BIOCHEMICAL GENETICS OF SELECTED COMMERCIALLY IMPORTANT PENAEID PRAWNS

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This is to certify that the thesis entitled **"BIOCHEMICAL GENETICS OF SELECTED COMMERCIALLY IMPORTANT PENAEID PRAWNS"** is the bonafide record of the work carried out by **Mr. P. PHILIP SAMUEL** under my guidance and superivision and that no part thereof has been presented for the award of any other Degree.

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PREFACE

Production of fishes and crustaceans through natural resources is on the decrease in several countries, especially in India. Mariculture is therefore, a fast developing field, in fisheries, in view of both decrease in natural production as well as the enhancing demand of cheaper protein resources to be produced with scientific manipulation methods to bring about large scale production It has gained momentum in all the developed and developing maritime countries. Especially in India, Crustaceans, Molluscs, fin fishes and seaweeds are the major important fields where much importance is given to improve the maximum return by culture methods. Keeping all this in mind the Central Marine Fisheries Research Institute (CMFRI), has taken up multidisciplinary programmes under the centre of advanced studies (CAS) in Mariculture of CMFRI funded by ICAR/UNDP/FAO Project.

After attaining M.Sc. degree in Zoology from the Madurai Kamaraj University in 1983 I joined in CAS in Mariculture as a Senior Research Fellow in the Ph.D. Programme in March 1984. During the first semester took up course work in Mariculture with a curriculum including

fishery and biological aspects of finfishes and shellfishes, culture methods of finfishes, prawn, lobster, crab, mussel, oyster, pearl, clam and sea weed along with site selection grow-out systems, production, economics and extension and environmental aspects.

Besides theory and practicals, study tours were undertaken to different Mariculture field laboratories of CMFRI. During the second semester a special subject "Fi fish and Shell Fish Genetics" was assigned for detailed study and I passed the Ph.D. qualifying examination conducted by the Cochin University of Science and Technology.

Afterwards the particular research project entitled "Biochemical genetics of selected commercially important penaeid prawns" alloted was carried out by collecting samples from different important fishing centres of India and the practical work was carried out in the Research Centre of CMFRI laboratories attached with those places. On the whole, in crustacea little importance has been given so far in finding out the genetic characteristics of different species, genetic variation within and between species and ontogenetic variations in lobsters, prawns and other crustaceans. Prawn is commercially important group

where very little attention had been given so far to find out the racial divergence which may exist in different species. With the increased foreign exchange earning and consequent indiscriminate over exploitation of existing resources of prawns resulting in depletion of the marine resources, alternative ways and augmenting production has become essential. In this connection genetic manipulation of the broodstock will surely bring about the heterogenous characters to multiply production. In order to understand racial fragmentation of some of the commercially important prawns such as Penaeus indicus and Parapenaeopsis stylifera the isozyme studies were carried out. Ontogenetic variation of P. indicus showed stage specific electrophoretic variation. Inter species variation studies was carried out for the closely aligned Penaeus species like P. merguiensis and P. penicillatus; P. japonicus, P. canaliculatus and P. latisulcatus. Metapenaeus sp. like M. brevicornis, M. affinis, M. monoceros and M. kutchensis, Parapenaeopsis species like P. stylifera, P. sculptilis and P. hardwickii.

These studies on inter species and intraspecies genetic variation along with morphometric variables and ontogenic genetic delineations carried out for the first time on Indian species of prawn would go a long way in delineating stocks in commercial populations and determining their genetic characteristics in order to use them for genetic engineering and manipulation.

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CHAPTER I

INTRODUCTION

In an estimated total marine fish production of 1.61 million tonnes in India during 1984-85, prawns constituted 2,04,497 tonnes. Apart from this, according to a survey conducted in 1985 the total area utilized at present for prawn farming is 42,650 ha with a total production of 21,119 tonnes (Rao 1987). From all these, the foreign exchange earning for the country by export of marine products contributed mostly by prawn products is Rs. 460.67 crores in the year 1986-87. This would indicate the importance of prawns in the economy of the country.

Shrimp resources along the 6100 Km long coast line of India are exploited by both artisanal as well as mechanised sectors. The existing stock within the 80 m depth zone along the different regions of the coast is subjected to much pressure due to additional efforts by various programmes of mechanisation of the boats and motorisation of country crafts being implemented by many maritime states and also by the attraction and entry of big business people into the field. The shrimp production

which reached the maximum of 220,000 tonnes in 1975 showed a downward trend in subsequent years. This declining trend is seen in the recent years also. This naturally points towards the necessity for proper management of the fishery. In this context study of biological features of the fishery from different aspects has become quite essential.

The penaeid shrimp resources of the country which constitutes the coastal shrimp fishery occupy different ecosystems such as estuaries, inshore and offshore waters having, different environments. So the fishery management requires proper study of various biological aspects of the fishery in these different environments. Added to these the coastal shrimp fishery is multispecies fishery with individual species having its own distribution patterns, sizes and breeding activities (Silas,George and Jacob 1984). This situation requires monitoring of the population characteristics of each species separatel to keep track of the effects of exploitation of the stocks.

Biology of economically important species of shrimp of the different regions of India are well

documented. Full bibliographies and reviews of the main features of shrimp biology are available in species synopsis papers and other publications by George (1970a, 1970b, 1970c, 1970d, 1972, 1978) Kunju (1970), Mohammed(1970a, 1970b, 1973), Rao (1970, 1973), Kurian and Sebastian (1975) and others. The important species contributing to the fishery are Penaeus indicus, Milne Edwards, P. monodon Fabricius, P. semisulcatus, De Haan, P. merguiensis De Man, Metapenaeus dobsoni (Miers) M. monoceros (Fabricius), M. affinis (Milne Edwards) M. brevicornis,(Milne Edwards) Parapenaeopsis stylifera (Milne Edwards), P. sculptilis (Heller) and P. hardwickii (Miers). Various aspects like distribution different stages of life history reproduction, spawning, larval history and adult history of most of these species are known.

Among other features, delineation of stock and population structure of each species remains important in fisheries management and aquaculture (Hedgecock et al, 1977 Ihssen et al, 1981, Wilkins, 1981). Mark recovery experiments conducted by CMFRI (Vijayaraghavan et al, 1982) showed that P. indicus migrates from Cochin to South east

coast. This further complicates the delineation of stocks of this prawn in the fishery at different places. At the same time proper understanding of the stock contributing to the fishery is very essential for the management of the fishery.

In aquaculture a life is closed and cultivated in a controlled environment. This will result in domestication of an animal due to shielding of that animal from unfavourable environmental condition and long term genetic adaptation to an artificial environment, (Doyle and Hunte 1981). This may result in an ever increasing divergence between domesticated stocks and wild populations due to reduction of variability. In this situation details about the genetics of changes in fitness of each cultivable species is urgently required, since more and more prawn species will be brought under cultivation. These little known effects of aquaculture and similar fields of fisheries activities such as breeding and hybridization on existing species and their populations can be best evaluated and managed only if the existing species and their population structures are known at molecular level of organisation which is most natural.

Recently electrophoresis has gained acceptance in the problem of stock delineation (Saila and Flowers 1969, Messieh and Tibbo 1971, Parsons and Hodder 1971, Johnson et al,1972, 1973, 1974; Messiah 1975, Smith et al, 1980, Lindsey 1981, Mulley and Latter 1981a, 1981b). However, very little work has been done in India to determine to what extent the prawn stocks differ genetically along their spatial range of distribution. Electrophoretic studies on planktonic juveniles and adults of P. indicus and P. monodon has been done by Sriraman et al, (1977). Protein patterns of different tissues of M. affinis, M. monoceros, P. hardwickii and P. stylifera has been studied by Kulkarni(1980). Thomas (1981) has shown the structure of different fraction of the muscle of P. indicus M. dobsoni, M. monoceros and M. affinis. Different proteins of tissues specific and species specific pattern of P. monodon was found out by Prathibha(1984).

The genetic structure of most economically important fish and shrimp populations still remains unknown in the absence of gene frequency data. These populations have been the object of intense fisheries [illegible] to the relative importance of natural selection and [illegible] races

on the pattern of genetic variation. Species can be subdivided into genetically differentiated populations. Constituent populations in a mixed population have to be traced out. Knowledge of stock composition is the fundamental tool for effective management on mixed stock fisheries (Larkin 1981). Absence of this knowledge will result in over exploitation. Patterns of gene flow within each species of rare alleles are also important. Rare alleles can be used in genetic tagging or marking of fish stocks (Mangaly and Jamieson 1978, Lester 1979). By specific pattern of enzymes a key can be produced in solving some identification problems (Johnson et al, 1974). By the paternal protein pattern the hybrid can be identified. Enhancement of inbreeding effect can be identified by the homozygosity estimation.

For the conservation of genetic resources, the United Nations Environment Programme has recommended consultation with experts for conservation techniques of the fish genetic resources, to establish a mechanism for monitoring changes in the genic diversity of fish production, to produce a catalogue of genetic material, to promote knowledge of fish genetics to enhance genetic

diversity and to promote the management of ecosystem with rich genetic diversity for a major socio-economic role. FAO has recommended to conserve genetic resources of fishes in man made or natural ecosystem and to have sample population in the genetic resources centre or in the form of gene pool of gamete storage and germ plasm banks.

In India the National Bureau of Fish Genetic resources Institute has been initiated with the aim of collection and classification of information of genetic resources, to maintain fish genetic material, introduction of new species and conservation of endangered species. Its main thrust is to find the ecological and taxonomic survey of natural habitats, to identify genetically distinct populations with advanced technique, cataloguing the genotype, developing methods to conserve exploited and endangered species.

Ontogenetic variations can be used in identification of different stages of a species with their characteristic protein fractions.

Considering the importance of these studies a detailed morphometric and electrophoretic investigation

was carried out for the separation of populations of two different species of commercially important penaeid prawns Penaeus indicus and Parapenaeopsis stylifera which occur along the Indian coast. The phenotype and genotype difference which may exist between populations were investigated by the studies of gene enzyme variation in natural populations of the two commercially important penaeid prawns (Penaeus indicus and Parapenaeopsis stylifera) This will quantify the amount of racial divergence, if any, among geographically separated natural populations. Thus subpopulation differences within each species of penaeid prawn can be elucidated.

In the biochemical genetic studies electrophoresis is a promising technique for the detection of individual protein variants on gel media such as starch polyacrylamide and agar coupled with histochemical staining procedures.

In 1807 the principle of electrophoresis was found out by Alexander Reuss a Russian physicist. When electricity was passed through a glass tube containing water and clay, colloidal particles moved towards the positive electrode. Tiselius (1937) cited by Brewer (1970) was the first to do the moving boundary electrophoresis and thus separated serum proteins using electric current in a solution. Subsequently zone electrophoresis was developed and the protein

was separated in a stabilized media rather than a solution. Other methods developed by crustacean workers include paper, (Hughes and Klinkler, 1966) agar gel, (Decleir 1961) cellulose accetate, (Lim and Lee 1970) Starch gel,(Whittaker 1959 Cowden and Coleman 1962) and polyacrylamide gel electrophosis (Dall, 1974, Alikhan and Akthar 1980).

In the present study polyacrylamide gel electrophoresis, having the following advantages was used. Sieving process in acrylamide can be adjusted by a varying proportion of cross linkage, by the addition of a proportion of bisacrylamide before polymerization. The bands formed by the larger proteins in acrylamide gels are considerably sharper than those of the same protein in starch gel. Acrylamide has an uncharged matrix in which separation is based on molecular seiving and mobility difference. But in starch proportion of CooH- group at neutral pH carry negative charge (Gordon 1978).

Genetic basis of electrophoretic variation is based on the known relationship between gene and structural protein band detected on the polyacrylamide gel (Crick 1963; Nirenberg et al,1963, Ochoa 1963). First the sub-unit composition and structural relationship of the isozymes were studied in the individual species.

Secondly this isozyme technique was studied on different populations to understand population genetics of a particular species. Detailed work of allozymic variation between different population of P. indicus collected in Cochin, Tuticorin, Madras and Waltair and P. stylifera collected in Cochin and Bombay was carried out in addition to ontogenetic variation in P. indicus.

General protein differences of closely allied species like Metapenaeus brevicornis, M. affinis, M. Kutchensis and M. monoceros; Parapenaeopsis hardwickii, P. stylifera and P. sculptilis; Penaeus latisulcatus, P. japonicus and P. canaliculatus; P. penicillatus and P. merguiensis were also studied in detail for detecting species specific genetic characteristics.

The results of these studies would give the necessary scientific and natural basis for the species verification and their genetically differentiated populations if any. Gene flow within each species also can be identified. This will be helpful to find out the rare alleles in the population, which will act as the genetic tag and also an indication of the movement of larval and adult prawns between areas.

Here electrophoresis of different enzyme protein has been adapted as an effective tool to quantify the amount of racial divergence among geographically separated natural populations of P. indicus and P. stylifera. This gives an insight for its taxonomic information by determining its degree of protein divergence between species and specimens of the same species from different populations where the identification is not clear and many of the discriminate quantitative characters overlap.

Genetic characterization of different species of prawns renders it possible to understand the extent to which prawn stocks differ genetically along their spatial range of distribution. In other words delineation of stocks, which is one of the most essential parameters necessary for effective management of a fishery, is made easier. The results obtained in the present study are expected to help in a big way in solving some of the problems envisaged.

CHAPTER II

MATERIALS AND METHODS

Collection of specimen

The specimens for extraction of organs and materials for study were collected live from the catches. Different species of prawns for analysis were collected from different centres as shown in Figure 1. For instance Penaeus indicus was collected from four different centres as shown in Table 1. In Cochin backwaters the white prawn was taken from cast net and chinese dip net catches. Live specimens were also collected from prawn culture laboratory at Narakkal. In addition collection of Penaeus indicus and Parapenaeopsis stylifera were made by operations of trawl nets from CMFRI Research Vessel Cadalmin. (Plate 13,14)

In Tuticorin material was collected from trawl net operated by CMFRI Research Vessel Cadalmin and in Waltair from the nets operated by research vessel there. Collection in Madras was made from the catches of local catamaran fisherman in Kovalam and from Pentakota fish landing centre at Puri.

Table 1: Distribution and collection sites of Penaeid prawn species studied.

Species	Common Name	Site of Collection	Distribution
Penaeus indicus H. Milne Edwards, 1837	Indian White Prawn	Cochin Tuticorin, Madras, Waltair	Kenya, Persian Gulf, Indo-Pacific, E. and S. Africa to S. China, New Guinea and Australia.
Penaeus japonicus Bate, 1888	Kurma prawn	Madras	Indo-west Pacific from the Red sea, E. and S.E. Africa to Korea, Japan and Malaya Archipelago, In India Bombay and Madras coasts.
Penaeus canaliculatus (Oliver, 1811)	Witch prawn	Madras	Indo-west Pacific, Madras, Cochin and Bombay in India.
Penaeus latisulcatus Kishinouye, 1896	King prawn	Madras	Mosambique, Southern Redsea, Somalia, Gulf of Aden and the Persian Gulf, Japan. South West coast of India.
Penaeus merguiensis De Man, 1888	Banana Prawn	Puri	Indo-west Pacific from Persian Gulf to Thailand, Malaya, Hong Kong, Philippines, India Karwar of W. India, Puri, and Paradeep.
Penaeus pennicillatus Alcock, 1905	Red tail prawn	Puri	Indo-west Pacific from Pakistan to Taiwan, Malaya and Indonesia Maharashtra coast.

Contd.....

Species	Common Name	Site of Collection	Distribution
Metapenaeus brevicornis (H. Milne Edwards,1837)	Yellow prawn	Bombay	Pakistan, N.W. India, Ganges, Delta of W. Bengal, Bangladesh, W. coast of Tailand, Malaya and Indonesia.
Metapenaeus affinis (H. Milne Edwards,1837)	Jinga prawn	Bombay	Indo-west Pacific, Arabian Sea to the Malaya, Archipelago and Hongkong. In India along the West coast of India and the Southern part of east coast
Metapenaeus kutchensis George, George & Rao, 1963	Ginger prawn	Bombay	Gulf of Kutch and N.W. India
Metapenaeus monoceros (Fabricius, 1798)	Speckled prawn	Bombay	Indo-west Pacific, E. and S.E. Africa, Red Sea to Bay of Bengal. In India most of the coast.
Parapenaeopsis stylifera (H. Milne Edwards, 1837)	Kiddi Prawn	Cochin, Bombay	Indo-west Pacific, Persian Gulf, All along the coast line of India.
Parapenaeopsis hardwickii (Miers,1878)	Spear prawn	Bombay	Indo-west Pacific, In India Bombay and Godavary estuary.
Parapenaeopsis sculptilis (Heller, 1862)	Rainbow prawn	Bombay	Indo-west Pacific from Pakistan to Malaya. In India Bombay and Ganges delta

Fig. 1

MAP SHOWING COLLECTION SITES

Penaeus indicus
Penaeus japonicus
Penaeus canaliculatus
Penaeus latisulcatus
Penaeus merguiensis
Penaeus penicillatus
Parapenaeopsis stylifera
Parapenaeopsis sculptilis
Parapenaeopsis hardwickii
Metapenaeus brevicornis
Metapenaeus affinis
Metapenaeus kutchensis
Metapenaeus monoceros

Tuticorin

Cochin

Madras

Waltair

Puri

Fig. 1

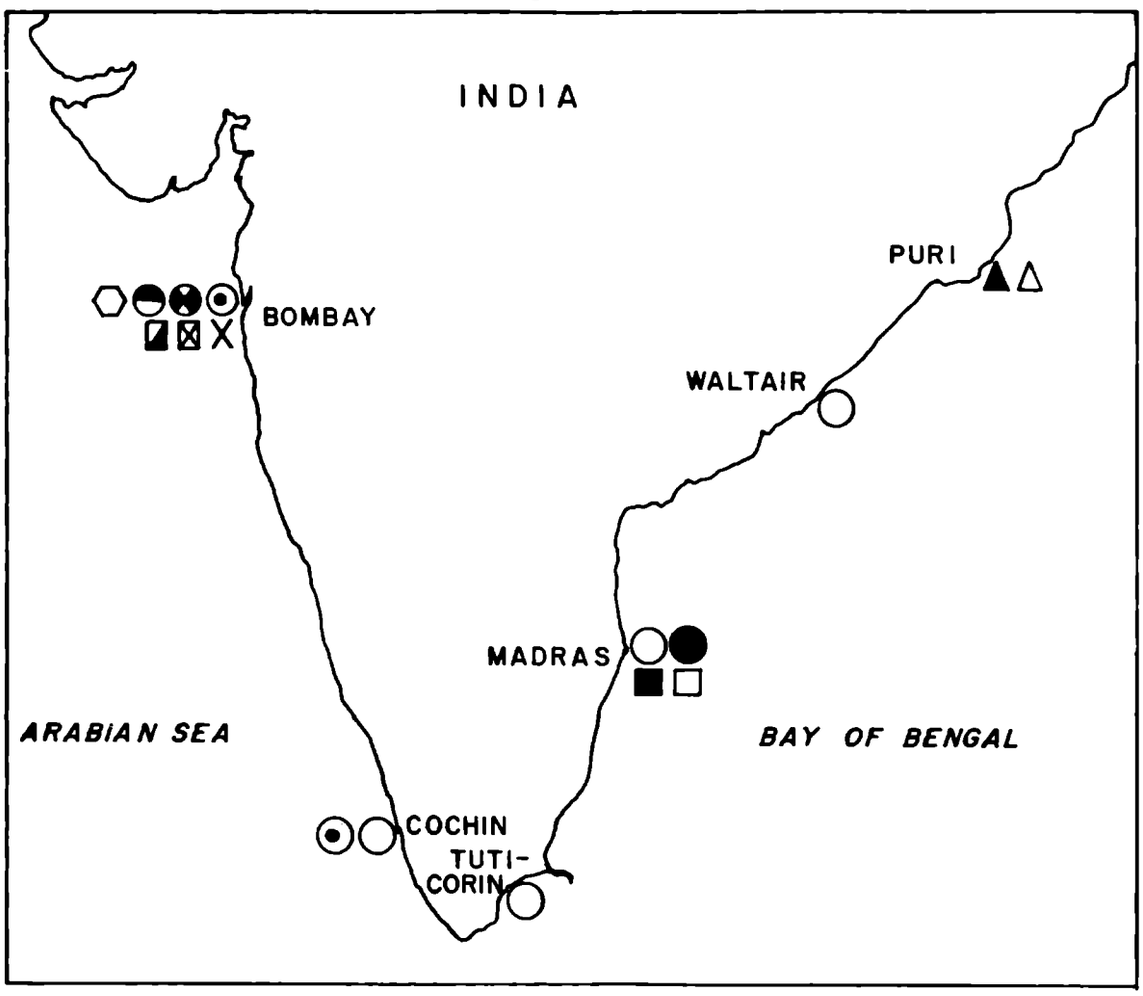

INDIA
PURI
BOMBAY
WALTAIR
MADRAS
ARABIAN SEA
BAY OF BENGAL
COCHIN
TUTI-
CORIN

Plate 13: *Penaeus indicus*


1 2 3 4 5 6 7 8 9 10 11 12 13
IDI
nku

Plate 14: *Parapenaeopsis stylifera*

Sample preparation

All enzymatic proteins were analysed in 3 different tissues. Viz. eye, hepatopancreas and muscles tissues. Prawns of intermoult stages with immature gonad conditions were collected. For larval stages whole animal homogenized tissue extract was taken. Tissues taken from freshly sacrificed prawns were dissected in ice cold condition. Tissue was washed with precooled distilled water and the water content was removed by wiping it with blotting paper. Definite quantity of tissue was measured and homogenized with ice cooled distilled water in machanical homogenizer at 80 rpm. inside ince box. Then it was centrifuged for 10,000 rpm at 4°C for 20 mts. The supernatant was taken and it was freezed for further use in electrophoresis. The quantity of protein used as the sample loaded for electrophoresis was determined by Lowry's method (1951).

Electrophoresis:

Simplified procedure of zone electrophoretic separation of serum protein is found to be the best method for separation of isoenzymes. The ability of a protein molecule to migrate in an electric field depends on its net electric charge and size. According to the pH of buffers protein can be made to travel towards either electrode. In alkaline

solution most protein are negatively charged and travel towards anode. Polyacrylamide gel medium was used here for electrophoresis.

Separating Gel preparation:

Polyacrylamide gel is stable, non-reactive with sample, inert and the pore size can be adjusted by addition of various concentrations of bisacrylamide. Different percentage of the polyacrylamide gel was prepared as given in the polyacrylamide gel electrophoretic method of Laemmli (1970).

30% of Acrylamide stock was prepared as follows:- 30 gm of acrylamide and 0.8 gm of bisacrylamide was dissolved in double distilled water and made upto 100 ml. This was filtered by multipore filterpaper No.42. For preparing 10% concentration of acrylamide from the 30% acrylamide stock, following calculation were made

$$V_1 N_1 = V_2 N_2$$

$$V_2 = 30 \text{ ml}$$

$$N_2 = 10\%$$

$$N_1 = 30\%$$

$$\text{So} \quad V_1 = \frac{30 \times 10}{30} = 10 \text{ ml.}$$

Likewise 5%, 7.5%, 10%, 12.5% and 15% concentration of acrylamide can be prepared from the above stock solution and bisacrylamid concentration can also be changed.

Volume of separating gel buffer was estimated as given below.

Separation gel buffer pH (8.9)

36.6 gm of Tris (Hydroxymethyl) was dissolved in double distilled water. The pH was adjusted with IN HCl and the final volume was made upto 100 ml with distilled water. The concentration is 3 molar. But for using 0.75 molar is necessary. So the following formula is used.

$$V_1 N_1 = V_2 N_2$$

$$V_1 \times 3 = 30 \times .75$$

$$V_1 = \frac{30 \times .75}{3} = 7.5$$

$$V_1 = 7.5 \text{ ml.}$$

So volume of separating gel buffer is 7.5 ml. for 30 ml. solutions. The amount of acrylamide and the total volume of distilled water will change according to different percentage of acrylamide. With this 20 µl of Tetramethylenediamine (TEMED) is usually added to serve as a catalyst of gel formation because it exist as a free radical. Oxygen inhibits polymerization of gels because it eliminates free radicals. Hence the solution is degased and gel is formed in air tight chambers. 90 µl

of 10% of Ammonium persulphate was added to the above solution to enhance the polymerization of the gel. This volume has to be subtracted from the total 30 ml. The remaining is made up with double distilled water.

For preparing 7.5% of acrylamide the following volume of solution were taken

Acrylamide 30% stock	7.5 ml
Separating gel buffer	7.5 ml
Water	14.89 ml
TEMED	20 µl
10% Ammonium persulphate	90 µl
Total	30 ml

Spacer gel preparation:

Large pore buffer, acrylamide solution and riboflavin were used to prepare this.

Large pore buffer.

Dissolve 5.98 gm of Tris (Hydroxymethyl) in double distilled water. Add 0.46 ml of TEMED and adjust pH to 6.7 with 1N HCl and make it upto 100 ml with distilled water.

Acrylamide solution:

Dissolve 10gm of acrylamide and 2.5 gm of methylene bisacrylamide in 100 ml of distilled water to get 3% concentration of spacer gel. 0.04% of riboflavin was prepared and used for polymerization of the gel in the presence of UV light to form free radicals. The above solution were mixed in 1:2:1 and the spacer gel is prepared. Main function of this gel is to arrange the different molecules depending on its size.

The acrylamide stock solutions, separating gel buffer and spacer gel buffer are prepared and stored in amber colour bottles in a refrigerator. Ammonium persulphate is prepared fresh every day before use.

Gel casting:

Gels are moulded in the form of gel rods. Polyacrylamide gels are made in gel tubes of desired size. In continuous gel electrophoresis gel pore size and buffer is one kind and in discontinuous gel electrophoresis pore size of the gel is of two kinds. In Disc gel electrophoresis some times two types of gels viz. separating gel and spacer gel are used for general proteins and separating gel only is used for enzymes. Glass tubes both end opened and having

uniform diameter (.5cm) and length of 7.5 cm was selected. They are placed in a suitable stand in vertical position. The gel tubes were placed in the gel stand and one end of the tube sealed by rubber cork.

Separating gel solution is prepared and it was poured in each gel tube from the sides of the tube with a filler upto the first scratch mark. Care was taken to avoid bubble formation while pouring the solution. After this one drop of water was added from the sides of the gel tube to avoid miniscus formation. When the polymerization is over remove the upper layer of water with the blotting paper completely and carefully. Spacer gel solution was prepared as mentioned above for general proteins. This solution was poured as before upto the second mark. Now also a drop of water is added from the sides to avoid miniscus and allow it for polymerization. The water layer is discarded after polymerization.

The gels were placed inside the refrigerator for half an hour before use. Then the sample for analysis in particular quantity was taken by a microliter syringe. This is mixed with 10 µl of 0.1% aqueous bromophenol blue and 40 µl of 40% sucrose. After the addition of all the above; the sample form the third layer in the gel. Remove the gel tubes from the cork and

place one drop of electrode buffer in the left out portion of tube to avoid bubble formation. Then the gel tubes are inserted into the grommets of the upper buffer tank. Electrode buffer is poured in the upper tank and lower tanks from the sides of the tank after proper dilution with the distilled water.

Then the electrical connections were made btween the disc electrophoresis which is placed inside the BOD incubator at 4°C and the Electrophoretic power pack. Power pack is adjusted in such a way to pass current of 4mA per gel tube or 200-240 V for general protein. This passage of current is adjusted in a different way for different enzymes. According to the charge and size of the protein it will move in the gel. After the bromophenol blue comes to the lower edge of the gel tubes the supply of current is terminated. Buffer is poured out. Gel tubes are removed from the grommet. Distance travelled by the bromophenol blue was found out. The gels in the tubes are removed by injecting water in between the gel and the tube with the help of a syringe. Staining was carried out for different proteins as given in the histochemical staining of proteins. Mobility of each fraction was measured from the point of application. Relative mobility was calculated. The gel was preserved in 7% acetic acid and photographed.

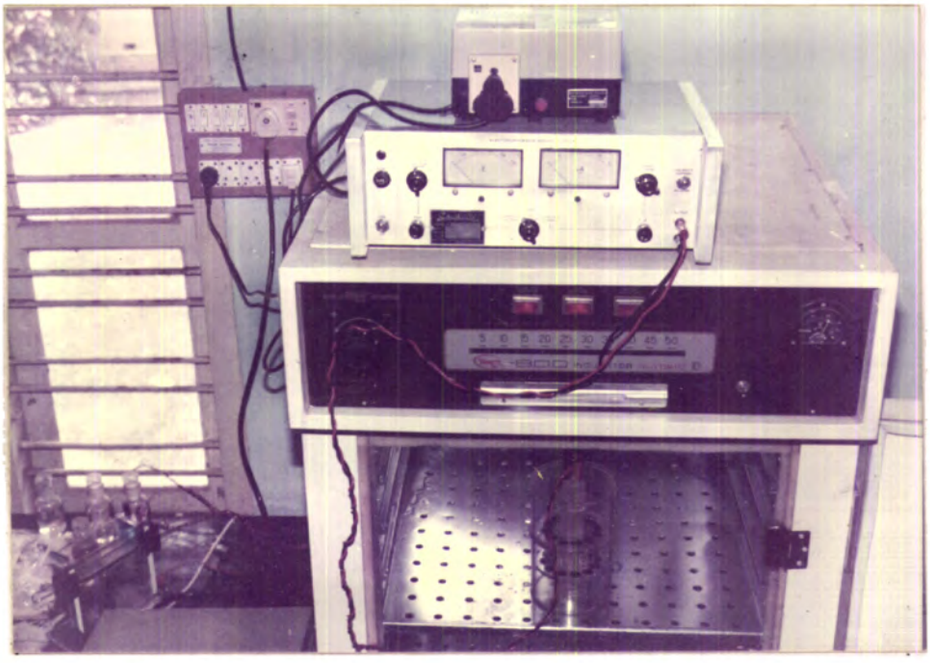
The enzyme which is separated by electrophoresis can be detected by stripping and incubating the test tube at 37°C. With the gel a solution containing substrate for each enzyme, cofactor NAD, electron acceptor PMS, electron indicator NBT one buffer to maintain pH were added.

Conditions were kept constant for all the studies. Same apparatus and power pack were used throughout this study to avoid any experimental error. The set up used for this purpose is shown in photograph (Plate 1).

