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BIOLOGICAL CHARACTERISTICS OF MILT AND FACTORS AFFECTING MOTILITY OF SPERMATOZOA OF INDIAN SAND WHITING *SILLAGO SIHAMA* (FORSSKÅL)

Revathy, S.,[1] Benno Pereira, F.G.[2] and Anu Thottappilly[3]

[1] Department of Zoology, St Xavier's College for Women, Aluva- 683101, Kerala, India
[2] *Department of Zoology, University of Kerala, Kariavattom Campus, Thiruvananthapuram -699581, Kerala,India*
[3] Department of Zoology, St Albert's College (Autonomous) Ernakulam-682018, Kerala,India
*Email:revathy@stxaviersaluva.com

Abstract: There is high need for superior quality gametes as fish farming expands and there is great need for gamete storage for artificial fertilization programmes. There is utmost need to have idea of milt of fishes to understand its reproductive potential. With this aim the present study is attempted to investigate various biological parameters of milt and factors affecting motility of *Sillago sihama,* a candidate species for mariculture. Milt of *Sillago sihama* showed high concentration of spermatozoa, viability, percentage of motile spermatozoa, duration of motility and motility score *Sillago sihama* exhibited wide range of motility from 30 to 100% sea water. Spermatozoa of *Sillago sihama* were motile in pH 7.5 to10, with a maximum duration of motility (91 sec) in pH 9. A gradual decrease in motility was observed with increasing temperature.

Key words: Biological parameters, milt, sperm, motility, salinity, pH, temperature, *Sillago sihama*

INTRODUCTION

The utilisation of superior quality gametes from fish brood stock is of great significance for producing viable and healthy larvae for fish culture and rearing (Kjorsvik *et al.,* 1990; Bromage and Roberts, 1995). Milt quality is the measure of the capability of sperm to successfully fertilize an egg. Milt quality is evaluated based on milt volume, spermatozoa concentration, viability, duration of motility, motility score, colour and nature of milt (Marshal and Bryson, 1989; Kazakov,1981 and Rurangwa *et al.,* 2004). Milt volume, spermatozoa concentration, viability, duration of motility, motility score are quantitative parameters while colour and nature of milt are qualitative parameters.

Sperm motility in fishes is dependent on several factors like temperature, pH,ions and their concentrations, osmolality etc (Morisawa *et al.,* 1999 and Alavi *et al.,* 2011).

Sillago sihama can be an attractive species for aquaculture sector. With this aim in the present study a preliminary attempt has been made to study and record various biological parameters of milt, and factors affecting motility of *Sillago sihama.*

MATERIALS AND METHODS

Sillago sihama (Forsskål, 1775) (Perciformes: Sillaginidae) specimens for the study were collected from the Chinese dip net operating areas of Cochin. Milt was collected from the live fishes after cleaning the genital area with dry cotton. At first gentle pressure was applied on the abdominal region posterior to get rid of faces and urine. Following which pressure was applied on both sides simultaneously in the posterior direction. Milt was stripped into 10 ml polypropylene storage vials and kept above crushed ice in Styrofoam box. Care was taken to avoid contamination of milt with blood, urine, faeces or mucus and also the stored milt was in no way allowed to come in contact with water. Colour and nature of milt, milt volume, spermatozoa concentration (Standard Clinical Method Buyukhatipogulu and Holtz,

1978, viability (Eosine –Nigrosine dye exclusion method Chao *et al.,*1975), duration of motility and percentage of motile spermatozoa, motility score (with slight modification of Goodall *et al.,*1989) were recorded.

Effect of salinity and pH on motility was carried out by preparing varying dilutions of sea water (10,20,30,40,50,60,70,80 and 90%), pH (5,5.5,6,6.5,7,7.5,8,8.5,9,9.5,10). Different temperatures from 5^0C to 30^0C with an interval of 5^0C were maintained to find out the effect of temperature on motility.

RESULTS

Biological Characteristics of Milt

Uncontaminated milt was mucilaginous in nature and white in colour. Milt volume (ml) recorded was 0.05 ± 0.019.The mean spermatozoa concentration ($x10^9$ ml^{-1}) of *Sillago sihama* was 74.44 ± 6.71.Viability indicates the physiologically functional spermatozoa and percentage of motile spermatozoa denotes the number of spermatozoa which can actively move in the medium. Viability of spermatozoa of *Sillago sihama* was 92% ± 4.99.Duration of motility is the time up to which spermatozoa remain motile in a particular medium. The duration of motility (seconds) of sperm of *Sillago sihama* was 104.33 ± 13.33. Motility score is also another way of expressing motility and is represented as grades I- V. Since *Sillago sihama* had high percentage of motile spermatozoa a score V was given. (Table 1)

Effect of salinity on sperm motility

Sillago sihama exhibited motility in wide range of salinity (from 30 to 100% sea water). The duration of motility was found decreasing with increasing salinities. The maximum duration of motility was 426.46 sec in 30% sea water and minimum duration of motility was in 100 % sea water. However percentage of motile spermatozoa was increasing with increase in salinity. Maximum percentage of motility (83.66%) was observed in 100 % sea water. (Table 2, Figure 1 & 2).

