CHAPTER 5

Neurosecretion
INTRODUCTION

The nervous and endocrine systems coordinate the activities of the various organs and the tissues in the body that the animals function as individuals. Majority of the neurones with glandular activity are known to be necessary for the transmission of transient impulses, with their highly localised production of chemicals such as neurohumsors which are rapidly destroyed. All neurones transmitting in this manner are gland cells secreting their chemical mediators into the synaptic cleft regions. Neurones have similar properties throughout the animal kingdom, although their morphology and arrangement may vary. Hagadorn (1967) and Highnam and Hill (1978) in their reviews stated that cytological display of neuronal secretory activity is indication of vigorous biosynthetic activity which is said to be associated with the formation of exoplasmic protein. The way in which nervous system operates may differ considerably between animals, depending on the number of neurones involved and the individual shape, size and spatial arrangement of the component. Upto 50 % of the
nervous system is composed of non-excitile satellite glial cells which are packed between and around the neurones. Lubet (1955a) and Umiji (1969) suggested that they transport neurosecretory substances. Leak and Walker (1980) stated that these cells provide physical support for neurones and can modify action of nerve cells as well as acting as barrier or reservoir for ions, metabolites and transmitters. The arrangement of neurones into ganglia allows an increase in number of synaptic contact for many particular cells and thus increases the integrative capacity of a number of neurones.

Amongst invertebrates, Mollusca show great variability in their nervous system ranging from primitive arrangement in chitons to the complex mass of fused ganglia forming "the brain" of cephalopods. Pharmacological or physiological aspects of molluscs using the effector organs have received considerable attention (Huddart, 1975; Bayne, 1976). Evidence for the occurrence of a wide variety of neurotransmitters in different tissues of bivalve molluscs including the nerve ganglia has been discussed from the functional point of view by Leak and Walker (1980).

In all major animal groups studied so far there occurs among the more 'conventional' neurones,
other nerve cells remarkably of different appearance, the neurosecretory cells (NSCs). These cells, in addition of displaying the cytological features common to all neurones, generally show prominent indication of glandular activity. With the light microscope they are characterised by the presence of abundant secretory material in their perikarya. This material is also seen in the axons which often end blindly adjacent to muscular spaces rather than innervating their target structures directly. These blindly ending terminals serve a storage-release function and in the more advanced groups of animals such as crustaceans such compact structures are termed as neurohemal organs by Knowles and Carlisle (1956). The structures like corpus cardiacum of insects, the sinus glands of crustaceans, and the pars nervosa of vertebrates are well known neurohemal organs. The neurosecretory chemical takes time to build an effective concentration and consequently must have a longer biological life than the chemicals of ordinary neurones, before they are eventually destroyed or excreted. Rothbaler (1957) stated that the brain, as a primary receptor and integrator of wide variety of internal or external sensory inputs, is faced with the problems of influencing activities with more diverse time courses; from phasic muscle twitches to the ionic, growth and
reproductive sequences. In order to achieve these sequences, it is not surprising that the nervous system should use hormonal outputs as well as conventional innervations where appropriate. The NSCs, with their combination of neuronal and glandular capabilities are perfectly suited to translate a neuronal input into the hormonal output best suited to a long term process. In this capacity the NSCs may produce hormones, which act directly upon the peripheral target or it may exert its effect indirectly by influencing the activity of other, non-neural, endocrine organs. In this later case, the NSCs again may act via the production of a blood-born hormone. Knowles and Bern (1966) stated that the significance of NSCs as connecting link between nervous and endocrine systems and neurosecretory neurones ‘participate either directly or indirectly in endocrine control and form all or part of endocrine organ’. Hormones are consequently well suited to exert their effects over a extended period of time and the endocrine system control long-term process within the body, such as the coordinated growth of organs or the maintenance of appropriate metabolite concentration in the blood or tissues.

Since Scharrers (1928, 1930, 1933) reported the occurrence of secretory cells like NSCs in the
hypothalamus of various vertebrates using different staining affinities, many investigators began to pay attention to the neurosecretory phenomenon in a wide variety of animals. In invertebrates several investigators have shown the occurrence of NSCs (in Echinodermata, Chaet, 1966; in Annelida, Gabe, 1966; Nagabhushanam and Kulkarni, 1983; in Mollusca, Highnam and Hill, 1978). Persual of literature reveals that the aspect of neurosecretion in bivalve molluscs has been reviewed by Gabe (1965,1966), Lubet (1966,1973), Martoja (1972) and Golding (1974). The development of the subject has been hampered by the presence of shell, by the diffused distribution of NSCs and by the ignorance of the chemical nature of the neurohormones. Presence of NSCs has been demonstrated by classic histological studies on a number of species. Their number and location have been shown to vary among species. NSCs are located in the central ganglia. In highly evolved bivalves, NSCs are less numerous and more localized. Their location in the central ganglia has been reported by Lubet (1955a, 1959), Nagabhushanam (1963, 1969), Nagabhushanam and Mane (1973) and Mane (1986). A close relationship between neurosecretion and sexual cycle in Mytilus and Chlamys (Lubet, 1956), Dreissena polymorpha (Fahrmann, 1961), Crassostrea virginica (Nagabhushanam, 1963), Katelysia opima (Nagabhushanam and Mane, 1973),
Crassostrea gryphoides (Mane and Nagabhushanam, 1976) and Mytilus viridis (Mane, 1986) has been established. Almost all the above investigators have shown that the NSCs from cerebral ganglia reveal a close relationship in their secretory activity with the maturation and release of gametes from gonads. However, further studies carried out by Herlin-Houteville and Lubet (1974), Lubet et al. (1976), Whittle et al. (1983), Kulkarni (1987) and Rao (1988) suggested that both cerebral and visceral ganglia in bivalves are responsible in the regulation of break down of reserve materials and gametes maturation. These authors attributed this to the role of neurosecretion from these ganglia. Comparatively very little work has been done on the role of neuroceretion in reproduction the freshwater species from India (Kulkarni, 1987; Rao, 1988).