Vertical gel electrophoresis

Polyacrylamide vertical slab system was employed for the separation of general protein patterns of Penaeus indicus. 12 cm x 12 cm length slabs having 1 mm thickness was used for this purpose. Spacers were kept in the 2 extreme ends of the slab in the parallel manner leaving 0.5 cm space in the ends. The slab was placed inside the lower buffer chamber and kept in position by the clips. Then the three sides of the slab were sealed off with agar gel. The preparation of acrylamide solution and other buffers are same as already explained in disc electrophoresis. Solution is poured with a filler from one side of the slab. The comb is placed in the anterior end of the

Plate 1: Showing the experimental set up of Disc gel Electrophoresis.



slab to form slots for loading the sample. After the polymerization the comb is removed and the sample was applied on the slots by microlitre syringe. Both the upper and lower tanks were filled with the buffer. This set up was kept in BOD at 4°C. The electrical connections were made with the power pack. For each slot 4 mA current was applied. When the bromophenol marker reaches the end the passage of electricity was terminated. The mobility distance of the bromophenol blue was measured. The gel is stained for gendral protein. The set up used for doing slab gel electrophoresis is shown in photograph.(Plate 2) This method was employed for various other enzymes but the separation and resolution was not good like what is resolved in disc electrophoresis. So disc gel electrophoresis method was employed for enzymes and general protein separation (Plate 3) of P. indicus was carried out using slab gel electrophoresis method.

Histochemical staining of gel:

After the electrophoresis the gels were incubated in the staining solution for the appearance of characteristic protein bands. Different staining components used for specific enzymes are given below:-

Plate 2: Showing the experimental set up of slabs gel electrophoresis.

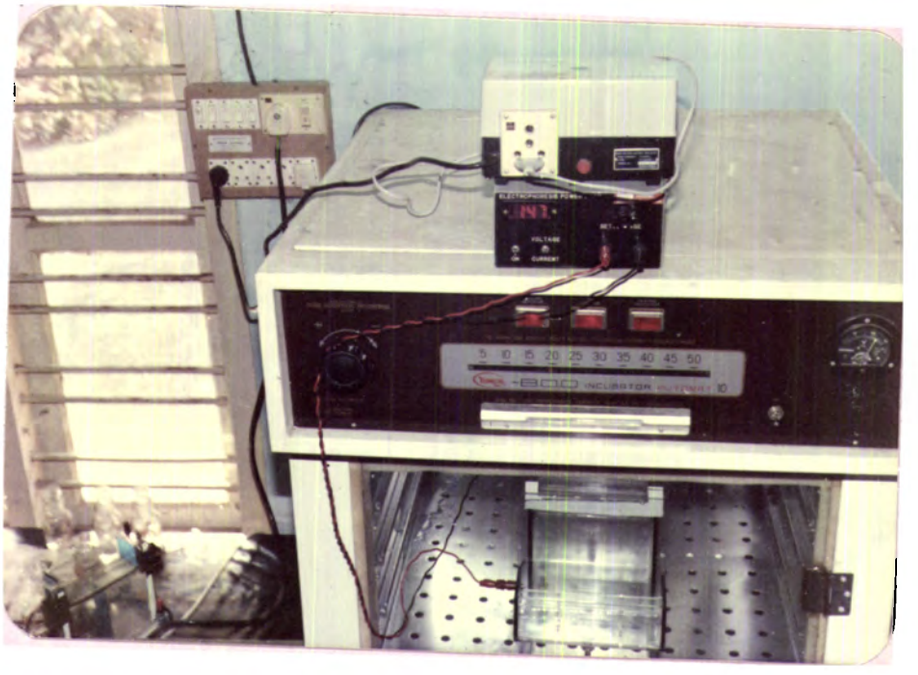
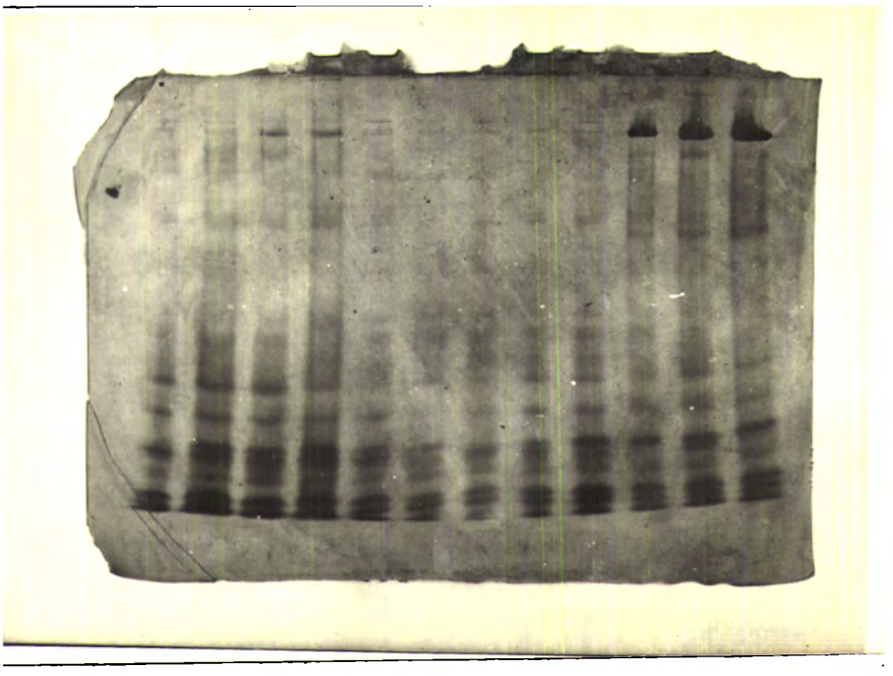

INT
VOLTAGE
CURRENT
5 10 15 20 25 30 35 40 45 50

Plate 3: Showing muscle myogen protein pattern observed in Penaeus indicus using slab gel electrophoresis.



Acid phosphatase (Siciliano & Shaw, 1976)

Combine the following in a flask/Sodium alpha napthylacid phosphate 50mg/Fast Garnet Blue Salt 50 mg/ water 50 ml/Put the gel in the above solution and incubate at 37°C for 30 minutes. Red bands indicate zones of activity.

Alcohol dehydrogenase (Siciliano & Shaw, 1976).

The following components were mixed and used.

95% ethanol	2 ml
NAD	25 ml
NBT	15 mg
PMS	1 mg
0.2 M Tris H cl pH 8.0	7 ml
Water	41 ml

The stain thus prepared was incubated at 37°C after soacking the gel in the stain and keeping it in dark. Dark blue band are the expression of enzyme on the gel.

Aldehyde oxidase (Redfield and Salini, 1980)

Benzaldehyde	1 ml
NAD	20 mg
MTT	10 mg

PMS	2 mg
Water	50 ml
Tris-H cl pH 8 buffer	50 ml

Dark blue bands appear as soon as the gel is placed inside the stain.

Aldolase (Siciliano & Shaw, 1976)

Following quantity of different chemicals were added and the staining solution of this enzyme was prepared.

Fructose 1-6 disphosphate tetrasodium salt		275 mg
NAD	25 mg	
NBT	15 mg	
PMS	1 mg	
Sodium arsenate	75 mg	
0.2 M Tris-HCl pH 8.0	10 ml	
Water	40 ml	
Glyceraldehyde 3 phosphate dehydrogenase		100 units

When the gel is immersed in the staining solution at 37°C dark blue bands appear as zones of enzyme activity.

Alkaline phosphatase (Redfield and Salini, 1980)

∝ Naphthyl acid phosphate	100 mg
Fast Garnet GBC Salt	100 mg
Water	50 ml
Tris-HCl, pH 8.5	50 ml

This stain is incubated at 37°C to get red coloured bands of enzyme activity.

Esterase (Redfield and Salini, 1980)

Alpha Napthyl acetate	15 mg
Fast blue RR salt	100 mg
Water	50 mg
Tris HCl pH 7.0	50 ml

Esterase activity are indicated by dark brown bands after incubating the gel in the stain at 37°C.

Glycerophosphate dehydrogenase (Siciliano & Shaw, 1976)

Sodium alpha Glycerophosphate	75 mg
NAD	25 mg
NBT	15 mg
PMS	1 mg
0.2 m Tris HCl pH 8.0	10 ml
Water	35 ml

Gels treated with above solution at 37°C will give dark blue bands of enzyme activity.

Lactate dehydrogenase (Siciliano & Shaw, 1976 modified)

Lithium lactate	100 mg
NAD	25 mg
NBT	15 mg

PMS	1 mg
0.2 M Tris-Hcl pH 8.0	10 ml
Water	35 ml

This stain is poured with gel in dark at 37°C to get dark blue bands.

Malate dehydrogenase (Siciliano & Shaw, 1976)

Malic acid	10 mg
NAD	25 mg
NBT	15 mg
PMS	1 mg
0.2 M Tris-HCl pH 8.0	10 ml
Water	35 ml

Surface of gels give dark blue bands hen the above solution was incubated at 37°C.

Malic enzyme (Siciliano & Shaw, 1976)

Malic acid	10 mg
NADP	15 mg
NBT	15 mg
PMS	1 mg
$MgCl_2$	50 mg
0.2 M Tris-HCl pH 8.0	10 ml
Water	35 ml

Gels were treated with this stain solution in dark at 37°C to get dark blue bands.

Octanol dehydrogenase (Redfield and Salini, 1980)

Octanol	3 ml
NAD	20 mg
MTT	10 mg
PMS	2 mg
Water	47 mg
Tris-HCl pH 8.5	50 ml

This gel was incubated inside the stain at 37°C to get pink coloured bands to note the presence of enzyme.

6-Phosphogluconate dehydrogenase (iciliano & Shaw, 1976)

6-Phosphogluconic acid Trisodium salt	100 mg
NADP	15 mg
NBT	15 mg
PMS	1 mg
Mgcl2	50 mg
0.2 M Tris HCl pH 8.0	10 ml
Water	40 ml

The gels were incubated at 37°C to get dark blue bands to indicate the presence of enzyme.

Peroxidase (Siciliano & Shaw 1976)

a. Add 1N HCl to 50 ml of electrode buffer until pH reaches 6.0. Soak gel in this solution for 45 minutes at 4°C and pour off.

b. Pour on solution containing

KI	1 g
Water	50 ml
Glacial acetic acid	1 ml

c. Soak gel in above for 60 seconds and wash throughly (3 rinses) with distilled water and add the following solution.

Water	50 ml
3% H_2O_2	1 ml

d. Incubate in 37°C until peroxidase appears as dark blue bands on a light blue background.

1-Pyrroline dehydrogenase (Redfield and Salini, 1980)

Alpha Pyroglutamic acid	50 mg
NAD	20 mg
MTT	10 mg
PMS	2 mg
Water	47 ml
Tris-HCl pH 3.0	50 ml

After adding the stain with the gel it is incubated at 37°C to get blue bands of this enzyme.

Sorbitol dehydrogenase (Redfield and Salini, 1980)

D-sorbitol	1 gm
NAD	20 mg

MTT	10 mg
PMS	2 mg
Water	47 ml
0.2 M Tris-HCl pH 8.0	50 ml

Gels were incubated at 37°C to get blue bands of enzyme presence.

Tetrazolium oxidase (Siciliano & Shaw, 1976)

NADP	9 mg
NBT	9 mg
PMS	4.5 mg
0.2 M Tris-HCl pH 8.0	6 ml
Water	24 ml

Colourless bands appear after the treatment of the gels with this stain at 37°C.

Standardisation:

Buffers: For genetic variation study particular protein system has to satisfy the following criteria. Bands of activity should be sharp and distinct in order to find the difference in migration rate and the protein has to be located in a tissue which is routinely tested. So a variety of buffers given in different literature have been used for electrophoresis. Buffers resist changes in H^+ and OH^- ion

concentration and maintain constant pH. Each has its own buffering capacity. Buffer system which gives optimum electrophoretic resolution for protein was selected from the following eight buffers.

Buffer system used on different proteins

I. Described by Siciliano and Shaw (1976)

Tris-citrate (TC)

Electrode buffer pH 7.0

Tris-16.35 g

Citric acid (granular monohydrate) - 9.04 g

Water upto 1 L.

Adjust pH upward with 10 N NaOH or downward with Con. HCl for final pH of 7.0

Gel buffer pH 7.0

Dilute 40 ml of electrode buffer upto 600 ml 4 mA per tube gave good resolution.

II. Described by Siciliano and Shaw (1976)

Tris-Versene-Borate (TVB) pH 8

Electrode buffer

Tris	60.6 g
Boric acid	40.0 g

Na_2 EDTA 2H 20	6.0 g
Water upto	1 L

Adjust pH upward with 10 N NaOH or doward with Conc HCl for final pH 8.0

Gel buffer pH 8.

Dilute 60 ml of electrode buffer upto 600 ml with water. 4 mA per tube gives good separation.

III. Described by Ferguson and Wallace (1961).

Tris-citric-boric acid (TCBL).

Electrode buffer pH	8.26
Lithium hydroxide	2.51 g/l
Boric acid	18.54 g/l
Gel buffer	pH 8.31
Tris	3.63 g/l
Citric acid	1.05 g/l
Electrode buffer	10 ml/l

Passage of current was restricted to 2 mA per tube.

IV. Described by Richardson (1982).

Stock solution (TM)

Tris	1.211 gm/L

pH adjusted to 7,8 with maleic acid. The solution was diluted to 1: 10 for gel preparation and used undiluted for electrode buffer.

Adjust current to 4 mA per tube.

V. Described by Brewer (1970.

Electrode buffer (TME)

Tris	12.114 g
Maleic acid	11.607 g
EDTA Na.2	3.7224 g
MgCl2	2.033 g

The pH is adjusted to 7.6 with 4 N NacH

Gel buffer:

Tris	1.2114 g
Maleic acid	1.1607g
EDTA Na2	0.37224 g
Mgcl2	0.2033 g

The pH is adjusted to 7.6 with 4 N NacH.

The gel buffer is a 1:10 dilution of electrode buffer.

Each gel tube was given 2 mA current.

VI. Described by Davis (1964)

Electrode buffer (TG) pH 8.3

Tris	6 g
Glycine	28.8 g
Water upto	1 L

1:10 dilution of this stock solution was used

Gel buffer (TH) pH 8.9

Tris	36.6 g
1N HCl	48 ml

Water upto 100 ml

VII. Described by Shaw and Prasad (1970).

Stock solution pH 6.8

NaH_2PO_4, H_2O	27.8 g/L	255 ml
$Na_2H\ PO_4, H_2O$	53.65 g/L	245 ml

Stock solution is undiluted and used as electrode buffer. 65 ml of Electrode buffer was diluted to 1L and used as gel buffer pH 7.0.

VIII. Described by Brewer (1970).

Electrode buffer pH 7

Sodium citrate 120.581 gm/L

pH is adjusted to 7.0 with 0.41 M citric acid

1:10 dilution was used.

Gel buffer

Histidine 0.7758 g/L

pH is adjusted to 7.0 with 2 N NaOH.

Extraction medium

Tissues were extracted in different solvents to find out the best solvent which gives good resolution without any denaturing effect. Definite quantity of tissues were homogenized in the mechanical homogenizer in ice cold condition.

Different solvents used for this purpose is given below:

1. Double distilled water.
2. Distilled water and sucrose
3. Tris-HCl mercaptoethanol pH 7.5 (Siciliano & Shaw 1970)
4. Tris-EDTA Na_2 pH 6.8 (Redfield and Salini 1980).

Quantity of sample

To find out optimum concentration of the sample different concentration of sample were tried. For this 50 mg of tissue was homogenized in 1 ml of precooled double distilled water From this 40 µl, 50 µl, 60 µl and 75 µl were taken for finding out optimum concentration of sample.

Polyacrylamide gel concentration

As given in gel preparation six different concentration of gels were prepared. viz. 5%, 7.5%, 10%, 12% and 15%. Definite quantity of eye, hepatopancreas and muscle were taken. and the electrophoresis was carried out in different gel concentration. This will be useful in finding out the percentage of acrylamide which gives good resolution and maximum number of bands in general proteins. This acrylamide percentag was tried for different bisacrylamide percentage from 0.8%, 2%, 3%, and 4%. In the above, the percentage which gives good resolution was used for further electrophoretic separation of proteins.

Staining method:

Staining for the general protein of eye, hepatopancreas and muscle was tried with three different stains. Amido black, coomassie Brilliant blue and Kenacid blue. Stains were prepared as given below:

Stain	Solvent
1. Amido black 0.25 gm	100 ml of methanol, Water and acetic acid in 5:5:1 ratio
2. Coomassie brilliant blue 0.25 gm	100 ml of methanol water and acetic acid in 5:5:1 ratio
3. Kenacid blue 0.25 gm	100 ml of methanol, water and acetic acid in 5:5:1 ratio
4. Kenacid blue 0.25 gm	100 ml of water

Storage effect

Effect of storage on different enzymatic proteins like, 1-Pyrroline dehydrogenase, Alcohol dehydrogenase, Aldolase, 6-Phosphogluconate dehydrogenase, Alpha Glycerophosphate dehydrogenase, Malate dehydrgnase and Acid phosphatase were done to find the effect of temperature variation on the activity of tissues. The samples were tried again and again after partial thawing.

Different Parameters analysed

Morphometric analysis: Eleven morphometric dimensions were taken for this work. All the morphometric variables were measured using vernier calipers. Circumference of the abdome was taken from metric tape. Weights of the specimens were measured by beam balance.

Code	Variable
SSL	Length of the sixth abdominal segment along the mid dorsal line.
FSL	Length of the first abdominal segment along the mid dorsal line with the prawn extended.
PCL	Partial carapace length from the margin of the orbit to the posterior edge of the carapace.
CW	Width of the carapace at the point of the last dorsal rostral tooth
FLF	Length of the fifth abdominal segment when the prawn is flexed ventrally.
SSD	Depth of the abdomen at the mid point of the sixth segment.
SAD	Depth of the abdomen at the intersection of the second and the third segments.
AAC	Circumference of the abdomen at the intersection of the second and third abdominal segments.

PAC	Circumference of the abdomen at the intersection of segment five and six
TW	Total weight of the prawn specimen.
TL	Total length from the tip of the rostrum upto the tip of the telson.

The above measurements were taken on Penaeus indicus collected from Cochin, Tuticorin, and Madras and Parapenaeopsis stylifera collected from Bombay and Cochin.

Fig 50. Univariate and multivariate analysis was carried out to find out the possible variation within the species.

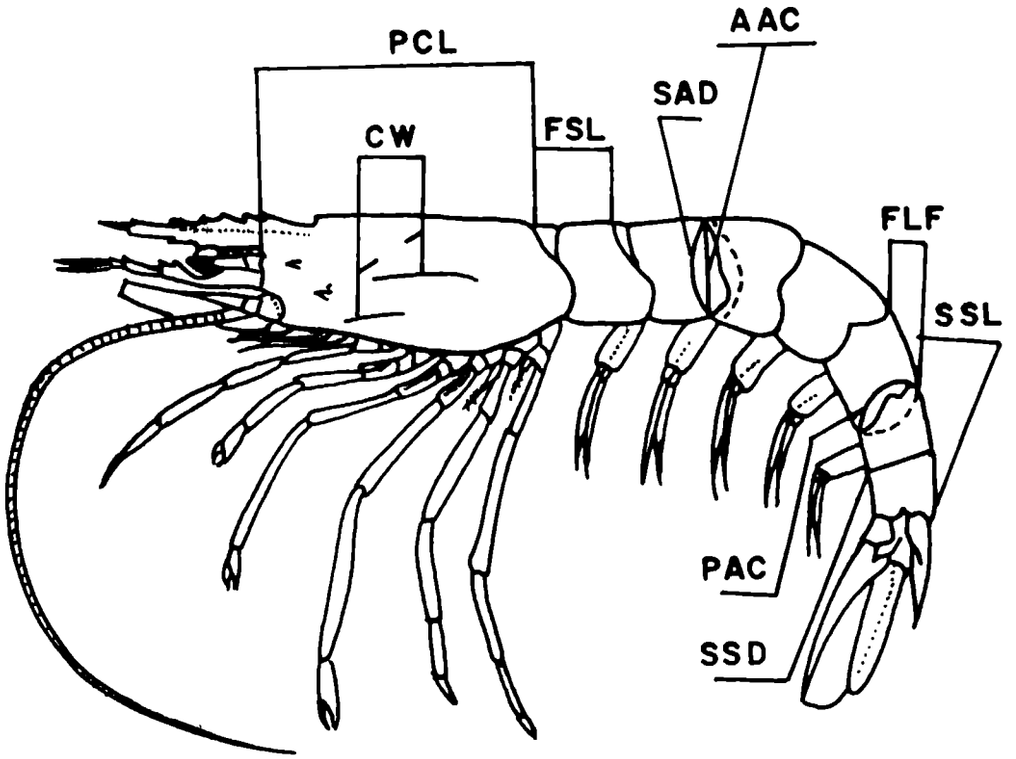
Genetic variation analysis

The enzymes examined for electrophoretic separation were:-

Acid phosphatase (ACPH, E.C.3.1.3.2), Alcohol dehydrogenase (ADH, E.C. 1.1.1.1), Aldehyde oxidase (AO, E.C.1.2.3. Aldolase (ALDO, E.C.4.1.2.13), Alkaline phosphatase (ALPH, E.C.3.1.3.1), Esterase (EST, E.C. 3.1.1.1), Alpha Glycerophosphate dehydrogenase (GPDH, E.C.1.1.1.8), Lactate dehydrogenase (LDH, E.C. 1.1.1.27), Malatedehydrogenase (MDH E.C. 1.1.1.37), Malic enzyme (ME, E.C. 1.1.1.40), Octanol dehydrogenase (ODH, E.C. 1.1.1.73), 6-Phosphogluconate dehydrogenase (6-PGDH, E.C. 1.1.1.44), 1-Pyrroline dehydrogenase (1-PYDH, E.C. 1.5.1.12), Peroxidase (E.C.1.11.1.7),

Fig. 50. Metric variables used in the multivariate analysis for *Penaeus indicus* and *Parapenaeopsis stylifera*.

Fig. 50



Sorbitol dehydrogenase (SDH, E.C. 1.1.1.14), and Tetrazolium oxidase (TO, E.C. 1.15.1.1).

Genetic expression and variation in different tissues were carried out on P. indicus and P. stylifera collected from the above localities. Electrophoretic separation of above mentioned enzymes were analysed in tissues like eyes, hepatopancreas and muscles.

Ontogeny:

Different larval stages and juvenile stages as given below have been collected for this study on P. indicus. In Larval stages whole tissue extract was taken and used as sample. But in juveniles the different tissues like eyes, hepatopancreas and muscle were taken and used for electrophoresis. The different proteins analysed were Acid phospha Aldehyde oxidase, Octanol dehydrogenase, Esterase, Alcohol dehydrogenase and Malate dehydrogenase. Different groups tested were: Protozoea, Mysis, Post larvae, Juveniles of 20-30mm, 40-50 mm and 70-80 mm and adults 90-120 mm. The larvae for study were collected from Prawn culture laborato Narakkal. Protozoea stage was collected for standardizatio procedure. Whole tissue extract was taken by 15% dilution. All these were done in ice cold condition. Then the extrac was used for electrophoresis of different enzymes. Particu

volume required for best resolution was determined by using different quantity of the sample. The particular concentration which gave best resolution was used for further studies on ontogeny. For the juvenile stages and adults particular tissues which gave best resolution was used.

Inter species variation

From Madras Muthukad backwaters three species of closely allied species like Penaeus japonicus, P. canaliculatus and P. latisculcatus were collected. The general protein pattern among these species were found out. Muscle tissue was used for this studies.

Penaeus merquiensis and P. penicillatus was collected from Pentakota fish landing centre at Puri. General protein pattern in muscle tissue was found out. The gels were photographed and scanned in Madurai-Kamaraj University, Madurai.

Allied species of Metapenaeus were collected from Bombay coast. Metapenaeus affinis, M. kutchensis, M. monoceros, M. brevicornis were collected from New Ferry Wharf. Electrophoretic separation of muscle tissue was carried out. Parapenaeopsis species of prawns were also collected from Bombay. P. hardwickii, P. sculptilis and P. stylifera were collected from seas on dock. General protein pattern of

muscle was found out for these species. Relative mobility was found out for each band and electrophorograms were prepared. The gels were photographed.

Analysis of electrophoretic data

Each protein fraction separated on the gel during electrophoresis were given numbers from slowest migrating cathodal end towards fastest migrating anodal direction in an increasing order. Rm values were calculated as using the following formula.

$$\text{Relative mobility} = \frac{\text{Distance migrated by the protein fraction X 100}}{\text{Distance migrated by the marker.}}$$

The frequency of an allele is calculated by $\frac{2Ho + He}{2N}$

Where Ho = Number of homozygotes for that allele

He = Number of heterozygotes for that allele

N = Number of individuals examined.

Hardy-Weinberg distribution

If a population is in Hardy-Weinberg equilibrium then the frequencies of genotypes will be in the ratio of p^2, 2 pg and q^2 for a 2 allele polymorphism where p is the frequency of allele A and q is the frequency of allele B. The difference between observed and expected values of allelic frequency was tested for Chi-Square method.

$$\chi^2 \text{ method} = \frac{(\text{observed frequency} - \text{expected frequency})^2}{\text{Expected frequency}}$$

Nei's coefficient of genetic identity(I)was used

$$I = \frac{x_i y_i}{x_i^2 \quad y_i^2}$$

Where x_i and y_i are the frequencies of the ith allele in populations x and y respectively. The genetic distance (D) is estimated by $D = -\ln I$
which can be obtained from tables of natural logorithms.

Genetic identity and genetic distance between population using Nei's method and Roger's method were carried out using a computer programme developed by Green (1979).

CHAPTER III

STANDARDIZATION OF METHODOLOGY

In the present study different procedures were modified and method which gives better result was used for further analytical work using P. indicus as a test species. For the extraction of enzymes four different homogenizing mediums were used as solvents. Double distilled water showed better results in P. monodon (Prathibha 1984) and in Homarus americanus (Odense and Anand 1978). Since best resolution was obtained using double distilled water it was used to extract protein and enzymes in all the experiments (Table 2).

Staining procedure for general protein was tried with different stains such as coomassie Brillient Blue, Amidoblack and Kenacid blue. Kenacid blue produced good result. When this was compared with coomassie Brillient blue Kenacid blue doesn't stain the gels. Whereas coomassie Brillient blue stains the surface of gel. Kenacid blue was considered to be giving superior staining than the other stains. A mixture of methanol water and acetic acid was used as solvent for dissolving the stain. Here without these solvent the stain Kenac blue dissolved in distilled water gave equally good results.

Table 2: Effect of different mediums on the resolution of muscle proteins of *Penaeus indicus*.

Extracting Medium	Total no. of bands	Resolution	Training
1. Double distilled water	13	Good	Nil
2. Double distilled water and sucrose	10	Poor	Heavy
3. Tris-Hcl, Mercapto-ethanol pH 7.5 (Siciliano & Shaw 1970)	11	Fair	Moderate
4. Tris-Edta Na_2 pH 6.8 (Redfield and Salini, 1980)	10	Fair	Moderate

So Kenacid blue was used for the staining of the general proteins (Table 3).

Quantity required for the optional resolution was determined. Since the quantity of protein content in different tissues vary, different concentration of tissues like eye, hepatopancreas and muscle were tried. For the penaeid prawns Sriraman and Reddy (1977) and Thomas (1981) used 50 mg. and 500 mg per ml. of solvents respectively. Here experiments were carried out, to find the desired quantity of sample which gives good resolution from the sampl got by homogenizing 50 mg. of tissue in 1 ml. of double distilled water. From the results it is concluded that 50 µl for eye and hepatopancreas tissues and 60 µl for muscle tissue gives good separation without trailing. So these quantity of sample was used in the forth coming experiments also (Table 4).

One more way of improving the resolution is by varyin the concentration of separating gel. The proteins of high molecular weight are separated using gels of larger pore s and the smaller proteins are separated in a better way using gels of smaller pore size. The upper range of Monom concentration for acrylamide gel has to be adjusted to get suitable gel concentration (Smith 1968). Most of the

Table 3: Muscle myogen protein pattern of *Penaeus indicus* in different stains

Staining Mixture	Staining Intensity				
	Dark	Medium	Light	Total number of bands	Background colour
1. Amido black in 100 ml of methanol, water and acetic acid in 5:5:1 ratio	5	6		11	Bluish black
2. 0.25 m of Coomassie brilliant blue GR-250	5	7		12	Pale blue
3. 0.25 gm of kenaeid blue in 100 ml of methanol, water and acetic acid in 5:5:1 ratio	5	8		13	Clear
4. 0.25 gm of kenaeid blue in 100 ml of water	5	8		13	Clear

Table 4: General Protein patterns of Penaeus indicus using different quantities of Eye, hepatopancreas and muscle tissues.

7.5%

Sample Volume	Protein µgm content	No. of bands	Resolution	Trailing
Eye				
40 µl	264	10	Good	Nil
50 µl	330	11	Good	Nil
60 µl	396	11	Fair	Moderate
75 µl	495	11	Poor	Moderate
Hepatopancreas				
40 µl	103	9	Good	Nil
50 µl	129	11	Good	Nil
60 µl	155	11	Fair	Moderate
75 µl	194	11	Fair	Moderate
Muscle				
40 µl	220	11	Good	Nil
50 µl	275	12	Good	Nil
60 µl	330	13	Good	Nil
75 µl	413	13	Fair	Moderate

investigation on prawns were carried out using 7% acrylamide (Sriraman and Reddy 1977, Thomas, 1981) whereas 10% acrylamide (Prathibha 1984) concentration was found to produce best resolution for proteins extracted from eye, hepatopancreas muscle and serum of P. monodon. Likewise here also 10% acrylamide with 0.8% bisacrylamide concentration gives best resolution than the other gel concentration which were experimentally tried and resulted in poor resolution (Table 5).

Storage effect:

Effect of storage on different enzymatic proteins like 1-Pyrroline dehydrogenase, Alcohol dehydrogenase, Aldolase, 6-Phosphogluconate dehydrogenase, Alpha Glycerophosphate dehydrogenase, Malate dehydrogenase and Acid phosphatase was tried. This was analysed for 3 days. Studies revealed that except Aldolase, 6-Phosphogluconate dehydrogenase, all the other enzymes didn't show any variation in their intensity. This proves that the enzymatic activity was not influenced by the storage. All the enzymes were tested with eight different buffers to find out their resolution. Acid phosphatase resolved well in Tris citrate buffer pH 7 (Table 6).

