ELECTROPHORETIC, BIOCHEMICAL AND MORPHOMETRIC STUDIES ON THREE BIVALVES OF COCHIN AND NEARBY WATERS

THESIS

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BY

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TO

My Beloved Father

and to

My Mother

CERTIFICATE

This is to certify that this thesis is an authentic record of the research work carried out by Smt. **REETHAMMA. O.V.,** under my supervision and guidance in the Division of Marine Biology, School of Marine Sciences, Cochin University of Science and Technology, in partial fulfilment of the requirements for the degree of Doctor of Philosophy of the Cochin University of Science and Technology, and no part thereof has been presented before for the award of any other degree, diploma or associateship in any University.

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DECLARATION

I, **REETHAMMA, O.V.,** do hereby declare that the thesis entitled "ELECTROPHORETIC, BIOCHEMICAL AND MORPHOMETRIC STUDIES ON THREE BIVALVES OF COCHIN AND NEARBY WATERS" is a genuine record of the research work done by me under the guidance of **Dr. C.K. RADHAKRISHNAN,** Senior Lecturer, Division of Marine Biology, Microbiology and Biochemistry, School of Marine Sciences, Cochin University of Science and Technology and has not been previously formed the basis of the award of any degree, diploma or associateship in any university.

Cochin-16.

REETHAMMA, O. V.

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PREFACE

Population genetics developed as a science with the intention of understanding the genetic basis of evolutionary process in both qualitative and quantitative terms. It is now obvious that in any widely distributed species, there is a tendency for divergencies to become established in different parts of the range with different environmental conditions. With few exceptions, the distribution of any organism is discontinuous and the degree of isolation of one population from the next depends upon a great variety of factors, including the mobility of the species and the nature and extent of the intervening habitats and the difference may be physiological, morphological or both and they may be phenotypic or genotypic in each case. However, even a species is continuously distributed, or the discontinuities in distribution are slight, it is still possible for differences to become established in parts of the range of the species. These may be of such magnitude that would bring forth sub-species, varieties or even species.

A description of the extent of variation among populations of the same species and of different species may be necessary but not sufficient for understanding the process of evolution. It is also necessary to understand the nature and magnitude of forces that change genotypic and phenotypic frequencies over time. In general, species assignments in molluscs are made on the basis of morphological criteria and this may not always help in the understanding of the exact taxonomical position of some species.

These were the important guiding principles for initiating the present

study which involved delineation of the systematic position of three bivalve species belonging to the phylum Mollusca and the extent of variation among selected populations of each species from a morphological and biochemical point of view. The information gathered during the present study would throw more light into our understanding of the nature of genetic variations that underly the process of speciation.

CONTENTS

Chapter 1

GENERAL INTRODUCTION

Like all successful groups of animals, the molluscs have explored with enterprise and ingenuity, the possibilities latent in their basic structure during the course of a long evolutionary history. Even when the extent of the plasticity of their basic form is seen, the variety of the 'Mollusca' remains 'exceptional and spectacular' among the various phyla of the animal kingdom (Morton and Yonge, 1964). Molluscs are the most numerous group of animals comprising a total of 100,000 species inhabiting all possible habitats except aerial (Abbot, 1954).

The class Bivalvia (Phylum : Mollusca) can be divided into two sub-classes - Protobranchia and Lamellibranchia, of which the latter comprises the clams, mussels and oysters (Newell, 1965; 1969; Boss, 1982). The unique pattern of distribution, polymorphism and other genetic variations among bivalves have been subject to extensive research in recent times. Over the past few years, several excellent discussions have appeared in scientific journals concerning the treatment of the lower taxonomic characters. The enormous diversity of Mollusca, which ranks about a third in the number of living species of animals makes it difficult to formulate a definition which will apply to all members of this group but none outside it, and at the same time be reasonably brief. Perhaps, this could be the reason why the phylogeny of this group have received so much attention in elucidating what the molluscs are.

The categorisation of phylum Mollusca as suggested by Burch (1956) seems inadequate on the basis of the statement that "sub-species should

be based on characters resulting from the environment". This implies that (1) the variations result entirely through natural selection of genotypes most compatible to the environments, (2) infraspecific variation results as a direct effect of the environment on the genetic system or (3) variations result by expression of the gene complex in different ways in different environments. Among molluscs, the third possibility appears to be more frequent and the fact that it leads to "ecotypes" and "ecophenotypes" and is well known to malacologists.

Molluscs in general and bivalves in particular are known to form isolated local populations because of their sedentary habits and wide distribution. Often these populations show a marked homogeneity among members of each population and in most cases, every local population may be morphologically distinguished from others. These "micro-geographical races" may either be genetically determined or ecophenotypically conditioned. The separation of an ecophenotype as a taxonomic unit with a sub-specific name is erroneous (Hubendick, 1951). This is because, if the morphological characters of populations are genetically determined, recognizing each population as a sub-species or species is absurd since there exists a clear-cut discontinuity of characters. Thus, there is more to systematics than merely the identification of the animals involved and it is essential to understand the underlying mechanisms and principles of variation, speciation and evolution, to realize the inadequacy of our system of nomenclature of animals.

India enjoys a luxuriant molluscan resource and has been widely used as food for man and as a source of lime besides other purposes. Among them, bivalves are of remarkable interest with regard to their

abundance and the growing importance in fisheries. Bivalves are by far the most important group compared to gastropods for commercial exploitation and utilisation as food. In recent years, there has been a spectacular advance in the utilisation of these forms in India and abroad. Hulse (1982) stated that the yield of high quality protein by bivalves per hectare of surface seawater far exceeds the protein that could be produced on a hectare of land by any known terrestrial plant or animal. Besides, they are of particular interest as indicator or sentinel organisms reflecting the level of environmental contamination in a manner amenable to both short term and long term monitoring.

Recent trends in aquaculture practice involve domestication of an animal by their shielding from unfavourable environmental conditions and long term genetic adaptation to an artificial environment (Doyle and Hunte, 1981). This may lead to increasing divergence between domesticated organisms and their wild population due to reduction of variability. Therefore, any information gathered on the genetics of change in fitness of each cultivable species would be useful as more and more bivalve species are being brought under cultivation. Although some success has been achieved in developing breeding technology of shellfishes especially bivalves, literature available on the genetic make up or genetic variability of these shellfishes is rather scarce. An understanding of the little known effects of aquaculture practices and related fields in fisheries on these species will help to promote their breeding and hybridization. The growing trend in the application of genetic principles envisages the necessity for scientific data pertaining to information on grounds of taxonomy and population genetics of bivalves.

Despite the fact that advanced researches have been initiated towards problems related to production, quality and maintenance of better stocks, scant attempts have been made towards the study of the systematic position and affinities of bivalve species. The present study was therefore taken up with a view to delineate the systematic position, interrelationships and variations among populations of three commercially important bivalve species representing the three major families of the class Bivalvia from Cochin and nearby waters. The species selected for the study are Villorita cyprinoides, Crassostrea madrasensis and Perna viridis belonging to the three major families Corbiculidae, Ostreidae and Mytilidae respectively.

Chapter 2

DESCRIPTION OF SPECIES BASED ON AVAILABLE LITERATURE

2.1. INTRODUCTION

In the analysis of evolution and classification of bivalves, earlier malacologists tended to emphasize one morphological feature above others as the key to the understanding of the broad evolution. As a result, there are a plethora of classifications in literature, not obviously related to one another, and a bewildering array of synonyms. Even the class name has got thirteen synonyms which has been used over the years as Acephala, Lamellibranchia, Pelecypoda, Conchifera and the most well known Linnaean name Bivalvia (Cox, 1969).

2.2. REVIEW OF LITERATURE

Systematics has been used as synonym for taxonomy by many but is probably best used in a more restrictive sense as "the scientific study of the kinds and diversity of organisms and of any and all relationships among them" (Simpson, 1961). The class Bivalvia comprises mainly three categories of organisms namely clams, mussels and oysters. Among clams, the genus Villorita, Griffith and Pidgeon (1833) has been the subject of interesting controversy. Although, some of the earlier workers did not consider it distinct from the genus Cyrena Lamarck, due to the differences in the shape of the shell and the hinge-teeth in the two genera, it was later separated as a distinct genus Villorita and the name was introduced for the first time by Griffith and Pidgeon (1833).

The type species of the genus was described by Gray as Cyrena

cyprinoides (1825) and figured by Wood as Venus cyprinoides (1828). Griffith and Pidgeon in 1833, published a figure of Gray's type specimen under the name Villorita cyprinoides following the meagre description in the alphabetical list of figures. They seem to be the first authors to introduce the name Villorita in literature. The same species was again reported as Velorita cyprinoides by a number of workers (Deshayes, 1854; Prime, 1869; Theobald, 1876; Sowerby, 1878; Clessin, 1879; Preston, 1915). Then in 1921, Prashad examined the type species and described it as Villorita cyprinoides followed by the latest revision of Sathyamurthi (1960). It appears from all authentic records available that the genus is of true Indian origin with a distribution confined only to the backwater areas of Malabar coasts of Peninsular India.

Villorita cochinensis has been described by Hanley (1866) from Cochin area of the Malabar coast, while Prime (1869) was not sure of the habitat of the group. Hanley had previously described them for the first time (1858) as Cyrena cochinensis while Prime (1860) identified them as Cyrena corbiculiformia and Benson (1860) reported them as Corbicula quilonica. Again Hanley's original description was found complete by Prime (1870), Sowerby (1878), Clessin (1879) and Preston (1915) and they all described them as Velorita cochinensis. But Prashad (1921) and Sathyamurthi (1960) found it impossible to consider Hanley's V. cochinensis as a species distinct from V. cypri-noides, since the minor differences between the two were quite sufficient only to consider V. cochinensis as a distinct variety of the species V. cypri-noides.

According to the literature available on the taxonomy of green and

brown edible mussels of the Indian coasts, they are undoubtedly treated under the genus Mytilus (Linnaeus, 1758; Chemnitz, 1785; Reeve, 1857; Annandale, 1916; Hornell, 1917, 1921; Rai, 1932; Gravely, 1941; Paul, 1942; Jones, 1951; Sathyamurthi, 1956; Kundu, 1965). The family Mytilidae has been included by Thiele (1931) in the order Anisomyaria based on the nature of the adductor muscles; the anterior adductor being very much reduced or more or less completely suppressed in members of the order. Pelsneer (1906) included Mytilus under the order Filibranchia comprising of bivalves with gills formed of parallel, ventrally directed and reflected filaments with ciliary interfilamentar junctions. Extensive collection and detailed studies on these mussels from all along the Indian coasts, however, failed to substantiate the occurence of characters of the genus Mytilus while they fully agreed with the distinguishing characters of the genus Perna. This indicated that the forms occurring along the coasts of India belong to the genus Perna. Thus, working on the systematics of the mussel species in India, Kuriakose and Nair (1976) came to the conclusion that the genus Mytilus seems to be absent in India and that the species which had hitherto been described as Mytilus viridis (green mussel) should be redesignated as Perna viridis.

Initially, as many as hundred species of living oysters and five hundred species of extinct ones were recognized by Korringa (1952). Later, Stenzel (1971) recognized, eight genera of living and fossilized oysters. Oyster biologists distinguishes four genera of living species of oysters - Ostrea, Crassostrea, Pycnodonta and Saccostrea and this has been widely accepted (Yonge, 1960; Galtsoff, 1964; Ahmed, 1975). Ranson (1948, 1950) included all the living species of oysters under three genera namely, Pycnodonta,

Ostrea and Grypheae based on the structural features of larval shell and adult. The genus Grypheae erected by Lamarck (1801) is not valid and the International Commission on Zoological Nomenclature (1958) in its opinion stated that the nominal species Gryphaea angulata was not type species of any nominal genus and the generic name Crassostrea (Sacco, 1897) was introduced for use of that species. Thus the species included under the genus Crassostrea is characterised by the presence of two teeth on the right valve and three teeth on the left valve in the larval shell. In the adult the irregularly shaped shell is generally attached to the substratum. Further, the adult is oviparous, rectum does not pass through the ventricle and a promyal chamber is present.

The taxonomy of Indian oysters has been studied by Hornell (1910, 1922), Annandale and Kemp (1916), Preston (1916), Moses (1928), Winkworth (1931), Awati and Rai (1931), Gravely (1941), Paul (1942), Sathyamurthi (1956), Rao (1956, 1958), Durve (1968) and Rao (1974). The Indian oysters were originally referred to the genus Ostrea (Awati and Rai, 1931) but later included under the genus Crassostrea (Rao, 1956, 1958; Durve, 1968). Awati and Rai (1931) have identified eight species of oysters including Ostrea cucullata, Crassostrea gryphoides and Crassostrea madrasensis and the description given in this chapter relates to the common backwater oyster Crassostrea madrasensis which is based on the diagnostic features as described by Rao (1974)

2.3. DESCRIPTION

2.3.1. CLAMS

Systematic position

Phylum : Mollusca

Class	:	Pelecypoda (Bivalvia)
Order	:	Eulamellibranchia
Sub-order	:	Heterodonta
Series	:	Sphaeriacea
Family	:	Corbiculidae
Sub-family	:	Corbiculinae
Genus	:	Villorita Griffith and Pidgeon 1833

The class Pelecypoda, Lamellibranchia or Bivalvia includes shelled animals popularly known as bivalves and is most abundantly represented in the sea or on the sea shores. But a small proportion of the animals inhabit freshwaters; the common freshwater mussel being a familiar example, but none occurs on land. However, the habits and habitats of freshwater pelecypods vary somewhat in the different families.

The members of the family Corbiculidae are found both in fresh and brackish waters. They were shells which are not pearly within, trigonal or ovately rounded, or more or less triangularly ovate and equivalve. Their umbones undergo gradual erosion.

The shell is more or less large and thick, triangular or ovate, with the outer surface concentrically striated and with yellowish, greenish or brownish periostracum. The hinge margin is well developed, usually with

three diverging cardinal teeth beneath the umbo and with both anterior and posterior lateral teeth. The ligament is external and the pallial line usually bears a sinus. This family includes the freshwater clams, found commonly in lakes, ponds and estuaries.

This family corresponds to the family Cyrenidae of Lankaster, which is the family name also used by Preston in his volume on the fresh water Gastropoda and Pelecypoda in the Fauna of British India series (1915), to include the genera Villorita and Corbicula. Thiele, however, adopts the name Corbiculidae for this family.

Genus : Villorita (Griffith and Pidgeon, 1833)

The shell is solid, moderately large, thick, triangularly and somewhat obliquely ovate, being rather inequilateral, with the hind margin being flattened and angular below. The umbones are prominent and well elevated. The hinge margin is very short and thick, always with three oblique cardinal teeth, of which the anterior in the right valve and the posterior in the left valve are obsolete. The anterior right lateral tooth is very short and situated close to the hinge margin. The posterior laterals are elongated and diverging. The pallial sinus is very small.

Species : Villorita cyprinoides (Gray)

The shell is fairly large and very thick, somewhat ovately triangular, heart-shaped, strongly oblique and inflated over the umbonal and central portions of the valves. The umbones are situated near the anterior margin

and are strongly recurved anteriorly and somewhat inwards, being closely approximated towards each other. The anterior margin is short, evenly curved above, almost straight in the middle and rapidly curving backwards below and continued into the ventral border which curves upwards posteriorly to meet the posterior margin in a more or less angular or narrowly rounded corner. The posterior margin is nearly straight and obliquely sloping, much longer than the anterior border and bears a low keel. The surface is traversed throughout by strong concentric ridges which are more strongly developed in the anterior half of the shell than towards the posterior border. The umbones are also striated, but are often found in a worn condition. The lunule in front of the umbo is narrow and the ligament is large and external, posterior to the umbo. The periostracum is dark olive-brown or blackish brown, somewhat entirely blackish, while the nacreous layer on the inside is whitish, light yellow near the margin with a violet tinge at the border. The hinge margin is very short and thick, always with three oblique cardinal teeth, of which the anterior in the right valve and the posterior in the left valve are obsolete. The anterior right lateral tooth is very short and situated close to the margin. The posterior laterals are elongated and diverging. The pallial sinus is very small.

The animal is some what trigonal, but the greater part of the umbonal region is occupied by a triangular structure formed by the union of the mantle flaps of the two sides only, the rest of the soft parts being somewhat elliptic in outline and lying below this hollow structure. Specimens preserved in spirit are of a whitish colour with dark brown black border in the region of the mantle papillae on the inner surface only, but seen through the translucent mantle flaps; the adductor muscles are dark yellow.

The mantle is very thin and translucent up to the pallial junction, below which, owing to the large numbers of radiating muscle fibres, it becomes much thicker; in the region of the papillae, it is very thick and opaque. The border is entire without any papillae on the edge, but a continuous row of small finger-like papillae of a dark brownish colour with whitish tips is present on the internal surface a little distance away from the margin. The papillae are of the same size throughout and are not reduced in the middle region of the bucco-pedal orifice. The papillae are also present on the line of union of the mantle flaps in the siphonal region above and below the two siphonal orifices. The two mantle flaps are united with each other anteriorly to a little above the anterior adductor muscle, the two then separate but in the region of the muscle itself, the free portion is not very broad owing to the muscle lying near the border; behind the muscle, however, the two flaps are quite separate forming the large bucco-pedal orifice where the siphonal orifice starts. The flaps of the mantle are united in this region in the situation of the mantle papillae except for the openings of the two siphons; the line of union is indicated by the row of papillae in this region. Above the siphonal orifice, which terminates about the middle of the posterior adductor muscle, the two flaps are again united intimately as on the anterior margin.

Of the two siphons, the upper or the anal siphon is about two thirds the size of the lower or the branchial siphon. Both the siphons are fully retracted in the preserved specimens, but from their structure appear to be sufficiently extensible. The anal siphon has a single circle of papillae surrounding the orifice, but the branchial has in addition, another circle of much larger papillae situated inside the smaller papillae. Both the siphons

are of a dark brown colour.

The two adductor muscles are of about the same size, but the posterior is more internally situated. The radiating muscles of the mantle arise from the pallial line and are connected with the papillae of the mantle. The siphonal retractor fibers are distinctly marked off from the rest and are connected with the siphonal sinus. The outer pair of gills is much narrower than the inner pair, particularly in the anterior half. A very narrow chink like opening is distinguishable between the united edge of the inner lamellae of the inner pair of gills and the foot.

The two pairs of palps are rather narrow, elongated, triangular in outline and are attached at the base with the apex pointing backwards and downwards. The surface of the palps is marked with very fine transverse ridges. The abdominal mass is comparatively small, while the foot is of a fair size, not very thick, triangular and acutely pointed at the apex.

Species : Villorita cyprinoides var. cochinensis (Hanley)

The shell is thick, solid, cordate, almost as high as broad and rather inequilateral and oblique, but the umbo is more centrally situated and less obliquely inclined than in the typical form. The valves are more or less inflated and the surface, which is covered by a blackish brown periostracum, is regularly and concentrically striated. These ridges are more markedly developed than in the typical form over the front and dorsal portions of the surface, but become quite obsolete towards the posterior and ventral margins. The anterior margin is regularly curved, but is much shorter

than in the typical form. The lunules are greatly reduced. The posterior margin is rather straight, obliquely sloping, and more or less narrowly and angularly rounded below where it joints the evenly curved ventral margin. The dorsal area behind the umbones is wide and bears the strong external ligament. The outer surface is generally glossy and deep reddish brown or blackish brown. The nacreous layer on the inner surface is whitish but the region outside the pallial line is fleshy pink and the extreme margin is smoky brown. The surface layer below the periostracum is reddish purple. The cardinal teeth tend to shelve outwards. The hinder and central ones bear a shallow linear grooving which makes them appear incompletely bifid.

Habitat:

Villorita cyprinoides, commonly known as 'black clam' is found in the west coast estuarine backwaters. They are found to form vast beds near the farthest ends of backwaters and the bar mouth. This clam is said to burrow deep into the soil to escape the adverse conditions when salinity rises during summer months.

2.3.2. MUSSELS

Systematic position

Phylum	:	Mollusca
Class	:	Pelecypoda (Bivalvia)
Order	:	Filibranchia
Sub-order	:	Mytilacea

Family : Mytilidae

Genus : Perna

Shell equivalve, very inequilateral with prosogyre umbones near the anterior end; ligament elongate, deep seated generally on nymphae, the resilial part typically connected with the nymphae by a calcareous white ridge; mantle lobes united below the anal siphonal opening; branchial opening confluent with the pedal opening; posterior part of the marginal edges pigmented and furnished with the papillae; anterior adductor muscle absent and posterior adductor very prominent; anterior byssal retractors small, fastened behind umbones; posterior retractor generally confluent with the posterior adductor; foot finger shaped with a posterior furrow; byssal gland behind the foot and highly functional; gills filibranch and ventricle embracing the rectum (Soot-Ryen, 1955).

Genus : Perna

Only one or two well developed hinge teeth; the absence of anterior adductor muscle; the wide separation of the two posterior byssal retractors; the recurrent loop of the mid gut lying at the left lateral side of the stomach; separation of the crystalline style sac from the mid gut.

Species : Perna viridis (Kuriakose and Nair)

Shell thick, equivalve, inequilateral, elongate and triangularly ovate in outline. Umbo terminal, hinge plate well developed extending slightly ventrally, provided with small teeth on the left valve and one large on

the right valve. Dorsal ligamental margin curved, mid-dorsal margin arcuate; posterior margin rounded and ventral margin highly concave. Periostracum thick, smooth and shining. Sculpture consisting of irregularly spaced concentric ridges and growth lines. Ligament very thick, white and pitted. External colour beautiful green, but in older specimens, bluish green at the anterior half. Interior of the shell is margaritaceous and shining; muscle scar deeply impressed.

Anterior adductor muscle absent. Posterior adductor large, cylindrical, surface slightly elongate and located in the posterior half of the shell a little above the antero-posterior axis of the body. Anterior byssal retractors cylindrical, thin and elongate, and join the shell a little behind the umbonal cavity; posterior byssal retractors arise as a common bundle from the base of the byssus apparatus which split into two short, thick bundles and diverge in the form of a 'V'; the anterior bundle inserting the shell below the posterior termination of the ligament and the posterior termination of the ligament and the posterior bundle joining the shell along with the posterior adductor bundle at its antero-dorsal side. Pedal retractor muscle thin and elongate, arises from the base of the foot and inserts the dorsal shell margin after crossing through the anteromesial aspect of the anterior bundle of the posterior retractor. Midgut or straight intestine lies at the left lateral side of the stomach. Crystalline style sac and midgut are widely separated , the former lying at the left ventral side of the latter. Mantle margin smooth, thin, slightly extensible and tentacles or papillae absent. The mouth of the exhalent aperture oval, wide and the passage into the mantle cavity very small being restricted by a septum; rectum and posterior adductor not visible through the opening. Foot is finger shaped, thick

and extensible. Byssus apparatus is large, situated at the posterior base of the foot; byssus threads emanate from the byssus stem. The threads are long, thick and strong with a well developed attachment disc at their distal ends.

Habitat:

In addition to open coasts and harbours, Perna viridis is distributed in the mouths of estuaries and rivers as they are capable of withstanding wide variation in salinity. The population extends from the low water mark to a depth of about ten fathoms and remain attached to rocks, pilings and other hard objects by means of byssus threads.

2.3.3. OYSTERS

Systematic position

Phylum	:	Mollusca
Class	:	Pelecypoda (Bivalvia)
Order	:	Eulamellibranchia
Sub-order	:	Ostraceae
Family	:	Ostreidae
Genus	:	Crassostrea

The family Ostreidae belongs to the order Eulamellibranchia and consists of bivalves with gills composed of branchial filaments united at regular intervals by vascular junctions. Members of the sub-order Ostraceae

are monomyarian or with a very small anterior adductor muscle. The mantle is open, the foot is reduced in size, the gills are folded and the shell is inequivalve. The family Ostreidae is characterised by very much reduced foot which does not have byssus gland. The gills are fused to the mantle and the shell is fixed to the substratum by the left valve which is larger than the right valve.

Genus : Crassostrea Sacco (1897)

The shell valves are variable in shape and are usually elongated. The left valve situated on the lower side is more or less cup-like and attached to the substratum while the right half is flat and functions like a cover for the left half. The hinge does not have teeth and the ligament is partly external. The adductor scar is situated dorso-laterally. The gill ostia are small and rectum does not pass through ventricle. Sexes are separate but sex reversal takes place in some individuals and hermaphrodite oysters occur. Members of the genus are oviparous and gametes are discharged into the water where fertilization occur. Eggs are small in size. The species of the genus are euryhaline and thrive well in turbid waters.

Species : Crassostrea madrasensis (Preston)

The shell straight, shape irregular, covered by numerous foliaceous laminae, left valve deep, right one slightly concave, hinge narrow and elongated. When spat set on flat surfaces and there is no crowding, flat shape is attained by the oysters. Those growing on uneven areas have shape of the niche where they are present and overcrowding leads to oysters

with very much twisted shells.

The numerous foliaceous laminae covering the outer surface of the shell have sharp edges giving the shell a width of 0.38 to 0.64 cm and thickness 0.14 to 0.36 cm. In some specimens, the narrow elongated hinge is elevated having a medial depression. The adductor muscle is situated sub-centrally, reniform and dark purple in color. The colour of the outer surface is grey, green or light purple depending on the area in which the oysters occur due to the presence of detritus, algae etc. The inner surface of valves is smooth, glossy and white in colour with purplish black colouration along the margins.

Habitat:

Crassostrea madrasensis is essentially a brackish water oyster. It occurs as extensive beds in estuaries, backwaters and sporadically on the open coasts. The oysters are found from the intertidal zone to a depth of about 4 meters. They colonize on rocky or concrete surfaces and also on hard muddy bottom where they thrive well. The general shape of the oysters varies in relation to the substratum and overcrowding may result in highly irregular form.

2.4. STUDY AREA

The bivalve species employed for the study were collected from selected habitats from Cochin and nearby waters. In order to understand the exact geographical position and hydrography of each station selected, a brief

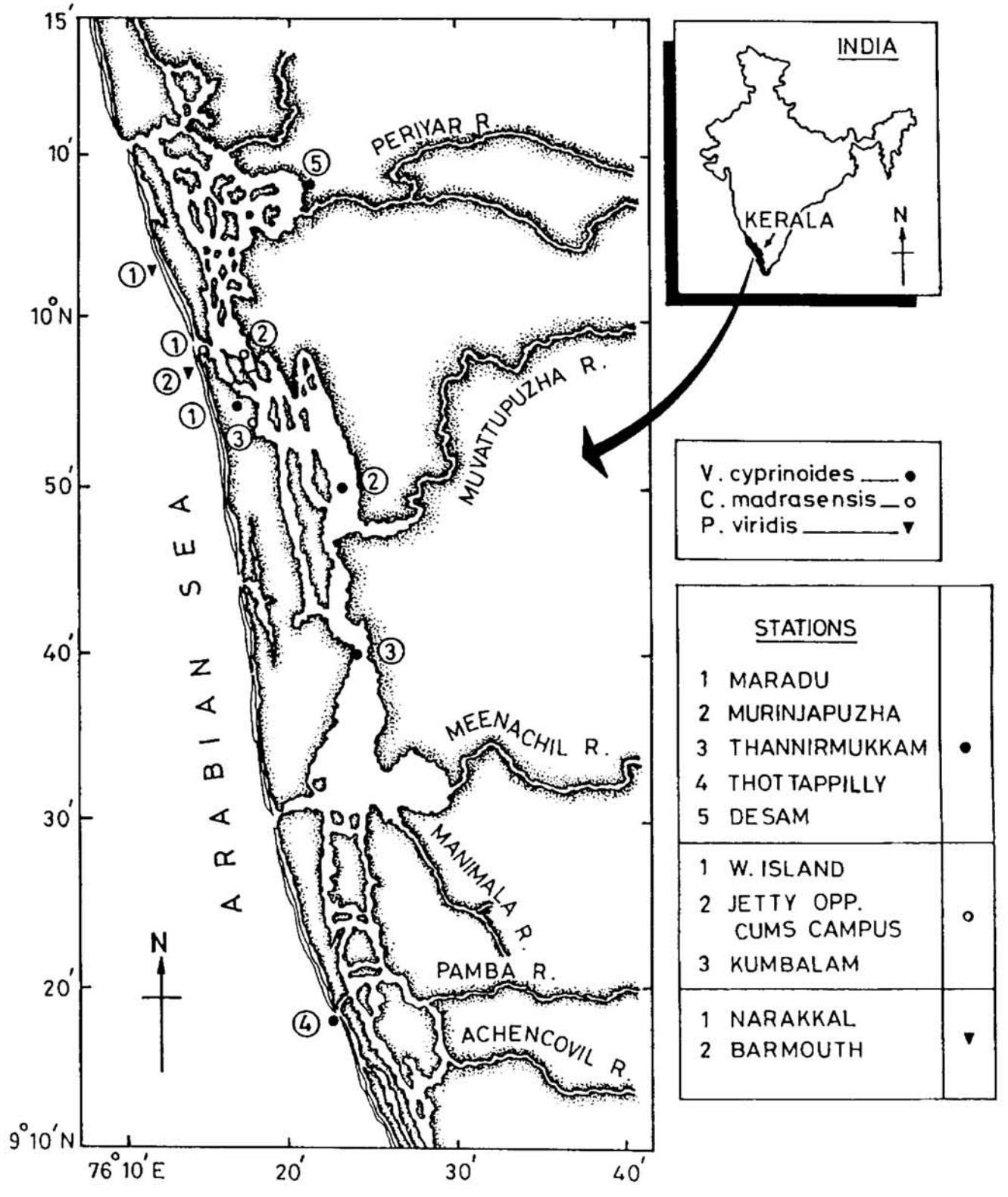


Fig. 1. Location map showing the different stations selected for the study.

description of the respective areas covering these stations would be worthwhile.

The Vembanad lake (Lat 9° 28' and 10° 10' N and Long 76° 13' and 76' 30' E) is the largest brackish water lake of the South west coast of India, extending from Alleppey in the South to Azheekode in the North. It has a total length of about 90 Km and an area of 270 Km^2 of total water spread. The depth varies between 1 to 8 mtrs. and the width varies from 100 mtrs to about 14.5 Kms. On the northern part there are two permanent openings to the Arabian Sea, one at Cochin - a 450 mtrs wide channel which forms the main entrance to the Cochin harbour, and the other at Azheekode. These regions are subjected to regular tidal influence and have all the characteristics of a tropical estuary. Tides of this area are mixed and semi-diurnal with substantial differences in range and time. A 1447 mtrs long bund has been constructed across the lake at Thannirmukkam during the year 1975 for preventing the penetration of salt water into the upper Kuttanad areas so as to enhance the paddy cultivation. The main sources of freshwater for the lake are two large rivers - Periyar in the North and Pamba in the South. Four small rivers viz. Achencoil, Manimala, Meenachil and Muvattupuzha also empty into the lake. The southern part of the Vembanad lake lodges two of the sampling stations namely Thannirmukkam and Murinjapuzha from where one of the test species namely Villorita cyprinoides was collected regularly. At the north-eastern border of the Vembanad lake is the Periyar river and one of the stations selected for the study - Desam is located here.

The Kayamkulam lake is a narrow stretch of backwater beyond the

southern end of Vembanad lake, between Lat 9° 7' and 9° 16'N and Long 76° 20' and 76° 28'E. The main body of the lake is about 14.5 Kms long and 0.8 to 2.5 Kms wide and runs parallel to the sea from which it is separated by a narrow strip of sandy beach, 0.2 to 0.6 Km wide. Two streams and a canal empty into the lake and during the monsoon months some of the flood waters of the Pampa and Achencoil rivers flow into the lake through the commercial canal at Thottappilly which links the Kayamkulam lake with these rivers. A number of irrigation channels and a few small streams open into the lake and drain the water from the adjoining paddy fields during the monsoon months. Thottappilly provides another station for the present study.

The Cochin backwater (Lat 9° 58'N and Long 76° 28'E) is a shallow semi-enclosed body of water in the tropical zone with all the characteristics of a tropical estuary. The topography and related features of the environment have been discussed in detail by various authors (Qasim and Gopinathan, 1969; Sankaranarayanan and Qasim, 1969; Wyatt and Qasim, 1973; Silas and Parameswaran Pillai, 1975). A narrow gut about 450 mtrs wide forms its main connection with the Arabian Sea and this region is subjected to regular tidal influence. It is a catchment basin for several rivers such as Periyar, Pamba and Muvattupuzha which empty either into the Vembanad lake or into the Cochin backwater which extends in the form of shallow brackish water lagoons, with a mean depth of about 3.5 mtrs. Along the main channel near Cochin harbour, the depth is about 10 to 14 mtrs which is maintained for navigational purposes. The inflow of freshwater and sea water into the estuary, bring about dynamic conditions which make the backwater extremely interesting and ecologically an intriguing environment.

Table 1. Details of geographical position and approximate salinity observed at the selected stations and the selected species of individuals.

