

CMFRI

bulletin 42

Part One

AUGUST 1988



NATIONAL SEMINAR ON SHELLFISH RESOURCES AND FARMING

TUTICORIN

19-21 January, 1987

Session - I

CENTRAL MARINE FISHERIES RESEARCH INSTITUTE
(Indian Council of Agricultural Research)
P. B. No. 2704, E. R. G. Road, Cochin-682 031, India

39 NEUROENDOCRINE REGULATION IN LAMELLIBRANCH MOLLUSCS

R. Nagabhushanam and U. H. Mane

University Department of Zoology, Marathwada University, Aurangabad-431 004.

ABSTRACT

Gametogenesis and spawning in lamellibranch molluscs may be controlled by either exogenous or endogenous factors. Though the factors like salinity or temperature in such studies have been widely elaborated by various workers, the endogenous regulation still remains in its infancy. Our experimental studies, based on the classic histological staining of the neurosecretory material, have revealed neurosecretory cells in the central ganglia of lamellibranch molluscs. The tinctorial properties of these cells may vary from species to species. The pyriform (pear-shaped) neurosecretory cells from cerebral ganglia revealed such endogenous regulation system affecting the release of sex products. There exists four successive stages in the passage of neurosecretory product within the shell body. This has been extensively worked out in case of *Perna* sp. from Ratnagiri coast. The data have been correlated with the gonad maturation stages and spawning.

INTRODUCTION

Reproduction in Indian lamellibranch molluscs has been studied extensively. Much of the literature is concerned with reports on annual breeding period. Reproduction is cyclical, and it may be annual, semiannual, or continuous. Reproductive phases such as gonad development, spawning and fertilization, and development and growth, functioning continually in coordination with seasonal environmental changes, produce the pattern characteristic of a species in a given area. The timing and duration of reproductive activity may be determined through interaction between endogenous and exogenous factors. The synchronization of breeding periods with the environmental conditions most favourable for the development and growth of the progeny is obviously significant for reproductive success. Though voluminous publications on the reproduction in lamellibranchs from Indian coastal waters exist, little attention has been given on endogenous regulating system during gonadal maturation and spawning in these animals. In this report we cover the work carried in different laboratories in abroad and India, including our laboratories on the endogenous regulating system during gonadal maturation and spawning of these molluscs.

In bivalve molluscs, nervous system and hormonal apparatus are not sharply separated and no endocrine glands have so far been

encountered. It is, therefore, possible that hormonal activity is restricted to the nervous system itself. Thus, nervous system and hormonal system are interrelated structurally and functionally, which is supported by the fact that the secretory cells occur in ganglia of these molluscs. The nervous system plays a role in neurotransmission as well as in the synthesis and discharge of secretion. Neurons secrete both neurohumors and neurohormones. Studies on neurohumors have involved their chemical nature (Milton and Gosselin 1960; Sweeney 1963; Zs Nagy et al 1965; Paparo 1972; Stefano and Aiello 1975), their control of ciliary activity (Aiello 1962, 1970; Paparo and Aiello 1970) and their influence on oxygen consumption (Moore et al 1961; Moore and Gosselin 1962).

Neurosecretion in bivalves has been reviewed by Gabe (1965, 1963), Lubet (1966, 1973), Martoja (1972), and Golding (1974). The development of subject has been hampered by the presence of shell, by the diffuse distribution of the neurosecretory cells, and by the ignorance of the chemical nature of the neurohormones. Presence of neurosecretory cells has been demonstrated by classic histological studies on a number of species. Their number and location vary among species. Neurosecretory cells are located in cerebral and visceral ganglia. In higher lamellibranchs, neurosecretory cells are less numerous and more localized. These cells are generally found in the dorsal caps of cerebral ganglia and the dorsal cell layer of

visceral ganglia (Lubet 1965a, 1959; Nagabhushanam 1963, 1969; Nagabhushanam and Mane 1973; Mane 1986). The neurosecretory cells were found to be less numerous in the cerebral ganglia than in the visceral ganglia. The presence of neurosecretory cells in the pedal ganglia is controversial. However, Gabe (1955) and Lubet (1955a) conclude that they are absent in *Mytilus edulis* or *M. galloprovincialis*, but Umiji (1969) in *Perna perna* and Nagabhushanam et al (1972) in *Mytilus (Perna) viridis*, have recorded them as present on the pedal ganglia. The neurosecretory cells have been reported to occur in all ganglia of freshwater mussels *Unio tumidus* (Fahrman 1961), *Dreissena polymorpha* (Antheunisse 1963), *Lamellidens marginalis* and *L. corrianus* (Muley 1985).

