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Reproductive biology of *Macrobrachium lar* (fabricius, 1798) in Andaman Islands

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Reproductive biology of the Monkey river prawn (*Macrobrachium lar*) was studied in the Rangat river, Andaman Islands, during 2007 to 2009, with the aim of determining the male: female ratio, maturity cycle, spawning seasons and the fecundity in those ecosystems. During 18 months period, 2078 individuals were collected, with a male: female ratio (M/F) of 1: 2.84. Total number of individuals caught per month ranged 65 -172. Males were generally larger than females. Percentage occurrence of reproducing and non-reproducing females, ovigerous females, seasonal occurrence of maturity stages, and maturity indices (GSI and HSI) exhibited that *M. lar* breeds twice in a year with two breeding peaks (June and November) every year. Highest reproduction indices (GSI) in female were observed in November (4.0 ± 0.07), December (3.88 ± 0.28), May (3.63 ± 0.08), and June (3.88 ± 0.16). In both the sexes, HSI showed inversely proportional relationships with GSI. With respect to average fecundity (F) by length classes, the lowest and highest number of eggs observed was 3090 and 8177, respectively. As for fecundity by weight classes, the lowest number of eggs observed was 4069 and the highest, 8543. Overall mean fecundity irrespective of the length and weight classes was 5402 (± 772.35) eggs per female. Diameter of non-eyed and eyed eggs ranged between 0.32 mm to 0.50 mm and 0.52 mm to 0.68 mm respectively.

[**Key Words**: Fresh water prawn, *Macrobrachium lar*, Andaman Islands, Sex ratio, Reproductive biology]

Introduction

Most of the prawn species of commercial interest belong to the *Macrobrachium* genus, distributed in the tropical and subtropical regions of the world[1]. Currently, there are nearly 210 species of the *Macrobrachium* genus known in the world[2]. The understanding of the reproductive biology of palaemonid prawns is imperative for development of management and culture programs as well as helping to describe sound strategies to preserve biodiversity[3-4]. Studies on the reproductive biology of other palaemonids have been reported by several workers[5-6] mainly *M. rosenbergii*[7-8], *M acanthurus* (Wiegmann, 1936)[9,7], *M. carcinus* (Linnaeus, 1758)[10,11] , *M. amazonicum* (Heller, 1862)[12-13] , *M. olfersii* (Wiegmann, 1836) [3], *M. iheringi* (Ortmann, 1897) [4],*Palaemon paucidens* (De Haan, 1844)[14], *P. longirostris* (Milne-Edwards, 1837)[15] and *Atya scabra* (Leach, 1815)[16]. However, information concerning the reproductive biology of *M. lar* is not available in any scientific literature. Present study is to investigate some aspects of the reproductive biology of the fresh water prawn *M. lar* such as the sex ratio, spawning period, its fecundity and egg size etc.

Materials and Methods

Study area is a stretch of Rangat River, Andaman Islands, flowing west to east is located in south east region of India. The river in its upper reaches flows through a rainforest zone, while its terrain in the vicinity of Rangat, small town, Andaman Islands where the study was carried out (Fig.1). A total of 18 collections of *M.lar* were collected with the assistance of fishermen using cast net from Rangat (lat.12° 38' 17.196" N and long. 92° 45' 55.635"E) during December 2007 to May 2009. Collected specimens were placed immediately in plastic jars containing clove oil solution. In the laboratory, specimens were segregated according to the size and sex and counted. Total length, TL of the prawn was measured with a meter rule to the nearest 0.1 mm, while the body weight was measured, to the nearest 0.01 g with an electronic balance. Prawns were grouped into male, female, ovigerous female and undifferentiated prawns. Determination of the maturity stages of *M.lar* was done by observing macroscopic characters as suggested by Singh and Roy (1994)[17] and the annual reproductive cycle was determined on