Considering the paucity of information on the freshwater species in determining whether there exists a close relationship between the neurosecretion and reproduction, the present study has been undertaken with the aim of adding our knowledge of neurosecretion in the freshwater bivalve, Lamellidens corrianum.
MATERIALS AND METHODS

For the histological examination of cerebral and visceral ganglia of the adult mussels, *Lamellidens corrianus*, the animals of 60-65 mm in shell length were dredged from the left bank of Godavari River 42 Kms away from Aurangabad at Kaygaon. The samples were collected on the fixed dates of 11th and 26th of each month for a period May 1986 to April 1987. A total of 360 animals were dissected for the cerebral and visceral ganglia. For this purpose, soon after collection the animals were kept in the laboratory in well aerated reservoir water to allow the shell valves to open. These animals were then immediately used to dissect the ganglia. The ganglia were fixed in Bouin's Hollande for 24 hours and then processed for histological preparations. The paraffin sections cut at 5-6 μ were stained with Gomori's chromium-haematoxylin phloxine (Gomori, 1941) and with thionine paraldehyde after permanganate oxidation (as shown by Illanes and Lubet, 1980). All the sections were observed under research binocular microscope and then photographed. The situation of the neurosecretory granules within the cell body of Cell Type I has been
examined to determine the successive stages of the secretory cycle (as described by Nagabhushanam et al., 1972). The measurements of the cells were also done for total length (including axon length), cell and nuclear diameters and length of the axonal part for cell Type I and for cell Type II the cell and nuclear diameters.

RESULTS

Cytological examinations of the serial sections of cerebral and visceral ganglia stained with chrome-haematoxylin-phloxine and thionine paraldehyde revealed the presence of groups of cells which are larger than the ordinary ganglion cells. Most of the large cells possess large nuclei and abundant cytoplasm; their perikarya and axons are filled with fine granules which stained conspicuously. The staining with thionine paraldehyde was the best to allow to distinguish the neurosecretory material and chromolipoids present in the neurones and NSCs. Two types of NSCs have been recognised. The size, general shape of the cell body, presence or absence of vacuoles in the cytoplasm, and staining properties of the secretory material were used as the basic criteria in distinguishing the types of NSCs. These two types of NSCs are distinguished as Cell Type I and Cell Type II. They are localized on the
dorsal and lateral periphery in cerebral and visceral ganglia.

**Cell Type I:** The cells are pyriform in majority of the cases but sometimes irregular in shape (Figs. 1 and 2). These cells measure $20.40 \pm 3.04$ to $13.0 \pm 1.54$ $\mu$ in length and $11.2 \pm 2.34$ to $10.8 \pm 2.21$ $\mu$ in width. Out of this total length axon length measured from $11.4 \pm 3.24$ to $9.0 \pm 3.0$ $\mu$. The nucleus is round or oval measuring about $7.2 \pm 1.52$ to $4.2 \pm 1.52$ $\mu$; it may be either central or eccentric in position. The nucleus generally contains a large nucleolus but in certain cases 2 to 3 nucleoli appear inside the nucleus. The secretory material stains blue-black with Gomori's and green with thionine paraldehyde. In some sections the secretory material appeared as extremely fine granules in the form of small particles. Furthermore, all the cells did not show the presence of large amount of secretory material at any one time. Apparently some cells are at the peak of their secretory process while others are devoid of granules. Vacuoles are generally absent but appear as the secretory products are released from cytoplasm. These cells are present in both the ganglia.

**Cell Type II:** These cells are smaller than cell Type I and are oval or round with diameter of $20.2 \pm 5.13$ to $9.2 \pm 2.11$ $\mu$ (Figs. 1 and 2). Their nuclei are similar to those of Cell Type I cells and measure $9.6 \pm 2.32$ to
Fig. 1: Histological changes in the neurosecretory cells of cerebral ganglia in *Lamellidens corrianus* show different stages during reproductive cycle. X 1000.

1. Gametogenesis
2. Active gametogenesis
3. M
4. Partially shed gametes
5. Many shed gametes
6. Fully shed gametes
7. Recovery

A – Type I cells;  B – Type II cells;  N – Nucleus;
N1 – Nucleolus;  V – Vacuoles;
NS – Neurosecretory material
Fig. 2: Histological changes in the neurosecretory cells of visceral ganglia in *Lamellidens corrianus* show different stages during reproductive cycle. X 1000.