In most species neurosecretory cells are small or medium-sized, with an approximate diameter of 20 μ m. Neurosecretory perikarya are avoid or pyriform. The general histological features are similar to those of plasmochrome cells, but neurosecretory perikarya can be distinguished by the marginal position of the Nissl bodies and the presence of acidophilic secretions in the cytoplasm (Gabe 1955, 1966). Different categories of neurosecretory cells have been distinguished, based on their size and morphology. In *M. edulis* and *Chlamys varia* some neurosecretory cells are pear-shaped, unipolar, and upto 25 μ m, while others are small and multipolar (Lubet 1959). Pear-shaped (type I) and oval-shaped (type II) neurosecretory cells were distinguished in *Crassostrea virginica* and *Meretrix casta* (Nagabhushanam 1963, 1969) and *Katelysia opima* (Nagabhushanam and Mane 1973). Different categories of neurosecretory cells have also been reported in the freshwater mussel, *U. tumidus* (Fahrman 1961).

The appearance and position of neurosecretory products within the perikarya vary with the stage of the neurosecretory cycle (Lubet 1955a, b; 1959; Gabe 1966; Gabe and Rancurel 1958; Blake 1972; Nagabhushanam et al 1972). In some cells neurosecretory granules are few, while in others they are abundant and remain discrete. In still other cells, neurosecretory products are present in lumps or pools. The discharge of neurosecretory products is characterized by cytoplasm and the presence of small quantities of secretory products between the

vacuole and axon hillock (Gabe 1966). Signs of axon transport are not very distinct in marine bivalves. Neurosecretory products have been observed in the axon hillock and proximal parts of the interganglionic paths of the axon, but they disappear in the neuropile and are not seen in the communicative branches, commissures or nerves leaving the ganglia. Endocrine glands or neurohemal organs have not been identified in bivalve molluscs. Umiji (1969) has reported that a neurohemal area exists on the cerebral commissure of *P. perna*.

The transport of neurosecretory substances by axons, intermediate cells, and possibly glial cells has been suggested by Lubet (1955b) and Umiji (1969). Several authors have suggested that glial cells play a role in storage and transport and that glial cells and epineurons can function as neurohemal organs (Fahrman 1961; Antheunisse 1963). However, the chemical nature transport, and fate of neurosecretory products is not clearly established in bivalves.

Cyclical activity in neurosecretory cells was observed by histological studies on *C. varia* and *W. edulis* (Lubet 1955a, 1959), *Splisula solidissima*, *M. casta*, *Yoldia* sp., *Modiolus demissus* and *Mullna lateralis* (Nagabhushanam 1963, 1969), *K. opima* (Nagabhushanam and Mane 1973), *P. perna* (Umiji 1969), and *Argopecten (= Acqu/pecten) irradians* (Blake 1972). Seasonal changes in cyclic activity have been related to the reproductive cycle. However, Welsh and Antheunisse (1963) have pointed out that the reproductive cycle is usually seasonal and in turn related to such environmental factors as temperature, food abundance, and light.

Investigations by Lubet (1956, 1959) demonstrated distinct annual neurosecretory cycle in the pear-shaped neurosecretory cells of the cerebral ganglia in temperate species, *M. edulis* and *M. galloprovincialis*. The annual neurosecretory cycle and gametogenesis cycle in these mussels appear to be closely correlated. Secretory material is accumulated in the cerebral ganglia during gametogenesis, and the evacuated from the cells when the gametes become fully mature. The small multipolar neurons and the neurosecretory cells of the visceral ganglion in these mussels showed continuous activity