the basis of characteristics such as percentage of sex ratio in sampling catch composition, preponderance of reproducing and non - reproducing females, percentage of occurrence of ovigerous females, percentage of maturity of female gonads and Gonado-somatic and Hepato- somatic indices etc. Sexual differentiation was assessed by the observation of secondary sexual characteristics such as the masculine appendix in the second pleopods of the males and its absence in females and in juveniles. Juveniles were not included in the study. To know the homogeneity of the distribution of males and females, Chi square test[18] was applied. The females undergo series of ovarian developmental stages, and the ovary exhibits variation in its coloration, which can be visualized through the carapace. Proportion of reproducing and non-reproducing females was distinguished; by the presence of dark brick red coloured ovaries, fertilised eggs in the brood pouch, before undergoing post-pertural moulting while all the immature females having white translucent ovaries, with light pink coloured ovaries are considered as non- reproducing females. Reproductive period was determined by the presence of ovigerous female during the sampling period.

Gonads and livers undergo regular seasonal and cyclical changes in weight in relation to the total weight of the prawn. Such changes are indicative of the spawning season of the prawn and the relationship is respectively termed as 'Gonado somatic index' (GSI) and Hepato somatic index (HSI). Both indices were calculated according to the method of Giese and Pearse [19].
The 'GSI' and 'HSI' were calculated as:

GSI = (weight of gonad X 100)/ Total body weight.

HSI = (weight of liver X 100)/ Total body weight.
In order to determine the spawning periodicity of *M. lar*, matured female prawns were caught: their total length and total weight were measured and preserved in 5% formalin for 3-4 days to harden it. Ovaries were dissected out from mature female to study the size distribution of the ova. Ova were spread on a glass slide and were observed under microscope and the diameter of about 140 ripe ova from each berried female (total six berried) were measured in micrometer division using ocular micrometer. Ova with diameter 0.02-0.08 mm were considered as immature and were ignored.
Fecundity was determined by counting the number of eggs of 30 berried females by following the methods described by Sarojini[20]. Specimens were randomly selected that carried eggs in the initial developmental stages only because egg loss may occur during the incubation period in caridean prawns [21]. The egg mass was completely removed from the pleopods with a small brush, and the eggs were placed in Petri dishes for counting under a stereoscopic microscope. Egg size measurement is done with the aid of a microscope using miotic software and eggs were classified by the absence and presence of eye spots in the embryo.

Results

Out of 2079 numbers of individuals examined during 18 months of sampling period, 550 were males and 1529 were females. Sex composition of *M. lar* in the examined samples varied monthly, But with an overall average (M: F) sex ratio of 1: 2.84 with a chi square value of 26.58 for both sexes. This indicates that the ratio of males to females was significantly different. Females predominated in all the months; the ratio varied between 1.90 (Jan. 2009) and 4.06 (Mar. 2008). High percentage of occurrence of females was observed in the month of March, and October whereas the same for males was observed in the month of December to February and again in the month of September and November. Total length of *M. lar* male and female ranged between 82.0 - 123.18 mm and 73.15-118.21 mm respectively. Males are relatively larger than female (Plate 1). Observations on percentage occurrence revealed that both the sexes were found throughout the year in natural water bodies of Andaman water.

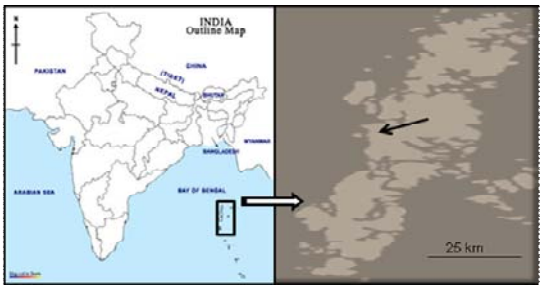

Fig.1.Map showing the location of sampling site (*arrow mark*) in Andaman Island, India

Plate1. Male and female individual of *M. lar*.

