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Male mating tactics and mating activity in freshwater prawn, *Macrobrachium dayanum* (Henderson, 1893) Paleomonidae: Caridae

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Abstract: *Macrobrachium dayanum* is a fresh water commercially important fish and highly preferred by the consumers. This study revealed that it was not a continuous breeder and showed two breeding peak periods in a year related to monsoon. Males were larger than the female. The largest male (largest carapace length and longest chelate legs) showed its dominance over recessive and non breeding males. Both male and female attained maturity at the age of about 5 months (male 5 cm in length, weight 1.3 gr in average; female 4.2 cm in length and weight 1.36 gr in average). GSI of prawn indicated the maximum size groups did not show maximum GSI. The male showed peculiar behavior or tactics during mating such as protection or guarding the female with the largest chelate leg, aggression, climbing over the female etc. Spawning took place after 20 ± 2 hours of mating. The fertilized eggs were deep green color, about 2.5-3.0 mm in length and 1.52-2.0 mm in breadth. The incubation period was 22-24 days. Hatching took place in the late afternoon and continued 1-2 hours. Hatching never occurred below 25 ^{0}C and above 30 ^{o}C. Hatchlings were very active after hatching.

Key Words: *Macrobrachium dayanum*, chelate leg, GSI, incubation period

Introduction

Macrobrachium dayanum (Henderson, 1893) is an important fresh water prawn, available in southern part of India, Bengal gulf, Kaira River and in Rajshahi area of Bangladesh (Parween *et al*., 2003). It is a resident of "beels" (large water bodies) and associated flood plains with rich aquatic vegetation. The market value of *M. dayanum* is quite high and is highly preferred by the consumers as dry powder (Parween *et al*., 2003). The local people used to catch them using different kinds of trap for consumption. This species can be reared in culture ponds along with other species. This prawn along with other species provides the animal protein to a great

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number of our poor people. But the production of the prawn is declining day by day due to habitat destruction and use of pesticides in paddy fields. So the importance has been given to increase the production potentiality of this species. Hence a thorough investigation of reproductive biology of this prawn is necessary. Reproduction is an important aspect of the biology of the species. Through this mechanism a species can continue to exist (Mayer and Contresas, 2009). Thus knowledge of reproduction aids in understanding their ecology and life cycles (Sastry, 1983). The biology of reproduction not only includes the production of hatchlings released by the species, but also the activity and behavior shown by male and female during mating, fecundity, breeding season, maturity age etc.

In many decapods crustaceans (shrimps, lobster, crabs) copulation is restricted to a brief period after the female molts (Bauer and Abdulla, 2001; Bauer and Holt, 1998).The guarding of females approaching a limited period of sexual receptivity is a common mating tactic of males. Typical mate guarding behaviors were observed in many decapods male (Ridley, 1983; Bauer, 1976). In decapods species in which precopulatory mate guarding occurs, males exhibit different characteristics that help them to guard females (Ridley, 1983; Jormalainen, 1998; Wilber, 1989; Grafen, 1983; Bauer and Abdulla, 2001). Mate guarding has not been reported in Penaedeae shrimp (Bauer, 1996). *M. dayanum* also showed mate guarding activity during copulation (this study).

Considering all these factors in mind, the main objective of our study was to determine **i**. breeding season, **ii.** age of maturity, **iii.** Gonado-somatic index, **iv.** behavior shown by male and female during, after and before mating, **v.** the mating tactics displayed by male during pre-copulation.

