Management of Scombroid Fisheries

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Studies on the quality, factors affecting motility and short – term storage of milt of the Indian mackerel, *Rastrelliger kanagurta*

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ABSTRACT

A detailed study was conducted on the monthly variation in milt-volume, spermatozoa concentration and duration of motility of spermatozoa of *Rastrelliger kanagurta*. Milt-volume showed peak (0.094 ml) in the month of May. Spermatozoa concentration did not vary much during the period of May to October (21.5-21.7 x $10^9 ml^{-1}$). Duration of motility in 100% seawater was observed to be maximum in the month of August (101.6 sec.) followed by October (96.53 sec.) and April (94.4 sec.). The effect of various dilutions of seawater on the duration of motility showed a decreasing trend with decrease in concentration. Maximum duration of motility (86.46 sec.) was observed in 100% seawater. Salinity below 70% seawater was found to be ineffective in inducing motility. The quality of stripped milt, milt in isolated testis and milt *in situ* in the body of the fish at room temperature were also studied and it was found that preservation of stripped milt gave the best result after storage. Another series of experiments were conducted to assess the effect of factors such as oxygen, air, water vapour, their combinations and milk of coconut on short-term storage. The milt stored in an atmosphere of oxygen was observed to maintain motility for a period of 20 days at 4^0C.

INTRODUCTION

Since the introduction of cryopreservation technology, extensive studies were going on worldwide which made clear that cryopreservation is an inevitable necessity for the development of aquaculture in the future. Most of the studies on this topic are restricted to the European fresh water species. Literature on the cryopreservation studies of spermatozoa of marine fishes is very limited which may be due to the difficulties in obtaining ripe specimens in time. Most of the studies in this line are on the fishes of temperate regions, whereas those on tropical fishes are limited, with pioneer studies by Blaxter (1953, 1955) and Mounib *et al.* (1968). Since then, cryopreservation technology has been standardised for a number of fishes such as Atlantic cod, Atlantic salmon, sea trout, Atlantic trout, plaices, mullet, sea bream, sea bass, crimson sea bream, barramundi, chinook salmon, rainbow trout, brown trout, yellowfin bream, curimbata, Atlantic halibut, Atlantic croaker etc.

The marine catch is declining day by day and the inland water bodies are under the threat of epidemics, pollution, and habitat destruction. Cage culture of marine fishes has become a prospective venture in this context. One of the major problems in mariculture is the unavailability of fries and

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fingerlings, which are difficult to be collected from the wild. The synchronous availability of spawners of both sexes may be difficult for artificial fertilization. Therefore, successful storage of male gametes for short periods can easily facilitate artificial fertilization on the availability of female spawners. It has been reported that storage of fish semen above freezing temperature for short duration can be used for the artificial propagation of cultivable species like carp (Hulatha *et al.*, 1979).

In the present study, year round data was collected on milt parameters such as milt-volume, duration of motility, and spermatozoa concentration. Effect of factors such as salinity and pH on the duration of motility was also studied. Another investigation was on variation in the duration of motility and percentage of motile spermatozoa of milt samples taken from the testis of dead fish, isolated testis and stripped milt stored at room temperature. Study was also conducted on the role of air, oxygen, water vapour, their combinations and milk of coconut on short-term unfrozen storage of milt of *R. kanagurta*. These basic studies may form the basis for further studies in this aspect.

MATERIALS AND METHODS

Live specimens of *R. kanagurta* were collected from Vizhinjam coast of Kerala by operating both shore seine and boat seine. Male specimens of uniform size only were selected for the present study. Monthly samples were collected from March 1993 to February 1994. Anal region was wiped clean and dry with towel and tissue paper, milt was stripped by gently pressing the abdominal region into 15 ml storage vials. The milt obtained in most of the months were paste like and not free flowing as in the case of many other fishes. Care was taken to complete the study on duration of motility within the shortest possible time. Spermatozoa concentration and milt-volume were measured as early as possible. In the months of December, January and February, fishes obtained were with poorly developed testis and without milt. A minimum of sixty specimens was used for the present study in each month.

Milt-volume was determined using graduated capillary tube. Care was taken to avoid any air bubble being trapped along with milt. Duration of motility was determined by activating spermatozoa with 100% seawater (salinity 34.14‰). A small drop of milt was placed on a clean glass slide and seawater was pipetted on the glass slide close to it (dilution 1:400) covered with a clear cover glass and observed under a pre-focussed microscope (450X). The time period was noted from the time of mixing to the time up to which at least 20% spermatozoa exhibited active forward movement. The spermatozoa concentration was determined using modified Neubar counting chamber (Buyukhathipogulu and Holtz, 1978).

