CHAPTER ONE

INTRODUCTION
Mussels have been popular research material for experimental studies. They have also been a subject of considerable amount of research effort, reflecting both their ecological and economic importance. Their geographical distribution is worldwide. They have, therefore, aroused interest not only in the factors controlling their own population ecology, but also in their role in the structure and function of the communities of which they are a characteristic and important component. Mussels form an important element in the shellfish industry. The harvesting of the mussels as food extends a long time into history but recent development in resource management and cultivation shows promise that the considerable potential of mussels as a source of protein may soon be realized in many countries. Their shells are used as poultry-feed and in the manufacture of lime and Ayurvedic medicines. Formerly the shells of the freshwater Bivalves were also used in the manufacture of buttons. Hence, in countries like the United States, Japan, India, England, France, Australia and Canada a good amount of work has been carried out in connection with the propagation of commercial species of mussels (Erman and Engelmann, 1833; Posner, 1875; Thiele, 1886; Mac Alpine, 1888; Lotsy, 1893; List, 1902; Wallengren, 1905a,b; Churchill and Lewis, 1924; Halstead, 1965; Robenson, 1968; Mason, 1972; Milne, 1972; Mostert,
Historical

An effort has been made to have the review of the literature as complete as possible; concerned with taxonomy, morphology, biology, sex-cycle along with biochemical and histochemical aspects of fresh water mussels. A reference is also made to the number of research papers related to the same subject in other species of mussels on account of their bearing upon certain principles involved.

Taxonomy, Morphology and Biology

In taxonomic studies the important features of the shell, gill and foot are considered by the earlier contributors (Goldfuss, 1820; De Balinville, 1825; Simpson, 1900; Ortmann, 1911; Preston, 1915). Accordingly, they have been designated as Bivalvia, Lamellibranchiata or Pelecypoda under phylum Mollusca. Simpson (1900) included the fresh-water mussels in the family Unionidae which includes genera like Unio, Anodonta, Lamellidens, Pseudodon and Parreysia having economic importance. Preston (1915), Prashad (1918a,b), Satyamurti (1960), Patil (1965, 1968) have taken systematic survey of freshwater mussels from Indian water.
Apart from the systematic studies, morphological and anatomical investigations appeared from time to time (Blanford, 1866; Lea, 1835, 1870; Simpson, 1900; Blanford and Godwin-Austin, 1908; Preston, 1915; Patil, 1968 and 1969). Anatomical work has thrown much light on the exact systematic position of bivalve species where true relationship could hitherto be correctly inferred from the study of their shell and external characters alone. Prashad (1918a,b, 1919a,b, 1925) has mainly concentrated his studies on marsupium and glochidium of freshwater mussels and tried to classify the different genera under family Unionidae on the basis of glochidial structure. The anatomy of Lamellidens marginalis has been studied by Ghosh (1918). Patil (1965) has studied the anatomy of various organ systems of a freshwater mussel, Lamellidens corrianus. Bloomer (1930, 1931), Patil (1967), Ghosh and Ghosh (1972) have studied the reproductive organs of freshwater mussels. Purchon (1956, 1957, 1958, 1959) has mainly studied the morphology of bivalve stomach and tried to differentiate it into five categories. Dinamani (1967) has reported variation in structural pattern of stomach and correlated it with interaction between stomach wall and digestive diverticula. Recently, Moueza et al. (1979) have studied fine structure of the gastric wall in lamellibranchs Donax trunculus.
The anatomical and histological work later on extended to digestive diverticula, crystalline style, reproductive organs and neurosecretory cells. List (1902), Potts (1923), Yonge (1926a) have studied the digestive diverticula of the mussels. Recently, Owen (1972) has described the fine structure of the tubule cells from the digestive diverticula in *Mytilus edulis*. The digestive diverticula have also been studied with reference to extra and intra cellular digestions. Kato and Kubomura (1954), Kamat (1955, 1957), Lobo and Kamat (1968), Reid (1968), Lomte (1973) have reported the activities of various digestive enzymes like amylase, cellulase, maltase, sucrase, lipase, etc. Payne (1973) has studied the lipid digestion and storage in the littoral bivalve *Scrobicularia plana*. The occurrence of the lysozyme enzyme in marine mussel, *Mytilus edulis* and its role in utilization of bacteria during digestion has been reported by McHenry et al. (1979).