Materials and Methods

Live specimens of adult *Macrobrachium dayanum* were collected from ponds of different localities of West Bengal, by a long handled hand net of 1 mm mesh size. Collections of Prawn were made twice in a week during pre-spawning period in the month of February 2009. On arrival at the laboratory, the prawns were given a dip bath in 0.5 ppm ($KMnO_4$) Potassium Permanganate solution for five minutes. After that they were released into a 1000 L tank containing 3/4 filled fresh water. The prawns were fed with cultured planktons (*Cyclops, Daphnia, Brachinus, Moina* etc.), frozen dried *Tubifex*, eggs of ants and crushed dry fish. The aquarium was provided with aquatic vegetation for natural environment and shelter for the prawn. To observe the reproductive conditions of females, basic observations on size and sex were made on all prawns and reproductive conditions of females were recorded. Cephalothoracic length (CL) measured from the post orbital margin to the posterior margin of the cephalothorax

(Bauer, 1986), was recorded for each specimen. Sex was determined by the presence (males) or absence (females) of the appendix masculana on the second pair of pleopods (Bauer, 2004). A reproductive condition of females was recorded according to the degree of ovarian maturation which can be seen through the translucent exoskeleton of the carapace and was measured on a scale of 1-4 (Bauer, 1986) with **i.** No ovarian development observable, **ii.** Ovary developing but not extending into the carapace above the cardiac stomach, **iii.** Ovary extending into and up to half of the carapace space, **iv.** Ovary usually filling more than half of carapace, just prior to spawning. Total lengths of males were 4.8-7.8 cm and females were 4.3-6.8 cm.

Ovary examination: The ovarian condition and gonadosomatic index (GSI) of about 25 females of *M. dayanum* were determined every week. Body weight was measured to the nearest 0.1 mg using an electrical balance; females were dissected under a microscope to investigate ovaries which were immediately preserved in 5% formalin for examination. The ovary weight was recorded to the nearest 0.01 mg. The gonado-somatic index was determined as follows: (Gupta and Roy, 2009)

$$GSI = \frac{\text{Ovary wet weight}}{\text{Female body wet weight}} \times 100$$

To observe the spawning and mating behavior, 25 pair ovigerous females and males were kept in a rectangular glass made aquaria measuring 45 cm × 25 cm with 45 litre water capacities, during June, 2009-July, 2010. Prawns were fed with appropriate diet.

Proper aeration, cleaning of aquarium to remove left over food and exchange of water were done daily to maintain the water quality of the aquarium. Observations of interactions among reproductive males and females were recorded by taking manual data and photograph by digital camera (Nikon Coolpix L110, 15 ×zoom, and 12.1 megapixels), video, time lapse by stop watch. Water temperature was recorded during the experiment. The light and dark cycle was maintained at 14h:10h.

To observe the hatching behavior and embryo incubation period: Duration of incubation period (spawning and egg attachment until embryo hatching) was recorded for females in the laboratory. Females were observed daily and the time from spawning to hatching was recorded. The fertilized female was taken into a rectangular aquarium (30 cm × 20 cm × 15 cm) covered with some aquatic vegetation. All the observations and data were recorded by digital camera (Nikon Coolpix L110, 15 × zoom, 12.1 mega pixel) and stop watch.

Statistical Analysis: The weight-length relation-ship was estimated using log transformed weight

and length data as:

log (w) = a+blog (TL)

"w" is the weight (gr), "TL" is the total length (cm), "a" is the intercept and "b" is the slope of regression line.

Results

Breeding Season: Breeding period was seasonal, one (Fig. 1) in summer beginning from April, maximum peak (87% ovigerous females) in June, and then ending in August. Another breeding period was in winter during December-January, when the number of ovigerous females found were only 15-20%. Spawning pattern of *M. dayanum* was not successive or it was not continuous like *M. lamarrei*. The appearance of eggs were rarely seen in the carapace during hatching period. If at all, only the first stage of ovary appeared in a few case. Therefore, *M. dayanum* seemed to be an alternative brooder. Further, molting was observed between two successive spawning. Interspawn interval was observed at about 15-20 days length.

