CHAPTER VI
6.1 INTRODUCTION

A thorough knowledge of the reproductive cycle of an animal is of vital importance in any biological investigation. The reproductive cycle of marine invertebrates may be continuous, annual, semi-annual or biennial (Giese and Pearse, 1974). In general, it is of continuous or extended type in most tropical marine invertebrates. Reproduction is a cyclic physiological process. The gametogenic cycle involves the initiation of gametogenesis, the growth of spermatocytes and oocytes including the process-eitelllogenesis in the female, the physiological "ripening" of the full-grown gametes, and their release from the adult or spawning (Bayne, 1975). Intimately linked with this cycle, the nutrients in the form of lipid, protein and glycogen are stored and subsequently utilized in the production of gametes when the metabolic demand is high (Gabbot, 1975; Bayne, 1976). The sequence of different stages of gametogenic cycle varies between species. This difference can be attributed to the variations in hydrographic conditions. Environmental differences produce different physiological responses in respect to timing of development and developmental pattern.

6.2 REVIEW

There is extensive literature on the breeding cycle of mytilid species from Indian waters as well as from other waters. Patil and Bal (1967) studied the seasonal gonadal changes in adult freshwater mussel, Parreysia favidens var. maroens based on the macroscopic and microscopic changes in the structure of the gonad. Wilson and Hodkin (1987) compared the reproductive cycles of five species of Western Australian marine mussels, namely Mytilus edulis,
Xenostrobus pulex, Septifer bilocularis, Brachidontes cf variabilis and Amygdalum glaberrimum. Reproductive cycle of the estuarine bivalve, Musculista arcuatula have been studied by George and Nair (1973). Kuriakose (1973) studied the reproductive cycle of Perna indica and Nagabhusanam and Mane (1975b) that of Mytilus viridis. Seed (1976) has reviewed the literature published on reproduction and settlement of different species of Mytilus in European waters and other parts of the world together with an account of the factors controlling the reproductive cycle. Ajithakumar (1984) has investigated the reproductive physiology of two Indian species of mussels, Perna indica and P. viridis. The ultrastructural alterations taking place within the developing oocytes in Mytilus edulis have been studied by Pipe (1987). Reproductive cycle of Brachidontes variabilis in Hong Kong had been elucidated by Morton (1988). King et al. (1989) have investigated the reproduction and settlement of Mytilus edulis in Galway Bay, west coast of Ireland. Jasim and Brand (1989) have made observations on the reproduction of Modiolus modiolus in Isle of Man waters. Barkati and Ahmed (1989-1990) have given an account of the reproductive cycle of the mussel, Mytilus edulis from Western Germany.

A great deal of work has been done on the breeding cycle of other bivalves like Katelysia opima (Mane, 1974a), Cardium edule and C. glaucum (Kingston, 1974), Mercenaria mercenaria (Keck et al., 1975), Meretrix casta (Harkantra, 1975), Donax cuneatus (Nagabhusanam and Talikhekar, 1977a), Paphia laterisulca (Nagabhusanam and Dhamne, 1977), Meretrix casta (Salih, 1977), Crassostrea madrasensis (Stephen, 1980a), Anadara senilis (Yankson, 1982), Argopecten irradians (Barber and Blake, 1983), Arctica islandica (Ropes et al., 1984), Crassostrea madrasensis (Joseph and Madhyastha, 1984), Meretrix

The reproduction of bivalves is influenced by several factors of the environment such as water temperature, salinity, availability of the food etc. Wilson and Hodkin (1967) investigated the role of temperature as the chief determining factor controlling the reproductive cycles of the marine mussels. Newell et al. (1982) observed differences in the gametogenic cycle of Mytilus edulis in response to altered availability of food in different environments. The relation of water temperature and food sources with the major difference in reproductive characteristics of Argopecten irradians (Barber and Blake, 1983) and Patinopecten caurinus (MacDonald and Bourne, 1987) were discussed. Borrero (1987) suggested that the level of occurrence in the intertidal zone, the length of submersion and potential feeding time influence the timing of reproductive cycle in Geukensia demissa. MacDonald and Thompson (1988) correlated latitudinal variation in growth and fecundity of Placopecten magellanicus with local environmental factors.

