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Seasonal Variation in Semen Characteristics and Biochemical Composition of Seminal Plasma of Mrigal, *Cirrhinus mrigala* (Ham.)

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Abstract

The milt quality parameters such as percentage of spermatozoa motility, duration of motility, sperm concentration, and seminal plasma composition of milt of *Cirrhinus Mrigala* (*C. mrigala*) varied throughout the breeding season i.e. from April to September. The motility duration (second) was low in the beginning of season ie. April (42 ± 4.32) and recorded a peak of 97.5 ± 4.12 during July and again started declining and reached to 39 ± 4.76 at the end of the season. The mean pH values of seminal plasma ranged from 8.05 ± 0.19 to 8.6 ± 0.12 with a maximum pH in July. The osmolality of seminal plasma was low during beginning and end of breeding season. The highest osmolality of seminal plasma 291.5 mOsm kg^{-1} was observed during July. The milt yield ml kg^{-1}, spermatocrit (%), spermatozoa counts (nos/ml) observed in July were 13.9 ± 3.47, 82.5 ± 4.43, and 33.5 ± 1.4, respectively. The same parameters declined to 3.07 ± 0.76, 69.75 ± 4.78, and 14.3 ± 3.3 at the end of the breeding season. The following are the range of ion concentration during breeding season: Na 88.92 ± 22.22 to 140.5 ± 3.7 m Eq/L, K^+ 29.25 ± 5.0 to 52.3 ± 19.28 m Eq/L, and Cl 64.82 ± 3.60 to 174 ± 5.88 m Eq/L. The seasonal declines of Na and Cl ion levels were observed when seminal plasma osmolality values showed lower values. The mean range of total protein, cholesterol, and glucose were 0.105 ± 0.03 to
0.515 ± 0.05 g/dl, 8.52 ± 0.77 to 22.97 ± 2.98 mg/dl, and 0.525±0.05 to 1.83 ± 0.125mg/dl, respectively, during the spawning season. The semen characteristics and biochemical composition of mrigal will help in development of the basic knowledge and the strategies during artificial spawning programmes.

Introduction

Both male and female brood fish share equal responsibility for seed production. In brood fish farming, there has been more focus on female brood fish rather than male brood fish. The systematic studies on the functional efficacy of the teleostean testis with reference to carp are meagre. Several parameters have been documented to evaluate the milt quality including motility, spermatocrit, sperm density, fertilizing capacity

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osmolality and pH of seminal plasma, chemical composition of seminal plasma, enzymatic activity, and several others. Reports on such studies related to seasonal variation are fragmentary (Billard & Cosson 1992; Billard et al. 1995).

High quality of semen is important to the fisheries industry and laboratory research. There are many factors contributing to individual variation in sperm quality (Rana 1995) such as genetic variability among fish, rearing conditions, fish handling, sperm collection methods, storage of milt, and sperm activation conditions. The constitution of milt in terms of performance and numbers of spermatozoa, chemical composition, and osmolality varies interspecifically and even within the same individual with time. Therefore, the time of collection of milt is significant for successful cryopreservation. Individual and seasonal variability of gamete quality is well known for carps (Billard et al. 1995; Linhart et al. 1995; Christ et al. 1996). Correspondingly, Lubzens et al. 1997 and Linhart et al. 2000 indicated that this variability might also influence the motility and the success of fertilization after cryopreservation.

The biochemical composition of teleost milt has been studied by many workers over the years (Piironen & Hyvarinen 1983; Billard & Menezo 1984; Linhart et al. 1991; Billard et al. 1995b). The seminal plasma analysis includes inorganic constituents (Na^+, K^+, Ca^{2+}, Mg^{2+}) involved in the process of inhibition or activation of sperm motility (Morisawa et al. 1983; Morisawa 1985). Organic compounds such as triglycerides, glycerols, fatty acids, and glucose are found in seminal plasma (Lahnsteiner et al. 1993).

The Indian major carp, mrigal, *Cirrhinus mrigala* (Ham.) is a widely farmed species in the Indo-Gangetic floodplains of India, Bangladesh, and Pakistan. It is an important component of carp polyculture system. Mrigal occupies normally 30–40% in polyculture of Indian major carps consisting catla, rohu, and mrigal. Literatures on the seasonal variation in the biochemical composition of seminal plasma of Indian major carps are scanty. These parameters play an important role in the sperm of Indian major carps. To have controlled and successful production in aquaculture systems, it is necessary to have adequate knowledge of the physical and chemical characteristics of the semen of cultivated fishes. In the present study, an attempt has been made to evaluate the seasonal changes of semen characteristics and the biochemical composition of seminal plasma of mrigal, *C. mrigala* (Ham.) in detailed and systematic manner.