No.	Station	Geographical position	Salinity (ppt)	Species
1	MARADU	9°55' Lat, 76°17' Long	15-25	VILLORITA CYPRINOIDES
2	MURINJAPUZHA	9°50' Lat, 76°23' Long	5-10	"
3	THANNIRMUKKAM	9°40' Lat, 76°24' Long	1-5	"
4	THOTTAPPILLY	9°18' Lat, 76°23' Long	0-2	"
5	DESAM	10°8' Lat, 76°21' Long	0	"
1	W. ISLAND	9°58' Lat, 76°15' Long	25-30	CRASSOSTREA MADRASENSIS
2	JETTY OPP. *CUMS CAMPUS	9°57' Lat, 76°17' Long	20-28	"
3	KUMBALAM	9°54' Lat, 76°18' Long	10-15	"
1	NARAKKAL	10°13' Lat, 76°12' Long	30-35	PERNA VIRIDIS
2	SEVENTH FIREBUOY OFF BARMOUTH	9°57' Lat, 76°14' Long	28-30	"

* CUMS : Cochin University Marine Sciences

For the present study, six stations were selected from various parts of the Cochin backwaters. They are Maradu, Kumbalam, Jetty opposite to the Cochin University Marine Sciences Campus, Willingdon Island, Narakkal and Seventh Fire buoy off barmouth.

All the stations which were selected for the study are characterised by slight variations in various ecological factors, chiefly salinity, which formed the basis of selection of the stations. The exact geographical location and the approximate salinity range observed at each station during the tenure of this study are presented in Table 1. For convenience, the selected stations are arranged in the descending order of salinity in the case of each species.

2.5. SALIENT FEATURES OBSERVED IN THE PRESENT STUDY

2.5.1. CLAMS

Organisms belonging to the genus Villorita cyprinoides were collected from the five selected stations (Table 1) and thoroughly examined to bring out their exact taxonomic identity. It was observed that features of specimens from stations 1 to 4 resembled those in the original description for the typical species Villorita cyprinoides. All features of the soft body parts coincided with those of the typical form. But they were found to differ from their counterparts at Desam (Table 1) in the following characters.

1. The shell is more conical than the triangular form.
2. Umbones are more anteriorly directed and are less oblique.

3. Lunules are more expressed.

4. Mantle margin is blackish-brown in colour.

Further, specimens from the more saline stations - Stn. 1 and 2 (Table 1) were found to be more tolerant than individuals from the other less saline or nearly fresh water regions like Stn. 3, 4 and 5 (Table 1); during the present study.

However, specimens collected from station 5 were significantly different from those sampled from the other four stations. They possessed features characteristic to the species Villorita cyprinoides var. cochinensis. The notable ones include,

1. More centrally situated umbones which are less oblique, thus giving a triangular form for the shell.
2. Much shorter and more regularly curved anterior side.
3. Greatly reduced lunules.

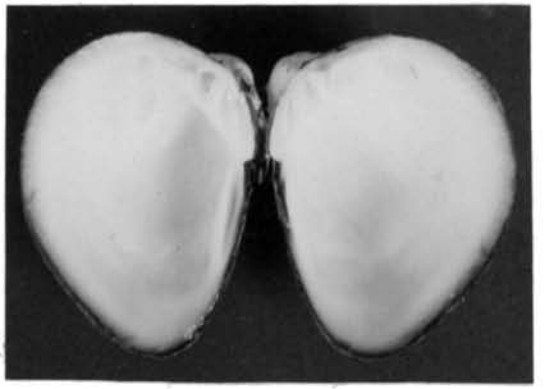
Besides these features, a peculiarity observed among the specimens was the creamy white or light yellow colour of the mantle margin.

From the observations made, it becomes clear that the specimens of genus Villorita collected from stations 1 to 5 belong to two distinct groups. The specimens sampled from the fifth station can be identified as Villorita cyprinoides var. cochinensis while those from the other four stations, Villorita cyprinoides. The dorsal and ventral views of the shells belonging to the two groups are depicted in Photomicrograph 1a-e. The Fig.

(a) Station 1

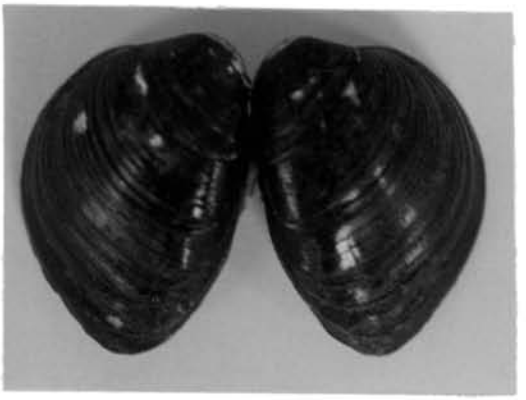
(i)

(ii)

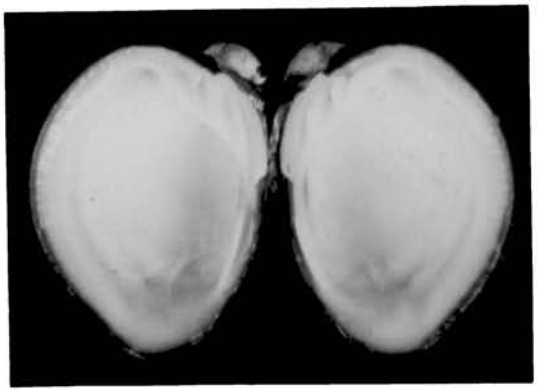


(b) Station 2

(i)



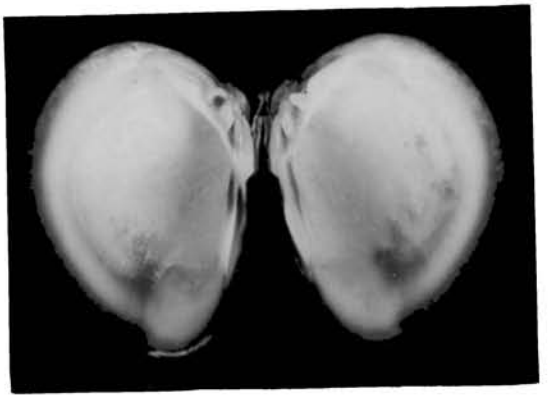
(ii)



(c) Station 3

(i)

(ii)

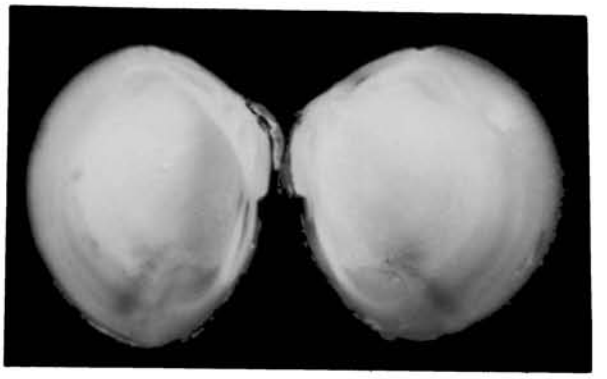


(d) Station 4

(i) (ii)

(e) Station 5

(i) (ii)



Photomicrograph 1 a-e. Villorita cyprinoides. Dorsal (i) and Ventral (ii) views of the shell of individuals from the five stations

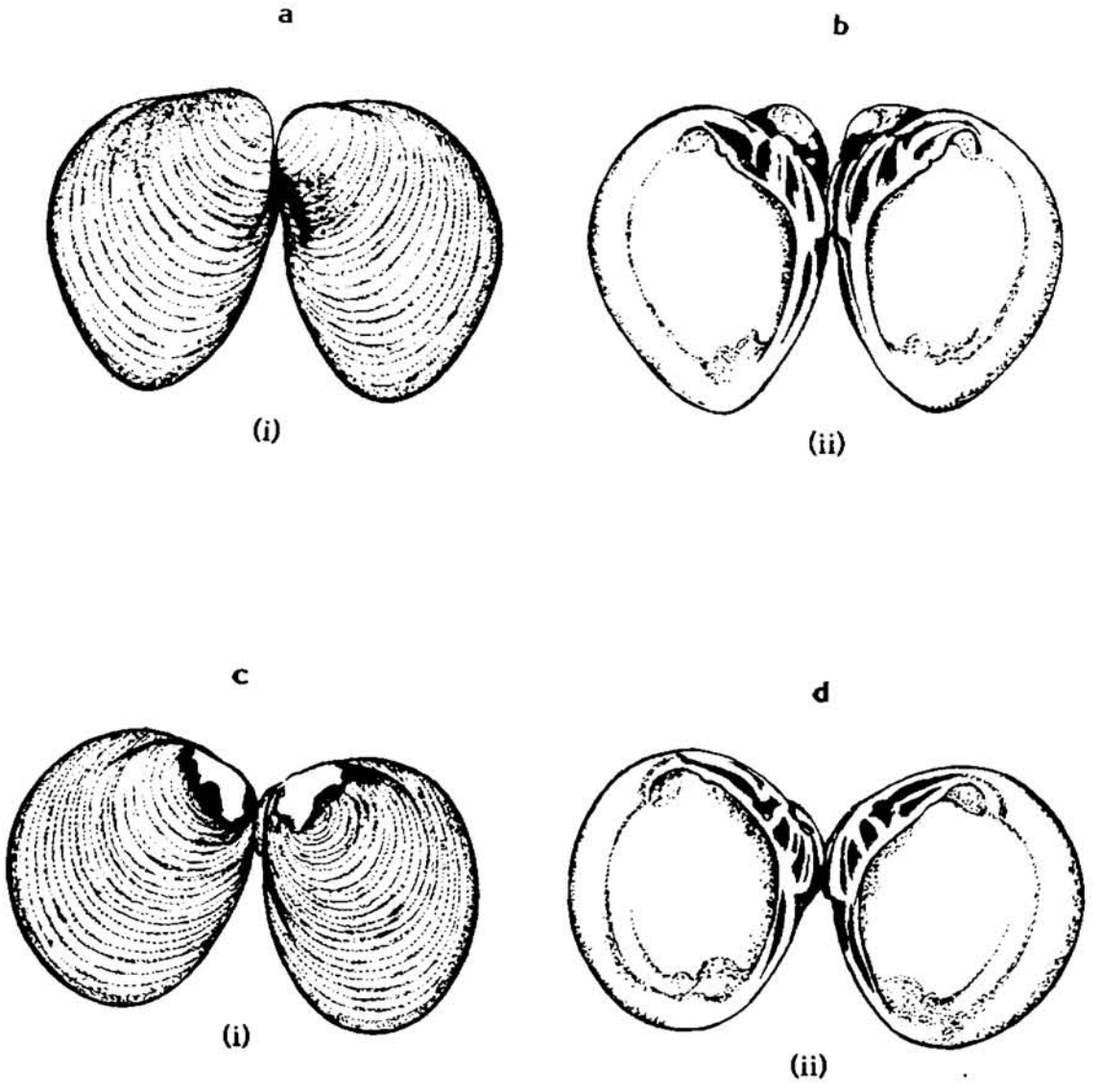


Fig. 2 a–d. Dorsal (i) and Ventral (ii) views of the shell of individuals of a and b - Villorita cyprinoides

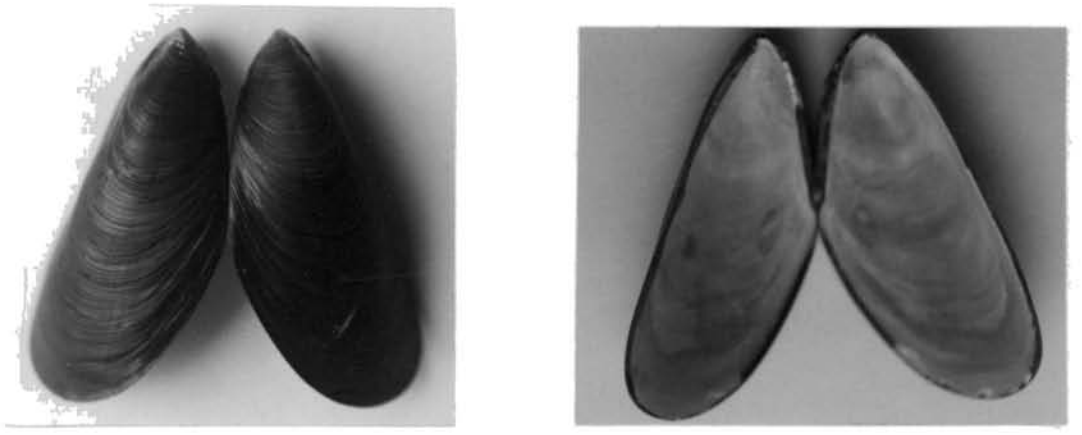
c and d - Villorita cyprinoides var. cochinensis

(a). Station 1.

(i) (ii)

(b) Station.2

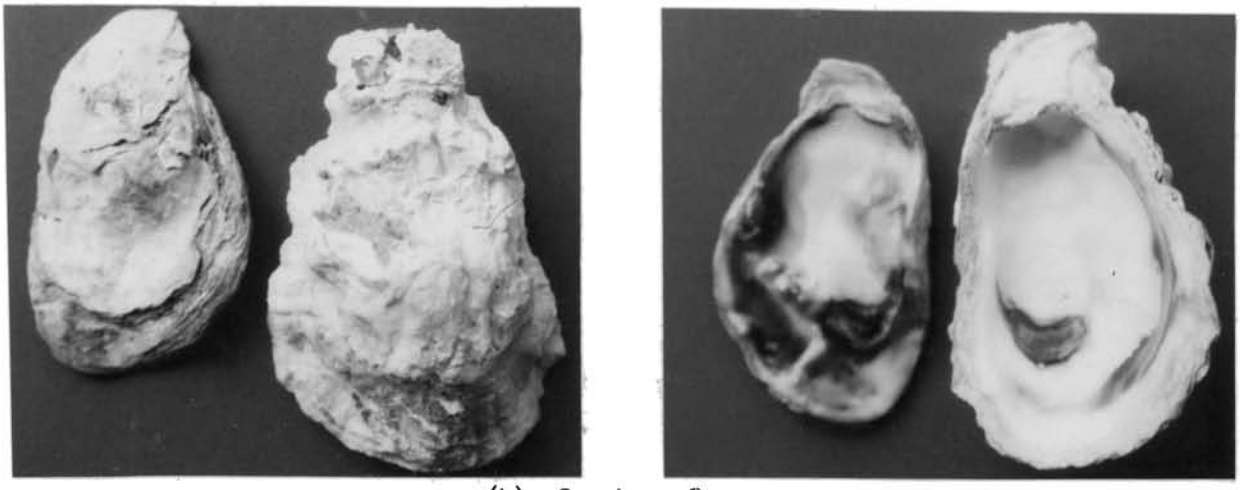
(i) (ii)



Photomicrograph 2 a-b. Perna viridis. Dorsal (i) and Ventral (ii) views of the shell of individuals from the two stations.

(a). Station 1.

(i) (ii)

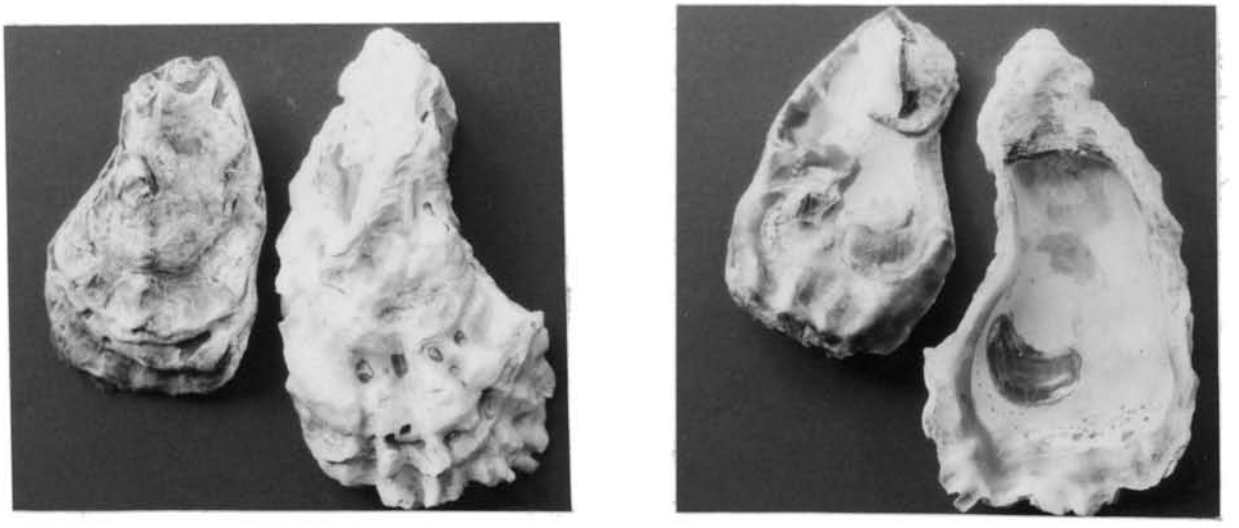


(b) Station 2.

(i) (ii)

(c) Station 3.

(i) (ii)



Photomicrograph 3 a-c. Crassostrea madrasensis. Dorsal (i) and Ventral (ii) views of the shell of individuals from the three stations.

2 a-d provides a more detailed description of the shells of Villorita cyprinoides (2 a and b) and Villorita cyprinoides var. cochinensis (2 c and d) and has taken into account the minor variations observed which cannot be pointed out in photograph.

2.5.2. MUSSELS

Individuals of the genus Perna were collected from the two stations (Table 1, Fig. 1) and identified using the available literature as Perna viridis. Owing to the differences in ecological parameters observed at the stations, the specimens sampled from the above stations were subjected to more detailed morphological study and their identification was confirmed. The dorsal and ventral views of the shell of Perna viridis from the two stations is depicted in Photomicrograph 2a-b.

2.5.3. OYSTERS

Specimens of the genus Crassostrea were collected from the selected habitats (Table 1, Fig. 1) and identified. All the specimens were found to have identical features permitting them to be included under the species Crassostrea madrasensis. The three stations were characterised by three different salinity grades. The less saline region, Stn. 3 (Table 1) was found to be inhabited by comparatively few specimens and the only notable difference in these organisms was the less glossy, less white appearance of the inner shell surface. The general appearance of the shell valves of the specimens from the three stations are depicted (Photomicrograph 3a-c).

Chapter 3

MORPHOMETRY

3.1. INTRODUCTION

In the analysis of population variations, geographic variation aims at description and summarisation of patterns of variation and covariation of characteristics of organisms distributed over an area. Such analyses are generally applied to species populations to study the variations of diverse characters. Most frequently, the characters employed for such studies are morphological but a more recent trend has been the utilisation of bio-chemical genetics and such other characters.

The basis for studies in geographic variation rest on the existence of population of comparable organisms at a number of localities with a set of characteristics observed for each organism sampled. Geographic variation studies may be univariate or multivariate. A univariate study considers only one character at a time, irrespective of other characters, while in multivariate study, one investigates not only the geographic variation of each character separately but also the covariation of all the characters together. Recent studies have tended to emphasize multivariate techniques (Sokal and Thomas, 1968; Thomas, 1968 a, b). Furthermore, attempts at explaining the pattern of variation in terms of underlying causes, frequently require a multivariate approach.

3.2. REVIEW OF LITERATURE

In any attempt to understand and measure the causes of fluctuations in the abundance of a species, it is imperative that the number and identity

of any sub-sets of species be established. The reason for this is obvious to any rational management plan in as much as each sub-set of the population or species may have a characteristic distribution and characteristic vital statistics. Saila and Flowers (1969) conducted a detailed analysis of morphologic measurements from geographically separated samples of the American lobster, Homarus americanus. The results of the study showed that definite profile differences were greater between inshore and offshore groups within geographically separated samples of both inshore and offshore lobsters.

A review of the statistical methodologies for comparison of morphological data has been provided by Royce (1957) and an explanation of multivariate technique is given by Kendall (1957). Jolicoeur and Mosimann (1960) are of the view that the shape of organisms may be affected by fluctuations in the external environment. Organisms occur in a more or less distinct or discrete population can be defined as stocks and such populations of a species would have one or more common characteristics depending on the type of environment of domicile (Kulkuhn, 1981). The existence of two or more unit stocks can be satisfactorily demonstrated by applying any one of the following techniques such as biochemical, immunological serological,behavioural, morphometric, electrophoretic etc.

Morphological variation studies by Schmidt (1917 a, 1918) in Zoarces viviparous collected from sixty one different locations demonstrated that the characters were heritable and were found to have direct environmental influence. Studies on morphological variations among organisms are plentiful (Boulva, 1972; Abidi et al., 1978; Choudhary and Dwivedi, 1981; Beacham, 1985). Morphometric technique has been successfully used for stock separation

in several species of fishes including Salmo villosus (Sharp et al., 1978), Salvelinus malma (Morrow, 1980) and Corregonus species (Ihssen et al., 1981; Todd et al., 1981). The main advantage of this technique is that it effectively isolates shape differences than most other traditional methods (Ihssen et al., 1981).

Geographical variations in morphological characters were observed in the genus Nematocelis (Crustacea : Euphausidae) (Gopalakrishnan, 1974); isopod Sphaeroma ruoicauda (Heath, 1975); Western Atlantic population of Gammarus oceanicus segerstrale (Amphipoda) (Croker and Gable, 1977); the dwarf cray fish (Chambers et al., 1979) and Pontinella dara (Flemi-nger and Hulseman, 1974).

Morphological differences were employed to delineate intraspecies variations in several marine and freshwater species of prawns (Lindenfelser, 1980; Morgan, 1982). Morphological relationships between Penaeus semisulcatus Metapenaeus and Parapenaeopsis stylifera was demonstrated by Farmur (1986). Several investigators have utilised electrophoresis and morphometry as valuable tools in the identification of different stocks of population and species (Kasinathan et al., 1972; Mickevich and Johnson, 1976; Salmon et al., 1979; Dando et al., 1979; Davidson et al., 1985; Samuel, 1987). Several environmental factors like salinity, temperature, oxygen, food, etc. are found to produce differences in prawn species (Johnson, 1960; Choudhary, 1971; Venkataramiah et al., 1975) with reference to morphometry.

Several workers have opined that among molluscs, the variations in environmental factors can influence the structure of organism (New

Combe, 1935, 1950; New Combe et al., 1938; Swan, 1953). Studies on the dimensional relationship among lamellibranchs by Galtsoff (1931) and Hamai (1934a,b) indicated that animals of different origin show variations in the ratio of their dimensions. Morphometric studies in Meretrix casta collected from two localities was the topic of scientific enquiry by Durve and Dharmaraja (1965). The results demonstrated the influence of environmental conditions on the extent of variation in their relationship. Measurements of shell dimension and their interrelationships in bivalve molluscs have been put to detailed study by several workers (Hamai, 1934a,b; 1935a,b; Abraham, 1953; Holme, 1961; Shafee, 1976; Mohan, 1980; Mohan and Damodaran, 1981; Nair and Nair, 1985; Mohan et al., 1986).

3.3. MATERIALS AND METHODS

3.3.1. SPECIMENS AND STUDY AREA

Details of the selected specimens and the study area have been described earlier (vide pages, Chapter 2).

3.3.2. COLLECTION AND CONDITIONING OF SPECIMENS

The selected animals namely Villorita cyprinoides, Crassostrea madrasensis and Perna viridis were collected from their respective habitats, using van Veen grab and other suitable devices. They were then transported to the laboratory in polyethylene carbuoys of 50 l capacity containing water from the site of collection. The animals were sorted out using vernier calipers and maintained under laboratory conditions for 48 h in aerated habitat water so as to clear them off their faeces.

3.3.3. METHODS

The various morphometric dimensions of the selected specimens were measured out using vernier calipers. All measurements were corrected upto the nearest 0.1 mm. The weight of each specimen was determined using a chemical balance. The morphometric dimensions chosen for each species were based on previous studies (Mohan, 1980; Nair and Nair, 1985; Mohan et al., 1986). Statistical analysis of the data were carried out and the results presented elsewhere.

a) Villorita cyprinoides

1. Length : The greatest dimension along the antero-posterior axis of the valves.
2. Height : The maximum distance between the hinge and the opposite end of the shell.
3. Depth : Greatest distance between the outer surfaces of the two valves, when they were closed.
4. Shell volume : The volume of water occupied by the shell, determined using displacement method.
5. Total weight of the organism.
6. Weight of the wet shell without flesh.
7. Weight of the flesh.
8. Weight of the dry shell.

b) Crassostrea madrasensis

1. Height : Maximum distance recorded from hinge to the opposite side of the shell.

2. Length : Greatest dimension of the antero-posterior axis of the valves.
3. Depth : Maximum distance between the two valves at a point where the axis of the other two dimensions crossed.
4. Shell volume.
5. Total weight of the organism.
6. Weight of the wet shell without flesh.
7. Weight of the flesh.
8. Weight of the dry shell.

c) Perna viridis

1. Length : The maximum distance along the long axis of the valves.
2. Height : Mean distance along the short axis of the valves.
3. Depth : Maximum thickness between the two valves when they were closed.
4. Shell volume.
5. Total weight of the organism.
6. Weight of the wet shell without flesh.
7. Weight of flesh alone.
8. Weight of the dry shell.

3.4. RESULTS

The present study has focused on the extent of variation in the shell characteristics of selected populations of the three species - V. cyprinoides, C. madrasensis and P. viridis. The data obtained had been analysed using various statistical procedures in testing for significance. The results are outlined in Tables 2a-j, 3a-h, 4a-h, 5a-h and 6a-c.

The matrix of correlation (Snedecor and Cochran, 1967) relating the

Table 2 a-e Villorita cyprinoides. Matrix of correlation coefficient among seven morphometric variables of individuals from stations 1 to 5.

2a. Stn. 1

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.000						
Hgt	0.7706	1.0000					
Dpt	0.4821	0.4437	1.0000				
Svl	0.5125	0.3532	0.1958	1.0000			
Twt	0.4980	0.5631	0.3108	0.3493	1.0000		
Fwt	0.1676*	0.1680*	0.2111	0.2821	0.4392	1.0000	
SdWt	0.4700	0.5257	0.2848	0.3093	0.9671	0.2840	1.0000

* Not significant at 5% level

2b. Stn. 2

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.7098	1.0000					
Dpt	0.4280	0.5421	1.0000				
Svl	0.6475	0.5422	0.2625	1.0000			
Twt	0.6916	0.6543	0.6821	0.4751	1.0000		
Fwt	0.2954	0.2939	0.1149*	0.2175	0.4726	1.0000	
SdWt	0.6887	0.6468	0.6329	0.4774	0.9718	0.2419	1.0000

* Not significant at 5% level

Len : Length Hgt : Height Dpt : Depth Svl : Shell Volume

Twt : Total Weight Fwt : Flesh Weight SdWt : Shell dry weight

2c. Stn. 3

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.8512	1.0000					
Dpt	0.7284	0.7985	1.0000				
Svl	0.8236	0.8335	0.7119	1.0000			
Twt	0.8011	0.7037	0.6880	0.6665	1.0000		
Fwt	0.6253	0.4656	0.5105	0.5135	0.5166	1.0000	
SdWt	0.5678	0.5512	0.5098	0.4763	0.8658	0.0262*	1.0000

* Not significant at 5% level

2d. Stn. 4

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.8338	1.0000					
Dpt	0.6612	0.7887	1.0000				
Svl	0.7031	0.7509	0.6131	1.0000			
Twt	0.6462	0.5776	0.6435	0.5764	1.0000		
Fwt	0.2883	0.1412*	0.1570*	0.1578*	0.4250	1.0000	
SdWt	0.6149	0.5819	0.6507	0.5733	0.9669	0.1851*	1.0000

* Not significant at 5% level

2e. Stn. 5

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.5966	1.0000					
Dpt	0.5093	0.4769	1.0000				
Svl	0.6231	0.4424	0.3591	1.0000			
Twt	0.7018	0.6387	0.6926	0.5243	1.0000		
Fwt	0.5249	0.4156	0.2764	0.3383	0.5536	1.0000	
SdWt	0.6150	0.5782	0.6929	0.4831	0.9382	0.2488	1.0000

Len : Length Hgt : Height Dpt : Depth Svl : Shell Volume

Twt : Total Weight Fwt : Flesh Weight SdWt : Shell dry weight

various morphometric characters of the three species is given in Tables 2a-j. Except for flesh weight with their length, height and depth, and shell volume with depth, all other correlations were found significant at 1% level for the specimens of V. cyprinoides sampled from station 1 (Table 1a). However, all correlations between the different morphometric variables were found significant ($p \leq 0.01$) with the exception of flesh weight with depth and shell volume, and between shell dry weight and flesh weight of individuals from station 2 (Table 2b). The individuals from station 3 were characterised by significant correlation between the different shell characteristics; the level of significance being 1%. The correlation existing between shell dry weight and flesh weight, however was found not significant (Table 2c). Table 2d depicts the matrix of correlation of the shell characteristics of specimens of V. cyprinoides sampled from station 4. Except for the correlations that existed between shell dry weight and flesh weight and those between flesh weight and the height, depth and shell volume which were statistically not significant, all other correlations depicted high statistical significance ($p \leq 0.01$). It was observed that at station 5, the correlations between the various shell measurements were highly significant at 1% level (Table 2e); no significant correlation existing between shell dry weight and flesh weight.

Table 2f-h illustrate the matrix of correlation of the shell characteristics of C. madrasensis from the three stations. It is evident from Table 2f that the correlations between total weight and shell volume and between flesh weight and length, depth and shell volume and between shell dry weight and shell volume were statistically not significant at 5% level for individuals sampled from station 1. Correlations between depth and height and between shell volume with height and length and between

Table 2 f-h. Crassostrea madrasensis. Matrix of correlation coefficient among seven morphometric variables of individuals from stations 1 to 3.

2f. Stn. 1

	Hgt	Len	Dpt	Svl	Twt	Fwt	SdWt
Hgt	1.0000						
Len	0.5212	1.0000					
Dpt	0.2188	0.5727	1.0000				
Svl	0.2204	0.2520	0.2539	1.0000			
Twt	0.5836	0.4123	0.2562	0.1648	1.0000		
Fwt	0.3298	0.1696*	0.0573*	0.1076*	0.4854	1.0000	
SdWt	0.5120	0.3538	0.2303	0.1292*	0.9783	0.3419	1.0000

* Not significant at 5% level

2g. Stn. 2

	Hgt	Len	Dpt	Svl	Twt	Fwt	SdWt
Hgt	1.0000						
Len	0.6999	1.0000					
Dpt	0.4873	0.4187	1.0000				
Svl	0.4174	0.4874	0.3033	1.0000			
Twt	0.6433	0.6412	0.4498	0.5038	1.0000		
Fwt	0.5709	0.6040	0.3249	0.5493	0.7931	1.0000	
SdWt	0.6590	0.6412	0.4833	0.4833	0.9749	0.7037	1.0000

2h. Stn. 3

	Hgt	Len	Dpt	Svl	Twt	Fwt	SdWt
Hgt	1.0000						
Len	0.5470	1.0000					
Dpt	0.6642	0.5245	1.0000				
Svl	0.5844	0.3463	0.6549	1.0000			
Twt	0.7641	0.4742	0.7392	0.5572	1.0000		
Fwt	0.6725	0.3991	0.6754	0.5550	0.7148	1.0000	
SdWt	0.6929	0.4154	0.6811	0.5018	0.9754	0.5536	1.0000

Hgt : Height Len : Length Dpt : Depth Svl : Shell Volume

Twt : Total Weight Fwt : Flesh Weight SdWt : Shell dry weight

shell dry weight and depth were also not significant at 5% level. All other characters were found to exhibit significant correlation even at 0.1% level. Contrary to the observations at station 1, all correlations between the different morphometric variables of individuals sampled from stations 2 and 3 were highly significant at 1% level.

The correlation matrix of shell characteristics of P. viridis from the two selected stations are illustrated in Table 2i and 2j. It is evident from Table 2i that, except for flesh weight with shell dry weight, all other correlations were highly significant at 1% level. On the other hand, with the exception of shell dry weight with height, depth and flesh weight and between depth and height, all other correlations between the various morphometric variables of individuals from station 2 were significant at 1% level.

To compare the various morphological characteristics of organisms inhabiting the different stations, analysis of variance (ANOVA) technique (Snedecor and Cochran, 1967) was employed. The model used was,

$X_{ij} = \mu + \alpha i + \epsilon_{ij}$ where,

X_{ij} - is the i^{th} morphometric character of the j^{th} specimen

μ - is the overall effect α_i, of the i^{th} morphometric effect

and ϵ_{ij} - is the random error.

The results of Analysis of Variance of the different morphometric

Table 2 i-j. Perna viridis. Matrix of correlation coefficient among seven morphometric variables of individuals from stations 1 and 2.