Sex ratio: In *M. dayanum*, males were larger than the females, as in *M. rosenbergii* (Kurian and Sebastian, 2005) and in *M. lamarrei* (Sharma and Subba, 2005). Mating was always preferred by 1:1 ratio i.e. between 1 male: 1 female. Mature males of this species range from 5.0-7.8 ± 0.83 cm in length (carapace length from 1.5-3.3 cm) while breeding females varied from 4.1-6.8 ± 0.77 cm (carapace length from 1.8-2.8 cm). The mean size of the species varied seasonally. Carapace length was defined as the mid-dorsal distance from the posterior edges of the eye orbits to the posterior edge of the carapace (Bauer, 1976). The water temperature varied from 20 ^{o}C to 30 ^{o}C throughout the experiment. The average value recorded during the study was 27.5 ^{o}C.

Statistical Analysis: Regression equation between length and weight in male female *M. dayanum* was done individually. Both male (y = 0.9907X-3.9143, R^2 = 0.8854) and Female (Y = 0.5421X-1.2758, R^2 = 0.6352) had shown the linear graphs (Fig. 2 and Fig. 3). A histogram was drawn between average length of carapace and length of 2nd chelate leg of three different morphotypes of male and female (Fig. 4). It was shown that, average length of carapace and 2nd chelate leg was the largest in dominant male than recessive male and female. The non-breeding male showed the smallest length of carapace and chelate leg. The regression equation between carapace length and chelate leg of all three males and females had shown linear relationship between these two organs (Fig. 5A, B, C, D). The equations of three males respectively and female were as follows:

Dominant male:

$Y = 1.7532 X + 0.0307$, $R^2 = 0.871$

Recessive male:

$Y = 1.1429 X + 0.6357$, $R^2 = 0.4781$

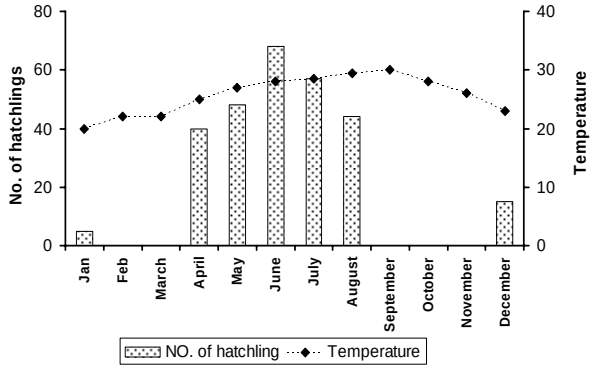

Fig. 1: Variation of number of hatchlings with different breeding season

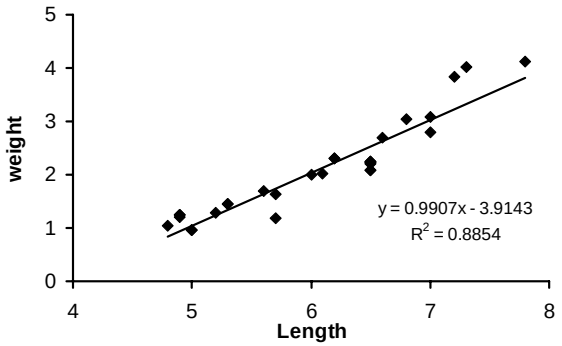

Fig. 2: Regression analysis of length (cm) and weight (gr) in male *M. dayanum*

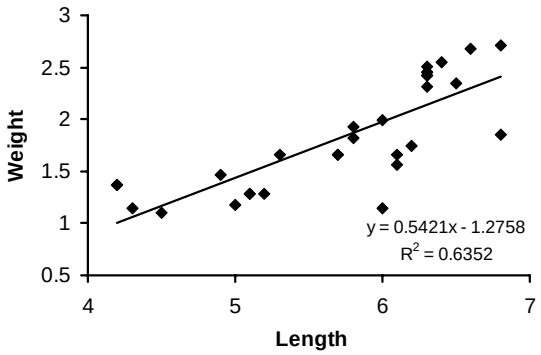

Fig. 3: Regression analysis of length (cm) and weight (gr) in female *M. dayanum*