Materials and Methods

Carp brood husbandry practices

The brood fish of Indian major carp, *Cirrhinus mrigala* used in this study was reared in earthen ponds of 0.2 ha of the farm facility of the Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar (Lat. 20° 11’ 06"-20°11’ 45" N., Long.

85° 50'52" – 85°51' 35"E.), Orissa, India during October 2005 to August 2007.

Collection of semen

For the study of seasonal changes in the semen characteristics and biochemical composition of seminal plasma of *C. mrigala*, the milt was collected every month of the spawning season from two-year matured male brood fish (1.6 ± 0.4 kg) after five hour of intraperitoneal administration of hormone (Ovaprim, Salmon GnRH + domperidone, Syndel Laboratories, Canada) at a rate of 0.2 ml/kg body weight. Milt samples were collected in ice cooled and sterilized test tubes. During milt collection, attention was paid to prevent contamination by fecal matter, urine, blood, or scales; to provide enough oxygenation to the sperm by maintaining enough head space in the tubes, and to maintain the temperature of the collected semen at 4°C until further analysis. The collected semen was evaluated for sperm yield/kg body weight, motility, pH, spermatocrit percentage. sperm count, and biochemical composition of seminal plasma.

Motility assessment

Spermatozoa motility assessment was carried out by diluting milt with sterile water (1:100) at room temperature (31°C) on glass slide and was observed immediately under an inverted microscope (200X) (Zeiss, Germany) that is attached with a CCD camera. Estimation of spermatozoa motility was started immediately (approximately 10 s) after dilution and the movement was observed for 3 min. The motility was recorded in a computer using computer aided motility software (Biovis motility software, M/S Expert Vision Pvt. Ltd, India). The percentage of rapid, vigorous, and forward motility was observed and calculated in relation to the total number of observed (immotile and poorly motile) spermatozoa in each field of vision from the time activator was added until the motility up to 0.

Estimation of sperm count and percentage of spermatocrit

Spermatocrit values (packed sperm cells) of all the semen samples were determined by micro haematocrit centrifuge (Hermle, USA) immediately after collection of milt to avoid abnormal reading due to cellular swelling induced by CO_2 release (Wedemeyer & Yasutake 1977). All semen samples were assessed under a microscope (Zeiss, Germany) using a computer assisted semen analyzer (Biovis motility software) on a 20 µm micro cell counting chamber. The sperm count was carried out by diluting it 1000 times with an extender solution and adding 20 µl of mixture to the hemocytometer slide and observed under an inverted microscope. Sperm density was also determined by measuring spermatocrit value and also through microscopic sperm counting. Microhematocrit

capillary tubes (75 mm length and 1.2 mm diameter) were filled (approximately 75%) with semen and one end of each tube was sealed for tube centrifugation in a microhematocrit centrifuge at 10000 xg. Measurements were taken in triplicate for each sample, and the average of the three measurements was used for the results.

Measurement of osmolality

The seasonal variations of osmolality of seminal plasma were studied through the spawning season**.** The osmolality of seminal plasma was measured by an osmometer (Model 3250, Advanced Instruments Inc, Massachusetts-02062, USA) using a freezing point depression and expressed as mOsmos.Kg^{-1}

Measurement of pH

The pH of the seminal plasma of mrigal was examined every month during spawning season. During the month of July, the pH of seminal plasma of Indian major carp and exotic carp were also studied. The pH was measured using a laboratory pH meter.

Biochemical analysis of seminal plasma

Milt samples were centrifuged (10,000 g, 10 min), and the supernatant (seminal plasma) was collected in a sterile container and stored at -20°C for further analysis in the laboratory of Department of Biochemistry, S.C.B. Medical College and Hospital, Cuttack, India. All electrolytes, metabolites, and enzymes were determined using an automated system with adequate standards (Flexor-XL ISE, Netherlands). The following parameters were measured and expressed in the following units: albumin (g/dl), glucose (mg/dl) (Srikanth et al. 2004), urea, uric acid (Fei et al. 2006), cholesterol, triglycerides (Sullivan et al. 1985), bilirubin, urea, creatinine (mg/dl); alanine aminotransferase **(**GPT), aspartate aminotransferase **(**SGOT), chloride, potassium, sodium (mEq/l), albumin, and total protein (g/dl) (Kingsley 1939).

Results

The semen characteristics of mrigal showed a clear variation throughout the breeding season starting from April to September. The milt parameters are shown in Table 1.