Nagabhushanam and Mane (1975b) and Harkantra (1975) correlated seasonal variation of reproductive cycle of Mytilus edulis and Meretrix casta respectively with changes in temperature and salinity of the habitat. Nagabhushanam and Talikhedkar (1977a) found that increase in salinity and temperature soon after the monsoon appears to promote gametogenesis and initiate
spawning in *Donax cuneatus*. Nagabhushanam and Dhamne (1977) observed similar effect in *Paphia laterisulca*. Stephen (1980a) opined that increase and decrease in salinity during different seasons synchronize the gametogenic pattern in *Crassostrea madrasensis*. Joseph and Madhyastha (1984) related the onset of gametogenesis in *C. madrasensis* with the rapid increase in salinity. Besides, they also reported the influence of some other factors like turbidity, temperature and pH variations. In *Donax cuneatus* Victor and Subramoniam (1988) observed an influence of low salinity and temperature on active gametogenesis, and high salinity and temperature on spawning. A contrary picture has been presented by Sukumar and Joseph (1988) for *Saccostrea cuneata* in which an increased salinity triggered maturation while low saline condition initiated spawning.

Although extensive studies on breeding biology have been made, only scattered information is available on the histochemical localisation of different components like protein, lipid and carbohydrate in gonad. Lubet (1959) has carried out histochemical studies in relation to reproduction of the mussel, *Mytilus edulis* and *M. californianus*. Some histochemical aspects of the male and female gonads of *Mytilus galloprovincialis* have been elucidated by Costanzo (1966). Bayne et al. (1982) studied ultrastructural details of glycogen utilization and gametogenesis in *Mytilus edulis*. Ajithakumar (1984) has investigated the changes in the localisation of proteins, lipids and carbohydrate in gonadal tissues of *Perna indica* and *P. viridis* during developmental cycle.

The main objective of the present investigation is to elucidate the gametogenic pattern of *Musculista senhousia* and its relation to changing salinity in Cochin backwaters. An attempt was also made to analyse the histochemical localization of the storage substances in the gonad.
6.3 MATERIALS AND METHODS

Sampling was conducted at fortnightly intervals for a period from February 1988 to February 1989. Specimens falling into different size groups were used for the study. The mature females could be distinguished macroscopically by its yellow colour of the mantle, while in mature males it is creamy white. The sex and approximate stage of gonad development were ascertained by examining fresh smears of gonad under microscope.

Histological preparations were used to assess the annual reproductive cycle. Approximately 20 individuals, arbitrarily selected with respect to age and visible stage of gonad development, were excised, fixed in Bouin's fixative and prepared for sectioning by dehydration in ethanol and embedding in paraffin wax of melting point 60-62°C (Humason, 1972). Sections were cut at 8 µ thickness and stained with Ehrlich's hematoxylin and counterstained with eosin, and were examined under microscope and classified in to different developmental stages. Examination of the sections at regular intervals (throughout the year when specimens were available) furnished detailed information on the reproductive cycle including the actual period of spawning in this locality. Mantle thickness and oocyte diameter were measured with an ocular micrometer.

Studies were conducted to follow the histochemical localisation of the organic components like glycogen, protein and lipid of the gonad during gametogenesis of Musculista senhousia. Main stages studied for this purpose were the developing, mature and spawning stages. The methods adopted for this study were Periodic Acid-Schiff technique for glycogen (Humason, 1972), Mercury Bromophenol Blue for protein (Humason, 1972) and Sudan
Black B for lipid (Pearse, 1968). Cryostat (5030 microtome) was also used for sectioning.