throughout the year. These observations were confirmed in oyster *C. virginica* by Nagabhushanam (1963, 1964). In oysters the activity of type I cells varies during the year; the neurosecretory material accumulates in January and reaches a maximum in March. The number of cells containing secretion decreases between April and September; the cells are emptying between October and December. In freshwater mussel, *D. polymorpha*, the neurosecretory material begins to accumulate in the cerebral ganglia in autumn; maximum activity takes place during winter. A period of inactivity occurs in summer. In the visceral ganglia, the cycle is similar to that in the cerebral ganglia, but emptying takes place during summer (Antheunisse 1963). Blake (1972) examined the effect of temperature and starvation on neurosecretory activity and oogenesis in *Argopecten* (= *Aegulpecten*) *irradians* population from Massachusetts, U.S.A. The annual cycle of neurosecretory activity of the population was divided into five stages based on changes in size, granulation, and vacuolization of the neurons. The neurosecretory cycle stages were found to coincide with the following stages in oogenesis: stage I, vegetative or resting stage with primary germ cells; stage II, oogonia and early oocytes; stage III, cytoplasmic growth phase; stage IV vitellogenesis and maturation; stage V, spawning. For the first 12 months, the neurosecretory cycle and reproductive cycle of the population were highly synchronous. Both cycles showed significant correlation with seasonal changes in temperature but not with each other.

in adult population of the tropical bivalve, *C. op/ma* from the west coast of India, gametogenesis, followed by spawning and resting phases, occurs twice each year. Neurosecretory product begins to accumulate in the type I cells with the initiation of gametogenesis and reaches a maximum when the animals are mature (Nagabhushanam and Mane, 1973). Secretory granules in the neurosecretory cells decrease with spawning and are not seen in resting animals. The neurosecretory cycle and reproductive cycle closely parallel each other (Fig 1). In another adult population of the bivalve *Crassostrea gryphoides* from the west coast, gametogenesis, followed by maturation and spawning, occurs once in each year but a phe-

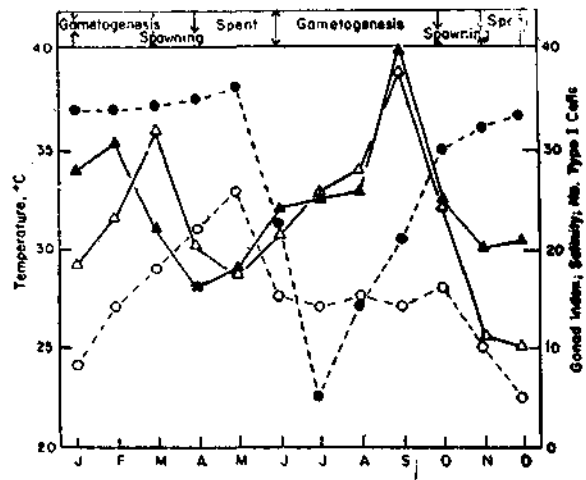


Fig. 1. Reproductive cycle and the number of Type I cells containing neurosecretory granules in *Katelaysia opima* relative to changes in temperature and salinity. O, temperature (°C); Δ , gonad index; \bullet , salinity (%); \blacktriangle , number of Type I cells. (From Nagabhushanam and Mane 1973, 1976; modified)

nomenon of sex reversal has been reported during July and September and again in April and May (Mane and Nagabhushnam 1976). Secretory granules in type I cells increase during August with mature gonads and by November most of the type I cells containing secretory granules decreased with emptying of gonads. From February onwards once again the type I cells with secretory granules increase in number. This clearly indicates parallel relationship in

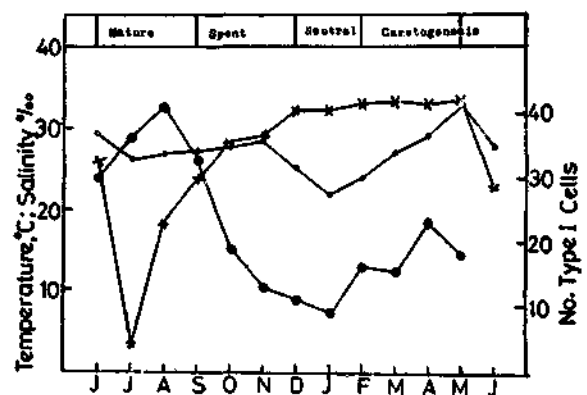


Fig. 2. The number of Type I cells containing neurosecretory granules in *Crassostrea gryphoides* relative to changes in temperature and salinity. \bullet , temperature (°C); X, salinity (‰); \blacktriangle , number of Type I cells. (From Mane and Nagabhushanam 1976; modified).