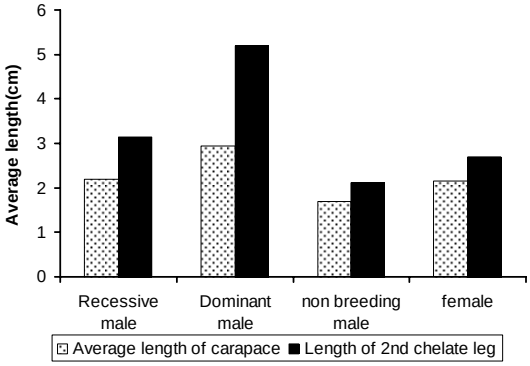

Fig. 4: A comparison among recessive, dominant, non-breeding male and female *M. dayanum*

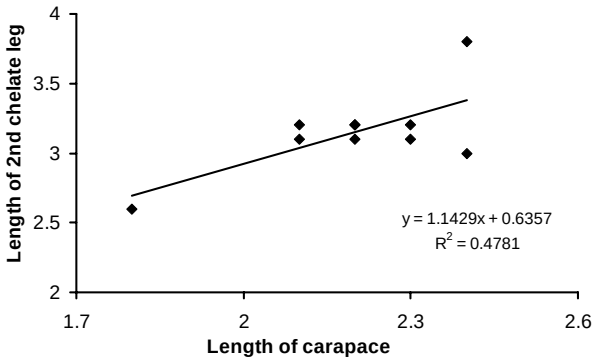

Fig. 5 (A): Regression analysis between length of carapace (cm) and second chelate leg (cm) in recessive male *M. dayanum*

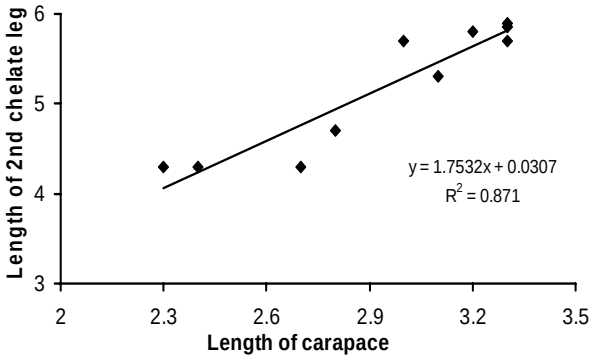

Fig. 5 (B): Regression analysis between length of carapace (cm) and second chelate leg (cm) in dominant male *M. dayanum*

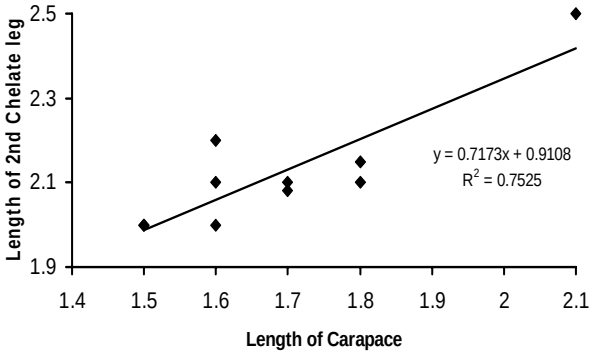

Fig. 5 (C): Regression b/w length of carapace (cm) and second chelate leg (cm) in nonbreeding male *M. dayanum*

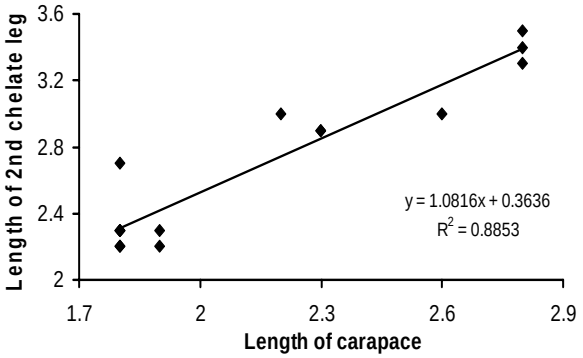

Fig. 5 (D): Regression between length of carapace (cm) and length of second chelate leg (cm) in female *M. dayanum*