6.4 OBSERVATIONS

6.4.1 Histology

Description of gonad development

The reproductive system of *M. senhausia* consists of numerous ducts which ramify throughout most of the body. During active phase of reproduction the mantle is occupied by reproductive tissue of different developmental stages. From histological preparation of the gonad four main types of gonad stages were recognised: developing, ripe, spawning and spent.

Indifferent or Inactive Stage

Gonads are in a state of quiescence. No gametogenesis is discernible and the sex is indistinguishable. Most of the gonad consists of numerous follicles with interfollicular connective tissues like adipogranular tissue and vesicular connective tissue in between (Fig.27). The follicle is usually expanded and the basal membrane and follicle wall are dominant. During this non-reproductive phase the mantle is generally thin and translucent.

Male: Developing or Active Stage

Each gonadal cycle begins with the proliferation and differentiation of the small earliest cells from stem cells which are distributed around the follicular wall. The proliferation of follicles become more apparent and the testicular follicles can be easily distinguished in the mantle tissue. In early stages large number of spermatogonia are present in early stages near the periphery of the lumen which may be found attached to the follicular wall,
but are more often free. Cell division proceeds and give rise to spermatocytes which are free in the follicular lumen in concentric band centripetal to the spermatogonia (Fig.17). In more advanced stages, spermatocytes and spermatids are predominant, making it difficult to see follicular cells or spermatogonia. Interfollicular connective tissue disintegrates with the progress of spermato genesis.

Male: Ripe or Mature Stage

In the ripe or mature male gonad (Fig.19) the follicles are densely packed and contain mainly spermatozoa. Spermatozoa aggregate in bands projecting into the lumen with their basophilic heads directed towards the periphery and eosinophilic sperm tails directed away from the follicular wall towards the centre (Fig.21). But spermatogonia, spermatocytes and spermatids appear as lightly stained band around the periphery of the follicle. Because of the rapid increase of the germ cells in the follicle, the walls of the adjacent follicle are apposed to each other. In this stage the mantle is found to be flabby and creamy white in colour.

Male: Spawning Stage

Spawning (Fig.23) takes place from the centre of the follicles where the oldest cells could be seen. In partially spawned individuals gonads are characterised by the shrinkage of the gonadal follicles. In some follicles the lumen is often seen empty due to the discharge of sperms while in other follicles gametogenesis continues and the central part of the follicle is still filled with spermatozoa.
**Figure 17.** Section of developing male showing a large number of spermatogonia (SG).

**Figure 18.** Section of developing female with stalked oocytes.
Figure 19. Section of ripe male gonad showing follicles densely packed with spermatozoa.

Figure 20. Section of female gonad with densely packed ova.
**Figure 21.** Ripe follicles at higher magnification showing densely packed spermatozoa (SZ).

**Figure 22.** Female follicle at higher magnification showing the presence of oocytes (OC).
Figure 23. Section of partially spawned male gonad with moderate quantity of spermatozoa.

Figure 24. Partially spawned female gonad with free ripe oocytes.
Figure 25. Late spawning stage of male gonad showing the presence of spermatozoa and infiltration of connective tissue (CT).

Figure 26. Late spawning stage of female gonad showing regressing oocytes with connective tissue in between.
Male: Spent Stage

In the spent condition (Fig. 23) the lumen of the follicles contains residual spermatozoa which are probably cytolysed by phagocytes. Gonads of the spent phase are characterised by contracted follicles, residual spermatozoa in the connective tissue, and semi-ovarian and semi-testicular follicles.

