

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 peculiarities 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. cyprinoides 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.