Table 1. Seasonal changes in semen characteristics of mrigal, *C. mrigala*

	April	May	June	July	August	Sept
Milt yield (ml/kg)	2.65 ± 0.66^{d}	5.7± 1.42 ^{c}	12.3 ± 3.07^{b}	13.9± 3.47^{a}	9.5± 2.37^{b}	3.07± 0.76^{d}
Spermatocrit (%)	65.2 ± 4.81^{e}	75.2 ± 3.19^{c}	81.0 ± 3.16^{a}	83.40 ± 2.19 ^{a}	78.60 ± 2.60^{b}	69.8 ± 3.63^{d}
Sperm count (X 10^{9})/ml	10.30 ± 1.53^{d}	20.1 ± 3.13^{b}	31.18 ± 2.34^{a}	33.52 ± 2.73^{a}	19.6 ± 2.10^{b}	14.38 ± 2.95^{c}
Motility (%)	74.40± 5.17^{c}	85.0± 4.58 ^{b}	93.0 ± 3.31^{a}	92.2± 2.68 ^{a}	84.2 ± 3.70^{b}	77.0 ± 4.0 ^{c}
Motility duration (Seconds)	42.2 ± 3.76^{e}	55.6 ± 2.96 ^{d}	78.4± 3.50^{b}	97.0 ± 3.74^{a}	69.0 ± 2.91^{c}	39.2 ± 4.14 ^{e}
Osmolality (mOsm/kg^{-1})	252.0± 3.16^{e}	259.0± 5.61^{d}	273.8 ± 4.38^{b}	291.8± 4.81^{a}	267.0± 9.21^{b}	261.0 ± 4.06^{d}
pH	8.04 ± 0.16^{e}	8.22 ±0.14^{d}	8.40 ± 0.122 ^{b}	8.6 ± 0.12 ^{a}	8.3 ± 0.1 ^{b}	8.12 ± 0.1 ^{d}

Note: Values are expressed as Mean ± SD (n=27). Values having different superscripts differ significantly in a row

The volumes of milt obtained after hormone induction during the spawning season are shown in figure 1a**.** The maximum volume of milt yield (12.3 ± 2.27 kg^{-1}) was recorded in July, whereas the minimum milt yield was 2.66 ± 0.38 ml kg^{-1} in the beginning and in the end of the season in September (3.07 ± 0.69 ml kg^{-1}). The milt yield during different months of spawning season was significantly different.

The values of sperm concentration in different months of breeding season showed a clear variation (Fig. 1b). The sperm cell count ranged from 10.3 x 10^{9} ml^{-1} to 33.5 x 10^{9} ml^{-1} during spawning season. The maximum sperm count of 33.5 x 10^{9} ml^{-1} was recorded during the peak spawning season. A minimum sperm count was observed in the months of June and July during the spawning season. The seasonal variation in percentage

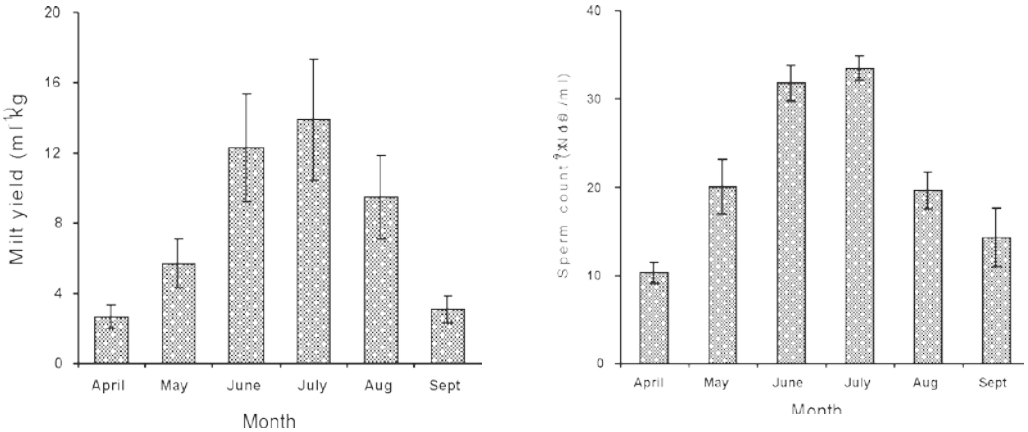

Figure 1. Seasonal variation in milt yield (a) and sperm count (b) in *C. mrigala*. Columns marked with the same letter are not significantly different at $P \leq 0.05$ (n=9)

of motile sperm and duration of motility during spawning periods are shown in figures 2 a and b. The maximum motility of 94% and 92% in the months of June and July, respectively, were observed. The percentage of motile sperm during the months of June and July was not significantly different. During other months, the motility percentage recorded was significantly different. The mean duration of sperm motility during milting period was in range from 39 ± 4.76 second to 97.5 ± 4.12 seconds. The maximum duration of motility was recorded in the month of July and minimum in the month of September (Fig. 2 b).