Effect of pH on sperm motility

Spermatozoa of *Sillago sihama* were motile in pH 7.5 to10, with a maximum duration of motility (91 sec) in pH 9. The least duration of motility (22 sec) was in pH 7.5. A gradual increase in the duration of motility was observed with increase in pH value. On observation the percentage wise spermatozoa, 91% of spermatozoa was motile in pH 9.5 while only 22% spermatozoa were motile in pH 7.5. (Table 3, Figure 3 & 4).

Effect of temperature on sperm motility

Sillago sihama exhibited a gradual decrease in motility from 145 sec at 5^0C to 87 sec at 30^0C. However the intensity of motility showed an increase with increasing temperature. (Table 4, Figure 5).

Table 1. Biological Characteristics of Milt of *Sillago sihama*

Parameter	Mean	SD
Milt volume (ml)	0.05	0.019
Spermatozoa concentration ($x10^9$ ml^{-1})	74.44	6.71
Viability (%)	92	4.99
% of Motile Spermatozoa	85.8	10.99
Duration of Motility (Seconds)	104.33	13.33

Table 2. Effect of salinity on sperm motility

Parameter	Mean	SD
Duration of Motility (Seconds)	228	124.439
% of Motile Spermatozoa	60	21.739

Table 3. Effect of pH on sperm motility

Parameter	Mean	SD
Duration of Motility (Seconds)	70.167	25.864
% of Motile Spermatozoa	52.333	28.061