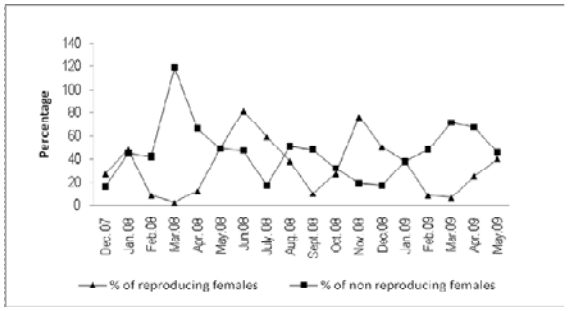

Fig.2. Percentage occurrences of reproducing and non reproducing females in Andaman waters.

During the present study, maximum percentage of reproducing females was observed in the months of June and November with two peaks. Preponderance of non-reproducing females was highest in the months of March and September (Fig. 2). These observations on preponderance of reproducing and non-reproducing females revealed that *M. lar* breeds twice in a year with two breeding peaks.

Plate 2.Ovigerous female of *M.lar* collected from Rangat River, Andaman.

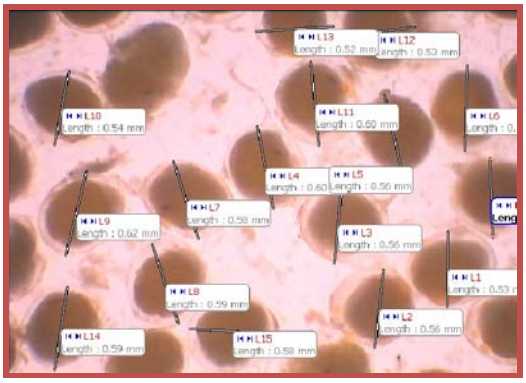

Plate 3. Measurement of ova diameter of *M. lar* using miotic soft ware.

During the study period, the maximum percentage of ovigerous females was observed in the months of December 2007, June and November 2008. The ovigerous females were found totally absent in the months from February - April 2008, September, 2008 and again in the months of February - April, 2009 (Fig. 3). These observations revealed that *M. lar* breeds twice in a year with two breeding peaks occurred in the months of June and November every year. Photograph of few specimens of ovigerous female is shown in the Plate 2.

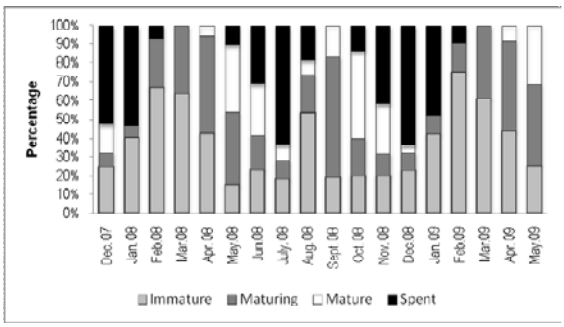

Fig.3. Percentage occurrence of Ovigerous female in Andaman waters.

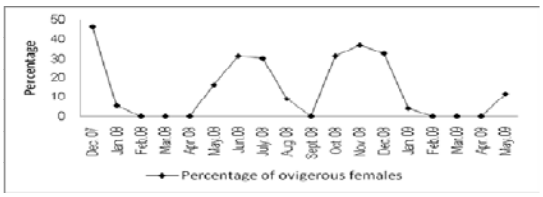

Fig. 4. Monthly stages of maturity of female of *M. lar* from Andaman waters.

During the study period, regular seasonal changes in the maturity occur in female gonads. The matured females were found all the months except January to March. The annual reproductive cycle showed two peaks periods. The first peak was observed in May followed by second peak in the month of October. Maximum percentage of immature females was found in the month of February, March and August. Maximum percentages of maturing females were found in the month of April and September. Similarly maximum numbers of spent females were found in the month of January, July and December respectively (Fig. 4).

