CHAPTER I

SEXUAL BIOLOGY
INTRODUCTION

Reproduction is one of the fundamental activities of the organism. The reproductive biology has become an interesting aspect of life because of its profound effect on anatomy, physiology and behaviour of the animals. The reproductive biology has been dealt by many workers in Crustacea (Pillay and Nair, 1970; Thomas, 1974; Ito, 1976). However, from the point of view of histology of gonads and gametogenesis, the crustaceans are studied with little emphasis.

A comprehensive study of the anatomy and histology of the reproductive system of the male crab, Callinectes sapidus, was given by Cronin (1947). An extensive account of the oogenesis and spermatogenesis was given by Estampador (1949) on the species of the portunid crab, Scylla. Later, Nishioka (1959) and Ryan (1965) studied the histology of the reproductive system of Portunus sanguinolentus. Cheung (1968) in stone crab, Menippe mercenaria studied the histology of the male reproductive system. Young (1974) examined the spermatogenesis in Uca pugnax.
The oogenesis in crayfish, *Cambarus clarkii* was studied by Suko (1954). Ewa (1976) has given an account of histological and morphological changes in the testis of crayfish, *Orconectes limosus*. Amato and Payen (1978) studied the spermatogenesis in *Pontastacus leptodactylus leptodactylus*.

Barnes and Stone (1973) in *Verruca stroemia* described the reproductive cycle. The oogenesis in *Balanus amphitrite* and *B. eburneus* was studied by Fyhn and Costlow (1977). Arsenault *et al.* (1979) have done an electron microscopic study of spermatogenesis in shrimp, *Crangon septemspinosa*.

However, very few contributory reports have been made on the reproductive system (histological study), spermatogenesis, oogenesis of the prawns (*King, 1948*; *Parameswaran, 1953*; *Pillai, 1960* and *Jyoti, 1974*).

There are also scanty studies on the histochemical nature of the oocytes and spermatozoa in crustaceans. Shyamsundari (1972 a,b) studied the cytochemistry of the oocytes of some amphipods. Bonina (1974) observed cytochemical

Though, the reproduction is a hormone controlled process (Otsu, 1963; Adiyodi and Adiyodi, 1970, 1974; De Leersnyder and Dhainaut, 1977; Goltzene and Porte, 1978), the environmental factors are also causative for reproduction, because the reproductive activities are often sensitive to, and synchronized by environmental changes. The photoperiod and temperature are two well known responsible factors, but tidal rhythms, food availability, rainfall and changes in the salinity also have some role in reproduction.

In planktonic crustaceans, the exogenous rhythmicities have pronounced effect on reproduction viz. branchiopod, *Daphnia schodleri* (Parker, 1966); isopod, *Ligia oceocnica* (Mocquard et al., 1971); copepod, *Tisbe cladiensis* (Fava, 1972) and *Limnocalanus macrurus* (Roff, 1972). Stephens (1952)

From the above cited literature it is clear that there is little information on the reproduction of freshwater prawns. Moreover, practically there is no literature on the reproductive cycles in male and female prawns along with histological and histochemical explanation for oogenesis and spermatogenesis. The aim of the present research on reproductive cycle is to correlate these cycles with environmental factors, such as photoperiod and temperature and to study the histological and histochemical changes in female and male reproductive systems of the prawn, *Macrobrachium kistnensis*. 
MATERIAL AND METHODS

The prawns, *Macrobrachium kistnensis*, were collected from Kham river, near Aurangabad, for a period of one year (May 1977 to April 1978). The prawns were kept in aerated water, in glass aquaria. They were acclimatized to laboratory conditions before the start of the experiment.

1) **Breeding behaviour**: The total number of berried females were counted for each month for one year and the percentage of berried females was calculated to study the breeding behaviour of the prawn, *M. kistnensis*.

2) **Carapace length and gonad index**: The prawns were towelled with blotting paper and the females and males handled separately for analysis. The total length of the body and carapace length was measured. The prawns were weighed nearest to milligram and then the gonads were removed. Each gonad was blot dried with filter paper and carefully transferred to previously weighed aluminium foil-pan. The wet weights of the gonad were noted. The gonadal index was calculated by the following formula (Giese, 1959).
Gonad index = \( \frac{\text{Wet weight of the gonad}}{\text{Wet weight of the prawn}} \times 100 \)

(3) **Histology**: In addition to the gonad indexing method adopted for assessing the reproductive cycle of *M. kistnensis*, more reliable method i.e. histological observations was adopted. This method is more reliable for estimating the size frequency distribution of the oocytes in the ovary. After counting the number of ova one can judge the reproductive stage of the animal. For this purpose the ovary was fixed in Bouin's fluid. The testis was also fixed for histological study. The tissues were dehydrated in alcohol grade series and were paraffin embedded. The sections were cut at 8-10 \( \mu \) and stained with Harri's Haematoxylineosin.

The stages in oogenesis and spermatogenesis were studied by scrutinizing the sections. The number of oocytes and seminiferous tubules (acini) were counted for each section and percentage was calculated for each month.

3.1 **Oocyte size classes and percent frequency distribution**

In a histological section of the ovary the frequency of different sizes of oocytes were determined by measuring
the diameter of oocytes. The measurements were made on the longest and shortest axes and both of them were added and the mean was taken. The oocytes were divided into nine classes according to their diameter ( µ ) which are given in the table below:

<table>
<thead>
<tr>
<th>Oocyte size classes</th>
<th>Division on oculometer</th>
<th>Oocyte diameter range</th>
<th>Stages of the oocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0 - 4</td>
<td>0 - 46.4</td>
<td>Oogonia</td>
</tr>
<tr>
<td>II</td>
<td>4 - 8</td>
<td>46.4-92.8</td>
<td>Oocytes</td>
</tr>
<tr>
<td>III</td>
<td>8 - 12</td>
<td>92.8-139.2</td>
<td>Previtellogenic</td>
</tr>
<tr>
<td>IV</td>
<td>12 - 16</td>
<td>139.2-185.6</td>
<td>Oocytes</td>
</tr>
<tr>
<td>V</td>
<td>16 - 20</td>
<td>185.6-232.0</td>
<td>Vitellogenic</td>
</tr>
<tr>
<td>VI</td>
<td>20 - 24</td>
<td>232.0-278.4</td>
<td>Oocytes</td>
</tr>
<tr>
<td>VII</td>
<td>24 - 28</td>
<td>278.4-324.8</td>
<td>Vitellogenic oocytes and spent oocytes.</td>
</tr>
<tr>
<td>VIII</td>
<td>28 - 32</td>
<td>324.8-371.2</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>32 - 34</td>
<td>371.2-394.2</td>
<td></td>
</tr>
</tbody>
</table>