Occurrence of crystalline style is one of the important diagnostic features of the mussel. It is gelatenuous, rod-like structure having mucoprotein. It protrudes from the style sac into the stomach and abuts against the gastric shield. Mitra (1901), Nelson (1918, 1925), Yonge (1926b), Kubomura (1958a, b), Goreau et al. (1966) have done major contributions regarding the morphology and development of the crystalline
style. Various views have been put forth in connection with its formation. Edmonston (1920), Nelson (1925), Goreau et al. (1966) have indicated that the style is secreted by the cells of typhlosole present in the intestine. Others have claimed that it is formed by the epithelial cells of the style sac (Yonge, 1926b). Kato and Kubomora (1954) have suggested that the underlying subepithelial tissue of the stomach along with typhlosole and style sac contribute to the formation of crystalline style. Kubomura (1958b) is of the opinion that the style is secreted by the special cells surrounding the stomach. Mane and Patil (1976) are of the opinion that the midgut-gland associated with the style sac mainly involves in the formation of the crystalline style. The association of β-glucuronidase with crystalline style has been reported by Varute (1969) in *Lamellidens marginalis*. Lipase activity of the crystalline style has been reported by Payne (1978) while association of lysozyme enzyme with crystalline style by McHenry et al. (1979).

The information concerning the food requirement and feeding habit became important in relation to the propagation of commercial species of mussels. Naturally, it is very essential to know the exact feeding methods and food requirements of the juveniles as well as adults. Erman and
and Engelmann (1833) have pointed out that the currents set of by the action of cilia are responsible for carrying the food material towards the mouth. Thiele (1886) has considered the structure and position of the labial palp while explaining the transport of food material, driven by the gills towards the mouth. The food material generally passes along the ciliated groove present at the ventral edge of the gill and then towards the mouth (Posner, 1875). Lotsy (1893) working with marine mussels has come to the conclusion that the mussels can discriminate between various sorts of food materials, but made no statement relating to the mechanism by which this is accomplished. Churchill and Lewis (1924), Patil (1965, 1976) have noticed wide varieties of diatoms, desmids, algal filaments, spores and fragments of animal tissue along with the debris in the stomach of bivalves. Experimental work of Kellogg (1915) and Patil (1965, 1976) suggested that the mussels do not select or separate food organism from other water-born particles. Allen (1914) has noticed that the particles brought into the mantle cavity get entangled into the mucus before reaching to the mouth. Kellogg (1915), Churchill and Lewis (1914) and Patil (1976) are of the opinion that the gills and labial palps are actively involved in the process of feeding. White (1937) has suggested that the primary function of gill is to collect the food rather
than respiration. Prashad (1918a, b), Lefevre and Curtis (1910), Bloomer (1931, 1934), Okada Katsuhiro (1935a, b, c) and Patil (1965) have studied the gill of freshwater mussels, with reference to the formation of marsupium during the breeding season. Sleigh (1969, 1974) have provided an upto date comprehensive review of the literature on bivalve gill.

Regarding the studies on labial palp, Alder and Hancock (1851) have attributed a role in active particle sorting to the palps of Pholas and Mya. In this respect further contributions are made by Menzel (1955a, b), Galtsof (1964), Reid and Reid (1969), Dwivedy (1972), Gilmour (1974) and Foster-Smith (1975).