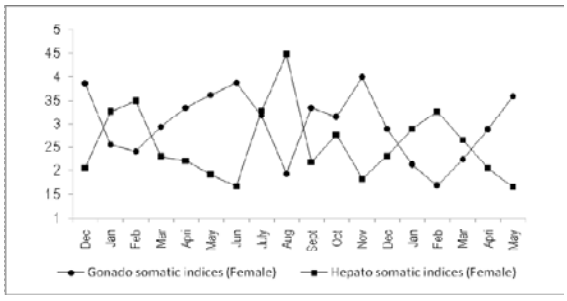

Fig. 5. Monthly variation in mean GSI and HSI for female *M. lar* from Dec '07 - May '09.

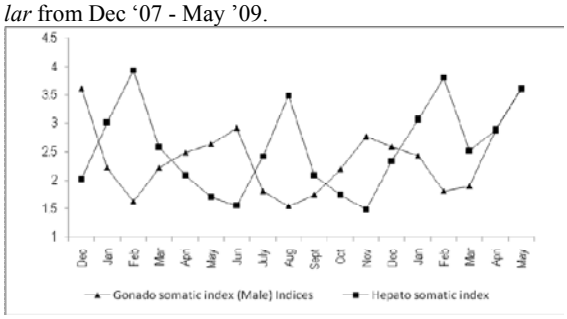

Fig. 6. Monthly variation in mean GSI and HSI for male *M. lar* from Dec '07 – May '09.

Maturity indices

Overall fluctuations in the reproductive cycle could be demonstrated through the cyclic changes in the mean values of GSI and HSI in *M. lar*. Monthly mean GSI values for females and males were estimated and graphical representation is given in the Fig. 5 and 6.

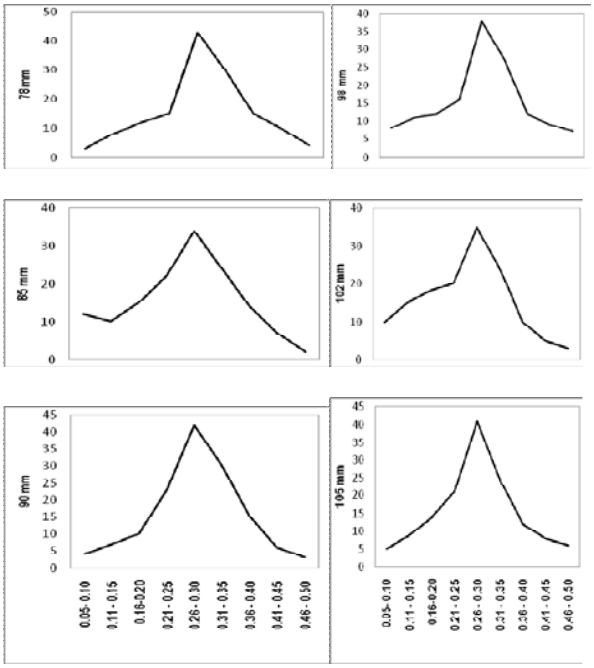

Fig.7. Frequency Polygons of Oocytes in 78 -105 mm female *M. lar.*

In males, maximum GSI was observed during the months of Nov. (2.76 ± 0.31), Dec. (3.61 ± 0.78), May (3.60 ± 0.12) and June (2.91 ± 0.15). Range of GSI during the whole year varied between 1.54 ± 0.24 to 3.61 ± 0.78. Minimum GSI was noticed during the month of Aug. (1.54 ± 0.24) and Feb. (1.63 ± 0.26). In females, maximum ovarian index was recorded during the month of Nov. (4.0 ± 0.07), Dec. (3.88 ± 0.28), May (3.63 ± 0.08), and June (3.88 ± 0.16). Range of GSI during the whole year in females varied between 1.68 ± 0.02 to 4.00 ± 0.07.This fluctuations in the mean values in both sexes exhibit two peak breeding periods in the annual reproductive cycle of *M. lar*. HSI showed inversely proportional relationships with the GSI in both sexes. In males, maximum HSI was observed in the months of Feb. (3.93 ± 0.71) and Aug. (3.48 ± 0.18) and minimum in the months of June (1.54 ± 0.12) and Nov. (1.48 ± 0.19) and similar trend was noticed in females also. It was also found that the negative correlation between the GSI and HSI in male is not significantly different ($P < 0.05$) whereas it was significantly different in female ($P > 0.05$) (Fig.5 and 6).