Non-breeding male:

$Y = 0.7173 X + 0.9108$, $R^2 = 0.7525$

Female:

$Y = 1.081 X + 0.3636$, $R^2 = 0.8853$

Mating

Mating behavior shown by Male prawn: Males of *M. dayanum* can be divided into these distinct morphological types (Raanan and Sagi, 1985). Behavioral and physical characteristics of all three morphotypes were examined with regard to mating behavior and reproductive probabilities.

Dominant Male: The male who participated in mating actively the largest of all three types in length (Carapace length 2.3-3.3 cm). The size of the 2nd chelate leg was the largest as compared to other males (4.3-5.9 cm), the male became bluish grey in colour with red claws during breeding season. They became glossy and attractive. Dominant male was only one in number in each population. It did not allow any other males near female and guarded the female with its extended chelate leg.

Recessive male: 1-2 recessive males were present in each population. They were comparatively smaller in size than dominant male (1.8-2.4 cm CL and length of 2nd chelate leg 2.6-3.2 cm). They showed reduced rate of reproductive activities in the presence of dominant male. They tried to get closer to the female, but dominant male defended its territory very actively with its large chelate legs, prior to mating.

Non-breeding male: The third type of male was marked as non-breeding males. They were 6-7 in number and very small in size (1.5-2.1 cm CL and length of 2nd chelate leg 2.0-2.5 cm). These small males were not involved in actual mating. They remained away from the copulating pair with a high frequency of movement around them.

Female: The female was usually smaller than male (1.8-2.8 cm Carapace length). Ovary was

fully matured and deep green in colour, which covered the whole carapace region. It was clearly visible from outside through transparent carapace. The ovary of female gradually matured in presence of dominant male and took 15-17 days. During this time, the dominant male actively guarded the female and helped to stimulate maturation of ovary.

Mating tactics in male: The following behavior or tactics showed by the dominant male during mating. Duration of pre mating behavior shown by the dominant male took 7 to 8.45 hours (Tab. 1).

Pre-mating activities shown by Male: Mating behaviors of the male which started about 2-3 hours prior to the actual mating could be categorized into followings.

Protection: The guarding of females approaching sexual receptivity was a common tactic of males (Bauer and Abdalla, 2001). In our experiment, the dominant male guarded the female with its large extended 2nd chelate leg at the corner of the aquarium and protected her from other recessive males. In between two chelate legs, the female showed no aggression. It was very calm and quite, not moving at all and standing still in position (Fig. 6A).

Aggression: The dominant male was very aggressive in this stage. They chased all the males vigorously who came closer to female. It ran after a long distance to chase other males and very quickly took its position to guard the female (Fig. 6B). Some time powerful attack led to break the rostrum or chela of recessive males.

Contact: Now the male approached closer to female to contact its chelate leg and hold it. The manner of holding the chelate leg looked like "hand shake". Upon the mutual contact of the chelate legs the behavior pattern changed immediately and abruptly. It became closer and attempted to seize the female with its walking legs (Fig. 6C).

Climb: The male attempted to ride to dorsal midline of the female. If the contact has been made at the anterior of the female, the female threw away the male from its body by the pereopods and third maxilliped. No such behavior was shown when the male approached the female from behind (Fig. 6D).