Information regarding the sex and reproductive activity is absolutely essential to work with reference to propagation of commercially important bivalve species and in other biological investigations. Sex in bivalves is subject of great interest as the most of the members of this group exhibit a peculiar phenomenon of sex change during their reproductive activity. Most of the work in this regard has been done on Oysters (Hornell, 1910, 1921; Amemiya, 1925, 1929a, b; Orton, 1927; Coe, 1932, 1936a, 1938; Loosanoff, 1937, 1941, 1942; Rao, 1951a, b, 1953), Teredo (Coe, 1936b, 1941; Grave, 1942), Venus (Loosanoff, 1937), Mya
(Coe and Turner, 1938) Lamellidens (Bloomer, 1931; Ghosh and Ghosh, 1972; Patil, 1974), Anodonta (Bloomer, 1930, 1934, 1935, 1939) and Parreysia (Patil, 1965). Hornell (1922) has noticed two spawning periods in Meritrix casta but Abraham (1953) has observed a prolonged spawning activity in the same clam. Prolonged breeding activity has been also noticed in Donax (Alagarswami, 1966), Parreysia (Patil and Bal, 1967), Lamellidens marginalis (Ghosh and Ghosh, 1972). On the contrary a short breeding has been observed in Crassostrea by Durve and Bal (1961).

Thus, the information regarding sex and sexual activity of organism open a new line of research in endocrinology. Naturally, attention is directed towards study of neurosecretory cells and neurosecretion in bivalve molluscs. The neurosecretory cells for the first time, have been described by Gabe (1955) in lamellibranch. The nerve ganglia present in the bivalve, contain neurosecretory cells (Lubet, 1955, 1956, 1957, 1959, 1966; Antheunisse, 1963; Nagabhushanam, 1963, 1964a, 1964b, 1964c, 1970; Baranyi and Salanki, 1963; Nagabhushanam and Lomte, 1972; Nagabhushanam and Mantale, 1972; Marchenko, 1976; Nagabhushanam and Lohagaonkar, 1976; Mane, 1978; Mane and Patil, 1979). In primitive species the neurosecretory cells are usually numerous and scattered but in more advanced forms they show a tendency
towards the formation of group (Gabe, 1955). Regarding the occurrence of neurosecretory cells in pedal ganglia there are controversial reports. Gabe (1955) and Lubet (1956) have informed the absence of neurosecretory cells in the pedal ganglia. A slight neurosecretory activity has been assigned to the pedal ganglia of Dreissena polymorpha by Antheunisse (1963). While Fahrmann (1961) has reported an intense neurosecretory activity in pedal ganglia of Unio tumidus. Mane and Patil (1979) have arrived at the same conclusion. They have further pointed out the presence or the absence of neurosecretory cells in pedal ganglia of mussel might be a response to the environment in which the animals are leading their normal life. From the literature concerning the neurosecretory cells and neurosecretion it appears that the neurosecretory cells in pedal ganglia are only present in freshwater bivalves.

**Biochemistry and Histochemistry**

Many of the recent studies concerning the biochemistry and histochemistry of mussels have physiological orientation, in the sense of being concerned with metabolic regulation at the level of 'whole' organism. The data obtained in the field discerns many physiological as well as biochemical gaps in our information. The histochemical and biochemical investigations give reliable information about the different
chemical constituents and their cytochemical architecture. The outcome of such studies provides sufficient background for wider researches in comparative morphology, anatomy and physiology. The literature on bivalves shows that only in recent years the biochemical aspects and their involvement in the life activities are being worked out. The biochemical studies mainly indicate the information regarding glycogen, protein, lipid and inorganic ions. The earlier contributions by the various investigators do not reveal much information concerning the seasonal variation in different chemical constituents.

Mitchell (1916) has studied seasonal variations in the glycogen content in Crassostrea virginica. He further suggests that the spring spawning might have been a factor for seasonal changes in the glycogen contents. In Ostrea circumpicta the glycogen content becomes maximum in spring, its level continued to be quite high through July. During breeding season, August-September, there is a slight decline to a distinct minimum in September (Okazaki and Kobayashi, 1928). These observations have been confirmed by Bierry et al. (1937) in Ostrea edulis and Crassostrea angulata.