Female: Development

This section of the female gonad is especially interesting because the initiation of the gametes involved is initiated by an increase in size of the germ cells proliferated from the large resting cells, the stem cells, found around the follicular wall. The primary oocytes developed by cell division are larger than the spermatocytes. As the development proceeds the oocytes increase in size, become elongated towards the centre of the lumen and retain an attachment with follicular wall by a slender stalk (Fig. 18) which has a measure of 14 μ. Nucleus of this oocyte migrates to the proximal end. Yolk materials get accumulated in the ooplasm and thus the stalked oocytes enter into a phase of vitellogenesis. Finally these cells detach from the follicular wall and lie free in the lumen. They become more regular in outline.
Male: Spent Stage

In the spent condition (Fig.25) the lumen of the follicles contains residual spermatozoa which are probably cytolyzed by phagocytes. Gonads of the spent phase are characterised by contracted follicles, residual spermatozoa in the process of being cytolyzed and extremely slight gametogenic activity. Connective tissue of the mantle appears to occupy the space between the follicles. The mantle of the spent individuals become soft, spongy, membranous and semi-translucent.

Female: Developing or Active Stage

This process in most respects is very similar to that of the male especially in early development. Sex differentiation starts with the differentiation of the germ cells in the connective tissue. With the formation of follicles the gametogenic process is initiated. The developing stage is characterised by an increase in the number and size of oocytes.

During early development, female follicles contain oogonia and primary oocytes which occur around follicle wall. Oogonia are the initial female germ cells proliferated from the large resting cells, the stem cells, found around the follicular wall. The primary oocytes developed by cell division are larger than the spermatocytes. As the development proceeds the oocytes increase in size, become elongated towards the centre of the lumen and retain an attachment with follicular wall by a slender stalk (Fig.18) which has a measure of 14 μ. Nucleus of this oocytes migrates to the proximal end. Yolk materials get accumulated in the ooplasm and thus the stalked oocytes enter into a phase of vitellogenesis. Finally these cells detach from the follicular wall and lie free in the lumen. They become more regular in outline.
The oocyte has an average diameter of 34.5 μ with a large nucleus occupying more than half the cell volume. As yolk accumulates the cytoplasmic volume increases so that the cytoplasm stains more deeply than the nucleus. The nucleolus differentiates into an amphinucleus and it is very prominent (Fig. 20).

**Female: Ripe or Mature Stage**

The mature gonad is characterised by the presence of large number of nearly round oocytes in the lumen of the follicles, but oogonia and oocytes are not infrequent (Fig. 22). The oocyte has an average diameter of 42 μ. Ooplasm is rich in yolk materials. Mantle of the mature female is thick, flabby and yellow in colour.

**Female: Spawning Stage**

This stage is characterised by the reduction in density of ova and rounding off, as the pressure within the follicles is reduced. Active discharge of the ripe ova take place, and as it proceeds the central portion of the follicles remain vacant (Fig. 24). The colour of the mantle gets reduced to a considerable extent owing to the release of the major part of the ova and finally becomes membranous and translucent.

**Female: Spent Stage**

Gonad of recently spawned individuals are characterised by shrinkage of the gonadal follicles and the spent females can be readily distinguished by the semi-translucent mantle. Spawning in females is not complete and a few ripe oocytes are always found in the spent gonad which are in different stages of phagocytosis (Fig. 26). Presence of large number of phagocytes is observed in this stage for the resorption of the residual eggs by cytolysis.
Table 22. Relationship between size and mantle thickness in males and females of *M. senhausen*