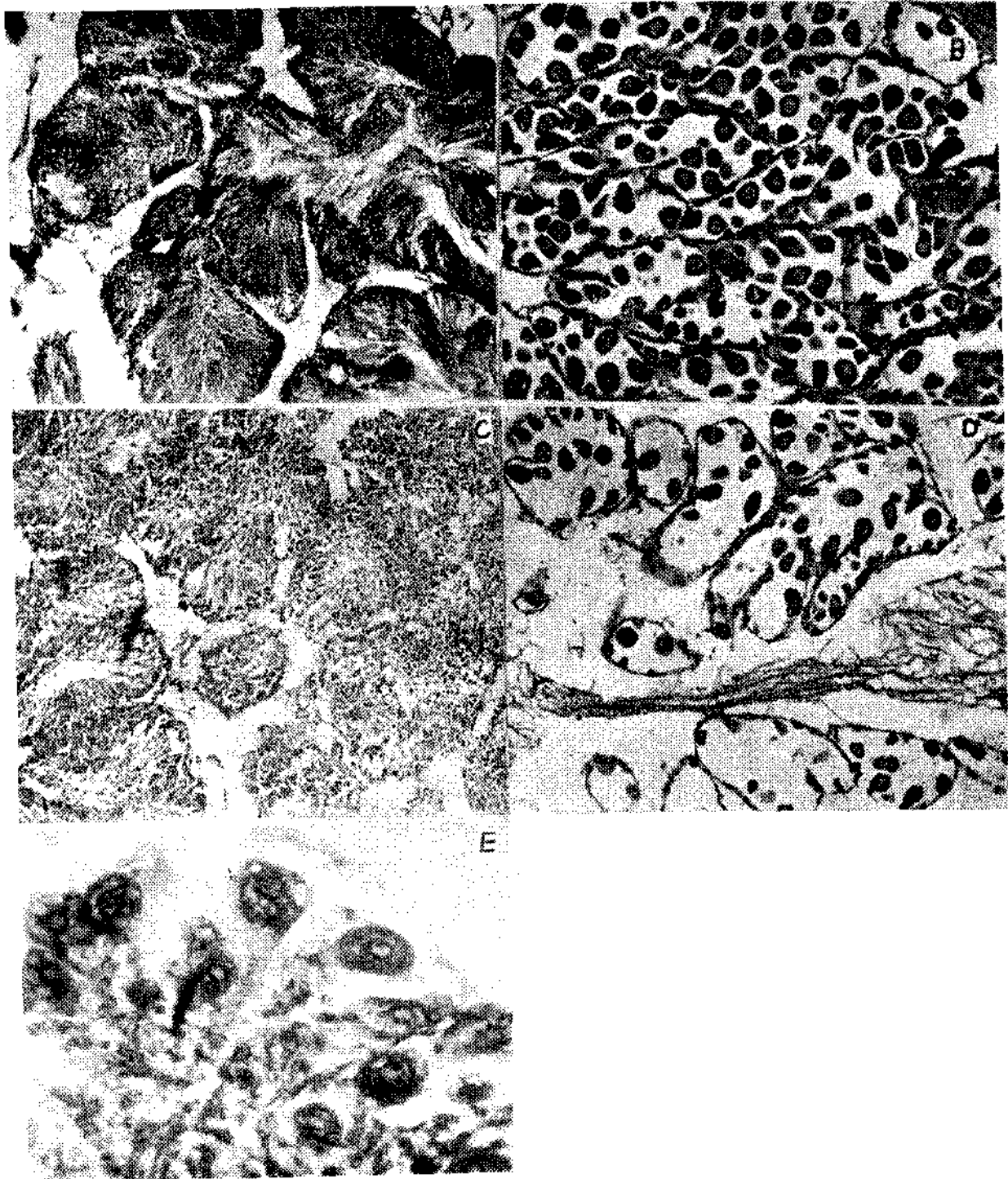


Plate 1, a-e. Effect of removal of cerebral ganglia in March in *Katelysia opima* on gametes from mature gonads. A, non-operated male; B, non-operated female; C, operated male; D, operated female; E, cerebral ganglion showing neurosecretory cells. (magnification X 400)

neurosecretory cycle and reproductive cycle but the authors could not ascertain the role of type I neurosecretory cells during sex reversal since only a small population was in hermaphroditic condition (Fig 2)

