Reproductive Cycle of the Freshwater Prawn
*Macrobrachium birmanicum choprai* (Tiwari)

S.R. SINGH and D. ROY

Department of Zoology
S.M.M.T.P.G. College
Ballia (U.P.)-277001
India

Abstract

The annual reproductive cycles of both sexes of the freshwater prawn *Macrobrachium birmanicum choprai* (Tiwari) were studied over a 2-year period (October 1989 - September 1991). The animals did not appear in the catches from late October to February probably owing to their migration into deeper zones of the rivers. The observations suggest that this species is a continuous breeder. While the male did not exhibit a distinct breeding peak, the female recorded a distinct reproductive peak in July/August. September was observed to be the extended spawning period for females, whereas all the males encountered during this month were found spent. Distinct histological changes were observed in the gonads of both sexes during the reproductive cycle.

The vitellogenic period of the ovary was divided into previtellogenic and vitellogenic phases, and each phase was further subdivided into three stages. Different entities of spermatogenesis were seen at the same time in seminiferous tubules.
Introduction

Studies on the reproductive cycles of freshwater prawns in India are few. Rajyalakshmi (1980) described the breeding behavior of *Macrobrachium malcolmsonii*. Reproductive biology of the freshwater prawn *M. kistensis* was described by Mirajkar (1980). Nagabhushanam et al. (1985) studied the histology and histochemistry of the ovary in a freshwater shrimp *Caridina weberi*, while Victor and Sarojini (1985) studied its oocyte differentiation and vitellogenesis. Sarojini and Rajani (1987) described the reproductive cycle of the female *C. rajdhari*. Jaylakshmi et al. (1986) also made a comprehensive study on the ovarian histology in relation to vitellogenesis in the freshwater prawn *M. lamarrei*.

The male reproductive cycle of freshwater prawns has received relatively less attention; among the few significant contributions are those of Langreth (1969), Reger (1970), Sengupta and Chatterji (1976) and Sarojini and Gyananath (1984).

*Macrobrachium birmanicum choprai* (Tiwari), which naturally inhabits middle and lower reaches of the Ganga River, ranks high among economically important freshwater prawns for its large size, high protein content and deli-
cacy. Unfortunately, its fishery, so luxuriant only a few years ago, has declined to a great extent.

The present paper describes observations made on the male and female reproductive cycles of the freshwater prawn *M. birmanicum choprai* (Tiwari).

**Materials and Methods**

In all, about 200 intermoult stage individuals of each sex were collected twice a month for two years (October 1989 - September 1991) from the Ganga River near Buxar and Ballia. Prawns did not appear in the commercial catches from middle of October to February owing to their migration into deeper layers due to excessive cold during this period.

Size, color and physical state of the gonads were noted and they were then fixed in Bouin's solution and processed under the usual histological techniques.

The reproductive stages of prawns during different months of the study were plotted against time to indicate the breeding period. Color of the ovary was indicative of the stage of maturity of female individuals. In males, the length, color and texture of the testes were taken into consideration to ascertain the maturity states, and this was further confirmed by histological observations.

**Results**

**Female Reproductive Cycle**

The female reproductive organs consist of a pair of compact, elongated and slightly flattened ovaries lying over the dorsal side of the stomach. The two ovaries closely approximate each other at the two ends leaving a small gap in the middle for the passage of the cardiovascular strand. The shape and size of the ovaries vary considerably with age and the season of the year. The oviduct, a short and thin walled tube, originates from the outer middle border of each ovary and runs vertically downward to open through a female genital aperture on the inner side of the coxa of the third walking leg of its side.

The following five stages of ovaries were recognized on the basis of color: 1. Premature - pink; 2. Early maturing - light green; 3. Mature - dark green; 4. Berried - greenish brown/yellowish brown; and 5. Spent - reddish brown.

**Maturity**

The percentage occurrence of female prawns in different stages of maturity is depicted in Fig. 1. The premature stages occurred in maximum from March (100%) to April (90%). Only 10% of the animals in April were observed to be mature. In May, 50% of the ovaries were noted in premature condition. This percentage, however, became considerably low (10%) in June and altogether absent from July to September.
Females with mature ovaries started appearing in significant number (50%) from May, with the highest percentage in June (65%). The percentage occurrence of females with mature ovaries was 35% in July, 50% in August, and 50% in September. July was observed as the principal spawning period with the highest percentage of berried (30%) and spent prawns (35%). In August and September, the percentage contribution of berried and spent animals was 40% and 10%, respectively.