<table>
<thead>
<tr>
<th>Size group (mm)</th>
<th>Mantle thickness (in μ)</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>99</td>
<td>128</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>114</td>
<td>142</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>128</td>
<td>129</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>132</td>
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<td></td>
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<tr>
<td>14</td>
<td>192</td>
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<td>16</td>
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<td>18</td>
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<tr>
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<td>24</td>
<td>399</td>
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<td>26</td>
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<td>317</td>
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<td>28</td>
<td>290</td>
<td>334</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>-</td>
<td>233</td>
<td></td>
</tr>
</tbody>
</table>
From the results obtained by direct observation and histological studies of the gonad it is revealed that relative thickness of mantle can also be regarded as useful index for assessing the reproductive cycle. The average mantle thickness (expressed in μ) of male and female specimens of different size groups are given in Table 22. The male and female specimens showed difference in thickness. The normal range of mantle thickness of the male was from 99 μ to 534 μ and the females the range was found to be between 128 μ and 653 μ. The maximum average thickness was attained at a length of 24-26 mm and above this, thinning of the mantle was resulted. This might be because of the spawning activity of the organisms. Thickest mantle observed during the study was in female (844 μ) having a length of 24 mm. But in males the thickest mantle (685 μ) was present in mussels of size 26 mm. These two were in mature stage. It indicates the fact that an increase in the mantle thickness generally denoted the development, and a decrease, the spawning. Besides with the gonad development, thickness of the mantle was observed to vary with the size of the animal.

6.4.2 Histochemistry

Histochemical analysis revealed that the developing (immature oocytes) stage of the female gonad gave a positive result with Mercury Bromophenol Blue (MBB). The cytoplasm of the oocyte was comparatively strongly stained than the other components. The staining was slightly deeper in mature ova. In the spawning stage also tissue showed positive but a mild reaction. The mantle epithelium showed a reduction in the intensity of staining in mature condition. In male the spermatogonia, spermatoocytes and spermatids showed
Figure 28. Pattern of staining of male gonad by Mercury bromo-phenol blue (MBB) for protein.

Figure 29. Pattern of staining of female gonad by MBB for protein.
**Figure 30.** Pattern of staining of male gonad by Periodic acid Schiff's reagent (PAS) for glycogen.

**Figure 31.** Pattern of staining of female gonad by PAS for glycogen.
Figure 32. Pattern of staining of male gonad by Sudan black B (SBB) for lipid.

Figure 33. Pattern of staining of female gonad by SBB for lipid.
higher stainability. But in the spawning stage the tissue gave a positive but milder reaction. The mantle epithelium also showed a positive result with MBB.

In the developing stage oogonia and oocytes gave a mild reaction with Periodic Acid Schiff's reagent (PAS). In mature stage, colour intensity was found to be slightly increased. The stainability was slightly reduced in the spawning stage. The male gonad was mildly positive to PAS. The mantle epithelium was positive to PAS. The follicle wall showed a little less stainability.

Generally female gonad was positive to Sudan Black B, (SBB) indicating the presence of lipid in the oocytes. The developing and mature oocytes showed positivity to SBB. The cytoplasm of these inclusions were found to be filled with sudanophilic substances. In stalked oocytes, especially in the region of the stalk, comparatively high staining intensity was noticeable. In the spawning stage the staining intensity, however was found to be faded. In males the staining of the gonadal tissue with SBB showed differential reaction at different stages of maturity. In the developing stage more sudanophilic substances could be observed in the mantle epithelium, spermatogonia and spermatocytes. The mature stage showed a little less stainability than the other stages. The mantle epithelium less stained in this stage. In the spawning stage more sudanophilic substances were found in the follicular wall and mantle epithelium.

6.4.3 Annual Reproductive Cycles

In the annual reproductive cycle of *M. senhausia* studied for a period from February 1988 to February 1989. Some sort of similarity in the develop-
ment of the gonads in the two sexes were observed. The frequency of different stages of the gonad in different months is given in Table 23.