1. Gametogenesis
2. Active gametogenesis
3. Partially shed gametes
4. Many shed gametes
5. Fully shed gametes
6. Recovery

A - Type I cells; B - Type II cells; N - Nucleus
N1 - Nucleolus; V - Vagules;
NS - Neurosecretory material
4.87±1.46 μ. Only one nucleus can be seen in this type. The secretory material stains grey with Gomori's stain and light green with thionine paraldehyde. The vacuoles are generally less abundant than in cell Type I. In both the ganglia it was observed that these cells are large in number compared to the Cell Type I.

The data on the measurements are presented in Table 1 and 2 for the two cell types.

Based upon the report of Nagabhushanan et al. (1972) on the secretory stages of the pyriform NSCs from *Mytilus viridis*, the average frequency of four different stages in neurosecretion of the NSCs from the cerebral and visceral ganglia of *Lamellidens corrianus* during different stages was determined throughout the study period. The four stages described by the above authors and considered in the present study are, 1. NSCs with uniform dispersed granules, 2. Cells with perinuclear concentration of granules, 3. Cells with accumulation of granules in the axon hillock, and 4. Cells with granules in proximal part of the axon. All these four stages were conspicuously recorded in both cerebral and visceral ganglia of *Lamellidens corrianus*. As could be expected many cells were in a transitional stage, 0-1, 1-2, 2-3 and 3-4. The observations suggest the following cycle: the secretory granules first appear
Measurements of NSCs from the cerebral ganglia of *Lamellidens corrianius* in different seasons correlated with the reproductive stages.

<table>
<thead>
<tr>
<th>Productive stages</th>
<th>Cell Type I</th>
<th>NSCs Types</th>
<th>Cell Type II</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Cell length</td>
<td>Cell diameter</td>
<td>Nucleus diameter</td>
</tr>
<tr>
<td>Metogenesis</td>
<td>18.60±2.820</td>
<td>11.20±2.396</td>
<td>5.20±2.111</td>
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<td></td>
<td>(P&lt;0.001)</td>
<td>NS</td>
<td>(P&lt;0.05)</td>
</tr>
<tr>
<td>Tive gametogenesis</td>
<td>18.14±2.229</td>
<td>11.00±1.851</td>
<td>6.40±1.919</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Ture</td>
<td>18.80±2.111</td>
<td>8.60±2.229</td>
<td>4.20±1.521</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>(P&lt;0.01)</td>
<td>NS</td>
</tr>
<tr>
<td>Tially shed</td>
<td>17.80±3.488</td>
<td>10.60±1.919</td>
<td>7.00±1.463</td>
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<tr>
<td></td>
<td>NS</td>
<td>(P&lt;0.05)</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>Hy shed</td>
<td>16.60±2.229</td>
<td>10.00±1.463</td>
<td>5.60±1.919</td>
</tr>
<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>(P&lt;0.05)</td>
</tr>
<tr>
<td>Aly shed</td>
<td>16.20±2.210</td>
<td>10.00±1.851</td>
<td>7.00±1.463</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>(P&lt;0.05)</td>
</tr>
<tr>
<td>Recovery</td>
<td>15.00±1.963</td>
<td>10.80±2.210</td>
<td>7.20±1.521</td>
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<tr>
<td></td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</table>
Table 2: Measurements of NSCs from the visceral ganglia of *Lammelidens corianus* in different seasons correlated with reproductive stages.

<table>
<thead>
<tr>
<th>Reproductive stages</th>
<th>NSCs Types</th>
<th>Cell Type I</th>
<th>Cell Type II</th>
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<tr>
<td></td>
<td></td>
<td>Cell length</td>
<td>Cell diameter</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(P&lt;0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>Fertile gametogenesis</td>
<td></td>
<td>19.80±3.166</td>
<td>15.40±3.180</td>
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<tr>
<td></td>
<td></td>
<td>(P&lt;0.001)</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>Maturity</td>
<td></td>
<td>20.40±3.042</td>
<td>11.00±2.699</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>(P&lt;0.001)</td>
</tr>
<tr>
<td>Partially shed</td>
<td></td>
<td>19.40±2.746</td>
<td>9.00±2.777</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Totally shed</td>
<td></td>
<td>17.40±2.823</td>
<td>10.60±1.919</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Overwintering</td>
<td></td>
<td>18.60±3.246</td>
<td>10.00±1.851</td>
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<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>Recovery</td>
<td></td>
<td>18.80±2.396</td>
<td>9.80±1.780</td>
</tr>
<tr>
<td></td>
<td></td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>
throughout the cytoplasm, then concentrate around the nucleus, followed by an accumulation in the axon hillock, and after and during this accumulation the granules can be found in the proximal part of the hillock. The stains used in the present study facilitated to study the secretory activity (stages of neurosecretory material in the NSCs) during every fortnight throughout the sampling period. The stages of neurosecretion in the NSCs were more conspicuously seen in the Cell Type I than in the Cell Type II. The results of the study are presented Tables 1 and 2, and the microphotographs are presented in Figs. 1 and 2 reveal the distinct neurosecretory stages with the reproductive stages (as given in Chapter 4) from both the ganglia.

Cerebral ganglia:

Stage I - This initial phase is distinguished by the presence of scattered neurosecretory material in the cytoplasm when compared with other phases. Moreover, the maximum number of cells in this stage were found during July first and second fortnights and August second fortnight. For another time the maximum number of the cells in this stage were also found during both the fortnights of January, February and March. The cells in this stage were more conspicuously seen during monsoon (July-August) than in the period of winter to
early summer.