Table 5: Protein separation using different ratios of Acrylamide and Bisacrylamide.

Tissues	Concentration of Acrylamide and Bisacrylamide % with no.of bands								
	5,0.8	7.5,0.8	10,0.8,	12.5,0.8	15,0.8	10,0.8	10, 2	10,3	10,4
Eye	3	10	14	7	5	14	11	5	6
Hepatopancreas	6	12	17	12	11	17	15	15	7
Muscle	5	10	20	10	9	20	18	17	15

Table 6: Phosphatase (ACPH 3.1.3.2) resolution of different tissues of Penaeus indicus in different buffers

Electrophoretic system	Tissues	No.of bands	Intensity of band					Distinction	Separation
			1	2	3	4	5		
TC I	E	2	xx	xx	0	0	0	+	+
	H	4	xx	xx	0	x	xx	+	+
	M	1	0	0	xx	0	0	+	+
TCB III	E	-	0	0	0	0	0	-	-
	H	2	0	0	xx	xxx	0	-	-
	M	-	0	0	0	0	0	-	-
TM IV	E	-	0	0	0	0	0	-	-
	H	2	0	0	xxxx	0	xx	-	-
	M	-	0	0	0	0	0	-	-
TME V	E	2	0	0	xx	0	x	-	-
	H	2	0	0	xxx	xx	0	-	-
	M	1	0	0	0	x	0	-	-
PH VII	E	1	0	0	0	xx	0	-	-
	H	4	0	x	xx	xxxx	xx	-	-
	M	1	0	0	0	xxx	0	-	-
HSC VIII	E	2	0	0	0	xx	xx	-	-
	H	2	0	0	0	xx	xx	-	-
	M	2	0	0	xx	xxx	0	-	-

No resolution in TG VI and TVB II

Distinction : Bands clear without trailing +
Bands diffused with trailing -

Separation : good clear and resolved well bands with sharpened ends +
Poor -

Band intensities:

Dark	xxxx	I	- Tris citrate pH 7 (TC)
Medium	xxx	II	- Tris-Versene-Borate pH 8(TVB)
Light	xx	III	- Tris-citric-Boric-LioH pH 8.31,8.2
Faint	x	IV	- Tris Maleic acid pH 7.8 (TM)
No activity	0	V	- Tris maleic acid Edta pH 7.6 (TME)
		VI	- Tris Glycine pH 8.3 (TG)
E - Eye		VII	- Phosphate buffer pH 7(pH)
H - Hepatopancreas		VIII	- Histidine pH 7 Sodium citrate pH 7
M - Muscle			

Alcohol dehydrogenase enzyme showed good resolution in Tris versene Borate buffer pH 8 (Table 7).

Table 8 showed that Aldehyde oxidase is giving good separation in Tris glycine buffer pH 8.3. Esterase enzyme gave good resolution when tested with Histidine pH 7 and Sodium citrate pH 7 buffers (Table 9). Alpha glycerophosphate dehydrogenase enzyme resolved well in Tris versene Borate.

pH 8 (Table 10). Table 11 showed lactate dehydrogenase to resolve in Tris citricacid (pH 8.31). Lithium Hydroxide buffer (pH 8.26). From the table No.12 it is learnt that malate dehydrogenase resolved well in Tris glycine buffer pH 8.3. Malic enzyme resolved well in Tris-maleic acid Edta buffer pH. 7.6 (Table 13), Octanol dehydrogenase resolved well in Tris Maleic acid buffer (Table 14).

6-Phosphogluconate dehydrogenase showed good resolution in Tris versene borate buffer pH 8 (Table 15).

1-Pyrroline dehydrogenase buffer expressed good resolution in Tris versene Borate pH .8(Table 16). Table 17 showed Tetrazolium oxidase to resolve well in Tris versene Borate buffer pH 8. Peroxidase showed good resolution in Histidine pH 7 sodium citrate buffer pH 7 since

Table 7: Alcohol Dehydrogenase (ADH 1.1.1.1) resolution of different tissues of *Penaeus indicus* in different buffers

Electrophoretic system	Tissue	No. of bands	Intensity of bands				Distinction	Separation
			1	2	3	4		
TC I	E	2	0	x	x	0	-	-
	H	2	x	xx	0	0	-	-
	M	1	0	xx	0	0	-	-
TVB II	E	2	0	xx	xx	0	+	+
	H	2	x	xx	0	0	+	+
	M	1	0	xx	0	0	+	+
TCBL III	E	1	0	0	xx	0	-	-
	H	2	xx	xx	0	0	-	-
	M	1	xx	0	0	0	-	-
TM IV	E	2	x	0	xx	0	-	-
	H	2	x	0	x	0	-	-
	M	-	0	0	0	0	-	-
TME V	E	1	0	0	xx	0	-	-
	H	2	xxx	0	0	xx	-	-
	M	2	xxx	0	x	0	-	-
pH VII	E	2	0	0	x	xx	-	-
	H	2	xxx	0	0	xx	-	-
	M	2	0	x	0	x	-	-
HSC VIII	E	2	0	0	xx	xx	-	-
	H	2	0	0	x	x	-	-
	M	2	xxx	xx	0	0	-	-

No resolution in TG VI

Table 8: Aldehyde Oxidase (AO 1.2.3.1) resolution of different tissues of Penaeus indicus in different buffer.

Electrophoretic system	Tissue	No. of bands	Intensity of bands			Distinction	Separation
			1	2	3		
TC I	E	-	0	0	0	-	-
	H	2	xxxx	0	xx	-	-
	M	1	0	x	0	-	-
TVB II	E	-	0	0	0	-	-
	H	1	0	xx	0	-	-
	M	-	0	0	0	-	-
TCBL III	E	-	0	0	0	-	-
	H	1	0	xxx	0	-	-
	M	-	0	0	0	-	-
TM IV	E	-	0	0	0	-	-
	H	1	0	xxx	0	-	-
	M	-	0	0	0	-	-
TME V	E	-	0	0	0	-	-
	H	1	0	xxxx	0	-	-
	M	-	0	0	0	-	-
TG VI	E	-	0	0	0	-	-
	H	2	xx	xx	0	+	+
	M	1	0	0	xx	+	+
pH VII	E	-	0	0	0	-	-
	H	1	0	xx	0	-	-
	M	-	0	0	0	-	-
HSC VIII	E	-	0	0	0	-	-
	H	2	0	xxx	x	-	-
	M	-	0	0	0	-	-

Table 9: Esterase (EST, E.C. 3.1.1.1) resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissues	No. of bands	Intensity of bands				Distinction	Separation
			1	2	3	4		
TC I	E	2	xx	0	0	xx	-	-
	H	2	0	xx	-	xxx	-	-
	M	2	xx	0	0	xx	-	-
TVB II	E	2	0	0	xx	xx	-	-
	H	2	0	x	0	0	-	-
	M	1	0	0	0	x	-	-
TCBL III	E	1	0	0	xx	0	-	-
	H	2	x	0	xx	0	-	-
	M	1	xx	0	0	0	-	-
TM IV	E	1	0	0	xx	0	-	-
	H	2	x	0	x	0	-	-
	M	-	0	0	0	0	-	-
TME V	E	1	0	0	0	xx	-	-
	H	2	xxx	0	0	xx	-	-
	M	2	xxx	0	0	x	-	-
pH VII	E	2	0	0	x	xx	-	-
	H	2	xxx	0	0	xx	-	-
	M	1	0	0	0	x	-	-
HSC VIII	E	1	0	x	0	0	+	+
	H	2	xx	0	xx	0	+	+
	M	3	xx	0	xx	x	+	+

Table 10: Glycerophosphate dehydrogenase (Gpdh 1.1.1.8) resolution of different tissues of Penaeus indicus in different buffers.

Electro-phoretic system	Tissues	No.of bands	Intensity of bands		Distinction	Separation
			1	2		
	E	-	0	0	-	-
TVB	H	1	xx	0	+	+
	M	-	0	0	-	-
	E	-	0	0	-	-
TCBL	H	1	x	0	-	-
	M	-	0	0	-	-

No activity in TC I, TM IV, TME V, TG VI, pH VII, HSC VIII.

Table 11: Lactate dehydrogenase (LDH 1.1.1.27) resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissues	No.of bands	Intensity of bands		Distinction	Separation
			1	2		
TC I	E	1	x	0	-	-
	H	-	0	0	-	-
	M	2	xx	xx	-	-
TVB II	E	1	0	xx	-	-
	H	1	xx	0	-	-
	M	1	0	xxxx	-	-
TCBL III	E	1	0	x	+	+
	H	1	x	0	+	+
	M	2	x	xx	+	+
TME V	E	1	0	xx	-	-
	H	1	xx	0	-	-
	M	1	0	xx	0	0
pH VIII	E	1	x	0	-	-
	H	-	0	0	-	-
	M	1	x	0	-	-

No resolution in TM IV, TG VI, HSC VIII

Table 12: Malate dehydrogenase (MDH 1.1.1.37) resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissue	No. of bands	Intensity of bands			Distinction	Separation
			1	2	3		
TVB II	E	1	x	0	0	-	-
	H	-	0	0	0	-	-
	M	2	0	xx	x	-	-
TCBL III	E	1	0	0	x	-	-
	H	-	0	0	0	-	-
	M	2	xx	x	0	-	-
TM IV	E	1	0	0	xx	-	-
	H	-	0	0	0	-	-
	M	1	x	0	0	-	-
TME V	E	1	x	0	0	-	-
	H	-	0	0	0	-	-
	M	2	0	xxx	x	-	-
TG VI	E	1	xx	0	0	+	+
	H	-	0	0	0	-	-
	M	2	0	x	x	+	+
pH VII	E	1	x	0	0	-	-
	H	-	0	0	0	-	-
	M	2	x	xx	0	-	-
HSC VIII	E	1	x	0	0	-	-
	H	-	0	0	0	-	-
	M	2	0	xxx	x	-	-

No resolution in TC 1.

Table 13: Malic enzyme (ME 1.1.1.40) resolution of different tissues of *Penaeus indicus* in different buffers.

Electrophoretic system	Tissues	No.of bands	Intensity of bands		Distinction	Separation
			1	2		
TC I	E	1	0	x	-	-
	H	-	0	x	-	-
	M	2	x	x	-	-
TVB II	E	1	0	x	-	-
	H	-	0	0	-	-
	M	1	0	xx	-	-
TCBL III	E	1	0	x	-	-
	H	-	0	0	-	-
	M	-	0	0	-	-
TM IV	E	1	0	xx	-	-
	H	-	0	0	-	-
	M	-	0	0	-	-
TME V	E	1	xx	0	+	+
	H	-	0	0	+	+
	M	1	0	x	+	+
TG VI	E	1	0	xx	-	-
	H	1	x	0	-	-
	M	1	x	0	-	-
pH VII	E	1	0	x	-	-
	H	-	0	0	-	-
	M	-	0	0	-	-
HSC VIII	E	2	x	xx	-	-
	H	1	0	x	-	-
	M	-	0	0	-	-

Table 14: Octanol dehydrogenase (ODH 1.1.1.73) Resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissue	No. of bands	Intensity of bands			Distinction	Separation
			1	2	3		
	E	-	0	0	0	-	-
TVB II	H	1	xx	0	0	-	-
	M	-	0	0	0	-	-
	E	1	x	0	0	-	-
TCB& III	H	1	x	0	0	-	-
	M	1	xx	0	0	-	-
	E	2	x	x	0	-	-
TM IV	H	1	0	0	x	-	-
	M	2	x	x	0	-	-
	E	1	0	xx	0	+	+
TME V	H	1	xx	0	0	+	+
	M	1	0	0	xx	+	+
	E	1	0	0	x	-	-
TG VI	H	1	x	0	0	-	-
	M	1	0	x	0	-	-

No resolution in TC I, HSC VIII and pH VII

Table 15: 6-Phosphogluconate dehydrogenase (6 PGDH 1.1.1.44) Resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissues	No. of bands	Intensity of bands		Distinction	Separation
			1	2		
TC I	E	-	0	0	-	-
	H	1	xx	0	-	-
	M	1	0	x	-	-
TVB II	E	2	x	x	+	+
	H	1	0	xx	+	+
	M	1	0	x	+	+
TCBL III	E	-	0	0	-	-
	H	1	xx	0	-	-
	M	1	0	x	-	-
TM IV	E	-	0	0	-	-
	H	1	x	-	-	-
	M	-	0	0	-	-
TME V	E	1	0	x	-	-
	H	-	0	0	-	-
	M	-	0	0	-	-
TG VI	E	1	xx	0	-	-
	H	1	xx	0	-	-
	M	-	0	0	-	-
pH VII	E	1	0	x	-	-
	H	-	0	0	-	-
	M	-	0	0	-	-
HSC VIII	E	1	0	x	-	-
	H	1	0	x	-	-
	M	1	0	x	-	-

Table 16 : 1-Pyrroline dehydrogenase (PYDH 1.5.1.12) Resolution of different tissues of *Penaeus indicus* in different buffers

Electrophoretic system	Tissues	No.of bands	Intensity of bands		Distinction	Separation
			1	2		
TC I	H	1	x	0	-	-
	M	-	0	0	-	-
TVB II	H	1	0	x	+	+
	M	1	xx	0	+	+
TCBL III	H	1	x	0	-	-
	M	1	x	0	-	-
TM IV	H	1	xx	0	-	-
	M	-	0	0	-	-
TME V	H	1	x	0	-	-
	M	-	0	0	-	-
pH VII	H	1	x	0	-	-
	M	-	0	0	-	-

No resolution in TG VI, HSC VIII

Table 17: Tetrazolium oxidase (TO 1.15.1.1.) resolution of different tissues of Penaeus indicus in different buffers

Electrophoretic system	Tissues	No.of bands	Intensity of bands				Distinction	Separation
			1	2	3	4		
TC I	E	2	0	x	x	0	-	-
	H	2	x	0	x	0	-	-
	M	1	0	0	x	0	-	-
TVB II	E	2	x	x	0	0	+	+
	H	2	0	0	x	x	+	+
	M	1	0	x	0	0	+	+
TCBL III	E	2	x	x	0	0	-	-
	H	1	0	x	0	0	-	-
	M	1	0	x	0	0	-	-
TG VI	E	1	0	x	0	0	-	-
	H	1	0	x	0	0	-	-
	M	-	0	0	0	0	-	-
HSC VIII	E	2	0	x	x	0	-	-
	H	1	0	x	0	0	-	-
	M	1	0	x	0	0	-	-

No resolution in TM IV, TME V, pH VII,

the bands disappeared very quickly, it couldn't be used for further studies (Table 18). Sorbitol dehydrogenase expressed good resolution in Tris glycine buffer pH 8.3 (Table 19).

Table 18: Peroxidase (PER 1.11.1.7) resolution of different tissues of *P. indicus* in different buffers.

Electrophoretic system	Tissues	No. of bands	Intensity of bands			Distinction	Separation
			1	2	3		
	E	3	xx	x	x	+	-
TC I	H	1	0	0	x	-	-
	M	1	0	0	x	-	-
	E	2	0	x	x	-	-
TVB II	H	1	0	0	x	-	-
	M	1	0	0	x	-	-
	E	1	0	0	x	-	-
TCBL III	H	1	0	0	x	-	-
	M	1	0	0	x	-	-
	E	2	0	x	x	-	-
TM IV	H	1	0	0	x	-	-
	M	2	0	x	x	-	-
	E	3	x	x	x	-	-
TME V	H	-	0	0	0	-	-
	M	1	0	0	x	-	-
	E	2	x	x	0	-	-
pH VII	H	2	x	x	0	-	-
	M	1	0	x	0	-	-
	E	3	x	x	x	+	+
HSC VIII	H	2	x	0	xx	+	+
	M	3	x	xx	x	+	+

No resolution in TG VI

Table 19: Sorbitol dehydrogenase (SDH, E.C.1.1.1.14) resolution of different tissues of Penaeus indicus in different buffers.

Electrophoretic system	Tissues	No. of bands	Intensity of bands			Distinction	Separation
			1	2	3		
	E	-	0	0	0	-	-
TC I	H	1	0	xx	0	-	-
	M	1	0	0	xx	-	-
	E	-	0	0	0	-	-
TVB II	H	1	0	xx	0	+	+
	M	1	x	0	0	-	-
	E	-	0	0	0	-	-
TCBL III	H	3	x	x	xx	+	-
	M	1	0	x	0	-	-
	E	-	0	0	0	-	-
TM V	H	1	xx	0	0	-	-
	M	1	x	0	0	-	-
	E	-	0	0	0	-	-
TG VI	H	1	0	0	xx	+	+
	M	1	0	x	0	+	+
	E	-	0	0	0	-	-
HSC VIII	H	-	0	0	0	-	-
	M	1	x	0	0	-	-

No resolution in pH VIII, TM IV.

CHAPTER IV

INTERSPECIES GENETIC VARIATION

Resume of literature:

Connel (1953 a, b) studied water soluble muscle proteins of fishes using Tiselius technique of electrophoresis for comparative purposes for the first time. Homoir (1955) also studied about fish muscle proteins. Water soluble muscle proteins were analysed in 20 species of Poeciliid fishes (Hewitt et al., 1963) and in hybrids of genus Xiphophorus (Greenberg and Kopac 1965) with the help of paper electrophoresis to find the difference in them. Rabaey (1964) used agar-gel electrophoresis for the separation of protein of 35 fish species.

Comparative muscle myogen electrophorogram study showed virtual constancy and species specific nature of myogen in 50 species of fishes (Tsuyuki et al., 1965). In species of the Petromyzontidae, (Uthe and Tsuyuki 1966) and in Rockfish scorpaenidae (Tsuyuki et al., 1968) muscle myogen pattern was used for the systematics studies.

Studies on muscle protein polymorphism within the genus Tilapia were conducted by Hines and Yashov (1970) and in the genus Merluccius by Jones and Mackie (1970).

Inter and intra species variation of muscle protein in Japanese Crucian carp was shown in cellulose acetate by Taniguchi and Ishiwatasi (1972) and in starch electrophoresis by Taniguchi and Sakata (1977). Herzberg and Pastear (1975) studied six species of grey mullets in the mediteranean coast of Israel with reference to muscle protein.

Electrophoretic studies on muscle proteins showed distinct patterns in Gobioids of Portonova (Natarajan et al., 1975) in Mullus surmuletus and M. barbatus (Arias and Morales 1977) in frigate tuna Auxis thazard (Yeh and Yang 1977) in Sarpa Salpa and Boops boops (Arias and Moralas 1980) and in four species of Sciaenidae (Garcia 1980).

Densitometric analysis was carried out and the crests found were proportional to the protein concentration which was worked out in Anodonta grandis (Saleuddin 1969) in cyprinid fishes (Haen and O' Rourke 1969) and in flat fishes (Menezes 1979).

In crustaceans, work done on this aspect is quite limited. Kannupandi and Paulpandian (1975) studied blood and muscle proteins of crabs and Cole and Morgan (1978) studied muscle protein of the blue crab Callinectes sapidus Rathbun.

Electrophoretic studies on muscle myogens of penaeid prawns like Metapenaeus mutatus, Parapenaeopsis hungerfordi, P. hardwickii, Metapenaeopsis stridulans, M. barbata, Penaeus monodon, P. semisulcatus, Metapenaeus ensis, and Parapenaeopsis affinis were carried out by Lim and Lee(1970) and Lee and Lim (1973). Sriraman and Reddy (1977) found out the characteristic muscle patterns of planktonic juveniles of Penaeus indicus and P. monodon. Kulkarni et al.,(1980) separated proteins of four penaeid prawns namely Metapenaeus affinis, M. monoceros, Parapenaeopsis hardwickii and P. stylifera in relation to their sex. Electrophoretic separation in marine prawns Penaeus indicus, Metapenaeus dobsoni, M. monoceros and M. affinis showed specificity in their protein patterns (Thomas 1981). Prathibha(1984) studied in detail protein patterns in different tissues of P. monodon. These studies show that electrophoretic separation of muscle myogen protein patterns confirm and classify the taxonomy of different species. In the present study, using this technique protein patterns of four species of prawns of the genus Metapenaeus namely, M. kutchensis, M. monoceros, M. affinis and M. brevicornis, 3 species of Parapenaeopsis such as P. stylifera, P. sculptilis and P. hardwickii and 5 species of Penaeus such as Penaeus merguiensi P. penicillatus, P. latisulcatus, P. canaliculatus and P. japonicus were compared.

Results:

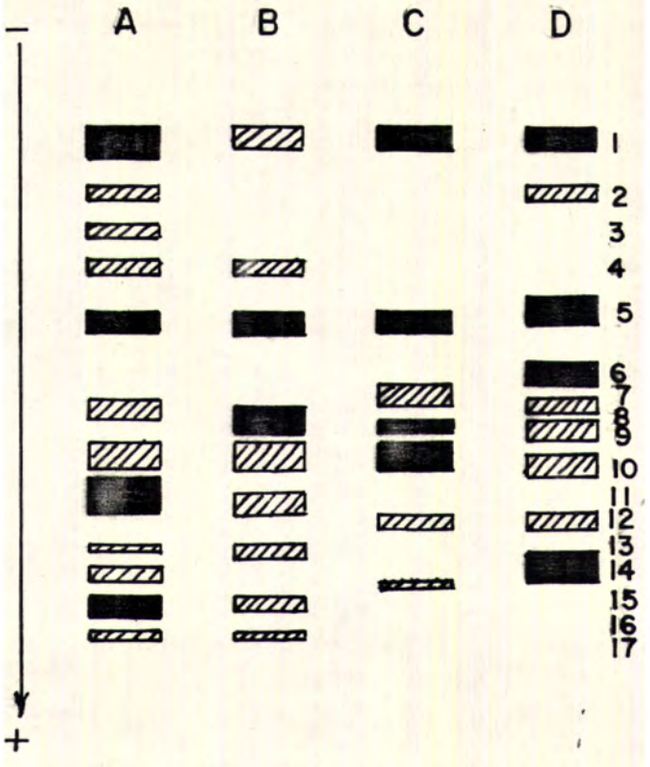
For comparison of each muscle protein band among the four species of Metapenaeus studied here each fraction was alloted a qualifying number obtained according to the electrophoretic mobility position of that particular band, thus the slowest moving and the fastest moving bands receiving the number one and the last number respectively. The bands in between receive the corresponding qualifying numbers. Thus the total number of protein bands present and the alloted numbers for these bands for each species need not be the same (Fig. 2 & 3).

Thus differences in the protein pattern was explained according to their mobility, number of fractions, staining intensities and with the width of each fraction.

Figure 2 & 3 shows the species specific protein patterns of muscle detected in penaeid prawns Metapenaeus kutchensis, M. monoceros, M. affinis, M. brevicornis, Parapenaeopsis sculptilis, P. stylifera and P. hardwickii These electrophoretic protein patterns help us to identify species which have greater similarity with each other.

Fig. 2. Comparative electrophorograms of abdominal muscle tissues of four *Metapenaeus* species of prawns. Different shades indicate the intensity of bands.

Fig. 2

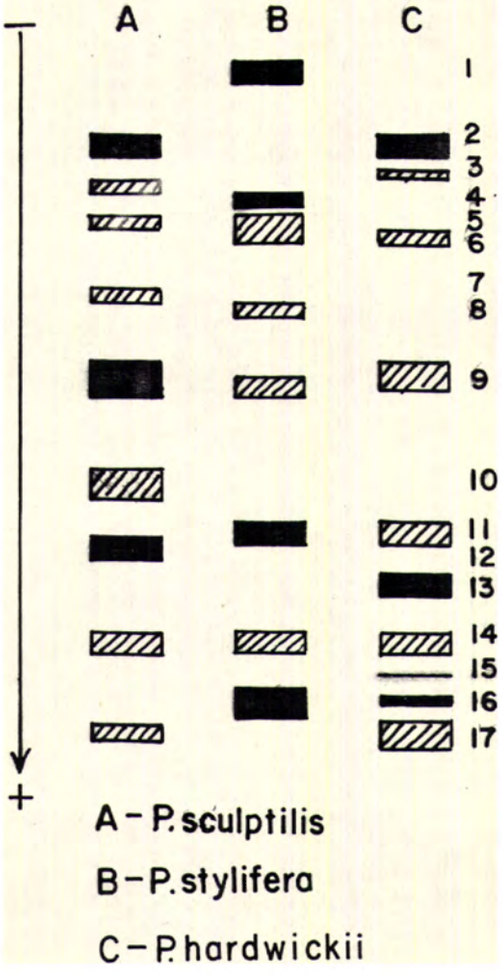


A-M. affinis, B-M. brevicornis

C-M. kutchensis, D-M. monoceros

Fig. 3. Comparative electrophorograms of abdominal muscle tissues of three *Parapenaeopsis* species.

Fig.3


A
B
C
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
A – P. sculptilis
B – P. stylifera
C – P. hardwickii

Metapenaeus species:

First the comparison was made between the species of prawns belonging to Metapenaeus. The protein fractions of Metapenaeus sp. were numbered serially from slowest moving cathodal band to the fastest moving anodal band, thus slowest moving fraction becoming band No.1 and the fastest moving fraction becoming band No. 17 (Fig. 2). Similarity observed in the relative mobility of some of the bands differed by their width and intensity of staining and thus gave a characteristic pattern for that particular species. M. affinis M. brevicornis, M. kutchensis and M. monoceros showed 12, 9, 7 and 9 muscle protein fractions respectively. The differences in the total number of bands between any of the two species except M. brevicornis and M. monoceros studied here indicated a specific number for muscle protein fractions. Though both M. brevicornis and M. monoceros showed 9 protein fractions each the distinct differences in the electrophoretic mobility, staining intensity and width of certain number of these 9 bands demonstrated a specific pattern for these two species also and thus all the four species showed their own specific muscle protein patterns (Table 21 & 22).

When the common bands found in these species were considered bands No.1, 5 and 10 showed similar relative mobility but their intensity of staining and width of the

Table 21: Relative mobility (RM) with intensity of muscle myogen proteins of Metapenaeus species of prawns.

No.	RM	Intensity
Metapenaeus kutchensis		
1	13.3 - 16.7	xx
2	38.3 - 41.7	xx
3	48.3 - 51.7	x
4	53.3 - 55	xx
5	56.7 - 60	xx
6	66.7 - 68.3	x
7	75 - 76.7	x
Metapenaeus monoceros		
1	13.3 - 16.7	xx
2	21.7 - 23.3	x
3	36.7 - 40	xx
4	45 - 48.3	xx
5	50 - 51.7	x
6	53.3 - 56.7	x
7	58.3 - 61.7	x
8	66.7 - 68.3	x
9	71.7 - 75.0	xx

Contd...

No.	RM	Intensity
Metapenaeus affinis		
1.	13.3 - 16.7	xx
2	21.7 - 23.3	x
3	26.7 - 28.3	x
4	31.7 - 33.3	x
5	38.3 - 41.7	xx
6	50.0 - 53.3	x
7	56.7 - 60.0	x
8	61.7 - 66.7	xx
9	70.0 - 71.7	x
10	73.3 - 75	x
11	76.7 - 80	xx
12	81.7 - 83.3	x
Metapenaeus brevicornis		
1	13.3 - 16.7	x
2	31.7 - 33.3	x
3	38.3 - 41.7	xx
4	51.7 - 55.0	xx
5	56.7 - 60.0	x
6	63.3 - 66.7	x
7	70.0 - 71.7	x
8	76.7 - 78.3	x
9	81.7 - 83.3	x

Table 22: Summary of muscle myogen patterns of Metapenaeus species based on Fig. 2.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total No.of bands	Common bands
M. affinis	+	+	+	+	+	-	-	+	-	+	+	-	+	+	-	+	+	12	3
M. brevicornis	+	-	-	+	+	-	-	-	+	+	+	-	+	-	-	+	+	9	3
M. kutchensis	+	-	-	-	+	-	+	-	+	+	-	+	-	-	+	-	-	7	3
M. monoceros	+	+	-	-	+	+	-	+	+	+	-	+	-	+	-	-	-	9	3

'+' represents presence of the protein band

'-' represents absence of the protein band

band varied to some extent. Similarly in relative mobility of Bands No.2, 8 and 14 showed the resumblance between species M. affinis and M. monoceros. Band No.12 is common for M. kutchensis and M. monoceros (Plate 4).

Parapenaeopsis species:

As stated above, the species comparison was made according to total number of band, the relative mobility, intensity of staining and width of the electrophoretic bands.

The species specific total number of bands observed in P. sculptilis, P. stylifera and P. hardwickii, was 9, 8 and 10 respectively (Plate 5). The bands which showed common relative mobility in these 3 species were bands No.6, 9 and 14 (Table 24, 25). At the same time band No. 2 and 17 found in P. sculptilis and P. hardwickii also showed similar fractions, P. stylifera and P. hardwickii expressed similar configration in band No. 11 and 16. Thus the muscle protein patterns of these 3 species of prawns indicated species specific differences (Fig. 3).

Penaeus species:

Penaeus merquiensis and P. penicillatus: Muscle myogen protein patterns of prawns belonging Penaeus penicillatus and P. merquiensis were compared, using the

Table 24: Relative mobility (RM) with intensity of muscle myogen proteins of Parapenaeopsis species of prawns.