Spawning periodicity

In the present study, 15 matured females of *M. lar* with matured ovaries were used for determination of ova diameter. Immature eggs in the size range of 0.02 – 0.08 mm, which formed the ground stock, were ignored. Distributions of ova in six ripe females in the size range of 78 mm to 105 mm were measured (Fig. 7). It is seen from the figure that in the case of all the six matured females, majority of ova were in the size range of 0.21 mm to 0.35mm with maximum numbers in 0.26 mm to 0.30 mm in size and they showed a single peak. Appearance of a single peak in the ovary clearly indicates that all the matured ova are shed at one instance.

Average fecundity

The berried females carrying fertilized eggs in the periopods were used for the estimation of fecundity. Present observations revealed that mean fecundity (F) varied from 3090 to 8177 in the individuals of length class of 6.0 to 11.5 cm, whereas by weight class of 5.1 to 21.0 g, it was ranged between 4069 and 8543. The estimated mean fecundity of *M. lar* was 5402 (± 772.35). Average fecundity/total length and average fecundity/total weight may be expressed by a linear relationship represented in the following equations:

$F = - 1697.47 + 811.34\ L\ (P > 0.01)$

$F = 5632.12 + 4.\ 439\ W\ (P < 0.01)$

Where F = average fecundity; L = total length (cm); and W = total weight (g).

The obtained result infers that there is no significant relationship between the fecundity (F) and the length classes (L) however, significant relationship exists between the fecundity (F) and the weight classes (W). Diameter of non-eyed eggs ranged between 0.32 mm to 0.50 mm whereas those of eyed eggs measured between 0.52 mm to 0.68 mm (Plate 3).

Discussion

The sex ratio in the natural population of *M. lar* collected in the Rangat River, Andaman was quite similar to that observed by Porto[22] and Sampaio[23] for the species *M. amazonicum* respectively collected in the Meir Ponte River, Goisa, and in the Jaguaribe River, Ceará with the female predominating over the males. Similarly Mantelatto and Barbosa[24] studied the species *M. brasiliense* in Serra Azul, Sao Paulo discovered the same, that is, and females predominated over males in a proportion of 1: 2.6. The sex ratio varied between the *Macrobrachium* species. Barros[25], Ammar[13] and Mossolin and Bueno[3] studied the *M. olfersii* population found a predominance of males over females at a proportion of 1: 0.53; 1: 0.71 and 1: 0.23, respectively. Antunes and Oshiro [26] studied samples of the species *M. potiuna* collected in the coastal waters of the State of Rio de Janeiro, Brazil found a ratio of 1: 0.94, that is, a predominance of males over females higher than *M. olfersii.* Silva[27] studied the species *M. amazonicum* collected in the State of Pará discovered the same, that is, males predominating over females. Male attained the largest size among all sexual categorical stages in *M. lar*. The studied sexual, size dimorphism could be resulted from differential growth among the sexes because females need to invest more energy into gonadal development. This change has been reported in several other palaemonid species[13, 28]. Mossolin & Bueno[29], Fransozo [4,] and Mantelatto &Barbosa [24] studied *M. olfersi* , *M. iheringi* and *M. brasiliense* respectively, also observed the similar differential growth rates between sexes. Mantelatto and Barbosa [24] also reported the additional fact for dimorphism that the larger size reached by males may be related to domination over females, as well pre-adult males, during the copulation process. In the present study, low numbers of adult prawns were collected. This could be probably related to large quantity of shelters available in the river edge, together with several aquatic macrophyte species, reducing their chance of capture. This truth was also observed by Galvao and Bueno [30] in *Atya scabra* (Leach, 1815). The four different stages of ovarian maturity for *M. lar* classified in this paper were very similar to the ones described by Singh and Roy [17]. Breeding period is assessed by the presence of