It is, thus, evident that the biological investigations
have been undertaken to know the variations in important chemical constituents like glycogen, protein and lipid. Recently, Holland and Gabbott (1971a,b) and Holland and Hannant (1973) have described microanalytical schemes for the determination of glycogen, protein and lipid. Most of the contributions concerning these chemical constituents mainly deal with their seasonal fluctuations in *Mytilus edulis* (Lubet and Le Feronde Longcamp, 1969; William, 1969; De Zwaan and Zandee, 1972; Dare, 1973 and Gabbott and Bayne, 1973), *Mytilus galloprovincialis* (Chapat et al., 1967; Pavalovic et al., 1970), *Parna parna* (Alvarez, 1968), *Ostrea gigas* (Masumoto et al., 1934; Venkatraman and Chari, 1951) *Ostrea edulis* (Collyer, 1957) *Teredo pedicelata* (Lane et al., 1952; Greenfield, 1953) *Corbicula sandi* (Hori, 1954), *Parreysia favidens var. marcens* (Patil and Bal, 1965). The seasonal fluctuations in glycogen, protein and lipid have been correlated with the reproductive cycle of bivalve (Venkatraman and Chari, 1951; Durve and Bal, 1961; Patil and Bal, 1965). It has been observed by these authors that in resting period these chemical constituents are at low level, during maturing stages they gradually increase and reach maximum in fully sexually matured individual and during spawning indicate gradual fall in their values. Curiously, an inverse relationship between lipid and glycogen contents of *Mytilus edulis* has been

The correlation between total nitrogen contents and feeding habits has been reported in *Teredo bartchi* and *T. pedecilata* by Laskar and Lane (1953) and Greenfield (1953). These authors have further pointed out that the bivalves derive their nitrogen requirements both from the plankton and wood. In addition to the above mentioned moieties, enzyme studies were undertaken especially with reference to digestive tract and other organ systems (Dogson and Spencer, 1953; Billett, 1954; Fripp, 1965; Inoue, 1965; Kapur and Gibson, 1968; Bennett and Nakada, 1968; Purchon, 1971 and Wojtowicz, 1972). Occurrence of digestive enzymes in the digestive diverticula and crystalline style has been reported by Purchon (1971). The activity of α-amylase, α-glycosidase, β-glucuronidase, β-galactosidase, laminarase and chitobiase have been reported in both digestive diverticula and crystalline style. George (1952) has studied the lipase activity in style and digestive diverticula of *Modiolus demissus*. Reid (1968) has determined the correlation between digestive enzymes and stomach morphology and diet.

The literature review referred above in connection with the biochemical studies indicates scanty information
especially from the point of view of individual organ and organ-systems. Naturally, the variations in the chemical constituents pointed out by different workers cannot be attributed to a particular organ-system. It appears that information available does not reveal clear picture of chemical changes occurring in the animal during various stages of its sexual behaviour. It is, therefore, absolutely essential to localize the different chemical constituents in various tissues at its various phases of life activities by employing histomorphological and histochemical studies. In recent years such studies have become a compliment to the biochemical investigations.