No.	RM	Intensity
Parapenaeopsis sculptilis		
1	15.0 - 18.3	xx
2	21.7 - 23.3	x
3	26.7 - 28.3	x
4	36.7 - 38.3	x
5	46.7 - 51.7	xx
6	61.7 - 65.0	x
7	76.0 - 73.3	xx
8	83.3 - 86.7	x
9	96.7 - 98.3	x
Parapenaeopsis stylifera		
1	15.0 - 18.3	xx
2	23.3 - 25.0	xx
3	26.7 - 30.0	x
4	38.3 - 40.0	x
5	48.3 - 51.7	x
6	68.3 - 71.7	xx
7	83.3 - 86.7	x
8	91.7 - 95.0	xx
Parapenaeopsis hardwickii		
1	15.0 - 18.3	xx
2	20.0 - 21.7	x
3	28.3 - 30.0	x
4	46.7 - 50.0	x
5	68.3 - 71.7	x
6	75.0 - 78.3	xx
7	83.3 - 86.7	x
8	88.3	x
9	91.7 - 93.3	x
10	95.0 - 98.3	x

Table 25: Summary of muscle myogen patterns of Parapenaeopsis species based on Fig.No.3.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total No. of bands	Common bands
P. sculptilis	-	+	-	+	-	+	+	-	+	+	-	+	-	+	-	-	+	9	3
P. stylifera	+	-	-	-	+	+	-	+	+	-	+	-	-	+	-	+	-	8	3
P. hardwickii	-	+	+	-	-	+	-	-	+	-	+	-	+	+	+	+	+	10	3

'+'represents presence of the protein band

'-'represents absence of the protein band

Plate 4: Muscle myogen patterns of four *Metapenaeus* species. a) *M. affinis*, b) *M. brevicornis*, c) *M. kutchensis* and d) *M. monoceros*

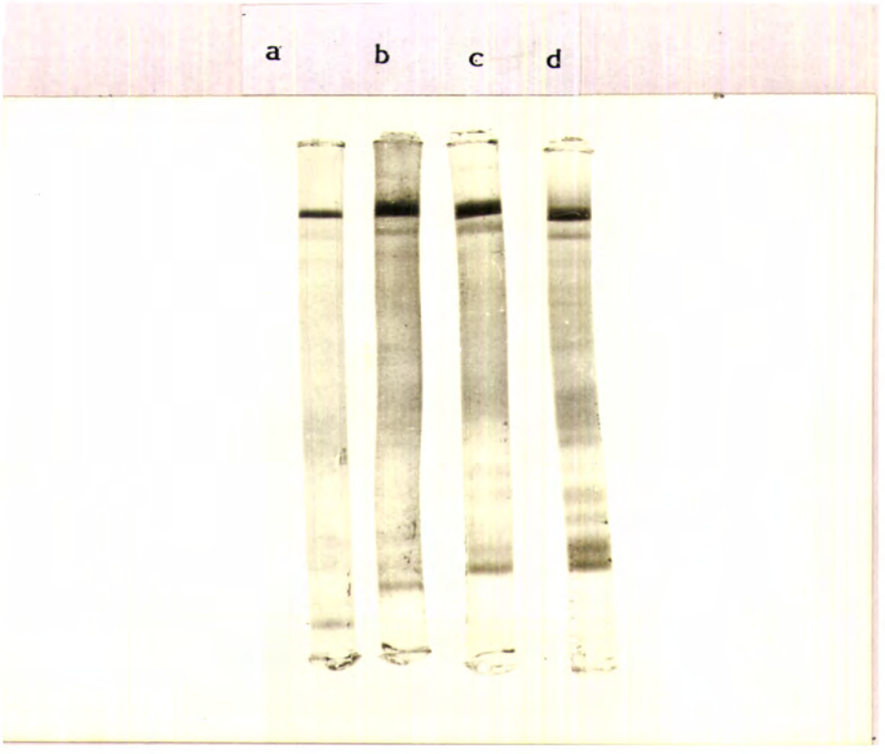
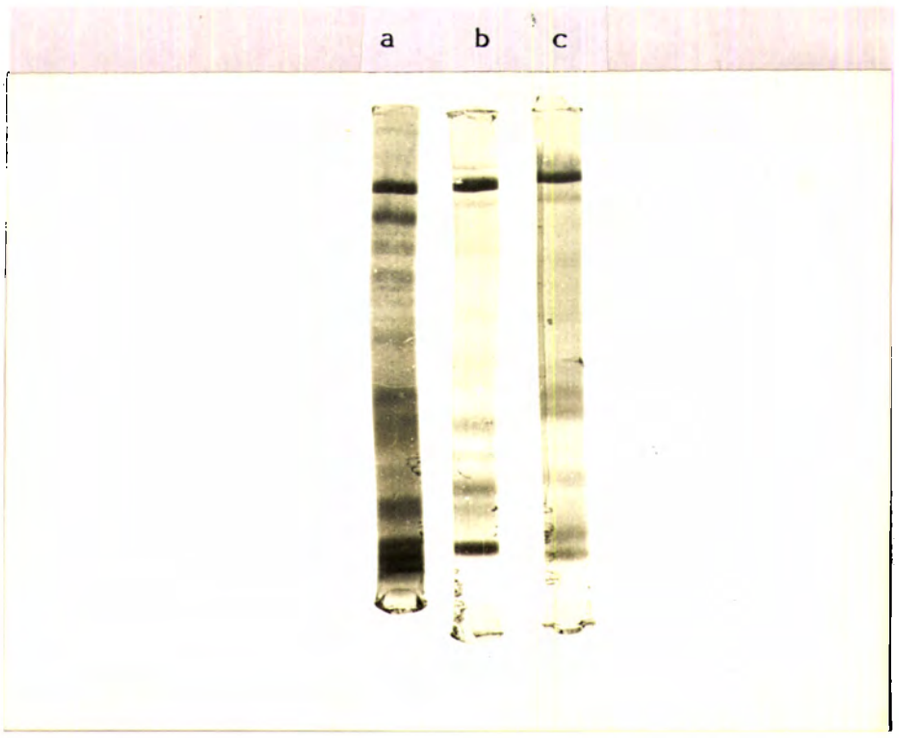

a
b
c
d

Plate 5: Muscle myogen pattern of three *Parapenaeopsis* species. a) *P. sculptilis*, b) *P. stylifera*, c) *P. hardwickii*.


a
b
c

gels photographed and scanned in ultra scanner. The electrophoretic fractions obtained were assigned numbers keeping in mind, the number of crests found to correspond to the number of distinct proteins and the areas under the crests were proportional to their concentrations.

For comparative studies of species, total number of protein bands were taken into consideration. P. penicillatu showed 13 muscle protein fractions whereas P. merquiensis showed only 9 protein fractions (Fig. 4). They both shared 8 common bands. The common fractions are No.1, 2, 3, 5, 6, 8, 9 and 14 (Fig. 4) P. penicillatus is found to have 4 additional bands namely the fraction Nos. 10, 11, 12 and 13, whereas in P. merquiensis fraction Nos. 10, 11, 12 & 13 were absent. Fraction No.4 present in P. penicillatus is absent in P. merquiensis. At the same time fraction No. 7 which is present in the later is absent in the former specie (Table 27).

According to the width, the bands may be divided into 3 types (a) thicker fractions (b) thinner fractions and (c) smaller fractions. Both species show 5 common thicker fractions which are 1, 2, 3, 5 and 14. When the smaller bands are compared P. penicillatus showed 2 fractions. (Band No. 6 & 8) whereas P. merquiensis showed 3 fractions

Table 27: Summary of muscle myogen patterns of *Penaeus penicillatus* and *P. merguiensis* based on Fig. 4.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Total No. of bands	No. of common bands	Deleted band	Additional bands
P. penicillatus	+ a	+ a	+ a	+ b	+ a	+ c	-	+ c	+ b	+ b	+ b	+ b	+ b	+ a	13	8	0	4
P. merguiensis	+ a	+ a	+ a	-	+ a	+ c	+ c	+ c	+ b	-	-	-	-	+ a	9	8	4	0

a-thicker fractions

b-thinner fractions

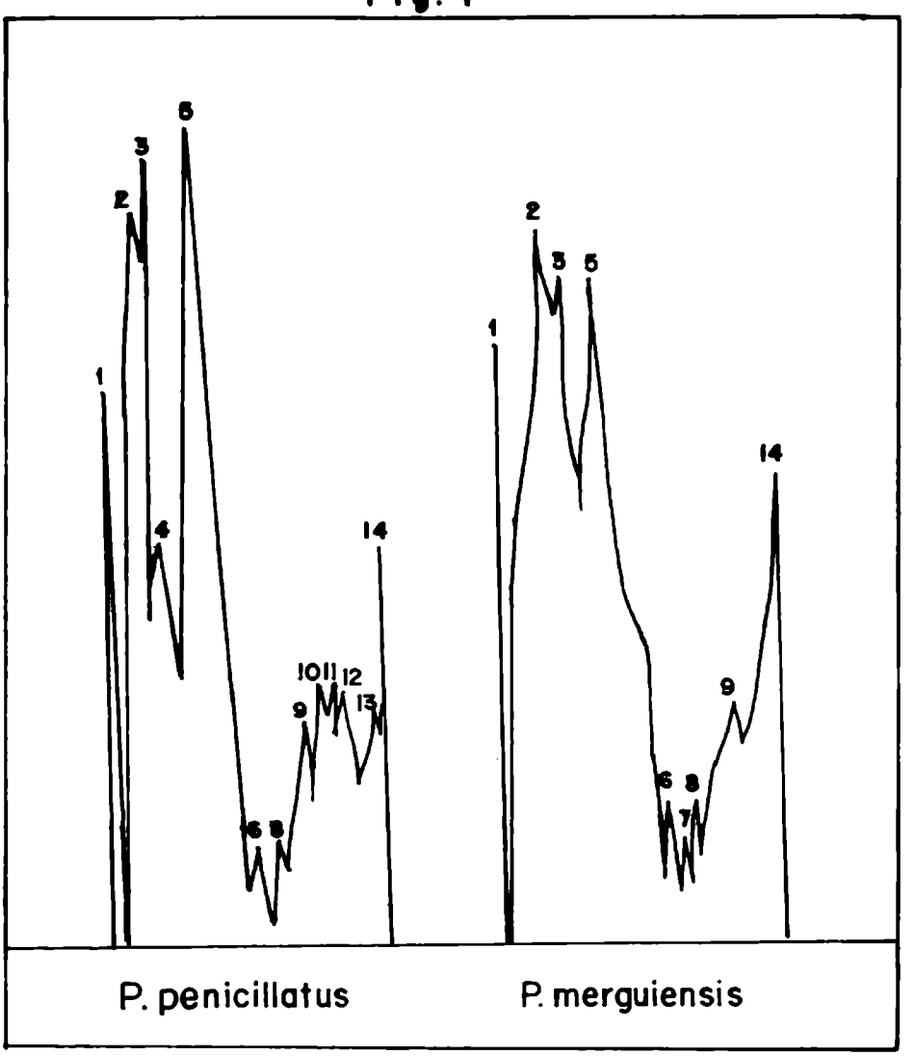
c-smaller fractions

'+' represents presence of the protein band

'-' represents absence of the protein band

Fig. 4. Comparative scanned pattern of abdominal muscle tissues of *Penaeus penicillatus* and *Penaeus merguiensis*.

Fig. 4



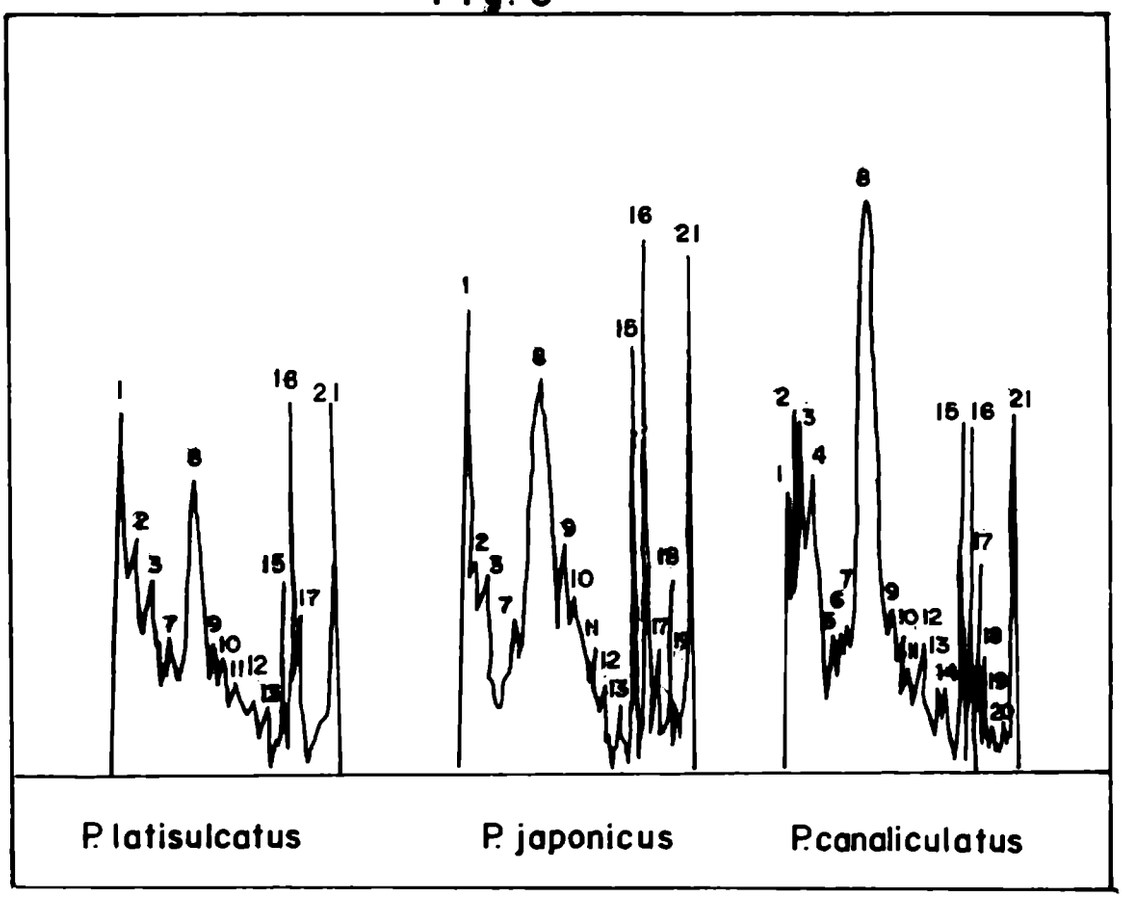
(Band No. 6, 7 and 8). When the second type of thinner bands are compared P. penicillatus showed band Nos. 4, 9, 10, 11, 12 and 13 and P. merguiensis showed band No. 9.

Penaeus latisulcatus, P. japonicus and P. canaliculatus: Muscle myogen proteins of closely related penaeus species of prawns like Penaeus latisulcatus, P. japonicus and P. canaliculatus were electrophoretically separated and thus biochemically distinguished from each other. Scanned pattern of this muscle myogen proteins is given in Fig. No. 5. As explained in P. merguiensis and P. penicillatus the bands were numbered according to the crests formed been which represent the concentration of protein bands separated on the gel.

Analysis of electrophorogram revealed total of 14 bands in P. latisulcatus, 16 bands in P. japonicus, and 21 bands in P. canaliculatus. Fourteen common bands (Band No. 1-3, 7-13, 15-17 and 21) were observed in these three species of penaeids. All common bands were seen in P. canaliculatus whereas 5 bands (Band No.4-6, 14 & 20) were absent in P. japonicus and 7 bands (bands No.4-6, 14, 18-20) were absent in P. latisulcatus. Thus P. japonicus and P. latisulcatus showed their distinctive distinguishing characters by the absence of the above mentioned bands.

Fig. 5. Comparative scanned pattern of abdominal muscle tissues of Penaeus latisulcatus, Penaeus japonicus and Penaeus canaliculatus.

Fig. 5



Scanned pattern observed can be divided into 3 groups. Band No.1-4 forms first group, Band No.5-14 forms second group and Band No.15-21 forms third group. When the comparison was made within first group of bands, band No.1-3 is seen in all the 3 species of prawns but Band No.4 is observed only in P. canaliculatus, P. japonicus and P. latisulcatus are found to be devoid of Band No.4. When group II type of bands were analysed all the 10 bands were present in P. canaliculatus contradictory to this Band No.5, 6 and 14 were absent in P. latisulcatus and P. japonicus. Thus group II showed only 8 bands in P. latisulcatus and P. japonicus. All the Group III Bands were present in P. canaliculatus but band Nos. 18-20 and band No. 20 were absent in P. latisulcatus and P. japonicus. According to the width of the band the scanned pattern is divided into 3 types. Thicker bands, thinner bands and smaller bands thus P. latisulcatus has one thicker band (band No.8) 4 thinner bands (band Nos.1, 15, 16, 17 and 21) and 8 smaller bands (Band Nos. 2, 3, 7 9-13) P. japonicus showed 2 thicker bands (band No.1 & 8), 4 thinner bands (15, 16, 18 and 21) a 10 smaller bands (band No.2, 3, 7, 9-13 17 & 19). P. canaliculatus expressed one thicker band (band No. 8), 9 thinner bands and 11 smaller bands (band Nos. 5-7, 9-14, 19-20). In this way all these three species which have morpl logy expressed species specific differences in their protein patterns. (Table 29)

Table 29: Summary of muscle myogen pattern of *Penaeus latisulcatus*, *P. japonicus* and *P. canaliculatus* based on Fig. 5.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	Total No. of bands	Number of common bands	Delected bands	Additional bands.
P. latisulcatus	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	14	14	7	0
P. japonicus	+	+	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	16	14	5	5
P. canaliculatus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	21	14	0	7

'+' represents presence of the protein band

'-' represents the absence of the protein band.

Discussion:

Accurate identification of organism at species level is a pre-requisite for the progress of scientific research in any field of biological science. Study of natural differences in the morphometrics and meristics has always been a popular traditional method for establishing species identity. Nevertheless, overlapping nature of morphological and meristic characteristics qualified among the animals to be identified may cause practical difficulties even for the expert taxonomist. Such inherent taxonomic problems cannot be easily solved by morphological comparisons alone (Wright 1966). A certain amount of innate plasticity of morphometrics and meristic characters present in fish has caused difficult taxonomic problems (Wilkins 1967). Hence, biologists began to introduce variety of modern experimental techniques developed in their period of research, particularly, in the field of medical sciences for improving the method of species identification.

Thus muscle protein of fishes were compared using classical Tiselius Technique of electrophoresis (Connel 1953, a, b Dingle et al., 1955), paper electrophoresis (Hewitt et al., 1963) and agar gel electrophoresis (Rabaey 1964). Buzzati-Traverso and Rechnitzer (1953) introduced

the use of chromatographic techniques in taxonomic studies. High resolution starch gel electrophoresis method established by Smithies (1955) found immediate application in various fields of biological research including fisheries. The efficiency of gel electrophoresis to separate and resolve biochemical properties of an individual or experimental animal at molecular level proved of immense help in solving even inherent taxonomic problems. The relationships of DNA molecule with structural proteins as explained by Crick (1963) and Nirenberg et al.,(1963) enabled the biologist to interpret the electrophoretically separated protein molecules in terms of genetics of the experimental animal, bringing taxonomy to most natural level. The taxonomists could label individual inherent differences at species and higher level of classification and coroborate and verify the traditional method of species differentiation. Thus the problem of taxonomic status of North Atlantic Sebastes fish was solved using agar gel electrophoresis (Altukhov and Nefyodov 1968). There are examples of unrecognized species being detected through the use of electrophoretic and biochemical techniques as in fish (Sage and Selander, 1975) and Snails, (Woodruff, 1978). Lester (1980) demonstrated biochemical genetic differences among P. aztecus, P. duorarum and P. setiferus.

Muscle is an important body tissue of all animals. It is commonly used for electrophoretic and biochemical investigations. Its protein is known as myogen. For comparative studies muscle myogen protein patterns was widely employed on fishes.

As the crustacean group of organism, particularly, prawns possess many overlapping and similar body characters, their acurate identification at species and even in generic level is difficult. Due to lack of easily observable species specific characteristics, species status is often subject to changes as seen in the case of P. subtilis and P. notialis each having related subspecies in Gulf of Mexico (Perez Farfante 1978). The Indian prawns M. kutchensis has a close resemblance with M. monoceros and M. affinis, particularly males (Table 20). Confusing taxonomic status of M. necopinans and M. mutatus was caused as they were probably synonymous of M. affinis (George 1979).

Though electrophoresis is a powerful analytical tool for solving problems of species identification, information on its application in crustacean group of organisms are limited. Lim and Lee (1970) separated muscle proteins of Metapenaeus mutatus, Parapenaeopsis hungerfordi, P. hardwickii, Metapenaeopsis stridulans, M. barbata, Penaeus

Table 20: Morphological variation between *M. kutchensis*, *M. monoceros*, *M. affinis* and *M. brevicornsis*

M. kutchensis	*M. monoceros*	*M. affinis*	*M. bravicornsis*
No expod on 5th perepod; pleurobranch on 7th thoracic somite present.	No expod on 5th perepod; pleurobranch on 7th thoracic somite present.	No expod on 5th perepod; pleurobranch on 7th thoracic somite present.	No expod on 5th perepod; pleurobranch on 7th thoracic somite present.
Ischial spine on 1st pereopod distinct.	Ischial spine on 1st pereopod distinct.	Ischial spine on 1st pereopod distinct.	Ischial spine on 1st pereopod small or absent.
Posterior extension of the anterior median thelycal plate not bound laterally by oval plate on either side; distomedian petasmal projections not overlying lateral projections.	Lateral thelycal plates with salient and parallel earshaped lateral ridges; distomedian petasmal projections hood-like.	Anterior thelycal plate longitudinally grooved, wider posteriorly than anteriorly, distomedian petasmal projections cresent-shaped.	Posterior part of rostrum with distinctly elevated crest; basial spine on male 3rd pereopod simple, apical petasmal filaments slender, slightly converging thelycum with large anterior and small lateral plates.

Source George (1979).

monodon, P. semisulcatus, Metapenaeus ensis and Parapenaeopsi affinis using cellulose acetate electrophoresis.

Later very high resolution giving polyacrylamide disc gel electrophoresis introduced by Davis (1964) was employed for separating tissue proteins for species identification of juveniles of Penaeus indicus and P. monodon (Sriraman and Reddy 1977), Metapenaeus affinis, M. monoceros, Parapenaeopsi hardwickii and P. stylifera in relation to sex (Kulkarni et a 1980) P. indicus, Metapenaeus dobsoni, M. monoceros and M. affinis (Thomas 1981) and P. monodon (Prathibha 1984).

The aim and objective of the present investigation was to discover natural and reliable species specific characteristics of selected species of Indian prawns like Parapenaeopsis stylifera, P. sculptilis, P. hardwickii, M. kutchensis, M. monoceros, M. affinis and M. brevicornis using polyacrylamide gel electrophoresis. All these species tested here can be distinguished easily on the basis of differences in the total number of muscle protein bands, their electrophoretic mobility and even staining intensity (Fig. No.2 & 3).

Metapenaeus species:

The present study has given 9 bands in M. monoceros, 12 in M. affinis, 9 in M. brevincornis and 7 bands in

M. kutchensis, from a location in Bombay on the north west coast of India showing a species specific nature in the number of bands. Total number of bands observed in different species of Metapenaeus by previous authors is 8 bands in M. dobsoni 11 fractions in M. affinis and 7 bands in M. monoceros by Thomas (1981), 8 bands each in M. mutatus, M. stridulans, M. barbata and M. ensis by Lim and Lee(1970), The latter study does not show any species specificity in the number of bands, all the species studied in the same genus showing similar bands in relation to number. However the study of Thomas (1981) do show difference in number of bands between M. monoceros, M. affinis and M. dobsoni, being 7, 11 and 8 bands in the three species respectively. However the present result of 9 bands in M. monoceros does not seem to agree with the observation of 7 bands by Thomas (1981). In M. monoceros also there is a difference of 1 band. The reason which could be attributed to this difference in the number of bands in the same species appears to be either geographic variation, the location of specimens collected being wide apart of the methodology applied in the finer analysis and standardisation. In the case of M. brevicornis which is preserved in 2% phenoxy ethanol showed 7 bands (Lim and Lee 1970) in cellulose acetate gel whereas M. brevicornis tested here showed 9 bands in acrylamide gel

which is known to give better resolution than cellulose acetate gel. Geographical differences in the species tested here may also account for the variation in the total number of band.

Since the specimens are collected in the immature gonad stage there is no difference observed in the male and female specimens analysed. Difference in the male and female sex is shown by Lim and Lee (1970) in the case of M. mutatus with 8 bands in female and 7 bands in male and M. ensis male with 8 bands female with 7 bands but M. brevicornis and M. stridulans which showed 7 and 8 band in both male and female specimens.

According to the relative mobility the comparison between Metapenaeus mutatus, M. stridulans and M. barbata. showed 3 common bands (Lim and Lee 1970) that shows probable generic relationship. M. affinis, M. brevicornis, M. kutchens and M. monoceros expressed 3 common bands (Band Nos. 1, 5 & 1 indicating their probable common generic relationship and the characteristic feature for the identification of this genus.

The comparison made between bands No.2, 8 & 14 showed the relationship between M. affinis and M.monoceros Band No. 4, 11, 13, 16 & 17 showed the similarly of protein pattern

seen between M. affinis and M. brevicornis Band No. 9 showed the similar mobility existed betweem M. brevicornis, M. kutchensis and M. monoceros. Band No. 12 expresses similarity between M. kutchensis and M. monoceros (Fig.2).

In this way above differences and similarities expressed by protein fractions can be applied for the biochemical identification of these species besides their morphological identification. Their species specific nature can also be utilized as a tool for the identification of these species and also to distinguish among themselves.

Parapenaeopsis species:

Earlier workers has pointed out the morphological characteristics of the species like P. hardwickii, P. stylifera and P. sculptilis and detail. (Rao 1970, George 1975, Fischer and Bianchi 1984) (Table 23).

To find out additional plausible evidence by means of biochemical analysis to reveal the species specific and distinguishing characters between these three species, electrophoretic studies on muscle myogen patterns were analysed. Biochemical systematics of this genus was carried out already in P. hungerfordi, P. hardwickii (Lim and Lee 1970), P. hardwickii and P. stylifera (Kulkarni et al., 1980).

Table 23: Morphological variation between *P. hardwickii*, *P. sculptilis*, and *P. stylifera*.

P. hardwickii	*P. sculptilis*	*P. stylifera*
3rd pereopod without epipodite.	3rd pereopod without epipodite.	3rd pereopod without epipodite.
Antenular flagella 0.7 length of carapace or longer; movable lateral spines present on telson.	Antennular flagella 0.5-0.6 length of carapace movable lateral spines absent on telson.	--
Petasma with pair of short spout-like distolateral projections and pair of cup like distal projections.	Distomedian projections of petasma large and flare out laterally anterior thelycal plate separated from the posterior sternal plate by a short intervening space.	Petasma long with distolateral projections divergent; appendix maxulina with distolateral projection.

Source: George (1979)

In the present study P. stylifera, P. hardwickii and P. sculptilis showed 8, 10, and 9 bands. Besides the total number of bands these three species vary by the electrophoretic mobility, staining and the width of the bands.

P. hardwickii analysed by Lim and Lee (1970) showed 8 bands and the present study showed 10 bands. This may be due to the usuage of polyacrylamide gel in the present study whic is superior to cellulose acetate employed by former and also probable geographical variation in the species.

Common bands seen in Parapenaeopsis genus by the present study showed their generic similarity, P. hungerfordi and P. hardwickii (Lim and Lee 1970) expressed 7 common bands whereas in the present study P. sculptilis, P. stylifera and P. hardwickii expressed only 3 common bands in band No. 6, 9 & 14. The wide differences observed in these above mentioned studies may be due to the better separation using polyacrylamide gel and the geographical variation expressed within thes species. This also reveals the greater differences within these species occurring in these area. Band number 2 and 17 found to have same relative mobility between the species P. sculptilis and P. hardwickii and band No. 11 and 16 expressed closeness between P. stylifera and P. hardwickii. With these electrophorograms patterns observed it is very ea

to distinguish these three species. The patterns observed also is species specific and the specific differences and the closeness between these three species were clearly seen.

Penaeus species:

P. penicillatus and P. merguiensis

Morphologically P. penicillatus and P. merguiensis are very closely allied, the only important difference being in the length of the dactyl of 3rd maxilliped of adult males. Thus it is very difficult to distinguish the two species when they are smaller in size. Therefore these two species were selected to study their muscle protein variation in order to use it as a taxonomic tool for identifying the 2 species.

Earlier workers used densitometric reading for the analysis of isoenzyme patterns of Anodonta grandis (Saleuddin 1969) serum patterns of flat fishes (Maria 1979) and muscle proteins of five Cyprinids (Haen and O'Rourke 1969b). Likewise here also the gels were scanned and the results were interpreted.

Polyacrylamide gel which gave good resolution was used as medium here for separation of proteins as reported in the species identification of P. indicus, P. monodon

(Sriraman and Reddy 1977), P. indicus (Thomas, 1981) and P. monodon (Prathibha 1984).

Differences in the total number of muscle protein bands namely 10 and 11 in P.indicus and P. monodon respectively (Sriraman and Reddy 1977) 8 bands in P. indicus (Thomas 1981) 7 bands in P. monodon, 9 bands in P. semisulcatus (Lim and Lee 1970) and 16 bands in P. monodon (Prathibha 1984) demonstrated species specific pattern of muscle proteins (Table 26).

Lim and Lee (1970) reported the presence of five bands of common electrophoretic mobility between P.semisulcatus and P. monodon as indicative of their close relationship at generic level. The present observation of eight common bands between P. penicillatus and P. merquiensis may also suggest greater generic relation between these two species studied here whereas four bands 10, 11, 12 and 13 present only in P. penecillatus demonstrates the species specific differences of these same two species (Fig. 4).

Lim and Lee (1970) reported only 7 muscle protein bands in P. merquiensis whereas 9 bands were obtained in the present study. This significant difference in the total number of bands as revealed in the above comparison may be due to slight difference in methodology adopted in

Table 26: Details of Muscle myogen patterns observed in different species of prawns.

Species	Total number of bands
P. sculptilis*	9
P. stylifera*	8
P. hardwickii*	10
M. kutchensis*	7
M. monoceros	9
M. affinis*	12
M. brevicornis*	9
P. indicus**	10
P. monodon**	11
P. monodon***	16

* Present study

** Sriraman and Reddy 1977

*** Prathibha 1984

the respective studies. The muscle tissue tested by Lim and Lee (1970) was preserved in 2% phenoxy-ethanol whereas the muscle in the present study was taken and tested from a fresh specimen. The effect of different geographical regions of the species may also account for the observed differences in the total number of proteins.

The important difference in the total number of muscle protein fractions detected between P. penicillatus and P. merquiensis in the present study clearly indicated the taxonomic identity of these two species, the number of fractions being 13 and 9 respectively.

The significant species specific muscle protein pattern differences between P. penicillatus and P. merquiens revealed in the present study proves the efficiency of electrophoretic techniques in solving the problems of species identity of morphologically very similar species of prawns.

P. latisulcatus, P. canaliculatus and P. japonicus

Morphologically P. latisulcatus, P. canaliculatus and P. japonicus closely resumable each other. Important morphological differences observed are given in table No.28. Because of these overlapping characters, ambiguous species

Table 28: Morphological variation between P. latisulcatus P. japonicus and P. canaliculatus.