2i. Stn. 1

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.5081	1.0000					
Dpt	0.5542	0.2124	1.0000				
Svl	0.6105	0.3433	0.6107	1.0000			
Twt	0.6591	0.4452	0.5208	0.4841	1.0000		
Fwt	0.5978	0.3819	0.4868	0.4086	0.8558	1.0000	
SdWt	0.3875	0.2323	0.2407	0.3208	0.6305	0.1861*	1.0000

* Not significant at 5% level

2j. Stn. 2

	Len	Hgt	Dpt	Svl	Twt	Fwt	SdWt
Len	1.0000						
Hgt	0.6148	1.0000					
Dpt	0.5340	0.3329	1.0000				
Svl	0.6745	0.4174	0.4293	1.0000			
Twt	0.6983	0.5727	0.4713	0.5773	1.0000		
Fwt	0.5796	0.4424	0.3311	0.4616	0.8322	1.0000	
SdWt	0.3978	0.3354	0.4042	0.3701	0.6214	0.2222	1.0000

Len : Length Hgt : Height Dpt : Depth Svl : Shell Volume

Twt : Total Weight Fwt : Flesh Weight SdWt : Shell dry weight

Table 3 a-h. Villorita cyprinoides. Analysis of variance (ANOVA) of the different morphometric variables of individuals from stations 1 to 5 along with their corresponding mean values and level of significance.

3a. Length

Source	SS	Df	Mean Sqr	F	Mean Length (cm)
Total	13.314	249	--		3.80
Station	1.462	4	0.366	7.56*	3.80 3.70 3.73
Error	11.852	245	0.048		3.91

* $P \leq 0.01$

3b. Height

Source	SS	Df	Mean Sqr	F	Mean Height (cm)
Total	30.171	249	--		3.63
Station	12.614	4	3.153	44.00*	3.69 3.13 3.23
Error	17.557	245	0.072		3.53

* $P \leq 0.01$

3c. Depth

Source	SS	Df	Mean Sqr	F	Mean Depth (cm)
Total	12.119	249	--		2.57
Station	2.162	4	0.540	13.30*	2.68 2.46 2.42
Error	9.957	245	0.041		2.57

* $P \leq 0.01$

3d. Shell volume

Source	SS	Df	Mean Sqr	F	Mean Shell vol. (ml)
Total	1557.222	249	--		10.11
Station	609.199	4	152.300	39.36*	8.28 6.62 6.90
Error	948.023	245	3.869		10.36

* $P \leq 0.01$

3e. Total weight

Source	SS	Df	Mean Sqr	F	Mean Total wt (gm)
Total	3722.610	249	--		19.07
					22.75
Station	2075.188	4	518.797	77.15*	14.53
					16.87
Error	1647.422	245	6.724		20.75

* $P \leq 0.01$

3f. Shell weight

Source	SS	Df	Mean Sqr	F	Mean Shell wt (gm)
Total	3021.301	249	--		16.09
					19.74
Station	1627.102	4	406.775	71.48*	12.20
					14.36
Error	1394.199	245	5.691		17.21

* $P \leq 0.01$

3g. Flesh weight

Source	SS	Df	Mean Sqr	F	Mean Flesh wt (gm)
Total	291.677	249	--		3.00
					2.66
Station	50.901	4	12.725	12.95*	2.17
					2.44
Error	240.776	245	0.983		3.46

* $P \leq 0.01$

3h. Shell Dry weight

Source	SS	Df	Mean Sqr	F	Mean Shell Dry wt (gm)
Total	2212.797	249	--		15.89
					20.06
Station	1784.547	4	446.137	76.53*	12.28
					14.31
Error	1428.250	245	5.830		17.56

* $P \leq 0.01$

variables of individuals of V. cyprinoides, C. madrasensis and P. viridis from the selected stations are outlined in Tables 3a-h, 4a-h and 5a-h. Wherever significant difference in mean was observed, the least significant difference at 1% level was calculated; the means are separated and presented along with the tables.

The length of V. cyprinoides from the five stations was subjected to 'ANOVA'. Depicting statistically significant variation between stations ($p \leq 0.01$), the individuals from station 5 showed higher mean length (Table 3a) when compared with those from the other stations. Notwithstanding the significant variations in height observed between stations at 1% level, the 'ANOVA' revealed that individuals from stations 1 and 2 had higher mean height than their counterparts from other stations (Table 3b).

Tables 3c,e,f and h illustrate the 'ANOVA' of depth, total weight, shell weight and shell dry weight of V. cyprinoides from the five stations. The results indicated statistically significant variation between stations ($p \leq 0.01$); the highest mean depth, total weight, shell weight and shell dry weight being characteristic to individuals from station 2. Table 3d outlines the 'ANOVA' of the shell volume of specimens of V. cyprinoides. When compared with other stations, individuals from station 1 and 5 recorded the highest mean shell volume and the observed difference between stations were statistically significant ($p \leq 0.01$). 'ANOVA' of the flesh weight showed significant variation between stations at 1% level and the highest mean flesh weight was depicted by individuals from station 5 (Table 3g).

Tables 4a-h illustrate the analysis of variance (ANOVA) of different

Table 4 a-h. Crassostrea madrasensis. Analysis of variance (ANOVA) of the different morphometric variables of individuals from stations 1 to 3 along with their corresponding mean values and level of significance.

4a. Height

Source	SS	Df	Mean Sqr	F	Mean Height (cm)
Total	19.125	149	--		6.78
Station	0.638	2	0.319	2.41	6.66
Error	19.487	147	0.133		6.64

4b. Length

Source	SS	Df	Mean Sqr	F	Mean Length (cm)
Total	12.691	149	--		4.81
Station	0.365	2	0.182	2.18	4.74
Error	12.326	147	0.084		4.69

4c. Depth

Source	SS	Df	Mean Sqr	F	Mean Depth (cm)
Total	11.657	149	--		2.92
Station	0.364	2	0.182	2.37	3.00
Error	11.293	147	0.077		2.88

4d. Shell Volume

Source	SS	Df	Mean Sqr	F	Mean Shell vol. (ml)
Total	811.549	149	--		12.54
Station	41.545	2	20.772	3.97	13.53
Error	770.004	147	5.238		12.32

4e. Total weight

Source	SS	Df	Mean Sqr	F	Mean Total wt (gm)
Total	3617.563	149	--		50.43
Station	133.625	2	66.813	2.83	49.86
Error	3473.938	147	23.632		48.20

4f. Shell Weight

Source	SS	Df	Mean Sqr	F	Mean Shell wt (gm)
Total	3071.828	149	--		39.14
Station	333.500	2	166.750	8.95*	37.57
Error	2738.328	147	18.628		35.50

* $P \leq 0.01$

4g. Flesh weight

Source	SS	Df	Mean Sqr	F	Mean Flesh wt (gm)
Total	367.983	149	--		12.12
Station	17.682	2	8.841	3.71*	12.92
Error	350.301	147	2.383		12.74

* $P \leq 0.01$

4h. Shell Dry weight

Source	SS	Df	Mean Sqr	F	Mean Shell Dry wt (gm)
Total	3541.657	149	--		37.23
Station	215.391	2	107.695	4.76*	37.76
Error	3326.266	147	22.628		35.00

* $P \leq 0.01$

morphometric variables of C. madrasensis from the three stations. Tables 4a, b, c, d and e explain the 'ANOVA' of height, length, depth, shell volume and total weight and no significant difference was observed between the three stations under study. Table 4f gives the 'ANOVA' of shell weight of C. madrasensis and specimens from stations 1 and 2 had higher mean shell weight when compared to the third station. The 'ANOVA' of flesh weight as explained in Table 4g revealed significant difference between stations at 1% level; stations 2 and 3 depicting higher mean flesh weight than those from station 1. Table 4h gives the 'ANOVA' of shell dry weight of C. madrasensis from the three stations. Here also, statistically significant difference was observed at 1% level. Individuals from station 3 had comparatively low shell dry weight.

Similarly, Table 5a-h represent the results of analysis of variance (ANOVA) of different morphometric characters of P. viridis sampled from the two selected stations. No significant difference between stations at 1% level was observed as far as the morphometric characters concerned.

The multiple regression of total weight on different parameters were worked out for all stations of the three test species using the mathematical model,

$Y = b0 + b1X1 + b2X2 + b3X3 + b4X4 + b5X5 + b6X6$, where,

Y is the total weight,

and X1, X2, X3, X4, X5 and X6 are the different variables.

The coefficient of multiple correlation (R^2) was highly significant

Table 5 a-h. Perna viridis. Analysis of variance (ANOVA) of the different morphometric variables of individuals from stations 1 and 2 along with their corresponding mean values and level of significance.

5a. Length

Source	SS	Df	Mean Sqr	F	Mean Length (cm)
Total	1.288	99	--		4.68
Station	0.002	1	0.002	0.17	
Error	1.286	98	0.013		4.67

5b. Height

Source	SS	Df	Mean Sqr	F	Mean Height (cm)
Total	0.278	99	--		1.99
Station	0.000	1	0.000	0.17	
Error	0.278	98	0.003		1.99

5c. Depth

Source	SS	Df	Mean Sqr	F	Mean Depth (cm)
Total	1.457	99	--		1.65
Station	0.009	1	0.009	0.61	
Error	1.448	98	0.015		1.63

5d. Shell Volume

Source	SS	Df	Mean Sqr	F	Mean Shell vol. (ml)
Total	19.249	99	--		5.55
Station	0.004	1	0.004	0.02	
Error	19.245	98	0.196		5.54

5e. Total weight

Source	SS	Df	Mean Sqr	F	Mean Total wt. (gm)
Total	117.667	99	--		7.43
Station	0.042	1	0.042	0.03	
Error	117.625	98	1.200		7.39

5f. Shell weight

Source	SS	Df	Mean Sqr	F	Mean Shell wt. (gm)
Total	11.861	99	--		3.07
Station	0.008	1	0.008	0.06	
Error	11.853	98	0.121		3.08

5g. Flesh weight

Source	SS	Df	Mean Sqr	F	Mean Flesh wt (gm)
Total	51.848	99	--		4.01
Station	0.128	1	0.128	0.24	
Error	51.720	98	0.528		4.08

5h. Shell Dry weight

Source	SS	Df	Mean Sqr	F	Mean Shell Dry wt (gm)
Total	11.451	99	--		3.00
Station	0.115	1	0.115	0.99	
Error	11.336	98	0.116		3.07

in all the stations. This indicates that the variable taken into account explains a significant part of the variability in the data. The fitted multiple regressions and the corresponding R^2 values are given below.

a) V. cyprinoides

Stn. 1

$$Y = 0.8034 + 0.1356\ X1 + 0.0089\ X2 + 0.0219\ X3 + 0.0935\ X4 - 0.0311\ X5 + 0.01970\ X6$$

$R^2 = 0.5597$; N = 100.

Stn. 2

$$Y = 0.0815 + 0.0393\ X1 - 0.0143\ X2 + 0.1855\ X3 - 0.0772\ X4 + 0.0994\ X5 + 0.2124\ X6$$

$R^2 = 0.7362$; N = 100

Stn. 3

$$Y = 0.8284 + 0.1668\ X1 + 0.0028\ X2 - 0.0026\ X3 - 0.0111\ X4 + 0.0403\ X5 + 0.1892\ X6$$

$R^2 = 0.9487$; N = 100.

Stn. 4

$$Y = 0.8336 + 0.1635\ X1 + 0.0038\ X2 + 0.0197\ X3 - 0.0178\ X4 + 0.0200\ X5 + 0.1869\ X6$$

$R^2 = 0.82234$; N = 100.

Stn. 5

$$Y = 0.8120 + 0.1746\ X1 - 0.0034\ X2 + 0.0238\ X3 + 0.0135\ X4 - 0.0252\ X5 + 0.1979\ X6$$

R^2 = 0.5367; N = 100. where

X1 = Length, X4 = Shell volume

X2 = Height X5 = Flesh weight

X3 = Depth X6 = Shell dry weight.

b) C. madrasensis

Stn. 1

$$Y = 0.6879 + 0.1947\ X1 + 0.0085\ X2 + 0.0109\ X3 + 0.0511\ X4 + 0.1234\ X5 + 0.2574\ X6$$

R^2 = 0.9445; N = 100.

Stn. 2

$$Y = 0.7441 + 0.2769\ X1 - 0.0274\ X2 - 0.0207\ X3 - 0.0230\ X4 - 0.0830\ X5 + 0.3356\ X6$$

R^2 = 0.8808; N = 100.

Stn. 3

$$Y = 0.6770 + 0.2677\ X1 - 0.0112\ X2 - 0.0215\ X3 + 0.0827\ X4 + 0.0707\ X5 + 0.2497\ X6$$

R^2 = 0.9632; N = 100, where,

X1 = Height X4 = Shell volume

X2 = Length X5 = Flesh weight

X3 = Depth X6 = Shell dry weight

c) P. viridis

Stn. 1

$$Y = 0.4918 + 0.3767\ X1 + 0.0702\ X2 + 0.0193\ X3 - 0.0863\ X4 + 0.5715\ X5 - 0.0305\ X6$$

$R^2 = 0.8644$; $N = 100$.

Stn. 2

$$Y = 0.5029 + 0.4336\ X1 - 0.0209\ X2 - 0.2738\ X3 + 0.1401\ X4 + 0.1257\ X5 + 0.1698\ X6$$

$R^2 = 0.8644$; $N = 100$, where,

X1 = Length
X2 = Height
X3 = Depth
X4 = Shell volume
X5 = Flesh weight
X6 = Shell dry weight

The relative importance of the different parameters in the fitted multiple regression model, for the different species were calculated using the formula, $b_i \sqrt{x_1^2/y^2}$ (Snedecor and Cochran, 1967) and are illustrated in Tables 6a-c.

Table 6a illustrates the relative importance of the different variables of V. cyprinoides. It is evident that in the first four stations, shell dry weight was the most important factor but in station 5, length was found to be more important than the other morphometric parameters. This difference may be explained as due to variations in the hydrological factors, mostly salinity, at station 5 when compared to other stations. Table 6b

Table 6a. Villorita cyprinoides. Relative importance of the different morphometric variables of individuals from stations 1 to 5.

	Stn. 1	Stn. 2	Stn. 3	Stn. 4	Stn. 5
Length (X1)	0.1241	0.0406	0.1661	0.1600	0.2324
Height (X2)	0.0077	-0.0413	0.0021	0.0031	-0.0045
Breadth (X3)	0.0148	0.2020	-0.0021	0.0191	0.0294
Shell vol (X4)	0.0355	-0.0477	-0.0057	-0.0056	0.0088
Flesh wt (X5)	-0.0151	0.0230	0.0171	0.0293	-0.0133
Sh. dry wt. (X6)	0.1847	0.2090	0.1831	0.1763	0.1894

Table 6b. Crassostrea madrasensis. Relative importance of the different morphometric variables of idividuals from stations 1 to 3.

	Stn. 1	Stn. 2	Stn. 3
Height (X1)	0.2129	0.3704	0.3641
Breadth (X2)	0.0065	-0.0337	-0.0128
Depth (X3)	0.0062	-0.0170	-0.0225
Shell vol (X4)	0.0192	-0.0200	0.0743
Flesh wt (X5)	0.0957	-0.0814	0.0629
Sh. dry wt. (X6)	0.2054	0.3090	0.2237

Table 6c. Perna viridis. Relative importance of the different morphometric variables of individuals from stations 1 and 2.

	Stn. 1	Stn. 2
Length (X1)	0.6102	0.7078
Height (X2)	0.0816	-0.0244
Breadth (X3)	0.0282	0.4422
Shell wt (X4)	-0.0945	0.1458
Flesh wt (X5)	0.3970	0.0907
Sh. dry wt. (X6)	-0.0262	0.1393

gives the relative importance of the various morphometric parameters of C. madrasensis. In all the three stations, height was found to be the most important parameter. Similarly, the relative importance of the different variables in the regression model of P. viridis is outlined in Table 6c. In both the stations, length was found to be the most important factor in comparison with other variables.

3.5. DISCUSSION

Morphological characters represent a series of measured variables and represent the synergism between shape and size. Effects of physiological and epigenetic constraints on morphology is directly related to certain environmental parameters such as temperature, salinity and oxygen. The nature of observed variations, however, suggested that morphometric differences are of adaptive significance. Univariate and multivariate analyses have been found to be effective in the analysis of geographic variation with respect to multiple characters. Further, analysis of the data employing the two methods would give a better understanding of the nature of variation within and between populations.

The results on univariate and multivariate analysis of the shell measurements revealed that variations did exist between populations of the three species the extent of variation although different in the three species. The difference in shell characters observed in respect of V. cyprinoides may in part be attributed to the changes in climate correlated with the latitudinal and longitudinal changes. Wilson and Summers (1966) found a clinal variation in relative width of the shell of Zoila friendi (Cowrie) around the southern

coast of Australia from Shark Bay in Western Australia to Southern Australia and deduced that water temperature was probably the environmental factor producing this cline. Similar observations on the effect of temperature on growth have been made by Barnes and Healy (1965, 1969) in some common cirripeds in which measurements of scuta and terga were found to be correlated with environmental temperature.

The variations in morphometric characteristics observed during the present study could possibly be due to changes in habitat salinity which was significantly different in the selected stations and may have exerted a major influence on the organism as a whole and shell characteristics in particular. It may be noted here that, the substratum of V. cyprinoides from stations 1, 2 and 3 was muddy in nature while those from stations 4 and 5 was sandy. Univariate analysis of the data revealed that variations did occur in the shell characteristics, among and between populations from stations of both sandy and muddy substrata (Table 3a-h). It has been suggested that the similarity of animals found on similar substrata is possibly not related to the substratum itself and it may be due to the food sources available or to a number of other factors as well. Further, it is reported that sites with similar substrata may offer similar food sources or other conditions (Phillips et al., 1973). The present results however, do not support the above contention.

In the case of C. madrasensis, despite the similarity in substratum, significant variations were observed at 1% level in the shell characteristics, especially with regard to shell weight, flesh weight and shell dry weight (Tables 4f, g and h), among the three selected stations. On the other

hand, in P. viridis, no significant variation in shell characters was evident, despite the similarity in substratum as evidenced by both univariate and multivariate analyses (Table 5a-h). Here also, the effect of substratum on shell characteristics seems doubtful.

Kitching et al. (1968) found that Nucella lapillus found on the open coast had a larger body weight, length and width of aperture and area of foot than in a sheltered area. Further, it was found that animals on the open coast had greater powers of adhesion under rough weather conditions. Moore (1936) found that an increase in mussels (Mytilus) in the food of Nucella lapillus tended to produce a fatter shell with a more open spiral and wider aperture. It was therefore assumed that food could play a significant role in causing the variation in shell characteristics of different populations. Further, studies on the development of Dicathais by Phillips et al. (1973) revealed that food may be particularly important as one of the selective forces of the habitat defining its development especially the shell characteristics. Although the study revealed a possible relationship between food and shell shape, it was suggested that what part of the food was more important at each of the selected sites is difficult to determine. They further pointed out that no single factor, but rather a combination of factors is the 'selective force' at any site. Moreover, since all geographic variations result from the adaptation of a local population to its 'environment', the clinal change in shell shape may be interpreted as a phenotypic expression caused by a change in local conditions. The marginal variations in morphometric characters observed among the different populations of C. madrasensis and V. cyprinoides may perhaps be explained on the basis of the above facts.

Phillips et al. (1973) further observed distinct differences in the structure of the shell between the two populations studied. They opined that among the factors which may have influenced these differences, the most important one would probably be the slight changes in the genetic condition of these populations over a long period of geographic separation. Moreover, majority of the phenotypic variability or morphovariance observed among areas would be due to the fact that structural gene evolution (measured by electrophoresis) proceeded independently at a different rate from evolution at more complex phenotypic level (King and Wilson, 1975; Wilson et al., 1974). Besides these, the rate of protein evolution appears to be proportional to time (Ayala, 1976). This is probably the reason why some morphovariance is noticed in specimens from different areas, although biochemically they may appear to belong to the same species. Perhaps the above interpretations may offer another possible explanation for the observed variations in morphometric characters of the different populations of V. cyprinoides, C. madrasensis and P. viridis in the present study.

It is therefore felt that, among the three species under study, V. cyprinoides represents a species of high phenotypic plasticity as evidenced from the present results. Since V. cyprinoides have a wide geographic distribution, they are more likely to produce locally adapted populations or ecophenotypes than the other two species. The results of the present study further indicate that any studies on morphometric variables of individuals of a population and to relate it with population variation has serious limitations. It may however be used in conjunction with other genetic studies and may be useful in bringing out the extent of such variations among populations as a result of environmental influence.

Chapter 4

ELECTROPHORETIC STUDIES OF MUSCLE PROTEINS

4.1. INTRODUCTION

Proteins, by virtue of their genetically controlled variations, have been subjected to extensive research in recent years. Studies involving protein biochemistry have often led to the formulation of new concepts and techniques which could be utilised to unravel the taxonomic and evolutionary relationships. The primary structure of protein molecule with its amino acid sequence, is genetically determined and the primary structure dictates the secondary, tertiary and quarternary structure in any given environment. Thus, the properties of proteins can indicate the ultimate biochemical makeup and relationships among different organisms.

Electrophoresis is the process of movement of charged particles through an electrolyte when subjected to an electric field. If the particles are charged differently, they will move in opposite directions; the positively charged particles migrating to the cathode and the negatively charged, to the anode. The rate of migration of particles of like charge will depend among other things, on the net charge each carries. A complex mixture such as tissue proteins can thus be separated into a number of fractions following electrophoresis due to this differential rate of migration of the component fractions. The sharpness of resolution depends upon the extent to which each fraction is homogeneous in its mobility. Since, protein synthesis in different living organisms occurs as a result of biosynthetic mechanisms controlled by species-specific genetic processes, the products of such reactions can be interpreted as a measure of genetic differences between species.

Such differences can be employed as a criterion for species classification as well as its population differences.

4.2. REVIEW OF LITERATURE

Electrophoretic techniques were first used by Thiselius in 1937 to distinguish multiple fractions of serum proteins migrating through a solution under the influence of an electric current (cited by Brewer, 1970). During the next twenty five years, the electrophoretic separation of specific proteins was one of the most widely used techniques to delineate specific biochemical characters in organisms. More recently, studies on the proteins of different tissues, and their patterns in relation to taxonomy have been receiving greater attention from among population biologists (Alston and Turner, 1963; Manwell and Baker, 1963). Finer separation of proteins has been achieved using the polyacrylamide disc gel electrophoretic technique (Davis, 1964; Ornstein, 1964).

The application of biochemical genetic techniques to geographically separated populations of marine and estuarine invertebrates has significantly enhanced our understanding of population differentiation in the marine environment. Differences in the muscle myogen components among various species of fishes has been studied utilising various methods which include the moving boundary technique (Connel, 1953; Dingle et al., 1955), paper electrophoresis, column chromatography and starch gel electrophoresis (Tsuyuki et al. 1962). Tsuyuki and Roberts (1965, 1966) and Tsuyuki et al., (1962, 1965a, 1967, 1968) reported that the electrophoretic muscle myogen patterns showed distinct species specificity in Salmonidae, Scorpanidae and Catastomidae,

and was quite independent of physiological factors such as sex, maturation and age. The species specific nature of the muscle myogens in various groups of teleosts was studied by Taniguchi (1969), Jones and Mackie (1970), Taniguchi et al., (1972), Herzberg and Pasteur (1975) and Taniguchi and Sakata (1977). This method has also been used to describe specific proteins from a variety of tissues including vertebrate eye lens proteins (Calhoun and Koeing, 1970) and the foot, adductor muscle and crystalline style of molluscs (Davis and Lindsay, 1967; Bedford and Reid, 1969).

Electrophoretic studies of muscle proteins have revealed distinct patterns in Gobioids of Porto Novo (Natarajan et al., 1975), Mullus surmuletus and M. barbatus (Arias and Morales, 1977), Sarpa salpa and Boops boops (Arias and Morales, 1980) and four species of Sciacnidae (Garcia, 1980). Skeletal muscle proteins often provide useful information in classifications at the generic, familial and higher taxonomic levels (Tsuyuki et al., 1965 a, b; Tsuyuki and Roberts, 1966; Uethe et al., 1966; Tsuyuki et al., 1967) and in several cases, at the species level as well. Examples of intraspecific polymorphism of muscle proteins have also been reported (Tsuyuki et al., 1965a). By virtue of their specificity, electropherograms of blood and tissue proteins show promise in the selection of biochemically uniform parental breeding stocks (Tsuyuki and Roberts, 1965) and in the diagnosis and genetic interpretations of natural and artificial hybrids (Whitmore, 1980). Systematic studies using electrophoresis in rockfishes demonstrated that the electropherograms were independent of size and sex (Tsuyuki et al., 1968). Similar observations of the independence of sex, physiological and ecological factors on muscle myogen patterns and their high degree of species sepcificity and constancy have been made

by Uethe et al., (1966) in a study of anadromous, marine and a few fresh water fishes. Tsuyuki et al. (1968) suggested that the myogen patterns can be regarded as an effective tool to differentiate between species and for such other purposes. Electrophoretic separation patterns of water soluble proteins were reproducible within each species and distinguishable from one another (Mackie and Jones, 1978). They are of the view that only the electrophoretic patterns gave an unequivocal identification of the species.

Studies on the blood and muscle proteins of crabs was carried out by Kannupandi and Paulpandian (1975) while Cole and Morgan (1978a) reported the muscle protein pattern of the blue crab Callinectes sapidus Rathbun. Maguire and Fielder (1975) investigating into the usefulness of electrophoretic pattern of the haemolymph proteins of some portunid crabs as taxonomic characters at the generic level, found that only haemocyanin bands are useful in defining related species and that the other bands are prone to individual variation. Haemocyanin patterns have been found to be species specific and are unaffected by the physiological state of the organism. On the contrary, crustacean haemolymph proteins were found to vary with nutritional state (Uglow, 1969; Busselin, 1970), parasitization (Manwell and Baker, 1963), moulting and reproduction (Busselin, 1970). Electrophoretic studies on the muscle myogen patterns of various penaeid prawns were carried out by Lim and Lee (1970) and Lee and Lim (1973) with a view to trace the interrelationship among closely related species. Sriraman and Reddy (1977) demonstrated the characteristic muscle protein patterns and its differences in planktonic juveniles of Penaeus indicus and P. monodon. Similar studies have been carried out by several others (Kulkarni et al., 1980; Thomas, 1981; Prathibha, 1984; Samuel, 1987). These studies have

indicated that electrophoretic separation of muscle myogens is an effective technique in the confirmation and classification of different species of organisms.

Relatively few attempts have been made towards the application of electrophoretic methods to molluscan systematics. Wright and Ross (1965) examined the usefulness of paper electrophoretic separation of blood and egg proteins as a taxonomic tool for planorbid snails. Davis and Lindsay (1967) used disc gel electrophoresis in order to study the systematics of the land pulmonate Helix pomatia and evaluated the reliability of this technique in molluscan systematics. They used mainly foot muscle and blood proteins and found them reliable in studies involving species and population differences. Pace and Lindsay (1965) looked into the electrophoretic pattern of some diploid and polyploid Bulinae. Studies on the electrophoretic patterns of foot muscle proteins of Pomatiopsis lapidaria and Oncomelania hupensis formosana was the topic of scientific study by Davis (1967). He found that the two taxa showed specific patterns. Davis (1968a,b) have also reported the specific patterns of Oncomelania hupensis chini, several populations of O. hupensis formosana, Semisulcospira trachea and S. libertina from Japan. Davis (1971) analysed the disc electrophoretic profiles of proteins of different populations of Brotia costula episcopalis from Malaysia. A preliminary study on the qualitative variation among the four different genera and five species of Cyclophoridae was carried out by Kasinathan (1974). Margulis and Pinaev (1976) investigated into the species specificity of the contractile proteins of bivalve molluscs and concluded that the electrophoretic data of proteins were species specific in molluscs and that these results may be useful in the analysis of systematic relations

in the class Bivalvia.

Since the first clear demonstration by Hubby and Lewontin (1966) that genetic polymorphism at structural loci can be easily detected by gel electrophoresis, there has been an explosion of information about genetic polymorphism at a number of loci (Wilkins, 1975; Ahmed et al., 1977; Skibinski et al., 1978; Beaumont and Beveridge, 1984). However, attempts to identify polymorphic loci from general protein zymograms have been carried out only in a limited number of bivalve species such as Ostrea lurida (Johnson et al., 1972), Crassostrea gigas (Buroker et al., 1975), Chlamys opercularis (Beaumont and Gruffydd, 1975; Beaumont, 1982a, b) and Crassostrea mardasensis (Ponniah, 1985).

Although a wealth of literature is available on the protein characteristics of bivalve molluscs, information on Indian species particularly that of Kerala waters are rather scarce. The present study centered around delineating the basic protein zymogram of various tissues especially the adductor muscle of three commercially important bivalve species from selected stations of Cochin and nearby waters. It involved the characterisation of intra and interspecific differences among populations of Villorita cyprinoides, Crassostrea madrasensis and Perna viridis inhabiting the selected locations.

4.3. MATERIALS AND METHODS

4.3.1. COLLECTION AND CONDITIONING OF SPECIMENS

The details of selected animals, study area, collection and conditioning

of specimens have been described earlier (vide pages, chapters 2 and 3).

4.3.2. PREPARATION OF STOCK SOLUTIONS

All the stock solutions were prepared according to the modified version of the procedure of Davis (1964) as described in CMFRI Special Publication No. 7.

4.3.3. PROCEDURE

1. Bring all the stock solutions to room temperature.
2. Fix the gel tubes in a gel tube stand.
3. Prepare running gel solutions by mixing small pore buffer, monomer, double distilled water and ammonium per sulphate in 1:2:1:4 ratio, and mix well.
4. Using a syringe, pour the solution gently along the sides of the gel tubes up to the first scratch mark, without interruption of air bubbles while pouring.
5. Add a few drops of distilled water over the solution to avoid meniscus formation.
6. Allow it to polymerise.
7. After completion of polymerisation, remove the overlying water carefully.
8. Prepare spacer gel solution by mixing large pour buffer, monomer solution, riboflavin and sucrose in 1:2:1:4 ratio.
9. Add few drops of this spacer gel up to a fixed level and overlay with a few drops of distilled water in order to avoid meniscus formation

and allow it to polymerise.

10. After the completion of polymerisation of spacer gel, the overlying water is removed in order to apply the test sample.

11. Add a fixed amount of supernatant of the centrifuged sample carefully over the spacer gel. Add equal amount of 40% sucrose solution above the sample and mix well carefully avoiding bubble formation.

12. Remove the gel tube gently and insert into the grommets of the upper buffer tank.

13. Dilute 60 ml of the buffer stock to 600 ml with distilled water and take 300 ml of this working buffer in the lower tank of the electrophoretic chamber.

14. Fill the remaining space of the gel tubes gently with tank buffer in the upper tank. Before adding, the buffer taken in the upper tank should be mixed with a few drops of indicator dye (Bromophenol blue).

15. Place the chamber system in a refrigerator and connect it with the power pack and start the electrophoresis at a very low temperature.

16. For the first ten minutes, the current should be 1 mA/tube. When the marker dye reaches the top level of the running gel, the current is increased to 3mA/tube and kept constant till the end of the run.

17. When the indicator reaches the lower edge of the gel tubes, the current supply is switched off.

18. Remove the gel from the gel tubes carefully using a syringe filled with the used buffer or distilled water.

19. Fix the gels in 10% TCA till the indicator turns to light yellow, rinse with distilled water and immerse in the staining solution for

30-45 minutes.

20. Destain in 7% glacial acetic acid till the bands become clear.

4.3.4. STANDARDISATION OF METHODOLOGY

During the present study, the electrophoretic procedure developed by earlier workers were adopted with necessary modifications and the one which gave the best result was employed for further analysis. The results obtained following standardisation are outlined in Tables 7 to 9.

During standardisation, four different tissues of the three species and four different homogenising media were put to test to judge their suitability in electrophoretic resolution of tissue proteins. Of the different tissues tested, the adductor muscle offered the most readily available source of extractable proteins (Ponniah, 1985) and hence, adductor muscle was chosen for standardization of the whole technique. The tested extraction media include double distilled water, 40% sucrose, 0.05% Sodium chloride (NaCl) solution and Tris-Phosphate buffer (pH-8). Of the different media employed, double distilled water was found to yield the best results. During extraction, 100 mg of tissue was asceptically removed and was homogenized in 1ml chilled double distilled water in a glass teflon test tube homogenizer. The homogenized tissue was then centrifuged for 15 minutes at 10,000 RPM and the supernatant solution containing the water soluble muscle myogens was immediately subjected to electrophoretic resolution.