Mating: Pre-mating moulting of female took place at first. Female became weak and sluggish. The male then clutched the female with its walking legs and mounted on the female's mid dorsal line with the anterior ends of both animal's facing the same direction (Fig. 6E). This posture allowed the female to settle down and accepted the male for next event. The male was successful in mating if it could achieve this position. Rejection by females took place most often in contact and climb phases. The male swinged its body underneath the female positioning the thoraco–abdominal junction. In this time, the male beat its pleopods and transferred the spermatophore. The female swam off quickly to other side of the aquarium,

Fig. 6A: Some behaviors shown by *Macrobrachium dayanum* (protection and guarding the female by dominate male)

Fig. 6B: Some behaviors shown by *Macrobrachium dayanum* (aggression and territory behaviors showed by dominate male with large red claw)

Fig. 6C: Some behaviors shown by *Macrobrachium dayanum* (pre-mating behavior; contact)

Fig. 6D: Some behaviors shown by *Macrobrachium dayanum* (mating behavior)

Fig. 6E: Some behaviors shown by *Macrobrachium dayanum* (copulation)

after receiving sperms.

The disengagement of male and female then occurred by jumping backward by both the species after the beating of pleopod of male- the female took rest, holding aquatic vegetation.

The only posture shown by female was pleopod lowering to take the spermatophore during mating. Duration of copulation lasted 45-55 seconds (Tab. 1)

Post-mating behavior of the female: Duration of post mating behavior shown by the female was 17-17.23 hours (Tab.1).

Cleaning: After copulation, the female immediately began to clean the posterior thoracic region. This function was done with the second chelipeds and undulation of pleopods. Ingle and Thomas (1974) also described the preening behavior of crayfish *Austropotamobius pallipes* (Lereboullet). Eggs of *Macrobrachium dayanum* slowly passed between the endopods and the abdominal sternite during spawning.

Spawning: Egg laying or spawning took place after 20 ± 2 hours of mating. Lee and Fielder (1982) reported that eggs usually lay within 12 hours of copulation in *M. australiense*, (Holthuis). Spawning took place within a few minutes reported by Bauer, 1976 in *Heptacarpus picturs* (Simpson). At the time of spawning, the body of female was observed to bend forward to keep contact with the ventral thoracic region to form a 'U' shaped structure. Therefore, the eggs were extruded directly into the brood chamber passing through the female genital pore. The eggs were held in bundles like grapes through some thin and elastic membranous substances and

adhered tightly to the fine ovigerous setae of the first to fourth pairs of pleopods (Prasad and Kanaujia, 2006). Now the female became berried. Before fertilization, eggs were deep green in color (length 1.18 mm breadth 1.0 mm).

Eggs: The length of the fertilized eggs were 2.5 to 3 mm and breadth were 1.5 to 2 mm .The color of the freshly spawned eggs were deep green (olive green) but it was green in *M. gangeticum* (Bate, 1868), yellow in *M. malcolmsonii* (H. M. Edwards) (Prasad and Kanaujia, 2006), yellow in *M. rosenbergii,* De Man, 1879 (Kurian and Sebastian, 2001) green in *M. lamarrei* (Uno and Sao, 1969)

Incubation Period: The berried female carried the eggs till hatching. The number of days that embryos were incubated i.e. from spawning to hatching was termed as incubation period.

During incubation period, the pleopods were observed to beat back and fourth intermittently, to provide aeration for developing embryos. The incubation period in *M. dayanum* was 22-24 days at room temperature of 26 ^{o}C–28 ^{o}C, but it may be extended to 30 days during winter season when temperature dropped below 25 ^{o}C. The incubation period varied in different species of *Macrobrachium* such as 14-17 days in *M. malcolmsonii* (H.M. Edwards), 12-14 days in *M. gangeticum*, Bate, 1868 (Prasad and Kanaujia, 2006). The female carefully removed the dead eggs and debris with the help of first pair of chelate legs. The color of the eggs became gradually lighter. When larvae became fully grown, the color became whitish grey.

Hatching: Hatching was observed in separate aquarium. When the larvae were fully developed, they were ready to hatch out from the egg shell. Eye - Spot was clearly observed from out side. Development of larvae carried on by the females up to Zoea stage. Hatching always took place at late afternoon or evening and was ended after 1.5-2.25 hours (Tab. 1) while it was observed during night (24 hrs) in *M. malcolmsonii*, completed before morning. In *M. gangeticum* it was prolonged and ended in morning. In *M. rosenbergii*, hatching started at night (24 hrs) and completed in 2nd night (Ling, 1969; Fujiinura and Okamoto, 1972; Kanaujia *et al*., 2005; Prasad and Kanaujia, 2006).