P. latisulcatus	P. japonicus	P. canaliculatus
1. Telson armed usually with 3 pairs of spinules.	1. Telson armed usually with 3 pairs of spinules.	1. Telson unarmed.
2. Adrostral sulcus as wide as postrostral carina.	2. Adrostral sulcus narrower than post-rostral carina.	--
3. Anterior plate of thelycum bifid at the apex.	3. Anterior plate of thelycum rounded at the apex.	--

Source: George (1979)

nature exists during their developmental stage. Thus these species were selected for discovering possible biochemical genetic differences which may exist in their muscle proteins.

Scanned patterns observed in these species also showed 14 common bands (Band No.1-3, 7-13, 15-17 and 21) expressing close ancestral relationship of P. latisulcatus, P. canaliculatus and P. japonicus. Present study revealed a total of 14 bands for P. latisulcatus due to the deletion of bands Nos. 4, 5, 6, 14, 16, 18, 19 & 20 from P.canaliculatus Deletion of the band Nos. 4, 5, 6 14 & 20 when compared with P. japonicus showed its biochemical difference from P. japonicus.

Intra species variation studies on P. latisulcatus was carried out by Richardson (1982b) Mulley and Latter 1980 used P. latisulcatus to find out the evolutionary relationships within a group of thirteen species of Penaeid prawns.and De Matthaeis et al., (1983) worked on the genetic difference between P. japonicus and P. kerathurus.

Characteristic species specific patterns observed using muscle myogen patterns can be used to solve the species identity in addition to the morphological characters.

The individual differences detected here are indicative of species specific nature of muscle myogen electrophoretic

fractions as established and reported in several other species of prawns (Table 30). These informations can now form a strong basis for understanding of these species at biochemical genetic level and further help in any hybridization and genic manipulation studies desirable for scientific management of these valuable cultivable resources.

Table 30: Groupwise comparison of muscle myogen patterns in different Penaeid prawns.

P. indicus*	4	3	3	10
P. monodon*	3	4	4	11
P. monodon**	8	5	3	16
P. penicillatus***	5	6	2	13
P. merquiensis***	5	1	3	9
P. latisulcatus	1	5	8	14
P. japonicus	2	4	10	16
P. canaliculatus	1	9	11	21

* Sriraman & Reddy 1977(According to electrophorogram)

** Prathibha 1984 (According to electrophoregrams)

*** Present study (According to scanning)

CHAPTER V

ONTOGENETIC VARIATION

Resume of literature

Development is the process by which a single fertilized cell becomes a complex organism. Ontological development leads to morphological changes in different organisms due to corresponding variation in the metabolic pathways and gene regulation patterns during the process of their growth. Isoenzymes and other protein types being specific gene products are efficient markers of cell types (Rider 1980). Hence these protein types can be used as control to assess the changes which occur during cellular protein enhancement during developmental changes. Thus observed differences in the isoenzyme activity can be related to developmental changes in isoenzyme synthesis. These changes can also be identified qualitatively by using electrophoretic separation of isoenzyme.

Artemia was intensively studied to understand the specific developmental changes occurring in them.(Hentschel and Tata 1976, Bagshaw and Warner 1979 and Clegg and Conte 1980). Ontogenetic changes were studied using isoenzymes patterns of Homarus americanus (Hedgecock

et al,1975) and in crabs (Gooch 1977, Morgan et al,1978 and Kannupandi 1980).

Lester (1980) using electrophoresis pattern of isoenzyms identified juvenile shrimps of different geographical areas. Different species of prawns has their own characteristic pattern of development.

In Penaeus species Lester and Cook (1987)compared gene expression in different developmental stages of four species of prawns viz; P. aztecus, P. setiferus, P. stylifrostris and P. Vannamei). Rizzotti et al,(1977) and Zoarces (Hjorth 1974) showed tremendous ontogenetic changes in haemoglobin of elver and adult stages of Anquilla anquilla.

Lactate dehydrogenase isoenzyme during ontogeny is visualized in two salmonids Salmo salar and S. trutta, (Kunz 1975) Palaemon serratus, (Thebault and Bernicard 1978) in Coho Salmon, (Marquez 1978) and in Liza parsia (Parag 1984) The same isoenzyme pattern in heart tissue expressed a change from predominantly LDH-5 to a predominant end of ieozyme LDH-1 during the development of an embryo of 9 days before birth to the adult mouse (Market 1983).

Alkaline phosphatase isoenzyme noticed in Drosophila melanogaster, showed changes, during its developmental stages like young larva, instar larva, pupa and adult (Beckman and Johnson 1964). Again Morgan et al,(1978) in Xanthid crab, Mary (1985) in Mugil cephalus and Lester and Cook (1987) in penaeid prawns showed the ontological changes in this enzyme.

Esterase isoenzyme pattern (Paul and Fottrell 1961) found in foetal human tissue resemble those of adults but Blanco and Zinkham (1966) reported an increase in both the number and activity of isozymes during development. Hunter et al,(1964) described enzyme changes in development of liver and kidney in the foetal and weanling mouse.

Esterase patterns of various Cavian tissues (Holmes and Masters 1967) show marked changes in the liver, kidney, and intestine.

Various other workers like Flowerdew (1976) in the Cirripede Balanus balanoides Gooch (1977) in crabs, Morgan et al,(1978) and Kannupandi (1980) in Xanthid Crabs and Lester and Cook (1987) in prawns of Penaeus species had worked on esterase to show their changes during development.

General protein patterns during ontogenetic development expressed gradual changes in Salmon (Nyman, 1967 Battacharya and Alfred, 1982) and in mullet (Herzberg and Pasteur 1975). Sriraman and Reddy (1977) showed changes in protein patterns during development of planktonic juveniles and adults of P. indicus and P. monodon Kannupandi (1980) in Xanthid crab, Prathibha (1984) in P. monodon and Lester and Cook(1987) in prawns P. aztecus, P. setiferus, P. stylirostris and P. vannamei.

Malate dehydrogenase enzyme changes during different stages of development is shown in crabs by Gooch (1977) and Morgan et al., (1978) and in plants (Rider 1980).

Aldehyde oxidase enzyme showed ontogenetic variation in penaeid spp. of prawns (Lester and Cook 1987).

Alcohol dehydrogenase showed ontogenetic changes which was correlated with liver maturation in the Brachydanio nigrofaciatus (Frankel 1981).

To find out the distinct biochemical variations taking place in the larval stages in the development of prawn, these were analysed using electrophoresis to get additional in sight for the larval identification of some species (Lester 1980) and to find out the changes taking place during its ontogenetic development.

The present work was aimed at finding out the isoenzyme expression and its specificity in different stages of development of P. indicus. It would help to attain characteristic band pattern in different stages due to the changes in the gene expression for the identification of larvae of P. indicus from other larvae.

There exist an inverse relationship between larval dispersal and the extent of population genetic differentiation (Gooch et al.,1972). To find out the population structure in marine environment nowadays efforts are focussed on species which have planktonic development stage with larval dispersal capacity (Burton 1983).

As these studies are very limited with reference to Indian prawns, the present investigation was undertaken to reveal probable ontological changes in the isoenzyme patterns of the prawn P. indicus.

Results:

Separation of isoenzymes using polyacrylamide gel electrophoresis revealed different enzyme patterns in different larval stages in the white prawn P. indicus collected from Narakkal Prawn Culture Laboratory, Narakkal. Various enzymatic and general proteins tested here showed stage specific protein patterns. Isoenzymes of acid phosphatase and aldehyde oxidase were studied in Protozoea, mysis and post larval stages, while, alcohol dehydrogenase, malate dehydrogenase, octanol dehydrogenase, esterase, and general protein patterns were analysed in protozoea, post-larva, different size groups of juveniles namely 20-30 mm, 40-50 mm, 70-80 mm and adults (90-120 mm).

Isoenzyme patterns detected in each of the above mentioned enzymes of different stages are given in Fig. Nos. 6-12. Exact position of all the bands obtained were drawn according to their relative mobility value. Bands were given serial numbers starting from the cathodal end towards the anodal end and in the order of increasing electrophoretic mobility.

Acid phosphatase:

Acid phosphatase isoenzyme patterns were detected in different stages of the life cycle of Penaeus indicus namely

protozoea, mysis, postlarvae, three different size range of juveniles namely 20-30 mm. 40-50 mm, 70-80 mm and adult (90-120 mm) (Plate 6).

Band Nos. 7 and 8 are present only in protozoea stage. Band No.6 is absent in protozoea and mysis and appears in post-larva, in all juvenile and adult stages. Band No.5 appears in juvenile I, II and III stages and disappears in other stages. Though Band No.4 is present in protozoea, mysis and post-larva stages, it is absent in all juvenile and adult stages. Band No.3 is found only in post-larva and juvenile stage I. Band No.2 is found to be expressed only in protozoea and mysis stages. Band No.1 is exhibited only in mysis stage. Band Nos. 1, 2, 4, 7 & 8 are expressed in larval stages only, whereas Band No.5 and 6 are found mainly in juvenile and adult stages (Fig. No.6). Thus the Isoenzymes of Acid phosphatase are found to be expressed in different electrophoretic mobility and in different numbers, indicating a stage-specific pattern for the enzyme (Table 31 & 32).

Aldehyde oxidase:

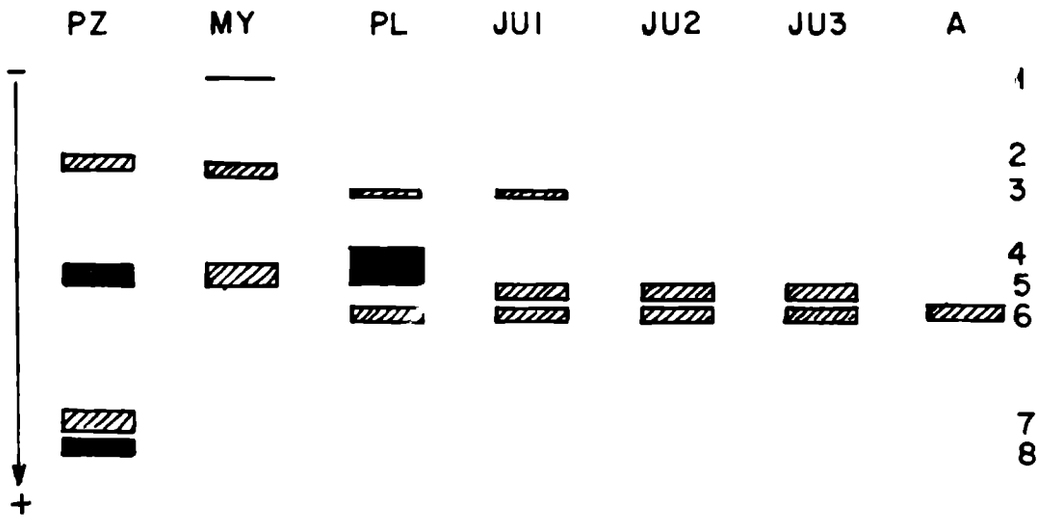
Aldehyde oxidase Isoenzyme patterns of different stages of Penaeus indicus mentioned earlier were found out (Fig. No.7) Protozoea stage had 4 distinct bands (Band No.3, 6, 8 & 9). Mysis showed 2 bands (Band No. 4 & 5), post-larva

Table 31: Relative mobility (RM) with intensity of Acid phosphatase bands separated in Penaeus indicus.

Stage	Band Nos.	RM Value	Intensity
Protozoea	2	11.7 - 13.3	x
	4	26.7 - 30.0	xx
	7	46.7 - 50.0	x
	8	51.7 - 53.3	xx
Mysis	1	1.7 - 3.3	x
	2	13.3 - 15.0	x
	4	26.7 - 30.0	xx
Postlarvae	3	16.7 - 18.3	x
	4	25.0 - 30.0	xx
	6	33.3 - 35.0	x
20-30mm (Juvenile)	3	15.7 - 18.3	x
	5	30.0 - 31.7	x
	6	33.3 - 35.0	x
10-50mm(Juvenile)	5	30.0 - 31.7	x
	6	33.3 - 35.0	x
70-80mm(Juvenile)	5	30.0 - 31.7	xx
	6	33.3 - 35.0	xx
90-120mm(Adult)	6	33.3 - 35.0	x

Fig. 6. Ontogenetic variation of acid phosphatase enzyme in *Penaeus indicus*.

Fig. 6



PZ - Protozoea, MY - Mysis, PL - Post larva

JU1 - 20 — 30mm, JU2 - 40 — 50mm, JU3 - 70 — 80mm

A - 90 — 120mm

Table 32: Summary of acid phosphatase patterns of Penaeus indicus based on Fig. 6.

Stages	1	2	3	4	5	6	7	8	Total No. of bands
Protozoea	-	+	-	++	-	-	+	(++)	4
Mysis	+	+	-	+	-	-	-	-	3
Post-larvae	-	-	+	(++)	-	+	-	-	3
20-30mm(Juvenile)	-	-	+	-	+	+	-	-	3
40-50mm(")	-	-	-	-	+	+	-	-	2
70-80mm(")	-	-	-	-	+	+	-	-	2
90-120mm(Adult)	-	-	-	-	-	+	-	-	1

'+' represent the presence of the Isozyme

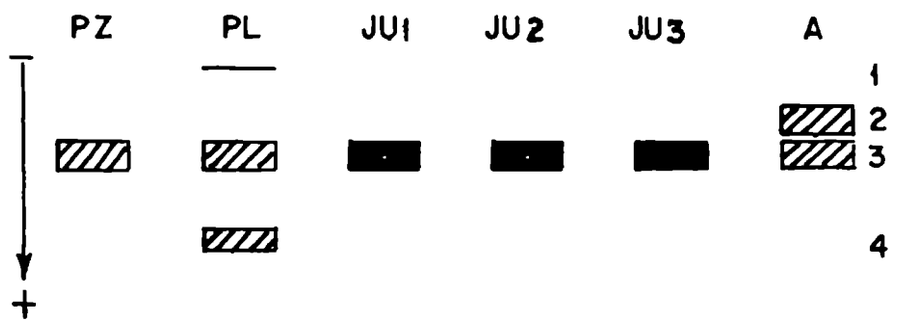
'-' represent the absence of the Isozyme

Table 35: Relative Mobility (RM) with intensity of Alcohol dehydrogenase bands separated in *Penaeus indicus*.

Stage	Band Number	RM	Intensity
Protozoea	3	13.3 - 16.7	x
Postlarva	1	3.3	x
	3	13.3 - 16.7	x
	4	25.0 - 28.3	x
20-30mm(Juvenile)	3	13.3 - 16.7	xx
40-50mm(")	3	13.3 - 16.7	xx
70-80mm(")	3	13.3 - 16.7	xx
90-120mm(Adult)	2	8.3 - 11.7	x
	3	13.3 - 16.7	x

Fig. 8. Ontogenetic variation of alcohol dehydrogenase enzyme in *Penaeus indicus*

Fig.8



PZ - Protozoea , PL - Post larva

JU1 - 20 − 30 mm , JU2 - 40 − 50 mm

JU3 - 70 - 80mm , A - 90 — 120 mm

Table 36: Summary of Alcohol dehydrogenase patterns of Penaeus indicus based on Fig. 8.

Stages	1	2	3	4	Total No. of bands
Protozoea	-	-	+	-	1
Post larva	+	-	+	+	3
20-30mm(Juveniles)	-	-	++	-	1
40-50mm(")	-	-	++	-	1
70-80mm(")	-	-	++	-	1
90-120mm(Adult)	-	+	+	-	2

'+' represents the presence of isozyme

'-' represents the absence of isozyme

Plate 6: Ontogenetic variation of acid phosphatase enzyme in Penaeus indicus a) Protozoea, b) Mysis, c) Post larva, d),e) juvenile, f) adult.

Plate 7: Ontogenetic variation of alcohol dehydrogenase enzyme in Penaeus indicus a) protozoea, b) post larva, c)-e) juveniles, f) adult


a
b
c
d
e
f


a
b
c
d
e
f

expressed 2 bands (Band No.1 & 2). Juvenile stages had 5 bands in Stage I, (Bands No.1, 2, 3, 5 & 6) 6 bands in Stage II, (Bands No.1, 2, 3, 5, 6 & 7), 4 bands in stage III, (Bands No.3, 5, 6 & 7) and 3 bands in Adult (bands No.3, 5 & 7). (Plate 8).

Bands No. 8 and 9 are found only in protozoea stage Band No.7 is expressed in juvenile II, and III and adult stages. Band No.6 is found in protozoea, juvenile and adult stages. Band No.5 is shown in mysis, in juvenile and adult stages. Band No.4 is seen in mysis stage only. Band No.3 is seen in protozoea, in juvenile and adult stages. Band Nos. 1 & 2 is found in post-larva and in juvenile stages I & II. Patterns obtained in juvenile and adult stages is only from hepatopancreas. Thus all the seven stages tested for aldehyde oxidase showed its stage specific patterns (Table No. 33 & 34).

Alcohol dehydrogenase:

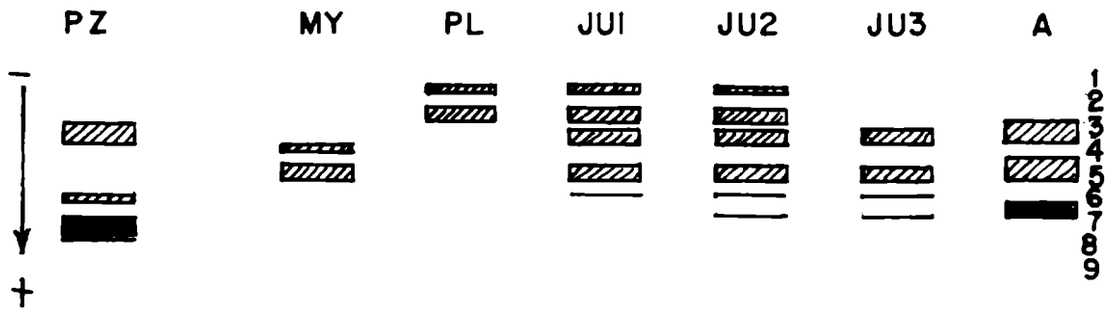
Alcohol dehydrogenase isoenzyme patterns were observed in Protozoea, Post-larva 3 different size groups of juveniles and adults (Fig. 8). Enzymatic activity of this particular enzyme was expressed by one band (Band No.3) in protozoea, 3 bands (Bands No.1, 3 & 4) in post-larva, one Band (Band No.3) in each stages of juveniles (Stage I-III) and 2 bands (Band No.2 & 3) in the adults stage (Plate 7).

Table 33: Relative Mobility with intensity of Aldehyde oxidase bands separated in Penaeus indicus.

Stage	Band	RM	Intensity
Protozoea	3	16.7 - 20.0	x
	6	26.7 - 28.3	x
	8	30.0 - 31.7	xx
	9	33.3	x
Mysis	4	20.0 - 21.7	x
	5	23.3 - 25.0	x
Post-larva	1	11.7 - 13.3	x
	2	15.0 - 16.7	x
20-30mm (Juvenile)	1	11.7 - 13.3	x
	2	15 - 16.7	x
	3	18.3 - 20	x
	5	23.3 - 25	x
	6	26.7	x
40-50mm (Juvenile)	1	11.7 - 13.3	x
	2	15.0 - 16.7	x
	3	18.3 - 20.0	x
	5	23.3 - 25.0	x
	6	26.7	x
	7	30.0	
70-80mm (Juvenile)	3	18.3 - 20.0	x
	5	23.3 - 25.0	x
	6	26.7	x
	7	30.0	x
90-120 mm(adult)	3	17.3 - 20.0	xx
	5	22.3 - 25.0	xx
	7	28.0 - 30.0	x

Fig. 7. Ontogenetic variation of aldehydeoxidase enzyme in *Penaeus indicus*.

Fig. 7



PZ-Protozoea , MY-Mysis , PL-Postlarva, JUI-20–30 mm

JU2–40–50mm, JU3-70–80mm, A-90–120mm

Table 34: Summary of Aldehyde oxidase patterns of Penaeus indicus based on Fig. 7.

Stages	1	2	3	4	5	6	7	8	9	Total
Protozoea	-	-	+	-	-	+	-	(++)	+	4
Mysis	-	-	-	+	+	-	-	-	-	2
Post-larva	+	+	-	-	-	-	-	-	-	2
20-30mm(Juvenile)	+	+	+	-	+	+	-	-	-	5
40-50mm(")	+	+	+	-	+	+	+	-	-	6
70-80mm(")	-	-	+	-	+	+	+	-	-	4
90-120mm(Adult)	-	-	+	-	+	-	+	-	-	3

'+' represents the presence of isozyme

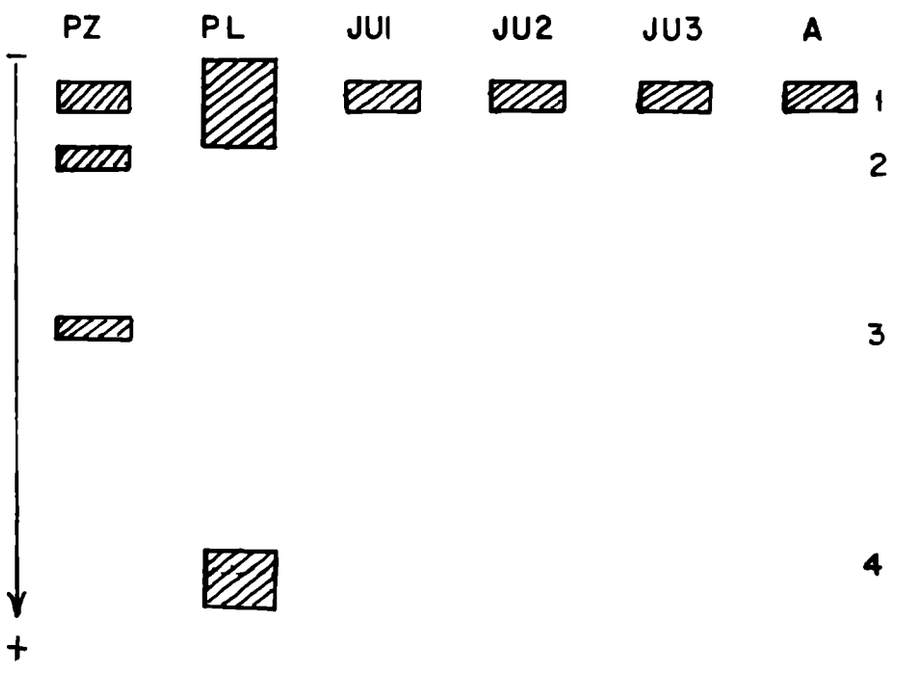
'-' represents the absence of isozyme

Table 37a: Relative mobility (RM) with intensity of Esterase bands separated in Penaeus indicus.

Stage	Band No.	RM	Intensity
Protozoea	1	3.3 - 6.7	x
	2	11.7 - 15.0	x
	3	35.0 - 38.3	x
Post larva	1	0 - 11.7	x
	4	66.7 - 75	x
20-30mm (Juveniles)	1	3.3 - 6.7	x
40-50mm (")	1	3.3 - 6.7	x
70-80mm (")	1	3.3 - 6.7	x
90-120mm(Adult)	1	3.3 - 6.7	x

Fig. 9. Ontogenetic variation of esterase enzyme in *Penaeus indicus*.

Fig. 9



PZ-Protozoea, PL-Post larva, JUI-20−30mm

JU2-40−50mm, JU3-70−80mm, A-90-120mm

Table 38: Summary of Esterase patterns of Penaeus indicus bands based on Fig. 9.

Stages	Position of band				Total No. of bands
	1	2	3	4	
Protozoea	+	+	+	-	3
Postlarva	+	-	-	+	2
20-30mm(Juvenile)	+	-	-	-	1
40-50mm(")	+	-	-	-	1
70-80mm(")	+	-	-	-	1
90-120mm (Adult)	+	-	-	-	1

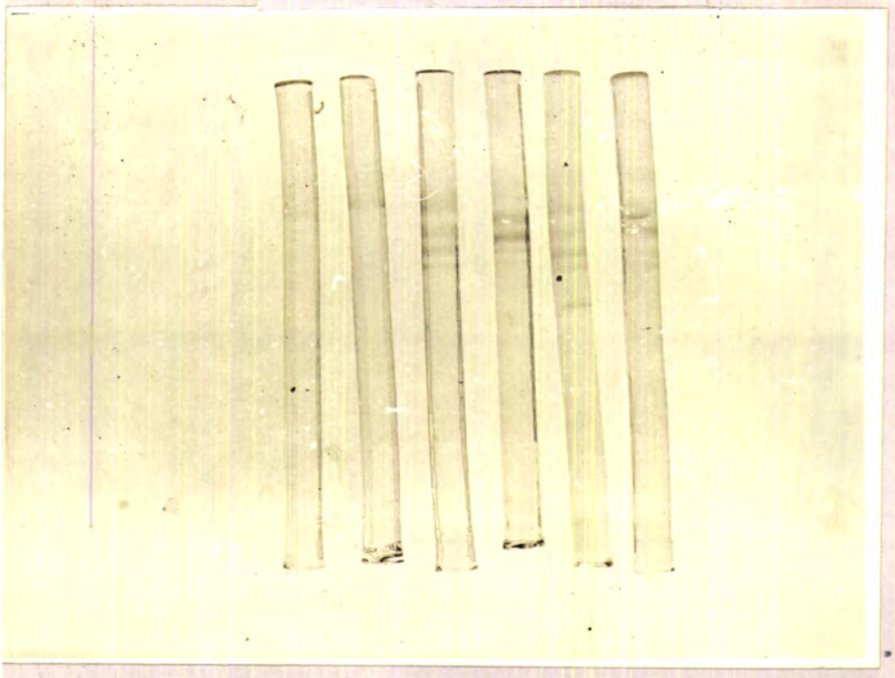
'+' represent the presence of the Isozyme

'-' represent the absence of the Isozyme

Plate 8: Ontogenetic variation of Aldehyde oxidase enzyme in Penaeus indicus a) Protozoea, b) Mysis, c) Post larva d),e) juveniles, f) adult.

Plate 9: Ontogenetic variation of esterase enzyme in Penaeus indicus. a) Protozoea, b) Post larva c)-e) Juveniles, f) Adult

a b c d e f



a b c d e f

Band No.4 was found only in post-larva. Band No.3 was expressed in all the stages of development. Band No.2 was active only in the adult stage. Though post-larva and adult stages showed distinct stage specific pattern for the enzyme, juvenile stages, and Protozoea had identical single banded pattern (Band No.3 and Table No. 35 & 36).

Esterase:

Esterase isoenzyme separated in protozoea stage of P. indicus showed 3 bands (Band No.1, 2 & 3) whereas post-larval stage showed only 2 bands (Band No.1 & 4). In juvenile stages the enzyme separation was carried out in eye tissue alone and the isozyme activity was found to be exhibited only by one common band (Band No.1) in all three juvenile stages and adult (Table No.37a & 38). Hence, the esterase enzyme was showing specific pattern only in protozoea, post-larva juvenile and adult stages (Fig. 9 and Plate 9).

Malate dehydrogenase:

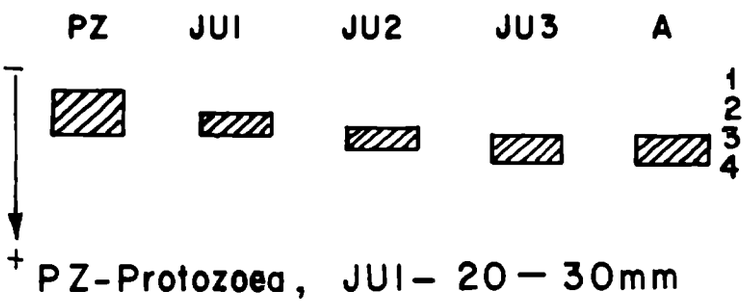
Malate dehydrogenase isoenzyme patterns were studied in detail only in post-larva, juvenile and the adult stages (Table No.37b). All stages expressed only one band with a

Table 37b: Relative mobility with intensity of malate dehydrogenase bands separated in Penæus indicus.

Stage	Band No.	RM Value	Intensity
Post larva	1	16.7 - 23.3	x
20-30 mm(Juvenile)	2	20 - 23.3	x
40-50 mm(")	3	21.7 - 25	x
70-80mm (")	4	23.3 - 26.7	x
90-120mm(Adult)	4	23.3 - 26.7	x

Fig. 10. Ontogenetic variation of malate dehydrogenase enzyme in *Penaeus indicus*.

Fig.10



PZ-Protozoea, JUI- 20 — 30mm

JU2 - 40 — 50mm, JU3-70-80mm

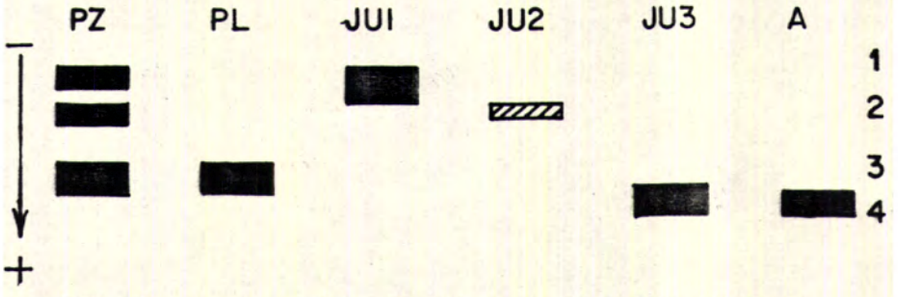
A - 90—120mm

Table 39: Relative mobility (RM) with intensity of Octanol dehydrogenase bands separated in Penaeus indicus.

Stage	Band No.	RM	Intensity
Protozoea	1	0 - 3.3	xx
	2	5 - 8.3	xx
	3	13.3 - 16.7	xx
Post-larvae	3	13.3 - 16.7	xx
20-30mm(Juvenile)	1	0 - 5	xx
40-50mm(")	2	5 - 6.7	x
70-80mm(")	4	16.7 - 20	xx
90-120mm(Adult)	4	16.7 - 20	xx

Fig. 11. Ontogenetic variation of octanol dehydrogenase enzyme in *Penaeus indicus*.

Fig. 11



PZ - Protozoea, PL - Postlarva, JUI - 20 − 30mm

JU2 - 40 − 50mm, JU3 - 70 − 80mm, A - 90 − 120mm

Table 40: Summary of Octanol dehydrogenase patterns of *Penaeus indicus* based on Fig. 11.

