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# Biological Parameters of Milt and Scanning Electron Microscopic Studies on Spermatozoa of Crescent Perch *Terapon jarbua* (Forsskål, 1775)

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## Abstract

This paper deals with the study of milt quality parameters and scanning electron microscopic studies on spermatozoa of a potent candidate species for aquaculture, *Terapon jarbua*. Since the spermatozoa quality parameters are highly variable, it is imperative that before any breeding attempt, one should ensure milt quality. Milt yielded from *T. jarbua* was 0.04±0.002ml. Spermatozoa concentration obtained was $7.11\pm3.13x10^{9}ml^{-1,}$ and viability was (91.13±0.99%). Motility and duration of motility were (83.4±7.81%) and 152.3±1.88, respectively. Spermatocrit value observed was 58.84± 11.83%. Milt was milky white in colour and thick fluid. Based on electron microscopic studies, spermatozoa can be differentiated into head, mid-piece and tail. The length of the head from tip to the mid-piece was 1.32±0.34µm and width was 1.65±0.32µm. Mid-piece measured 0.42±0.13 µm in length and 1.05±0.174 µm in diameter. The length and breadth of the tail were measured as13.8±1.16 and 0.26±0.06 µm, respectively.

**Keywords:** Spermatozoa, Motility, Viability, Motility score

## 1. Introduction

Fish spermatozoan generally differ in morphology and ultrastructure (Jamieson, 1991; Psenicka *et al.*, 2007). Even though minimal work is available on the spermatozoa morphology, most of the available literature on spermatozoan biology of teleost fishes show wide variations in sperm morphology and physiology (Psenicka *et al*., 2007; Cosson, 2019). The Crescent Perch or Tiger Bass *Terapon jarbua* (Forsskål, 1775) is brackish water to deeper water species, occurring in Indo-Pacific regions (Kuiter and Tonozuka, 2001). This species is a catadromous fish, in which the adult fishes spawn in deeper water and the juveniles move to the shallow sandy bottom area near the estuarine and Barmouth region (Chanthran *et al*., 2020). The species is considered as a commercially important marine ornamental as well as a food fish (Sirajudeen and Biju Kumar, 2011).

Sperm quality is an index of the ability of sperm to successfully fertilise an egg (Rurangwa *et al*. 2004). It includes motility induction, motility duration, percentage of motility, motility score and spermatocrit value. Hence, milt volume, spermatozoa concentration, viability, duration of motility, motility score and spermatocrit value are quantitative measures whereas, colour and nature of milt are qualitative parameters. Billard (1969) studied the morphology of spermatozoa of teleost fishes (common carp, rainbow trout, brown trout and guppy) using a scanning electron microscope (SEM). The high-quality sperms are essential for captive fish bloodstock and excellent production of valuable offspring for aquaculture (Kjørsvik *et al*., 1990; Bromage and Roberts, 1995; Rurangwa *et al*., 2004). Morphology of spermatozoa is one of the primary methods for assessing its performance. This study is aimed to determine the biological parameters of milt and morphology of spermatozoa of *Terapon jarbua*.

## 2. Materials and Methods

Live specimens of *Terapon jarbua* was collected from Vizinjum, Poonthura and Veli coastal areas operating shore-seine, hook and line or traps. Live fishes were brought and kept under the laboratory conditions, and semen samples were collected from fully mature males. Live fish was stripped to collect milt, and the milt volume was measured using a graduated capillary tube. The concentration of the spermatozoa was assessed using an improved Neubauer counting chamber following standard clinical methods (Buyukhatipoglu and Holtz, 1978). Viability was determined using eosin-nigrosin dye exclusion method (Chao *et al.*,1975). One drop of milt was placed in a slide and mixed with two drops of 10% nigrosine and one drop of 5% eosin; then thoroughly mixed, and a thin uniform smear was prepared on the slide. The slides were air-dried and observed under a pre-focussed compound microscope (450X). Dead spermatozoa were found as pink or red coloured, and live ones in grey or ash colour. From each field, live, dead and total spermatozoa were enumerated.