To study the effect of salinity on duration of motility, different con-

centrations of seawater samples were prepared (10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%) using sea water of salinity 34.14‰. Duration of motility was determined for each concentration. Similarly seawater samples of various pH were prepared and duration of motility was studied.

Milt was taken in a watch glass and kept at room temperature (28^0C) to study the effect of storage on room temperature. Percentage reduction in the duration of motility, viability and also the percentage of motile spermatozoa in 100% sea water were determined from a small drop taken from the sample at an interval of 30 minutes.

To study the effect of storage of milt in isolated testis, testis was isolated from the fish after killing it and kept in a petridish at room temperature. A slice of testis was taken in an interval of 30 minutes, the milt was extracted and the duration of motility and percentage of motile spermatozoa were determined.

The fish with testis was kept at room temperature to study its storage in the body of fish. At intervals of 30 minutes, a small drop of milt was stripped and duration of motility, viability and percentage of motile spermatozoa were determined.

Experiments were devised to check the effect of anaerobic storage of milt. Milt samples were taken in 0.25ml polypropylene screw cap storage vials, by filling without air space. Vials were kept in refrigerator at 4^0C (± 0.5). In each experiment three samples were used. Samples were drawn at every 24-hour interval and were analysed for spermatozoa qualities like duration of motility, viability and percentage of motile spermatozoa.

Experiments were conducted to study the role of air on the storage of milt. 0.25 ml milt was taken in a petridish and placed at the base of a 1.5L polythene bag filled with air and tied at the top. Care was taken to avoid falling of condensed water drops into the milt. Petridish and samples were kept in the refrigerator at 4^0C (±0.5). At every 24 hours intervals, sample was drawn and analysed.

Another experiment was devised to evaluate the role of air and water vapour on the storage of milt. 0.25 ml milt was taken in a petridish and placed at the base of 1.5-L polythene bag. Wet cotton and filter paper were placed at the base outside the petridish. Bag was then filled with air and tied well. Three samples each were placed in the refrigerator at 4^0C (±0.5). Samples were drawn at every 24 hour intervals and analysed. Similar experiments were done by adding 500mg streptomycin to 1ml of milt.

Experiments were conducted with milt diluted with milk of coconut in the ratio 1: 3 and stored in 10 ml screw capped storage vials and kept at 4^0C (±0.5). Samples were taken at every 24 hour interval and analysed.

RESULTS AND DISCUSSION

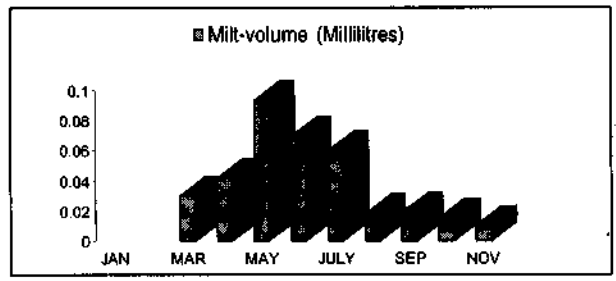

Fig. 1. Monthly variation in the milt-volume of *R.kanagurta*

Milt-volume was found to be increasing from March with a maximum value of 0.094ml recorded in May (Fig. 1). Species dependent variation has been observed in milt-volume. Milt-volume of 0.2-2.2 ml in turbot (Sequet *et al.*, 1994), 1.5 ml in perch and 0.5-1.7 ml in white fish (Piironen, 1995), 0.5 ml in *Epinephelus malabaricus* (Chao *et al.*, 1992) are comparable.

Fig.2 represents the monthly variation in the duration of motility, which was found to be maximum in August (101.6 sec.) followed by October (96.53 sec.) and the least was observed in November (62.33 sec.). Duration of motility of spermatozoa shows wide variation among species and was observed to be 1-17 minutes in Turbot (Fauvel *et al.*, 1993), 1-120 seconds in Atlantic cod (Trippel and Nielson, 1992) and 2-3 minutes in *Esox lucius* (Ginsburg 1992).