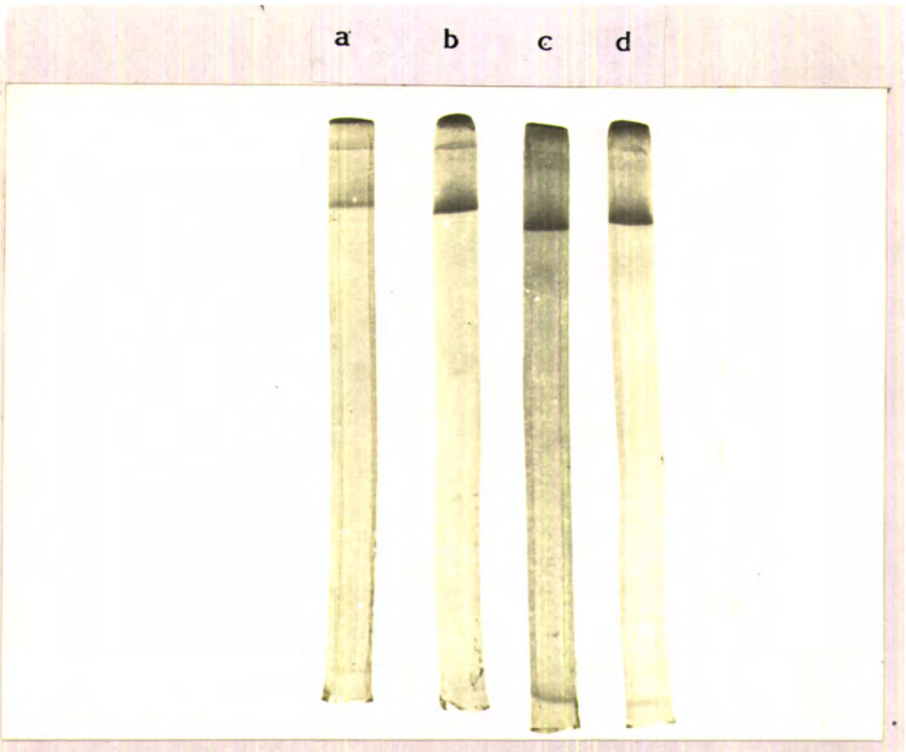
Stage	Position of Band				Total No. of bands
	1	2	3	4	
Protozoea	++	++	++	-	3
Post larva	-	-	++	-	1
20-30mm(juvenile)	++	-	-	-	1
40-50mm(")	-	+	-	-	1
70-80mm(")	-	-	-	++	1
90-120mm(Adult)	-	-	-	++	1

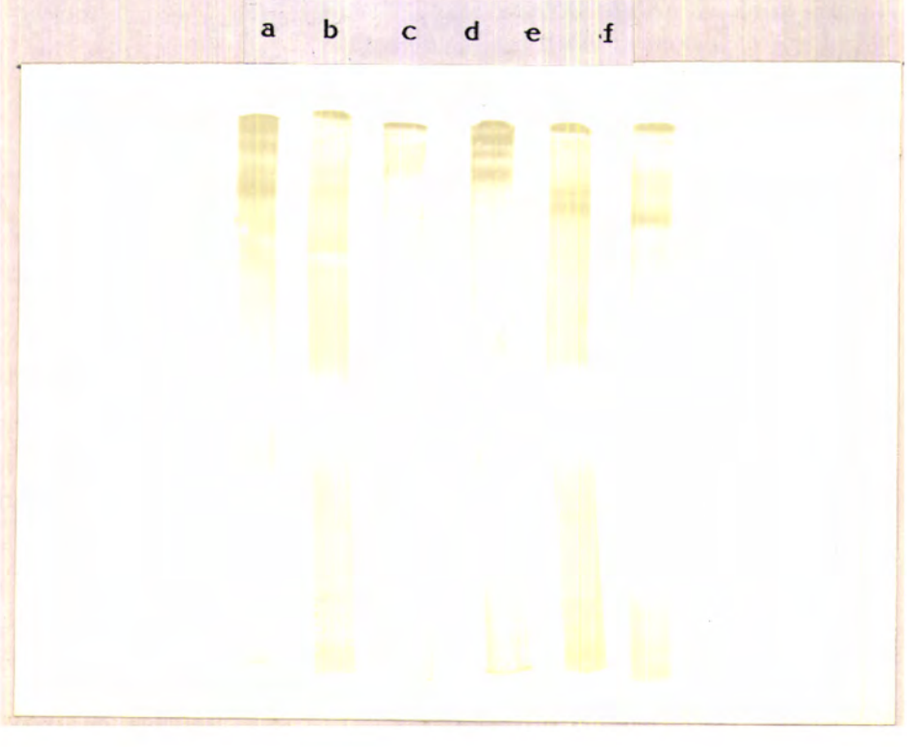
'+' represents the presence of the isozyme

'-' represents the absence of the isozyme

Plate 10: Ontogenetic variation of malate dehydrogenase enzyme in Penaeus indicus
a) Protozoea, b), c) Juvenile, d) Adult

Plate 11: Ontogenetic variation of octanol dehydrogenase enzyme in Penaeus indicus a) Protozea, b) Postlarva, c)-e) juvenile f) Adult.


a
b
c
d


a
b
c
d
e
f

slight variation in their relative mobility, width and intensity, indicating a lesser degree of stage specific pattern for the enzyme (Fig. 10 and Plate 10).

Octanol dehydrogenase:

Octanol dehydrogenase analysed in protozoea stage of P. indicus showed 3 bands (Band No.1, 2 & 3) whereas it showed only one band in post-larva (Band No.3), in all the juvenile (Band No.1 in stage I, band No.2 in stage II, Band No in Stages III) and in adult stages (Band No.4) but the relative mobility, width and staining intensity varied. For all juvenile stages the eye tissue only was used for separation of this isozyme. Thus protozoea, post-larva and juvenile stages of 1, 2 & 3 and adult could be distinguished by this enzyme pattern (Table No. 39 & 40 and Plate 11).

General Protein:

Protein patterns analysed in muscle tissue of P. indicus showed an enhancement in the number of fractions as the development progresses from juvenile to adult stages giving 12 bands in stage I (20-30 mm) 14 bands in stage II(40-50 mm) 15 bands in Stage III(70-80 mm) and 17 bands in adult stage (90-120 mm) (Fig. 12 and Plate 12).

Table 41: Relative mobility (RM) with intensity of muscle myogen patterns of *Penaeus indicus*

Band No.	RM	Intensity
20-30mm (Juvenile)		
1	0	x
2	4.3 - 5.7	xx
3	14.2 - 15.7	xx
4	27.1 - 28.6	x
5	32.9	x
6	34.3	x
7	35.7	x
8	44.3 - 47.1	xx
9	55.7 - 57.1	xx
10	64.3 - 65.7	x
11	77.1 - 80	x
12	84.2 - 85.7	x
50-60mm (Juvenile)		
1	0	x
2	4.3 - 5.7	x
3	14.2 - 15.7	xx
4	27.1 - 28.6	x
5	32.9	x
6	34.3	x
7	35.7	x
8	44.3 - 47.1	x
9	51.4 - 52.9	x
10	54.3 - 57.1	xx
11	64.3 - 65.7	x
12	70.0 - 72.9	x
13	77.1 - 80.0	x
14	85.7 - 90.0	x

Band No.	RM	Intensity
70-80 mm (Juvenile)		
1	0	x
2	4.3 - 5.7	xx
3	14.2 - 15.7	x
4	20.0 - 21.4	x
5	25.7 - 27.1	x
6	32.9	x
7	34.3	x
8	35.7	
9	40.0 - 42.9	x
10	45.7 - 48.8	x
11	52.9 - 54.3	x
12	55.7 - 57.1	x
13	64.3 - 65.7	xx
14	71.4 - 72.9	x
15	77.1 - 80.0	x
16	87.1 - 90.0	
90-120 mm(Adult)		
1	0	x
2	5.7 - 7.1	x
3	11.4	x
4	14.2 - 15.7	x
5	20.0 - 21.4	x
6	25.7 - 28.6	x
7	32.9	x
8	34.3	x
9	35.7	x
10	40.0 - 42.9	x
11	45.7 - 48.8	xx
12	52.0 - 54.3	x
13	55.7 - 57.0	xx
14	64.3 - 65.7	xx
15	71.4 - 72.9	x
16	77.1 - 80.0	x
17	85.7 - 88.6	x

Fig. 12. Ontogenetic variation of general protein in *Penaeus indicus*.

Fig. 12

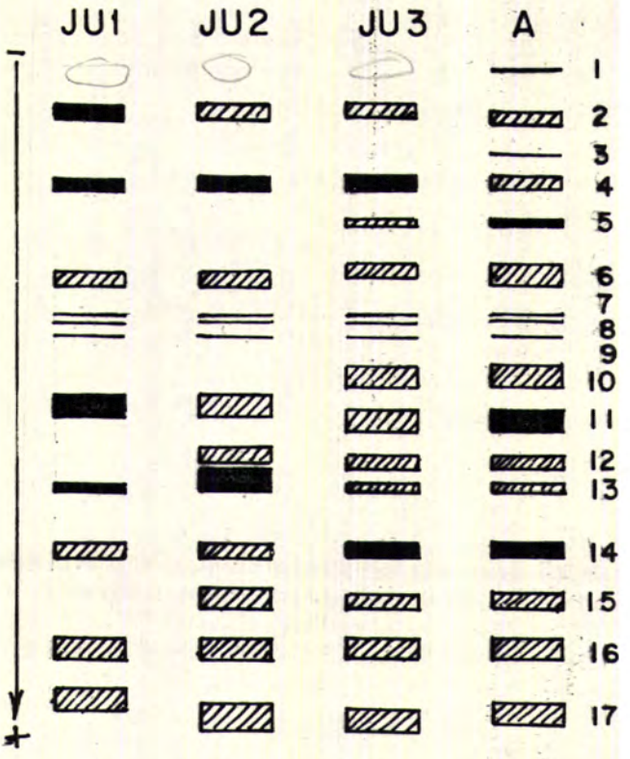

JU1
JU2
JU3
A
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
JU1 — 20 — 30 mm
JU2 — 40 — 50 mm
JU3 — 70 — 80 mm
A — 90 — 120 mm

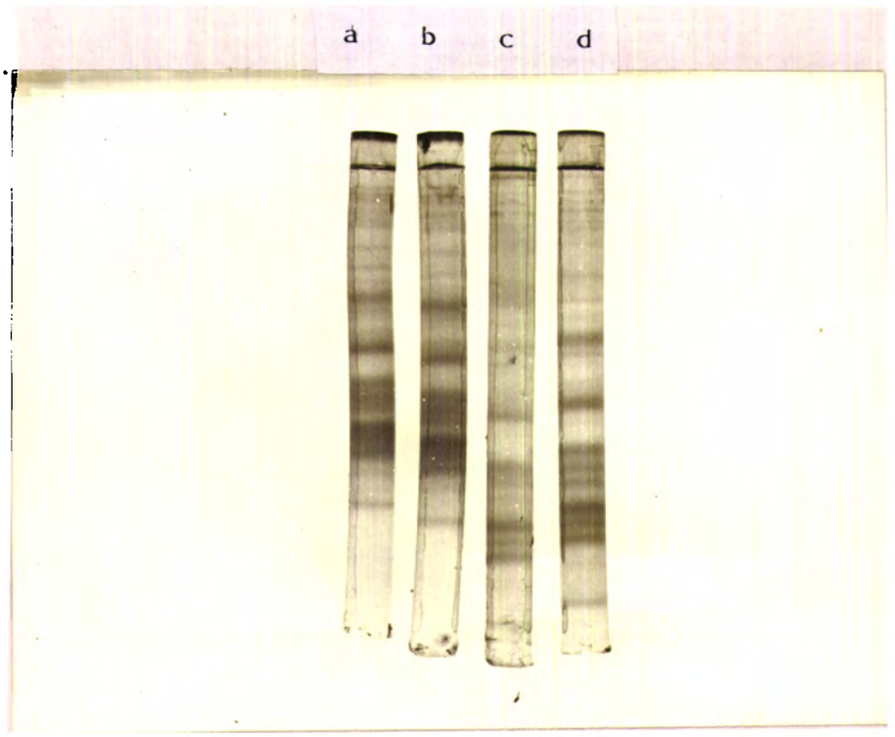
Table 42: Summary of muscle myogen pattern of General protein based on their ontogeny Fig. 12.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	Total No. of bands	Common bands	No. of deleted bands	Total	No. of additional bands
P. indicus																						
20-30 mm (Juveniles)	+	+	-	+	-	+	+	+	+	-	+	-	+	+	-	+	+	12	12	5	0	
40-50 mm (")	+	+	-	+	-	+	+	+	+	-	+	+	+	+	+	+	+	14	12	3	1	
70-80 mm (")	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16	12	1	3	
90-120 mm (Adult)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	17	12	0	5	

'+' represents the presence of the protein band

'-' represents the absence of the protein band

Plate 12: Ontogenetic variation of general protein in *Penaeus indicus* a)-c) Juveniles, d) Adult.


a
b
c
d

When the analysis was done according to the relative mobility of these bands, the common bands seen in all stages, the total deleted bands and the exact number representing their location can be compared. Thus 12 bands are seen representing their common nature in all stages. Stage I is found to lack 5 numbers of bands viz. band No. 3, 5, 10, 12 & 15, stage II lacks band No.3, 5, & 10 and stage III lacks band No.3. Though there are more number of common bands, absence or presence of a few additional bands again indicated specific general protein pattern for the different stages tested here.(Table No. 41 & 42).

Discussion:

Due to commercial importance, studies on Larval development of penaeid prawns gained much importance in the last 3 decades. Rearing of penaeid shrimp from eggs to post-larvae and their culture practice is explained in detail by Cook and Murphy (1966, 1969, 1971). Information regarding developmental stages of various prawns like Penaeid duorarum (Dobkin, 1961), Penaeus setiferus(Heegaard 1953), Penaeus japonicus (Hudinaga 1935), Metapenaeus joyneri (Lee and Lee 1968, 1969), Metapenaeus dobsoni (Menon 1951), commercially important Penaeus sp. of prawns (Williams 1953, 1955, 1959), Metapenaeus sp. of prawns (Muriel and Bennett 1951) and penaeid prawns of south west coast of India (Mohamed, Rao and George 1967) are available. Rao(1973) had explained clearly the different stages of Parapenaeopsis stylifera, Metapenaeus monoceros, M. affinis, M. dobsoni and Penaeus indicus. Paulinose (1982) has given the key for the identification of larvae and postlarvae of the penaeid prawns. Artificial hatchery production and rearing of penaeid prawn seed of P. indicus is attempted in many parts of the country and the techniques adopted for this at Narakkal Prawn Culture Laboratory CMFRI is given in CMFRI (1985). Some of the morphological

differences between the same stages of different species are very minute, making it often difficult to identify them in the field. So an alternate method for identifying would be advantageous.

Genetic codes in DNA are translated into structural proteins of each organism. Some of these proteins exist in the form of isoenzymes. Electrophoretic separation of isoenzymes such as acid phosphatase, aldehyde oxidase, alcohol dehydrogenase, malate dehydrogenase, octanol dehydrogenase, esterase, and general protein were carried out in different developmental stages in Indian White Prawn Penaeus indicus.

Cellular components increases during development due to differential synthesis of isoenzymes bringing out certain natural changes in the quantity and quality of each enzyme in each stage of development. Therefore, the isoenzymes which are specific gene products can be identified and used as efficient genetic markers at a particular developmental stage. Biochemical identification of each stage will give their extent of, differentiation, relationship and closeness of different stages of development. Changes observed in different developmental stages are measured quantitatively and qualitatively by separating different enzymes using electrophoresis.

Acid phosphatase:

Ontogenetic changes in Acid phosphatase in different developmental stages like young larva, 3rd in star larvae, pupa, of Drosophila melanogaster showed faster mobility as it reached adult stage with only one band in each atage (Beckman and Johnson 1964) whereas in Xanthid crab Morgan et al,(1978) found 2 bands in Stage III & IV, one common band and one with varying mobility in zoea stage, 3 bands in megalopa stage with varying mobility and 2 bands in young crab with same mobility. In the fish Liza parsia, Mary (1985) found 3 bands in the eye tissues of fingerlings and 4 bands (2 bands showing different mobility) in the eye tissues of adult. Thus different organisms indicated ontogenetic changes in the expression of acid phosphatase isoenzymes.

Similarly, Lester and Cook (1977) reported definite ontological changes in the electrophoretic patterns of acid phosphatase in four species of Penaeus. The ontological patterns of the enzyme in each of these species indicate an inter species ontological patterns for each larval stage besides ontological changes in each species. The different developmental stages of P. indicus studied here for the first time also showed distinct ontological patterns for the acid phosphatase (Fig. 6).

Thus protozoea stage showed 2 bands in P. aztecus, one band in P. setiferus, 3 bands in P. stylirostris 2 bands in P. vannamei (Lester and Cook 1987) and 4 bands in P. indicus. Mysis stage expressed 3 bands in P. aztecus, 2 bands in P. setiferus, 2 bands in P. stylirostris 2 bands P. vannamei (Lester and Cook 1987) but in the present study 3 bands in P. indicus. The relative mobility showed difference from P. aztecus which alone has equal number of bands as P. indicus. Post larval stage showed one band in P. aztecus, 2 bands in P. setiferus, 3 bands in P. stylirostris and one band in P. vannamei (Lester and Cook, 1987) and expressed 3 bands in P. indicus. But here again the relative mobility showed variation from P. stylirostris which has 3 bands as in P. indicus.

P. indicus showed 4 bands in protozoea, 3 bands each in mysis and post-larva, 3, 2, 2 and 1 bands in three different stages of juvenile and adult stages respectively indicating stage-specific acid phosphatase patterns either in terms of the number of bands, electrophoretic mobility of different bands or even width and intensity of certain bands.

Though mysis, post larva and juvenile stage I of P. indicus had three bands, each of these stages could

still be separated on the basis of difference in electrophoretic mobility pattern of the enzyme. A comparison of Acid phosphotase patterns observed by Lester and Cook (1987) and the present observations shows that even each larval stage of different species can be clearly identified on the basis of species specific number of bands or where similar number of bands may exist difference in the electrophoretic mobility of the bands.

Aldehyde oxidase:

Ontological changes in the Aldehyde oxidase of P. indicus described for the first time here also showed a developmental stage specific pattern particularly in the expression of number of bands as different larval stages progress from protozoea to adult.

Aldehyde oxidase in P. indicus studied here also showed a pattern different from that of Penaeus setiferus, P. stylirostris, P. aztecus and P. vannamei of Lester and Cook (1987). Protozoea stages of P. aztecus, P. stiferus, P. stylifostris, and P. vannamei showed 1, 4, 2 and 1 band respectively whereas P. indicus expressed 4 bands. Mysis stage of P. aztecus, P. setiferus, P. stylirostris and P. vannamei showed 2, 2, 4 and 1 bands whereas P. indicus

was found to have 2 bands. Post larval stage of P. aztecus P. setiferus, P. stylirostris and P. vannamei had 2, 3, 1 and 1 bands. P. indicus also showed 2 bands. In the adult pattern P. aztecus, P. setiferus, P. stylirostris, and P. vannamei expressed 1, 2, 3 and 2 bands whereas P. indicus has 3 bands (Table 34).

Therefore, aldehyde oxidase detected and described here for the first time in P. indicus possess Ontological as well as species specific patterns as also indicated by the pattern of acid phosphatase discussed earlier.

Alcohol dehydrogenase:

Ontogeny changes of Alcohol dehydrogenase observed in leopard danio showed activity in liver cells (Frankel 1981) and this was correlated with the changes over in metabolic pathway to liver due to depletion of yolk in the later stage of development (Shaklee et al,1974).

Present investigation carried out in the different stages of P. indicus showed for the first time the stage specific patterns of alcohol dehydrogenase. In the protozoea and first three juvenile stages it expressed a single zone of enzyme activity whereas post-larval stage expressed 3 bands which are distinct and the adult stage showed only 2

bands. As already explained post larva stage showed maximum of 3 bands whereas all other stages except the adult stage showed only one band. Though band No.3 is common to all stages specific pattern of alcohol dehydrogenase can also be used for separating different larval stages of P. indicus.

Esterase

Ontogenetic study of esterase patterns of various cavian tissues showed marked changes to occur in the liver, Kidney and intestine (Holmes and Masters 1967). Hunter et al,(1964) described changes occurring in the development of liver and kidney of the foetal and weaning mouse. Paul and Fottrell (1961) found foetal human tissue esterase isoenzyme pattern to resemble those of adult but Blanco and Zinkhan (1966) reported an increase in both the number of isoenzymes and their activities during development.

Various workers like Gooch (1977), Morgan et al,(1980) and Kannupandi (1980) have investigated on the ontological changes of esterase in the different stages of crabs. Morgan et al,(1980) found 2, 3, 3 and 7 bands during the zoeal stages I-IV, 5 bands in megalopa stage and 4 bands in young crab stage. Kannupandi (1980) found out a gradual increase in number of esterase bands upto Zoea stage IV and thereafter the zone of activity decreased in the later stages.

Lester and Cook (1987) reported ontological changes in esterase in the different stages such as protozoea, mysis, postlarva and adult of Penaeus species of prawns like Penaeus aztecus, P. setiferus, P. stylirostris and P. vannamei. Similarly, the esterase patterns in P. indicus studied here also showed characteristic bands for each larval stages (Fig. 9).

When the esterase of different larval stages of P. indicus detected here are compared with that of other species of Penaeus as reported by Lester and Cook (1987), definite species specific esterase patterns are indicated in different larval stages of each species Protozoea of P. aztecus, P. setiferus, P. stylirostris and P. vannamei showed 4, 5, 4 and 3 bands respectively (Lester and Cook 1987) whereas P. indicus expressed 3 bands.

Postlarva stage expressed 9, 8, 9 and 1 bands respectively in P. aztecus, P. setiferus, P. stylirostris and P. vennamei whereas P. indicus expressed only 2 bands, Esterase showed only one band in all the juvenile and adult stages of P. indicus probably due to the analysis of this enzyme in Eye tissue.

Hence ontological changes in the esterase of P. indicus studied here are also comparable with that of other species as well as with that of other enzymes discussed earlier.

Malate dehydrogenase:

Ontological changes were observed in malate dehydrogenase enzyme in crabs by Gooch (1977) and Morgan et al,(1978 and in plants by Rider (1980). Morgan et al,(1978) showed a increase in number of bands as the animal becomes adult. In P. indicus the expression of malate dehydrogenase enzyme was studied in Postlarva, three different stages of juveniles and adult. Though the malate dehydrogenase consisted of onl a single band in all the stages slight variation in the relative mobility of the single band indicated its own stage specific nature of the enzyme as described in other enzymes but to a lesser degree.

Octanol dehydrogenase:

The octanol dehydrogenase enzyme in P. indicus tested in the present study also exhibited stage specific pattern of the enzyme.

Protozoea with 3 bands, could be easily distinguished from postlarva, all juvenile and adult stages with a single band. Though postlarva juvenile and adult stages had only

one band, differences in the relative mobility and staining intensity showed expected ontological changes (Fig. 11). Band No.1 present in protozoea is present in juvenile stage I and the Band No.2 present in protozoea is also present in juvenile stage 2 whereas the band No.3 found in protozoea and postlarvae is absent in all juvenile stages and the band No.4 which is present in juvenile stage 3 and adult is found to be absent in all other stages.

Thus ontological changes in the acid Octanol dehydrogenase showed a specific pattern particularly in the number of bands between protozoea and other larval stages.

General protein:

Ontological studies of general protein in salmon showed changes with gradual transition of the protein pattern showing an increase in number from newly hatched having 9 bands to 12 bands in sexually matured salmons (Nyman 1967). Bhattacharya and Alfred (1982) observed an increase in quantity of protein in the tissues of brain and muscle and a decrease in protein content of liver with the increase in size of Channa stewartii and Danio dangila. Sriraman and Reddy (1977) found out 9 bands in the size group 13-15 mm, 10 bands in size group 18-20mm and 10 & 11 bands in the adult stage of P. indicus and P. monodon. Whereas Prathibha

(1984) found out 11, 14 and 16 bands in 10-15 mm post-larva, 30-70 mm juvenile and 70-190 mm adult.

Lester and Cook (1987) got 1, 2, 1, 0 bands in protozoea, 3, 2, 4 & 4 bands in Mysis, 8, 8, 6 & 5 bands in postlarvae and 11, 8, 10 & 12 bands in adult of P. aztecus, P. setiferus, P. stylirostris and P. vannamei respectively. In the present study 11, 12, 14, 16 & 17 bands were obtained in juveniles of size groups of 20-30 mm, 40-50 mm, 70-80 mm and Adult 90-120 mm in P. indicus (Table 43).

Penaeus indicus studied here showed an I-III increase in the number of fractions from juveniles to adult showing 12, 14, 16 and 17 numbers respectively. This type of striking increase in band diversity alongwith development is also noticed in other Penaeus spp.(Lester and Cook 1987) and also in Xanthid crab by Kannupandi (1980).

Though 12 out of 17 general protein bands in all the larval stages of P. indicus studied here were similar in electrophoretic mobility, absence or presence of a few additional bands, again, clearly separated different larval stages.

The separation of different isoenzymes like, acid Phosphatase, alcohol dehydrogenase, aldehydeoxidase, esterase,

Table 43: Total number of Muscle myogen protein patterns found in different penaeid prawns.

		Total
Sriraman & Reddy 1977*		
P. indicus	13-15 mm	9
	18-20 mm	10
	Adult	10
P. monodon	13-15 mm	9
	18-20 mm	10
	Adult	11
Prathibha 1984**		
P. monodon	10-15 PL mm	12
	Juvenile 30-70 mm	14
	Adult 70-190 mm	16

Lester & Cook 1987***	Protozoea	Mysis	Postlarvae	Adult
P. aztecus	1	3	8	11
P. setiferus	2	2	8	8
P. stylirostris	1	4	6	10
P. vannamei	-	4	5	12

Present study		
P. indicus	20-30 mm	12
	40-50 mm	14
	70-90 mm	16
	Adult	17

protein, octanol dehydrogenase and malate dehydrogenase in different developmental stages of the prawn P. indicus studied here revealed stage and species specific characteristic bands as seen in Xanthid crab (Kanmupandi 1980) and in P. monodon (Prathibha 1984). Lester (1980) used these patterns for identification of juveniles of different species of prawns and Morgan et al,(1980), for the identification of different stages of crab. Lester and Cook (1987) expressed abundant changes in isozyme pattern among different development stages of penaeid prawn. Hedgecock et al,(1982) stated that there is constancy of Zymogran pattern throughout the development of decapods species and Gooch (1977) found no change in enzyme expression of life cycle of crabs. This is in contradiction with the present results as well as some of the earlier reports, suggesting that ontological changes are either not essential for all species or not sufficiently expressed as to detect it biochemically.

On comparison of the total number of bands possessed by protozoea and adult stages of P. indicus studied here it is interesting to note that ontological changes in the acid phosphatase reduced the three banded pattern of protozoea to a single banded pattern in the adult, whereas the number of bands were found increased in P. setiferus, P. stylirostri and P. vannamei while retaining the same number of P. aztecus

Therefore the ontological reduction in the total number of acid phosphatase enzyme bands as noticed in the present study of P. indicus is not a general phenomenon of ontological changes. Such ontological shift in the total number of bands and even electrophoretic mobility of certain bands is also noticeable to some extent in other enzymes of P. indicus. The species specific and stage specific biochemical complexity of each species alone could be accounted for the above phenomenon.

The protein present in initial stage of development migrate to a different position due to changes in the cytoplasmic milieu or post-translational modification as found in Penaeus sp. of prawns (Lester and Cook 1987) and thus ontogenetic modification of gene action leading to increased differentiation of tissues and organs with increasing age of the organism (Morgan et al,1978).

Increased isozyme complexity corresponds to enhancement in dietary requirement (Frank et al,1975) and also changes occur for metabolic preparation for the metamorphic period (Costlow 1968, Oconnor and Gilbert 1968, Yamoka and Scheer 1970).

Characteristic changes observed in isozyme patterns may thus reflect changes in cell components and synthesis of

isoenzyme in relation to gene expression. This may also be due to essential physiological adaptive changes brought about during habit shift from marine to brackish water as reported in crabs when changing from Pelagic to benthic environments (Morgan et al, 1978 and Kanmupandi 1980).

To conclude the distinct ontological patterns of different isoenzymes and general proteins discovered and described here should enable one to easily identify different larval stages of P. indicus from that of other prawn species.

CHAPTER VI

INTRASPECIES ENZYME LOCI AND THEIR VARIATION

Resume of literature:

Biochemical genetics is an effective tool in detecting single gene variation and this paved way to resolve several problems faced in fish population studies.

It aims at establishment of the pattern of population structure of the species, analysis of genetic differences between populations, demarkating boundaries between adjacent populations to show the extent of isolation, for the study of evolutionary processes in populations, possible side effects due to modern techniques on population structure, and to protect the natural resources of the commercially important fishes (Avise et al., 1975, Kirpicknikov 1981). Loss of genetic variation found in the artificially cultured population can be traced out and possible genetic manipulation can be recommended (Allendorf et al.,1979). Genetics is being employed in aquaculture to select diverse parental stocks and to produce progeny which show hybrid vigour (Hedgecock et al.,1976). Genetic differentiation without isolation in the American eel was found out (Williams et al, 1973, Koehn and Williams 1978). Biochemical variants were

used in Pacific salmon and Rainbow trout among populations in identification and characterization of population (Utter et al.,1973). Geographic patterns of Zoarces viviparus was identified by Christiansen et al(1974). Population analysis on German trout was done by electrophoresis (Keese and Langholz 1974). May et al.,(1975) examined inter and intra specific genetic variation in pink salmon (Oncorhynchus gorbuscha) and Chum Salmon (Oncorhynchus keta). Avise et al., (1975) found out the genetic change due to adaptive differentiation between two native California minnows. Genetic variation studies in Scandinavian brown trout (Salmo trutta L.) showed sympatric populations (Allendorf et al,1976) Christianaen and Simonsen (1978) found out the geographic variation in protein polymorphisum in the eelpout Zoarces Viviparus (L). Rodino and Comparini (1978) found out the genetic variability in the European eel Anguilla anguilla L. Ward (1977) worked on the protein variation in plaice Pleuronectes platessa L. Mangaly & Jamieson (1978) used three genetic tags to find out the unit stock nature of the European hake, Merluccius merluccius (L). Koehn and Williams (1978) traced the genetic difference in the American eel Anguilla rostrata without any isolation. Enzyme polymorphisms in the Atlantic Mackerel, Scomber Scombrus L. was found out by Smith and Jamieson (1978; 1980). Genetic variation and

population structure of New Zealand snapper Chrysophrys auratus (Forster) was found out by Smith et al,(1978).