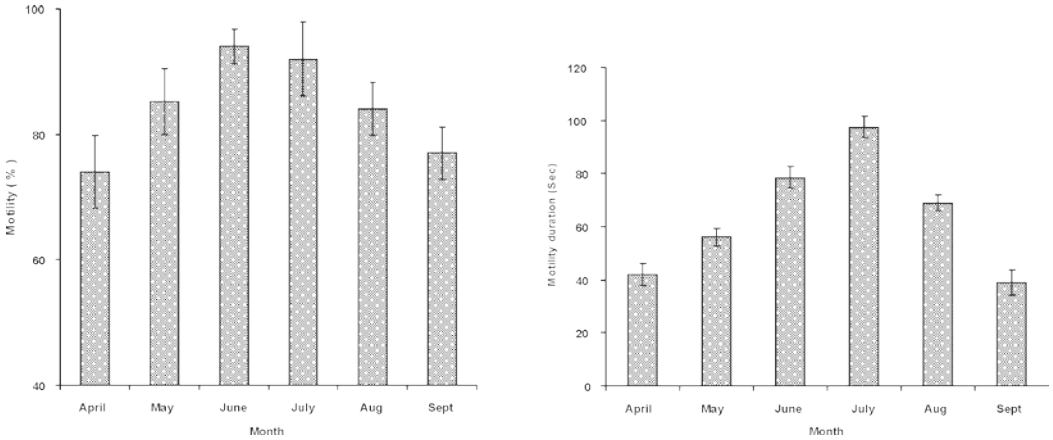

Figure 2. a & b. Seasonal variation in sperm motility (a) and duration of motile sperm (b) in *C. Mrigala* Columns marked with the same letter are not significantly different at $P < 0.05$ (n=9)

The spermatocrit values from April to September are shown in figure 3a. In the beginning of the breeding season, the spermatocrit value was minimum (65.2 ± 4.81%) and maximum value was observed in June (81 ± 3.16%) and July (83.40 ± 2.19%). The spermatocrit values during the month of May were 75%. The spermatocrit values were significantly different during the different months of breeding (milting) season.

The seminal plasma osmolality, which is the main factor that regulates sperm motility of mrigal, varied throughout the breeding period (Fig. 3 b). The mean range of osmolality of seminal plasma observed from April to September was from 252.0 ± 3.16 mOsm kg^{-1} to 291.8 ± 4.81 mOsm kg^{-1}. The maximum osmolality was observed in the month of July and minimum was studied in the month of September. The mean osmolality of seminal plasma observed was 252, 259, 273, 291, 267, and 261 mOsmol kg^{-1} during April, May, June, July, August, and September, respectively. The osmolality of the seminal fluid studied in the milt of mrigal was significantly different during the different month of spawning season.

The pH values of seminal fluid of mrigal are shown in figure 3c. The mean pH

range of seminal plasma of *C. mrigala* was also recorded varying during the different month of spawning season. The pH of seminal fluid ranged from 8.04 ± 0.16 to 8.6 ± 0.12 (figure 3c). During April, May, June, July, August, and September, the pH was 8.0, 8.2, 8.4, 8.6, 8.3, and 8.12, respectively. The pH of seminal plasma during different months of breeding season was significantly different ($P < 0.05$).

The mean concentration of the seminal ions (Na, K, and Cl) in mrigal during different months is given in Table 2.

Table 2. Seasonal changes in biochemical composition of seminal plasma of mrigal, *C. mrigala*