Hatching was accompanied by continuous vibration of the mouth of the larva and stretch-ing of its rolled (coiled) body, forcing the egg shell to elongate gradually. Suddenly the egg shell was broken and telson thrashed out followed by head, and the larvae hatched out (Fig. 6F).

Hatchlings: Hatchlings (Fig. 6F) were very active just after hatching. They moved to and fro in the aquarium and were big enough to be seen by the naked eyes as compared to the *M. lamarrei lamarrei* (Sarkar *et al*., 2010).

Tab. 1: Different parameter of mating

Pre-mating Behavior		Mating Behavior		Post mating Behavior		Hatching (Duration)
Duration of male guarding the female	Duration of Pre-Spawning moult	Duration of mating	Time Taken in between Pre-Spawning mode and mating	Duration of Post-mating	Gap between mating and spawning	
7-8.45 hours	45-50 Second	45-55 Seconds	1-2 minutes	17-17.23 hours	20-23.25 hours	1.5-2.25 hours

Fig. 6F: Some behaviors shown by *Macrobrachium dayanum* (female with many hatchlings)

Tab. 2: Gonado-somatic Index of *Macrobrachium dayanum*

Size Group	Average Length (cm)	Average Weight (gr)	Average GSI
4-5	4.58	1.188	13.69
5-6	5.6	1.507	17.02
6-7	6.57	2.44	11.73

Gonado-somatic index (GSI): Table 2 showed the three size groups of *M. dayanum* including its length, weight and average GSI. Maximum GSI showed by the size groups (5-6 cm). But maximum size groups did not show the maximum GSI. Regression analysis between GSI and length, weight, ovary length, and ovary weight were drawn, all showed negative correlation with GSI (Fig. 7,8,9,10).

Discussion

M. dayanum (Henderson, 1893) showed two breeding periods in a year, though the peak season was always related to monsoon and late monsoon. But it was not a continuous breeder. There was a distinct gap between hatching and maturation of ovary. By contrast Hossain and Parween (1982) stated that it was a continuous breeder in the wild, having two peak periods, one at the later part of the monsoon and the other before the onset of full monsoon. In mating behavior, single largest male with massive claw showed its dominance and participated in copulation. Recessive males never got any chance to participate in mating. Only when dominant male was dead, one of the recessive males became dominant to take part in copulation. Non breeding males had no role in mating. A high competition between dominant male and recessive males for copulation with ripe female resulted in successful mating or in fatal injuries to the recessive males (Raanan and Sagi, 1985).The largest carapace length and weight and longest chelate leg always identified the dominant male in comparison to other males. Both male and female attained maturity at the age of 136 to 142 days. i.e. about 5 months when the female became 4.2 cm on length and weight 1.36 gr in averages. This was very similar to 3.5-4.0 cm length reported by Parween *et al*., 2003. The male was about 5 cm in length and weight 1.23 gr in average. Breeding or hatching never occurred below 25 ^{o}C and above 30 ^{o}C.

In *M. dayanum* the ova measured from 0.34 to 1.18 mm and the berried eggs ranged from 2.1 to 2.5 mm when Parween reported the ova size from 0.14 to 1.21 mm and the berried eggs ranged from 1.6 to 2.1 mm.

GSI of prawn clearly indicated that maximum size groups did not show maximum GSI. It significantly declined in higher size groups. The reason may be attributed to that after certain age group, the degree of increase of weight of ovary were not high as degree of increase of body weight. It was restricted at certain size groups. For this reason, all the regression analysis of GSI with body length and weight, ovary weight and length showed strongly negative correlation (Ibrahim, 1962; Singh and Srivastava, 1991; Gupta and Roy, 2009.)