Avise and Felley (1979) showed the population structure of freshwater fish Bluegill (Lepomis macrochirus). Kirpicknick and Selander (1979) traced the supporting evidence of speciatic in lake white fish (Coregonus clupeaformis). Protein variation studies revealed subspeciation in cutthroat trout Salmo clarki (Loudenslager and Gall, 1980). Population structure of lake white fish Coregonus clupeiformis was analysed using 4 populations by Imhof et al.,(1980). Wishard et al.,(1980) worked on biochemical genetic characteristics of native trout population of owyhee country, Idaho. Gary (1980) showed geographic variation in milk fish Chanos chanos with biochemical evidence using 38 loci from 14 locations. Electrophoretic variation studies in four strains of rainbow trout (Salmo cairdneri Richardson) was done by Guyomard (1981). Ecological, morphological and electrophoretic variation among allopatric Ontario lake white fish(Coregonus clupeaformis) stock was studied by Ihssen et al.,(1981). Population structure of spanish mackerel Scomberomorus maculatus was found out by Johnson (1981).

The existence of taxonomically distinct strains of Brook trout (Salvelinus fontinalis) was investigated by

Mark et al.,(1981). Genetic distinctness of large mouth bass (Micropterus salmoides) populations from different geographic regions were studied by Philip et al.,(1981). Origin of Rainbowtrout population was traced by exposing them in different ecological niches (Reichle 1981). Gene frequency difference of New Zealand **loki**. Macruronus novaezelandiae from different locations were analysed (Smith et al.,1981). Krieg and Guymard 1984 gave an account of the genetic differentiation between brown trout populations. Electrophoresis analysis of Australian barramundi suggested the existance of multiple stocks (Shaklee and Salini, 1983). Biochemical genetics of Atlantic herring(Clupea harengus)and Pacific herring (Clupea Pallasi) was studied by Grant and Utter(1984)and Grant(1984) Milner et al.,(1985) identified the naturally occurring mixed population of Pacific salmon Oncorhynchus sp. Electrophoretic assessment of stocks of brown trout Salmo trutta L. suggested a supplemental stocking programme (Taggert & Ferguson 1986). Biochemical genetic variation in angler fish Lophius piscatorius was done by Crozier (1987).

A review of the literature available on electrophoretically detectables genetic variations in natural populations shows considerable work, on this line on several isoenzymes in crustaceans. Details of these studies are summarised in the table given below:-

Electrophoretically Detectable Genetic Variation in Natural Populations of Crustaceans[a]

Subphylum Class Order Species[b]	Group 1															
	ALDO	FUM	GDH	GOT	G-3PDH	α-GPDH	HK	IDH	LDH	MDH	ME	MPI	PGM	6-PGDH	PGI	TPI	XDH
Branchiopoda Diplostraca Conchostraca Daphnia magna(1)									2M	.53 3							1M
Daphnia pulex(2)		1M		1M						2M .53					1M		1M .06
Simocephalus serrulatus (3)										3							2
Maxillopoda Copepoda Harpacticoidea Tisbe holothuriae(4)										.14 2	.29 3		.42 3		.39 3		.22 3
Cirripedia																	
Thoracica Chthamalus stellatus (Poli)(5)	.45 3				1M					.05 2		.13 5	.09 4		.05 4		
Chthamalus montagui(5)	1M				1M					.32 2		.15 3	.46 8		.27 7		
Chthamalus dalli Pilsbry(6)		1M				1M		.33 2	1M	.07 3	3M		.48 3	.33 2	.46 3		.08 2
Chthamalus fissus Darwin(6)		.03 2	1M	.07 3		1M	.02 2	.16 3		.05 2		.06 3	1M	.33 3	.51 4		1M
Chthamalus anisopoma Pilsbry(6)		.10 2	1M	.12 2			.38 2	1M		.10 2		.05 2	.14 2	.35 3	.50 3		
Chthamalus sp.(6)		1 M	1M	.14	1M		.02 2	1M		.04 2		.15 3		.36 3	1M		.14 2
Chthamalus sp.(6)		.28 3	1M	2M	.08 2		.02 2	1M		.04 2		.21 3	1M	1M	.40 3		1M
Balanus amphitrite Darwin(7)		1M		.37 3	.11 2			1M		.05 2		.31 3	.52 3		.14 3		
Balanus amphitrite inexpectatus Pilsbry(7)		1M		.11 2	1M		1M			.03 2		.54 4	.08 3		.43 3		
Balanus glandula Darwin(7)		.18 2		.50 2	1M			.06 2	1M	.08 3		.75 6	.51 4		.64 6		
Balanus crenatus Brugiere(7)		1M		.36 3	.1M			1M		2M	1M	.70 4	.09 3		.60 4		
Semibalanus cariosus (Pallas)(7)		1M		.36 2						.06 3	1M	.64 5			.14 4		
Hoplocarida Hoplocarida Stomatopoda Squilla nepa(8)		.10 2	1M	2M	1M		1M	1M		2M	1M	.03 2	1M	.56 3	.38 2	1M	.16 2
Squilla woodmasoni (8)				1M							1M	1M	.25 2		1M	1M	

Contd

Subphylum Class Order Species[b]	Group 1 ALDO	FUM	GDH	GOT	G-3PDH	α-GPDH	HK	IDH	LDH	MDH	ME	MPI	PGM	6-PGDH	PGI	TPI	XDH
Malacostraca																	
Peracarida																	
Isopoda																	
Excirolana caidii(7)		1M			1M		1M	.41 2	1M	.22 2	1M	1M		1M	.39	1M	
Excirolana Sp.(7)		1M	1M		1M		1M	1M		2M	1M	.44 3	1M	1M	.54 3	1M	
Eucarida																	
Euphausiacea																	
Euphausia superba(9)	.28 2	2M		.49 3	1M	2M	.06 3	1M		.22 3	2M		1M	1M	.35 4	1M	1M
Euphausia mucronata(10)	.32 3	.25 4		.11 3	1M	1M	.40 3		.22 4	.13 2	.32 4	.37 4		.11 3	.20 6	.06 3	
Euphausia distinguenda (10)	.46 4	.26 3		.13 5	1M	1M	.55 4		.20 3	.41 3	.20 5		.42 6	.19 4	.33 6	.13 4	
Decapoda																	
Penaeidae																	
Penaeus merguiensis de Man(8)	1M		1M	1M	1M	1M			1M	2M	1M	.11 5	.03 3	1M	.04 4	1M	
Penaeus azecus Ives (11)	1M			1M	1M		.28 3			.03 3		.40 6	.33 4	.06 3	.41 4		
Penaeus duorarum Burkenroad(11)			1M	1M	1M		.02 3	1M		.26 3		.31 7	.09 4	.45 3	.10 3		
Penaeus setiferus Linnaeus(11)			1M		1M		.17 3	1M		1M		.37 6	.33 5	.18 3	.26 5		
Penaeus stylirostris (23)										1M				1P			
Penaeus Vannamei (23)										1M				1P 3			
Metapenaeus macleayi(25)						1M				2M				1M			
M.bennett-ae(25)						1M				1P				1M			
M.endeavou-ri (25)						1M				2M				1M			
M.ensis(25)						P 2				2M				1M			
M.insolitus (25)						1M				2M				1M			
M.eboracen-sis(25)						P 2				1P				1M			
Penaeus se-misulcatus (25)						1M				2M				1M			
P. monodon (25)						1M				2M				1M			
P.esculen-tus(25)						1P 2				2M				1M			
P.merguien-sis(25)						1M				1P				1M			
P.plebejus (25)						1M				2M				1M			
P.latisulca-tus(25)						1M				2N				1M			
P.longisty-lus(25)						1M				2M				1M			
P.latisulc-atus(26)									1M	1M	1M			1M			
P.japonicus (27)	.125 2								2P								
P.kerathurus (27)	.083 2								.036 2	.206 2							
Palaemonidae																	
Palaemonetes Pugio(12)				1M			1P	1M	1M	1M		.43 7	.30 5		.08 5		

Contd...

Subphylum Class Order species[b]	Group 1														
	ALDO	FUN	GDH	GOT	G-3PDH	α-GPDH	HK	IDH	LDH	MDH	ME	MPI	PGM	6-PGDH	PGI	TPI
Macrobrachium rosenbergii de Man (13)	1M	1M	.05 2	1M	.02 2	.09 2	1M	1M	2M	1M	1M	.26 4	1M	1M	1M
Pandalidae															
Pandalus danae(14)				1M		.35 2		1M	1M	1M	.31 2	1M		1M	1M
Pandalus jordani(14)				1M		.02 3		.15 3	.02 3	1M	1M	1M		.03 3	1M
Pandalus platyceros(14)				1M		.33 3		1M	1M	1M	.02 2	.11 2		.02 2	1M
Pandalopsis ampla(15)					1P 2			1P 3	1M			1P 3		1M	
Crangonidae															
Crangon franciscorum(16)				.49 2	1M		2M	.11 2	1M	1M			.27 3		.45 3	1M
Crangon nigricauda(16)				.04 2	1M		2M		1M	1M			.10 3		.47 2	1M
Nephropidae															
Homarus americanus(17)	2M			1M	1M		3M	1M		.12 2	.50 2		.39 2		.20 3	.03 3
Homarus gammarus (18)	2M			1M	1M		3M	1M		2M	.51 4		.11 2	1M	.08 2	.02 2
Astacidae															
Orconectes propinquus(19)										.18 2			.04 2		.28 2	
Orconectes virilis(19)										1M			2M		1M	
Orconectes immunis(19)										2M			2M		1M	
Cambarus robustus(19)										1M			2M		1M	
Cambarus bartonii(19)										1M			2M		1M	
Cambarus latimanus(19)										1M			2M		.02 2	
Palinuridae															
Panulirus interuptus(16)				1M	1M					.20 3			1M		.12 2	.04 2
Panulirus cygnus(16)	1M			.04 2	1M		.33 2			.12 2			.04 2		.49 2	1M
Jasus edwardsii(24)						1M				1P 2	1M			1M		
Jasus novaehollandiae(24)						1M			1P 2	1P 2	1M			1M		
Scyllaridae															
Thenus orientalis(16)	1M				1M		1M		1M	.04 2	1M		1M	1M	.16 3	1M
Callianassidae															
Callianassa californiensis (16)				.46 2	1M		.26 2	1M	1M	.06 3	1M		.13 2	.06 3	.08 2	1M
Callianassa sp.(16)				.07 2	.41 2			1M	1M	.08 2		1M	.25 2	.25 3	.07 2	
Upogebia pugeitensis(16)	1M			2M	1M	1M	.03 2		1M	1M	1M		.08 3	1M	.07 2	1M
Galatheidae															
Galathea californiensis(16)					1M		1M		1M	1M			1M		1M	
Munida hispida(16)					1M		2M		1M	2M	1M		.49 2	1M	.05 2	
Munidopsis diomedea(15)						1P 2			1M	1P 3			1M		1P 5	
Munidopsis hamata(20)							.36 4	.27 2	1M	1M	1M		.27 3	.42 3	1M	
Porcellanidae															
Pachycheles rudis(16)	1M			.50 2			1M	.04 2	2M	1M	2M		.47 2	1M	.08 2	1M

Subphylum Class Order Species[b]	Group 1														
	ALDO	FUM	GDH	GOT	G-3PDH	G6PDH	HK	IDH	LDH	MDH	ME	MPI	PGM	6-PGDH	PGI	TPI	XDH
Petrolisthes cinctipes(16)		1M	1M	2M	1M		1M	1M		.04 2	2M		.37 3		.26 3	1M	
Hippidae																	
Emerita analoga(16)		1M		.04 3	1M		.10 2	1M		.47 2	1M	.25 3	.52 4		.20 2	1M	
Hippa pacifica (16)			1M					1M		2M	1M	.08 2	2M	.08 2	1M		
Paguridae																	
Pagurus granosimanus(16)		1M					.45 2	1M		1M	1M	1M	.10 2		.05 2	1M	1M
Coenobitidae																	
Coenobita compresus (16)		1M					1M	1M		2M	1M	.04 2	.36 3	.14 3	.33 2		
Coenobita clypeatus (16)							1M		1M	.40 2		1M	.12 3		.48 3	1M	
Diogenidae																	
Calcinus obscurus(16)		.04 2		1M			1M		.44 2	.06 2		1M	.12 2	2M	.04 2	1M	
Calcinus tibicen(16)		1M		1M	1M		1M			1M		1M	.46 2	.04 2	.04 2		
Clibanarus panamensis(16)				.13 2	1M		1M	1M	1M	1M		1M	.61 3		.15 2	1M	
Clibanarius albidigitus(16)		1M					1M		1M	.03 2	.09 2	1M	.11 2		.06 2	1M	
Clibanarius antillensis(16)		.48 2			1M		1M	1M	1M	.04 2	.15 2	.04 2	.11 2	1M	.04 2	1M	
Calappidae Matutalunaras(16)		1M		1M	1M		.57 3	1M	1M	2M	.26 2	1M	1M	1M	1M	1M	
Matuta planipes(16)	1M	1M		.04 2	1M			1M	1M	1M	1M	1M	.04 2	1M	.08 3	1M	
Cancridae																	
Cancer gracilis (16)			1M		1M		1M	1M		1M			1M		.16 3	.06 2	1M
Cancer magister (16)	.05 2	1M	1M	2M	1M		2M	1M	1M	2M	1M		1M	1M	1M	.24 2	
Portunidae																	
Callinectes arcuatus(16)		1M		.42 3				1M	2M	.23 3		.12 2			.19 2	1M	1M
Callinectes sapidus(16)		.43 2		.44 3	1M		.41 2	1M		.04 2	1M	1M	1M		.08 2	1M	
Portunus sabguinolentus(16)		1M	1M	2M	1M			.31 3	1M	2M	1M	1M	.06 3	1M	.04 2	1M	
Charybdis callianassae(16)		1M			1M		1M			2M	1M		1M		.30 2	1M	
Charybdis sp.(16)		1M			1M					2M	1M		.27 2		1M	1M	
Xanthidae																	
Panopeus purpureus(16)				.18 2				.12 2		.04 2	1M	1M	1M	1M	.08 2	1M	
Rhithropanopeus harrisii(21)				1M				1M	1M	2M			1M	1M	1M		
Xanthodius sternbergii(16)				.05 2				1M		1M		1M	.04 2	1M	1M	1M	
Ocypodidae																	
Ocypode occidentalis(16)				.36 3	1M					1M		1M	1M	2M	1M	1M	
Ocypode quadrata(16)				2M	1M					2M		1M	.05 2	2M	.05 2	1M	
Uca musica(16)				.05 2	1M		1M			.05 2	1M	1M	.61 4	1M	.71 6	1M	
Uca princeps (16)		1M		2M	1M		2M			1M	.09 2	1M	.16 3	1M	.34 3	1M	
Uca spinicarpa(22)	1M		1M		1M			1M	1M	3M		1M	.09 2	1M	.56 3		1M

Contd.....

Subphylum Class Order Species[b]	Group 1															
	ALDO	FUM	GDH	GOT	g-3PDH	GPDH	HK	IDH	LDH	MDH	ME	MPI	PGM	6-PGDH	PGI	TPI	XD
Grapsidae																	
Hemigrapsus oregonensis (16)		1M		1M	1M		1M	2M		2M	.06 2		.26 2	1M	.08 3	1M	.2
Pachygrapsus crassipes (16)		1M		1M	1M		1M			1M	1M	1M	.12 2	1M	1M	1M	
Pachygrapsus transversus (16)		1M		1M			1M	1M		2M	1M	1M	.10 2		.05 2	.02 2	
Gecarcinidae																	
Gecarcinus quadratus (16)				.04 2			1M			1M	.41 2	1M		1M	.04 2	.08 2	.0
Sesarma Ginereum (21)				1M				1M	1M	2M			1M		1M		

Subphylum Class Order Species[b]	Group II													
	ACPH	ALPH	AMY	AO'	EST	LAP	ODH	PEP	ADH	AO[c]	SDH	TO	PT	PYDH
Brachiopoda														
Diplostraca														
Conchostraca														
Daphnia magna(1)		.02 2			.64 3	1M						1M	1M	
Daphnia pulex(2)	2M	.61 4			.34 2	.68 3						1M	1M	
Simocephalus serrulatus(3)		.15 2		.57 3										
Maxillopoda														
Copepoda														
Harpacticoidea														
Tisbe holothuriae(4)		.39 3			5M	.23 2		.59 3			.32 3			
Cirripedia														
Thoracica														
Chthamalus stellatus (Poli)(5)												.03 2		
Chthamalus montagui(5)												1M		
Chthamalus dalli Pilsbry (6)	1M		1M	.18 2	.08 2		1M				1M		1M	
Chthamalus fissus Darwin(6)	.03 2		1M	.02 3	.03 2						.53 3	2M		
Chthamalus anisopoma Pilsbry (6)	.06 2		1M	.48 2	1M						1M	1M		
Chthamalus sp. (6)	1M		1M	0.02 2	.09 2						.24 2	1M		
Chthamalus sp. (6)	1M		1M	.02 2	.04 3						.08 2	.02 2		
Balanus amphitrite amphitrite Darwin(7)	1M			4M	1M		1M					1M		
Balanus amphitrite inexpectatus Pilsbry(7)				.38 2	.46 2		1M				.04 2	.06 3		
Balanus glandula Darwin(7)	2M		.15 2	.74 5	.22 3	1M	.40 3				.24 3	.04 2		
Balanus crenatus Brugiere(7)	1M			.33 2	.38 5		.12 2				.20 4			
Semibalanus cariosus(Pallas) (7)				2M							.50 2	.04 3		
Hoplocarida														
Hoplocarida														
Stomatopoda Squilla nepa(8)	.08 2			.03 2	.05 2		1M				1M		.10 2	
Squilla woodmasoni(8)	3M			1M			1M				1M			
Eumalacostraca														
Peracarida														
Isopoda														
Excirolana kincaidii(7)		.22 2			.04 2	.10 2	1M							
Excirolana sp.(7)		1M			2M		1M							
Eucarida														
Euphausiacea														
Euphausia superba(9)	3M	1M		.08 3	.26 3	1M	.08 3					.02 2		
Euphausia mucronata(10)	.13 3	.33 2		.13 2	.22 3	1M	.06 3					2M		
Euphausia distinguenda (10)	.27 5	.60 3		.23 4	.23 3	1M	.13 4					2M		

Subphylum Class Order Species[b]	Group II													
	ACPH	APLH	AMY	AO'	EST	LAP	ODH	PEP	ADH	AO²	SDH	TO	PT	PYDH
Decapoda														
Penaeidae														
Penaeus merguiensis de Man (8)	1M	1M		1M		1M							4M	
Penaeus aztecus Ives (11)	1M			2M	.35 3	.06 3		.08 3					2M	
Penaeus duorarum Burkenroad (11)	1M			2M	.52 5	1M		.09 3					2M	
Penaeus setiferus Linnaeus (11)	1M			2M	.22 4	1M		.07 3					2M	
Palaemonidae Palaemonetes pugio(12)		1P?			.39 2	1M							2M	
Penaeus stylirostris(23)	2M				1P					2M				
Penaeus vannamei(23)	1M				2 P 2					1M				
Metapenaeus macleayi(25)		2M			1M		P				P 2	M		
M.bennettiae (25)		2M			1M		P 2				1M	P 2		
M. endeavouri (25)		1M			1M		P 3				M	P 2		
M. ensis(25)		1M			1M		1M				P 2	M		
M. insolitus (25)		2M			1M		P 2				P 2	M		
M. eboracensis (25)		2M			1M		P 2				M	M		
Penaeus semisulcatus(25)		2M			1M		P 2				M	M		1M
P. monodon(25)		1M			1M		1M				M	M		1M
P. esculentus(25)		1M			1M		P 3				P 2	M		1M
P. merguiensis(25)		2M			1M		M				M	M		1M
P. plebejus (25)		2M			1M		P 2				M	M		P 2
P. latisulcatus(25)		1M			1M		P 2				M	M		M
P. longistylus (25)		2M			1M		P 2				M	M		M
P. latisulcatus(26)							1M		1M					
P. japonicus (27)					0.429 2					.077 2				
P. kerathurus (27)					.125 . 2									
Macrobrachium rosenbergii de Man(12)	.07 3			1M		.38 2						1M	7M	
Pandalidae														
Pandalus danae(14)	3M			3M	4M	.14 2		1M				1M	5M	
Pandalus jordani(14)	.02 3			2M	.02 2	.12 4		2M				1M	4M	
Pandalus platyceros(14)	.25 3			.07 2	.07 2	011 2		1M				1M	4M	
Pandalopsis ampla(15)					3M	1M		1P 2				1M	4M	
Crangonidae														
Crangon franciscorum(16)	2M			.10 2	.10 2	1M	.10 2				.11 2	3M	4M	

Subphylum / Class / Order / Species[b]	Group II												
	ACPH	ALPH	AMY	AO[1]	EST	LAP	ODH	PEP	ADH	AO[2]	SDH	TO	PT
Crangon nigricauda (16)	.47 2			.13 2	.35 2	1M	,03 2				1M	3M	4M
Nephropidae													
Homarus americanus (17)	.31 2			.33 2	.37 3	1M						5M	6M
Homarus gammarus(18)	.42 2			1M	.47 2							5M	6M
Astacidae													
Orconectes propinquus(19)	1M		2M	.15 2	3M	,46 3	1M					2M	5M
Orconectes virilis(19)	1M		.02 2	4M	1M	.44 2	1M					1M	2M
Orconectes immunis(19)	1M		2M	.23 4	.42 3		1M					1M	2M
Cambarus robustus(19)	1M		1M	.50 4	2M	.18 2	1M					1M	4M
Cambarus bartonii(19)	1M		1M	.50 2	1M	.49 2	1M					.16 2	4M
Cambarus latimanus(19)	1M			.46 3		.44 2	1M					1M	5M
Palinuridae													
Panulirus interruptus(16)	3M				.19 2	1M	.06 2					.44 3	6M
Panulirus cygnus(16)	4M			.49 2	.19 2	1M						1M	6M
Jasus edwardsii(24)	P 2				P 2						1M	2M	
Jasus novae hollandiae(24)		P 2			P 2						1M	2M	
Scyllaridae													
Thenus orientalis(16)	2M			.04 2	.48 2	1M					1M		.09 2
Callianassidae													
Callianassa californiensis (16)	2M			.16 2	.66 4	1M	1M				.16 2	3M	5M
Callianassa sp.(16)		3M		.13 2	.46 4	.67 4						2M	
Upogebia pugettensis (16)	3M			.49 2	.33 3	1M	.6 2				.22 2	.43 3	4M
Galatheidae													
Galathea californiensis(16)	.58 4			.30 2	.12 2	.42 4					1M		3M
Munida hispida (16)	.27 3			.05 2	.25 2	.90 2	.13 2					1M	4M
Munidopsis diomedea(15)					2M			1M					3M
Munidopsis hamata(20)	2M	.47 2		1M	.07 2	.52 3		3M				3M	
Porcellanidae													
Pachycheles rudis(16)	2M		1M		.04 2	2M						1M	4M
Petrolisthes cinctipes(16)	.49 2			1M	.32 2		1M					3M	3M
Hippidae													
Emerita analoga(16)	1M			.09 2	.28 2							2M	2M
Hippa pacifica(16)	1M	2M			4M	1M						1M	
Paguridae													
Pagurus granosimanus(16)	.05 2			1M	.44 2	1M	.05 2					1M	2M
Coenobitidae													
Coenobita compressus(16)				2M	3M	.55 5							3M
Coenobita clypeatus(16)				2M	1M								1M
Diogenidae													
Calcinus obscu-	.04	1M	1M	1M	6 3								

Subphylum Class Order Species[b]	Group II													
	ACPH	ALPH	AMY	AO[1]	EST	LAP	ODH	PEP	ADH	AO[2]	SDH	TO	PT	PYDH
Calcinus tibicen(16)	.04 2	1M	1M	1M	.25 2	1M							1M	
Clibanarius panamensis(16)	3M	.47 2	1M	.54 3			1M					1M	1M	
Clibanarius albidguis(16)	.05 2	3M	1M	.03 2	2M		1M						1M	
Clibanarius antillensis(16)	2M		1M	1M	6M	.06 2							1M	
Calappidae Matuta lunaris (16)	1M			1M	4M		1M						6M	
Matuta planipes (16)	.05 2			2M	3M		1M						3M	
Cancridae Cancer gracilis (16)				.28 2	.52 3	1M	1M				1M	2M	3M	
Cancer magister (16)				1M	.11 2	1M	1M					3M	5M	
Portunidae Callinectes arcuatus(16)	1M			.45 2	.48 3		.23 2					1M	2M	
Callinectes sapidus(16)	1M		1M	2M	.46 3	1M	1M					1M	2M	
Portunus sanguinolentus(16)				.48 3	.23 2	.66 3	1M					3M	.17 2	
Charybdis callianassae (16)	1M			3M	4M	.06 2							.24 2	
Charybdis sp. (16)				1M	.11 2								.33 2	
Xanthidae Panopeus purpureus(16)	.54 4	.26 3		.29 2	6M							1M	3M	
Rhithropanopeus harrisii(16)		1M			3M			2M						
Xanthodius sternbergii(16)		1M	.32 2	.27 2			1M					2 M	2M	
Ocypodidae Ocypode occidentalis(16)		2M		.18 2	.18 2	.19 3							2M	
Ocypode quadrata (16)		2M		.13 2	.05 2	3M							2M	
Uca musica(16)	1M	.77 6		.04 2	2M	1M							2M	
Uca princeps(16)	1M	1M		1M	.08 2	1M	1M						3M	
Uca speciosa(16)	1M				4M							1M	.42 2	
Uca spinicarpa (22)	1M				4M							1M	.09 2	
Grapsidae Hemigrapsus oregonensis(16)	.06 2		2M	2M	.04 2	1M	1M				1M		.43 2	
Pachygrapsus crassipes(16)	.23 2	.04 2	2M	2M	2M								3M	
Pachygrapsus transversus(16)	.42 2	.02 2	2M	4M	.34 4								2M	
Gecarcinidae Gecarcinus quadratus(16)		.05 2		3M	.09 2	1M							4M	
Sesarma cinereum(21)		1M			3M			1M				1M		

[a]Proportion of individuals heterozygous and number of alleles given for loci classified as polymorphic, P; monomorphic loci designated as "M" with prefix indicating number of loci. For each polymorphic protei the most heterozygous locus is listed in the body of the table. Enzymes grouped according to Gillespie and Kojima (1968; see text for additional references). Summary measures of variation defined in text: n_a, average number of allozymes detected per locus; P, proportion of loci polymorphic; H, averace proportion of heterozygous individuals per locus. Abbreviations for proteins are as follows: Group I-ALDO, Aldolase; FUM Fumarase; G6-PDH, Glycose-6-Phosphate dehydrogenase; GDH, glylamate dehydrogenase; GOT glutamate-oxaloacetate transminase; G-3PDH, glyceraldehyde-3-phosphate dehydrogenase; GPDH, glycerophosphate dehydrogenase; HBDH, hydroxybutyrate dehydrogenase; HK, hexokinase; IDH-isocitrate, dehydrogenase; LDH, lactate dehydrogenase; MDH, Malate dehydrogenase; ME, malic enzyme(NADP-dependent MDH); MPI, mannose-phosphate isomerase; PGM, Phosphoglucomutase; 6-PGDH, 6-phosphogluconate dehydrogenase; PGI phosphoglucose(phosphohexose) isomerase; TPI, triosephosphate isomerase; XDH, xanthine dehydrogenase. Group II - ACPH, acid phosphatase; ADH, alcohol dehydrogenase; ALPH, Alkaline phosphatase; AMY, amylase; AO, acetaldehyde oxidase; EST, esterase; LAP, leucine aminopeptidase; ODHOctanol dehydrogenase, PEP pept ADH, alcohol dehydrogenase; ALPH, alkaline phosphatase; AMY, amylase; AO, acetaldehyde oxidase; EST, esterase; LAP, leucine aminopeptidase; ODH, octanol dehydrogenase, PEP peptidase; PER peroxidase, SDH; sorbitol (inositol) dehydrogenase. Other-TO, letrazolium oxidase (superoxidase dismutase); PT, proteins revealed by nonspecific general protein stain.

[b]References: (1) Hebert, 1974c; (2) Berger and Sutherland, 1978; (3) Smith and Fraser, 1976; (4) Battag et al., 1978a; (5) Dando et al., 1979, (6) Hedgecock, 1979; (7) D. Hedgecock and K. Nelson, unpublished data cited in Hedgecock et al., 1982 (8) Redfield et al., 1980; (9) Ayala et al., 1975; (10) Ayala and Valentine, 1979; (11) Lester, 1979; (12) Fuller and Lester, 1980; (13) Hedgecock et al., 1979; (14) Berthelemy, 1978; (15) Gooch and Schopf, 1973; (16) Nelson and Hedgecock, 1980; (17) Tracey et al., 1957; (18) Hedgecock et al., 1977; (19) Nemeth and Tracey, 1979; (20) Costa and Bisol, 1978; (21) Gooch, 1977; (22) Salmon et al., 1979; (23) Lester, 1983; (24) Smith et al., 1980; (25) Mulley and Latter, 1980; (26) Richardson, 1982; (27) Dematthaeis et al., 1983;

[c]These averages include summary measures of variation reported for four harpacticoid copepods and a gammarid amphipod (Battaglia et al., 1978a), another gammarid amphipod (Battaglia et al., 1978a), another gammarid amphipod (Gooch and Hetrick, 1979) and three thoracican barnacles (Nevo, 1978).

The table would show that only little work has been carried out on shrimps to find out the genetic structure of their populations.

Out of 31 enzymes studied so far in crustaceans as given in the above table, 15 enzymes have been related for the present study on shrimps and the results on these isozymes studies are given in the following sections.