**Oogenesis**

The ovary has an outermost epithelial layer. The germinal zone is in the ventrolateral region of the ovary. The oogonial cells are in clusters near the germinal zone which increases in number through repeated mitotic divisions. The meiotic division follows, then primary oocytes and finally ova. All the stages of oocyte growth as well as the accessory cells and nutritive phagocytes are seen in a fully matured ovary. The developing ova are in radially disposed strings with the immature ones towards the germinal zone and the mature ones towards the periphery.

The distinction of different stages of oocytes was based on the size of the oocytes, their staining affinity, changes in nucleus and the presence and absence of the yolk globules. Oocytes were broadly divided into two principal categories - previtellogenic and vitellogenic. A brief description of the different stages of oogenesis follows:

**Oogonia**

These small, spherical, basophilic cells with large and round nuclei, were surrounded by a thin rim of oocortex. They lacked stainable yolk materials. In the immature ovary, oogonial cells were highly aggregated near the germinal zone. Some residual oogonia were also present in the mature ovary. Oogonia developed into previtellogenic oocytes.
**Previtellogenic Oocytes**

A large amount of basophilic cytoplasm was acquired by previtellogenic oocytes. Yolk formation had not yet begun. The nucleus appeared vesicular possessing chromatin clumps arranged peripherally with 2-6 nucleoli which stained black with hematoxylin. Based on the size, the previtellogenic oocytes were further divided into three groups: PVO 1 (17-32 $\mu$m x 400); PVO 2 (32.1-45 $\mu$m x 400); and PVO 3 (45.1-54 $\mu$m x 400).

**Vitellogenic Oocytes**

This was the synthetic phase of the oocytes in which yolk synthesis took place. Follicle cells also started appearing. This stage was marked by considerable changes in nucleus, nucleolus and ooplasm, and was divided into three substages:

I. **PRIMARY VITELLOGENIC OOCYTES**

Small yolk droplets appeared in the peripheral ooplasm which stained purple to black with hematoxylin. The nucleus was solid, central in position and without nucleoli. Small, round follicular cells appeared around oocytes.

II. **SECONDARY VITELLOGENIC OOCYTES**

The oocytes further increased in size. Small unstainable vacuoles appeared in the ooplasm which later fused together to form large unstainable yolk vesicles. Small eosinophilic yolk granules started accumulating in the peripheral region of the oocortex. The follicle cells remained as seen in the earlier phase. Advancement in the vitellogenic phase led to the disappearance of the perinuclear ring of eosinophilic granules surrounding the nucleus.

III. **TERTIARY VITELLOGENIC OOCYTES**

The oocytes attained their utmost size in this phase. Yolk droplets converted into yolk vesicles which were strongly eosinophilic. Most of the ooplasm including the perinuclear region was occupied by yolk globules. The entire ooplasm thus became acidophilic.

Maturation of ova was followed by their ovulation and oviposition in the brood pouch of the female.

**Degenerating Oocytes**

Crowding and competition among oocytes led to simultaneous degeneration of some of the oocytes. Failure of ovulation due to unfavorable conditions could also cause degeneration of oocytes. Degeneration of oocytes was initiated by appearance of the vacuoles and disintegration of the nucleus. There was, however no remarkable decrease in size of the degenerating oocytes. They
were seen surrounded by nutritive phagocytes which increased gradually in size with growing vacuolization.

Degeneration of oocytes did not occur simultaneously in all parts of the ovary. Therefore, spent ovaries were observed in different stages. Some were completely degenerated, some filled with eosinophilic degenerating oocytes, and others completely basophilic with no detectable oocyte.

**Reproductive Cycle**

**MARCH-APRIL**

Ovaries showed a large number of oogonia near germinal zone and previtellogenic oocytes. No vitellogenic oocyte was observed (Fig. 2A).

**MAY-JUNE**

The ovaries showed few oogonia, which were probably the residual oogonia, and three types of previtellogenic oocytes were encountered. Apart from this, some primary vitellogenic oocytes were also observed showing yolk droplets in the peripheral region (Fig. 2B).

**JULY-AUGUST**

The ovaries were full of mature (secondary and tertiary vitellogenic) oocytes (Figs. 2C-D). Large eosinophilic vesicles were observed throughout the ooplasm. In some of them, the nucleus was invisible due to overlapping yolk material. Degenerating oocytes were also encountered. A large number of ovaries were spent (Figs. 2E-F). Among spent ovaries, some were partially spent showing two or three distinct regions - one region showing completely degenerated oocytes, the others with growing oocytes. Some ovaries were completely degenerated. In the latter case, some were filled with eosinophilic degenerating oocytes while others contained no eosinophilic material. Thus it is obvious that the months of July and August constituted the major spawning period of the animal.