(4) **Histochemistry**: For histochemical study, the gonads were fixed in different fixatives. For detection of carbohydrates, the testis and ovary were fixed in alcoholic
Bouin’s and Susa’s fixative. Carnoy’s fixative was used for lipids. The stains used for histochemical tests are tabulated in Table - 1 (Pearse, 1968).

(5) Environmental factors

The role of environmental factors in maturation of gonads was studied in M. kiihnensis. The breeding activity and natural photoperiod and temperature fluctuations in the environment were compared from May 1977 to April 1978.

The following experiment was performed in the laboratory. The male and female prawns were separated. The prawns ranging between 3.4-4.1 mm in length were selected for the experiment. Each group had ten females and ten males. They were exposed to the following schedules of photoperiod in the laboratory by providing artificial light by using 60 Watt bulb.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Groups</th>
<th>Photoperiod</th>
<th>Natural photoperiod (11:13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td></td>
<td>6 : 18</td>
</tr>
<tr>
<td>3</td>
<td>B</td>
<td></td>
<td>9 : 15</td>
</tr>
<tr>
<td>4</td>
<td>C</td>
<td></td>
<td>12 : 12</td>
</tr>
<tr>
<td>5</td>
<td>D</td>
<td></td>
<td>18 : 6</td>
</tr>
<tr>
<td>6</td>
<td>E</td>
<td></td>
<td>24 : 0</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td></td>
<td>0 : 24</td>
</tr>
<tr>
<td>No.</td>
<td>Tests</td>
<td>Reference</td>
<td>To demonstrate</td>
</tr>
<tr>
<td>-----</td>
<td>--------------------------------------------</td>
<td>-----------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td></td>
<td>For Proteins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1)</td>
<td>Mercuric Bromophenol Blue (after Bonhag)</td>
<td>Pearse, 1968</td>
<td>General protein</td>
</tr>
<tr>
<td>(2)</td>
<td>The Millon Reaction (Bensley and Gersh modification)</td>
<td>Pearse, 1968</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>(3)</td>
<td>Millon Reaction (Baker modification)</td>
<td>Pearse, 1968</td>
<td>Tyrosine</td>
</tr>
<tr>
<td>(4)</td>
<td>Nihydrin-schiff method</td>
<td>Yasuma and Itchikawa, 1953</td>
<td>Protein bind amino groups</td>
</tr>
<tr>
<td>(5)</td>
<td>Ferric ferricyanide method for - SH group</td>
<td>Chevrement and Frederic, 1943</td>
<td>Sulphydryl groups</td>
</tr>
<tr>
<td>(6)</td>
<td>Aldehyde fuchsin</td>
<td>Ewey, 1962</td>
<td>Cystine, Cysteine</td>
</tr>
<tr>
<td></td>
<td>For carbohydrates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(7)</td>
<td>Best's Carmine</td>
<td>Best, 1906</td>
<td>Glycogen</td>
</tr>
<tr>
<td>(8)</td>
<td>The performic Acid schiff method</td>
<td>Pearse, 1968</td>
<td>Keratin (amount of -SS groups)</td>
</tr>
</tbody>
</table>

Contd...on....2
<table>
<thead>
<tr>
<th>No.</th>
<th>Tests</th>
<th>Reference</th>
<th>To demonstrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>The periodic Acid-schiff technique (PAS)</td>
<td>After Hotchkiss 1948</td>
<td>glycogen, 1-2 glycol groups, Hexose containing mucosubstance</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>For lipids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Sudan black B (after McManus, 1946)</td>
<td>Pearse, 1968</td>
<td>Lipid or lipoproteins</td>
</tr>
</tbody>
</table>
Here 'L' refers to hours of light and 'D' refers to hours of darkness in 24 hours of a day. Along with these groups a group of twenty prawns (ten females + ten males) was subjected to natural photoperiod (11:13). The experiment was performed from January to March 1978.

Two groups of twenty prawns each (ten females + ten males) were kept at low temperature (18°C) and high temperature (30°C) regimes. The experiments were conducted simultaneously in the laboratory for forty five days. On the 45th day all prawns were sacrificed to calculate gonad index. The gonad index was calculated by using formula given previously (Giese, 1959). For histological study the ovary and testis were fixed in Bouin's fluid. They were dehydrated, paraffin embedded and sectioned at 8-10 μ. The sections were stained with Harri's Haematoxylin eosin method. The measurements of the diameters of oocytes and testicular acini were made.

The results obtained were statistically analysed with students 't' test, wherever required. The results are summarized in Tables 2-5 and Figures 1-11.
RESULTS

(1) Breeding behaviour

From Text fig. 1 it is clear that the maximum berried females were present in February 1978 (42.24%). The collection data showed that there were two peaks in breeding cycle of prawn, *M. kistnensis*. The major peak was from January to April 1978 and minor peak was in September - October 1977.

(2) Carapace length and gonad index

The maximum size attained by female prawn was in April 1978. The carapace length value for April 1978 was 21.67 mm. The range for carapace length for female was 9.67 mm to 21.67 mm (Text fig. 2).

The male prawns measured 19 mm, a highest carapace length, in January 1978 and April 1978. The minimum carapace length was in May 1977, 10.33 mm (Text-Fig.3).

The gonad index for female prawns ranged between 0.71 to 4.21. The gonad indices for major peak were 3.61, 4.21, 3.73, and 2.40. For minor peak values were 1.15 and 1.31 (Text-fig.4).
Text Fig. 1: Percentage of prawns *Macrobrachium kistnensis* with eggs, for the period May 1977 - April 1978.
Text-fig. 1.