**Stage II** - In this intermediate stage, the neurosecretory material got concentrated around the nucleus. This stage was particularly seen during August second fortnight to November first fortnight and also, but in less number, during November second fortnight to April second fortnight.

**Stage III** - The accumulation of neurosecretory material in the hillock is the main characteristic of this stage. The maximum number of cells in this stage was observed during November second fortnight to December second fortnight. This stage again reached maximum number during February and April.

**Stage IV** - During this end phase the neurosecretory material is found not only in the axon hillock but also in the proximal part of the axon. The maximum number of cells in this stage was observed during the first and second fortnights of October, November, December and also during March and April, however, this time only a few cells fell in this stage.

Based on the above results it can be stated that a distinct cycle exists in the neurosecretory activity which can be correlated with the gametogenesis, active gametogenesis, mature, partially shed gametes, many shed gametes, fully shed gametes and recovery as
described earlier in Chapter 4. The photographs represented in Fig. 1 reveal the majority of NSCs of Cell Type I falling under four different secretory stages through different reproductive stages in a cycle. The average number of NSCs of Cell Type I in each secretory stage during each fortnight is given in Fig. 3. This cycle begins with stage I in June, July, August, January, February and March at the time many animals showed gametogenesis and active gametogenesis in both male and female follicles. The number of cells in this stage were more in July and August than during January, February and March. Thereafter, stage II followed which showed maximum number during August second fortnight to November first fortnight and also in less number for a longer period during November second fortnight to April second fortnight as well as in July. This period can be correlated with subsequent maturation of gametes in maximum number during August second fortnight to October second fortnight, during December first fortnight, January second fortnight and March second fortnight. From September second fortnight to December second fortnight a maximum of stage III was seen. This stage again appeared in February and April. This stage was prominently noticed during both the fortnights of November and December at the time of release of almost all mature gametes. This stage followed by stage IV
Fig. 3: The average numbers of Cell Type I neurosecretory cells of cerebral ganglia in *Lamellidens corrianus* successive stages I-IV during different fortnights. 

I - Stage I; II - Stage II; III - Stage III 
IV - Stage IV.
showed a maximum number during November, December and again in April which could be correlated with the maximum emptying of gametes.

Visceral ganglia:

The photographs taken for majority of NSCs of Cell Type I falling under four different stages of reproduction are presented in Fig.2. The average number of NSCs of Cell Type I in each secretory stage during every fortnight is given in Fig.4. Stage I to stage IV did not show any maximum or minimum numbers in any given season of monsoon, post monsoon, winter and summer or during any given reproductive stage. Thus, the fortnightly variations in the number of various stages could not be correlated to either the fluctuations in the environmental parameters or to the physiological state of the animal for any fortnight.

Comparing the secretory activity of the NSCs of the cerebral and visceral ganglia, a striking difference exists between them; the cerebral ganglia show the variations in the secretory stages of NSCs correlated with the gametogenesis, maturation and release of gametes, whereas visceral NSCs do not show such correlation in a strict sense.

The data on measurements of cell Type I and cell Type II during different fortnights have been
Fig. 4: The average numbers of Cell Type I neurosecretory cells of visceral ganglia in *Lamellidens corrianus* successive stages I-IV during different fortnights. I - Stage I; II - Stage II; III - Stage III IV - Stage IV.
pulled for seven reproductive stages viz. gametogenesis, active gametogenesis, mature, partially shed, many shed, fully shed and recovery as described in Chapter 4. Majority of the cells showed similar stage of the neurosecretory accumulation in the cytoplasm in the given fortnight and only those cells revealing such stages were considered for measurement. The data are presented in Table 1 and 2 for cerebral and visceral ganglia. It can be seen that there is significant increase in the length of cell Type I (P<0.001) with the commencement of gametogenesis in June and the nuclear diameter significantly decreases (P<0.01) as the animals pass from active gametogenesis to mature stage. The cell diameter significantly increases (P<0.05) as the animals pass from mature to partially shed gametes stage. Regarding nuclear diameter, it decreases significantly (P<0.05) with the commencement of gametogenesis but it increases significantly (P<0.001) with the onset of shedding of gametes. As the gametes are shed more the nuclear diameter significantly decreases (P<0.05) but as all the gametes are shed it attains the diameter similar to that seen in commencement of shedding gametes. No significant difference in the nuclear diameter occurs as the animals pass from fully shed stage to recovery or from gametogenesis to mature stage. Regarding the axon
length no significant change in the diameter occurs throughout different stages of gonad development. It is probable that since the role of axon is to carry gradual release of the secretion from the cytoplasm of the cell body, there occurs no significant change in its length. Regarding oval cells the diameter significantly increased (P<0.001) as the animals pass from recovery to gametogenesis (P<0.001) and from partially to many shed (P<0.001) but as the gonad reaches fully shed and recovery stages it decreases significantly (P<0.001 and P<0.05, respectively). The nuclear diameter also increases significantly (P<0.01) as the gonad pass from recovery to gametogenesis and from partially shed to many shed (P<0.01) but decreases significantly as the gonad pass from many shed to fully shed stage (P<0.001).