**SEPTEMBER**

September was the extended spawning period. Mature, berried, as well as spent ovaries were observed.

**Male Reproductive Cycle**

A pair of testes, a pair of vas deferens and a pair of vesicula seminalis constitute the male reproductive organs. The testes are soft, white and elongated structures lying above the posterior half of the dorsal surface of the hepatopancreas and beneath the pericardial sinus and the heart. The two testes are fused together anteriorly, forming a common lobe; whereas at the posterior
end, though remaining separate, they closely approximate each other. A gap between the two testes provides passage for the cardio-pyloric strand connecting the heart to the pyloric stomach.

From the posterior end of each testis arises the vas deferens, a long, coiled and narrow tube. Soon after emerging, the vas deferens forms a highly coiled mass, runs down vertically between the thoracic wall on the outer side and the

Fig. 2. Sectional views of the ovary of *M. birmanicum choprai* (Tiwari). Hematoxylin-eosin (x400)
A. T.S. Ovary showing clusters of oogonia.
B. T.S. Ovary showing previtellogenic oocytes.
C. T.S. Ovary showing vitellogenic oocytes.
D. T.S. Ovary showing matured oocytes.
E-F. T.S. Ovary in degenerated state.

FC - follicle cells, MO - matured oocyte, N - nucleus, NU - nucleolus, OG - oogonia, PNR - perinuclear ring, PVO - previtellogenic oocytes, RO - residual oogonia, VO - vitellogenic oocyte, YD - yolk droplets.
abdominal flexor on the inner side. On reaching the coxa of the fifth pair of walking legs on the ventral side, it forms a club shaped vesicula seminalis. The vesicula seminalis stores the spermatophores. It opens to the exterior through a male genital aperture in the inner side of the coxa of the fifth walking leg of its side.

The testes varied in length from 1.0 to 3.8 cm. They were almost always white with pink or brown spots. Testes of maturing and mature individuals had a turgid texture, whereas those of spent animals had a loose texture. Based on size, color, texture and histological differentiations, four stages of testes were recognized as depicted in Table 1.

Table 1. Testes characteristics of *M. birmanicum choprai* at various stages of maturity.

<table>
<thead>
<tr>
<th>State of maturity</th>
<th>Length group (cm)</th>
<th>Texture</th>
<th>Color</th>
<th>Histological examination</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premature (Stage I)</td>
<td>1 - 1.9</td>
<td>Turgid</td>
<td>White</td>
<td>Tubule diameter short, compactly packed, mostly with spermatogonia; wall of tubule thick.</td>
</tr>
<tr>
<td>Early maturing (Stage II)</td>
<td>2 - 2.9</td>
<td>Turgid</td>
<td>White with pink spots</td>
<td>Tubule diameter large, spermatocytes and spermatids are dominant, spermatogonia near the germininal epithelium only; wall of tubule thin.</td>
</tr>
<tr>
<td>Mature (Stage III)</td>
<td>3 - 3.8</td>
<td>Turgid</td>
<td>White with pink spots</td>
<td>Tubule diameter very large, filled with spermatids and spermatozoa.</td>
</tr>
<tr>
<td>Spent (Stage IV)</td>
<td>1.2 - 2.2</td>
<td>Loose</td>
<td>Dull white, sometimes with brown spots</td>
<td>Lumen of tubules irregular, a gap between germininal epithelium and germ cells which are few and include residual spermatozoa.</td>
</tr>
</tbody>
</table>

**Histological Observation**

Testes of *M. choprai* are made up of a large number of seminiferous tubules of varying size held together by connective tissue. A cross section of a tubule clearly shows the lumen bounded by a germinative zone and the germ cells in various stages of development. The various entities of spermatogenesis, viz., spermatogonia, spermatocytes, spermatids and spermatozoa, can usually be observed. A brief description of the organization of these cells is given in Table 2.

Spermatogonia are the first group of cells to appear during the process of spermatogenesis and hence are most populous near the germinative zone of maturing or mature testis. These are circular and basophilic structures with a network of chromatin material and nucleoli but indistinct nuclear wall. Their average diameter was 8.5 µm. However, under light microscopy no distinction
could be made between primary and secondary spermatogonia. These cells undergo further mitotic divisions to form primary spermatocytes.