Since the quantity of protein varies in different tissues, different concentrations of the extract were tried, with a view to determine the

quantity of sample that would produce optimal resolution. Following standardisation, 80 µl of the adductor muscle tissue extract was found to produce the best resolution, while 50 µl, 60 µl and 80 µl of gill, mantle and foot tissue extract respectively were necessary to produce good resolution in the three species

The resolution of protein fractions can be further improved by varying the concentration of separating gel. Proteins of high molecular weight are usually separated using gels of larger pore size while smaller proteins get separated in a better way in gels of smaller pore size. Most investigations in invertebrates have been carried out using a 7% monomer solution although a 10% monomer has also been found to yield good resolution of proteins (Sreeraman and Redy, 1977; Thomas, 1981; Prathibha, 1984; Samuel, 1987). The present study revealed that of the three gel concentrations (5%, 7% and 7.7%) tested, a 7% gel concentration produced the maximum resolution, when compared to the other two concentrations.

The staining techniques for general proteins were standardised employing three different stains such as Amido black, Coomassie brilliant blue and Kenacid blue. The results indicated that amido black was more ideal than the other two stains, for the different tissues under study. While, Amido black stains the gel with a superior staining property, both Coomassie brilliant blue and Kenacid blue were found to act only on the gel surface. The results obtained are detailed in Tables 7 to 9.

Relative mobility of each fraction is calculated as,

$$rm = \frac{\text{distance travelled by each fraction}}{\text{Total distance of gel column}} \times 100 \text{ (Davis, 1964)}$$

Table 7. Effect of different extracting media on the resolution of muscle proteins.

Extracting Media	Total Number of Bands			Resolution			Trailing		
	Clam	Oyster	Mussel	Clam	Oyster	Mussel	Clam	Oyster	Mussel
Double distilled water	13	14	14	Best	Best	Best	Nil	Nil	Nil
40% Sucrose solution	12	13	12	Better	Better	Good	Mode-rate	Nil	Moderate
0.05% $NaCI_2$ solution	10	12	13	Good	Good	Better	Mode-rate	Moderate	Nil
Tris phosphate buffer	11	11	10	Poor	Poor	Poor	Mode-rate	Moderate	Moderate

Table 8. Muscle protein patterns in different stains.

Stains used	Staining Intensity									Total Number of Bands			Backgroun colour
	Clam			Oyster			Mussel			Clam	Oyster	Mussel	
	Dense	Moderate	Thin	Dense	Moderate	Thin	Dense	Moderate	Thin				
Amido black (.1%)	7	3	3	6	6	2	6	7	1	13	14	14	Clear
Coomassie Brilliant blue (0.25%)	7	2	3	5	6	2	6	5	2	12	13	13	Pale blue
Kenacid blue (0.25%)	6	3	4	6	2	6	5	7	2	13	14	14	Bluish black

Table 9. General protein patterns using different quantities of adductor, foot, mantle and gill tissues.

Sample value	Number of Bands			Resolution			Trailing		
	Clam	Oyster	Mussel	Clam	Oyster	Mussel	Clam	Oyster	Mussel
Adductor muscle									
50 ul	10	12	12	Moderate	Moderate	Poor	Heavy	Nil	Medium
60 ul	8	10	12	Poor	Poor	Poor	Nil	Medium	Poor
70 ul	12	12	13	Moderate	Moderate	Moderate	Medium	Poor	Medium
80 ul	13	14	14	Good	Good	Good	NIl	Nil	Nil
Gill									
50 ul	12	10	10	Good	Poor	Poor	Nil	Nil	Medium
70 ul	10	13	12	Good	Moderate	Moderate	Medium	Medium	Medium
80 ul	10	15	12	Moderate	Good	Good	Medium	Nil	Nil
Mantle									
50 ul	9	10	12	Poor	Poor	Poor	Heavy	Heavy	Heavy
60 ul	11	10	14	Good	Moderate	Moderate	Nil	Medium	Medium
70 ul	11	11	15	Moderate	Moderate	Moderate	Medium	Medium	Medium
80 ul	10	12	15	Moderate	Good	Good	Heavy	Nil	Nil
Foot									
50 ul	7	-	13	Poor	-	Moderate	Heavy	-	Medium
60 ul	7	-	14	Moderate	-	Poor	Nil	-	Heavy
70 ul	8	-	16	Moderate	-	Good	Medium	-	Nil
80 ul	10	-	16	Good	-	Good	Nil	-	Nil

4.4 RESULTS

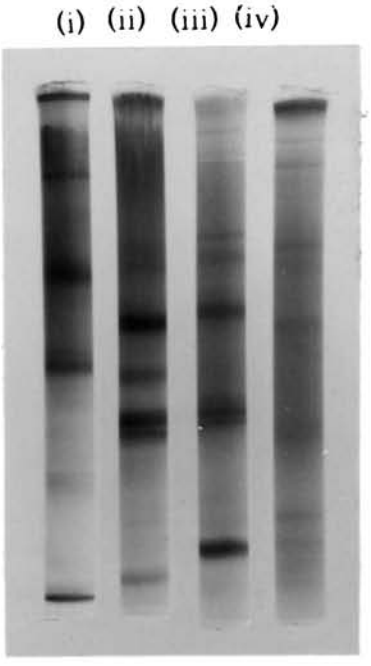
The comparative electrophoretic patterns of different tissues of the tree bivalve species - Villorita cyprinoides, Crassostrea madrasensis and Perna viridis are depicted in Photomicrographs 4a-7b, Figures 3a-6b and Tables 10a-16.

Each fraction obtained has been assigned a qualifying number according to their electrophoretic mobility and position in the electropherogram. Thus, the slowest and the fastest moving bands would receive the first and the last number respectively; the bands in between being denoted by the corresponding serial numbers. Therefore, the total number of protein fractions obtained and their allotted numbers may not necessarily be the same for each population and species. Analysis of electrophoretic patterns help to ascertain the biochemical similarity between species and the populations from which they were sampled.

The tissue specific banding pattern of various tissues of the three test species, following electrophoretic separation is outlined in Photomicrographs 4 a-c , Figs. 3 a-c and Tables 10 a-c . Variations were evident in the number of bands, their relative mobility, thickness and staining intensity. Compared to other tissues, the adductor muscle was found to produce less variations between individuals with regard to staining intensity and thickness of bands. Moreover, adductor muscle was found to yield greater number of clear bands in comparison to other tissues.

4.4.1. VILLORITA CYPRINOIDES

The tissue specific banding pattern of adductor muscle, foot, gill and mantle tissues of V. cyprinoides are explained in Photomicrograph 4a,



Photomicrograph 4a. Villorita cyprinoides. Electrophoretic protein banding pattern of the various tissues.

(i) Adductor muscle (ii) Foot (iii) Gill (iv) Mantle.

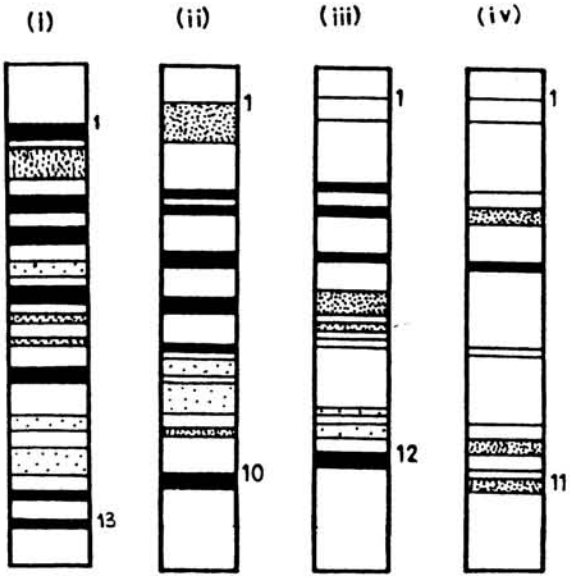


Fig.3a. Villorita cyprinoides. Electropherogram of the various tissue proteins (i) Adductor muscle (ii) Foot (iii) Gill (iv) Mantle.

Table 10a. Villorita cyprinoides. Relative mobility (rm) with staining intensity (I) of the protein fractions of various tissues. (1) Adductor muscle (2) Foot (3) Gill (4) Mantle.

(1)			(2)			(3)			(4)		
No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity
1.	12.31-15.38	xxx	1.	7.69-15.38	xx	1.	6.15	xxx	1.	6.15	xxx
2.	16.92-23.08	xx	2.	24.69-26.15	xxx	2.	10.77	xxx	2.	10.77	xxx
3.	26.15-29.23	xxx	3.	27.68-29.23	xxx	3.	23.08-24.62	xxx	3.	24.62	xxx
4.	32.31-35.38	xxx	4.	36.92-40.00	xxx	4.	27.69-29.23	xxx	4.	27.69-30.77	xx
5.	38.46-41.54	x	5.	46.15-49.23	xxx	5.	36.92-38.46	xxx	5.	38.46-10.00	xxx
6.	43.08-47.69	xxx	6.	55.38-50.92	xxx	6.	44.62-49.23	xx	6.	55.38	xxx
7.	49.23-50.77	xx	7.	58.46-61.54	x	7.	50.77-52.31	xx	7.	56.92	xxx
8.	53.85-55.38	xx	8.	63.08-69.23	x	8.	53.85	xxx	8.	70.77	xxx
9.	60.00-63.08	xxx	9.	72.31-73.85	xx	9.	55.38	xxx	9.	73.85-76.92	xx
10.	69.23-72.31	x	10.	81.54-84.62	xxx	10.	67.69-69.23	x	10.	80.00	xxx
11.	75.38-81.54	x				11.	70.77-73.85	x	11.	81.54-84.62	xx
12.	84.62-86.15	xxx				12.	76.92-80.00	xxx			
13.	90.77-92.31	xxx									

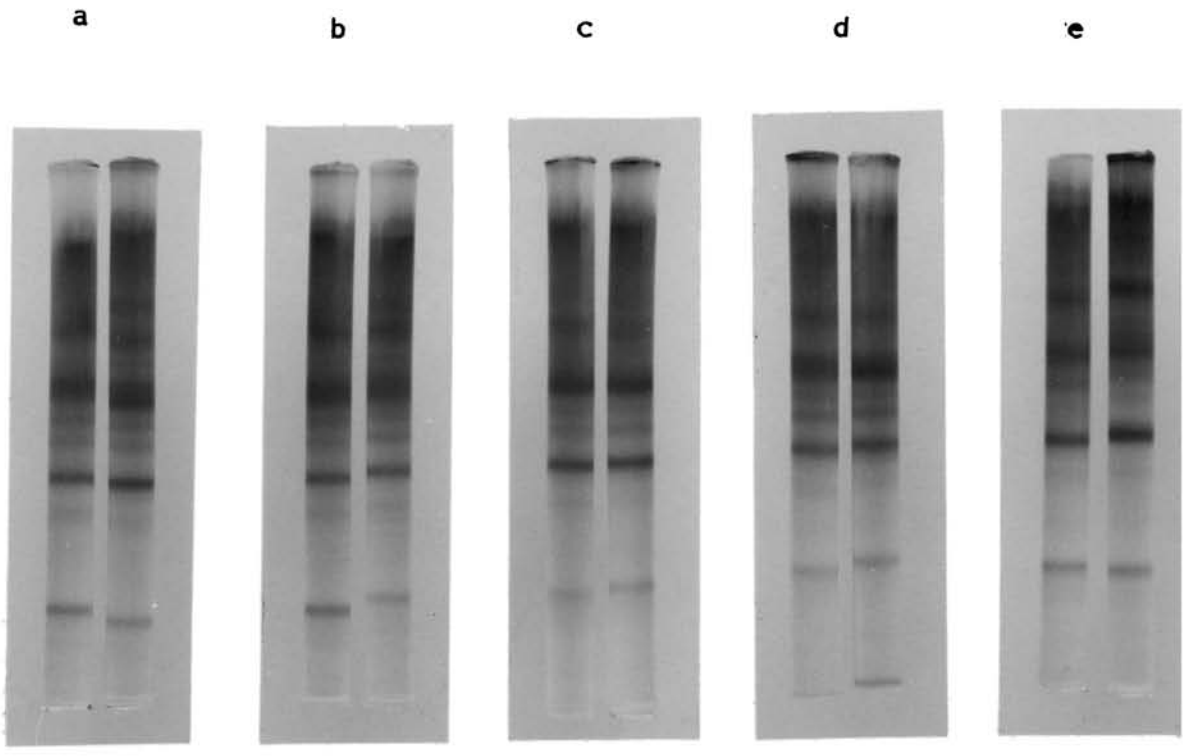
xxx : Thickly stained xx: Moderately stained x : Lightly stained

Fig. 3a and Table 10a. The electropherogram of the adductor muscle revealed 13 bands while the foot tissue had 10 bands. Marginal variations were evident among the different individuals with reference to staining intensity and thickness of some of the fractions. The gill and mantle tissues were characterised by 12 and 11 bands respectively. Both the tissues depicted variations in the staining intensity among individuals, but not in the thickness of fractions.

Photomicrographs 5 a-e , Figs. 4 a-e and Table 11 a-e illustrate the myogen banding patterns of the adductor muscle of individuals of V. cyprinoides sampled from the selected stations. Examination of the data obtained on the relative mobility (Table 11 a-e) of the various protein fractions indicated that individuals from stations 1,2,3 and 4 possessed 13 number of fractions while those from station 5 had 14 distinct fractions. However, the position of these bands in the gel may be arranged in 22 ways (22 bands) based on their relative mobility (rm) (Fig. 4a-e and Table 11 a-e).

Specimens from station 1 had typically 13 fractions comprising of 7 dense (no. 2, 5, 6, 11, 16, 20 and 22), 3 less dense (No. 3, 13 and 14) and 3 diffuse bands (no. 9, 17 and 18) as shown in Fig. 4a. The muscle myogen pattern of individuals from station 2 was found to be of a similar nature (Photomicrograph 5b, Fig. 4b and Table 11b). Despite the similarity observed in the number and relative mobility of the various fractions, differences were evident in the staining intensity and width of some fractions (no. 9, 14 and 22).

The results obtained following electrophoretic analysis of the muscle



Photomicrograph 5 a-e. Villorita cyprinoides. Electrophoretic banding pattern of the adductor muscle proteins of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn. 4 (e) Stn. 5

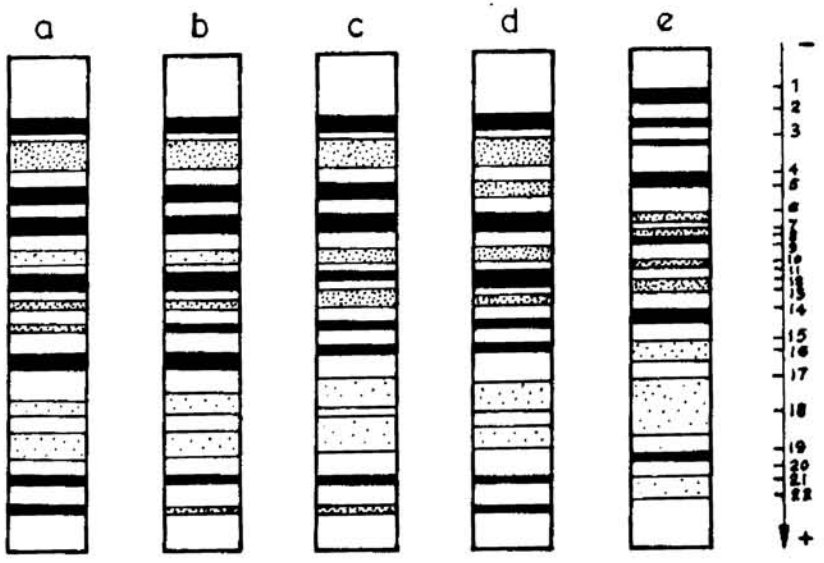


Fig. 4 a-e Villorita cyprinoides. Electropherogram of the adductor muscle proteins of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn.3 (d) Stn.4 and Stn.5

Table 11 a-e. Villorita cyprinoides. Relative mobility (rm) with staining intensity (I) of the adductor muscle proteins of individuals from stations 1 to 5 (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn. 4 (e) Stn. 5.

(a)			(b)			(c)			(d)			(e)		
No.	rm(mm)	Inten-sity	No.	RM(mm)	Inten-sity	No.	RM(mm)	Inten-sity	No.	rm(mm)	Inten-sity	No.	rm(mm)	Inten-sity
1.	-	-	1.	-	-	1.	-	-	1.	-	-	1.	7.69-10.77	xxx
2.	12.31-15.38	xxx	2.	12.31-15.38	xxx	2.	12.31-15.38	xxx	2.	12.31-15.38	xxx	2.	13.85-15.38	xxx
3.	16.92-23.08	xx	3.	16.92-23.08	xx	3.	16.92-23.08	xx	3.	16.92-23.08	xx	3.	18.46-20.00	xxx
4.	-	-	4.	-	-	4.	-	-	4.	-	-	4.	24.62-27.69	xxx
5.	26.15-29.23	xxx	5.	26.15-29.23	xxx	5.	26.15-29.23	xxx	5.	26.15-29.23	xxx	5.	-	-
6.	32.31-35.38	xxx	6.	32.31-35.38	xxx	6.	32.31-35.38	xxx	6.	32.31-35.38	xxx	6.	32.31-33.85	xx
7.	-	-	7.	-	-	7.	-	-	7.	-	-	7.	35.38-36.92	xx
8.	-	-	8.	-	-	8.	-	-	8	-	-	8.	36.92-38.46	xxx
9.	38.46-41.54	x	9.	38.46-41.54	xx	9.	38.46-41.54	xx	9.	38.46-41.54	xx	9.	-	-
10.	-	-	10	-	-	10	-	-	10	-	-	10.	41.53-43.08	xx
11.	43.08-47.69	xxx	11.	43.08-47.69	xxx	11.	43.08-46.15	xxx	11.	43.08-47.69	xxx	11.	-	-
12.	-	-	12.	-	-	12.	-	-	12.	-	-	12.	46.15-49.23	xx
13.	49.23-50.77	xx	13.	49.23-50.77	xx	13.	49.23-50.77	xx	13.	49.23-50.77	xx	13.	-	-
14.	53.85-55.38	xx	14.	53.85-55.38	xxx	14.	53.85-55.38	xxx	14.	53.85-55.38	xxx	14.	52.31-55.38	xxx
15.	-	-	15.	-	-	15.	58.46-60.00	xxx	15.	58.46-60.00	xxx	15.	58.46-63.08	x
16.	60.00-63.08	xxx	16.	60.00-63.08	xxx	16.	-	-	16.	-	-	16.	-	-
17.	69.23-72.31	x	17.	69.23-72.31	x	17.	64.62-70.77	x	17.	66.15-72.31	x	17.	66.15-78.46	x
18.	75.38-81.54	x	18.	75.38-80.00	x	18.	72.31-80.00	x	18.	75.38-80.00	x	18.	-	-
19.	-	-	19.	-	-	19.	-	-	19.	-	-	19.	81.54-83.08	xxx
20.	84.62-86.15	xxx	20.	84.62-86.15	xxx	20.	84.62-86.15	xxx	20.	84.62-86.15	xxx	20.	-	-
21.	-	-	21.	-	-	21.	-	-	21.	-	-	21.	86.15-90.77	x
22.	90.77-92.31	xxx	22.	90.77-92.31	x	22.	90.77-92.31	x	22.	90.77-92.31	x	22.	-	-

xxx : Thickly stained xx : Moderately stained x : Lightly stained

proteins of V. cyprinoides sampled from station 3 is outlined in Photomicrograph 5c, Fig. 4c and Table 11c. Notwithstanding the minor variations in the 'rm' values of some of the fractions, the banding pattern was more or less similar to those obtained from stations 1 and 2. Fractions denoted by no. 2, 3, 5, 6, 9, 11, 13, 14, 17, 18, 20 and 22 were identical to those recorded from stations 1 and 2, although band width evinced marginal differences in the case of bands represented by No. 9, 14 and 22 from stations 1 and 3. These three bands, on the other hand, were similar in their intensity and relative mobility with those obtained from stations 2, 3 and 4.

Specimens from station 4 were characterised by an almost identical electrophoretic pattern when compared with those obtained from the first three stations (Photomicrograph 5d, Fig. 4d and Table 11d). However, marginal difference was evident with respect to the staining intensity and relative mobility of some of the fractions (no. 9, 17 and 18). Fraction no. 15 was common to stations 3 and 4 while fraction no. 16 was identical to stations 1 and 2. Apart from these variations, the other fractions were comparable to those obtained from stations 1, 2 and 3.

Contrary to the observations at stations 1 to 4, the nature of the zymogram of adductor muscle tissue of specimens from station 5 depicted marked variations. Conspicuous differences were evident with respect to the number of protein fractions, their staining intensity and relative mobility (Photomicrograph 5e, Fig. 4e and Table 11e). The fraction denoted by no. 1 present at the anodal region was absent in all the other stations.

Table 12. Villorita cyprinoides. Summary of the adductor muscle protein pattern of individuals from stations 1 to 5 based on Fig. 4 a-c.

Stations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	No. of total bands	No. of common bands
Station 1	-	+	+	-	+	+	-	-	+	-	+	-	+	+	-	+	+	+	-	+	-	+	13	5
Station 2	-	+	+	-	+	+	-	-	+	-	+	-	+	+	_	+	+	+	-	+	-	+	13	5
Station 3	-	+	+	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	-	+	-	+	13	5
Station 4	-	+	+	-	+	+	-	-	+	-	+	-	+	+	+	-	+	+	-	+	-	+	13	5
Station 5	+	+	+	+	-	+	+	+	-	+	-	+	-	+	+	-	+	-	+	-	+	-	14	5

(+) Presence of that fraction
(-) Absence of that fraction

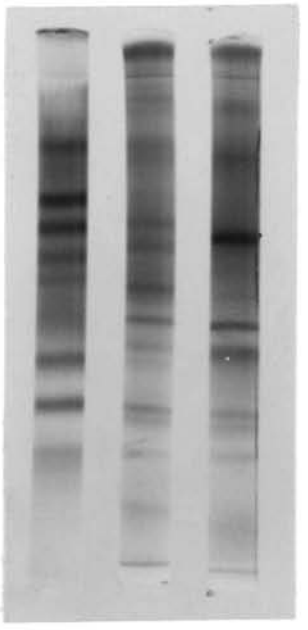
Further, bands no. 4, 7, 8, 10, 12, 19 and 21 indicated in Fig. 4e and Table 11e were found incomparable with the zymogram of the other four stations. On the other hand, fractions no. 2, 3, 6, 14 and 17 were found to occur in the electropherogram of individuals from the other stations but they showed difference in the staining intensity, relative mobility and band width. However, individuals from this station was found to share band no.15 (Table 11 a-e) with their counterparts from station 4. In fact, there was no band in common with exactly the same relative mobility, staining intensity and thickness in the five stations. A summary of the pattern obtained is presented in the table 12 based on fig. 4a-e.

4.4.2. CRASSOSTREA MADRASENSIS

Tissue specific variations in the general protein zymogram were observed with regard to their relative mobility, thickness and staining intensity (Photomicrograph 4b, Fig. 3b and Table 10b). While the gill tissue of C. madrasensis was characterised by 15 bands, the mantle tissue had only 12 bands in the electropherogram of different individuals. Compared to these tissues, the adductor muscle had 14 bands and showed less variation among individuals of the same population with respect to the staining intensity and thickness of the bands. For the purpose of comparison of different populations, only adductor muscle pattern was therefore taken into account.

Studies on the electrophoretic resolution of the protein fractions of the adductor muscle of individuals of C. madrasensis sampled from the three stations were carried out and the results obtained are detailed out in Photomicrographs 6 a-c , Fig. 5 a-c and Table 13 a-c .

(i) (ii) (iii)



Photomicrograph 4b Crassostrea madrasensis. Electrophoretic protein banding pattern of the various tissues.
(i) Adductor muscle (ii) Gill (iii) Mantle.

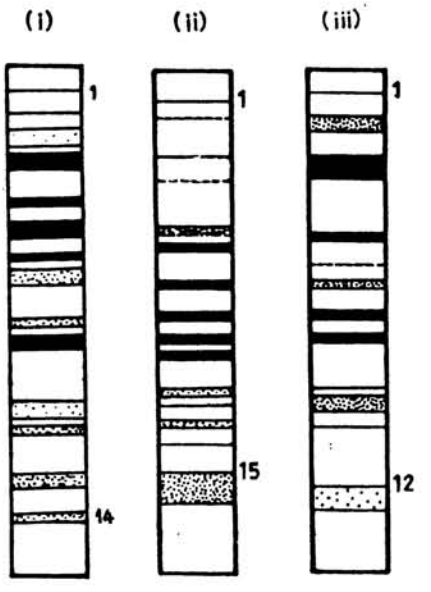


Fig. 3b. Crassostrea madrasensis. Electropherogram of the various tissue proteins. (i) Adductor muscle (ii) Gill (iii) Mantle.

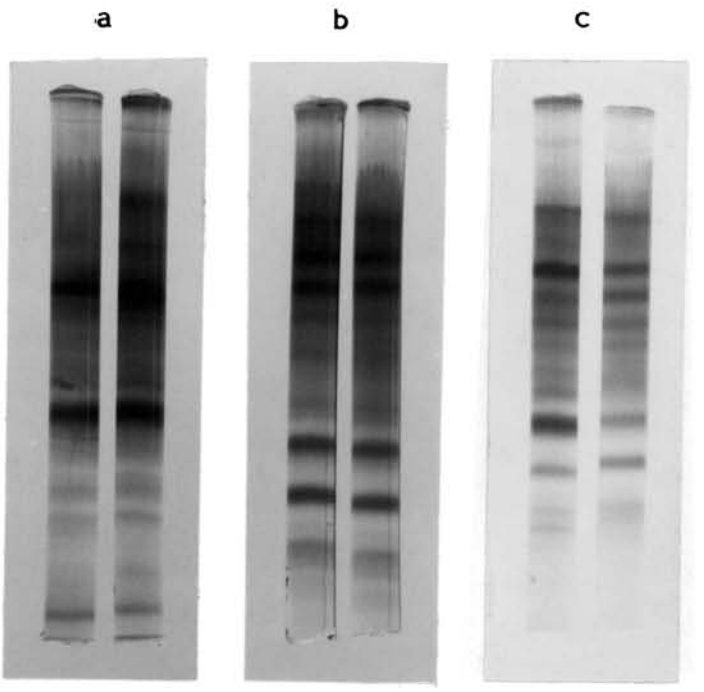
Table 10b. Crassostrea madrasensis. Relative mobility (rm) with staining intensity (I) of the protein fraction of various tissues. (1) Adductor muscle (2) Gill (3) Mantle

(1)			(2)			(3)		
No.	rm	Inten-sity	No.	rm	Inten-sity	No.	rm	Inten-sity
1.	4.62	xxx	1.	6.15	xxx	1.	4.62	xxx
2.	9.23	xxx	2.	7.69	x	2.	9.23-12.31	xx
3.	12.31-15.38	x	3.	9.23	xxx	3.	16.92-21.54	xxx
4.	16.92-20.00	xxx	4.	10.77	x	4.	32.31-33.85	xxx
5.	26.15-27.69	xxx	5.	16.92-21.54	xx	5.	38.46	xx
6.	30.77-33.85	xxx	6.	30.77	xxx	6.	41.54-43.08	xx
7.	36.92-38.46	xxx	7.	33.85-35.38	xxx	7.	47.69-49.23	xxx
8.	40.00-43.08	xx	8.	41.54-43.08	xxx	8.	52.31-53.85	xxx
9.	49.23-50.77	xx	9.	47.69-49.23	xxx	9.	63.08	xxx
10.	52.31-55.38	xxx	10.	52.31-53.85	xxx	10.	64.62-67.69	xx
11.	66.15-69.23	xx	11.	55.38-56.92	xx	11.	70.77	xxx
12.	70.77-72.31	xx	12.	63.08	xxx	12.	83.08-87.69	x
13.	80.00-83.08	xxx	13.	64.62-66.15	xx			
14.	87.69-89.23	xx	14.	70.77	xxx			
			15.	80.00-86.15	xx			

It is evident from Photomicrographs 6 a-c , Fig. 5 a-c and Table 13 a-c that, specimens collected from the three stations have altogether 14 fractions. Although, 14 bands could be identified in all the three populations studied, on the basis of the differences showed in the relative mobility and thickness, they could be arranged in such a way that there were 24 band positions for comparison. Of the 14 fractions observed among individuals from station 1, 7 were densely stained, while 5 were moderately dense and 2 lightly stained (Photomicrograph 6a, Fig. 5a and Table 13a-c).

The protein banding pattern of the adductor muscle tissue of individuals from station 2 depicted 14 bands (Photomicrograph 6b, Fig. 5b and Table 13 a-c). When compared to station 1, the anodal region of the electropherogram from station 2 had only a single band (no. 2) in place of the two bands from station 1 (no. 1 and 3) and station 3 (no.. 1 and 2) respectively. The electropherogram had 7 dense bands, 3 moderately dense bands and 4 light bands (Fig. 5b). The individuals from this station were found to share 6 bands (no. 4, 5, 6, 8, 10 and 14) with those from station 1 while 10 bands (no. 2, 4, 5, 12, 14, 15, 17, 19, 20 and 22) were found common to station 3. However, differences were evident with regard to the relative mobility, staining intensity and width of some of the common bands (Fig. 5 a-c).

The banding pattern obtained from station 3 is depicted in Photomicrograph 6c, Fig. 5c and Table 13 a-c . Individuals from station 3 had 14 bands in the electropherogram, of which 8 were dense bands, 4 moderately dense and 2 light bands. The position of the first two bands obtained from this station appeared to be a combination of the first band



Photomicrograph 6 a-c Crassostrea madrasensis. Electrophoretic banding pattern of the adductor muscle proteins of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3

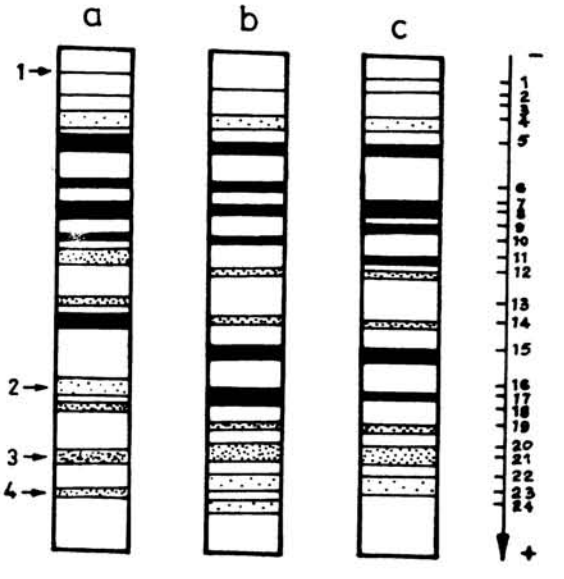


Fig. 5 a-c Crassostrea madrasensis. Electropherogram of the adductor muscle proteins of individuals from stations 1 to 3. (a) Stn. 1 (b) (b) Stn. 2 (c) Stn. 3

Table 13 a-c Crassostrea madrasensis. Relative mobility (rm) with staining intensity (I) of the adductor muscle proteins of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3.

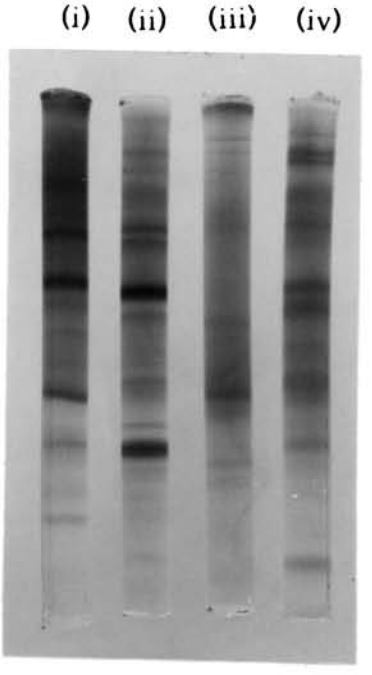
(a)			(b)			(c)		
No.	Relative mobility (rm)	Inten-sity (I)	No.	Relative mobility (rm)	Inten-sity (I)	No.	Relative mobility (rm)	Inte sity (I)
1.	4.62	xxx	1.	-	-	1.	4.62	xxx
2.	-	-	2.	7.69	xxx	2.	7.69	xxx
3.	9.23	xxx	3.	-	-	3.	-	-
4.	12.31-15.38	x	4.	12.31-15.38	x	4.	12.31-15.38	x
5.	16.92-20.00	xxx	5.	18.46-20.00	xxx	5.	18.46-20.00	xxx
6.	26.15-27.69	xxx	6.	26.15-27.69	xxx	6.	-	-
7.	-	-	7.	-	-	7.	29.23-32.31	xxx
8.	30.77-33.85	xxx	8.	30.77-32.31	xxx	8.	-	-
9.	-	-	9.	-	-	9.	33.85-35.38	xxx
10.	36.92-38.46	xxx	10.	36.92-38.46	xxx	10.	-	-
11.	40.00-43.08	xx	11.	-	-	11.	40.00-41.54	xxx
12.	-	-	12.	43.08-44.62	xx	12.	43.08-44.62	xx
13.	49.23-50.77	xx	13.	-	-	13.	-	-
14.	52.31-55.38	xxx	14.	52.31-53.85	xx	14.	52.31-53.85	xx
15.	-	-	15.	58.46-61.54	xxx	15.	58.46-61.54	xxx
16.	66.15-69.23	x	16.	-	-	16.	-	-
17.	-	-	17.	61.69-70.77	xxx	17.	67.69-69.23	xxx
18.	70.77-72.31	xx	18	-	-	18.	-	-
19.	-	-	19.	73.46-81.54	x	19.	73.85-75.38	xx
20.	-	-	20.	78.46-81.54	xx	20.	78.46-81.54	xx
21.	80.00-83.08	xx	21.	-	-	21.	-	-
22.	-	-	22.	84.62-87.69	x	22.	84.62-87.69	x
23.	87.69-89.23	xx	23.	-	-	23.	-	-
24.	-	-	24.	89.23-92.31	x	24.	-	-

from station 1 with the first band from the station 2 indicating the existence of polymorphism among populations of Crassostrea from these two regions. Of the 14 bands obtained, 10 bands (No. 2, 4, 5, 12, 14, 15, 17, 19, 20 and 22) were common for this station and station 2, while 5 bands were identical with those from station 1 (No. 1, 4, 5, 11 and 14) despite minor variations in intensity and band width. Notwithstanding the marginal variations in relative mobility, staining intensity and width of the fifteenth fraction, three bands (no. 4, 5 and 14) were common for the three different stations. Thus, all individuals from each of the selected stations gave a slightly different pattern characteristic to the respective population. This observed variation can however be considered as only those within the range of possible flexibility.