In case of visceral ganglia the cell length of cell Type I decreases significantly as the animals pass from recovery to gametogenesis (P<0.05) but increases as they pass from gametogenesis to active gametogenesis (P<0.001). The nuclear diameter also significantly increases in this later process (P<0.001) but decreases (P<0.001) as they pass from active gametogenesis to mature. The cell diameter increases significantly during active gametogenesis (P<0.001) but decreases during mature stage (P<0.001). The nuclear diameter on
the other hand, decreases during the fully shed stage (P<0.05), i.e. as the animals pass from many shed to full shed stage but increases during recovery (P<0.01) after the gametes are fully shed. Axon length significantly decreases as the animals pass from fully shed to recovery (P<0.001) but increases significantly during gametogenesis (P<0.001). Regarding cell Type II it is observed that the diameter significantly increases with the commencement of shedding of gametes (P<0.001) but decreases (P<0.001) as the shedding of gametes continues, i.e. during many shed stage. It also decreases further as the animals pass in recovery stage (P<0.001). The nuclear diameter significantly increases (P<0.01) with the commencement of gametes shedding but decreases (P<0.05) as the gametes shedding continues, i.e. during many shed stage. The diameter further decreases (P<0.05) as the animals pass in recovery stage.

**DISCUSSION**

With a steadily increasing number of NSCs being described, particularly as new histological procedures are adapted, it is desirable to prevent unnecessary duplication on terminology. Consequently, extended comparison of the NSCs described herein with
types described by other workers in bivalves is somewhat appropriate. With regard to the location of the NSCs in bivalves some similarities could be observed. The dorsal position of NSCs in *Lamellidens corrianus* corresponds with the situation of these cells in *Modiolus demissus*, *Crassostrea virginica* and *Meretrix casta* (Nagabhushanam, 1964, 1968a,b), *Katelysia opima*, (Nagabhushanam and Mane, 1973) and *Lamellidens marginalis* (Rao, 1988). Gabe (1955) recorded NSCs on the dorsal part of the cerebral ganglia in *Nucula nucleus*, whereas in higher lamellibranchs these cells were also seen in medial region. Lubet (1955a, 1956) in *Mytilus edulis* and *Chlamys varia* observed NSCs in lateral, dorsal and rostral position of the ganglia. In the present study *Lamellidens corrianus* showed NSCs in dorsal and lateral periphery in cerebral and visceral ganglia. The two types of cells present in these ganglia are in accordance with those observed in *Teredo* (Gabe and Rancurel, 1958), *Mytilus* and *Chlamys* (Lubet, 1955b), *Crassostrea virginica* (Nagabhushanam, 1963), *Meretrix casta* (Nagabhushanam, 1969) and *Katelysia opima* (Nagabhushanam and Mane, 1973) for Cell Type I and in *Crassostrea virginica* (Nagabhushanam, 1963), *Katelysia opima* (Nagabhushanam and Mane, 1973) and *Lamellidens marginalis* (Rao, 1988) for Cell Type II.
It should be kept in mind that the nervous system and the hormonal apparatus are not as shapely separated in invertebrates as they are in vertebrates and that no endocrine gland has so far been encountered in molluscs. It is, therefore, possible that hormonal activity is restricted to the nervous system itself, a theory supported by the fact that the secretory cells occur in all the ganglia of the freshwater mussels (Kulkarni, 1987; Rao, 1988). Additional physiological and morphological investigations are necessary for the elucidation of the biological role played by the neurosecretion.

The physiological study under the present investigation revealed that in *Lamellidens corrianus* Cell Type I shows a distinct annual cycle of neurosecretory activity which can be correlated with the reproductive activity. The tinctorial properties of these cells also revealed a close relation with reproductive stages. The cell length of Type I through different reproductive stages showed significant increase as the gonads commenced gametogenesis. The cell diameter, on the other hand, decreased significantly as the gonads reached mature stage but significantly increased, along with the nuclear diameter, with the onset of gametes shedding. The nuclear diameter increased as the gametes were shed. The axon length did
not significantly change with the different reproductive stages. The cell length of Cell Type I from visceral ganglia decreased significantly as the gonads began gametogenesis soon with onset of monsoon but increased significantly with the active gametogenesis. The diameter of this cell type also significantly increased with the onset of active gametogenesis but decreased as the gonads matured. The nuclear diameter significantly decreased as the gametes were fully shed but increased significantly during recovery. Thus, these changes in Cell Type I can be attributed due to the changes in the secretory stages described earlier in the results. It is of interest to note the significant changes in the cell diameter and the nuclear diameter of cell Type II from both the ganglia with the reproductive stages. In cerebral ganglia these cells showed significant increase in diameter of cell as well as of nucleus as the gametes continued to shed and at the time of fully shed gametes they showed significant decrease in the diameter. During recovery the cell diameter further decreased significantly. With the onset of gametogenesis both the cell and nuclear diameters increased significantly. In visceral ganglia these cells showed significant increase in both the cell and nuclear diameters at the commencement of gametes shedding but these diameters
decreased significantly as the gametes continued to shed. The cell diameter significantly increased with the onset of gametogenesis. These changes from Cell Type II observed in both the ganglia revealed that these cells may also play some role in completion of reproductive events. However, the secretory material form these cells revealed no such strict correlation. This opens a new topic to investigate in detail the nature of the role played by these oval cells in the reproduction. Cyclic activity in NSCs was observed by histological studies in many species of bivalve molluscs (Lubet, 1955a, 1959; Nagabushanam, 1963, 1969; Umiji, 1969; Blake, 1972; Nagabushanam and Mane, 1973; Mane, 1986). However, a few studies do indicate that there is a direct involvement of endogenous regulation through this secretory system in controlling the mobilization of organic reserves providing energy for gonad development (Herlin-Hauteville and Lubet, 1974; Lubet et al., 1973; Whittle et al., 1983; Rao, 1988). Hence, there is a necessity for taking precaution on to correlate directly whether the neurosecretion from Cell Type I is only responsible for the reproduction or the ganglion as a whole (including both the types of NSCs) is the essential in controlling many of the physiological, including reproductive, processes. Before drawing any conclusion it is necessary to evaluate the exact
chemical nature of the neurosecretory material as has been described for neurotransmitters from the ganglia of several bivalve species (Leak and Walker, 1980).