Spawning took place 8 ± 2 hours after copulation. The female did not accept any food during incubation period. Incubation period depended on the room temperature and size of

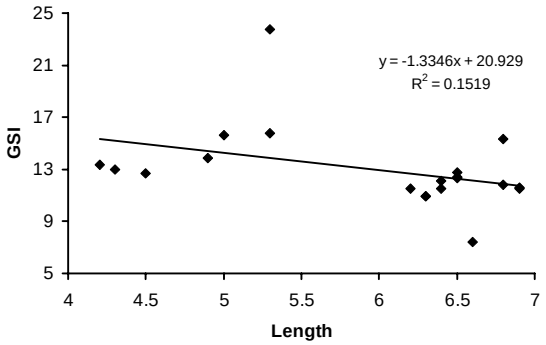

Fig. 7: Regression between body length (cm) and GSI

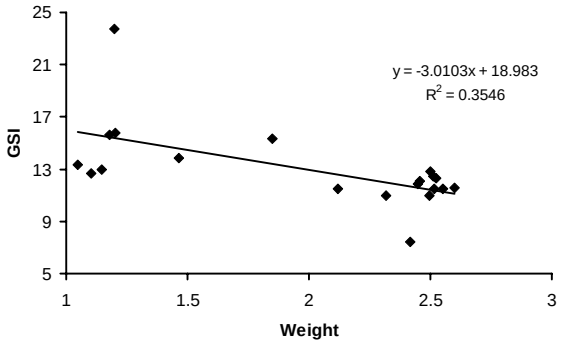

Fig. 8: Regression between body weight (gr) and GSI

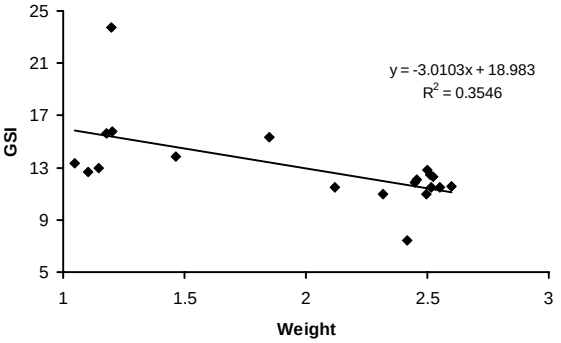

Fig. 8: Regression between body weight (gr) and GSI

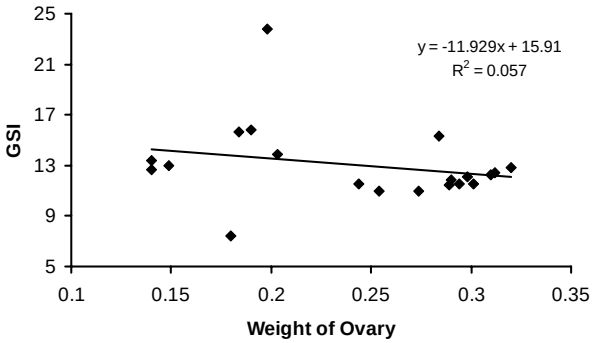

Fig. 10: Regression between ovary weight (gr) and GSI

the egg. The smaller size of egg required a short incubation period, favoring a greater number of spawning during the reproductive period (Bauer, 1991; Sarkar, 2011). The larger size of egg in *M. dayanum* required longer incubation period.

In post mating behavior, the cleaning was due to the arrangement of setae and the removal of debris. The spread of spermatophore in that area was another consequence of cleaning behavior (Hoglund, 1943; Bauer, 1976). Development of eggs started immediately after fertilization. Hatching of larvae was also depended on temperature (26 to 28 ^{o}C) as compared to winter temp. (25 ^{o}C) when hatching took place rarely. The mother took 1-2 hrs to release its hatchlings, but sometimes it may be extended to the next afternoon.

This investigation thus demonstrated a very high level of hatching success (95%) and survival rate of hatchlings of *Macrobrachium dayanum* under laboratory conditions.

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