Viability was calculated as follows:

$$\text{Viability} = \frac{\text{no. of live spermatozoa x 100}}{\text{total number of spermatozoa}}$$

Duration of motility of spermatozoa is the period up to which the spermatozoa show vigorous and rapid forward movement. To assess the duration of motility, 100% seawater was considered as the standard activating media. A small drop of milt was taken in a glass slide and mixed with the activating media (dilution approximately 1:400), and the transparency was viewed under a pre-focussed microscope (450X). Duration of motility was assessed from the time of mixing up of milt with activating media to the time up to which at least 20% of spermatozoa show active forward movement.

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### 2.1 Percentage of motile spermatozoa (Motility)

Activated spermatozoa were viewed under a pre-focussed microscope and the percentage of spermatozoa exhibiting active forward movement in the field, and the total immovable spermatozoa were enumerated.

Motility was calculated as follows:

$$\text{Motility} = \frac{\text{number of motile spermatozoa} \times 100}{\text{total number of spermatozoa}}$$

### 2.2 Motility Score

Motility Score was assessed based on Goodall *et al.* (1989), with slight modification following an arbitrary scale.

| SCORE | DESCRIPTION |
|---|---|
| 0 | 100% spermatozoa immotile |
| I | <30% spermatozoa actively motile |
| II | 30-50% spermatozoa actively motile |
| III | 50-70% spermatozoa actively motile |
| IV | 70-80% spermatozoa actively motile |
| V | >80% spermatozoa actively motile |

### 2.3 Spermatocrit value

The number of spermatozoa (sperm density) was counted following Buyukhatipoglu (1977), and it was measured by haematocrit centrifuge.

### 2.4 Colour and nature of milt

Colour and nature of the milt were observed at the time of stripping.

### 2.5 Scanning Electron Microscope (SEM) Studies

Milt was rinsed with marine ringer solution to get rid of debris and centrifuged at 3000 rpm for five minutes and then fixed in 3% Glutaraldehyde (in phosphate buffer) for 3hours. The samples were dehydrated in ascending series of ethanol, critical-point-dried, gold-coated, mounted on SEM sample mounts and examined with a scanning electron microscope (S 2400, HITACHI and Carl Zeiss) at an acceleration voltage of 15kv and micrographs were taken.

## 3. Results

Biological parameters, such as milt volume, Spermatozoa concentration, viability, duration of motility, percentage of motile spermatozoa, motility score and spermatocrit value are given in table1. Mean milt volume observed was 0.04±0.002ml in *Terapon jarbua*.

The mean spermatozoa concentration was 7.11± 3.13 x $10^9$ $ml^{-1}$. The viability of freshly collected milt was observed to be 91.13± 0.99 % (Table. 1). The spermatozoa of *T. jarbua* in 100% seawater showed a duration of motility of 152.3 ± 1.88s. (Table. 1). The percentage of motile spermatozoa observed was 83.4 ± 7.81 % in 100% seawater. The motility score of spermatozoa of fresh milt of *T. jarbua* was V (Table. 1). The mean spermatocrit value of freshly collected milt was 58.84± 11.83% (Table. 1).

Milt was found to be homogeneous. The colour of *T. Jarbua* milt was observed milky white. Contaminated milt appeared yellowish or pinkish. This is due to the mixing of faecal matter, blood or urine.

Spermatozoa of *T. jarbua* are generally flattened oval in shape and acrosome was absent. It is uniflagellated and differentiated into head, mid-piece, and a long tail (Fig.1, 2). Head was located in the anterior portion of the spermatozoa, and the dimensions were 1.32±0.34µm in length and 1.65±0.32µm in width. The anteroposterior length was found to be less than the width or diameter of the head. Sperm has a well-differentiated mid-piece or neck (Fig. 2). Mid-piece was located between the head and tail. Mid-piece consists of five radially placed spherical mitochondria around the central axis which make it asymmetrical. Its dimensions measured were 0.418±0.013µm length and 1.049±0.174µm width. Long-tail was inserted at the posterior region of the mid-piece, which measures 13.8±1.16µm in length.