However, a comparison of gel position of certain specific bands in the Fig. 5(a-c) appear to indicate protein polymorphism in the species of C. madrasensis. For example, in gel region marked 1, the presence or absence of the first, second and third bands indicates three phenotypes, namely, 'SF'(a), 'FF'(b) and 'SF'(c). Similar comparison in region marked 2, 3 and 4 appears to indicate phenotypic variations. However, it is interesting to note that each region showed only one particular phenotype. In other words, the expected variable phenotypes usually present at a polymorphic locus were absent. Moreover, the gel positions of the rest of the bands in these three regions are not comparable. Certain bands differed in thier gel position, staining intensity and band width. A summary of the pattern depicted is given in Table 14 based on fig. 5a-c.

4.4.3. PERNA VIRIDIS

Electrophoretic analysis of the adductor muscle of specimens of



Photomicrograph 4c. Perna viridis. Electrophoretic protein banding pattern of the various tissues. (i) Adductor muscle (ii) Foot (iii) Gill (iv) Mantle.

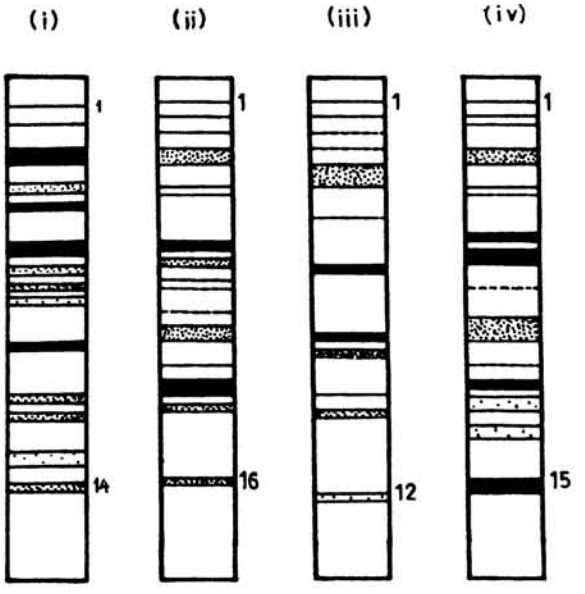
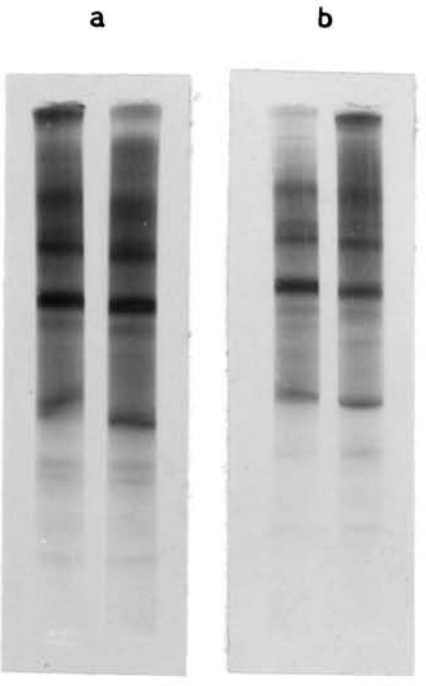


Fig. 3c. Perna viridis. Electropherogram of the various tissue proteins. (i) Adductor muscle (ii) Foot (iii) Gill (iv) Mantle

Table 10c. Perna viridis. Relative mobility (rm) staining intensity (I) of the Protein fractions of various tissues. (1) Adductor muscle (2) Foot (3) Gill (4) Mantle

(1)			(2)			(3)			(4)		
No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity	No.	rm (mm)	Inten-sity
1.	6.15	xxx	1.	4.62	xxx	1.	4.62	xxx	1.	4.62	xxx
2.	9.23	xxx	2.	7.69	xxx	2.	7.69	xxx	2.	7.69	xxx
3.	13.85-16.92	xxx	3.	10.77	xxx	3.	10.77	x	3.	9.23	xxx
4.	21.54-23.08	xx	4.	13.85-16.92	xx	4.	13.85	x	4.	13.85-16.72	xx
5.	24.62-26.15	xxx	5.	21.54	xxx	5.	16.92-21.54	xx	5.	21.54	xxx
6.	32.31-35.38	xxx	6.	23.08	xxx	6.	27.69	x	6.	23.08	xx
7.	36.92-38.47	xx	7.	32.31-33.85	xxx	7.	36.92-38.46	xxx	7.	30.77-32.31	xxx
8.	40.00-41.54	xx	8.	35.38-36.92	xx	8.	50.17-52.31	xxx	8.	33.85-36.92	xxx
9.	43.08-44.62	x	9.	40.00	x	9.	53.85-55.38	xx	9.	41.54	xx
10.	52.31-53.85	xxx	10.	41.54	x	10.	63.08	xxx	10.	47.69-52.31	xx
11.	63.08-64.62	xx	11.	46.15	x	11.	66.15-67.69	xx	11.	56.92	x
12.	66.15-67.69	xx	12.	49.23-52.31	xx	12.	83.08-84.62	x	12.	60.00-61.54	xxx
13.	75.38-76.91	xx	13.	56.92	xxx				13.	63.08-66.15	x
14.	80.00-81.54	xx	14.	60.00-63.08	xxx				14.	67.23-70.17	x
			15.	64.61-66.15	xx				15.	80.00-83.08	xxx
			16.	80.00-81.54	xx						

xxx : Thickly stained xx : Moderately stained x : Lightly stained



Photomicrograph 7 a-b. Perna viridis. Electrophoretic banding pattern of the adductor muscle proteins of individuals from stations 1 and 2. (a) Stn. 1 (b) Stn. 2.

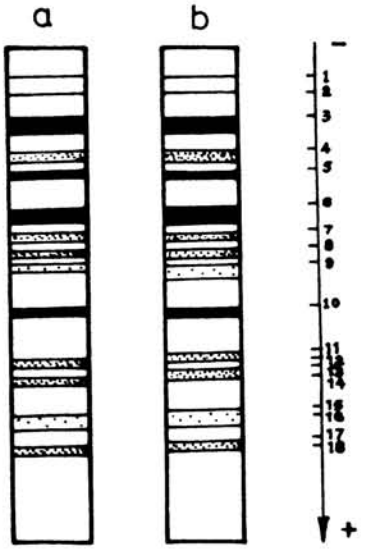


Fig. 6 a-b. Perna viridis. Electropherogram of the adductor muscle proteins of individuals from stations 1 and 2. (a) Stn. 1 (b) stn. 2.

Table 15 a-b. Perna viridis. Relative mobility (rm) with staining intensity (I) of adductor muscle proteins of individuals from stations 1 and 2.

(a) Stn.1 (b) Stn. 2

(a)			(b)		
No.	Relative mobility (rm)	Intensity (I)	No.	Relative mobility (rm)	Intensity (I)
1.	6.15	xxx	1.	6.15	xxx
2.	9.23	xxx	2.	9.23	xxx
3.	13.85-16.92	xxx	3.	13.85-16.92	xxx
4.	21.54-23.08	xx	4.	21.54-23.08	xx
5.	24.62-26.15	xxx	5.	24.62-26.15	xxx
6.	32.31-35.38	xxx	6.	32.31-35.38	xxx
7.	36.92-38.47	xx	7.	36.92-38.46	xx
8.	40.00-41.54	xx	8.	40.00-41.54	xx
9.	43.08-44.62	x	9.	43.08-44.62	x
10.	52.31-53.85	xxx	10.	52.31-53.85	xxx
11.	-	-	11.	61.54-63.08	xx
12.	63.08-64.62	xx	12.	-	-
13.	-	-	13.	64.62-66.15	xx
14.	66.15-67.69	xx	14.	-	-
15.	-	-	15.	72.31-75.38	xx
16.	75.38-76.91	xx	16.	-	-
17.	-	-	17.	78.46-80.00	xx
18.	80.00-81.54	xx	18.	-	-

Table 14. Crassostrea madrasensis. Summary of the adductor muscle protein pattern of individuals from stations 1 to 3 based on Fig. 5a-c.

Stations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	No. of Total bands	No. of Common bands
Station 1	+	-	+	+	+	+	-	+	-	+	+	-	+	+	-	+	-	+	-	-	+	-	+	-	14	3
Station 2	-	+	-	+	+	+	-	+	-	+	-	+	-	+	+	-	+	-	+	+	-	+	-	+	14	3
Station 3	+	+	-	+	+	-	+	-	+	-	+	+	-	+	+	-	+	-	+	+	-	+	-	-	14	3

(+) Presence of that fraction
(-) Absence of that fraction

Table 16. Perna viridis. Summary of the adductor muscle protein pattern of individuals from Stations 1 and 2 based on fig. 6 a-b.

Stations	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	No. of total bands	No. of Common bands
Station 1	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+	14	10
STation 2	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	-	+	-	14	10

(+) Presence of that fraction
(-) Absence of that fraction

P. viridis sampled from the two selected stations yielded results as detailed in Photomicrograph 7a-b, Fig. 6a-b and Tables 15a-b. The tissue-specific variation in the electrophoretic pattern of adductor muscle, foot, mantle and gill tissues were also carried out (Photomicrograph 4c, Fig. 3c and Table 10c). The results indicated 14, 16, 15 and 12 number of protein fractions in the case of adductor muscle, foot, mantle and gills respectively. All the tissues evinced marginal variations in the staining intensity of protein fractions between individuals.

With regard to the general protein banding pattern of adductor muscle, both the populations of P. viridis indicated 14 bands, of which bands no.1 to 10 were identical and common to the two stations. Although the last four bands (no. 11, 13, 15 and 17) from station 2 and 4 bands (no. 12, 14, 16 and 18) from station 1 differed in their relative mobility, the staining intensity and thickness, the protein fractions from the two populations could be clearly demarcated into 6 dense, 6 moderately dense and 2 lightly stained fractions. The overall pattern in both the stations were almost identical (Table 16).

A comparison of the electrophoretic protein pattern of the different tissues tested clearly indicate that these species may be easily identified using any of the tissue patterns. The species-specific pattern was found to differ in the number of bands, their relative mobility and even staining intensity as evidenced from the results obtained.

4.5. DISCUSSION

Polyacrylamide gel electrophoresis can be employed to understand

the intra-specific and inter-specific differences among populations of different species of bivalves. Further, the species-specific and tissue-specific nature of the electrophoretic patterns can also be clearly demonstrated using this technique. Various tissues like foot, gill, mantle and adductor muscle of of the three bivalve species were put to electrophoretic analysis during the present investigation. The results indicated 10, 12, 11 and 14 number of fractions in the general protein zymogram of the foot, gill, mantle and adductor muscle tissues of V. cyprinoides (Fig. 3a, Table 10a) and were found species-specific. The results obtained for C. madrasensis (Fig. 3b and Table 10b) and P. viridis (Fig. 3c and Table 10c) further established the tissue-specific and species-specific nature of electrophoretic patterns. Commenting on the electrophoretic pattern of Helix pomatia, Davis and Lindsay (1967) opined that since muscle tissue yields reliable and reproducible qualitative and quantitative results, foot muscle extract may be chosen to establish 'fingerprint' patterns for population and species. Species and tissue specificity of electrophoretic pattern with reference to muscle myogens have also been reported in crabs, prawns and fishes (Tsuyuki et al., 1962, 1965; Jones and Mackie, 1970; Lim and Lee, 1970; Kannupandi and Paulpandian 1975). Studies on oysters by Ponniah (1985) revealed that adductor muscle is the best for detecting population differences, since the variations between individuals with respect to relative mobility, staining intensity and thickness are minimum and yields reliable and better reproducible qualitative results. Besides, adductor muscle is characteristic to the class Bivalvia. The adductor and foot muscle tissues have been consistently found to give better resolution during electrophoretic separation. The results obtained during the present study are found to confirm these previous observations. Further, the observed patterns were found independent of sex and size. This agrees well with

the earlier findings of Tsuyuki et al. (1965a) in marine and fresh water fishes.

Comparison of the general protein zymogram of individuals of the three species and their populations from the selected habitats was made on the basis of the specific pattern produced by the adductor muscle tissue protein pattern following electrophoresis. The zymogram of individuals of V. cyprinoides sampled from the five selected stations indicated that individuals from station 5 were distinctly different from their counterparts from stations 1 to 4. This is evident from the fact that specimens from this site had no fraction in common with those from the other four stations. This is further qualified by the observations that populations from stations 1 to 4 shared 12 fractions (no. 2, 3, 5, 6, 9, 11, 13, 14, 17, 18, 20 and 22) in common with only marginal variations in their staining intensity, relative mobility and thickness. This indicates that, electrophoretically, the populations from station 1 to 4 are very similar while those sampled from station 5 are distinctly different from all the other populations. Wright and Ross (1965) investigating into the species-specific nature of electrophoretic pattern in species of Bulinus and Biomphalaria found that the patterns of different populations exhibited variations although they were morphologically similar. The results obtained during the present investigation seem to support the view that electrophoretic patterns provide objective criteria for characterising inter and intraspecific differences.

The present electrophoretic analyses of different populations of Villorita cyprinoides seem to strongly support the conclusions arrived at earlier during the present study on morphological features and morphometry.

The individuals from station 5, may thus be described as Villorita cyprinoides var. cochinensis, while those from stations 1 to 4 as Villorita cyprinoides following the description by Prashad (1921). The observed variation among individuals from station 5 may in part be due to the peculiarities of the local habitat especially with reference to habitat salinity (vide pages, Chapter 2).

Despite their morphological similarity, individuals of Crassostrea madrasensis from the three selected stations exhibited some variations in the general protein zymogram pattern, when compared to the species V. cyprinoides. Ponniah (1985) reported the presence of two polymorphic loci in C. madrasensis. During the present study, the electrophoretic pattern revealed 14 bands instead of the reported 9 bands. Moreover, there was no clearcut evidence for polymorphic loci reported earlier by Ponniah (1985). A comparison of the protein bands positions 18 to 24 in populations of C. madrasensis from stations 1, 2 & 3 seems to indicate that some form of polymorphism at 3 presumed loci as explained in Fig. 5 a-c . However, all the three populations showed only one specific phenotype at each of the three apparent loci even after testing about 200 individuals of each population. If genetic polymorphism is expected at a particular locus, different phenotypes are expected to manifest itself at that region. The present results however revealed only a single definite phenotypic pattern (Fig. 5 a-c) for each of the regions suggesting that, the observed variations may be due to non-genetic factors like salinity, temperature etc. Further, the variations observed in the two studies may be regarded as the outcome of the methodological differences adopted during the two studies. Besides, the number of protein fractions obtained for gill and

mantle tissues were also more than that reported earlier by Ponniah (1985). Further the present study demonstrated altogether 14, 12 and 15 fractions in the electropherogram of the adductor muscle, mantle and gill tissues respectively, including the faint bands which were discarded in the earlier study.

Individuals from station 1 shared 6 hands (no. 4, 5, 6, 8, 10 and 14) with those from station 2 and 5 bands (no. 1, 4, 5, 11 and 14) with their counterparts from station 3. Comparison of the protein fractions between stations 2 and 3 revealed 10 identical fractions (no. 2, 4, 5, 12 14, 15, 17, 19, 20 and 22). It follows that populations from stations 2 and 3 are more closely related than those from station 1. However, the three stations had 3 bands in common (no. 4, 5 and 14). Thus, the three populations showed intra-specific differences with respect to their electrophoretic mobility, thickness and staining intensity. Their resemblances being manifested in protein zones of similar mobilities which indicate the presence of protein biosynthetic systems under similar genetic control, although the similarity in the mobilities of proteins need not necessarily indicate the identity in the primary structure of proteins. Among the protein fractions examined, those indicated by no. 1, 16, 21 and 23 (Fig. 5a) reflect chances of polymorphism in the three populations compared, thus reflecting their genetic similarity.

Despite the lack of morphological variation between individuals of C. madrasensis from the three stations, data on the electrophoretic resolution of muscle myogen indicate that C. madrasensis may be capable of adaptive

radiation. This may be explained on the basis of the 'niche width variation hypothesis' or 'environmental amplitude hypothesis' (Van Valen, 1965; Somero and Soule, 1974; Karlin and Guttman, 1979; Karlin, 1982). According to this hypothesis, the amount of genetic variation may be regarded as an adaptive strategy for increasing population's fitness in a spatio-temporally heterogeneous and uncertain environment. Several studies have supported this hypothesis (Hedrick et al., 1976; Powell and Taylor, 1979). Thus, morphologically indistinguishable populations can become adapted to local environmental conditions, manifested as variations in the protein composition. Further, the observed changes might possibly be due to post-translational changes, that do not have any direct genetic basis.

Electrophoretic analysis of the muscle tissue proteins of two populations of P. viridis however, gave results of a similar nature. Notwithstanding the minor variations in the relative mobility of a few fast moving cathodal bands (between band positions 11 and 18), the pattern remained more or less uniform. It may be concluded that the two populations under investigation belong to the same species and that the evidences for sharp molecular variations are rather negligible. The tissue-specific and species-specific nature of the protein banding pattern is noteworthy.

The data on relative mobility of the protein fractions of adductor muscle tissue from the three species studied, further demonstrate their affinities and variations that are supposed to occur among the three species representing the three major families of the class Bivalvia. There were 7 fractions which were relatively similar in their relative mobility and staining intensity for clams (V. cyprinoides) and oysters (C. madrasensis) whereas

only 3 fractions were common for clams and mussels (P. viridis). Notwithstanding the slight differences in the staining intensity and relative mobility, 9 fractions were found similar among oysters and mussels. From the above observation, it may be assumed that clams are perhaps more closely related to oysters than to mussels while oysters show more affinity towards mussels than to clams.

In the light of the present observation, it appears that polyacrylamide gel electrophoresis can be applied with great success in elucidating any intra-specific and inter-specific relationship among natural populations of organisms. Analysis of the zymogram of individuals of V. cyprinoides sampled from the fifth station revealed their distinct difference from their counterparts at stations 1 to 4. Further, the zymograms from the five stations had no electrophoretic fraction in common. The data thus strongly supports the earlier observations made during the morphological studies of individuals from station 5 which revealed that they possessed features characteristic to Villorita cyprinoides var. cochinensis. On the other hand, morphologically similar individuals of the species Crassostrea madrasensis sampled from the three stations exhibited only marginal variations in the protein banding pattern, the adaptive significance of which is quite explainable. Specimens of Perna viridis sampled from the two selected stations also had an almost identical electrophoretic pattern with only minor variations in the relative mobility of a few fast moving cathodal bands. It thus becomes clear that any studies on protein polymorphism would only add to our understanding of the more intricate mechanisms involved in the genetic differentiation of aquatic populations.

Chapter 5

ELECTROPHORETIC STUDIES OF ISOENZYMES

5.1. INTRODUCTION

The relative contribution of genetic and non-genetic (environmental) influences on the phenotype of organisms in a population is an interesting field of scientific study. Often, the interpretation of the genetic basis of variations in morphological characters is complicated through the influence of various environmental factors. The detection of enzymes and other proteins, which are primary gene products, using electrophoretic techniques, have enabled investigators to observe genetic variations within and between species, minimising the effects of environmental modulation (Ferguson, 1980). Studies have shown that in most organisms, about one third of the proteins exist in variant, genetically determined states, in different individuals of a species, ie, they are polymorphic. These separate electrophoretic phenotypes of a given protein, which are often the products of alternative alleles segregating at the controlling locus, has provided useful markers in investigations of population genetics of diverse organisms including invertebrates. Further, the frequencies of these genotypes and phenotypes are often used to describe the genetic structure of populations.

The detection of genetic variation by gel electrophoresis and histochemical staining of proteins is an indirect estimate of the changes in the genetic (DNA) structure. In most cases, the variations in banding pattern can be directly equated to variations in the gene coding for the variant protein. The electrophoretic banding patterns of isoenzymes may be treated as phenotypes and investigated through genetic tests that determine which

bands are coded by allelic genes and which of those are specified by genes at different loci. The observed variation on gels may be employed to elucidate the genetic structure of any given population. When alleles at a locus are products of similar base sequence, they are called homozygotes and a combination of two or more different alleles are referred to as heterozygotes. A locus is considered to be polymorphic when the frequency of the most common allele was not greater than 0.95 and thus may reveal electrophoretic variants. The enzyme loci which appear as a single band are considered monomorphic while those with two or more well separated zones of activity are taken to be the products of two or more loci.

In the present investigation, an attempt has been made to evaluate the genetic differences that exist, if any, within and among populations of the three bivalvespecies namely, V. cyprinoides, C. madrasensis and P. viridis. The isoenzymes studied are alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and tetrazolium oxidase (TO).

5.2. REVIEW OF LITERATURE

Isoenzymes as allelic markers have been particularly useful in investigations involving issues such as the extent of genetic heterogeneity in population (Whitt, 1970). After the original population analyses by Lewontin and Hubby (1966), many evolutionary biologists have used the allelic isoenzymes to measure the amount of genetic variation in both natural and laboratory reared populations and then to relate the changes observed to the difference in various environmental parameters. Application of these techniques in

the taxonomic problems of marine invertebrates were initiated by Manwell and Baker (1963) to be later exploited by several others (Manwell et al. 1967; Manwell and Baker, 1970; Selander et al., 1970; Gooch and Scoph, 1971).

The use of electrophoretic techniques in the study of geographically separated populations of marine and estuarine invertebrates, over the past decade has significantly enhanced our understanding of population differentiation in the marine environment (Gooch, 1975; Battaglia and Beardmore, 1978; Burton and Feldman, 1982). Utilising gel electrophoresis as a tool to identify gene loci among populations of different species of marine ectoprocts, Gooch and Scoph (1971) found that the genetic structure of an ectoproct population appears to be fundamentally like that of the genetically well known terrestrial animals such as Drosophila. Gooch et al., (1972) in an attempt to characterise six gene loci by polyacrylamide gel electrophoretic technique of five protein systems in Nassarius obsoletus observed a remarkable geographic homogeneity of allele frequency and suggested that this could possibly be due to (1) mechanisms of balanced polymorphism that are insensitive to local environmental perturbations or (2) extensive gene flow. Genetic differences in microhabitats of Modiolus demissus was the topic of scientific enquiry by Koehn et al. (1973). They observed significant difference in the proportion of heterozygotes at the tetrazolium oxidase locus between micro-habitats within the intertidal zone and heterozygosity was found to increase with age. The observed genetic differences according to these authors could be due to active site selection of larvae or more probably due to differential survival of heterozygotes.

Following investigations into the genetic variation in Tridacna maxima,

Ayala et al. (1973) came to the conclusion that this species is one of the genetically most polymorphic organisms. The results on allelic variations at twenty five polymorphic loci failed to support the hypothesis that some massive extinctions registered in fossil record may have been due to the scarcity of genetic variations in populations adatped to stable environments.

Electrophoresis of extracts of the white skeletal muscle of Stizostedion istreum istreum revealed a total of six phenotypes of malate dehydrogenase (MDH) isoenzyme (Clayton et al., 1971). The individual MDH phenotype was found to consist of four or more MDH isoenzymes. Further studies on the heritability of the six phenotypes provided evidence for the existence of three non-dominant alleles varying from far different locations. Assessment of the effects of varying levels of larval dispersal on the genetic structure of three littorinid species was the topic of scientific enquiry by Berger (1973). He is of the view that in species endowed with limited dispersal capabilities as Littorina saxatilis and L. obtusata, one may find both geographic differentiation with respect to allele frequencies and in certain cases, alleles unique to a specific geographical region.

Studies by Buroker et al. (1975) revealed that the considerable genetic variability exhibited as enzyme polymorphism in the Pacific oyster Crassostrea gigas can be of immense use in the oyster culture industry. Different alleles or combination of alleles can be used as permanent biological 'tags' to identify particular strain of artificially bred oysters. In the polychaete Neanthes arenaceodentata, the activity of MDH was over four times greater than that of LDH and a lower dissolved oxygen concentration resulted in an increase in salinity with a concomittant decrease in LDH activity (Cripps

and Reish, 1973).

The patterns and mobilities of lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and tetrazolium oxidase (TO) have provided further support for the previously assumed monophyletic origin of the genera Percinia and Etheostoma (Page and Whitt, 1973). Geographic variations were limited and the most significant variation was the dichotomous nature of the LDH pattern of Percinia copelandi suggesting that the nominal species may actually consist of two or more taxonomically recognizable forms. Further, the results supported several of the accepted relationships among darters and new relationships could be suggested. Electrophoretic analysis of the LDH and MDH isoenzyme patterns in tissues of temperature acclimated Carassius auratus by Wilson et al. (1973) revealed no detectable qualitative or quantitative alterations in the isoenzyme patterns as a result of temperature acclimation. The marginal variations in LDH isoenzyme pattern observed were attributed to genetic polymorphism and to the differences in ratios of red and white skeletal muscles. Environmental factors such as temperature, salinity etc. affect the isoenzyme expression in such a way that these isoenzyme systems may afford an organism with greater capacity to respond to environmental changes at high latitude (Bolaffi and Booke, 1974). Commenting on the outcome of comparative electrophoretic studies of tissues from Alosa aestivalis and A. pseudoharengus Mc Kenzie (1973) opined that LDH and muscle myogen electrophoretic patterns are of little use as biochemical markers, because of the identical mobilities of the five LDH isoenzymes in the two species. However, the muscle myogen pattern in the two species was different and species specific. It has been shown that, in certain echinoderms, a greater genetic variability occurs among species

inhabiting deep sea environment when compared to those from shallow waters (Scoph and Murphy, 1973).

An analysis of evolutionary relationships among shiners of the sub-genus Luxilus, (Teleosti, Cypriniformes, Notropis) employing the LDH and MDH isoenzyme systems permitted the resolution of three distinct phyletic groups within the sub-genus (Rainboth and Whitt, 1974). They further observed extensive polymorphism at the LDH c locus, the function of which is restricted primarily to the liver. Ayala (1975) hypothesized that the genetic variability in deep sea populations could be maintained by the selective pressures of environmental biotic factors. Investigating into the stereo-specific distribution and evolutionary significance of invertebrate LDHs, Long (1976) opined that every organism contained enzyme activity for only one lactate stereo-isomer although several cases of multiple molecular forms have been observed. He further suggested that the minimum number of changes in stereo specificity to accommodate the evolution of the major invertebrate classes are four.

The distribution of LDH isoenzymes have been found to vary from species to species in homologous tissues (Wieland et al. 1959) and even from tissue to tissue within the same species (Pfleiderer and Jeckel, 1957). In vertebrates, the LDH may have five isoenzymes while in many invertebrates this does not seems to be true. Some fishes have only one form of LDH (Markert and Faulhaber, 1965) while several others have been reported to have upto fifteen isoenzymes (Massaro, 1972). LDH locus occur in a multiple molecular form (Markert and Moller, 1959) and different tissues show different isoenzyme patterns (Markert, 1965). LDH has been reported

to exist only in one form in penaeid prawns (Samuel, 1987) but as one, three or five forms in other crustaceans. Electrophoretic analysis of three dehydrogenases from various tissues of the Atlantic crab, Callinectes sapidus revealed that LDH occurred as only a single band and there was no difference between the banding pattern of enzymes at premoult and intermoult stages. Narang (1974) looked into the physico-chemical characterisation of LDH in various tissues of Biomphalaria glabrata. LDH was found to exist as a multiple molecular form, possessing five isoenzymes in most of the tissues; each tissue having a characteristic LDH isoenzyme pattern with one or two tissue specific principal enzymes in high concentration.

Cole and Morgan (1978a,b) examined twenty enzymes in Callinectus sapidus and found eleven to be polymorphic. Non-genetic variations of LDH isoenzymes in the snail, Cepaea nemoralis was the topic of scientific investigation by Gill (1978). Different isoenzyme patterns were obtained at different times of the year depending on whether the snails were feeding or aestivating. Investigating into the activities of LDH and MDH in the pyloric caeca of Luida clathrata, Duranko et al., (1979) found that MDH can function during periods of reduced oxygen levels or during periods of organic synthesis. The strategies of adaptation are the result of interaction between environmental factors and biological characters of the species (Costa and Bisol, 1978). An electrophoretic study of selected enzymes of the coconut crab Birgus latro revealed only a single anodal band of LDH (Massaro and Cohen, 1978).

In a preliminary study of the electrophoretic variation of enzymes in the tunicates, Clavelina picta and C. oblonga, Lyerla and Lyerla (1978)

observed that the zymogram patterns of the different isoenzymes were species-specific. Presumptive allelic variants of MDH was found in C. oblonga whereas none was observed in C. picta and it was concluded that this technique could be effectively applied in the selection of tunicate species which may provide a unique material for population genetic studies. Qualitative and quantitative changes in the activity of isoenzymes have been observed in relation to temperature (Bolaffi and Booke, 1974; Wilson et al. 1974; Tsukuda, 1975).

Studies on the comparative electrophoretic patterns of LDH in three species of trout led Bouck and Ball (1964) to suggest that LDH isoenzyme patterns could be used for taxonomic and diagnostic purposes. They observed that the patterns were species-specific; the tissue-specificity of the isoenzymes being low except for the skeletal muscles. Investigations directed towards an understanding of the population genetics of Salmonids have also been carried out (Ryman et al., 1979; Ryman and Stahl, 1981).

Electrophoretic technique has been used as a powerful means in elucidating the systematic relationships among organisms especially in cases where classical taxonomic techniques have proved insufficient (Avise, 1974). Analysis of biochemical genetic variations may sometimes prove to be the best means of distinguishing cryptic species (Murphy, 1978; Dando et al., 1979; Bucklin and Hedgecock, 1982).

Gel electrophoretic analysis of gene-enzyme systems constitute a powerful tool in empirical studies on population genetics, systematics and evolutionary biology (Singh et al., 1982; Ravi Prakash and Yadav, 1989).

The data on enzyme phenotype may be used to describe the genetic structure of natural populations (Nei, 1975; Hedrick, 1983). The technique of electrophoresis has also been used to demonstrate the genetic variation among populations of bivalve species (Wilkins and Mathers, 1973; Levinton and Koehn, 1976; Morgan et al., 1978 and Brock, 1978), fishes (Grant and Utter, 1984; Skaala et al., 1990), crabs (Beckwitt, 1985) and squids (Carvalho and Loney, 1989). Investigations on the extent and patterns of electrophoretically detectable genetic variations as well as biochemical systematics have been carried out in a wide variety of gammaridean amphipod species inhabiting marine, brackish and fresh water environments (Borowsky et al., 1985; Siegismund et al., 1985; Mc Donnald, 1985; Bulnheim and Scholl, 1986).

Description of variation between size classes within a single population are rather scarce. Koehn et al., (1973) reported that small size classes of Modiolus demissus exhibited a significant deficiency of heterozygote at tetrazolium oxidase (TO) locus, but the heterozygosity increased with an increase in shell size. Analysis by electrophoretic methods of 23 enzyme systems of Salmo trutta by Taggart et al. (1981) revealed polymorphism at 13 of the 23 loci examined with a relatively high level (22%) of polymorphism. The tissue-specificity of LDH isoenzyme has been examined in a wide variety of teleostian fishes (Frankel and Harth, 1977 and Frankel, 1980).