**Male**

In February 1988 the mussels were in active growth phase. Most of the mussels were in the developing (59.09%) and mature (36.36%) condition. In March and April most of the mussel were in mature and spawning phase. Animal with mature gonads were more (62.51%) in March. In April 21.43% of the male were in developing stage. Others were in mature (32.14%) and spawning (42.86%) phase of the gonad. In this month spawning was at its peak. It was indicated by the presence of few spent mussels (3.57%) with residual gametes. During this period shells of large size group were present in the collection, indicating the mortality of large specimens. In May developing mussels (50%) increased in number. Mature (25%), spawning (18.75%) and a few spent (6.25%) mussels were also present in the sample. In June, that is in low saline period due to southwest monsoon, the development and spawning took place in smaller sizes compared to that in other months. During this period 66.67% of the males were in the developing stage of the gonad, 23.81% in mature stage and 9.52% in spawning condition. In July also the same trend was maintained. But animals with mature gonad (51.85%) increased in number. Developing (37.04%) and spawning (11.11%) were also observed. After a break of three months, in November most of the mussels collected were in mature stage (41.18%). A few mussels with developing (35.29%) and spawning (23.53%) gonads could be seen in the population. During December 44.44% of the population were in developing, 27.78% in mature,

<table>
<thead>
<tr>
<th>Month</th>
<th>Gonadal stages of male</th>
<th>Gonadal stages of female</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Feb.1988</td>
<td>59.09</td>
<td>36.36</td>
</tr>
<tr>
<td>March</td>
<td>4.17</td>
<td>62.51</td>
</tr>
<tr>
<td>April</td>
<td>21.43</td>
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<tr>
<td>November</td>
<td>35.29</td>
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<tr>
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<td>Jan.1989</td>
<td>15.38</td>
<td>61.54</td>
</tr>
<tr>
<td>February</td>
<td>45.00</td>
<td>30.00</td>
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</table>
Figure 34. Percentage distribution of different stages of gonad development (Dg - developing, M - mature, Sg - spawning and S - spent) in *M. senhausia* (Male - M and Female - F)
16.67% in spawning and 11.11% in spent stage. In January 1989 most of the mussels were in mature (61.54%) stage, but a few were observed to be in developing (15.38%) and spawning (23.08%) condition. In February all the stages of development could be observed in the population. That is, 45% developing, 30% mature, 20% spawning and 5% spent mussels could be observed.

Female

In the case of females almost the same trend as in males could be observed. In February 1988 most of the females (50%) were in developing stage of the gonad. Individuals with mature (40%) and spawning (10%) gonad were also encountered in this month. During March about 54.17% of the mussels were in mature stage and a few with developing (20.83%) and early spawning (25%) condition. In April there was an increase in the number of spawning females (42.31%) in the population, while mature females registered only 38.46%. A few mussels in developing (11.54%) and spent (7.69%) stages were observed during this period. In May mussels of developing stage constituted 41.67%. Mussels with all the other stages of gonad development also could be seen in this month. The mortality of older specimens observed during these months resulted in the occurrence of the fewer number of fully spent mussels in the population. In June most of the mussels were in developing (76.47%) and only 23.53% of the mussels were in maturing condition. In July all the four stages were present in the population. After monsoon season, in November most of the mussels were with mature gonad (37.5%). Besides, 25% of the mussels showed developing, 31.25% spawning and 6.25% spent state of the gonad. In December mature (43.75%) mussels increased
Table 24. Distribution of male and female during the period of study

<table>
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<th>Month</th>
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<td>17</td>
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<td>November</td>
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<tr>
<td>December</td>
<td>18</td>
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<tr>
<td>February</td>
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<td>24</td>
<td>0.3636</td>
</tr>
<tr>
<td>Total</td>
<td>222</td>
<td>227</td>
<td>0.0557</td>
</tr>
</tbody>
</table>
in number and developing (37.5%) and spawning (18.75%) mussels also could be seen. During January 1989 along with the developing (25%), mature (35%) and spawning (30%) mussels, few mussels were found to be in spent (10%) stage. In February also mussels with all the four stages of gonad development were present in the population. Developing (25%) and mature (25%) mussels were more in number.