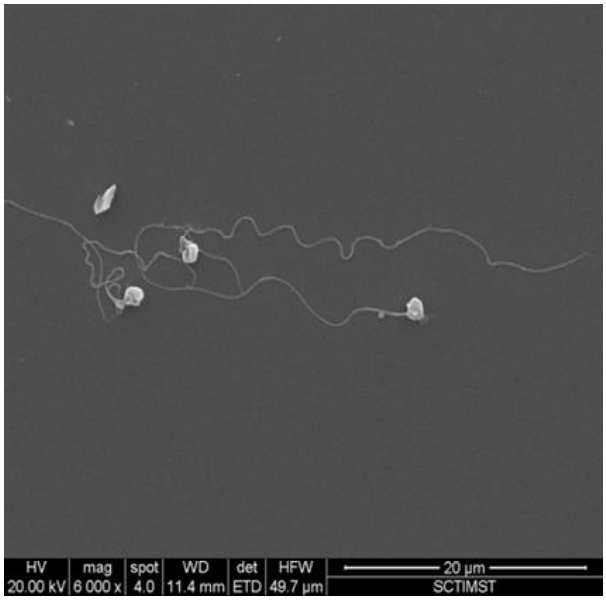


**Fig. 1.** Scanning electron micrographs of sperms of *Terapon jarbua*

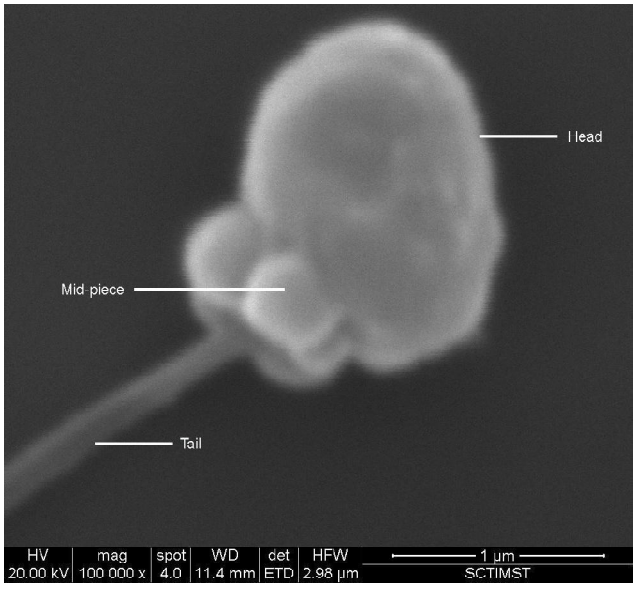


**Fig. 2.** Scanning electron micrograph of sperms head of *Terapon jarbua*

**Table 1.** Spermatological parameters of *Terapon jarbua*

| Sperm quality parameters | Mean | ±SD |
|---|---|---|
| Milt volume (ml) | 0.04 | 0.002 |
| Spermatozoa con:(x$10^9$ml) | 7.106 | 3.131 |
| Viability of sperm (%) | 91.133 | 0.993 |
| % of motile spermatozoa | 83.4 | 7.806 |
| Duration of motility (s) | 152.3 | 1.889 |
| Motility score | V | |
| spermatocrit value(%) | 58.84 | 11.83 |
| Colour of milt | milky white | |

## 4. Discussion

The quality of spermatozoa is determined by its capacity to fertilize (Rurangwa *et al*. 2004). Sperm quality is determined by a number of parameters like induction of motility, duration of motility, speed of spermatozoa and fertilising capacity of sperm. Milt consists of sperm and seminal plasma (Islam and Akhter, 2011). The milt quality is assessed mainly by biological parameters such as milt-volume, spermatozoa concentration, viability, duration of motility and spermatocrit value.

In the present study, the milt volume observed in *Terapon jarbua* was 0.04±0.002ml. Large-sized fishes usually contain large amounts of milt, and it is species dependent (Pereira. 2000). The milt volume ranges from 0.1 ml in Pike (de Montalembert*et al*., 1980) rainbow trout (Sanchez Rodriguez *et al*., 1978) to 92 ml. in Atlantic halibut. The variation in milt-volume indicates variable requirements for the minimum number of spermatozoa required for fertilisation in different species of fishes (Piironen and Hyvarincn, 1983; Piironen, 1987; Erdahl and Graham, 1987). Moccia and Munkittrick (1987), Baynes and Scott (1985), Marshall and Bryson (1988) and Marshall *et al*., (1989) observed that milt volume varies with different species of fishes.