Experiments by Morrissey (1981) to determine the ADH activity in tissues of house cricket Acheta domesticus demonstrated that ADH activity was present in the fat body and testes but not in malphigian tubules or central nervous system; the specific activity being greater in the fat body

than in testes. Achaval (1984) is of the view that the variability of isoenzyme patterns in fishes is an important source of information about the non-genetic and genetic mechanisms responsible for the synthesis of isoenzymes.

5.3. MATERIAL AND METHODS

5.3.1. SELECTED SPECIMENS

The details of selected animals, study area, laboratory conditioning and electrophoretic technique employed are described earlier (vide pages, Chapter 2, 3 and 4).

5.3.2. METHODS OF STAINING

The isoenzymes namely alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and tetrazolium oxidase (TO) were visualized in the gel using the method of Siciliano and Shaw (1976) with necessary modifications.

a) Alcohol dehydrogenase (ADH)

Alcohol dehydrogenase isoenzyme is highly non-specific capable of reacting with a number of primary, secondary, straight and branched chain aliphatic and aromatic alcohols. For staining this isoenzyme, the gels after electrophoresis were immersed in a specific staining medium containing,

(a) Ethyl alcohol (95%) - 2 ml

(b) Nicotinamide adenine dinucleotide (NAD) - 25 mg

(c) Nitroblue tetrazolium (NBT) - 15 mg

(d)	Phenazine methosulphate (PMS)	-	1 mg
(e)	0.2 M Tris-HCl (pH - 8)	-	7 ml and
(f)	Distilled water	-	41 ml

The gels were then incubated at 37°C for 20 minutes to get the violet-blue bands of diformozan, indicating the presence of ADH in the tissue sample applied in the gel.

b) Lactate dehydrogenase (LDH)

The method is based on the differences in electrophoretic mobilities of LDH isoenzymes whose separation in polyacrylamide column is identified by means of electron carriers like NAD from lactate via phenazine methosulphate on to nitroblue tetrazolium dye. The ensuing reaction yields a violet-blue precipitate of diformozan at the gel column site to which the LDH fraction has migrated. After electrophoretic run, the gels were immersed in a staining medium containing,

(a)	Lithium lactate	-	100 mg
(b)	Nicotinamide adenine dinucleotide (NAD)	-	25 mg
(c)	Nitro-blue tetrazolium (NBT)	-	15 mg
(d)	Phenazine methosulphate (PMS)	-	1 mg
(e)	0.2 M Tris-HCl buffer (pH - 8)	-	10 ml and
(f)	Distilled water	-	35 ml

The gels were then incubated at 37°C for 20 to 30 minutes to get the violet-blue colour.

c) Malate dehydrogenase (MDH)

The staining medium for malate dehydrogenase contains,

(a)	Malic acid	- 10 mg
(b)	Nicotinamide adenine dinucleotide (NAD)	- 25 mg
(c)	Nitro-blue tetrazolium (NBT)	- 15 mg
(d)	Phenazine methosulphate (PMS)	- 1 mg
(e)	0.2 M Tris-HCl buffer (pH - 8)	- 7 ml and
(f)	Distilled water	- 35 ml

and following electrophoretic separation, the gels were immersed in the above solution and incubated at 37°C for 15 to 20 minutes to get the violet-blue bands of diformozan at the gel column site to which the MDH fraction has migrated.

d) Tetrazolium oxidase (TO)

Achromatic zones, operationally termed tetrazolium oxidase (Baur and Schorr, 1969) can be visualized along with the LDH and MDH staining systems with substrate omitted and staining at 37°C for 2 to 3 hours.

5.4. RESULTS

The adductor muscle of individuals from selected populations of Villorita cyprinoides, Crassostrea madrasensis and Perna viridis were subjected to electrophoretic resolution of four isoenzymes namely alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and

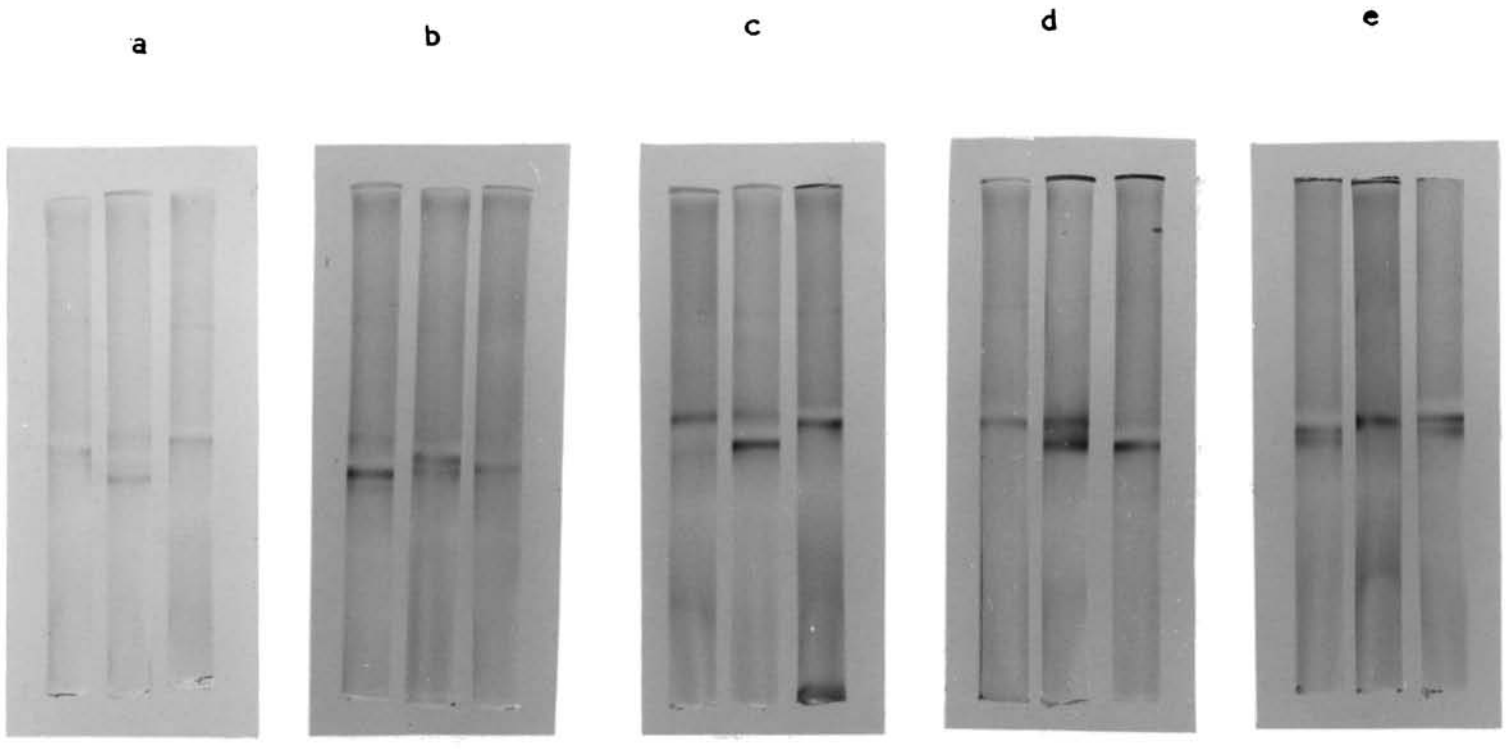
tetrazolium oxidase (TO) with a view to evaluate the genetic structure of each population of the species based on isoenzyme patterns. The results obtained are outlined in Photomicrographs 8a-10b, Figures 7 a-e and Tables 17 a-b to 19 a-b and Figures 7a-9b. A minimum of 40 individuals from each population were utilized for enzyme analysis.

5.4.1. VILLORITA CYPRINOIDES

a) Alcohol dehydrogenase (ADH)

The isoenzyme patterns of individuals of V. cyprinoides collected from the five sampling stations are given in Photomicrographs 8 a-e , Fig. 7 a-e and Table 17 a-e . The position of the bands in the electropherogram were based on their relative mobility (rm).

Three phenotypic patterns were observed among individuals sampled from stations 1 to 5 as evidenced in Photomicrograph 8 a-e . Polymorphism was found to occur only at one locus (Fig. 7 a-e). The anodal end had only a single lightly stained fraction (rm: 28.33) and was common in all the populations tested. The ADH (II) (Fig. 7 a-e) locus was found to be polymorphic having two to three alleles in the range of populations sampled. Homozygotes were characterised by a single major band alongside a light band (among populations from stations 1 to 4) and heterozygotes displayed a two banded pattern characteristic of a monomeric enzyme in which sub-unit polypeptides associate at random. Of the forty individuals tested from station 1, 12 were found homozygous for the ADH (II) locus (102/102), while 17 others were homozygous for the same locus expressed



Photomicrograph 8 a-e. Villorita cyprinoides. Phenotype of alcohol dehydrogenase (ADH) isoenzyme in the adductor muscle of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn. 4 (e) Stn. 5.

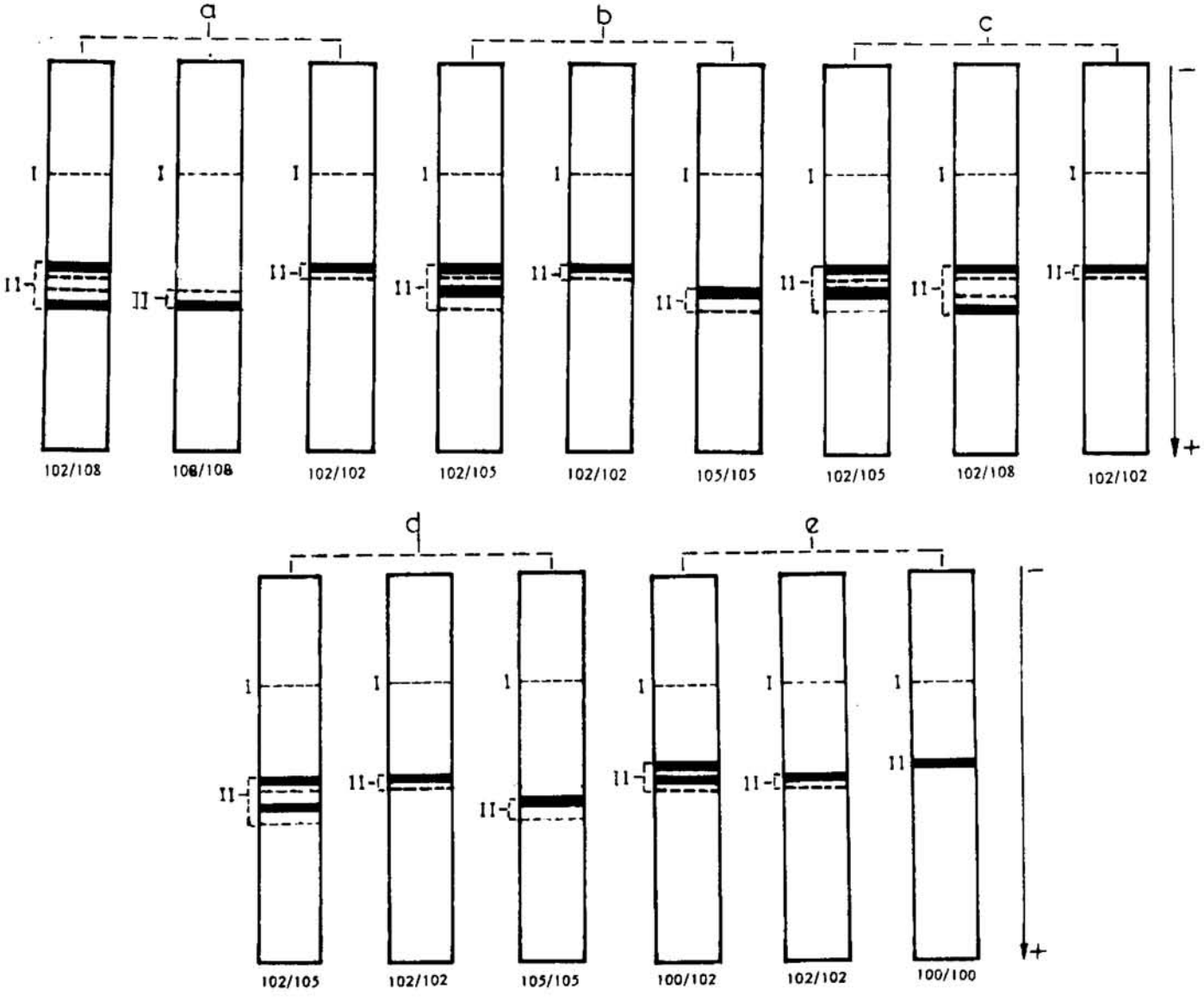


Fig. 7 a-e. Villorita cyprinoides. Electropherogram of ADH - observed phenotype in the adductor muscle of individuals from stations 1 to 5.

(a) Stn. 1 (b) Stn.2 (c) Stn.3 (d) Stn.4 (e) Stn. 5

Table 17 a-e. Villorita cyprinoides. Relative mobility (rm) with stainig intensity (I) of four isoenzymes (ADH, LDH, MDH and TO) in the adductor muscle of individuals from Stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (b) (c) stn. 3 (d) Stn. 4 (e) Stn. 5

	(a)		(b)		(c)		(d)		(e)	
Enzyme	rm	I	rm	I	rm	I	rm	I	rm	I
ADH	28.33	x	28.33	x	28.33	x	28.33	x	28.33	x
	51.67-53.33	xxx	51.67-53.33	xxx	51.67-53.33	xxx	51.67-53.33	xxx	48.33-50.00	xxx
									51.67-53.33	xxx
	55.00	x	55.00	x	55.00	x	55.00	x	55.00	x
	58.33	x	58.33-60.00	xxx	58.33	x	58.33-60.00	xxx		
					58.33-60.00	xxx				
	61.67-63.33	xxx			61.67-63.33	xxx				
			63.33	x	63.33	x	63.33	x		
LDH	36.67	x								
	55.00-56.67	xxx	55.00-56.67	xxx	50.00-51.67	xxx	50.00-51.67	xxx	50.00-51.67	xxx
									56.67-58.33	xx
MDH	53.33-55.00	xxx	53.33-55.00	xxx	50.83-52.50	xxx	50.88-52 50	xxx	50.83-52.50	xxx
TO	23.33-26.67						23.33-26.67			
			26.67-30.00		26.67-30.00					
	38.33-41.67		38.33-41.67		38.33-41.67					
							40.00-43.33		40.00-43.33	
	51.67-65.00		51.67-65.00		51.67-65.00		51.67-65.00			
									58.33-73.33	

by another genotype (108/108). However, 11 individuals possessed both the above bands (102 and 108) and may be considered as heterozygotes containing both the alleles. Thus, this enzyme appears to be a monomer.

Comparison of the isoenzyme patterns of individuals from station 2 revealed that of the 45 individuals examined, 19 were homozygous for the allele (102/102) while 10 others were found to have a different homozygous phenotype of (105/105). Among the different individuals tested, heterozygosity (102/105) was manifested in 16 specimens (Photomicrograph 8b and Fig. 7b).

Curiously enough at station 3, there were two heterozygous phenotypes denoted by (102/105) and (102/108) at the ADH (II) locus. The homozygous allele (102/102) was observed only in 11 out of the 42 individuals sampled from this station (Photomicrograph 8c and Fig. 7c).

The data on the isoenzyme patterns of individuals from station 4 indicated that the phenotype included both homozygous (102/102), (105/105) and heterozygous (102/105) patterns (Photomicrograph 8d and Fig. 7d). The results from station 5 (V. cyprinoides var. cochinensis) indicated an additional allele (100/100) in 16 individuals tested , besides the more common allele (102/102) observed among individuals from the other four populations and also a heterozygous phenotype of (100/102) in 6 individuals (Photomicrograph 8e and Fig. 7e.

The data on the allele frequency distribution including the observed and expected frequency, along with the respective Chi-square values from

Table 18. Villorita cyprinoides. ADH : Observed and expected frequency with their chi-square value.

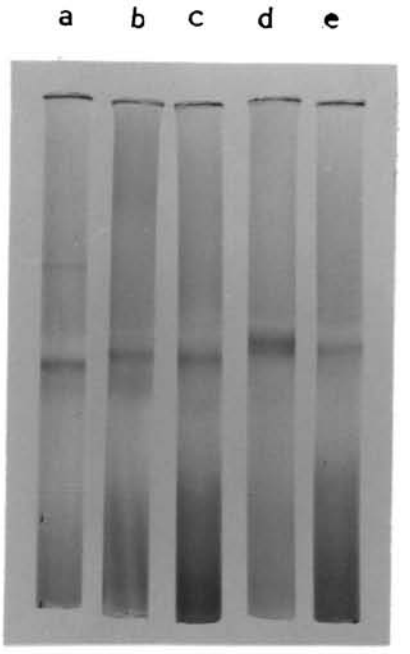
lation	No. of samples tested	Different alleles	Gene frequency	Observed frequency	Expected frequency	χ^2
I		102	0.56	12	12.54	1.59
laradu	40	108	0.44	17	7.74	2.34
		102/108	-	11	19.71	3.85
						Total : 7.78
II		102	0.60	19	16.20	0.48
urinjapuzha	45	105	0.40	10	7.20	1.09
		102/105	-	16	21.60	1.45
						» 3.02
III		102	0.63	11	16.67	1.93
hannirmukkam	42	105	0.17	-	1.21	1.21
		108	0.20	-	1.68	1.68
		102/105	-	14	10.58	1.11
		102/108	-	17	9.00	7.12
		105/108	-	-	2.86	2.86
						» 15.91
IV		102	0.60	20	14.40	2.18
ıottappilly	40	105	0.40	12	6.40	4.90
		102/105	-	8	19.20	6.53
						» 13.61
V		100	0.48	16	9.03	5.39
ɜsam	40	102	0.52	18	11.03	4.41
		100/102	-	6	19.91	9.72
						» 19.51

the five sampling stations are detailed in Table 18. It is evident from the table that ADH (II) locus showed ADH $(II)^{100}$, ADH $(II)^{102}$, ADH $(II)^{105}$ and ADH $(II)^{108}$ alleles of which ADH $(II)^{102}$ was the most common allele in the five stations; the proportional frequency varying from 0.52 in station 5 to 0.63 in station 3. However, for ADH $(II)^{105}$ which was the next predominant allele at stations 2, 3 and 4, the proportional frequency varied from 0.17 in station 3 to 0.40 in stations 2 and 4. The third allele ADH $(II)^{108}$ exhibited a frequency of 0.20 in station 3 and 0.44 in station 1. The additional allele (100/100) observed at station 5 was characterised by a frequency of 0.48 (Table 18).

The overall estimate of average frequency of heterozygotes per locus in the five populations of V. cyprinoides was also calculated by averaging the observed frequency of heterozygotes over all loci sampled. It was found that the average heterozygote proportion at station 1 was the least among the five populations (ie 0.15) while that of population from station 4 was the highest(0.405). The average heterozygote proportion for populations from station 2, 3, and 5 were 0.275, 0.356 and 0.20 respectively

b) Lactate dehydrogenase (LDH)

The results obtained during the present study indicated three different LDH patterns for individuals from the five stations (Photomicrograph 9 a-e, Fig. 8 a-e and Table 17 a-e. A dark fraction of relative mobility 55.00-56.67 was obtained among all individuals from stations 1 and 2 while in individuals from station 3 and 4, the results indicated the occurrence of a different



Photomicrograph 9 a-e. Villorita cyprinoides. Phenotype of lactate dehydrogenase (LDH) isoenzyme in the adductor muscle of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn. 4 (e) Stn. 5

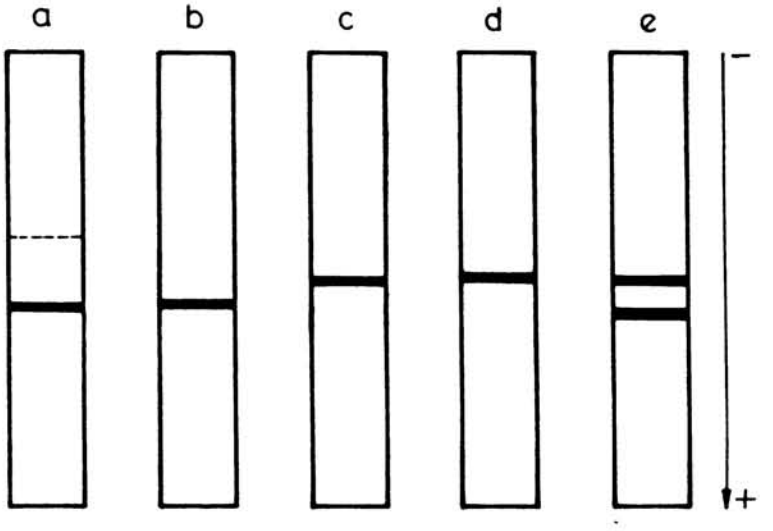


Fig. 8 a-e. Villorita cyprinoides. Electropherogram of LDH - observed phenotype in the adductor muscle of individuals from stations 1 to 5 (a) Stn.1 (b) Stn.2 (c) Stn. 3 (d) Stn.4 (e) Stn. 5

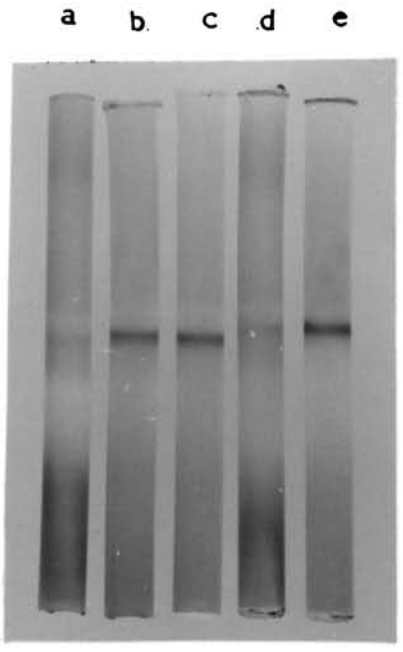
dark fraction of 'rm' 50.00-51.67. Individuals from station 5 (V. cyprinoides var. cochinensis) had the same fraction of 'rm' 50.00-51.67 besides a fast moving fraction of 'rm' 56.67-58.33 as seen in Fig. 8e. Likewise, a light band of relative mobility 36.67 was found in the electropherogram of individuals from station 1. It is evident from the results that individuals from station 1 and 2 are more or less similar while those from stations 3 and 4, although similar, were distinctly different from their counterparts from stations 1, 2 and 5 with regard to the LDH isoenzyme pattern. Although, individuals from station 5 had the first fraction in common with those from stations 3 and 4, the second dense fraction was rather unique to this population.

c) Malate dehydrogenase (MDH)

The MDH locus appeared to be monomorphic among individuals from the five stations compared. Photomicrograph 10 a-e , Figure 9 a-e and Table 17 a-e illustrate the details of the MDH phenotype of V. cyprinoides from the selected stations. Individuals from station 1 and 2 were characterised by a similar phenotype for MDH while those from stations 3, 4 and 5 had similar phenotype but differing from those observed at stations 1 and 2.

d) Tetrazolium oxidase (TO)

The electrophoretic pattern of tetrazolium oxidase isoenzyme are depicted in Photomicrograph 11 a-e , Fig. 10 a-e and Table 17 a-e . The species was characterised by a specific pattern for TO, with a characteristic



Photomicrograph 10 a-e.. Villorita cyprinoides. Phenotype of malate dehydrogenase (MDH) isoenzyme in the adductor muscle of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn. 4 (e) Stn. 5.

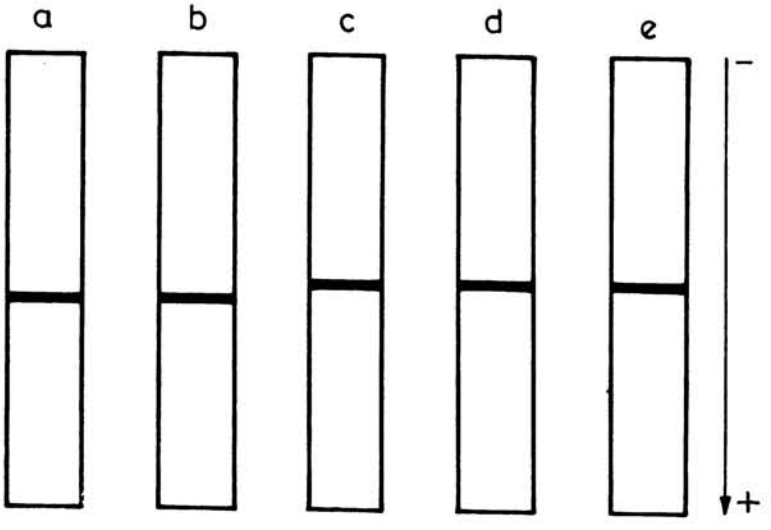
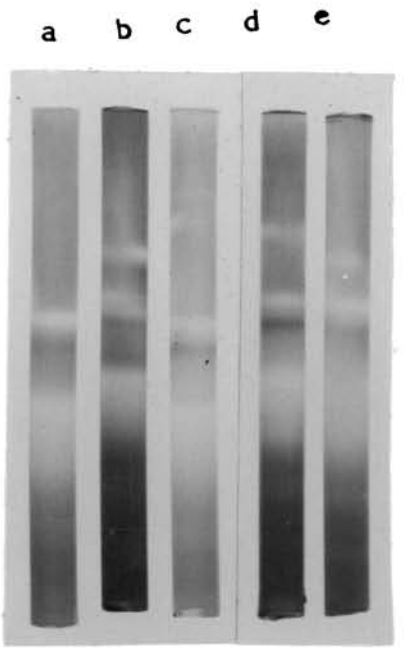


Fig. 9 a-e. Villorita cyprinoides. Electropherogram of MDH - observed phenotype in the adductor muscle of individuals from Stations 1 to 5. (a) Stn.1 (b) stn.2 (c) Stn.3 (d) Stn.4 (e) stn.5



Photomicrograph 11 a-e. Villorita cyprinoides. Phenotype of tetrazolium oxidase (TO) isoenzyme in the adductor muscle of individuals from stations 1 to 5. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3 (d) Stn.4 (e) Stn.5

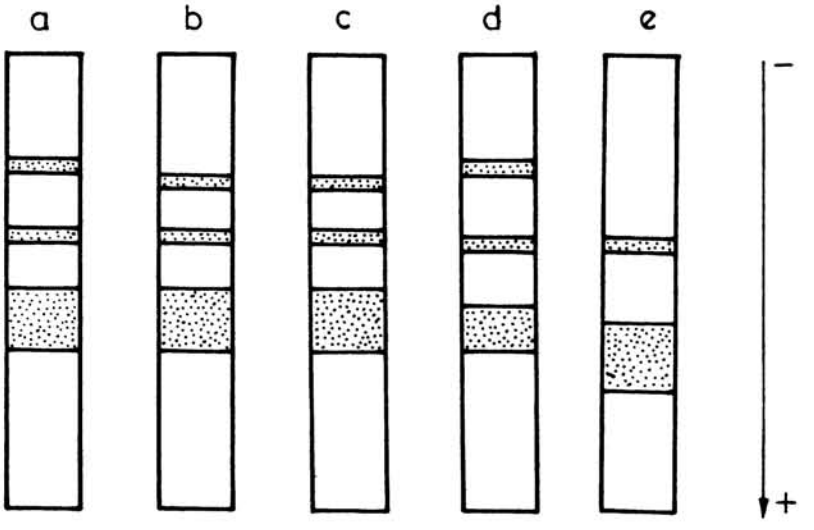


Fig. 10 a-e. Villorita cyprinoides. Electropherogram of TO - observed phenotype in the adductor muscle of individuals of stations 1 to 5. (a) Stn.1 (b) Stn. 2 (c) Stn. 3 (d) stn.4 (e) Stn. 5

electrophoretic mobility. Although variation was not found among individuals of the same population, some interpopulation variations were evident with regard to the relative mobility of the fractions. Individuals from station 2 and 3 evinced an almost identical electrophoretic pattern, while those from stations 1 and 4 had a similar but different banding pattern with marginal variations in the relative mobility of the second and third fractions (Photomicrograph 11 a-e , Fig. 10 a-e and Table 17 a-e . However, individuals from station 5 were characterised by a rather unique electrophoretic pattern as evidenced in Fig. 10e; the two fractions differing from those obtained from stations 1 to 4 in their band width and relative mobility.

5.4.2. CRASSOSTREA MADRASENSIS

a) Alcohol dehydrogenase (ADH)

The adductor muscle of C. madrasensis collected from the three salinity regimes were subjected to electrophoretic resolution of ADH isoenzymes. The results obtained are outlined in Photomicrograph 12 a-c, Fig 11 a-c and Table 19 a-c. The ADH isoenzymes showed two zones of activity; a zone of fast moving dark band and a zone of slow moving minor band. The zone of dark band was identical in all the three populations, except for the minor differences observed in the relative mobility of the same in individuals from station 3. However, the zone of minor band depicted variation between stations. While individuals from station 1 had only one minor band, those from stations 2 and 3 revealed three minor bands in comparable positions (Fig 11 a-c).

Thus, the pattern obtained from station 1 was characterised by a minor fraction (rm : 16.67) and a major dark fraction (rm : 41.67-43.33). Individuals from stations 2 and 3 depicted 3 similar minor fractions (rm : 20.00, 28.33 and 30.00) while with respect to the dark fraction, they showed difference in its relative mobility. (Fig 11 a-c, Table 19 a-c)

b) Lactate dehydrogenase (LDH)

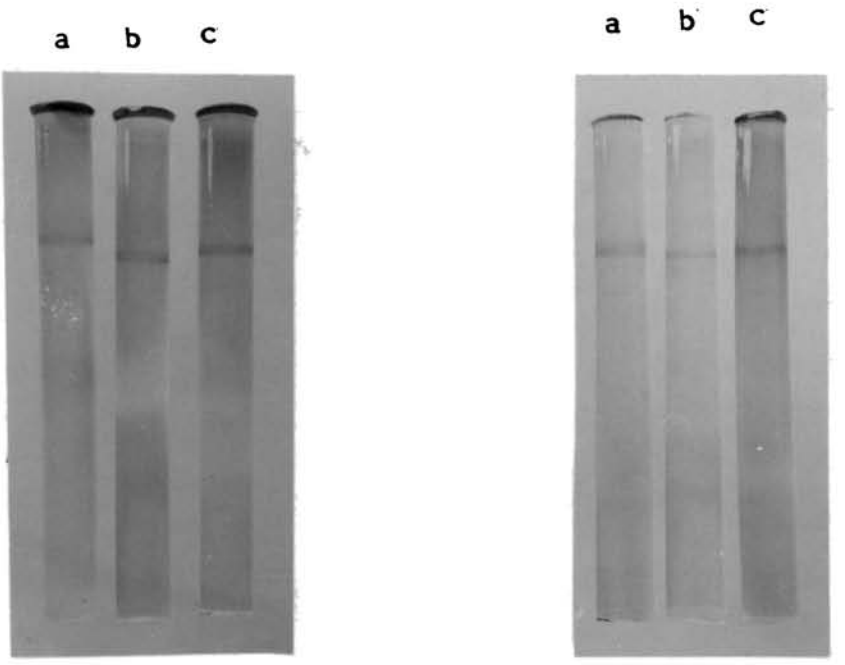
The single banded nature of the electrophoretic pattern of LDH isoenzyme was the same in the three populations of C. madrasensis tested. and was independent of sex and size. The data obtained are outlined in Photomicrograph 13 a-c , Fig. 12 a-c and Table 19 a-c . All individuals were presumably homozygous for the single allele observed of relative mobility 21.67.

c) Malate dehydrogenase (MDH)

The isoenzyme patterns of MDH obtained from the three stations are detailed out in Photomicrograph 14 a-c , Fig. 13 a-c and Table 19 a-c . No variations were observed in the single banded electrophoretic pattern among individuals from the three stations. Here also, the test species was found to be homozygous for the single allele characterised by a relative mobility value of 23.33.

d) Tetrazolium oxidase (TO)

Unlike the identical monomorphic banding pattern obtained for LDH and MDH isoenzymes, those obtained following electrophoretic resolution



Photomicrograph 12 & 13 a-c. Crassostrea madrasensis. Phenotype of alcohol dehydrogenase (ADH) (12 a-c) and lactate dehydrogenase (LDH) (13 a-c) isoenzymes in the adductor muscle of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3.