Breeding season

Percentage of prawns with eggs

1977

1978

M O N T H S

Percentage of prawns with eggs
Text - Fig. 2: Carapace length increase in female prawn, *Macrobrachium kistnensis*, for the period May 1977 - April 1978.
Text-Fig. 3: Carapace length increase in male prawn, *Macrobrachium kiihnensis*, for the period May 1977 - April 1978.
Text - Fig. 4: Gonad index of female prawn, *Macrobrachium kistnensis*, for the period May 1977 - April 1978.
In male prawns the gonad index range was 0.29 to 2.07. The gonad indices for January, February, March and April 1978, which was major peak, were 2.07, 1.34, 1.21 and 0.95 (Text-fig.5). In September and October 1977 the gonad indices were 0.85 and 0.93.

(3) Histology

3.1 Oogenesis: In early stages the ovary has two kinds of cells; the oogonia and follicle cells (Plate I, fig. 1). These cells are small with large nuclei and measure in between 12 to 46.4 μ. The oogonia increase in number by cell division to become oocytes which measure 46-93 μ. This growth continues till the end of the oogenesis and hardly perceptible. This process is known as premeiotic phenomena and at the end of this process the nucleus swells into the germinal vesicle. Some of the oocytes grow rapidly by absorbing the nutrients. These grown oocytes lie at the periphery and develop in the center. The size range for oocytes is between 96 and 140 μ. (Plate I, fig.2). The follicle cells in this stage increase in number by repeated cell divisions and arrange round the oocyte. Upto this stage the period of oocyte growth is termed as generative phase, because yolk
Text-Fig. 5: Gonad index of male prawn, *Macrobrachium kistnensis*, for the period May 1977 - April 1978.
Breeding season

Text-fig. 5

GONAD INDEX

M O N T H S 1977

J A S O N D A N 1978

2 0 2
Oogenesis stages of prawn, *Macrobrachium kiihnensis*.

**Fig. 1:** Transverse section of the immature ovary showing oogonia and follicle cells (x150).

Harri's Haematoxylin cosin stain.

OG = Oogonia,
FC = Follicle cells.

**Fig. 2:** Transverse section of the maturing ovary showing oocytes of previtellinoc stage (x 400)

PO = Primary oocyte
N = Nucleus
C = Cytoplasm.

**Fig. 3:** Transverse section of the mature ovary with mature oocytes showing yolk globules (x 150).

MO = Mature oocyte
N = Nucleus
YG = Yolk globules.

**Fig. 4:** Transverse section of the ovary showing degenerating ova with vacuoles and collapsed nucleus
(x 150).

DO = Degenerating ova
N = Nucleus
V = Vacuole
is hardly formed during this phase. Therefore, this phase may be called the period of previtellogenesis.

Finally oocyte enters into vegetative phase. Here growth of oocytes rises abruptly in short time and oocyte grows to its final size due to accumulation of deutoplasmic material or yolk in the ooplasm (Plate I, Fig.3). Here the vitellogenic oocytes measure 140-290 μ. This phase of rapid growth corresponds to the period of vitellogenesis. After completion of yolk deposition the mature oocyte is termed as ovum.

These ova are ovulated and oviposited in the brood pouch of the female. But sometimes due to unfavourable conditions the oocytes are reabsorbed. The degenerating ova (290-390 μ) are with foamy vacuoles in the ooplasm. These vacuoles increase in number and size and form a branch-like large vacuole and nucleus collapses (Plate I, Fig.4).

Thus the oogenesis is summarized like, oocoonia→primary→oocytes (previtellogenic period)→mature oocytes→(vitellogenic period)→ova→ovulation→oviposition or oosorption.
3.2 Spermatogenesis

The testis consists of a number of seminiferous tubules or testicular acini. The germinal ridge which is situated at one corner of acini proliferates the spermatogonia. The spermatogonia are round structures and the outermost layer is of primary spermatogonia and inner one is of secondary spermatogonia (Plate II, Fig. 1).

The secondary spermatogonia after mitotic divisions convert into the spermatocytes (Plate II, Fig. 2). The spermatocytes then form the spermatids or immature spermatozoa (Plate II, Fig. 3). The mature spermatozoa are flagellate structures with typical cup-shaped nuclei and a spine; each measures 3-5 μ (Plate III, Fig. 4). The spermatophore formation takes place in the vas deferens. The degenerating testis showed the primary, secondary spermatogonia and spermatocytes (Plate III, Fig. 2). They were completely degenerated in the next step (Plate III, Fig. 3). The spermatogenesis stages are primary spermatogonia → secondary spermatogonia → spermatocytes → spermatids → spermatozoa.
PLATE II

Spermatogenesis stages in the prawn, *Macrobrachium kistnensis*.

Fig. 1: Transverse section of immature testis with primary and secondary spermatogonia. (x400)

GR - Germinal ridge
PS - Primary spermatogonia
SS - Secondary spermatogonia

Fig. 2: Transverse section of testis showing spermatocytes. (x 400)

SC - Spermatocytes

Fig. 3: Transverse section of testis showing spermatids or immature spermatozoa (x400).

ST - Spermatids
PLATE III

Spermatogenesis stages in the prawn, *Macrobrachium kiiatensis*.

**Fig. 1** Transverse section of testis with spermatozoa (x 400).

SZ - Spermatozoa.

**Fig. 2** Transverse section of degenerating testis, showing degenerated primary, secondary spermatogonia and spermatocytes (x 400).

**Fig. 3** Transverse section of testis with completely degenerated acini (x 400).
3.3 Oocyte size classes and percent frequency distribution

The percent frequency of oocyte size classes and percentage of oocytes and ova which were present in the ovary, for each month, May 1977 to April 1978, are plotted in Text Fig. 6.