Lubet (1955a, 1956) on Mytilus edulis and Chlamys varia showed that the spawning corresponded to the period of excavation of products from the cerebral and visceral NSCs. The secretion was produced just before gametogenesis and was maximal at the time of gametes maturation. Just before each release of gametes during spawning, some of the NSCs emptied their secretion. In the freshwater zebra mussel, Dreissena polymorpha, Antheunisse (1963) also observed a parallel relationship between neurosecretory cycle and reproductive cycle. He observed both the cycles to start at the end of summer and the beginning of the autumn. A slight discharge of neurosecretory substance at the end of autumn and winter was observed with a moderate growth of oocytes, whereas a maximum discharge of secretory material occurred in spring with the intensive growth of gametes, ripening of gametes and spawning process. Nagabhushanam (1968b) in Crassostrea virginica observed a distinct seasonal fluctuation in the secretory activity of cells of Type I. He correlated this fluctuation with reproductive activity of the oyster. During October to December most of the
Type I cells contained no secretory granules and at this time gonads were spent. In adult population of the esturine bivalve, *Katelysia opima* from Ratnagiri, gametogenesis followed by spawning and resting phases occur twice in a year. Neurosecretory product begins to accumulate in the cell Type I with the initiation of gametogenesis and reaches a maximum when the animals are mature (Nagabhushana and Mane, 1973). Secretory granules in the NSCs decrease with the spawning and are not seen in resting animals.

The fact that is to be kept in mind at this time is that both these cycles could be independently controlled by a factor related, for instance, to a change in the environmental factors, like temperature, food, rainfall etc. But to show it is the secretory product from the NSCs that stimulates the spawning periodicity of the field population, the periodic sampling of the field population at fortnight intervals for a complete reproductive cycle can give a better insight for some definite answer.

The appearance and position of neurosecretory products within the perikarya vary with the stage of the secretion (Lubet, 1955a,b, 1959; Gabe, 1966; Gabe and Rancural, 1958; Blake, 1972). In some cells neurosecretory granules are few, while in other they are abundant and remain discrete. In still other cells,
neurosecretory products are present in lumps and pools. The discharge of neurosecretory products is characterised by cytoplasm with the presence of small quantities of secretory products between vacuoles and axon hillock (Gabe, 1966). In the light of such stages in neurosecretory product, Nagabhushanam et al. (1972) confirmed four different stages of secretory activity in Mytilus viridis, which were distinctly correlated with reproductive activity of the same species by Mane (1986). Similar attempt was made in the present study by correlating the data on dominant secretory stage and dominant reproductive stage of the histological sections prepared and observed in every fortnight. The present study on Lamellidens corrianus revealed that all the four stages of neurosecretory activity from cell Type I of cerebral ganglia correlate with seven stages involved in reproductive cycle. The stage I has been correlated with scattered material in the cytoplasm during gametogenic activity, with stage II in which the neurosecretory material got concentrated around the nucleus at which time the gonad subsequently pass into mature condition. In stage III and stage IV the material accumulated in the hillock and released through axon as the mature gametes are shed.

Demonstration of the role of neurosecretion in
the reproduction of bivalves has been difficult with
standard surgical procedure. In a few marine bivalves
like mussels and oysters a window is to be prepared by
drilling the shell valves at appropriate places to
remove cerebral and visceral ganglia but for many
species of freshwater bivalves the removal of these
ganglia is facilitated by just keeping a wedge between
the valves (Kulkarni, 1987; Rao, 1988). Based on the
surgical data, cerebral ganglia have been shown to be
responsible for gametes maturation and spawning (Lubet,
1959; Antheunisse, 1963; Nagabhushanam and Mane, 1973;
Mane, 1986). In the light of this manipulation, the
experiments performed by Kulkarni (1987) and Rao (1988)
suggested that the cerebral ganglia play a vital role in
the physiological functionings of freshwater mussels
which include aspects like respiration, mobilization of
biochemical reserves from different body components and
stage of maturity of gonads. The study with surgical
operations of ganglia on the gonad were not performed on
*Lamellidens corrianus* in the present study. However, it
has been established from our laboratory that cerebral
and visceral ganglia in marine and freshwater bivalves
are responsible in modifying the physiological
activities of the individual species of Bivalvia. This
aspect has been nicely covered by Rao (1988) on
*Lamellidens marginalis*. Further insight in the
secretory product, either neurotransmitter or neurohormonal, requires a detailed examination. In this connection it is appropriate to state that there is now considerable evidence from the experiments conducted on a large number of invertebrates to suggest that neurosecretory mechanism actually do have the general purpose of coordinating environmental changes with developmental and physiological events within the animals. The environmental cues to which the NSCs response usually precede the situation which necessitate particular development and physiological changes. The animal is thus preadapted to take the advantage of favourable or to avoid unfavourable conditions.
SUMMARY

1) The freshwater bivalve, Lamellidens corrianus, 60-65 mm in shell length from Godavari River at Kaygaon near Aurangabad, was subjected for histological examination of cerebral and visceral ganglia to understand the neurosecretory cycle of neurosecretory cell (NSCs). Fortnight collections were subjected for the study.