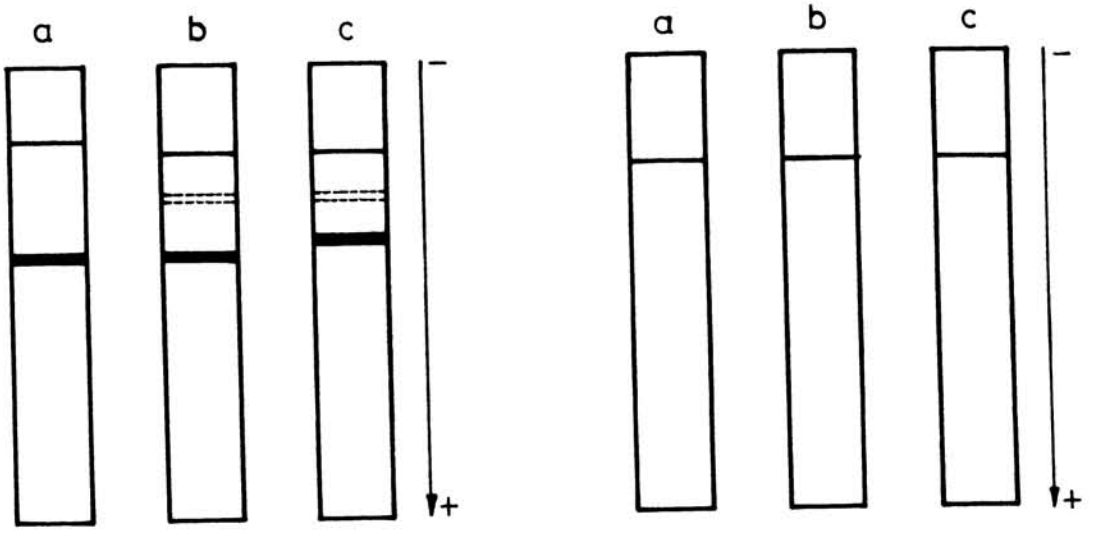
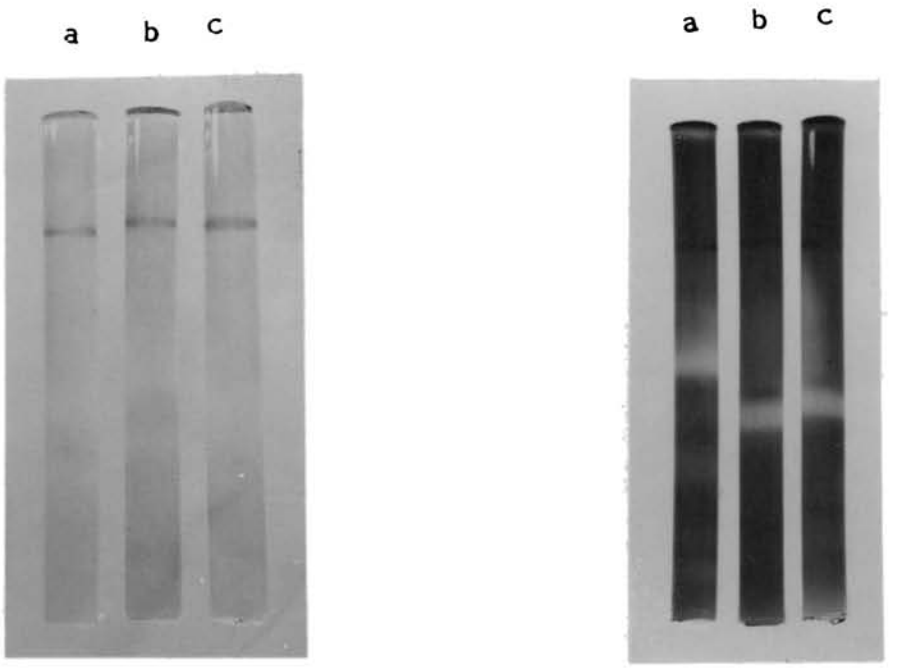


Fig.11 & 12 a-c. Crassostrea madrasensis. Electropherogram of ADH (11a-c) and LDH (12a-c) - observed phenotype in the adductor muscle of individuals from stations 1 to 3. (a) Stn.1 (b) stn.2 (c) Stn.3



Photomicrograph 14 & 15 a-c. Crassostrea madrasensis. Phenotype of malate dehydrogenase (MDH) (14 a-c) and Tetrazolium oxidase (TO) (15 a-c) isoenzymes in the adductor muscle of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3

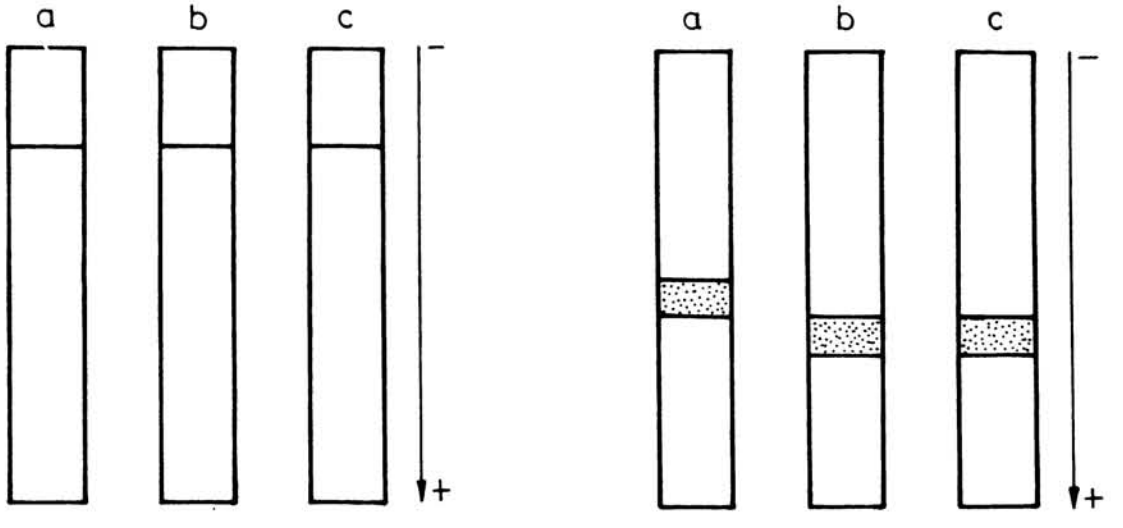


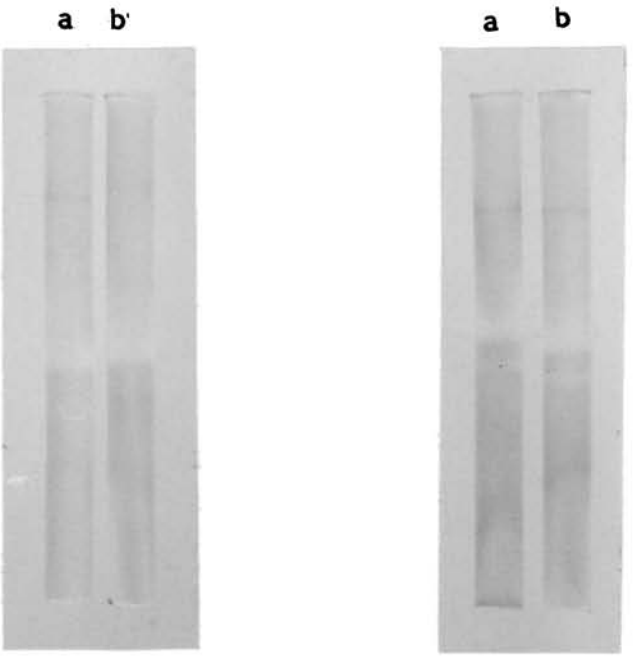
Fig. 13 & 14 a-c. Crassostrea madrasensis. Electropherogram of MDH (13 a-c) and TO (14 a-c) observed phenotype in the adducter muscle of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn.2 (c) Stn. 3

of tetrazolium oxidase (TO) of the adductor muscle revealed minor variation among individuals of C. madrasensis sampled from the three stations. Although monomorphic, the single allele of relative mobility, 58.33-66.67 was common only among individuals from stations 2 and 3. The phenotype of TO among individuals from station 1 was found to differ from their counterparts from stations 2 and 3 with respect to relative mobility (50.00-58.33) of the band (Photomicrograph 15 a-c , Fig. 14 a-c and Table 19 a-c).

5.4.3. PERNA VIRIDIS

The isoenzyme patterns of alcohol dehydrogenase, lactate dehydrogenase, malate dehydrogenase and tetrazolium oxidase of the adductor muscle of individuals of P. viridis from the two selected stations are detailed out in Photomicrograph 16 to 19 a-b , Figures 15 to 18 a-b and Table 20a-b. Individuals of P. viridis sampled from both the selected stations showed ADH activity which was visualised as a single densely stained band of relative mobility 18.33-20.00 (Photomicrograph 16 a-b , Fig. 15 a-b and Table 20 a-b). Similarly, LDH and MDH isoenzyme activity was expressed as monomorphic bands characterised by relative mobility values 21.67-23.22 and 25.00 respectively as illustrated in Photomicrograph 17 to 18 a-b , Fig. 16 to 17 a-b and Table 20 a-b . On the other hand, individuals of P. viridis sampled from the two stations showed TO activity which was expressed as two achromatic zones of relative mobility 41.67-48.33 and 53.33-56.67 (Photomicrograph 19 a-b , Fig. 18 a-b and Table 20 a-b).

It is evident from the data obtained that no variation in isoenzyme patterns exist between individuals from the two stations. Both the populations



Photomicrograph 16 & 17 a-b. Perna viridis. Phenotype of alcohol dehydrogenase (ADH) (16 a-b) and lactate dehydrogenase (LDH) (17 a-b) isoenzymes in the adductor muscle of individuals from stations 1 and 2. (a) Stn. 1 (b) Stn. 2

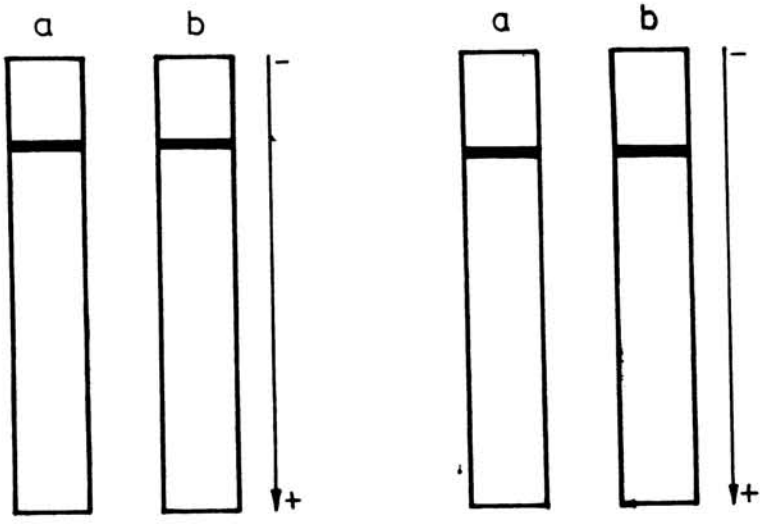
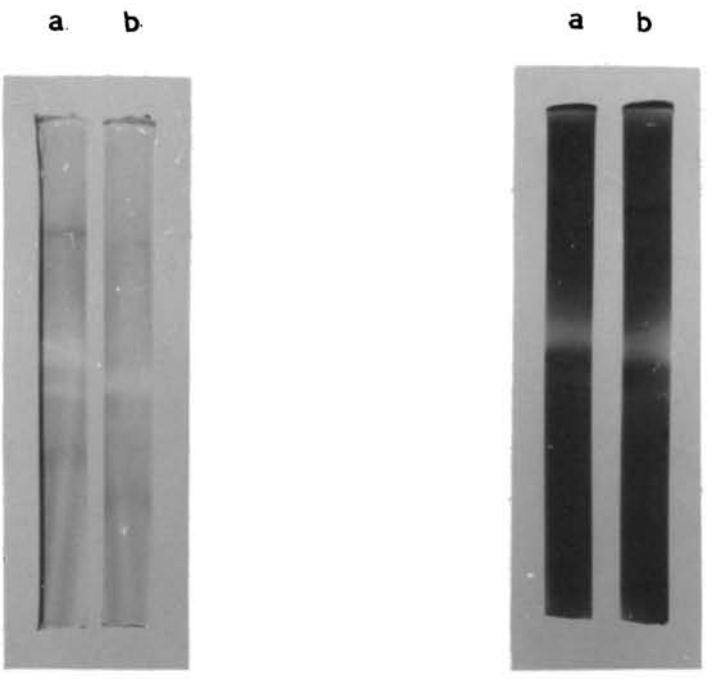


Fig. 15 & 16 a-b. Perna viridis. Electropherogram ADH (15 a-b) and LDH (16 a-b)isoenzymes - observed phenotype in the adductor muscle of individuals from stations 1 and 2. (a) Stn. 1 (b) Stn. 2



Photomicrograph 18 & 19 a-b. Perna viridis. Phenotype of malate dehydrogenase (MDH) (18 a-b) and tetrazolium oxidase (19 a-b) isoenzymes in the adductor muscle of individuals from stations 1 and 2. (a) stn.1. (b) Stn.2.

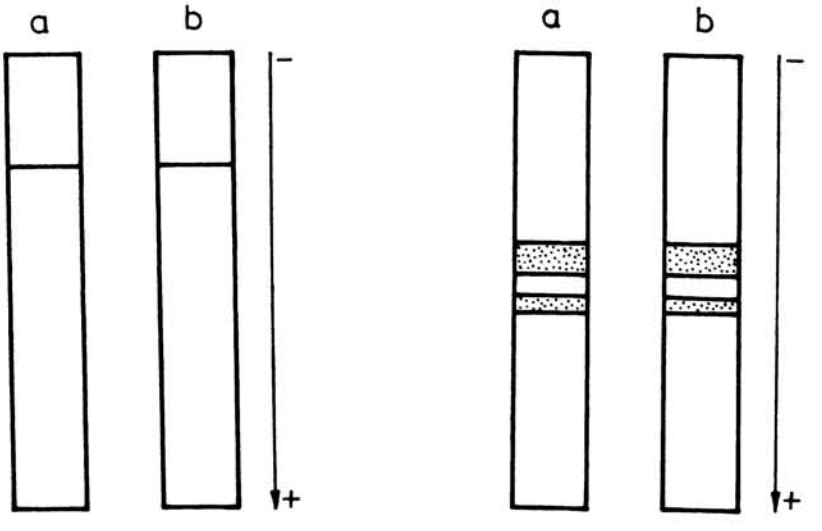


Fig. 17 & 18 (a-b). Perna viridis. Eletropherogram of MDH (17 a-b) and TO (18 a-b) isoenzymes - observed phenotype in the adductor muscle of individuals from stations 1 and 2.((a) Stn. 1 (b) Stn. 2)

Table 19 a-c. Crassostrea madrasensis. Relative mobility (rm) with staining intensity (I) of four isoenzymes (ADH, LDH, MDH and TO) in the adductor muscle of individuals from stations 1 to 3. (a) Stn. 1 (b) Stn. 2 (c) Stn. 3

	(a)		(b)		(c)	
Enzyme	rm	I	rm	I	rm	I
ADH	16.67	x				
			20.00	xx	20.00	xx
			28.33	xx	28.33	xx
			30.00	xx	30.00	xx
					38.33-40.00	xxx
	41.67-43.33	xxx	41.67-43.44	xxx		
LDH	21.67	xxx	21.67	xxx	21.67	xxx
MDH	23.33	xxx	23.33	xxx	23.33	xxx
TO	50.00-58.33		58.33-66.67		58.33-66.67	

Table 20 a-b. Perna viridis. Relative mobility (rm) with staining intensity (I) of four isoenymes (ADH, LDH, MDH and TO) in the adductor muscle of individuals from stations 1 and 2 (a) Stn. 1 and (b) stn. 2

	(a)		(b)	
Enzyme	rm	I	rm	I
ADH	18.33-20.00	xxx	18.33-20.00	xxx
LDH	21.67-23.22	xxx	21.67-23.33	xxx
MDH	25.00	xxx	25.00	xxx
TO	41.67-48.33		41.67-48.33	
	53.33-56.67		53.33-56.67	

of P. viridis were homozygous for the four isoenzymes with reference to their relative mobility, staining intensity and band width.

5.5. DISCUSSION

The present study has attempted an evaluation of the electrophoretic characteristics of four isoenzymes namely alcohol dehydrogenase (ADH), Lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and tetrazolium oxidase (TO) in three bivalve species and has revealed important information which have not been hitherto reported elsewhere. An overall comparison of the zymogram patterns of the four isoenzymes obtained during the present study clearly demonstrated the species-specific genetic characteristics of the three species beyond doubt. The species-specific isoenzyme pattern existed either in the form of differences in the number of enzyme bands or their distinct electrophoretic positions or both. Such species-specific isoenzyme characteristic of bivalve species and other invertebrate and vertebrate species have been well documented and reported by several authors (Gooch, 1975; Battaglia and Beardmore, 1978; Levinton, 1980, 1982; Burton and Feldman, 1982; Sameul, 1987).

Another equally important information that has emerged from the present study is that some of these enzymes that revealed species specific genetic characteristics, are also capable of revealing the apparent inter-population differences existing within each species. Thus phenotypic patterns of ADH, LDH and TO showed population specific characteristics for V. cyprinoides (Fig. 7 a-e , 8 a-e and 10 a-e) and C. madrasensis (Fig. 11 a-c and 14 a-c). The individuals of V. cyprinoides analysed from station 5

possessed a unique two banded LDH and TO isoenzyme patterns distinguishing it clearly from their counterparts from stations 1, 2, 3 and 4. The individuals from station 5 also appears to be genetically different from all other populations on account of the presence of a unique allele (100/100) at 0.48 frequency level which was absent in all the other populations.

Although populations of V. cyprinoides from stations 1 and 2 are almost similar in many aspects of enzyme characteristics studied; station 1 could be separated from all other stations by the absence of ADH (II) allele (105/105). The absence of ADH (II)^{108} allele among population from station 2 and 4 is also indicative of their genetic difference to a certain extent.

Molluscs have been extensively used in population genetic studies involving allozyme variants (Zouros and Foltz, 1984) and have often revealed an excess of homozygosity with a corresponding heterozygote deficiency in many species. The results obtained following the electrophoretic resolution of ADH from the adductor muscle of V. cyprinoides was in accordance with this observation. In all the populations tested, except those from station 3 and excess of homozygosity was observed with a corresponding heterozygote deficiency (Table 18). Several possible explanations have been suggested for such observations (Singh and Green, 1984) and they fall into four biological categories; each individually or in combination has been preferred by different authors. These include (1) presence of null alleles, allelozymes devoid of enzyme activity (Milkman and Beaty, 1970), (2) inbr-eeding and self fertilisation (Hornbach, et al., 1980), (3) wahlund effect ie. population mixing (Tracey et al., 1975; Koehn et al., 1976) and (4) selection (Koehn

and Mitton, 1972; Koehn et al., 1973; Gartner-Kepkay et al., 1980; Colgen, 1981). Ayala et al. (1973) and Buroker et al. (1975) suggested the possibility of missclassification of heterozygotes as homozygotes due to poor electrophoretic resolution and the technical possibility may explain excess of homozygosity.

One aspect that has emerged from multilocus studies of bivalve species is that within a single species, different loci do not show the same deficiency of heterozygotes. This result appears to rule out self fertilization or other forms of close inbreeding as an explanation of heterozygote deficiency in bivalves (Zouros and Foltz, 1984). Further, V. cyprinoides is an external fertilizer, no hermaphroditic individuals have hitherto been reported and no reproductive incompatibility are known. Therefore, it is logical to assume that deviation from Hardy-Weinberg equilibrium condition, ie, an excess of homozygosity over heterozygosity in the case of ADH is most likely be due to mechanisms other than inbreeding. It may be pointed out here that no polymorphism was evident in the case of LDH, MDH and TO in V. cyprinoides from the different stations.

The relationship of genetic polymorphism to the amount of temporal variation in the environment still remains open to question. Theoretical expectations suggest that a temporally constant environment with parameters such as temperature, salinity, dissolved oxygen etc. showing little or no changes over long life spans should select for reduced genetic polymorphisms (Grassle, 1972). It has been observed that species inhabiting deeper layers of sediment have low levels of genetic polymorphism than species living epifaunally on the sediment-water interface (Levinton, 1973). The observed

lack of polymorphism with respect to LDH, MDH and TO in all populations of the three test species, except ADH in the case of V. cyprinoides (no polymorphism was observed among C. madrasensis and P. viridis in the case of ADH) may be attributed to this factor. The observed variation among V. cyprinoides from the different stations with reference to isoenzyme patterns is in agreement with the results obtained during the morphometric and electrophoretic studies of general proteins of the species detailed earlier (vide pages, Chapter 3 and 4). Thus, individuals from station 5 may be regarded as V. cyprinoides var. cochinensis, while those from stations 1-4 belong to the species V. cyprinoides (Prashad, 1921).

A comparison of enzyme patterns of ADH, LDH, MDH and TO in the three populations of C. madrasensis indicated that population from station 1 is considerably different from those of the other two stations with respect to ADH and TO activity (Fig. 11 a-c and 14 a-c). The same enzyme patterns from station 3 and 2 demonstrated that those population were almost identical. However, the pattern obtained for LDH and MDH were more or less similar in the three populations of C. madrasensis. The only rational explanation that can be put forward to explain the variations observed among individuals of C. madrasensis and V. cyprinoides, is the influence of some specific environmental factors characteristic to each location indicating some form of adaptive radiation (Van Valen, 1965; Somero and Soule, 1974; Karlin, 1982).

More or less identical isoenzyme patterns were obtained with reference to individuals of P. viridis sampled from the two stations (Fig. 15 to 18 a-b). All the four isoenzymes namely ADH, LDH, MDH and TO showed similarity

in their nature among individuals of the two stations. Despite the fact that P. viridis is an epifaunal species, inhabiting areas of high environmental variability, the expected genetic polymorphism was however, not evident. Similar lack of isoenzyme polymorphism have been reported earlier at several enzyme loci studied among invertebrates species (Cole and Morgan, 1978 a,b; Gill, 1978).

The enzyme polymorphism is believed to have a physiological role in organisms. Gillespie and Kojima (1968) suggested that levels of polymorphism may reflect environmental variation in substrates. Among loci, whose enzymes utilize substrates originating from the environment (substrates such as dietary proteins, alcohols, esterase etc.) considerable variation in available substrate types or concentration or both would be expected. The relationship as observed by Gillespie and Kojima (1968) suggest that enzyme polymorphism increase fitness by providing a means of metabolic compensation for a varying environment. Of the four enzymes under study, LDH, MDH and TO utilize 'internal' metabolic substrates while ADH act upon 'external' substrates. The fact that LDH, MDH and TO were monomorphs in all populations of the three bivalve species underlines the earlier proposition that enzymes utilizing 'internal' substrates rarely exhibit more than one allele. Further, ADH was found to exhibit polymorphism in the different populations of V. cyprinoides which is in accordance with the above suggestion. However, the lack of polymorphism of ADH in the two other species is inexplicable.

It is therefore felt that although environmental heterogeneity may regulate genetic polymorphism, it does not do so equally over all loci,

as evidenced by the present study. This inequality calls into question the validity of investigating genetic variation over many loci and attempting to correlate the overall variation with environmental heterogeneity. The finding of Lewontin (1984) is significant in that niche breadth is indeed correlated with genetic variation at some part of the genome but not at others and a study of only a few enzymes is insufficient to draw any firm conclusion.

The present study also revealed that mussels are more related to oysters than to clams based on both protein and isoenzyme zymogram patterns. This is evidenced by the relative similarity in the monomorphic, homozygous nature of the different isoenzymes under study. It is also evident that no single explanation can offer a satisfactory interpretation to all the variation in isoenzyme pattern observed among the different populations during the present study. However, the various explanations that have been suggested may either be used singly or in combination with others before a conclusion can be arrived at regarding the underlying mechanisms involved in isoenzyme variation in populations.

It may be concluded that certain isoenzyme variations like that of ADH, LDH and TO in V. cyprinoides and ADH and TO in C. madrasensis may be useful as potential markers to differentiate individual population characteristics.

Chapter 6

BIOCHEMICAL COMPOSITION

6.1. INTRODUCTION

Molluscs in general, and bivalves in particular, have conquered a wide variable environment for their successful inhabitance and a varied environment presupposes important adaptations at the morphological, physiological and biochemical levels, which may be of ecological singificance. An understanding of the biochemical composition of marine and estuarine organisms is highly essential, since an understanding of the metabolism of different populations provide an estimate of their energy content and an insight into the understanding of the biogeochemical circulation of elements.

India has a rich and varied bivalve resource and has been the subject of extensive biochemical investigations not only because of their importance as food for man but also because of their significant role in the economy of many littoral states. Although a considerable body of information has been accumulated on the biochemical composition of bivalves in relation to sex, size and season, data on the biochemical composition of the same species inhabiting different areas so as to estimate the relative nutritive value of the species with reference to geographical distribution, are rather scarce. Studies by earlier workers have shown that seasonal metabolic cycles in bivalves are a reflection of complex interactions between food availability, environmental parameters, growth and reproductive activities (Bayne, 1976; Gabbot, 1983). Similarly, owing to variation in hydrographical conditions of the environment, there is a distinct seasonal fluctuation in

the abundance of bivalves and the present estimations have therefore been restricted to months during which the specific groups are available in sufficient quantities.

Further, the present study envisages an evaluation of the biochemical composition from more of a commercial view point than a physiological or sex wise determination, since in commercial utilisation, generally, greater stress is given to better "quality" of the meat. Hence if estimation such as these are based on sex, will be little appreciated. Moreover, species enjoying a wide geographical distribution are often likely to give rise to locally adapted populations or ecotypes and a comparative account on the biochemical status of such individual populations would be worthwhile. Hence in the present study, pooled estimations were made of the various biochemical components without regard for the sex of individuals.

6.2. REVIEW OF LITERATURE

Much of the early work on the seasonal changes in biochemical composition of Mytilus edulis and M. galloprovincialis have been reviewed in detail by Giese (1969) and the more recent ones are of De Zwaan and Zande (1972), Gabbot and Bayne (1973) and Bayne et al. (1982) in Mytilus edulis and Ansell et al. (1980) in Donax trunculus L. These studies have demonstrated that the changes in body weight are mainly due to changes in carbohydrate or glycogen content.

Seasonal variations in quality and quantity of tissues in Mytilus have been investigated in different geographic areas by several authors

(De Zwaan and Zande, 1972; Telembici and Dimoftache, 1972; Gabbot and Bayne, 1973; Dare and Edwards, 1975; Pieters et al., 1980). These studies have demonstrated that seasonal variation in biochemical composition have different patterns depending on the different latitudes and are strongly influenced by temperature and phytoplankton availability. It is fairly well established that, in fishes, marked changes do occur in the chemical constitution of tissues from season to season (Love, 1970; Shreni, 1980).

Studies on the biochemistry of Indian bivalves have chiefly focused on the biochemical composition, seasonal changes in composition and calorific values. Analyses have mostly been made on pooled, homogenized animals with little or no distinction of sex, gonadal condition or environmental parameters (Venkataraman and Chari, 1951; Durve and Bal, 1961; George and Nair, 1975; Shafee, 1978). Studies on the biochemical constituents and food values of five commercially important edible bivalves of Kerala by Suryanarayan and Alexander (1972) revealed that the bivalve meat compared favourably with the common food fishes with regard to their calorific value and hence would be an excellent and economic source of nutrition for man. Ansell (1974a, b, c) has reported that spawning in Abra alba, Chlamys septemradiata and Nucula sulcata is accompanied by a rapid decline in the total carbon and calorific value and the highest values of energy content coincided with the maximum lipid content of the tissue. Supporting these findings, Nair and Shynamma (1975) and Ansari et al. (1981), working on the seasonal variations in calorific value and lipid content of Villorita cyprinoides var. cochinensis reported that the calorific values are directly proportional to the lipid content. Similar studies have been carried out by several others

(Krishnakumari, et al., 1977; Nagabhushanam and Mane, 1978; Shafee, 1978). As pointed out by Giese (1969), such data are only of limited use in the study of the relation of biochemistry of the animal to its nutritional stages.

Studies on the seasonal variations in biochemical composition have been carried out in a number of bivalve species from the Indian sub-continent. Durve and Bal (1961) investigated into the seasonal variation in all the biochemical components including inorganic constituents and the calorific values of Crassostrea gryphoides and inferred that this species is good for consumption from late October to June. Similar studies on other Crassostrea species have also been reported by several others (Krishnakumari et al., 1976; Joseph, 1979; Joseph and Madhyastha, 1986; Easterson and Kandasami, 1988). In Meretrix meretrix, Nagabhushanam and Deshmukh, (1974) found that the glycogen content was related to gonad development and depicted an increase during active gametogenesis. Studies by Venkataraman and Chari (1951) revealed an increase in fat content in Meretrix casta during periods of gonad development. In Meretrix meretrix, the glycogen and protein content showed a steady fall during premonsoon period with an equally steady rise during postmonsoon period; the fat content depicting a reverse trend (Salih, 1979). Lakshmanan and Nambisan (1980) observed a significant negative correlation between carbohydrate and protein in Meretrix casta and Villorita cyprinoides var. cochinensis. In Meretrix casta, Balasubramanyan and Natarajan (1988) recorded a high percentage of protein in the mantle while the gonad and digestive gland had higher percentage of carbohydrate and lipid respectively. Similar observations have also been made in other Meretrix species (Durve and George, 1973; Krishnakumari et al, 1976; Jayabal and Kalyani, 1986). Biochemical analyses were carried out

in Katelysia marmorata (Joshi and Bal, 1965) and K. opima (Mane, 1974) with a view to estimate their nutritive value. Two distinct periods of variation in biochemical composition were observed in Cellana radiata while estimating their nutritive value (Suryanarayan and Nair, 1976). Krishnakumari et al. (1976) estimated the variations in nutritive value and the suitable periods for harvesting Mytilus viridis and such estimations have also been made in the species of Paphia (Nagabhushanam and Dhamne, 1975; Mane and Nagabhushanam, 1979), Perna viridis (Ramachandran, 1980) and Sunetta scripta (Katticaran, 1988).

6.3. MATERIALS AND METHODS

6.3.1. SPECIMENS EMPLOYED

Details of the specimens employed, sampling stations, collection and laboratory conditioning etc. have been described earlier (vide pages, chapter 2 and 3). Sexually mature individuals were avoided and only immature specimens were utilised.

6.3.2. ESTIMATION OF BIOCHEMICAL CONSTITUENTS

a) Water content

To estimate the percentage water content in unit weight of adductor muscle of the three species, the specimens were first forced open with a scalpel, washed with distilled water and the excess water was blotted using filter paper. The adductor muscle was then removed asceptically,

transferred to previously weighed aluminium foil dishes and weighed immediately to determine the wet weight of the tissue. They were then dried at 70-80°C for 48 h and the dry weights taken to constancy. The difference between the fresh wet weight and dry weight yields the water content of the tissue and is expressed as percentage wet weight, or

$$\text{Water content} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100 = \% \text{ wet weight}$$

b) Protein

Protein content in the tissue was determined by the method of Lowry et al. (1951). A unit weight of the adductor muscle was dissolved in 0.1 N Sodium hydroxide with gentle warming in a water bath. The following procedure was adopted for analysis of the extract.

1. Add 0.2 ml of the sample to 1 ml of 10% trichloro acetic acid.
2. Centrifuge at 3000 RPM for 15 minutes and decant the supernatant.
3. Dissolve the precipitate in 1 ml of 0.1 N Sodium hydroxide.
4. Pipette out 0.5 ml of this into another test tube and add 0.5 ml of distilled water to make up to 1 ml.
5. Add 5 ml of alkaline copper reagent to this and mix well.
6. After 10 minutes, add 0.5 ml of Folin's reagent and mix well. Leave the resulting solution for 30 minutes at room temperature.
7. Using bovine serum, prepare standards similarly. Read the optical density (OD) of the blank, standard and samples at 500 nm in a Spectrophotometer (Hitachi, Model 200 - 20).

c) Glycogen

To estimate the total glycogen content in the adductor muscle, Montgomory's method (1957) was employed. The extract was prepared as described for protein estimation and later analysed according to the following procedure.

1. Add 0.2 ml of the extract to 1 ml of 10% trichloro acetic acid.
2. Centrifuge at 2500 RPM for 10 minutes. Take the supernatant.
3. To 1 ml of the supernatant, add 3 ml of 95% ethyl alcohol and shake well.
4. Keep that undisturbed in a refrigerator for about 12-24 h.
5. Centrifuge at 2500 RPM for 15 minutes. Remove the supernatant gently and take the pellet.
6. Add 0.1 ml of 80% phenol to the pellet.
7. Then add, 2 ml of distilled water followed by 5 ml of Con. Suphuric acid and shake well.
8. Standards are prepared similarly using glucose.
9. Prepare a 'blank' that contains 2 ml of distilled water, 0.1 ml of 80% phenol and 5 ml Con. Sulphuric acid.
10. Allow the preparations to stand for 30 minutes at room temperature.
11. Read the OD of the blank, standards and samples, at 490 nm in a Spectrophotometer.

d) Lipid

The method of Barnes and Blackstock (1973) was used to estimate

the lipid level in the adductor muscle. Weighed tissue samples were extracted with chloroform : methanol (2:1) mixture and subjected to further analysis as follows:

1. To the extract, add 1 ml of methanol, 2 ml of chloroform and 2 ml of chloroform : methanol mixture again.
2. Add 0.2 volume of 0.9% Sodium chloride solution. Pour this into a separating funnel and mix thoroughly. Allow it to stand for few hours.
3. Separate the lower phase into a clean test tube. Make up the volume of the lower phase to the original quantity of chloroform added before.
4. Measure out 0.5 ml of extract into a clean test tube and allow it to dry in a vacuum desiccator over silica gel. Dissolve in 0.5 ml of Con. Sulphuric acid and mix well. Plug the tubes with non-absorbent cotton wool, place in boiling water bath for 10 minutes and cool.
5. Pipette out 0.2 ml of this acid digest into a dry test tube and add 5 ml of sulphophospho-vanillin reagent. Mix well and allow to stand for 30 minutes.
6. Prepare standards using cholesterol (8 mg/4 ml of chloroform : methanol mixture).
7. Read the blank, standard and samples at 520 nm using a spectrophotometer.