The primary spermatocytes have eosinophilic cytoplasm. They undergo first maturation division to give rise to secondary spermatocytes. The latter have poorly stainable cytoplasm. The primary and secondary spermatocytes did not show any marked difference in size. The average diameter of primary and secondary spermatocytes was 5.7 µm. The secondary spermatocytes, followed by second maturation division, gave rise to spermatids.

The spermatids are small rounded bodies 3.0 µm in diameter. They have a little cytoplasm and most of their volume is occupied by a large nucleus. The nuclei show uniform condensed chromatin material deeply stained with hematoxylin.

Finally the spermatids undergo certain morphological changes to produce spermatozoa. The transformation of spermatids into spermatozoa appeared to be a quick process in *M. choprai* as evidenced by the presence of meager spermatids in the maturing and mature seminiferous tubules. The spermatozoa are crescent shaped structures bearing a short tail. The two ends of the crescent measured 4.0 µm, its widest part in the middle measured 1.0 µm.

### Seasonal Testicular Cycle

The percentage of animals in different stages of maturity observed during different months was computed and depicted in Fig. 3.

In March, 50% of the animals had immature testes and the rest were on the way to maturity. In April, most of the animals had mature or early maturing testes. Only 10% had immature testes. May showed a marked increase in the percentage of maturing animals (70%). Mature as well as spent animals were contributing equally (10% each). Some immature animals were also found.

From June to September, no immature individual was observed. In June 50% of the individuals had early maturing testes, 25% had fully mature testes,

Table 2. General organization of germ cells at light microscopic level in testes of *M. birmanicum choprai*

<table>
<thead>
<tr>
<th>Germ cells</th>
<th>Shape</th>
<th>Size (µm)</th>
<th>Cell boundary</th>
<th>Cytoplasm</th>
<th>Nucleus</th>
<th>Nucleoli</th>
<th>Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spermatogonia</td>
<td>Circular</td>
<td>8.5</td>
<td>Distinct</td>
<td>Clear</td>
<td>Nuclear wall indistinct</td>
<td>Distinct</td>
<td>Forming a network</td>
</tr>
<tr>
<td>Spermatocytes</td>
<td>Circular</td>
<td>5.7</td>
<td>Distinct</td>
<td>Clear</td>
<td>Nuclear wall indistinct</td>
<td>Distinct</td>
<td>Indistinct</td>
</tr>
<tr>
<td>Spermatids</td>
<td>Circular</td>
<td>3.0</td>
<td>Distinct</td>
<td>Indistinct</td>
<td>Distinct</td>
<td>Invisible</td>
<td>Condensed</td>
</tr>
<tr>
<td>Spermatozoa</td>
<td>Crescent with short tail</td>
<td>L-4.0 W-1.0</td>
<td>Indistinct</td>
<td>Indistinct</td>
<td>Indistinct</td>
<td>Indistinct</td>
<td>Indistinct</td>
</tr>
</tbody>
</table>
the rest had spent ones. Maturity states in July were more or less similar as in June. However, August was equally shared by mature and spent animals. In September, all the individuals met with were spent.

The premature testes seen in March had tubules compactly packed with germ cells in different stages of development (Figs. 4-5). However, spermatogonia were dominant in this stage. Primary and secondary spermatocytes were also seen in the center. Testes of the early maturing stage showed tubules dominated by primary and secondary spermatocytes, spermatids and few spermatozoa (Fig. 6). The thin walled tubules in fully mature testes (size group 3-3.8 cm) exhibited varying dimensions packed either with spermatozoa only or with both spermatids and spermatozoa (Figs. 7-8). In some cases, two seminiferous tubules were closely associated with each other showing a common lumen.

In spent testes (Figs. 9-10), the germ cells had left the germinal wall of the tubules. The germ cells which included residual spermatids and spermatozoa were scarcely distributed inside the tubules.
Fig. 6. Cross section of a maturing testis (stage II) showing tubules filled with spermatids and spermatozoa. Hematoxylin-eosin (X400)

Fig. 7. Cross section of a mature testis (stage III) showing tubules filled with spermatozoa. Hematoxylin-eosin (X400)

Fig. 8. Cross section of a mature testis showing a single tubule filled with spermatozoa and clusters of spermatozoa. Hematoxylin-eosin (X1000)

Fig. 9. Cross section of a partially spent testis (stage IV) showing deshaped tubular lumen with residual spermatozoa. Hematoxylin-eosin (X400)

Fig. 10. Cross section of a completely spent testis with some residual spermatozoa. Hematoxylin-eosin (X400)

ST - spermatids
CSZ - clusters of spermatozoa
SZ - spermatozoa
RSZ - residual spermatozoa
Discussion

In March, when the females started appearing in the catches, all were immature. A significant percentage of mature ovaries (65%) was observed only from May onward, climaxing in June. This indicates that ovary maturation is closely correlated with rise in temperature and increasing day length. Sarojini and Rajani (1987) also suggested a possible relationship between reproductive activity and increased temperature and longer day lengths in female freshwater prawn, *Caridina rajdhar*. A direct correlation between longer duration of photoperiod and maturation of ovary in freshwater prawn *Macrobrachium kistensis* has been established by Patil et al. (1987).