2) Two types of cells have been observed in both the ganglia, designated as Type I and Type II. Both the cells differ in the measurements and a distinct neurosecretory cycle is seen in Type I cells.

3) In the neurosecretory cycle the secretory granules first appear throughout the cytoplasm, then concentrate along the nucleus, followed by an accumulation in the axon hillock, and during this accumulation the granules can be found in the proximal part of the hillock. These stages are conspicuously seen in Cell Type I of the cerebral ganglia than visceral ganglia.

4) Neurosecretory activity has been correlated with gametogenesis, maturation and release of gametes. This cycle begins in June, July, August, January, February, and March during the period of gametogenesis. Accumulation of neurosecretory material was more during
September second fortnight to December second fortnight and again during February and April. This stage was prominently noticed in both the fortnights of November and December at the time of release of almost all mature gametes. With the maximum emptying of gametes during November, December and April the neurosecretory material was released and traces were seen in the hillock and proximal part of the axon.

5) Distinct neurosecretory cycle correlating reproductive cycle in Cell Type I from visceral ganglia was not seen.

6) Measurements of the cells of Type I from both the ganglia revealed significant changes in cell length and nuclear diameter during the annual reproductive cycle.

7) The results are discussed in the light of the animals reproductive cycle.
BIBLIOGRAPHY


General summary and conclusion
GENERAL SUMMARY AND CONCLUSION

The bivalve shellfishes in India are exploited for various purposes. The need for popularising these shellfishes as food is great. Apart from their edibility value they are used as bait for fishing, and shells for multiple use like preparation of toys, ornaments, utility articles, and also in lime, cement and paint industries. Personal literature reveals that considerable attention has been paid on the shellfishes from the coastal areas in understanding their eco-physiological and biological aspects. Comparatively the work in the similar direction on the shellfishes from the freshwater areas are less. In fact these freshwater shellfishes are fished in our country for multiple use but they have less edibility value. For scientific basis of management decision, the application of research data for benefit of fisheries should be considered worthwhile. Thus the development of any fishery is dependent on the physiological and ecological status of the animal in its local environment. Amongst the several aspects studied by many investigators, the subject of reproduction has received considerable research interest. Studies on
reproduction are important in ecological investigations since they provide important data relating to distribution and population structure and also enable accurate predictions to be made concerning recruitment to the population. Amongst the freshwater bivalves Lamellidens corrianus is abundantly distributed along the banks of the rivers in Maharashtra State. This species has received comparatively little attention in understanding the reproductive physiology. Hence, the present study was directed in understanding the seasonal variations in the aspects of heart beats, gill ciliary activity, respiration, reproduction, neurosecretory cycle and biochemical compositions of different soft tissues. These aspects were studied on the freshly collected samples of adult Lamellidens corrianus from the banks of the Godavari River at Kaygaon 42 Km away from Aurangabad during every fortnight. The data were collected over a period from May first fortnight of 1986 to April second fortnight of 1987.