6.4. RESULTS

The approximate biochemical composition of the adductor muscle

of the three bivalve species under investigation is presented in Tables 21-23 and Fig. 19-21. 'One way ANOVA' was carried out to assess whether there were any significant variation in the biochemical composition analysed between the different stations from where each species were sampled. The results thus obtained has been treated under species heads to make the presentation more meaningful. The different biochemical constituents such as protein, glycogen and lipid are expressed in µg per mg (µg/mg) wet weight of the tissue, while water content in the tissue is expressed as percentage wet weight.

6.4.1. VILLORITA CYPRINOIDES

The adductor muscle of V. cyprinoides sampled from the five stations (vide pages, chapter 2) were found to have protein as the major biochemical constituent, despite the minor variations between sampling sites. The highest protein concentration was observed at station 3 (53.32 µg/mg) while individuals from station 2 had a low value of 42.50 µg/mg. Specimens from stations 1, 4 and 5 had protein levels of 43.40 µg/mg, 52.10 µg/mg and 50.12 ug/mg respecively as shown in Table 21 and Fig 19. Analysis of variance (ANOVA) revealed that the protein concentration was significantly different only between stations 1 and 3, 1 and 4, 2 and 3, and 2 and 4 ($P \leqslant 0.01$) (Table 24a).

Glycogen represents the most variable biochemical component in an organism. Clearcut variation in the glycogen content in the adductor muscle was evident among the different populations tested. Significant

muscle of individuals from stations 1 to 3.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	43.40	31.21	14.05	82.00
Stn. 2	42.50	36.94	14.86	82.21
Stn. 3	53.32	38.03	17.70	80.50
Stn. 4	52.10	31.48	18.75	79.38
Stn. 5	50.12	29.49	17.78	82.62

Table 22. Crassostrea madrasensis. Biochemical composition of the adductor muscle of individuals from stations 1 to 3.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	56.82	28.92	9.83	80.58
Stn. 2	56.98	28.73	10.42	80.38
Stn. 3	60.69	31.39	11.19	81.83

Table 23. Perna viridis. Biochemical composition of the adductor muscle of individuals from stations 1 and 2.

	PROTEIN (µg/mg)	GLYCOGEN (µg/mg)	LIPID (µg/mg)	WATER CONTENT (%)
Stn. 1	62.68	29.03	17.24	77.80
Stn. 2	66.27	34.13	20.61	79.10

difference was observed between stations 1 and 2, 1 and 3, 2 and 5, 3 and 4, and 3 and 5 (Table 24b). Specimens from station 3 depicted the highest glycogen content (38.03 µg/mg) while the lowest concentration was observed among individuals from station 5 (29.49 µg/mg). Among the other three populations, those from station 2 had 36.94 µg/mg glycogen in their muscle tissue while individuals from station 2 and 4 recorded an almost identical concentration of 31.21 µg/mg and 31.48 µg/mg (Table 21).

It is evident from Table 21 that the lipid level in the adductor muscle of V. cyprinoides from the five stations were more or less identical and that the variation was not statistically significant ($P \leq 0.01$) (Table 24c). Individuals from station 4 recorded the highest lipid level (18.75 µg/mg) although their counterparts from stations 1 and 2 had only 14.05 µg/mg and 14.86 µg/mg respectively. A moderate concentration of 17.70 µg/mg and 17.78 µg/mg were reported in respect of individuals sampled from stations 3 and 5.

As with the other biochemical constituents, the water content of the muscle tissue was also found to vary in a similar manner. The water content varied from 79.38 to 82.62% in the different populations of the species. Stations 1 and 4, 2 and 3, 2 and 4, and 4 and 5 showed statistically significant variation in the amount of water in the adductor muscle of individuals (Table 24d). A water content of 82.62% was obtained for individuals from station 5; the corresponding value being 82.00%, 82.21%, 80.50% and 79.38% for individuals from stations 1, 2, 3 and 4 respectively (Table 21).

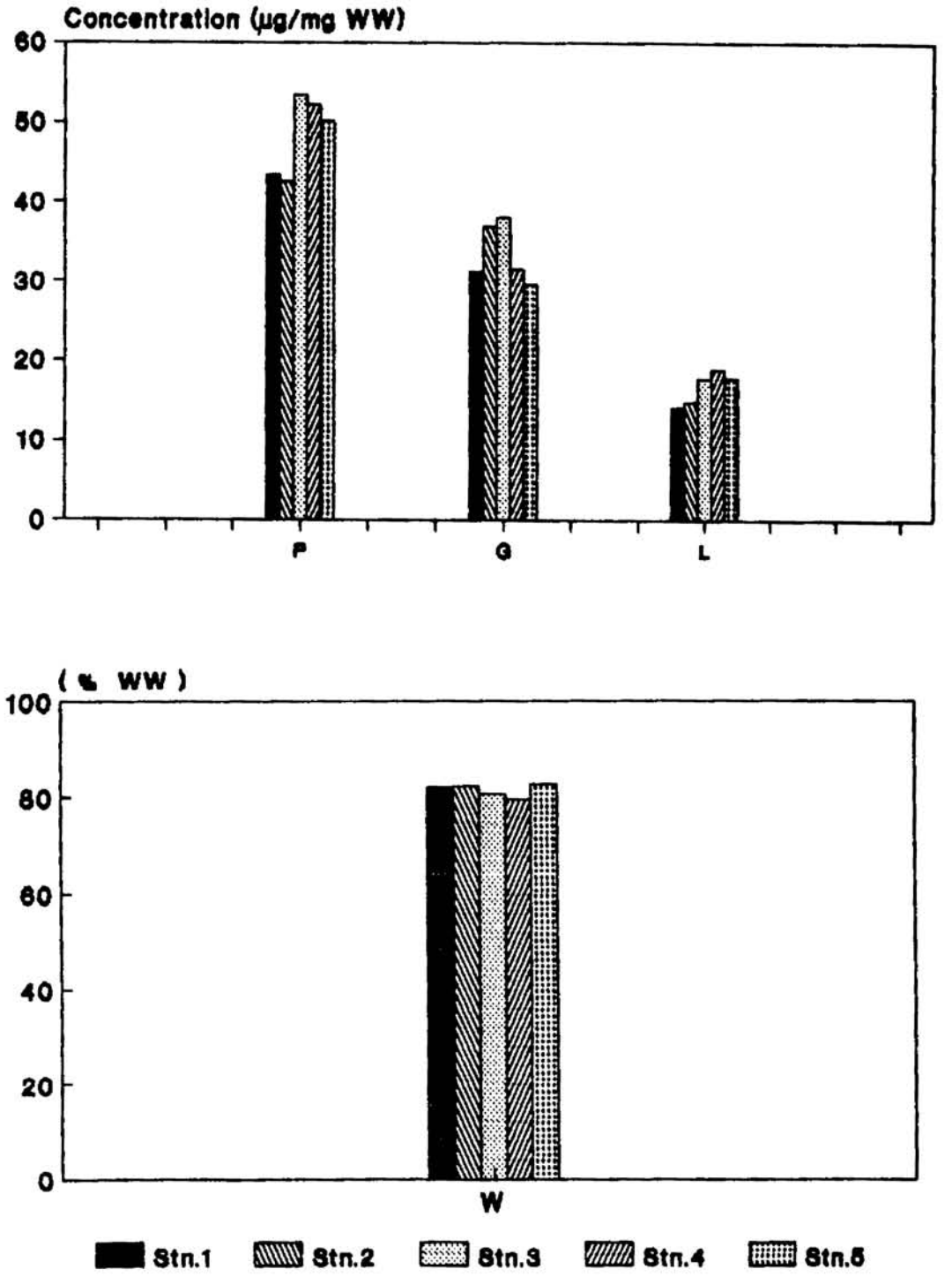


Fig. 19 Villorita cyprinoides. Various biochemical constituents ((P) Protein (G) Glycogen (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 to 5.

Table 24 a-d. Villorita cyprinoides. Analysis of variance (ANOVA) of the various biochemical components in the adductor muscle of individuals from stations 1 to 5, along with their level of significance.

24a. Protein

Source	SS	Df	Mean Sqr	F
Total	5846.38	149	--	
Station	3017.25	4	754.313	3.89*
Error	28129.13	145	193.994	

* $P \leq 0.01$

24b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	19645.39	149	--	
Station	1734.125	4	433.531	3.51*
Error	17911.27	145	123.526	

* $P \leq 0.01$

24c. Lipid

Source	SS	Df	Mean Sqr	F
Total	7711.183	149	--	
Station	419.191	4	104.798	2.08
Error	7291.992	145	50.29	

24d. Water content

Source	SS	Df	Mean Sqr	F
Total	977.064	149	--	
Station	151.25	4	37.813	5.49*
Error	825.813	145	6.882	

* $P \leq 0.01$

6.4.2. CRASSOSTREA MADRASENSIS

No significant variation was observed in the biochemical composition of the adductor muscle of C. madrasensis collected from the three stations. Individuals from station 3 were characterised by a relatively high concentration of the different components : the values being, protein 60.69 µg/mg, glycogen 31.39 µg/mg, lipid 10.19 µg/mg and water content 81.83%. On the other hand, individuals from the most saline area (station 1) had the following biochemical composition : protein 56.82 µg/mg, glycogen 28.92 µg/mg, lipid 9.83 µg/mg and water content of 80.58%, the same being 56.98 µg/mg, 28.73 µg/mg, 10.42 µg/mg and 80.38% for the moderately saline station 2 (Table 22 and Fig 20). The results of the analysis of variance (ANOVA) of the various components are detailed in Tables 25 a-d.

6.4.3. PERNA VIRIDIS

Despite the statistically significant variation ($P \leq 0.01$) in lipid and water content, individuals of P. viridis collected from the two stations had an almost identical glycogen and protein levels. The protein content of the adductor muscle of specimens from station 1 was 62.68 µg/mg while that for station 2 was 66.27 µg/mg; glycogen content being 29.03 µg/mg and 34.13 µg/mg respectively for individuals from stations 1 and 2. On the other hand, the lipid levels in the tissue were 17.24 µg/mg and 20.61 µg/mg and water content was 77.80% and 79.10% respectively (Table 23 and Fig 21). No significant variation was observed in the protein and glycogen levels (Table 26 a-d).

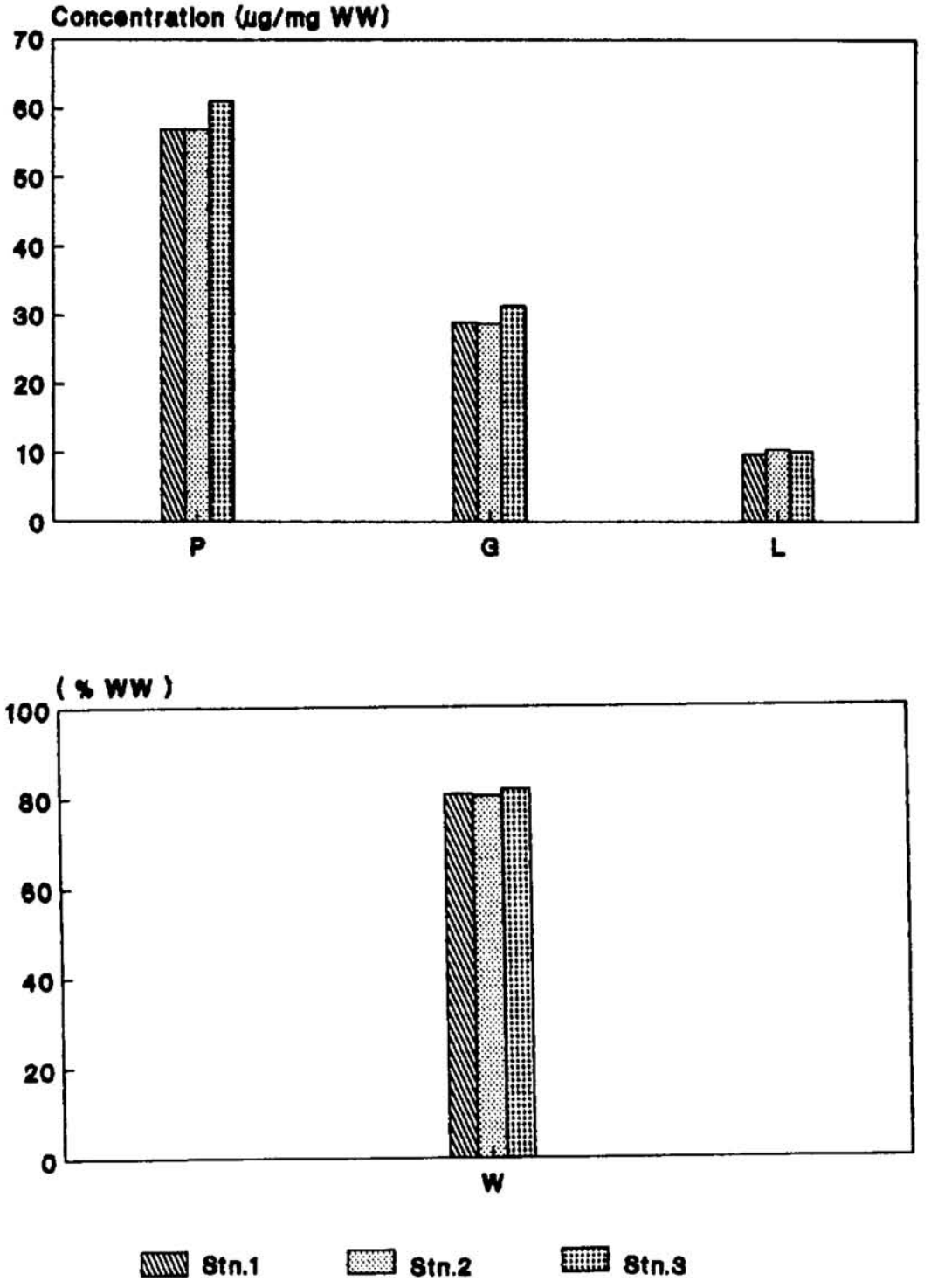


Fig.20 Crassostrea madrasensis. Various biochemical constituents ((P) Protein (G) Glycogen (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 to 3.

Table 25 a-d. Crassostrea madrasensis. Analysis of variance (ANOVA) of the biochemical components in the adductor muscle of individuals from stations 1 to 3, along with their level of significance.

25a. Protein

Source	SS	Df	Mean Sqr	F
Total	23346.93	89	--	
Station	287.219	2	143.609	0.54
Error	23159.72	87	266.204	

25b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	12687.24	89	--	
Station	132.422	2	66.211	0.46
Error	12554.82	87	144.308	

25c. Lipid

Source	SS	Df	Mean Sqr	F
Total	1945.081	89	--	
Station	27.785	2	13.893	0.63
Error	1917.296	87	22.038	

25d. Water content

Source	SS	Df	Mean Sqr	F
Total	903.313	89	--	
Station	29.313	2	14.656	1.21
Error	874	87	12.139	

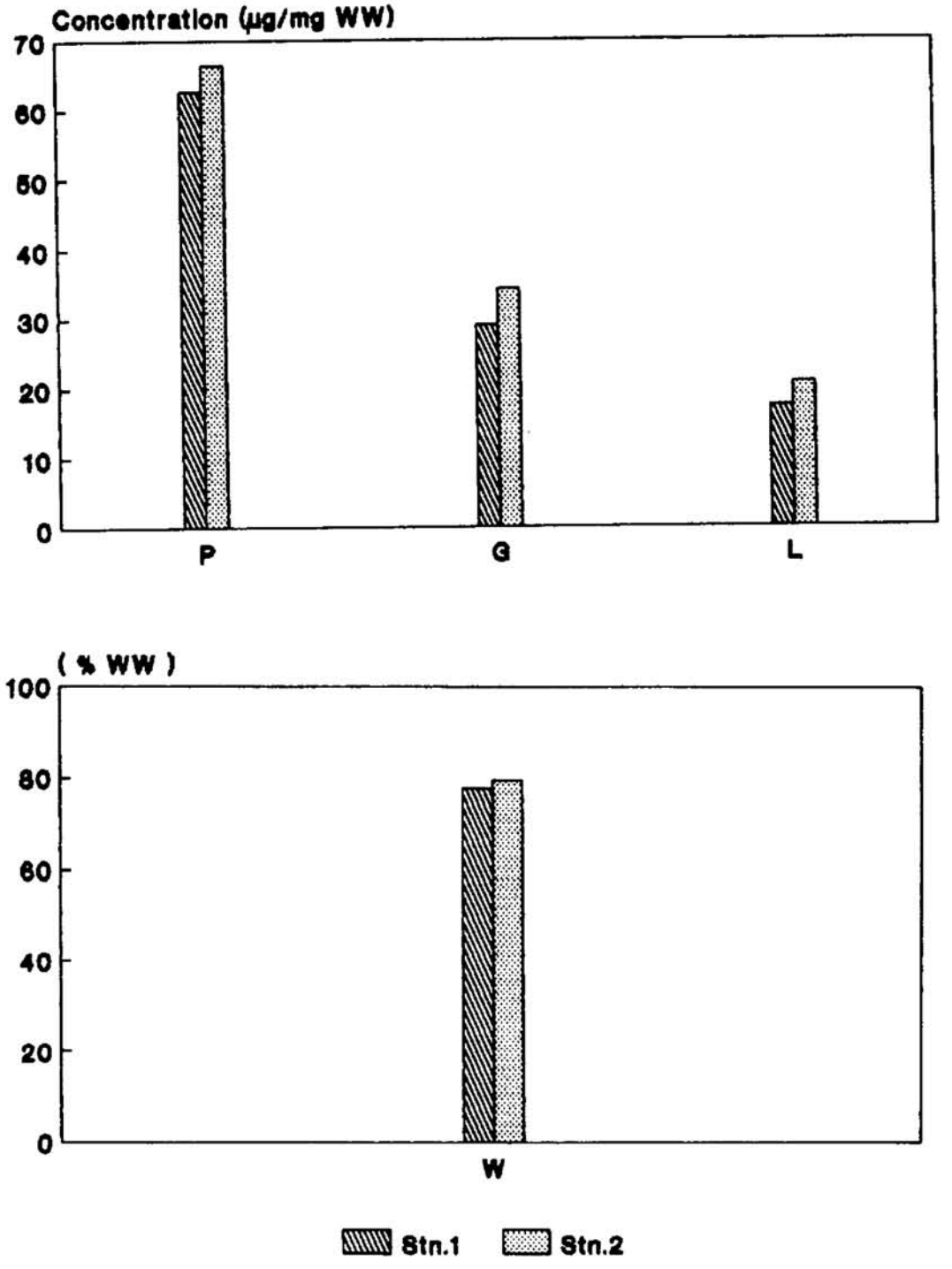


Fig. 21 Perna viridis. Various biochemical constituents ((P) Protein, (G) Glycogen, (L) Lipid (W) Water content) in the adductor muscle of individuals from stations 1 and 2.

Table 26 a-d. Perna viridis. Analysis of variance (ANOVA) of the various biochemical components in the adductor muscle of individuals from stations 1 and 2, along with their level of significance.

26a. Protein

Source	SS	Df	Mean Sqr	F
Total	15634.95	59	--	
Station	193.594	1	193.594	0.73
Error	15441.34	58	266.23	

26b. Glycogen

Source	SS	Df	Mean Sqr	F
Total	7084.004	59	--	
Station	393.465	1	393.465	3.41
Error	6690.539	58	115.354	

26c. Lipid

Source	SS	Df	Mean Sqr	F
Total	1328.549	59	--	
Station	170.684	1	170.684	8.55
Error	1157.965	58	19.965	

26d. Water content

Source	SS	Df	Mean Sqr	F
Total	287.001	59	--	
Station	30.188	1	30.188	5.64
Error	256.813	58	5.35	

6.5. DISCUSSION

Organisms store reserve energy in the form of lipid, glycogen or protein. Regarding fat and glycogen levels, some molluscs especially bivalve species have a glycogen economy with a corresponding low lipid level (Giese, 1966). Synthesis and accumulation of protein have been regarded as the main denominator of true growth by several investigators (Giese, 1969). Further, the nutrient substances accumulated in the form of protein may serve as an energy source during periods of starvation (Giese, 1966; Love, 1970). Detailed comparisons and broad generalizations are difficult in such biochemical studies. This is mainly due to variations in the habitat, season and breeding periodicity of the species concerned. Morevoer, aspects like age and physiological state of such experimental animals are also found to have significant influence.

It is evident from the results obtained that the biochemical constituents of the adductor muscle exhibited some degree of variation among the different populations of the three species sampled from areas of varying salinity regimes. Further, in all the three species, the protein content was relatively high when compared to lipid and glycogen. Thus, of the five populations of V. cyprinoides compared, the highest levels of protein and glycogen in the muscle tissue (53.32 µg/mg and 38.03 µg/mg respectively) were reported from station 3;the lipid content was highest at station 4 (18.75 µg/mg) while the highest water content (82.62%) was reported from station 5 (Table 21). In the case of C. madrasensis, the highest values of protein, glycogen, lipid and water content were reported in individuals from station

3 (Table 22); in P. viridis, the highest value being recorded from station 2 (Table 23).

The storage and utilisation of glycogen reserves reflect the complex interactions between food supply and temperature, and between growth and annual reproductive cycle. The form of the reproductive cycle varies considerably between species and with geographical locality : some species having definite annual cycles while others may breed more or less continuously (Nair and Shynamma, 1975; Ramachandran, 1980; Reddy, 1983; Easterson and Kandasami, 1988). Biochemical studies in bivalves have revealed that glycogen accumulates mainly during the non-reproductive period in the summer (Easterson and Kandasami, 1988) and some bivalves are known to store large amounts of glycogen (11-37%) in their tissues. While in Martesia fragilis values as high as 52% has been reported for glycogen (Srinivasan and Krishnaswamy, 1963), values ranging from 10-35% of glycogen has also been observed in the soft parts of the mussel Mytilus edulis (De Zwaan and Zande, 1972) with extremes of about 60% in Ostrea edulis (Walne, 1970). On the other hand, in most lamellibranchs, the protein content remains at a relatively high level throughout the year and decreases during the period of gametogenic activity and breeding season. The maximum glycogen content in tissues has been observed during the period of gametogenic cycle and decreases with an increase in the fat content. Both glycogen and fat are found to decrease during the spawning period (Naghabhushanam and Thalikhedkar, 1977). Despite all these facts, the results obtained during the present study involving immature individuals of uniform size revealed that the variation found among populations of the same species can be attributed to factors other than those

associated with maturation.

Several workers have reported on the effect of salinity on the water content of marine bivalves (Joshi and Bal, 1965; Deshmukh, 1972; Nagabhushanam and Mane, 1978). In oysters, the water content in the adductor muscle was found to increase up to 92% during periods of low salinity and this increase was ascribed to the loss of salts and gain of water (Nagabhushanam and Mane, 1978). Similar observations were made in the case of Meretrix meretrix (Deshmukh, 1972). Katelysia opima (Nagabhushanam and Mane, 1978), Donax cuneatus (Nagabhushanam and Talikedkar, 1977) and several other species. The increase in water content observed among populations of V. cyprinoides, C. madrasensis and P. viridis sampled from the comparatively less saline areas, in the present study may be explained as due to the effects of salinity on the salt concentration of tissues. It has been observed that in some bivalves, the seasonal fluctuations in the chemical constituents of tissues are reciprocal with the variation in water content (Venkataraman and Chari, 1951; Joshi and Bal, 1965; Deshmukh, 1972). The decrease in glycogen content reported during the present study could be attributed to the utilisation of carbohydrate reserves which are beleived to occur during periods of stress, such as low salinity, rather than to a mere increase in water content. Similar observations were made in respect of C. madrasensis and P. viridis (Table 22 and 23). The marginal variations in moisture content observed between the different populations of the test species may however be explained as due to variations in the habitat salinity.

Besides an inverse relationship between the water content and lipid

levels were evident in almost all populations of the three species. Thus, populations of V. cyprinoides which were characterised by high water content, had a reduced lipid level in their tissues (Table 21). Similar observations were made in the case of C. madrasensis also (Table 22). However, no such relationship was evident among populations of P. viridis (Table 23).

The present series of analyses were carried out employing only immature specimens and avoiding sexually mature ones with a view to minimize the possible fluctuation in the organic constituents associated with reproductive cycle. It is therefore reasonable to assume that the variations in biochemical constituents observed during the present study may perhaps be due to the resultant effect of fluctuations in salinity of the ambient water, nutritional conditions available at different areas and the physiological status of the animal (Suryanarayan and Nair, 1976). A perusal of the data further reveals that mussels are more nutritious than clams and oysters (Fig. 19, 20 and 21) with a relatively high protein content than other biochemical constituents.

Chapter 7

SUMMARY

The subject matter of the thesis relates to the investigations involving delineation of the systematic position and enumeration of the affinities and differences, if any, among natural populations of three bivalve species. The organisms selected for the study are Villorita cyprinoides, Crassostrea madrasensis and Perna viridis belonging to the three major families Corbiculidae, Ostreidae and Mytilidae respectively, of the class Bivalvia from Cochin and nearby waters.

A short preface synoptically describes the importance of the present study. The thesis is presented in six chapters comprising of INTRODUCTION, DESCRIPTION OF SPECIES, MORPHOMETRY, ELECTROPHORETIC STUDIES OF MUSCLE PROTEINS, ELECTROPHORETIC STUDIES OF ISOENZYMES and BIOCHEMICAL COMPOSITION. The general introduction embodies the present status of the work from the Indian context. It also explains the main objectives and scope of the work.

The second chapter on description of species provide a detailed review of the systematics of the three species and a key to the identification of each species along with a description of the study areas. All stations selected for the study were of different salinity regimes, which formed the basis for the selection of stations. Accordingly, the stations which were selected for the study of the genus Villorita are Maradu, Murinjapuzha, Thannirmukkam, Thottappilly and Desam, in the descending order of salinity. Similarly, individuals of C. madrasensis were collected from three stations namely, Willingdon Island, Jetty opposite to Cochin University Marine Sciences laboratory campus and Kumbalam while the individuals of P. viridis were studied from Narakkal and the Seventh fire buoy off Barmouth.

. The present study also involved detailed observation on the minor variations and pecularities characteristic to each species and their populations. Accordingly, individuals of the genus Villorita sampled from the five stations revealed that the fresh water area (Station 5) was inhabited by the variety namely Villorita cyprinoides var.cochinesis while the other four stations were found inhabited by the typical species Villorita cyprinoides. Despite the lack of striking differences in morphological features of C. madrasensis collected from the three stations, those from the less saline habitat (Station.3) were found to exhibit minor variations in respect of size and shell texture. On the contrary, no such variations were evident among individuals of P. viridis from the two stations.

The chapter on morphometry examines the usefulness of this parameter as a taxonomic tool. The review of literature has taken into account the published information available on morphometric studies in relation to systematics. The details of sampling, laboratory conditioning of the selected animals and the morphometric variables employed to assess the possible variations, if any, among populations of the three species are explained. The statistical significance of the data obtained has also been worked out. The results indicated variations in morphometric variables among populations of V. cyprinoides from the five stations; being more pronounced among individuals of station 5. However, no such variations were evident among populations of C. madrasensis sampled from the three stations. This was true in the case of the two populations of P. viridis also. The results obtained has been discussed and conclusions drawn accordingly. The most important finding is that the factors which may have influenced the variations in morphometric characters could be the differential growth rate influenced by the difference in environmental factors in relation to geographical conditions rather than direct genetic influences. It is also assumed that, the

influence on morphometric characters of slight changes in the genetic condition of populations over a long period of geographic separation is also considerable.

Studies on the electrophoretic banding pattern of the muscle proteins of the three species formed the subject matter of the fourth chapter. Polyacrylamide gel electrophoresis, which is a well established technique for the delineation of electrophoretic banding pattern was employed for the purpose. The details of experimental procedure, standardisation of methodology and data analysis are explained under materials and methods. Following standardisation, it was found that a 7% acrylamide concentration would produce the best results for different tissues of the three species. Further, for better resolution of total proteins, Tris-Glycine buffer at a pH range of 8.2-8.6 and 0.1% Amido black stain prepared in methanol : water : acetic acid in the ratio 5:5:1 were found more ideal.

Electrophoretic banding patterns of foot, gill, mantle and adductor muscle tissues were analysed with a view to understand the extend of species specificity and tissue specificity of the three test species. It was found that in all the three species, each tissue exhibited a characteristic electrophoretic pattern with regard to number, staining intensity and relative mobility of each fraction. In V. cyprinoides, the adductor muscle, foot, gill and mantle tissues were characterised by 13, 10, 12 and 11 number of bands while C. madrasensis had 14, 15 and 12 number of bands for their adductor muscle, gill and mantle tissues respectively. On the other hand, the various tissues in P. viridis exhibited 14, 16, 12 and 15 number of bands which were specific to their adductor muscle, foot, gill and mantle tissues respectively. Further, the adductor muscle was found to yield uniform pattern with least variations among individuals of the same population belonging to the same species emphasizing the suitability of the tissue

for further investigations. Studies on the banding pattern of the adductor muscle of individuals of the test species revealed distinct differences between the three species and their populations. Thus, V. cyprinoides from station 5 were characterised by 14 bands, while those from the other four stations had only 13 bands in the zymogram pattern. Marginal variations in the nature of banding pattern were also evident among the three populations of C. madrasensis. Contrary to these observations, hardly any difference in the protein banding pattern was evident among populations of P. viridis. The obvious conclusion that can be drawn from the above observations is that, clams are more closely related to oysters than to mussels while oysters show more affinity towards mussels. No clearcut evidence could be obtained to hypothesize the existence of protein polymorphism among individuals of each population.

The chapter on isoenzyme studies have focused on the analysis of the electrophoretic patterns of four isoenzymes namely, alcohol dehydrogenase (ADH), lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and tetrazolium oxidase (TO) and to relate it with population structure of the three species. The interpretation of electrophoretic phenotypes of isoenzymes is generally unambiguous, allowing genotype assignments to be made directly. The materials and methods utilized are detailed and the techniques followed, listed. In V. cyprinoides of the four isoenzymes studied, only ADH exhibited polymorphism in the phenotypic distribution and allele frequency between stations.The lack of goodness of fit as per Hardy-Weinberg equilibrium in the distribution of the different phenotypes in the various populations may be attributed to an excess of homozygosity. The other isoenzymes depicted only marginal variations in relative mobility as evidenced from the figures. The significant difference in the gene frequencies at the polymorphic ADH locus and the distinct phenotypic pattern differences at non-polymorphic loci

namely LDH, MDH and TO and other muscle protein loci found between populations of V. cypriinoides from station 5 and those from stations 1, 2, 3 and 4 clearly indicate that individuals of Villorita from station 5 is a distinct variety as suspected earlier during the morphometric studies. The results on isoenzymes further confirmed the existence of the variety V. cyprinoides var. cochinesis in the fifth station and is in agreement with other information gathered during the present study. With regard to C. madrasensis and P. viridis, the isoenzyme patterns of their adductor muscle were rather uniform, indicating no marked variations between populations of the two species studied.

Information gathered on the biochemical composition of the adductor muscle of individuals of selected populations of the three species is presented in the sixth chapter. The details of the methodology adopted are described and the data obtained has been analysed for statistical significance. The results are presented under species heads and discussed in the light of available literature. It is evident from the results obtained that, the biochemical components of the adductor muscle exhibited some degree of variation among the different populations of the same species sampled from areas of varying salinity regimes. Moreover, in all the three species, the protein content was relatively high when compared to lipid and glycogen. Besides, an inverse relationship between water content and lipid was evident in most habitats of the three species treated. Further, the results obtained revealed that mussels are more nutritious than clams and oysters with a relatively high protein content when compared to other biochemical constituents. The variations in biochemical constituents observed during the present study, even between the populations of the same species, may, perhaps be due to undetected genetic variations existing between these populations or due to the resultant effect of variations in salinity of the

ambient water, nutritional conditions prevailing at the different areas and the physiological status of the individuals tested.

The literature utilised for the study are listed under references, at the end of the thesis.

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