Secretion of mucus is one of the important secretory activities of molluscs (Tarao, 1935; Masamune et al., 1947; Bacila Ronkin, 1952). Freshwater gastropods travel from substrate to substrate along the mucoid tight ropes. In filter feeding gastropods and mussels the food material gets covered with mucus and then directed towards mouth by ciliary action, resulting in mucociliary mode of feeding mechanism (Allen, 1914; Hyman, 1967 and Patil, 1976). The mucus has also been assigned a functional role in the courtship behaviour of pulmonates (Hyman, 1967). The paucity of information on mucus in mussels has been pointed out by Jakowaska (1965, 1966), Jeanloz (1966) and Kale and
Patil (1976). Recent workers with aid of more sophisticated techniques have analysed the mucus secretion of gastropods (Tarao, 1935; Masamune et al., 1947; Bavelender and Benzer, 1948; Bacila and Ronkin, 1952; Kwart and Shashoua, 1958; Shashoua and Kwart, 1958; Lash and Whitehouse, 1960; Masamune and Yosizawa, 1956; McMahon, Von Brand and Nolan, 1957; Arcadi, 1965, 1967; Smith, 1965; Hollande, 1968; Anderson, 1969; Fantin and Vigo, 1968; Minakshi and Scheer, 1969; Rangaraao, 1963; Hunt, 1967; Nanaware and Varute, 1973 and Patil, 1977). Curiously, scanty histochemical data related to bivalve mucopolysaccharides is available. Reedham (1958) has detected the mucous cells in the mantle of Anodonta cygnea. Hillman (1968, 1969) has noticed two types of mucus secreting cells in the mantle of clam Mercenaria mercenaria. Recently, Kale and Patil (1976, 1977) have noticed three types of mucous cells elaborating neutral mucopolysaccharides, sialomucins and sulphomucins in the mantle of freshwater mussel. These observations have been confirmed by Mane and Patil (1980) in Lamellidens consobrinus. Patil (1976) has pointed out the absence of mucous cells in the outer marginal fold of mantle in Lamellidens corrianus. Patil (1977) and Mane and Patil (1980) have noticed that the mucus secreting cells, in addition to the acid mucopolysaccharides, also contain protein and the cells from the ventral edge of the mantle which elaborate
sulphomucins are PAS negative after pepsin digestion.

Histochemical investigations on the gill are very few. Eble Albert (1968) demonstrated presence of glycogen, glycogen phosphorylase and branching enzymes in the gill of Oyster. Pasteels (1971) has demonstrated the acid phosphatase activity and golgian polarity in the gills of bivalve. With the help of alcian blue technique the presence of acid mucosubstance secreting cells has been reported by Reid and Reid (1969). Kale and Patil (1977) extended the histochemical studies on the gill of freshwater mussel and pointed out the presence of different types of mucous cells, secreting sulphated and non-sulphated acid mucopolysaccharides.

Histochemical studies on the lamellibranch alimentary canal with reference to mucous cells are scanty. Attempts have been made to locate the mucus secreting cells in the alimentary canal (Owen, 1955, 1956, 1966; Lomte, 1972). Suzuki (1968) reported the presence of three cell types in the ducts of digestive diverticula of Patinopecter vesseoensis. Mane (1979) has studied the distribution of mucous cells in the alimentary canal of Lamellidens consubrinus. He has pointed out the presence of mucous cells elaborating acid mucopolysaccharides in oesophagus. He further gives the regional differentiation of mucous cells
in the alimentary canal. Sumner (1969) has described the
distribution of hydrolytic enzymes in the cells of digestive diverticula of some lamellibranchs.

Attempts have been also made to study the neurosecretory cells by employing recent histochemical techniques. Nagabhushanam (1963, 1964b) has observed the neurosecretory cells containing PAS positive polysaccharides in the nerve ganglia of Crassostrea. In Parreysia the neurosecretion is of glycoprotein nature (Nagabhushanam and Lomte, 1972). Mane (1978, 1979) has differentiated the two types of neurosecretory cells by employing histochemical methods involving PAS and Millon's techniques.