Based on the situation of neurosecretory granules in the pear-shaped cells, in another tropical species *M. (= P) viridis* Nagabhushanam et al (1972) determined successive stages of the neurosecretory cycle. Four stages recognized are, a) cells with uniform dispersed granules, b) cells with perinuclear concentration of granules, (c) cells with accumulation of granules in the axon hillocks, and d) cells with granules in the proximal part of the axons. The authors found that the functioning of the neurosecretory cells in the same individual is not strictly at the same time but in any chosen individual most of the neuro-secretory perikarya are more or less in the same stage. Thus, it has been suggested that the secretory granules first appear throughout the cytoplasm, then concentrates around the nucleus, followed by accumulation in axon hillock and in the proximal part of the axon. On the basis of these findings Mane (1986) reported seasonal changes in the stages of the neurosecretory cycle of cerebral and visceral ganglia of *P. viridis*. Stage (a) is distinguished by the scattered neurosecretory material in the cytoplasm when compared with other stages and this stage is found in maximum number of neurosecretory cells during post-monsoon and early part of summer, especially during October and April. Stage (b) is the intermediate stage wherein the neurosecretory material gets concentrated around the nucleus. This stage dominates in the cells during late summer and late post-monsoon. Stage (c) is distinguished by the accumulation of neurosecretory material in the hillock and this is seen in maximum number of cells during early monsoon and early winter. Stage (d) is so-called end phase of the neurosecretory cycle in which the neurosecretory material is found not only in the axon hillock but also in the proximate part of the axon. The maximum number of cells in this stage are found in late monsoon and late winter. This cycle showed that immediately after the reproduction maximum number of neurosecretory cells in

stage (a) occur in April and October, thereafter stage (b) reaches peak in May and November and during July and January stage (c) dominates followed by stage (d) in August and February, Thus, discharge of neurosecretory material is much increased just before and during spawning. In visceral ganglia the neurosecretory cells also show the maximum of various stages succeeding each other during the seasons of the year. Stage (a) and stage (b) have maxima during late summer and early monsoon and winter, whereas stage (c) and stage (d) during post-monsoon to early summer. Comparing the seasonal secretory stages of the neurosecretory cells from cerebral and visceral ganglia, a striking difference exists in the discharge of neurosecretory material during monsoon and late winter, whereas in visceral ganglia discharge continues to early summer (Fig 3).

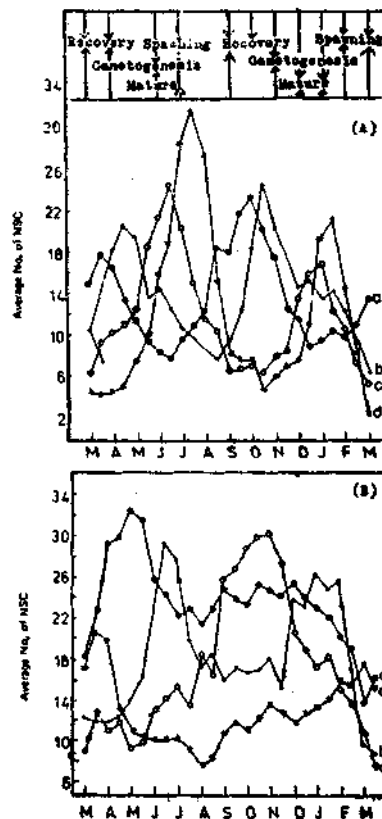


Fig. 3. The average number of dorsal neurosecretory cells of cerebral ganglia (A) and visceral ganglia (B) in *Perna viridis* in successive stages 1-4 during different months relative to changes in gonadal condition. a, stage 1; b stage 2; c, stage 3; d, stage 4. Nagabhushanam and Mane 1976; Mane 1986; modified.