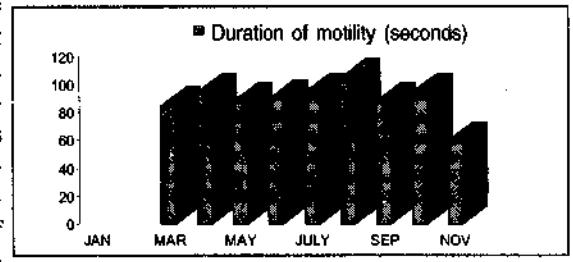

Fig. 2. Monthly variation in the duration of motility of spermatozoa of *R. kanagurta*

Monthly record of spermatozoa concentration is presented in Fig. 3. The maximum value recorded was $27x10^9ml^{-1}$ in the month of August followed by June ($25.2 x10^9ml^{-1}$). The minimum value was in March ($13.5x 10^9ml^{-1}$). Spermatozoa concentration also shows species-wise variation. Spermatozoa concentration as reported by Chauvaud *et al.*(1995) in turbot ($0.7-11 x10^9ml^{-1}$) and Gwo (1982) in Atlantic croaker (23.4 –36.4

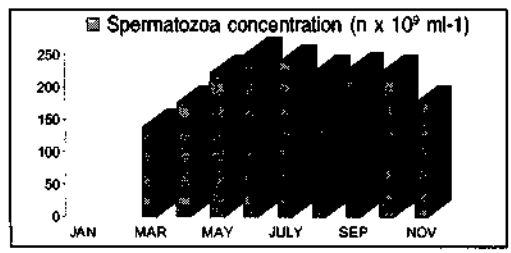

Fig.3.Monthly variation in the spermatozoa concentration of milt of *R. kanagurta*

$x10^9ml^{-1}$), are also contrasting. Comparatively high spermatozoa concentration was observed in the milt of mackerel. The chance for spermatozoa to enter into micropyle is very limited owing to its brief life span and the short distance that can be covered during this time. To overcome this handicap, there is no other way than to increase the number of spermatozoa (Billard,1992). Milt was not available in the specimens collected in January, February and December.

Duration of motility was found to be decreasing with decrease in salinity up to 70% seawater. Maximum duration of motility was recorded in 100% seawater (Table 1) which was gradually decreased with decrease in salinity up to 70% seawater (75 seconds). Salinity below 70% seawater was found to be ineffective in inducing motility. Spermatozoa exhibited the fastest movement in 100% seawater, which was found decreasing with decrease in salinity. Spermatozoa of mackerel was found to be motile only in alkaline pH *i.e.*, in media of pH 9 (106 ± 13.11 sec.). Motility of mackerel spermatozoa in a short range *i.e.*, in pH 9 may be due to the special conditions of its spawning ground.

Table 1. Analysis of variance comparing duration of motility and percentage of motile spermatozoa of *R. kanagurta* in various dilutions of sea water

Dilutions of seawater	Duration of motility		Percentage of motile spermatozoa	
(%)	Mean	±SD	Mean	±SD
70	75	4	51	4
80	78	18	75	1
90	82	9	75	3
100	87	17	81	2
	F ratio - 2.0496		F ratio - 186.3474	

Of the factors affecting the duration of motility, salinity is a major factor where marine fish spermatozoa are concerned. According to Hines and Yadshou (1971), neither freshwater nor seawater will give the optimum duration of motility. The variation showed by mackerel spermatozoa *i.e.*, reduction in the duration of motility with decrease in salinity may be connected with the specialities of the spawning ground of the species. Spermatozoa motility is initiated by hypertonic media as in many marine fishes (Oda and Morisawa, 1993) as is also observed in mackerel. It has also been reported that hypertonocity initiates motility in fresh water fishes such as *Cyprinus carpio, Carassius auratus,* and *Tribolodon hakonensis* (Morizava and Suzuki, 1980). The intensity of motility was found to be high in media of high osmotic pressure. Chambeyron and Zohar (1990) also reported simi-

lar observation in the spermatozoa of *Sparus auratus*.

Of the storage conditions tried for milt of mackerel, stripped milt stored at room temperature was found to be superior, which maintained motility after 180 minutes followed by milt stored in isolated testis (motility up to 120 minutes) and the least was with milt stored inside the fish (motility up to 90 minutes). The duration of motility and percentage of motile spermatozoa are presented in Fig. 4 and 5.

Successful storage of milt in these conditions and the superior results in the stripped milt sample may be due to the availability of oxygen by diffusion from the atmosphere. The drastic decline in the quality after sometime can be due to the drying up of the outer layer making it impermeable to air. Inside the isolated testis, there is absolutely no supply of oxygen, which makes the viable period very short. Furthermore, autolysis takes place in the body of dead fish soon after death.