From monthly histological observations it is clear that the maximum frequency of oocytes class VII was 29.77 ± 1.93 and of size class IV was 27.83 ± 3.26 in the month of February 1978. This month comes under the major peak of reproductive cycle. In October 1977, the minor peak month, the percentage frequency of oocytes of size class IV was 28.57 ± 2.6 and the percentage of ova i.e. size class VII was 7.19 ± 1.05. It is also clear from observations that the oocytes of all classes were present in the ovary. In September and October 1977 as well as from January to April the outermost few oocytes in the follicles were under osorption stages.

3.4 Histological observations of the testis (Table-2)

The testis showed cyclic changes with the reproductive cycle. The number of testicular acini was higher (39.2 ± 5.04) in February 1978. In March and April 1978 few acini
Text - Fig. 6: Frequency histogram of mean diameter of oocytes-ova, in the ovary of *Macrobrachium kistnensis* for the period May 1977 - April 1978.
<table>
<thead>
<tr>
<th>Month and Year</th>
<th>Total number of acini</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1977</strong></td>
<td></td>
</tr>
<tr>
<td>May</td>
<td>-</td>
</tr>
<tr>
<td>June</td>
<td>-</td>
</tr>
<tr>
<td>July</td>
<td>3.2 ± 1.16</td>
</tr>
<tr>
<td>August</td>
<td>5.0 ± 1.41</td>
</tr>
<tr>
<td>September</td>
<td>14.2 ± 3.06</td>
</tr>
<tr>
<td>October</td>
<td>16.4 ± 1.02</td>
</tr>
<tr>
<td>November</td>
<td>*3.4 ± 1.04</td>
</tr>
<tr>
<td>December</td>
<td>7.6 ± 2.06</td>
</tr>
<tr>
<td><strong>1978</strong></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>19.8 ± 1.72</td>
</tr>
<tr>
<td>February</td>
<td>39.2 ± 5.04</td>
</tr>
<tr>
<td>March</td>
<td>21.2 ± 2.48</td>
</tr>
<tr>
<td>April</td>
<td>*12.8 ± 1.72</td>
</tr>
</tbody>
</table>

* Indicate the presence of few degenerating acini.
were in degenerating stages and the vas deferens was full of spermatozoa. In December 1977 and January 1978 the acini were only with primary and secondary spermatagonia and spermatocytes. In February 1978 the acini were with numerous spermatozoa.

In October 1977 the acini number was 16.40 ± 1.04. They were in ripe stages i.e. with spermatozoa. In November 1977 the degenerating acini were found in the testis.

(4) Histochemistry

The results of the histochemical tests for proteins, carbohydrates and lipids are summarized in Table 3 and 4.

The ovary showed positive tinctorial affinity with Bromophenol blue (Plate IV, Fig. 1). The ooplasm was uniformly blue in colour; but in some sections a dark ring was found around the nucleus. With Aldehyde fuchsin also a dark blue ring around nucleus was noticed (Plate IV, Fig.2). The Millon's reactions gave magenta colour to the peripheral oocytes only (Plate IV, Fig.3). Ninhydrin-schiff stained the protein bind amino groups as dot-like spherules.
### Table 3

Results of histochemical reactions of the ovary to different histochemical tests in *M. kistnensis*

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tests</th>
<th>Ovary</th>
<th>Ova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oocytes</td>
<td>Ova</td>
</tr>
</tbody>
</table>

#### For proteins

1. **Mercuric Bromophenol blue**
   - ++
   - +++
2. **The millon reaction (Bensley and Gersh modification)**
   - ±
   - ++
3. **Millon reaction (Baker modification)**
   - ±
   - ++
4. **Ninhydrin-Schiff method**
   - +
   - +++
5. **Ferric ferricyanide method**
   - +
6. **Aldehyde fuchsine (AF)**
   - ++
   - ++

#### For carbohydrates

7. **Best’s carmine**
   - +
   - +
8. **The performic acid-schiff method (PFAS)**
   - ±
   - ++
9. **The periodic acid-schiff method (PAS)**
   - ±
   - +++

Contd... on...2
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Tests</th>
<th>Ovary</th>
<th>Ova</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oocytes</td>
<td>Ova</td>
</tr>
<tr>
<td>For lipids</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(10)</td>
<td>Sudan black B</td>
<td>++</td>
<td>+++</td>
</tr>
</tbody>
</table>

- = negative reaction
+ = doubtful reaction
+ = positive reaction
++ = moderate reaction
+++ = strong reaction
Table 4
Results of histochemical reactions of the testis to different histochemical tests in *H. kistnensis*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Tests</th>
<th>Testis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Pri. and Secondary spermatogonia tozoa</td>
</tr>
</tbody>
</table>

For proteins

1. Mercuric Bromophenol blue + +
2. The Millon reaction (Bensley and Gersh modification) + ++
3. Millon reaction (Baker modification) + ++
4. Ninhydrin-schiff method + +
5. Ferric ferricyanide method for-SH group - -
6. Aldehyde fuchsia (AF) ++ +

For carbohydrates

7. Best's carmine + +
8. The performic acid schiff method (PFAS) - +
9. The periodic acid–schiff method (PAS) - +

For lipids

10. Sudan black B ++ +

- = absence,
± = doubtful,
+ = presence
++ = intensity moderate
PLATE IV

Fig. 1 Transverse section of ovary showing presence of proteins. Bromophenol blue (x 60)

OV - Ova
OC - Oocytes

Fig. 2 Transverse section of ovary showing perinuclear ring in maturing oocytes. Aldehyde fuchsin (x 150)

PN - Perinuclear ring

Fig. 3 Transverse section of ovary showing the peripheral oocytes stained with Millon's reaction and immature oocytes showing absence of Tyrosine Millon's reaction (x 60)

OV - Ova
Best's carmine determined the presence of glycogen in the oocytes. The performic acid schiff (PFAS) and periodic acid schiff (PAS) showed positive reaction with matured peripheral oocytes which stained deep magenta in colour (Plate V, Fig. 1,2).

Sudan black B detected high lipid content (++++) in mature oocytes, and comparatively less lipid (+) in immature ones. From these histochemical tests it is confirmed that the ooplasm is glyco-lipo-protein. (Plate V, Fig.3).

