261**: 122-131.
6. **BimalLal, T. S. 1993**. Studies on certain aspects on the motility and viability of spermatozoa of four species of carps. M. Phil Dissertation, University of Kerala, 109 pp.
7. **Bindu, L. 1999**. Studies on certain factors influencing the preservation of spermatozoa of four species of fishes. Ph.D Thesis, University of Kerala, Thiruvananthapuram.
8. **Carpentier, P.** and **Billard, R. 1978***. Conservation of a courte-terme des gamete de salminides a destemperatures voisines de 0^0 C. *Ann. Biol. Anim. Bioch. Biophys.*, **18**: 1083-1088.
9. **Chambeyron, F. and Zohar, y. 1990**. A diluent for sperm cryopreservation of gilthead sea bream, *Sparus aurata*. *Aquaculture*, **90**: 345-352.
10. **Chao, N. H., Chen, H. P.** and **Liao, I. C. 1975**. Study on the cryogenic preservation of grey mullet sperm. *Aquaculture*, **5**: 386-406.
11. **Chao, N. H., Tsai, H. P.** and **Liao, I. C., 1992**. Short and long term cryopreservation of sperm and sperm suspension of the grouper, *Epinephelus malabaricus* (Bloch and Schneider). *Asian Fisheries Science*, **5**: 103-116.

12. **Das, P.** and **Pandey, A. K. 1998**. Endangered fish species: Measures of rehabilitation and conservation. *J. Fishing Chimes*, **19**: 16-18.

13. **Erdahl, A. W., Erdahl, D. A.** and **Graham, E. F. 1984**. Some factors affecting the salmonid spermatozoa. *Aquaculture*, **43**: 341-350.

14. **Goryezko, K.** and **Tomasik, L. 1975**. An influence of males on the variability and fertilization degree of trout eggs. *Acta. Ichtyol. Piscat*., **5**: 3-11.

15. **Hara, S., Canto, J. T.** and **Alemendras, J. M. E. 1982**. A comparative study of various extenders of milkfish (*Chanos chanos*) sperm preservation. *Aquaculture*, **28**: 339-546.

16. **Harvey, B., Kelley, R.N. and Ashwood- Smith, M.J. 1982.** Cryopreservation of zebra fish spermatozoa using methanol. *Can. J. Zool*., **60**: 1867-1870.

17. **Jaechnichen, H., 1992**. Further improvement of artificial propagation of cyprinidae by short term preservation of sperm. *Fortschr-fischereiwiss. Adv. Fish. Sci.*, **10**: 61-67.

18. **Jayaprakas, V.** and **BimalLal, T. S. 1996**. Factors affecting the motility and short-term storage of spermatozoa of Indian major carps, *Labeo rohita* and *Cirrhinus mrigala*. *J. Aqua. Trop*., **11**: 67-78.

19. **Kerby, J. H. 1983.** Cryogenic preservation of sperm from striped bass. *Trans. Am. Fish. Soc.*, **112**: 86-94.

20. **Palmer, P. J. 1994**. Chilled storage of Pickey bream (*Acanthopagrus berda*) sperm and activation in different salinities. *Asian. Fish. Sci.* **7**: 35-40.

21. **Scott, A. P.** and **Baynes, S. M. 1980.** A review of the biology, handling and storage of salmonid spermatozoa. *J. Fish. Biol.*, **17**: 707-739.

22. **Terner, C. 1986**. Evaluation of salmonid sperm motility for cryopreservation. *Prog.Fish cult.*, **48**: 230-232.

23. **Thorogood, J.** and **Blackshaw, A. 1992**. Factors affecting the activation, motility and cryopreservation of the spermatozoa of yellowfin bream, *Acanthopagrus australis* Gunther. *Aquaculture and Fisheries Management*, **23**: 337-334.

24. **Withler, F. C.** and **Humpreys, R. M., 1967**. Duration of fertility of ova and sperm of sock eye salmon (*Onchorhynchus nerka*) and pink salmon (*Onchorhynchus gorbuscha*). *J. Fish. Res. Bd. Can*., **24**: 1573-1578.