Parameter	April	May	June	July	August	September
Protein(g/dl)	0.516 ± .043^{a}	0.108± 0.030^{c}	0.112±0.022^{c}	0.104 ± 0.029^{c}	0.414±0.029^{b}	0.112±0.022^{c}
Albumin (g/dl)	0.102±0.014^{c}	0.112± 0.016^{c}	0.107±0.019^{c}	0.212 ± 0.019^{a}	0.158±0.016^{b}	0.114±0.024^{c}
Creatinine (mg/dl)	0.702± 0.042^{e}	0.906± 0.220^{d}	2.216±0.116^{a}	1.730 ± 0.050^{b}	1.352±0.059^{c}	0.456±0.037^{f}
Total bilurin (mg/dl)	0.114 ±0.036^{b}	0.034± 0.015^{c}	0.102±0.022^{b}	0.034 ± 0.005^{c}	0.222±0.031^{a}	0.254±0.029^{a}
Urea (mg/dl)	7.020±0.303^{a}	3.160± 0.698^{b}	3.080±0.327^{b}	2.520 ± 0.389^{c}	3.460±0.296^{b}	2.480±0.130^{c}
Uric acid (mg/dl)	0.524± 0.055^{a}	0.294 ± 0.143^{b}	0.506±0.069^{a}	0.316 ± 0.037^{b}	0.434±0.053^{a}	0.536±0.047^{a}
HDLc(mg/dl	14.500±2.150^{c}	6.200± 2.049^{d}	20.60±2.509^{b}	24.82 ± 2.268^{a}	24.80±2.679^{a}	18.76±3.426^{b}
SGOT(IU/L)	36.580±1.613^{c}	44.820±1.200^{b}	87.32± 2.572^{a}	20.92 ± 1.466^{e}	22.36±1.352^{e}	32.70±1.036^{d}
SGPT(IU/L)	6.620 ±0.356^{c}	9.100± 1.072^{b}	6.360±0.736^{c}	5.860 ± 0.378^{c}	2.420±0.354^{d}	11.46±1.023^{a}
Na (mEq/L)	89.98 ± 7.40^{d}	107.42 ± 4.82^{c}	124.6 ±5.90^{b}	140.4 ±1.58^{a}	104.10±6.04^{c}	80.40 ± 3.33^{e}
K (mEq/L)	28.20±4.91^{d}	37.20 ± 3.63^{c}	40.76 ± 4.54^{c}	52.30 ± 16.7^{a}	48.84 ± 3.34^{a}	33.10 ± 2.41^{c}
Chloride (mEq/L)	64.86±3.11^{f}	103.46± 11.47^{d}	122.68 ± 4.32^{c}	174.2 ± 5.21^{a}	156.30 ± 3.19^{b}	93.12 ± 3.28^{e}
Glucose (mg/dl)	1.508 ±0.10^{b}	1.0 ± 0.20^{c}	0.52 ±0.04^{d}	0.626 ± 0.06^{d}	1.838 ± 0.10^{a}	1.538 ± 0.06^{b}
Cholesterol (mg/dl)	8.540 ± 0.67^{d}	7.16 ± 1.03^{d}	58.0 ± 2.91^{b}	56.68±2.58^{b}	22.98 ± 2.58^{c}	102.32 ±2.96^{a}
Tryglyceride (mg/dl)	12.02 ± 0.77 ^{c}	12.36 ± 3.43^{b}	20.92 ±1.71^{a}	10.016±0.99^{c}	19.48 ± 0.69^{a}	14.58 ± 1.24^{b}

Note: Values are expressed as Mean ± SD (n=27). Values having different superscripts differ significantly in a row

The mean concentration of sodium in the seminal plasma ranged between 80.45 ± 3.33 mEq/L to 140.4 ± 1.58 mEq.L^{-1} from April to September during milting period. The maximum concentration of sodium ion in seminal fluid was observed in July and a minimum was observed in September. The sodium concentration in the months of June and July was significantly different, whereas during the months of May and August, it was not significantly different (Fig. 4a.). The mean concentration of potassium ions in seminal fluid during milting period was in the range of 28.20 ± 4.91 mEq/L to 52.30 ± 16.7 mEq.L^{-1} Seasonal variation of potassium ions in seminal plasma was observed throughout the breeding period (Fig. 4b). The potassium concentration was

observed to be in increasing trend during the beginning of the season, and then it reached peak in July and started declining towards the end of the breeding season (September).

High concentration of chloride was observed during milting period from April to September that ranged from 64.86 ± 3.11 $mEq.L^{-1}$ to 174.2 ± 5.21 $mEq.L^{-1}$ (Fig. 4 c). Increasing trend of the mean concentration of chloride in seminal fluid was observed initially reaching peak in July and chloride concentration declined till end of the September. The chloride concentration in the seminal fluid during different months of milting period was significantly different ($P < 0.05$).

The mean concentration of glucose, albumin, and protein of seminal plasma in *C. mrigala* during milting period was found varying throughout the season. The glucose in the seminal plasma was in a range from 0.52 ± 0.05 mg/dl to 1.83 ± 0.12 mg/dl (Fig. 5a), albumin was found in a range from 0.10 ± 0.01 mg/dl to 0.21 ± 0.02 mg/dl, (Fig. 5c), and Protein was recorded in a range of 0.105 ± 0.03 mg/dl to 0.515 ± 0.05 mg/dl (Fig. 5b) during the spawning season. The glucose concentration in the month of August is significantly different. The albumin concentration was also significantly different in the month of July. The protein concentration was not significantly different in May, June, July, and September.