The testis also showed positive reaction to bromophenol blue. With Ninhydrin-Schiff the testis was uniformly stained pink in colour. Whereas, in Millon's reactions only the outer acini of the testis showed affinity. The Aldehyde fuchsin gave positive reaction to the primary and secondary spermatogonia.

The Best's carmine stained the primary and secondary spermatogonia and PFAS, PAS stained the spermatozoa. With Sudan black B the primary and secondary spermatogonia showed '+' affinity and spermatocytes '0 to +' affinity (Plate VI, Fig. 1,2).
PLATE V

Fig. 1  Transverse section of ovary showing amount of ss groups in the vitellogenic mature oocytes and its absence in immature oocytes.

Performic acid schiff method
(PFAS)  (x60)

OV  -  Ova
OC  -  Oocytes

Fig. 2  Transverse section of ovary showing substances in the mature oocytes and immature without it.
Periodic acid schiff method (PAS) (x 60)

Fig. 3  Transverse section of ovary showing the lipid globules in the yolk of mature oocytes.
Sudan black B (x 150)

LG  -  Lipid Globules
PLATE VI

**Fig. 1** Transverse section of testis showing primary and secondary spermatogonia stained with carmine (x 400)

- **PS** - Primary spermatogonia
- **SS** - Secondary spermatogonia

**Fig. 2** Transverse section of testis showing the black stained primary and secondary spermatogonia.

Sudan black B (x 400)

- **PS** - Primary spermatogonia
- **SS** - Secondary spermatogonia
(5) **Environmental factors**

5.1: **Annual reproductive cycle and environmental factors**

( Table - 5, Text fig. 11).

The temperature and day length fluctuations during the period May 1977 - April 1978 are given in the Fig. 11 and Table-5. The temperature during major peak period ranged from 23°C to 30°C, (mean values). The photoperiod for January 1978 to April 1978 was 10.58 (hours of light in 24 hours) to 12.38. During major peak period, the day length was 11.24 - 12.22 and temperature was less fluctuated 24°C - 24.5°C. (Data obtained from Government day length charts).

5.2: **Artificial photoperiod changes**

5.2.1: **Effect of artificial photoperiod schedules on gonad index**

During photoperiodic schedules 6:18 (group A) and 9:15 (group B), the gonad index of testis and ovary was increased, 1.57, 1.62 and 1.64, 2.95 respectively. The control ovarian and testis indices were 1.37 and 0.73. Here the animals were facing a natural photoperiod (11:13). For 12:12 (group C) photoperiod, the ovary index was decreased almost near to the control value. However, in the testis there was no such decrease in gonad index (Text Fig. 7).
Table 5

Range of the day light hours in a month for the period, May 1977 - April 1978.

<table>
<thead>
<tr>
<th>Month and year</th>
<th>Range of day light hours</th>
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<tr>
<td><strong>1977</strong></td>
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<tr>
<td>May</td>
<td>12.40</td>
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<td>June</td>
<td>12.59</td>
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<tr>
<td>July</td>
<td>13.03</td>
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<tr>
<td>August</td>
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<td>12.22</td>
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<td>December</td>
<td>11.02</td>
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<td><strong>1978</strong></td>
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<td>January</td>
<td>10.58</td>
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<td>February</td>
<td>11.15</td>
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<td>11.40</td>
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<tr>
<td>April</td>
<td>12.11</td>
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</table>
Text-Fig. 11: Monthly variations in the minimum and maximum (average values) temperature at the habitat of prawn, *Macrobrachium kistnensis* for the period between May 1977 to April 1978.
Text-Fig. 7: *Macrobrachium kijtnensis*: Gonad index for groups, control and A to F exposed to various photoperiod schedules.
Text-fig. 7

Gonad Index

- Female (ovary)
- Male (testis)
- L:D

Open area — hours of light
Lined area — hours of darkness

Photoperiod Schedules

- Control
  - 11L:13D
- A
  - 6L:18D
- B
  - 9L:15D
- C
  - 12L:12D
- D
  - 18L:6D
- E
  - 24L:0D
- F
  - 0L:24D
In group D (18:6) the ovarian index was tremendously increased (7.69) and testis index also showed greater value (2.41). Gonad indices for group E (24:0) in both sexes decreased suddenly whereas group F(0:24) showed high gonad indices for both sexes (Text - Fig. 7).

5.2.2 : **Effect of artificial photoperiod schedules on spermatogenesis**

The spermatogenesis was enhanced during short day photoperiod 6 L : 18 D and 9 L : 15 D. The primary and secondary spermatogonia were proliferated and the spermatocytes were formed. The acini were increased in number (30 ± 5.25, 29 ± 4.26) than that of control (15 ± 6.97) respectively. The diameters of the acini was increased (Plate, VII, Fig.2,3 and Text Fig.8). In 12 L: 12 D photoperiod group most of the acini were full of spermatozoa and the outer acini were disappeared and only few inner acini with less diameter were observed (Text Fig.8, Plate VII Fig.4). The increased photoperiod (18 : 6 D and 24 L: 0 D) accelerated the spermatogenesis, which was revealed by increase in diameter of acini to that of control and more production of spermatozoa.
The effect of artificial photoperiodic schedules on the spermatogenesis of male prawn, *Macrobrachium kistnensis*.

**Fig. 1** T.S. of control testis (11 L: 13 D) Harri's Haematoxylin eosin (x 400)

**Fig. 2** T.S. of the testis group 'A' (6 L : 18 D) x 150.

**Fig. 3** T.S. of the testis group 'B' (9 L : 15 D) x 400

**Fig. 4** T.S. of the testis group 'C' (12 L : 12 D) x 400
Text-Fig. 8: *Macrobachium kiftnensis*: Histological data of gonads, for groups control and A to F, exposed to various photoperiod schedules.
Text-fig. 8

- Female (ovary)
- Male (testis)
- L:D

Open area hours of light
Lined area hours of darkness

Diameter of Oocytes and seminiferous tubules

<table>
<thead>
<tr>
<th>Photoperiod Schedules</th>
<th>Control</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
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<td></td>
<td>11 L:13 D</td>
<td>6 L:18 D</td>
<td>9 L:15 D</td>
<td>12 L:12 D</td>
<td>18 L:6 D</td>
<td>24 L:00</td>
<td>0 L:24 D</td>
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</table>
(Text Fig. 8, Plate VIII and Fig. 1-2). The 0 L : 24 D i.e. total darkness group showed an increase in the acini diameter as well as its number (25 ± 3.98) (Text Fig. 8, Plate, VIII, Fig. 3).