Table 4. Effect of Temperature on sperm motility

Parameter	Mean	SD
Duration of Motility (Seconds)	122	23.409

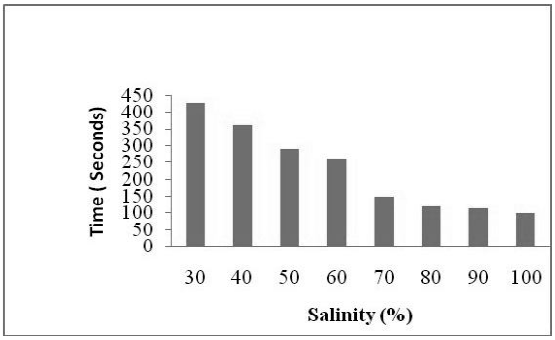

Fig. 1. Effect of Salinity on duration of sperm motility of *Sillago sihama*

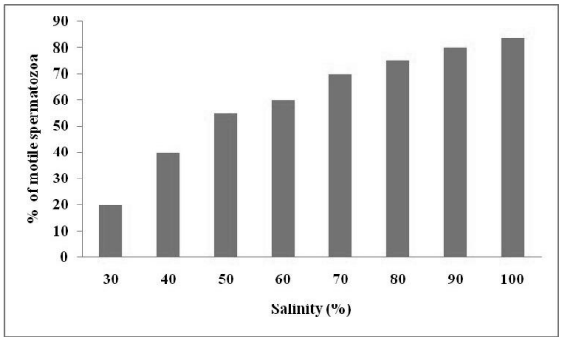

Fig. 2. Influence of Salinity on % of motile spermatozoa of *Sillago sihama*

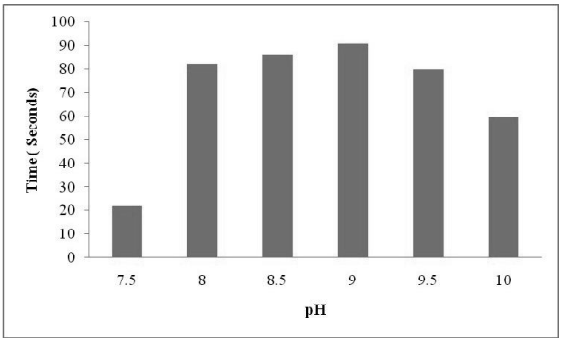

Fig. 3. Effect of pH on duration of sperm motility of *Sillago sihama*

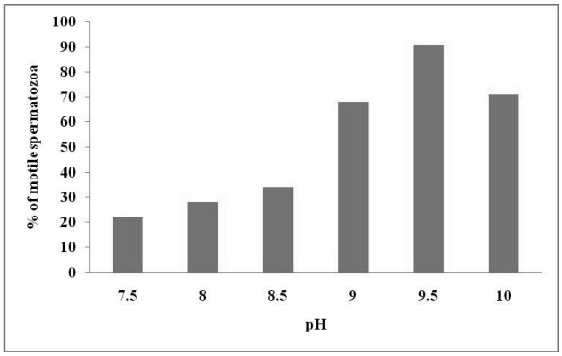

Fig. 4. Influence of pH on % of motile spermatozoa of *Sillago sihama*

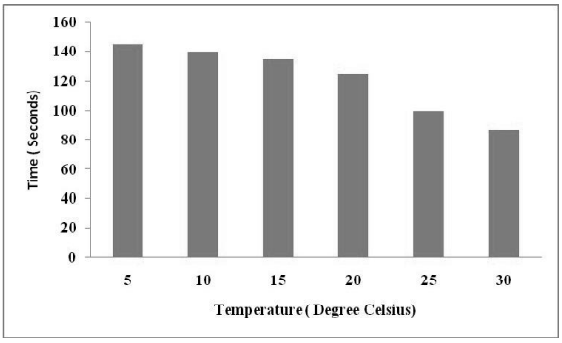

Fig. 5. Effect of Temperature on duration of sperm motility of *Sillago sihama*

DISCUSSION

Appearance, colour and nature of milt can be used to assess the quality of milt. The milt volume of fishes varies with species. Marshall and Bryson (1989) reported that there exists large variability among fish populations on milt volume. Spermatozoa concentration was considerably very high. In *Sillago sihama* the fertilization is external, the milt is released to external media *viz*., water and gets diluted and it is very difficult for the spermatozoa to meet the egg in such a short lifespan. The practicable adaptation is to have high concentration of spermatozoa. According to Aas *et al.* (1991) spermatozoa concentration has direct effect on fertilization. High spermatozoa concentration is required to get good fertilization in natural environment, while in artificial fertilization comparatively low number of spermatozoa as low as 10^3 spermatozoa per egg (Billard, 1992) and 75,000 spermatozoa per egg (Erdahl and Graham, 1987) is only required to get 100 % fertilization success. Chao *et al.* (1974) and Billard (1992) considered viability as reliable index of fertility. The mean percentage of motility observed in the study was also very high (85.8 ± 10.99). Greater the percentage of motility, greater is the fertilization success. Relationship between percentage motility and fertilization capacity of spermatozoa has been

reported in many fishes (Harvey and Kelley, 1984; Billard and Cosson 1992; Ohta *et al.,* 1995; Morisawa *et al.,* 1983; Stoss 1983; Cosson *et al.,* 1985). The motility score was V and duration of motility was also good. Longer the duration of motility, higher will be the rate of fertilisation (Van Heerden *et al.,* 1993). The semen characteristics vary between fish species and these parameters are important for initiating breeding programmes and formulating cryopreservation protocols (Routray *et al.,* 2006; Routray *et al.,* 2007).

Salinity can be a prime factor ensuring male reproductive success in fishes (Atse *et al.,* 2002). In altered and varying salinities there could be an osmotic inhibition of motility in freshwater and marine fishes (Morisawa *et al.,* 1992). Sperm motility, velocity, and fertilizing ability vary with seasonal variations in osmolality of seminal plasma (Babiak *et al.,* 2006). The differences in motility of *Sillago sihama* in varying salinities may be linked to this osmotic inhibition and variations in osmolality of the activating medium.

Extracellular and intracellular pH has an influential role in initiation and duration of sperm motility (Marian *et al.*, 1997). The intracellular proton concentration is influenced by external pH, which in turn modifies the membrane potential, and motility behaviour (Boitano *et al.,* 1991, Boitano *et al.,* 1992). Chao *et al.* (1992) found that spermatozoa are active when pH values were at 7.5-8. Too low or high pH inhibits motility. In the present study an alkaline pH at the range of 9 was suitable for the species.

The duration of motility duration, fertilizing capacity and sperm velocity is dependent on temperature of the activation medium as per the investigations of Ginsburg (1968), Billard *et al.* (1995) and Stoss (1983).The duration of motility in *Sillago sihama* was found to be decreasing with increasing temperature while the intensity of motility was higher at high temperatures. Similar results were reported by Alavi & Cosson, 2005, motility increases with temperature while duration of motility decreases with increasing temperature. Several scientists have reported an inverse relationship between sperm motility duration and temperature in salmonid fishes (Billard and Cosson, 1992; Vladic and Jarvi, 1997).

CONCLUSION

The biological parameters of milt of *Sillago sihama* showed high concentration of spermatozoa, viability, percentage of motile spermatozoa, duration of motility and motility score. Salinity, pH and temperature also have an influential in sperm motility. The study will serve as baseline data helpful for artificial breeding methods and short term and long term storage of sperm of *Sillago sihama*.

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