The concentrations of cholesterol, triglyceride, and high density lipoprotein cholesterol (HDLc) in seminal fluid of *C. mrigala* varied throughout the milting period. During the period from April to September, the mean range of the concentration of cholesterol was observed to be from 7.1 ± 1.24 mg/dl to 102.3 ± 3.21 mg/dl (Fig. 6a.), triglyceride was observed to be from 9.97 ± 1.14 mg/dl to 14.475±1.41 mg/dl (Fig. 6b), and HDLc was found to be in a mean range of 6.0 ± 2.309 mg/dl to 25.0 ±3.05 mg/dl (Fig. 6c). The concentration of cholesterol in the seminal fluid was significantly different in September, whereas in June and July, it was not significantly different. The triglyceride concentration was not significantly different in June and August, whereas in April and July, it was also not significantly different. The concentration of HDLc was not significantly different in July and August

The concentrations of creatinine, SGPT, and SGOT during milting period are shown in Figure 7 a, b, and c. The mean range of the concentration of creatinine, SGPT, and SGOT in seminal fluid was 0.457 ± 0.04 mg/dl to 2.21 ± 0.13 mg/dl, 2.412 ± 0.41 IU/L to 11.45 ± 0.18 IU/L, and 20.75 ± 1.63 IU/L to 87.32 ± 2.97, respectively, during the breeding season starting from April to September. Creatinine concentration was significantly different ($P < 0.05$) during different months of the season. The SGPT concentration was not significant during April, June, and July. However, SGOT concentration in the seminal fluid was significantly different in April, May, June, and September.

Variations were observed in the mean concentrations of total bilurin, urea, and

uric acid in the seminal plasma throughout the breeding season. The mean range of total bilurin in seminal fluid was from 035 ± 0.01 mg/dl to 0.257 ± 0.03 mg/dl (Fig. 8a.), mean concentration of urea was from 2.45 ± 0.13 mg/dl to 7.05 ± 0.341 mg/dl (Fig. 8b), and uric acid was from 0.28 ± 0.165 mg/dl to 0.535 ± 0.05 (Fig. 8c).

Discussion

The study of seasonal variation in milt characteristics and biochemical composition of Indian major carps is scanty. Billard et al. (1995), Linhart et al. (1995), and Christ et al. (1996) reported seasonal variation in quality of male gamete in some carps. Many other factors contributing to individual variation in sperm quality have been reported (Rana 1995; Rurangwa et al. 2004), and these factors are genetic variability among fish, rearing conditions, brood stress, sperm collection methods, and storage of milt and sperm activation conditions. The seasonal variation of gamete quality also influences motility and fertilization success (Lubzens et al. 1997; Linhart et al. 2000). Seasonal variation affects the keeping quality of spermatozoa in vivo (Baynes & Scott, 1987). Knowledge of physical and chemical constituents of spermatozoa and seminal fluid is a pre-requisite for the successful evaluation of the reproductive ability of different fish species. This may also lead to the better understanding of the mechanisms of fertilization and to detect anomalies. Changes in the quality of milt during the spawning season have been reported in teleosts (Billard et al. 1977).

In this study, the maximum milt yields were recorded in the peak of season and the yields declined toward the end of season. Similar variation in milt yield has also been reported by various workers in other fishes (Billard & Marcel, 1980). The variation in milt yield reported are due to the seasonal changes, the age of milter, the maintenance circumstances of the milters (Turkadov 1968; Ginzburg 1972; Kazakov 1978, 1979, 1981; Buyukhatipoglu & Holtz 1984; Piironen 1985), and the inducing agents (Billard & Marcel 1980; Wei & Crim 1983; Wohlfarth 1994; Lin et al. 1996). Billard & Marcel (1980) reported that injections of crude gonadotropin preparation of pike, carp, and partially purified salmon gonadropin in pike, *Esox lucius*, resulted in a significant increase in volume of milt compared with the control (Saline injected). In addition, they observed a significant increase in the collectable milt volume from the untreated males of common carp exposed to females undergoing ovulation after carp pituitary extract injection. This may be due to the release of sex pheromones i.e. C_{21} steroids.