In the mussels of the size group 4-5 mm onwards, developing stages of the gonad were observed. The smallest animal with mature gonad was in the size of 13-14 mm. Spawning was observed to commence in the mussels of length 22-23 mm. There was no distinct difference between male and female at the commencement of different stages, however similar observation could not be found throughout the year. Especially during monsoon season the animal of 2-3 mm size group was found to start the gonad development and the maturity attained in a size group of 8-9 mm. This may be due to the fact that sexual maturity may be attained irrespective of size denoting that the size at which maturity occurred varied most probably according to changes in the environmental condition.

The sex ratio of *M. senhausia* observed during different months is presented in Table 24. Of the 449 mussels studied during one year period 222 (49.44%) were males and 227 (50.56%) were females. In general sex ratio in different months was more or less the same. The chi-square test of the monthly sex ratio were showed that the male: female ratio was not significantly different from the expected 1:1 ratio.
6.5 DISCUSSION

The mussel *M. senhusia* is dioecious and showed no trace of sex reversal and hermaphroditism. Bivalves are regarded as a group characterised by gonochorism and 96% of the species included in the class are of separate sexes (Coe, 1943).

*M. senhusia* showed different prominent gonadal stages like indifferent, developing, mature, spawning and spent. Here the classification is almost in agreement to that of the Seed (1976). He recognised four main stages of gonad development in *Mytilus edulis* : developing, ripe, spawning and spent. He divided developing and spawning stages into four substages based on minor changes in the development which is not followed in the present study.

In the indifferent and spent condition the mantle is found to be composed of connective tissues like adipogranular tissue and vesicular connective tissue (Leydig cells). According to Lubet et al. (1976) the vesicular cells store large amounts of glycogen and adipogranular cells contain lipid droplets and protein granules. These cells have the common ability to stretch or contract as occasion demands (Tranter, 1958). Several authors have pointed out about the possibility of various cell types that form the mantle tissue. Lubet (1959) described that the mantle of *Mytilus galloprovincialis* is composed of various blood cells, gametes, adipogranular cells (AG) and vesicular connective tissue (VCT). Lunetta (1969) reported the existence of both AG and VCT cells in *Mytilus* Spp., but in *M. (=Perna) perna* he observed only VCT cells which he referred to as interfollicular connective tissue. The occurrence of AG and VCT in the mantle connective tissue has been recorded in *Mytilus*
edulis (Bayne et al., 1982; Lowe et al., 1982), M. californianus (Kelley et al., 1982), Perna indica and P. viridis (Ajithakumar, 1984).

M. senhousia showed an extended breeding period with a major peak in April-May with intermittent spawning in other months. According to Giese (1959) breeding season of organisms in tropical seas is the most prolonged one. Studies on the marine and estuarine bivalves have revealed the presence of continuous and discontinuous breeding in bivalves of the Indian coasts. George and Nair (1973) observed protracted and asynchronous breeding period with three synchronous peaks in Musculista areuatula. A prolonged breeding period with two major peaks have been observed in Katelysia opima (Nagabhushanam and Mane, 1975a), Paphia laterisulca (Nagabhushanam and Dhamne, 1977) and Mytilus edulis (King et al., 1989). An extended breeding period with a distinct period of intense activity was noticed in Perna indica (Kuriakose, 1973) and Donax cuneatus (Nagabhushanam and Talikhedkar, 1977a). Extended breeding period was reported in some bivalves like Meretrix meretrix, M. casta, Katelysia opima (Jayabal and Kalyani, 1986a) and Modiolus modiolus (Jasim and Brand, 1989). In Mytilus viridis (Nagabhushanam and Mane, 1975b) and Crassostrea madrasensis (Stephen, 1980a) only two spawning periods were observed in a year.

The bivalve gonad usually enters into a resting stage after spawning (Loosanoff, 1962). But in the present study no resting stage was observed. Throughout the year germ cells in different developmental stages could be seen in follicles of the gonad. Likewise no resting stage was observed in Pecten maximus (Mason, 1958), Musculista areuatula (George and Nair, 1973),
Perna indica (Kuriakose, 1973), and Paphia laterisulca (Nagabhushanam and Dhamne, 1977). Stephen (1980a) observed a gametogenic cycle of nine months followed by an inactive phase for the rest of the period in Crassostrea madrasensis. In Donax cuneatus an inactive period of three months after spawning was reported by Victor and Subramoniam (1988).