Demonstration of the role of neurosecretion in the reproduction of bivalves has been difficult with standard surgical procedures. Bilateral ablation of cerebral ganglia during the resting phase and at the beginning of gametogenesis delays gametogenesis in *M. edulis*; the few gametes formed may undergo lysis before spawning (Lubet 1959, 1965). Removal of ganglia at latter stage of gametogenesis accelerates gamete maturation. After ablation of ganglia during the resting phase the amount of RNA and lipid is decreased and glycogen is increased in gonadal connective tissues. Antheunisse (1963), who worked with freshwater animal *Dreissena polymorpha*, has suggested that premature spawning may be a function of the intensity of the operative shock when ablating the ganglia. Antheunisse concluded that, in spite of a parallelism between the neurosecretory and reproductive cycles in this species, a direct causative relationship did not operate. Umiji (1969) has found that ablation has no effect on follicle development nor on the intrafollicular connective tissue. Removal of cerebral ganglia in March, just before spawning, of *K. opima* show release of gametes; females are much more responsive than males and immediately release gametes (Nagabhushanam and Mane 1973) PII, a-e. Similarly, ablation of cerebral ganglia in oyster, *C. gryphoides* in July at the time of mature gonads also release gametes. (Mane and Nagabhushanam 1976). In *P. viridis*, a large number of mussels operated in late June and early July at the time of mature gonads release gametes after cerebralelectomy, in contrast to those operated during previous and next period (Mane 1986). Operation of mussels in similar way in late winter give spawning reaction in few mussels compared to those operated in monsoon. The author further stated that comparing the spawning reaction in cerebralelectomized and visceralectomized mussels, the latter do not show accelerated spawning as cerebralectomized ones. Although cerebral ganglia appear to secrete neurosecretory substances during gametogenesis, it is impossible to decide the mechanism is nervous or hormonal in nature. If the activities of the neurohormones are important, removal of an internal inhibition, such as the neurosecretory product of the cerebral ganglia, may allow the animal to become

receptive to external stimuli which then induce the release of the gametes,

REFERENCES

- AIELLO, E. L. 1962. Identification of the cilio-excitatory substance present in the gill of the mussel, *Mytilus edulis*. *J. Cell. Comp. Physiol.*, 60 : 17-21.
- AIELLO, E. L. 1970. Nervous and chemical stimulation of the gill cilia in the bivalve mussel. *Physiol. Zool.*, 43: 1-11.
- ANTHEUNISSE, L. J. 1963. Neurosecretory phenomena in the zebra mussel *Dreissena polymorpha* Pallas, *Arch. Neerl. Zool.* 15 : 237-314.
- BLAKE, N.J. 1972. Environmental regulation of neurosecretion and reproductive activity in the bay of scallop, *Aequipecten irradians* (Lamarck), Ph.D. Thesis, University of Maryland.
- FAHRMANN, W. 1961. Licht und Electronenmikroskopische Untersuchungen des Nervenstems von *Urtio tumidus* (Phillipsson) unter besonderer Berücksichtigung der Neurosekretion. *Z. Zellforsch. Mikrosk Anat.*, 51: 1-10.
- GABÉ, M. 1955. Particules histologiques de cellules neurosecrétices chez quelques lamellibranches, *C. R. Acad. Sci.*, 240 : 1810-1812.
- GABÉ, M. 1965. La neurosecretion chez les mollusques et ses rapports avec la reproduction. *Arch Anat. Microsc. Morphol. Exp.*, 54: 371-385.
- GABÉ, M. 1966. Neurosecretion", Pergamon, Oxford.
- RANCURREL 1958. Caracteres histologiques des cellules neurosecrétices chez quelques. *Teredo. Bull.* 20 : 73-78.
- GOLDING, D. W. 1974. A survey of neuroendocrine phenomena in nonarthropod invertebrates. *Biol. Rev. Cambridge Philos. Soc.*, 49 : 161-224.