The mean sperm count of milt during June and July was significant compared with the other months of reproductive season ($P < 0.05$). The sperm count declined as the spawning season advanced. Similar result has been observed by various workers. The sperm concentration increased during spermiation period in turbot, Atlantic halibut (Suquet et al. 1998) and decreased at the end of reproductive season of rainbow trout (Buyukhatipoglu & Holtz 1884). The spermatozoa concentration declines as the spawning

season advances in rainbow trout, *O. mykis* (Buyukhatipoglu & Holtz 1884; Billard & Marcel 1980; Wei & Crim, 1983; Wohlfarth 1994; Lin et al. 1996; Suquet et al. 1998) and carp, *Cyprinus carpio* (Christ et al. 1996; Lubzens et al. 1997), and Billard et al. (1977), Buyukhatipoglu & Holtz (1984) and Munkittrick & Moccia (1987) reported that sperm density declined as the season advanced (in rainbow trout).

The mean spermmatocrit value of milt studied during the month of July was significantly higher than other months during milting period in *C. mrigala*. The observation of spermatocrit value showed a clear seasonal variation during reproductive periods. Similar seasonal variation has also been reported in salmon (Piironen 1985) in rainbow trout (Munkittrick & Moccia 1987). Piironen & Hyvarinen (1983) observed that spermatocrit increased over the stripping season. Piironen 1985 reported seasonal variation of spermatocrit value in *Salmo salar.* The seasonal variation of spermatocrit value in Atlantic cod *Gadus morhua* has been reported (Rakitin et al. 1999).

The maximum motility of spermatozoa in mrigal was observed in June (94 ± 2.82%) and July (92 ± 5.88 %). The mean duration of sperm motility during milting period was in range from 39 ± 4.76 sec to 97.5 ± 4.12 sec. The maximum duration of motility was recorded in July and minimum was recorded in September. Similar result has been reported in rainbow trout, brown trout (*Salmo trutta* [*S. trutta*]), brook trout *(Salvelinus fontanalis)*, and Atlantic salmon (*Salmo salar*) (Benau & Terner 1980). During the peak spawning season, activated rainbow trout spermatozoa remained motile for 30–55 sec. By the end of the spawning season, the duration of the motility declined to 15 sec.

The mean pH value of the seminal plasma of *C. mrigala* during breeding season ranged from 8.05 ± 0.19 to 8.6 ± 0.12. The alkaline pH of the seminal plasma found in the mrigal is similar to the results observed by Billard (1981) who reported that alkaline pH gives better motility in rainbow trout spermatozoa. Optimum sperm motility has been reported at pH 9.0 in *Oncorhynchus mykiss* (Billard & Cosson, 1988) and *Scaphthalmus maximus* (Chauvaud et al. 1995) and pH 7.0 and 8.0 in *Cyprinus carpio* (Cosson et al. 1991). Alternation of the internal pH as possible mechanism interfering with motility was described for spermatozoa from different species.

In the present study, the osmolality of seminal plasma was found to be varying throughout the milting period from April to September. It has shown increasing trend until July (291.5 ± 5.5 mOsm kg $^{-1}$) and started declining towards the end of breeding season. These findings are in agreement with the observation of osmolality of seminal plasma by Kruger et al. (1984), Billard (1988), Aas et al. (1991), and Lahnsteiner et al. (1997) who reported variation of seminal osmolality in different seasons. Osmolality of seminal plasma of Atlantic salmon showed wide variation among 27 males ranging from 117 to 320 mOsmol kg^{-1}. Much variation of osmolality of seminal fluid observed

with extreme values 178–282 mOsm kg^{-1} by Kruger et al. (1984) for males were sampled at various times throughout the year. It was found that variations in the lower ranges were due to contamination of semen by urine (Perchec et al. 1995). The osmolality of semen had been studied in cyprinids (Cruea, 1969; Kruger et al. 1984; Billard & Cosson, 1992; Billard et al. 1995a, b; Lahnsteiner et al. 1996 Linhart et al. 1991, 2003a, b, c.). The osmotic pressure can vary between individuals, and this is correlated with the thinning (hydration) of the semen (Morisawa et al. 1979*)*. In addition, variation in osmotic pressure observed in the literature might be due to hormonal induction of spermiation outside the natural reproductive season (Redondo Muller et al. 1991).