The pattern of reproductive periodicity of organisms shows a relationship with the climatic conditions to which they are exposed. In the present investigation major spawning was observed to be in April-May indicating the fact that increase in salinity facilitated the rapid development of the gonad and spawning. Later sudden decrease in salinity of the ambient medium due to southwest monsoon and resulting freshwater inflow resulted in the mortality of major part of the population in this region. Later at the close of the monsoon, the presence of the mussels in this region helped to reach the conclusion that the population might get replenished by the larvae of the population that exist at the outer end of the barmouth near sea. During this period, with the gradual increase in salinity the mussel started its development. Spawning reached its peak with further increase in salinity.

Studies on reproductive cycle of bivalves reveal that the temperature influences the spawning in temperate waters (Wilson and Hodkin, 1967; Barber and Blake, 1983; MacDonald and Bourne, 1987). But in tropical waters, salinity, rather than temperature appears to play an important role in the breeding cycle. The factors influencing spawning may be quite different from those inducing annual reproductive cycle (Giese, 1959). Increase in salinity has been found to trigger spawning in many bivalves such as Paphia
laterisulca (Nagabushanam and Dhamne, 1977), Donax cuneatus (Nagabushanam and Talikhedkar, 1977a), Crassostrea madrasensis (Joseph and Madhyastha, 1984), and Donax cuneatus (Victor and Subramoniam, 1988). In Mytilus viridis (Nagabushanam and Mane, 1975b), Crassostrea madrasensis (Stephen, 1980a), and Saccostrea cucullata (Sukumar and Joseph, 1988) peak spawning was observed with decline in salinity. But in some other bivalves like Meretrix meretrix, M. casta, and Katelysis opima spawning was found to occur in moderate salinity (Jayabal and Kalyani, 1986a).

The histochemical studies on gonad helps to locate changes of organic components like glycogen, protein and lipid occurring at different periods of the reproductive cycle. The present study revealed that the mantle of M. senhausia is glycolipo-protein in nature. The gonadal tissue showed slight increase in protein content in mature condition. This may be due to the build up of protein in the developing oocytes and spermatoocytes. In the spent gonad, the staining property was diminished because of the depletion of protein due to the release of gametes.

Glycogen was found to accumulate in the cytoplasm of the oocytes. During later stages glycogen content showed a reduction. In Mytilus edulis Lowe et al. (1982) and Bayne et al. (1982) observed the transfer of yolk precursor substances from the connective tissue to the developing oocyte in the follicles. In male, only a lesser quantity of glycogen could be noticed inside the spermatozoa, but it occurred in more quantity in the mantle epithelium.

Histochemical observations on the lipid revealed that in males the accumulation of the lipid was little more than in females in different stages
of development. The mature follicles showed only moderate stainability. In the case of females, as the gametogenesis proceeded, the oocytes showed an increase in lipid content. The stalk of the stalked oocytes was found to be more stainable. This may lead to the conclusion that other than the lipid stored in the connective tissue, lipid is also contributed from some other tissue. The mature oocytes contained more sudanophilic granules. Lubet (1959) also observed the appearance of granules in mature Mytilus edulis. Ajithakumar (1984) got almost similar results in the distribution of glycogen, protein and lipid components during the period of gametogenesis of Perna indica and P. viridis.

From the different studies to elucidate the reproductive cycle and its changes in different condition revealed that the M. senhausia is gonochoristic and shows different prominent gonadal stages according to the development. It shows a protracted spawning period with a peak during April-May. The development and spawning is influenced by salinity of the ambient medium. Besides these different organic components like protein, glycogen and lipid also shows variation according to the development of the gonad.