5.2.3: Effect of artificial photoperiod schedules on oogenesis

The control ovary (11 L : 13 D) was with primary oogonia and primary oocytes (Plate IX, Fig. 1). The short day photoperiod caused ovary to remain in preliminary stages of maturation. Though the oocyte diameter was increased the active vitellogenesis was not observed (Text Fig. 8, Plate IX, Fig. 2, 3). The oocyte number for 6 L : 18 D and 9 L : 15 D photoperiodic schedules was 20 ± 6.29 and 30 ± 7.23 respectively. During 12 L : 12 D photoperiod schedule the ovarian growth was increased in all respects and the ova were oviposited (Text Fig. 8, Plate, IX, Fig. 4).

The long day photoperiod (18 L : 6 D and 24 L : 0 D) group ovaries reached maturation earlier and ovulation of mature oocytes was observed (Plate, X, Fig. 1, 2) and 24 hrs. of darkness showed an overall increase in ovary weight but vitellogenesis did not take place (Text Fig 8, Plate X Fig 3).
PLATE VIII

The effect of artificial photoperiodic schedules on the spermatogenesis of male prawn, *Macrobrachium kastnensis*.

**Fig. 1**  T.S. of the testis, group 'D'
(18 L : 6 D) x 400

**Fig. 2**  T.S. of the testis, group 'E'
(24 L : 0 D) x 400.

**Fig. 3**  T.S. of the testis group 'F'
(0 L : 24 D), x 400.
PLATE IX

The effect of artificial photoperiod schedules on the oogenesis of female prawn, *Macrobrachium kistnensis*.

**Fig. 1**
T.S. of the ovary, group control (11 L : 13 D) x 400

**Fig. 2**
T.S. of the ovary, group A (6 L : 18 D), x 150.

**Fig. 3**
T.S. of the ovary, group B (9 L : 15 D), x 150.

**Fig. 4**
T.S. of the ovary, group C (12 L : 17 D), x 150.
The effect of artificial photoperiod schedules on the oogenesis of prawn, *Macrobrachium kistnensis*.

**Fig. 1**  
T.S. of ovary, group 'D'  
(18 L : 6 D)  
$\times 150$

**Fig. 2**  
T.S. of ovary group 'E'  
(24 L : 0 D)  
$\times 80$

**Fig. 3**  
T.S. of ovary group 'F'  
(0 L : 24 D)  
$\times 150$. 
5.3: Temperature

It was found that the gonad indices for ovary and testis at 18°C temperature were 0.96 and 0.52 respectively (Text Fig. 9). These values were less than the control values.

At 30°C temperature the gonad index for ovary was 1.88 and for testis was 0.79 (Text - Fig. 9).

The oocytes and testicular acini number was increased with increase in temperature. The oocyte diameter was 153.7 μ at 30°C, which was a high value, whereas, the maximum diameter of acini was 155.15 at 18°C. All these results are shown in Text - Fig. 10 and Plate XI, Figs. 1 - 4.

DISCUSSION

The marine crustaceans show wide variety in their breeding periods. Menon (1952) showed that Neptunus sanquino lentus, from the west coast of India, was an annual breeder, whereas Portunus pelagicus (Rahaman, 1967) and Charybdis variegata (Chandran, 1968) were continuous and biannual breeders. Like marine invertebrates the freshwater invertebrates also show variations in their breeding cycle inspite of their similarity in geographical distribution (Rajyalakshmi, 1961 and Mallikarjuna Rao, 1966).
Text-Fig. 9: *Macrobrachium kisstnensis*. Gonad index of prawns subjected to low (18°C) and high (30°C) temperature regimes.

Text-Fig. 10: *Macrobrachium kisstnensis*. Histological data of the gonads of prawns subjected to low (18°C) and high (30°C) temperature regimes.
The effect high (30°C) and low (18°C) temperature on the gonadogenesis of prawn, *Macrobrachium kistnensis*.

**Fig. 1**  T.S. of ovary (30°C), x 50

**Fig. 2**  T.S. of ovary (18°C), x 150

**Fig. 3**  T.S. of testis (30°C), x 150

**Fig. 4**  T.S. of testis (18°C), x 150
The crustaceans of Marathwada region showed variations in their breeding cycles. The prawn, *Caridina weberi* had June – October as major peak and January as minor peak for breeding (Chinnayya, 1968; Jyoti, 1974). Diwan (1971) reported that the crab, *Barytelphusa cunicularia*, breed during the months of June – September, which was dependent on the rainfall. *Macrobrachium kistnensis* showed major peak from January to April and minor peak in September – October. The water flow in these months was moderate. June to August 1977 was the monsoon season for Marathwada region. It can be said that in the case of *Macrobrachium kistnensis* it is the temperature and photoperiod (discussed in the latter part of this chapter) which regulated the reproductive cycle.

Chacko and Thyagarajan (1952) observed that *Paratelphusa jacquemontii*, generally breeds from February to April when water level is low and calm. Ali (1955) mentioned that in *Paratelphusa querini*, the rainfall determines the breeding activity. Pillai and Nair (1971) found that rainy season reduces the breeding activity in marine and shore crabs. Subramoniam (1979) in mole crab, *Emerita asiatica* reported a steady increase in the breeding intensity in the post -
monsoon months from January to March, on the east coast.

Knudsen (1960) in Xanthidae of California and Pillai (1960) in Caridina laevis studied the reproductive cycle by recording the ovigerous females in the field. Along with collection data, the gonad indexing was precise method adopted by many workers in assessing the reproductive cycle (Jegla, 1966; Rahaman, 1967; Chandran, 1968; Haley, 1972; Thomas, 1974).