The rate of respiration of the whole animal significantly alters from one fortnight to another in all the months and the total variation for the entire study period accounts for 78.43 %. The respiration varies in relation to the rise in temperature and increase in the day length during summer months.
Variations in nutritional levels along the banks of the Godavari River influencing the rate of respiration are discussed. According to the timing of completion of reproductive events and the growth of the animals itself the rate of respiration also varies. The fluctuations in the rate of respiration are correlated with the changes in the biochemical metabolites from different body parts. Possibilities of involvement of hormones, neurotransmitters and gonadal steroids in changing the rate of respiration during the seasonal cycle has also been discussed. Measurements of the rate of heart beats (diastolic) and the gill ciliary activity (of isolated entire inner demibranch) also significantly alter from one fortnight to another throughout the study period. The data on these two aspects have been seperately compared with the data on respiration during discussion of the results. Probable rate of increased total carbonates in the water at the habitat of the animal altering the rate of gill ciliary activity is also discussed. Correlation between the low food availability, decreased rate of respiration, exhaust of organic reserves and the field population undergoing starvation stress, and gill ciliary activity has been made. Probable rate of brooding of glochidia in the outer demibranch in altering the gill ciliary activity, particularly during many gametes are shed, is also
discussed. Endogenous regulation via neurotransmitters is also considered while discussing the results. Changes in the rate of heart beats are compared with low oxygen content of the water. The rates of heart beats have been correlated with the starvation and active feeding in the field population. Probable involvement of neurotransmitters in regulating the heart rates is also discussed. The data on gill ciliary activity and heart rates more or less follow a parallel trend rather than with respiration. The changes in the whole body weight and of different body parts like mantle, foot, hepatopancreas and gonad show significant fluctuation in relation to the gonad development and release of gametes, as well as with the changes in the biochemical composition in different fortnights. Maximum and minimum accumulation of glycogen in mantle and foot during certain periods of the year have been discussed to their functional significance. Similar functional significance of lipid and protein in these organs is discussed in the light of alternative source of energy utilization. Mobilization of glycogen from hepatopancreas to gonad has been correlated with the gametes development and to meet the energy demand of other tissues during the starvation in the field population. Correlation between the changes in the
lipid content from gonad with the gametes development has been made. The data on the seasonal variation in the biochemical composition are discussed in the light of environmental impact and reproduction. Study on the gross reproductive system reveals that the animal is a hermaphrodite, and male and female follicles are regionally distinct in separated zones, however mixed follicles of both the sexes are seen in the region of zonation. A single gonoduct opens anterior to renal opening near the exhalent siphon. Outer demibranch creates ovisac for brooding glochidia, whose thickness varies with the loading of glochidia. Gonad follicles are innervated with connective tissue and muscles. Follicles consist of outer thin epithelial layer and inner muscular strands. Fortnight histological preparations of gonad tissue show that males mature first than females during July and August but such distinction is not so clear from September onwards. Male follicles undergo recovery earlier than females which is seen first in males in May and females in June. Seven stages are differentiated from the histological preperations - 1) Gametogenesis, 2) Active gametogenesis, 3) Mature, 4) Partially shed gametes, 5) Many shed gametes, 6) Fully shed gametes and 7) Recovery. Gametogenesis occurs in males during June first fortnight and in December second fortnight, where
as in females during June second fortnight and January first fortnight. Active gametogenesis occurs in males during July first fortnight, August second fortnight, January second fortnight and February second fortnight. In females this activity is seen in July second fortnight, August second fortnight, January second fortnight and March first fortnight. Gonad matures in August second fortnight, October second fortnight, December first fortnight, January second fortnight and March second fortnight in males, while in females in September first fortnight, October second fortnight, December first fortnight, January second fortnight and March second fortnight. Partially shed gonad follicles of both the sexes is seen in September first fortnight (male follicles), September second fortnight (female follicles), November first fortnight, December second fortnight, February (first fortnight in male follicles and second fortnight in female follicles) and April first fortnight. Many shed condition of the gonad follicles of both the sexes is seen in October first fortnight, November first fortnight, December first fortnight, February first fortnight and April first fortnight. Fully shed gonad follicles of both the sexes are seen in November first and second fortnights, December second fortnight, March first fortnight and
April second fortnight. Thus, the animal spawns during October-November, the intensity of which decreases from December to April. The results are discussed in the light of exogenous factors affecting the gonad maturation and shedding of gametes. The histological examination of cerebral and visceral ganglia has been made by using signalatic staining reactions for neurosecretory cells to understand the neurosecretory cycle of the neurosecretory cells. In both the ganglia two types of NSCs viz. Type I and Type II are seen whose measurements differ and a distinct neurosecretory cycle occur in Type I cells. The neurosecretory cycle starts with the appearance of secretory granules throughout the cytoplasm which concentrate along the nucleus, followed by its accumulation in the axon hillock, and during this accumulation the granules can be found in the proximal part of the hillock. Such cycle is conspicuously seen in cerebral ganglia than visceral ganglia. Neurosecretory activity has been correlated and discussed with the stages of gametogensis, maturation and release of gametes. Measurements of tinctorial properties of both the type of cells revealed that significant changes in the cell length and nuclear diameter of Type I cells occur during the annual reproductive cycle. The results are discussed in the light of annual reproduction of the species.
It is concluded that the physiological aspects of the regulation of rate of respiration, heart beats and gill ciliary activity are interrelated both in respect to the impact exogenous and endogenous factors. The animals can increase or decrease these rates according to the need of body maintenance metabolism, growth and reproduction. The reproduction takes place with the advent of favourable environmental conditions at which time the body reserves are also build up. During certain periods of the year body parts having functionally importance store more biochemical composition to meet with the drastic conditions. According to the favourable situation the gametes are released and the body weight declines but is regained as the gametes develop. The biochemical composition like glycogen, protein and lipid vary from hepatopancreas and gonad according to the need of gametes development. Food available to the animal from the surrounding water appears to determine the period of gametes shedding and build-up of biochemical reserves. Apart form exogenous factors affecting the physiological aspects of reproduction endogenous factors via neurotransmitters and neurohormones appear to play important role. Amongst endogenous factors, the aspect of neurosecretion has been elaborated in this study and it is found the
cells of Type I from both cerebral and visceral ganglia play an important role in reproductive physiology of this animal.

From the present study it can be further stated that the distribution and biology of the bivalves shellfishes is influenced by local ecological factors like temperature, pH, oxygen content, rainfall, carbonates, draught conditions, presence of suitable micro-organisms, type of soil, water flow system, fishes as host for glochidia etc. Such parameters require special attention while elaborating the impact of exogenous factors upon the ecophysiology of freshwater bivalve species. Amongst the endogenous factors wide occurrence of Ach, 5-HT and catecholamines (predominantly dopamine) in the nervous system, and neurohormones in the NSCs require special attention since these two endogenous factors have a considerable influence on the physiological activity of the bivalve shellfishes.