The freshwater prawns, *M. kistensis* (Mirajkar et al. 1983) and *M. lamarrei* (Sarojini and Gyananath 1984), have been reported to be biannual breeders. In the present study, *M. choprai* did not withstand a wide range of thermal variation. Immediately after the fall in temperature in November, it enters the deeper zones of the river - a comparatively warmer part that is least liable to thermal changes. No evidence could be traced for its breeding during this period. Its activities resumed only after considerable rise in temperature from March onward. The presence of more than one size range of oocytes in the ovaries, however, suggests a continuous breeding pattern of this prawn species.

Spawning was observed from June to September as indicated by the presence of an appreciable number of females in berried condition. Spent ovaries were observed from July onwards. July to August formed the major spawning period as evidenced by the presence of the maximum number of berried and spent animals in these months. September, which also showed a good percentage of berried and spent animals, may be regarded as the extended spawning period. It may therefore be inferred that, though being a continuous breeder, the major breeding period of *M. choprai* lies in the monsoon season. This observation does not coincide with the findings of Nagabhushanam et al. (1987) who observed that rainy season reduces breeding activity in *M. affinis*. Pillay and Nair (1971) also found that the rainy season is a constraint to breeding in estuarine and shore crabs. However, observations by Gyananath (1982), Victor (1984) and Sarojini and Rajani (1987) on various freshwater prawn species support the present investigation which concludes that rainfall has little to do with the breeding activities of freshwater prawns. Further, Nagabhushanam et al. (1987) suggested that reduced availability of food may limit breeding in *M. affinis*. However, in the present study, a number of mature and berried prawns were found with empty stomachs or traces of food (Singh and Roy, in press). Feeding in *M. choprai* increased only after the animals had become spent.

In *M. choprai*, spermatogenic tubules have been observed to contain two to three developmental stages together in the same tubules as is observed in different species of crab (Fasten 1926; Ryan 1967; Sengupta and Chatterji 1976). Sengupta and Chatterji (1976) noted the presence of both spermatocytes and spermatids in the same tubule and observed that all spermatocytes were at the same stage of differentiation. Sarojini and Gyananath (1984) made a similar observation in *M. lamarrei*. However, in the crab *Portunus sanguinolentus*, the seminiferous tubules have been reported to carry spermatogenic cells all at the same stage of development (Ryan 1967).
Marked differences in the morphology and organization of spermatozoa in different groups of crustaceans have been recorded. In the present investigation, the sperm was observed to have a crescent shaped head with a short tail. Joshi (1980) reported sperm of penaeid shrimp *Parapenaeopsis stylifera* and *P. hardwickii* having a round head and a short tail. On the other hand, Sarojini and Gyananath (1984) observed sperm of *M. lamarrei* to be aflagellate having no pseudopodial rays on distal filaments.

Different crustacean groups exhibit variations in appearance, location, formation and transfer of spermatophores. In *M. choprai*, formation of spermatophores which were found attached with the periopods by means of a sticky substance, seemed to have been initiated in the testis itself as evinced by the clustering of spermatozoa in the seminiferous tubules of mature testis (Fig. 8). In penaeid shrimp *P. stylifera* and *P. hardwickii*, Joshi (1980) also reported formation of the spermatophores in the testis. On the other hand, Sarojini and Gyananath (1984) observed the vas deferens to be the site of spermatophore formation.

During the entire period of study except in September (March-August) maturing as well as mature individuals were observed. Animals in premature condition were observed maximum in March, few in April and May, and altogether absent from June onwards. Thus it can be stated that the male breeding period varies from March to August. No distinct peak could be observed during this period. In September, all the individuals were found to be spent. It can thus be presumed that the period from September/October to February is the resting period for male individuals, during which they migrate deeper into the river.

Temperature was noted to be associated with the process of sexual maturity of the animals. In *M. choprai*, spermatogenesis seems to be a continuous process. Once the individual attains sexual maturity, the testes remain in a permanent state of readiness for the release of spermatophores.

References


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