In the present study for the determination of reproductive cycle, various methods such as percentage of berried females in monthly collections, gonad indexing, relation of carapace length with maturity of gonads, histological observations of testis and ovary with frequency of size classes of oocytes were used.

The percentage of egg bearing females for the duration of one year clearly indicated that there are two breeding peaks in the annual reproductive cycle of M. kistnensis.

The major peak included the months of January, February, March and April, and minor peak in September-October. The maximum reproductive activity in these months is again confirmed by gonad indices of these months. Subramanyan (1963)
in *Penaeus indicus*, observed two peaks in breeding activity. The major peaks were in August-September and May-July and minor peak in March. He also found that the gonad index for males was always less than that of females. In *M. kistnensis* the male gonad index was less (2.07) than the female gonad index (4.21).

The carapace length is another parameter to ascertain the reproductive maturity. For *M. kistnensis* the mature female carapace length range was 18-21 mm. and for males it was 15-19 mm. The prawns ranging between these values were maximum in the breeding peaks. Butler (1960) found that male *Cancer magister* with carapace width of 100 mm and female with 116 mm were sexually mature. Ryan (1965) recorded 42-56 mm carapace length range for sexually mature instars of crab, *Portunus sanguinolentus*. Diwan (1971) in *Barytelphusa cunicularis* observed that it entered into puberty after attaining the carapace length of 40 mm for male and 44 mm for female crabs. Hakey (1972) reported that at 25-34.9 mm carapace length *Ocyopoda quadrata* obtained sexual maturity. Subramoniam (1977) gave sexual maturity range for male and female *Emerita asiatica*, from 3.75 to 11.0 mm and 20 mm respectively.
A good indicator of an annual reproductive cycle is the oocyte formation and percentage of ova in the ovary. From the results obtained in the present investigation on *M. kistnensis*, it is clear that the total number of oocytes and percentage of ova were maximum in the month of February 1978. This coincided with the high gonad index in that month. In October 1977, which comes under minor reproductive peak, a similar relationship was observed.

Jagla (1966) did similar studies in the crayfish, *Orconectes pellucidus inermis*. He observed that the frequency of mature oocytes was correlated with the frequency of ovigerous females in the field. Jagla found February - March as the peak for reproductive cycle. Barnes and Stone (1973) in *Verruca stroemia* found that majority of ova in February were related to maximum number of animals with egg masses in habitat.

The testicular acini number was highest (39.2) and the spermatozoa were numerous in February 1978. In *M. kistnensis* oviposition occurs after mating of the prawns. Thus, presence of highest number of testicular acini (with spermatozoa) and
berried females in February 1978 suggest that mating perhaps takes place during this month. For minor peak same relationship was observed. In March and April 1978 as well as November 1978 the degenerating acini were present in testis. Similar results were obtained by Wood and Hobbs (1958) in *Cambarus montanus acuminatus* and by Ewa (1976) in *Orconectes limosus*.

The histochemical observations in *M. kiestnensis* revealed that the lipids, carbohydrates and proteins are rich in ooplasm. It was found that as the oocyte was reaching to the advanced stages of maturation the cytochemical nature of the oocyte also changed. The primary oocytes were positive to proteins. The presence of glycogen in ooplasm was confirmed by Best's carmine. But Sudan black B stained ooplasm weakly. These histochemical reactions of the developing oocytes are mostly in agreement with description given for other crustaceans, where, immature oocytes were with carbohydrates and proteins in moderate amounts, and RNA was in large amount and Sudan black B stained the oocytes weakly indicating the less quantity of lipid in the ooplasm.

A perinuclear ring stained with Bromophenol blue and aldehyde fuchsins in maturing oocytes was found. Though the protein and carbohydrate tests revealed the presence of yolk globules around the nucleus, staining with Sudan black B showed the bound lipids in these globules. This perinuclear ring disappeared with the advancement of vitellogenesis. Such a perinuclear ring was also observed in cirriped, Trypetes (Turquier, 1972), barnacles, Balanus amphitrite and B. eburneus (Fyhn and Costlow, 1977) crab, Xantho bidentatus (Erribabu et al. 1978) and in anomuran crab, Clibanarius clibanarius (Varadarajan and Subramoniam, 1980).

The mature oocytes or vitellogenic oocytes were completely filled with yolk globules and granules. The PFAS and PAS reactions stained both types of granules. These tests confirmed that in ova the-S5 groups and acid mucosubstances were in high quantities. The Millon's reactions detected the presence of tyrosine in the deutooplasm of the ova. Ninhydrin-Schiff positive reaction (Protein bind amino groups) was given by yolk. The Sudan black B obtained maximum intensity for
vitellogenic oocytes, inferring the high amounts of lipid in them.

In *Xantho bidentatus*, oocytes showed carbohydrate-protein complex bodies and lipid bodies particularly in the third and fourth developing stages (Erribabu et al., 1978). Varadarajan and Subramoniam (1980) in crab, *Clibanarius clibanarius*, described augmentation of protein and carbohydrate, yolk globules along with phospholipids and neutral lipids in mature stages (3).

The formation of yolk in the oocytes of *M. kistnensis* may be autosynthetic, heterosynthetic or autoheterosynthetic process. But the biochemical studies performed in the same prawn (see chapter four page 149) showed that during ovary maturation there was a drift of organic materials from other tissues, mostly from hepatopancreas. So, the autoheterosynthesis may be proposed for *M. kistnensis* but for confirming this conclusion further studies are required.

The *Bromophenol and ninhydrin schiff tests gave elementary affinity with the testis. Millon’s reactions detected tyrosine in the outer acini and aldehyde fuschin detected cysteine or cystine in the primary and secondary spermatogonia.
Best's Carmine gave positive results for the presence of glycogen in testis. Sudan black B showed less affinity with spermatogonia whereas spermatozoa gave feasible affinity.