The critical review of the work done on bivalve molluscs especially with reference to the taxonomy, biology and biochemical and histochemical study indicates that the mussels have been given more attention from the taxonomy and biological points of view. The biochemical work has been mainly carried out with reference to the nutritive value of bivalves and hence, whole animals have been taken for the studies involving fluctuations in different chemical constituents. Thus, the various metabolites seem to have been studied at organ or tissue level. Histochemical investigations on mussels are mainly with reference to the enzyme activity. Recently, contributions
are made regarding mucus secretory activity. Moreover, the
organ and organ-systems which have received the attention
from the biochemists and histochemists are alimentary canal,
gill and mantle. Other organs seem to have not been
investigated thoroughly. To understand the biochemical
aspect in detail histochemical information not only on sex
organs but also on associated glands and organs is absolute­
ly essential. It appears that the gonads and digestive
diverticula of marine bivalves have lavishly received
attention. Secondly, all the organs from the mussel have
not been investigated from the histochemical point of view.

Reasons that led to take the present problem

1) Most of the contributions referred to above are
on marine bivalves. To our surprise the freshwater
mussels though having economic value, have been paid
attention only from the taxonomic point of view and seem
to be neglected for the histochemical studies. Hence, a
freshwater mussel, *Parreysia corrugata* var. nagpoorensis
available in abundance, has been selected for the present
study because there is hardly any work on this species in
the area of histochemistry. The histochemical investiga­
tions can be extended to study the various metabolites but
they are mainly concentrated towards the mucopolysacchari-
des and protein as secretion of these metabolites seems
to be an important secretory activity of mussels. In addition to the mucopolysaccharides and protein, lipid is also studied because most of the biochemical contributions are with reference to the total value of carbohydrates, proteins and lipids. The histochemical studies are further extended to characterize different types of proteins and neurosecretory cells.

2) Mucopolysaccharides

Occurrence of mucins in bivalve molluscs is a prominent feature. Hence, the mucopolysaccharides have been investigated mainly in mantle and digestive diverticula of some bivalves. Beedham (1958), Hillman (1964, 1968, 1969), Kale and Patil (1976, 1977), Patil (1976), Mane and Patil (1980) have taken a keen interest in mucopolysaccharides from the mantle of mussels and their efforts are mainly directed towards finding out the phylogenetic basis for the distribution of mucopolysaccharides and their histochemical characterisation. Though mucopolysaccharides have been studied in mussels, lacunae still remain in their understanding from the various organ-systems of mussels. They are:

i) Complete histochemical characterization of mucopolysaccharides from the various organ and organ-systems
in mussels is yet to be worked out. Such a study is essential to know the cytochemical nature of the various organs from the secretory point of view.

ii) Except in a few, cellular sites of secretion of mucopolysaccharides are not known. There is no information regarding the type of glandular cells in different organs of alimentary canal which are responsible for synthesis of different types of mucopolysaccharides. Whether all the glandular cells in different organs are endowed with the capacity of secretion of different mucopolysaccharides or whether their mucopolysaccharide synthetic capacities differ on the basis of which they can be classified into different types is also far from full understanding.

iii) There is no information regarding seasonal alteration in the mucopolysaccharides during the breeding cycle. If the organ secretes different types of mucopolysaccharides, it is not known whether the same type of glandular cells are activated in different seasons.

iv) For such studies on mucopolysaccharides histochemical techniques are ideal. Since they provide information on the tissue and cellular localization and variation in staining techniques, can be taken as reflections of
alterations in their concentrations.

3) Protein

Major contributions concerning the protein are limited towards the studies in gonad. Organwise distribution of protein and its further characterisation has been paid less attention. Hence, in the present investigation it was decided to study different types of proteins in different organs, mantle, gill, gonad, etc. and their importance in the life activities of mussels.

4) Lipid

The literature review referred to earlier indicates that the lipids have been studied in entire body of the mussel especially in reproductive organs. It is, hence, desired to study the lipids in different organs.

5) Neurosecretory Cells and Neurosecretion

Most of the contributions regarding neurosecretory cells deal with their morphological characteristic features and that too in marine species. It is, hence, desired to study the chemical nature of neurosecretory cells by employing histochemical techniques and to find out their probable role during the sex cycle.