In the case of mammals and birds, fertilization is internal and needs comparatively less number of spermatozoa. Whereas in the case of fishes, fertilization is external and requires a very high number of spermatozoa since milt is diluted in the infinitely high concentration of water. High spermatozoa concentration is required to get good fertilisation rates. The rate of fertilisation is affected by spermatozoa concentration (Aas *et al*., 1991). Spermatozoa concentration of *Terapon jarbua* (7.11± 3.13 x $10^9$ $ml^{-1}$) was compared with other fishes *Ctenopharyngodon Idella* (94.45 x$10^9$$ml^{-1}$), *Cirrhinus Mrigala* (94.08 x $10^9$ $m1^{-1}$), *Labeo rohita* (93.08 x $10^9$ $m1^{-1}$ ), *Cyprinus carpio* (93.93 x $10^9$ $m1^{-1}$ ), *Mystus gulio* (82.73 x $10^9$ $m1^{-1}$ ), *Amblypharyngodon mola* (89.01 x $10^9$ $m1^{-1}$), *Anabas testudineus* (93.12 x $10^9$$m1^{-1}$), *Ambassis commersoni* (94.85 x $10^9$$m1^{-1}$), *Clarias batrachus* '(95.10 x $10^9$$m1^{-1}$), *Puntius filamentosus* (93.56 x $10^9$ $m1^{-1}$) and *Heteropneustes fossilis* (94.26 x $10^9$ $m1^{-1}$ ) (Chao *et al*., 1974; Billard 1992; Bimal lal 1993; Sunitha and Paul 1998).

Viability determines the biochemically functional spermatozoa. In the present investigation, the mean viability was 91.13%, which is somewhat low in contrast with different reports. Bimal lal (1993) observed the viability of spermatozoa of various species such as *Ctenopharyngodon Idella* (94.45%), *Cirrhinus mrigala* (94.08%), *Labeo rohita* (93.08%) and *Cyprinus carpio* (93.93%). According to Sunitha (1995), 82.73% of spermatozoa are viable in the milt of *Mystus gulio*, whereas, in the present study, the value was comparatively high.

Percentage of motile spermatozoa means the number of spermatozoa, which can move forward in the medium. According to Terner (1986), sperm motility is the simple and best index of sperm quality. The mean percentage of motile spermatozoa observed in the present study was 83.4%±7.81, which is very high. A high percentage of motile spermatozoa implies greater fertilisation success. Geffen and Frayer (1993) suggested that about 76.3% of turbot (*Scophthalmus maximus*) spermatozoa become motile with the help of seawater. Levanduski and Cloud (1988), reported reliable fertilization results with motile spermatozoa as low as 10%. According to Harvey and Kelley (1984), Billard and Cosson (1992), Ohta *et al*. (1995), Morisava *et al*. (1983), Stoss (1983), and Cosson *et al*. (1985), there is a relationship between percentage motility and fertilization capacity of spermatozoa in many fishes. Harvey and Kelley (1984) suggested that tilapia milt with 20% motile spermatozoa would be supposed to yield about 80% fertility. Ohta *et al*. (1996) reported 89.6% of fertility using milt, having only 33.5% of motile spermatozoa.

The duration of motility is the period up to which the spermatozoa show active forward movement in a particular medium. Longer is the duration of motility, higher will be the rate of fertilisation (Van Heerden *et al*., 1993). In the present study, the duration of motility was 152.3±1.88s. The observed duration of motility ranges from 2s. in Peejerry, *Odontesthes bonariensis* (Strussman *et al*., 1994) to 120 min. in the guppy (Billard, 1986). In viviparous fishes, the duration of motility of spermatozoa was comparatively longer (Billard, 1986) than those fishes with external fertilization. The short period of spermatozoa motility appears to be overcome by the high spermatozoa concentration.