berried female[15]. The studies related to reproductive characteristics such as percentage occurrence of reproducing and non-reproducing females, percentage of occurrence of ovigerous females, seasonal occurrence of maturity stages, and maturity indices (GSI and HSI) exhibited that *M. lar* breeds twice in a year with two breeding peaks occurred in the months of June and November every year. Presence of ovigerous females only during those monsoon months indicate that this species breeds seasonally. Walker and Ferreira [31] studied the samples of *M. nattereri* (Heller, 1862) and *M. inpa* (Kensley and Walker, 1982) species collected in the Taruma-Mirim River in central Amazon recorded the pattern of seasonal reproduction. Presence of ovigerous females in those species were found only during rainy season or monsoon months. Chaves and Magalhães [32], Porto [22] and Silva [27] observed that ovigerous *M. amazonicum* females are more abundant in the rainy season than at other times of the year. Sastry [33] reported that there are two main strategies for reproduction in decapods, that is, continuous strategy, when spawning occurs at roughly constant levels throughout the year and seasonal strategy, when spawning is seasonal or restricted in a certain period of the year. In the latter one, there is a close link between reproduction, environmental conditions and food supply, which are fundamental for the survival of larvae and juveniles. *M. lar* is coming under the latter case. However, in general, it is accepted that the reproduction is continuous in the tropical regions whereas it is seasonal and more and more restricted in species inhabiting subtropical to temperate [34-35]. Occurrence of non-ovigerous females during most of the month of collection could be related to a difficulty in catching ovigerous females as they migrate to lower regions during the reproductive phase where egg incubation take places or they try to find shelter in protected areas, that is, in marginal plants to keep away from predation pressure [36]. Fecundity is an important parameter to know the population ecology and life history of any species [37]. Different fecundity indexes are differentiated: potential fecundity (number of oocytes in the ovary), realized fecundity (number of eggs attached under the pleopods) and actual fecundity (number of larvae hatched) [38]. Generally realized fecundity is illustrated in studies of crustacean reproductive biology [16]. In the present study, realized fecundity was found to be ranged between 4069 and 8543 with estimated mean fecundity of 5402 (± 772.35). Fecundity of *Macrobrachium sp* prawns is highly unpredictable. The female of *M. rosenbergii* and *M. carcinus* can lay eggs between 80,000 and 100,000 numbers in each spawning during the fully mature condition. During the first spawning, they can lay the eggs between 5,000 and 20,000 numbers [36]. Third highest fecundity in species of this genus is observed in *M. acanthurus* females, which can hatch about 18, 000 eggs [7-8], whereas Shokita, cited by Scaico [39] hatched bout 13, 600 eggs. Although fecundity of *M. lar* collected in lower stream of river is smaller compared to those above species, it shows higher fecundity than *M. amazonicum* whose fecundity is lower than 2193 eggs [40] and *M. iheringi, M. Borelli, M. australiense, M. potiuna*, and *M. jelski*, whose absolute fecundity is lower than 200 eggs [39]. Fecundity of *M. lar* was found higher than those of *M. equidens* [41] but it was quite related to that observed by Krishna-Murthy [42] for the same species. Egg size of decapods that reproduce in fresh water is frequently enlarged compared with marine relatives [43]. Egg size measured in the present study was lower than those of *M. potiuna* and *M. olfersi* whose mean size was 1.79 ± 0.13 (long axis) and 2.17 ± 0.17 (long axis) respectively [44] but fecundity was found more than those species. Nazari [44] studied in *M. olfersi* and *M. potiuna* reported that the strategy of carrying a large number of small eggs by *M. olfersi* allows a greater egg loss without affecting the reproductive success of the species whereas in *M. potiuna* has a reduced egg loss because this species spawns smaller number of voluminous eggs and a larger loss could compromise its reproductive investment.

Conclusions

In *M. lar*, Male attained the largest size than female but female predominant over male throughout the entire year. Occurrence of ovigerous females with mature ovaries twice in a year, that is, June and November, reinforces the fact that this species breed seasonally during rainy season with low realized fecundity.

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