- LUBET, P. 1955a. Cycle neurosecretoire chez *Chlamys varia* Mytilidae (Mollusques Lamellibranches). *C R Acad. Sci.* 241 : 119-121.
- LUBET, P. 1955b. Effects de l'ablation des centres nerveux sur l'émission des gametes chez *Mytilus edulis* L. et *Chlamys varia* L. (Moll. Lamellibranches). *Ann. Sci. Nat. Zool. Biol. Anim.*, 2: 1-9 3
- LUBET, P. 1956. Effects de l'ablation des centres nerveux sur l'émission des gametes chez *Mytilus edulis* L. et *Chlamys varia* L. (Mollusques, Lamellibranches). *Annales Sciences Naturelles* 18:175-183.
- LUBET, P. 1959. Recherches sur le cycle sexuel et l'émission des gametes chez les Mytilides et les Pectinides. *Rev. Trav. Inst. Perches Merit.* 23 : 389-548.
- LUBET, P. 1965. Incidences de l'ablation bilaterale des ganglions cerebroides sur la gametogenese et le developement du tissu conjonctif chez la moule, *Mytilus galloprovincialis* Lam. (Moll. Lamellibranches). *C. R. Soc. Biol.*, 159: 397-399.
- LUBET, P. 1966. Essai d'analyse experimentale des perturbations produites par les ablations des ganglions nerveux chez, *Mytilus edulis* L. et *Mytilus galloprovincialis* Lam. (Mollusques Lamellibranches). *Ann Endocrinol.*, 27 : 353-365
- LUBET, P. 1973. "Expose synoptiques des Donnees Biologique sur la Moule *Mytilus galloprovincialis* Lamarck 1819". Synopsis FAO sur les Perches No. 88 (SAST-Moule, 3, 16 (1970), 028108 pag. var.), FAO, Rome.
- MANE, U. H. 1986. Neurosecretory phenomena of Indian Green Mussel, *Perna viridis* (L). Proceedings of the 8th International Malacological Congress, Budapest, 1983(1986); 151-156.
- MANE, U. H. AND NAGABHUSHANAM. R. 1976. A study on the Reproductive Biology of Indian Oysters, *Crassostrea gryphoides*. *Natural Sciences Journal Marathwada University, Aurangabad.* 15 (8); 245-258.
- MARTOJA, M. 1972. Endocrinology of Molluscs. In *Climical Zoology* (M. Florin and B. F. Scheer, eds.), pp. 349-392. Academic Press, New York.
- MULEY, D. V. 1985. Effect of Pollutants on Freshwater Molluscs, from Godavari River at Paithan. Ph. D. Thesis, Marathwada University (India).
- MILTON, A. S. AND GOSSELIN R. E. 1960. Metabolism and cilio-accelerator action of 5-hydroxytryptamine (5-HTP) in gill plates of *Mytilus* and *Modiolus*. *Fed. Proc Fed Am. Soc Exp Biol.* 19; 126.
- GOSSELIN R. E. 1962. 5-hydroxytryptamine on the anaerobic metabolism and phosphorylase activity of lamellibranch gill. *J. Pharmacol. Exp. Ther.* 138 : 45-53.
- MOORE, K E., A. S. MILTON AND GOSSELIN R. E. 1961. Effect of 5-hydroxytryptamine on the respiration of excised lamellibranch gill. *Br J. Pharmacol.*, 17-278-285
- NAGABHUSHANAM, R. 1963. Neurosecretory reproduction in the bivalve *crassostrea virginica*. *Indian J. Exp.* 151-152
- NAGABHUSHANAM, R. 1964. Neurosecretory changes in the nervous system of the *Crassostrea virginica*, induced by experimental conditions. *Indian J. Zool.*, 2: 1-4
- NAGABHUSHANAM R. 1969. Studies on neurosecretion in mollusca. *Proc Symp. Mollusca, Marine. Biological Association of India, Pt. 2* : 572-579.
- NAGABHUSHANAM, R. AND U. H. MANE. 1973. Neurosecretion in the clam, *Katylsia opima*. *Marathwada Univ. J. Sc.*, 12 :193-203.

- NAGABHUSHANAM, R. B. M. MANTALE, U. H. MANE, AND R. S. DESHMUKH. 1972. On the Neurosecretory cells in the central ganglia of Mussel, *Mytilus viridis*. R. Natarajan (ed), *Recent Researches In Estuarine Biology*, Hindustan Publishing Corporation, Delhi" 105-108
- PAPARO, A. 1972. Innervation of the lateral cilia in the mussel, *Mytilus edulis* L. *Biol Bull. (Woods Hole, Mass)*, 143: 592-604.
- PAPARO, A. AND E. AIELLO 1970. Cilio-inhibitory effects of branchial nerve stimulation in the mussel, *Mytilus edulis*. *Comp. Gen. Pharmacol.*, 1 : 241-250.
- STEFANO, G. B., AND E. AIELLO 1975. Histofluorescent of serotonin and dopamine in the nervous system and gill of *Mytilus edulis* (Bivalvia). *Biol Bull. (Woods-Hole, Mass.* 148: 141-156
- SWEENEY, D. 1963. Dopamine : Its occurrence in molluscan ganglia. *Science*, 139: 1051.
- ^MIJI, S. 1969. Neurosecrecao em *Mytilus pgma* Molusco- Lamellibranchio). *Zool. Bol. Mar, Saopaulo Nova Ser.*, 26: 181-254.
- ZSNAGY, I., K. ROSA, I. FOLDES, L. PÉREZ, J., AND D. DEMETER 1965. Subcellular localization of 5-hydroxytryptamine in the central nervous system of lamellibranchiates. *J. Neurochem.*, 12:245-251.