There are no reports on the cytochemistry of spermatogenesis. Vishwanath (1965) described cytochemical nature of the spermatozoa of scorpions, *Palamnaeus bengalensis* and *P. fulvipes*. He detected the proteins, lipids, glycogen, -SH groups and nucleic acids. Recently Kandhaswami and Ramalingam (1980) gave a report on histochemistry of gametes of *Canesque tigrinum*. They described basic proteins, bound and free lipid and mucopolysaccharides in the ova and sperms.

Uma and Subramoniam (1979) reported the histochemical characterization of the spermatophore of *Scylla serrata*. Both outer and inner layers of the spermatophores are rich in mucopolysaccharides containing sulphated and carboxylic groups. The proteins of the two layers show tryptophan, but lack tyrosyl, sulphhydryl and disulphide groups. No phenols or phenol oxidases could be detected in both layer.
Menaker (1971) pointed that photoperiod (day length) is employed by animals as a seasonal cue to control the annual reproductive cycle. The adaptive advantage of this factor appears to be that it serves as a reliable predictor of seasonal changes. Thus, reproductive activity can be initiated by photoperiod at an appropriate time, so that the resulted offsprings can withstand the forthcoming harsh climatic conditions. Like photoperiod, the temperature is one of the environmental factors to which the organism must adjust, if it has to exist successfully in its habitat. The rhythmic activities of these two factors control the biological clock of an organism (Palmer, 1973).

In many crustaceans photoperiod plays a major role in timing moulting and ovarian development (Parris and Jenner, 1952; Stephens, 1952; Aiken 1969 a, b; Perryman, 1969; Armitage et al., 1973).

Stephens (1952) in Cambarus virilis showed that increased daylength accelerated the oogenesis and effected weight loss in the ovary. She further demonstrated that constant darkness interrupted the cyclic activity and trapped the oocyte
development of maturing stages, the animals did not show egg laying during dark adaptation.

Suko (1956, 1958) studied the development of winter eggs and found that darkness influenced the histological changes in the ovary of crayfish, Procambarus clarkii. He concluded that the darkness affected the oogenesis but it depended upon the reproductive stages of the animal when it was kept in the darkness.

Lowe (1961) in Cambarillus shufeldti, demonstrated that increased photoperiod induced a more rapid cycling of the maturation and resorption of oocytes, and decreased day length tends to stabilize the ovary in mature condition. The lowered temperature in C. shufeldti, tended to slow maturation of oocytes while elevated temperature cause a quick maturation followed by disintegration of the ovary.

In Orconectes virilis, Aiken (1969 b) assumed that when both temperature and photoperiod were in proper proportions the complete maturation occurred, particularly it was the temperature which triggered the egg laying.
The photoperiod and ambient temperature have got synchronous impacts on gonad maturation in *M. kistnensis*.

From January to April 1978 the photoperiod was continuously increased from 10.58 to 12.11. The Sudan rise (23°C to 30°C) in the temperature followed by increased photoperiod resulted in rapid growth of the gonads. This period (January to April 1978) was denoted as major peak in the reproductive cycle of prawn. In September - October 1977 the photoperiod was 11.24 - 12.22 and temperature was comparatively low (24.0°C - 24.5°C) than the previous month. Therefore, September - October 1977 was minor peak for annual reproductive cycle of *M. kistnensis*. From these observations it can be concluded that though photoperiod or day length initiated the rapid maturation of gonads, it is the temperature which governs this cycle. The artificial illumination and temperature controlled experiments conducted in the laboratory strengthened these conclusions. In *M. kistnensis*, increase in dark hours, caused rapid maturation of the ovary. This was confirmed by an increment in gonad indices of Groups A (6:18), B (9:15) and F (0:24) as compared to control groups (11:13). The oocytes diameter range for
these groups was 138.04 to 146.4 μ. Testis showed similar pattern of photoperiodic effect. The acini diameter ranged in between 150.8-238.96 μ.

For group C (12:12) the ovary and testis were under normal reproductive cycle. The increased photoperiod in group D stimulated the quick maturation and yolk deposition in the oocytes which resulted into an increment in gonad index of this group. In group F (24:0) the ovary was spent due to rapid cyclic changes in the oocytes and all the eggs were ovulated. The acini also showed rapid cycling in spermatogenesis.

The results obtained on other crustaceans such as *Daphnia schodleri* var. (Parker, 1966), *Balanus balanoides* (Barnes, 1963; Barnes and Barnes, 1967), *Ocypode quadrata* (Naley, 1972) and *Orconectes nais* (Rice and Armitage, 1974) agree with the present findings.

In amphipod, *Hyalidea asteca*, the photoperiod determined whether reproduction was to be continued or discontinued but that temperature influenced the rate of all changes (De-March, 1976).
(4) 21.67 mm was maximum carapace length attained by the female prawns and 19 mm by the male prawns.

(5) The gonad index for female prawn was higher (4.21) than male (2.07).

(6) In February, which is a peak month for reproductive activity, the higher number of oocytes (29.27) and ova (27.83) were present in the ovary. In same month the testicular acini number was high (39.2).

(7) Histochemical changes during oogenesis and spermatogenesis were studied. The primary oocytes gave positive tests to proteins and glycogen. But Sudan black B detected feable lipid in the primary oocytes. The mature oocytes were with yolk globules. The various tests performed detected the presence of acid mucosubstances, lipid, protein, tyrosine and protein bind amino groups.

The Bromophenol blue and ninhydrin-schiff method detected less proteins in the testis. The primary and secondary spermatogonia showed positive affinity with carmine but less affinity with Sudan black B.
Along with annual reproductive cycle, the effect of environmental factors on the reproductive activity of the prawn was studied.

The photoperiod and temperature regulated the reproductive cycle of the prawn. It was found that long day photoperiod (18:6) induced rapid cycling of the ovary maturation and resorption of the oocytes and short day photoperiod (9:15) tended to stabilize the ovary in mature condition.

The low temperature (18°C) slowed maturation of the oocytes while elevated temperature (30°C) caused quick maturation and disintegration of the ovary. The male prawn showed similar results.
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<th>Author(s)</th>
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<td>Suko T.</td>
<td>1954</td>
<td>Studies on the development of the crayfish. II. The development of egg cell before fertilization.</td>
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