Seminal plasma of Fish contains mainly mineral compounds and low concentrations of organic substances. There is no information available on the seasonal changes in biochemical composition of seminal plasma of Indian major carps prior to and beyond spawning time. The composition of seminal fluid of fish has been reviewed by Billard & Cosson (1990) and Linhart et al. (1991). The constitution of milt in terms of performance and chemical composition varies interspecifically and even within the same individuals with time. The various available data show considerable intra- and inter-species variability in the composition of the seminal fluid. Ionic composition is reportedly changing during reproductive season **(**Linhart et al. 1992**)**. It was reported that organic component of seminal plasma in *Salmo salar* underwent specific changes throughout the spawning season (Piironen 1985). Variation in the inorganic and organic composition of seminal plasma may affect the preservation properties of milt (Benau & Terner 1980; Piironen & Hyvarinen 1983, Kruger et al. 1984).

The Na^+ and K^+ concentration in the seminal plasma of *C. mrigala* is shown in Table 9. The high values of ions are believed to be responsible for the suppression of sperm motility. The Na^+ and K^+ concentrations of seminal plasma in mrigala were in the range of 80.45 ± 3.84 mEq/L to 140.5 ± 3.7 mEq/L and 29.25 ± 5.0 mEq/L to
52.3 ±19.28 mEq /L, respectively. The ionic composition in the range of 103–140 mM Na^+, 20–66 mM K^+ , 0.8–3.6 mM in Salmonid (Morisawa et al. 1983) and 94–107 mM Na^+, 39–78 mM K^+, 0.02–1.2 in cyprinids (Kruger et al. 1984) were reported. The seasonal variation of sodium and potassium concentrations in seminal plasma of cyprinids has been reported and similar results were obtained in the present experiments.

Limited information is available on the organic composition of the carp semen. The variability in organic composition of the seminal plasma is wide and changed according to season, to gonadotropin treatment given to stimulate spermiation, and during semen storage (Belova 1982; Kruger et.al. 1984). Some energetic substrates such as glucose and fructose are found in the seminal plasma and the sperm but in small amounts (Kruger et al. 1984) and are generally 10 times lower than in mammals (Ford & Rees 1990). Organic constituents of seminal plasma has been reported, and it was variable in the interspecies and the range ($mg.l^{-1}$) was 8-220 for glucose, 0-218 for fructose, 0-40

for cholesterol, 0-1316 for lipids, 35-391 for glycerol, 0.4-280 for protein, 84-136 for amino acids, and 12-136 for urea (Billard & Cosson 1990). The protein content is highly variable throughout the year (Billard et al. 1995). Similar result was also obtained in the protein content of seminal plasma, which showed variations throughout the season, and the range of amount of total protein was from 0.105 ± 0.03 to 0.515 ± 0.05 g/dl. In the present study, the mean concentrations of triglyceride and HDLc from April to September were found to be from 9.97 ± 1.14 mg/dl to 14.475 ± 1.41 mg/dl and 6.0 ± 2.309 mg/dl to 25.0 ± 3.05 mg/dl, respectively. The mean concentrations of creatinine, SGPT, and SGOT in seminal fluid (ranges) were 0.457 ± 0.04 mg/dl to 2.21 ± 0.13 mg/dl, 2.412 ± 0.41 IU/L to 11.45 ± 0.18 IU/L, and 20.75 ± 1.63 IU/L to 87.32 ± 2.97, respectively. Total bilurin in seminal fluid was 035 ± 0.01 mg/dl to 0.257 ± 0.03 mg/dl, and the mean concentration of urea was 2.45 ± 0.13 mg/dl to 7.05 ± 0.341 mg/dl and uric acid was 0.28 ± 0.165 mg/dl to 0.535 ± 0.05.

The mean ranges of cholesterol and glucose studied in the present experimental fish were 8.52 ± 0.77 to 22.97 ± 2.98 mg/dl and 0.525±0.05 to 1.83 ± 0.125 mg/dl, respectively during the spawning season. Kruger et al. (1984) reported that the cholesterol and glucose contents of carp seminal fluid were in the range from 0 to 40 mg/l and 9 to 100 mg/l, respectively. Stein & Bayrle (1985) found the highest glucose content in the seminal plasma of *S. trutta fario*, at 12.2 mg/100 ml compared with 3.7, 1.8, and 8.8 mg/100 ml for *Salmo gairdneri, S. trutta lacustris*, and *Coregonus* sps, respectively.

Although the milting in *C. mrigala* has been found to be started from April, it has two reproductive peaks i.e. June and July during the breeding season. The result indicates that in June and July, spermatocrit value, sperm count, milt volume, and duration of total spermatozoa motility were comparatively higher than in other months of spawning season, indicating a better quality of milt. Therefore, the information of the normal physical and chemical characteristics of seminal plasma of the *C. mrigala* presented in this study will help to optimize in selection of high quality male donors for aquaculture and artificial spawning performances.

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