Motility score according to Goodall *et al.*, (1989) is one of the different methods of expressing motility, which is expressed as grades from I-V. Semen samples having a greater percentage of motile spermatozoa exhibit a high score. In the present study, the fish semen exhibited a high score, ie., V, indicating high motility.

Spermatocrit value for milt varies with species of fishes (Piironen and Hyvarinen, 1983). In *Terapon jarbua* the spermatocrit (SP) value was 58.84%, and the sperm concentration was 7.11 x $10^9$ $ml^{-1}$. The spermatocrit value and concentration in rainbow trout are lower at the beginning of spawning. The high values observed in perch at the end of spawning may be caused by the reabsorption of seminal plasma (Piironen and Hyvarinen,1983). The sperm density increases with the increase of spermatocrit value. Thus the eloquent relationship observed between spermatocrit value, and sperm density allows the usage of spermatocrit as an estimate of sperm density in *T. jarbua*.

Colour and nature of milt differ from fish to fish (Pereira, 2000). Fresh and uncontaminated milt was considered as homogeneous. In the present study, the colour of milt was milky-white and homogenous having thick consistency, thereby indicating its efficiency. The present study is a first of its kind on the morphology of *Terapon jarbua* spermatozoa using scanning electron microscopy (SEM). For the reproduction, most of the marine and freshwater fishes adopt external fertilisation, and their spermatozoa become motile once released into seawater or freshwater. The primary function of spermatozoan is to swim through the extremely hostile environment to find and to fertilize the egg successfully.

The shape of the head of sperm in different fishes are spherical in carp (Gwo et al., 1993), (Lin et al., 1996), *Anabas testudineus*, *Ambassis commersoni* and *Puntius filamentosus* (Paul, 1998). Ovoid sperm head has been observed in Perch (*Perca fulvescens*) (Lahnsteiner et al.,1995), Wolf fish (*Anarhichas lupus*) (Pavlov et al., 1997), rainbow trout and marine puffer (Gwo et al., 1993). In the present study, the spermatozoa head was ovoid. Jamieson (1991) reported that elongated heads are an excellent characteristic than spherical heads.

The length and width of sperm head of different fishes reported are; *Cyprinus carpio* 3 µ (length) and 2.5 µ (width) (Billard, 1969) *Anabas testudineus* 2.3 µ (length) (Paul, 1998), *Cyprinus carpio* 2.5 µ (length) (Billard, 1970), *Mugil cephalus* 2.3 µ (length) and 1.4 µ (width) (Chao et al., 1975), *Puntius filamentosus* 2.9 µ (length) (Paul, 1998), *Perca flavescens* 2.5 µ (length) and1.8 µ(width) (Koenig et al., 1978). In the present study, the length and width of the sperm head were 1.3 and 1.7, respectively.

In wolf fish, spermatozoa have a mid-piece measuring 1.5 x 1.5ì (Pavlov et al., 1997). The mid-piece is comparatively more significant in the case of fishes having internal fertilisation. A collar-like globular shape has five mitochondrial sections observed in the mid-piece. Usually, the smaller size of midpiece has a short period of motility (Ginsburg, 1968; Fribourgh *et al*., 1970; Gardiner, 1978; Pavlov *et al*., 1997). In the present study, also a small or distinct midpiece was present. Tail or flagella is the most important organ for the motility of spermatozoa. The insertion of the tail is the primary factor responsible for the symmetry of spermatozoa. In perch and turbot, spermatozoa are asymmetrically shaped because of the mediolaterally inserted flagellum and the structure of midpiece (Stein, 1981; Lahnsteiner *et al*., 1994). In turbot, the insertion of the flagellum is mediolateral and hence asymmetric (Lahnsteiner *et al*., 1994). In comparison, the insertions are caudolateral in Cyprinidae (Baccetti, 1984) and Esocidae (Billard, 1970). In the present study, spermatozoa of *T. jarbua* were found to be asymmetrical In conclusion, the data obtained on the milt quality parameters guarantee that the breeding programme can be implemented with a high rate of success if healthy eggs of *T. jarbua* are made available. Morphological studies of the spermatozoa can be used as a confirmation for functional integrity.

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