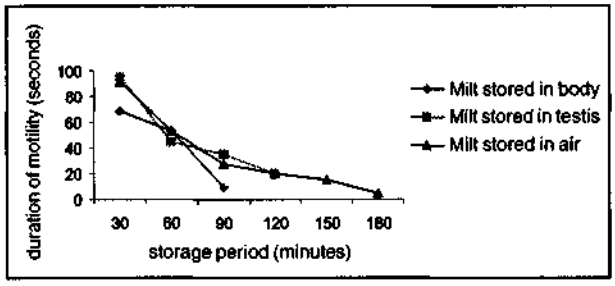

Fig.4 Duration of motility of spermatozoa of mackerel milt stored at room temperature

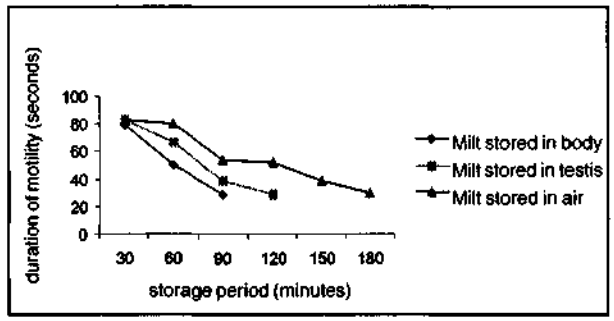

Fig. 5. Percentage of motile spermatozoa of mackerel milt stored at room temperature

In the eight conditions tried for short-term storage of milt of mackerel, storage of milt with oxygen was found to be superior which maintained motility up to 20th day (Fig. 6). Stoss and Refstie (1983) maintained fertility in stored undiluted Atlantic salmon milt up to 10 days at 0^0C under oxygen and with the addition of penicillin and streptomycin. Investigators like Carpentier and Billard (1978), Scott and Baynes (1980) obtained longer survival period *i.e.*, more than one week when stored at temperature around 0^0C in salmon spermatozoa. Scheuring (1924) reported survival of undiluted rainbow trout spermatozoa at 0-6^0C for five days.

Extended survival period on samples stored at 4°C than that stored at room temperature may be due to the reduction in the rate of metabolism (Stoss and Holtz, 1983). Storage of spermatozoa at -2°C by Stoss *et al.*(1978) was found to be detrimental due to the formation of ice crystals. The increase in survival in the samples stored with water vapour may be because of the very low rate of evaporation, which prevents dehydration and subsequent osmotic disturbances. These findings are supported by the reports of Stoss and Holtz (1983). Many authors like Stoss and Refstie (1983), Harvey and Kelley (1984), Stoss *et al.* (1978) and Billard (1981) reported that the addition of antibiotics is essential for the better survival of spermatozoa whereas in the present study, the survival of spermatozoa was poor when stored with antibiotics than with oxygen. This indicates that species-specific modifications are essential for the storage conditions of spermatozoa. Tom and Mitra (1994) observed that the aerated samples of milt of *Cyprinus carpio* were motile up to 140 hours. The survival of spermatozoa in samples added with milk of coconut may be because of its isotonicity with milt.

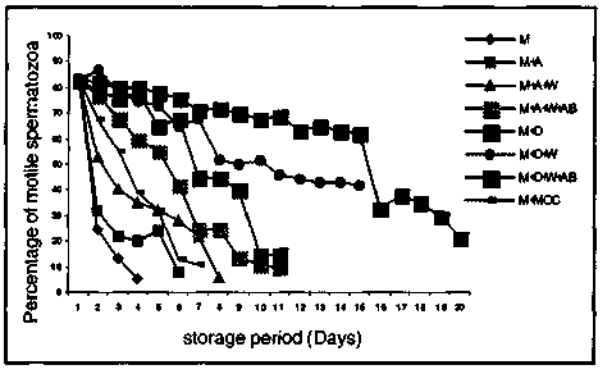

Fig. 6. Percentage of motile spermatozoa of milt of mackerel stored under different storage conditions at 4°C.

CONCLUSION

Month-wise variation was observed in the milt-volume, spermatozoa concentration and duration of motility. Spermatozoa of mackerel were found to be motile in 100-70% seawater, the maximum duration being in 100%. Motility was observed only in seawater of pH 9. Storage of stripped milt was found to be advantageous than storage either in isolated testis or *in situ* in fish. For the short-term storage of spermatozoa, storage of milt with oxygen was found to be surviving for the maximum period *i.e.* 20 days with 20% motile spermatozoa. Milt stored with oxygen and water vapour and milt stored with oxygen, water vapour and antibiotics resulted in survival up to 15 days with 42 and 79% motility.

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