Results:

Genetic basis of the observed electrophoretic variation:

DNA (Deoxyribonucleic acid) is a giant molecule composed of a sequence of subunits called nucleotides attached to a sugar-phosphate backone. Triplet combination of these nucleotides are the genetic code for each aminoacid, which forms the back bone of protein molecule. A segment of DNA which codes for a single amino acid sequence is called a gene. The location of that segment of DNA in relation to total DNA is called the locus for that gene. These segments of DNA which codes for specific polypeptides are grouped together to form chromosomes. In diploid organism two copies of chromosomes are present and result in two genes per locus per individual. Different genes that occur in the same locus are called alleles. Alleles differ from one to other by minor amino acid substitutions in the polypeptides. When alleles in a locus are products of similar base sequence it will have similar allele combination termed homozygote. However if the base sequence differed slightly in a locus, the product will be a combination of two different alleles called heterozygote. A locus is considered to be polymorphic when frequencies of the common allele was not greater than 0.95 and thus reveal electrophoretic variants. Enzyme Loci in which all specimens

tested appeared as a single monomorphic band were classified as monomorphic loci. Those with two or more well separated zones of activity are assumed to be the products of two or more loci. The enzyme loci in different tissues tested were numbered consecutively from the most cathodal to the most anodal and alleles were designated alphabetically.

Electrophoresis conducted in the present investigation helped to detect allelic and non-allelic forms of proteins in each individual of the species tested and thus enabled to find out the differences between heterozygotes and homozygotes and to quantify the number of individuals with each respective genotypes for the analysis of the genetic structure of each population sample drawn from Penaeus indicus and Parapenaeopsis stylifera species. (Table 44-47)

Genetic variation studies were carried out in fifteen enzymes, such as acid phosphatase, alcohol dehydrogenase, aldehydeoxidase, aldolase, alkaline phosphatase, esterase, alpha-glycerophosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, malic enzyme octanol dehydrogenase, 6-phosphogluconate dehydrogenase, 1-pyrroline dehydrogenase, sorbitol dehydrogenase, and tetrazolium oxidase in different tissues like eye, hepatopancreas and muscle of Penaeus indicus and Parapenaeopsis stylifera (Table 69, 70). Alternative

enzyme forms derived from different loci are isozymes where as multiple enzymes encoded by alternative alleles at a locus are allozymes.

In the case of monomeric proteins each homozygote showed one protein band in the gel after staining. When single banded allozymes were electrophoretically different, the homozygotes differed in their location in the gel. In heterozygote both products of the homozygotes were formed and thus 2 bands were observed in the gel.

In the dimeric proteins two polypeptide chains are present in the protein globule and hence three bands are generally found in the heterozygous individuals.

Acid phosphatase:

Penaeus indicus

Expression of this enzyme in eyes, hepatopancreas and muscle was analysed. According to their relative mobility, and staining intensity of the bands five zones of enzyme activity were observed. Numbering of bands was done from the cathodal end ie. from slowest migrating band, to anodal end i.e. fastest migrating band. Thus four loci (Acph 1, 2, 4, 5) were identified in hepatopancreas whereas muscle showed Acph-3 locus and eye Acph-1 and Acph-2 locus. Expression

observed in different tissues tested is given in Fig. 13. Genetic variation study was carried out in muscle and eye tissues of the P. indicus population samples collected from four different areas viz. Cochin, Tuticorin, Madras and Waltair. But Acph-3 locus was found to be polymorphic only in muscle tissue analysed in all the localities. A two banded phenotype of presumed heterozygotes is consistent with a monomer subunit structure of the enzyme found with 2 alleles (Table 48). These two alleles, one slow moving and the other fast moving were designated as A and B respectively (Fig. 14; Plate 15). A and B frequencies of these two alleles are given in the table 71. Gene frequency of dominant allele is some what similar.

Lack of Hardy-Weinberg equilibrium was noticed due to heterozygote deficiency and excess of homozygotes at all stations. Observed and expected frequency of heterozygote were calculated (Table 73).

P. stylifera:

Acid phosphatase was analysed for genetic variation in P. stylifera collected from Cochin and Bombay. Four different zones of activity two regions in hepatopancreas and one region in muscle and eye tissues was observed (Fig.15). Thus the two regions seen in hepatopancreas represent Acph-3

Table 44: Relative mobility (R.M.) values with their intensities of different enzymatic proteins analysed in different tissues of Penaeus indicus.

Enzyme	Tissue	RM	Intensity
Malic enzyme	Eye	8.3-10	xx
	Muscle	21.7-26.6	x
Malate dehydrogenase	Eye	23.3-28.3	xx
		26.7-33.3	x
		40-50	x
Lactate dehydrogenase	Eye	25-28.3	x
	Hepatopancreas	8.3-11.7	x
	Muscle	8.3-11.7	x
		25-28.3	xx
Sorbitol dehydrogenase	Hepatopancreas	18.3-22	xx
	Muscle	13.3-16.7	xx
Aldehydeoxidase	Hepatopancreas	17.2-20	xx
		28.3-30	xx
	Muscle	33-36.7	xx
Alcoholdehydrogenase	Eye	13.3-18.3	xx
		28.3-35	xx
	Hepatopancreas	8-12	x
		13.3-16.7	xx
	Muscle	13.3-16.7	xx
Acid Phosphatase	Eye	6.7-10	xx
		26.7-33.3	xx
	Hepatopancreas	6.7-10	xx
		26.7-33.3	xx
		66.7-75	x
		80-86.7	xx
	Muscle	33-36	xx
Tetrazolium oxidase	Eye	5-7	
		23.3-28.3	
	Hepatopancreas	33.3-38	
		41.7-46.7	
	Muscle	23.3-26.7	

Contd....

Enzyme	Tissue	RM	Intensity
1-Pyrroline dehydrogenase	Hepatopancreas	36.7-40	xx
	Muscle	16.7-20	xx
6-Phosphogluconate dehydrogenase	Eye	8.3-11.7	x
		30-40	x
	Hepatopancreas	31-33.3	xx
	Muscle	33-41.7	x
Octanol dehydrogenase	Eye	17-20	xx
	Hepatopancreas	15-18.3	xx
	Muscle	23.3-30	xx
Alpha Glycerophosphate dehydrogenase	Hepatopancreas	20-23	xx
Aldolase	Muscle	18.3-20	xx

Table 45: Relative mobility (RM) with their intensities for the different genotypes of various enzymes analysed in Penaeus indicus.

Enzyme	Locus	Genotype	RM	Intensity
Acid Phosphatase	Acph-3	AA	25-27.3	xx
		AB	25-27.3	x
			34-36.3	x
		BB	34-36.3	xx
Aldehyde oxidase	Ao-1	AB	16.7-20	x
			21.7-25	x
			28.3-30	xx
		AA	16.7-20	xx
			28.3-30	xx
		AB	16.7-20	x
			21.7-25	x
			28.3-30	xx
		BB	21.7-25	xx
			28.3-30	xx
Aldolase	Ald-1	AA	18.3-20	xx
		AB	18.3-20	x
			21.0-23.3	x
		BB	21.0-23.3	xx
		BC	21.0-23.3	x
			25-26.7	x
		CC	25-26.7	x
Alkaline phosphatase	Alph-	AA	50-53.3	xxx
		AB	50-53.3	xx
			55-58.3	xx
		BB	55-58.3	xxx
Octanol dehydrogenase	Odh-2	AA	17.7-20	xx
		AB	17.7-20	x
			23.3-25.3	xx
			28-30.3	x
		BB	28-30.3	xx

Contd.....

Enzyme	Locus	Genotype	RM	Intensity
6-Phosphogluconate dehydrogenase	6-Pgdh-2	AA	31.0-33.3	xx
		AB	31.0-33.3	x
			35-36.7	x
		BB	35-36.7	xx
Malate dehydrogenase	Mdh-2	AA	31.7-36.7	xx
			23-28	x
			31.7-36.7	x
		BB	23-28	xx
Malic enzyme	Me-1	AA	8.3-10	xx
		AB	8.3-10	x
			15-16.7	x
		BB	15-16.7	xx

Table 46: Relative mobility (RM) with their intensities of different enzymatic proteins analysed in different tissues of *Parapenaeopsis stylifera*.

Enzyme	Tissue	RM	Intensity
Alcohol dehydrogenase	Eye	15-18.3	xx
	Hepatopancreas	30-33.3	xx
	Muscle	15-18.3	xx
Octanol dehydrogenase	Eye	30-33.3	xx
	Hepatopancreas	30-33.3	x
		40-45	xx
	Muscle	13.3-16.7	xx
		28.3-31.7	x
Glycerophosphate dehydrogenase	Hepatopancreas	16.7-20	xx
Malate dehydrogenase	Eye	20-25.3	xx
	Muscle	41.7-48.3	x
Acid Phosphatase	Eye	8.2-13.3	xx
	Hepatopancreas	30-33.3	xx
		66.7-70	x
	Muscle	15.2-17	xx
Alkaline phosphatase	Eye	21.7-25	x
	Hepatopancreas	46.7-50	xx
	Muscle	30-33	xx
Tetrazolium oxidase	Eye	3.3-8.3	
	Hepatopancreas	3-5	
		25-30.3	
	Muscle	3.3-8.3	
Aldehydeoxidase	Hepatopancreas	18.3-21.7	xx
		31.7-35	x
		6.7-8.3	x
Malie enzyme	Eye	8.3-10	x
	Muscle	10-13.3	x

Contd...

Enzyme	Tissue	RM	Intensity
Esterase	Eye	10–13.3	xx
		18.3–20	xx
		30–35.3	x
	Hepatopancreas	8–12.3	xx
		30–33.3	x
	Muscle	18.3–20	xx
1-Pyrroline dehydrogenase	Hepatopancreas	16.7–20	x
	Muscle	16.7–20	x

Table 47: Relative mobility (RM) with their intensities for the different genotypes of various enzymes analysed in *Parapenaeopsis stylifera*

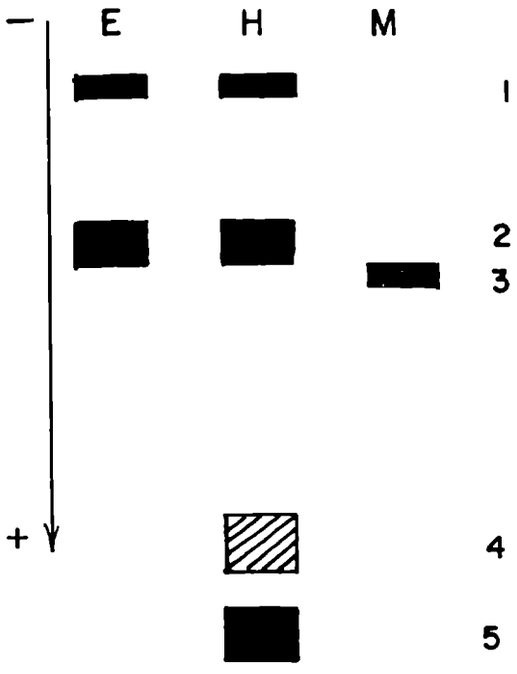
Enzyme	Locus	Genotype	RM	Intensity
Acid Phosphatase	Acph-2	AA	15.3-17	xx
		AB	15.3-17	x
			18.4-20	x
		BB	18.4-20	xx
Alkaline phosphatase	Alph-2	AA	25-27.2	xx
		AB	25-27.2	x
			29-30.5	x
		BB	29-30.5	xx
Alcohol dehydrogenase	Adh-2	AA	30-32.2	xx
		AB	30-32.2	x
			34-36.2	x
		BB	34-36.2	xx
Esterase	Est-1		10-13.3	xx
	Est-2	AA	22.2-24	xx
		AB	18.3-20	x
			22.2-24	x
		BB	18.3-20	xx
	Est-3		30-35.3	x
Malatedehydrogenase	Mdh-2	AA	20-25.2	xx
		AB	20-25.2	x
			38.3-43	x
		BB	38.3-43	xx
Tetrazolium oxidase	To-1	AA	3.3-5	
		AB	3.3-5	
			6.2-8	
		BB	6.2-8	
	To-2	CC	25.2-30	
		CD	25.2-30	
			33.3-38	

Table 48: Observed and expected phenotype frequency of acid phosphatase (Acph-3) observed in *Penaeus indicus* with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	18	2	16	28.6
	Expected	10	18	8	
Tuticorin	Observed	17	2	17	28.4
	Expected	9	18	9	
Madras	Observed	21	1	14	30.6
	Expected	13	17	6	
Waltair	Observed	23	1	12	32.1
	Expected	25.3	16.3	4.3	

Fig. 13. Expression of acidphosphatase in different tissues of *Penaeus indicus*.

Fig. 13



E – Eye

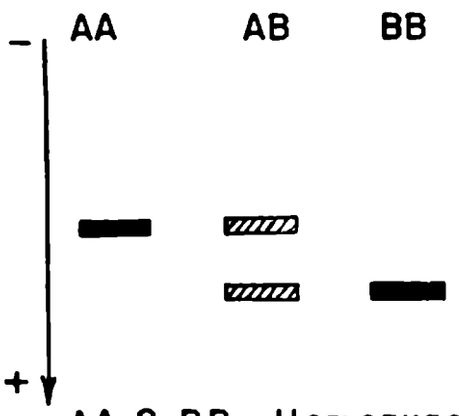
H – Hepatopancreas

M – Muscle

Fig. 14. Expression of different genogypes of acid phosphatase (Acph-3) in muscle tissue of *Penaeus indicus*

Fig. 15. Expression of acid phosphatase in different tissues of *Parapenaeopsis stylifera*.

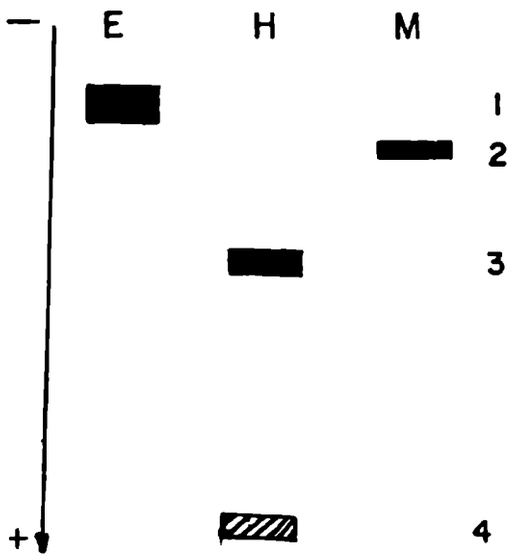
Fig. 14



AA & BB – Homozygote

AB – Heterozygote

Fig. 15



E-Eye

H-Hepatopacreas

M-Muscle

Fig. 16. Expression of different genotypes of acid phosphatase (Acph-2) in muscle tissue of *Parapenaeopsis stylifera*.

Fig. 17. Expression of alcohol dehydrogenase in different tissues of *Penaeus indicus*.

Fig. 16

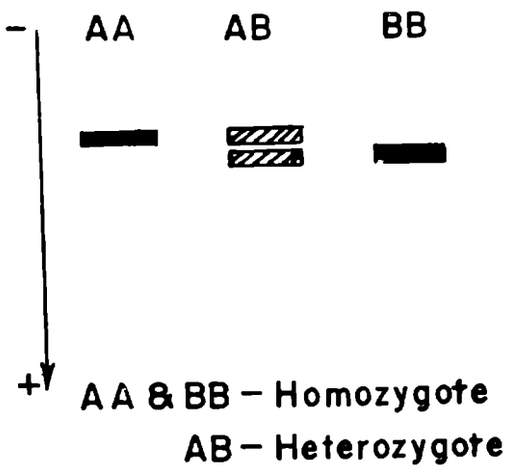

−
AA
AB
BB
+
AA & BB – Homozygote
AB – Heterozygote

Fig. 17

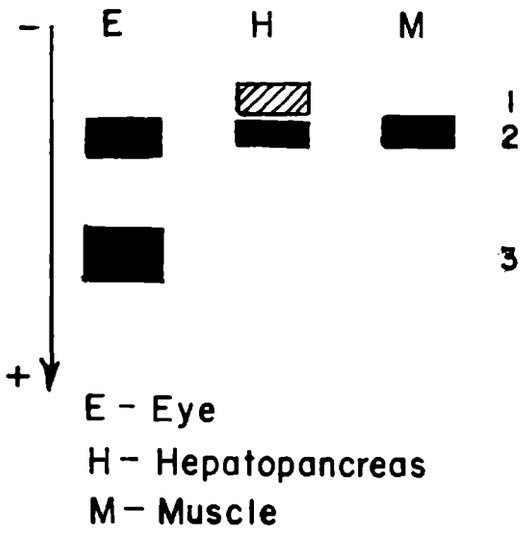
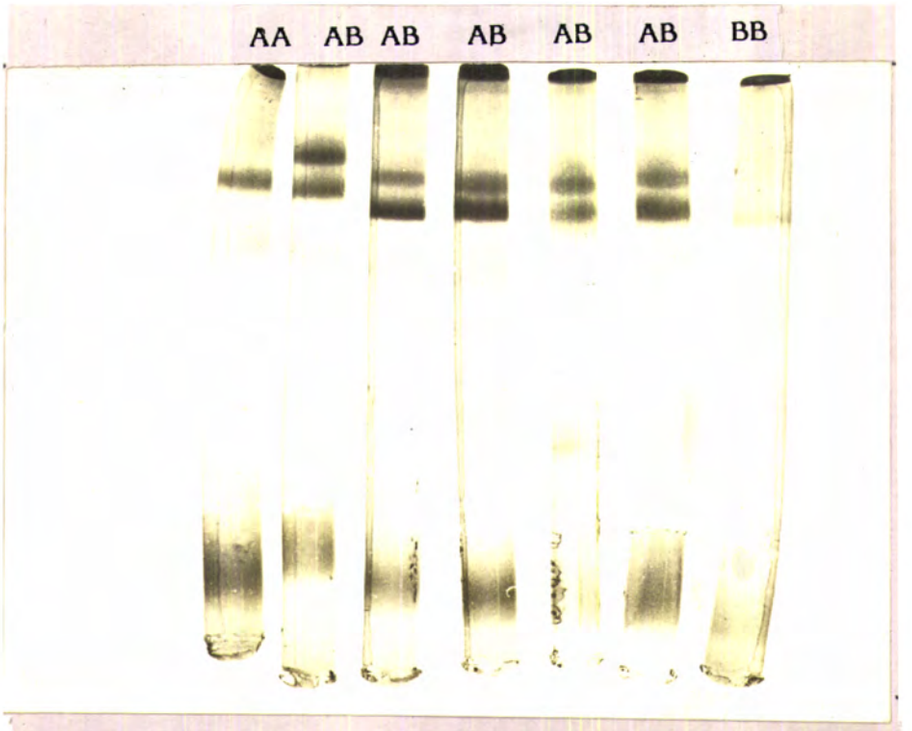
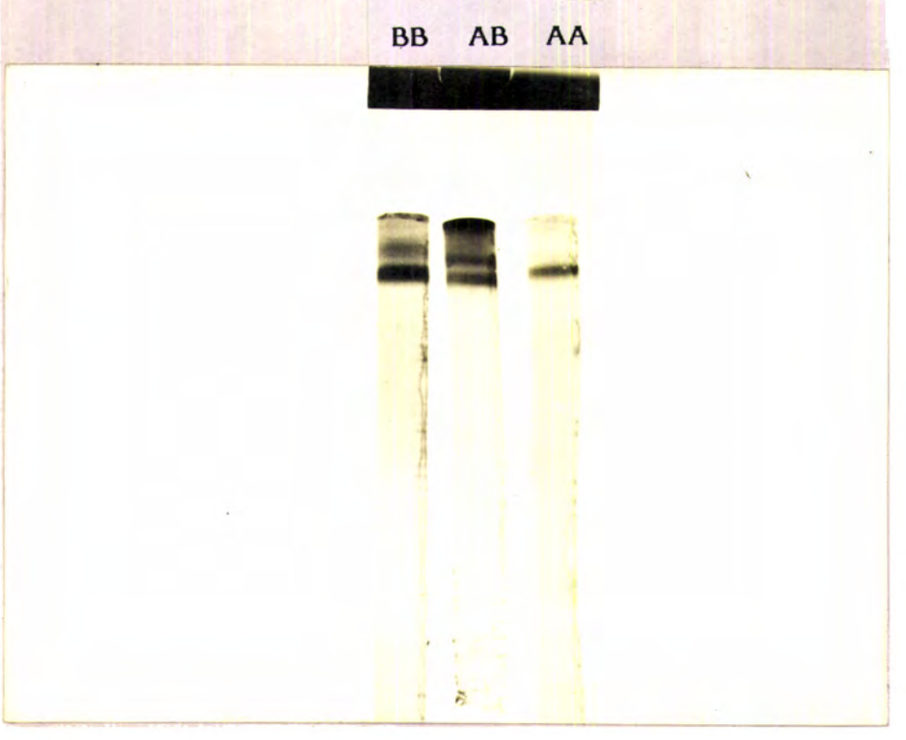

−
E
H
M
1
2
3
+
E – Eye
H – Hepatopancreas
M – Muscle

Plate 15: Showing the different genotypes of acid phosphatase (Acph-3) in muscle tissues of Penaeus indicus AA, BB,-homozygotes AB-heterozygote.

Plate 16: Showing the different genotypes of acid phosphatase (Acph-2) in muscle tissues of Parapenaeopsis stylifera, AA, BB-homozygotes, AB-Heterozygote.


AA
AB
AB
AB
AB
AB
BB


BB
AB
AA

and Acph-4 whereas in muscle and eye showed Acph-2 and Acph-1 respectively. Genetic analysis was carried in eye and muscle tissues. Acph-2 locus in muscle tissue exhibited two banded heterozygote expression indicating monomer structure of the enzymes. Genetic variation studies in these tissues revealed Acph-2 found in muscle to express polymorphism in all the localities (Fig. 16; Plate 16).

The allele frequency observed is given in Table 72. There seems to be significant difference in the phenotypic distribution and allele frequency between the samples of Cochin and Bombay. Expected and observed phenotypic expression were seen in Table 49. Observed and expected of heterozygote was also found out (Table 74).

Alcohol dehydrogenase:-

Phenaeus indicus

Samples for genetic variation studies of P. indicus were collected from Cochin, Tuticorin, Madras and Waltair. Alcohol dehydrogenase activity appeared in three zones which were coded by three genetic loci (Fig. 17). Eye tissue showed 2 loci one fast migrating Adh-3 locus and one slow migrating Adh-2 locus whereas hepatopancreas expressed the Adh-1 and Adh-2 locus and muscle with Adh-2 locus. The Adh-2 locus represented in all tissues. Genetic variation analysis was

Table 49: Observed and Expected phenotype frequency of acidphosphatase (Acph-2) observed in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	25	3	8	22.3
	Expected	19.5	14	2.5	
Bombay	Observed	11	2	22	24.2
	Expected	4.3	16.3	15.3	

Table 50: Observed and expected phenotype frequency of alcohol dehydrogenase (Adh-2) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	34	0	2	40.025
	Expected	32	3.8	.1	
Bombay	Observed	32	1	3	29.04
	Expected	29.3	6.3	0.3	

carried out in muscle and hepatopancreas tissues. Allele frequency calculated showed the common allele to have value more than 0.95. Thus these loci were considered to be monomorphic.

Parapenaeopsis stylifera:

In Parapenaeopsis stylifera also alcohol dehydrogenase was employed for the genetic variation analysis for the samples collected from two localities viz. Cochin and Bombay. Two zones of enzymes activity expressed the presence of loci Adh-2 in hepatopancreas and Adh-1 in eye and muscle tissues (Fig.18) Genetic variation studies in eyes and hepatopancreas confirmed Adh-2 locus in hepatopancreas to be polymorphic. Different genotype pattern observed is given in Fig. 19(Plate 17).

Phenotypic expression observed is given in Table 50. The allele frequency was calculated and didn't express much difference between the two places (Table 72). Observed and expected frequency of heterozygote is given in Table 74.

Aldehyde oxidase:

Penaeus indicus

Genes controlling this enzyme was identified in the samples which were collected from four different locations

Fig. 18. Expression of alcohol dehydrogenase in different tissues of *Parapenaeopsis stylifera*.

Fig. 19. Expression of different genotypes of alcohol dehydrogenase in hepatopancreas tissues of *Parapenaeopsis stylifera*

Fig. 18

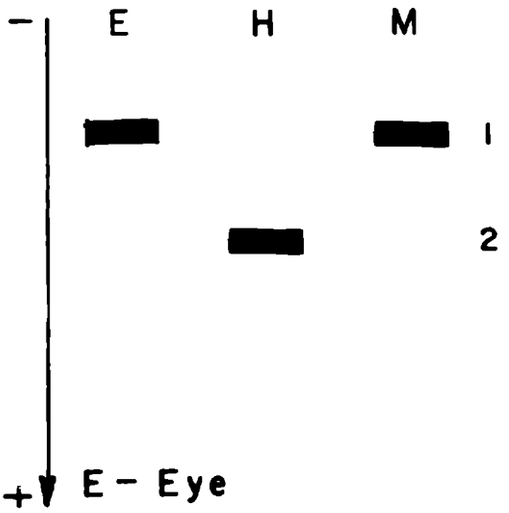

−
E
H
M
1
2
+
E – Eye
H – Hepatopancreas
M – Muscle

Fig. 19

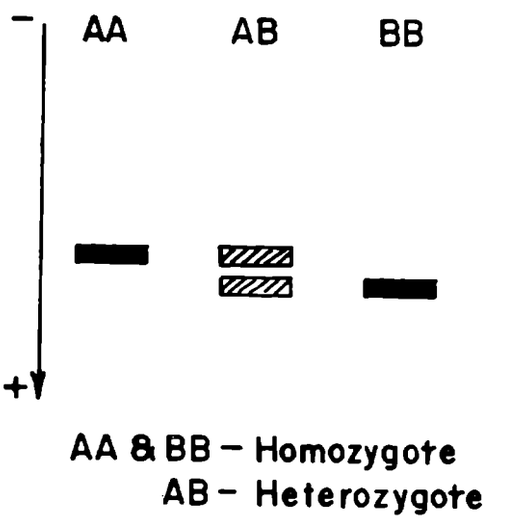
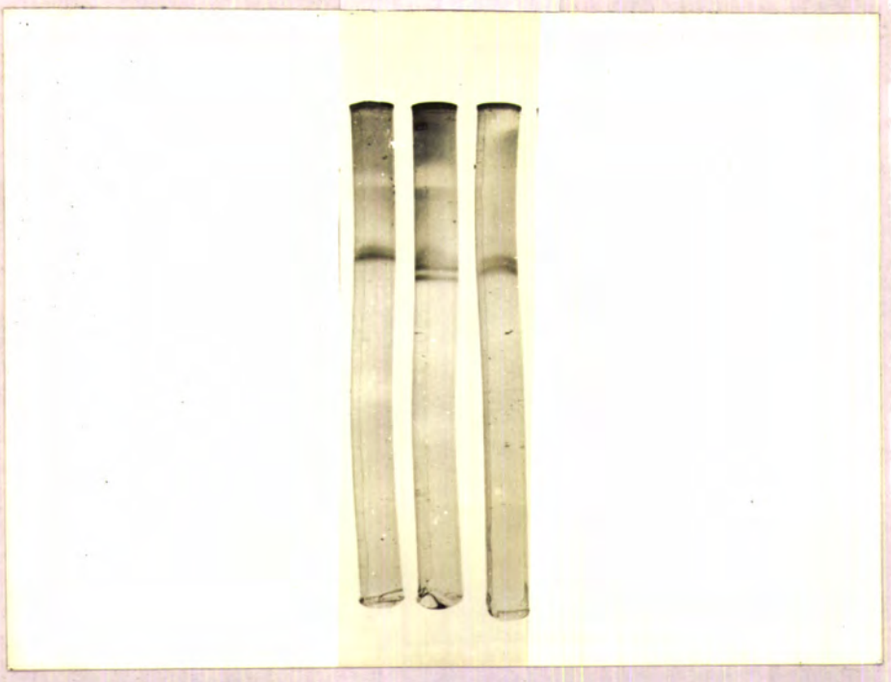

−
AA
AB
BB
+
AA & BB – Homozygote
AB – Heterozygote

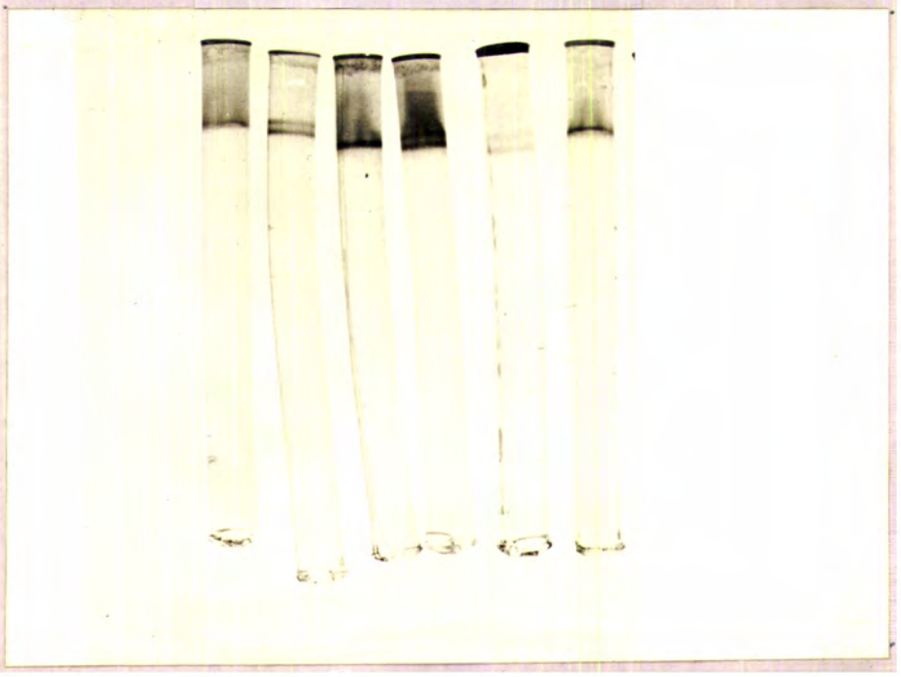
Plate 17: Showing the different genotypes of alcohol dehydrogenase (Adh-2) in hepatopancreas tissue of Parapenaeopsis stylifera AA, BB-homozygotes, AB-heterozygote.

Plate 18: Showing the different genotypes of aldolase (Ald-1) in muscle tissues of Penaeus indicus AA, BB, CC-hemozygotes, AB, BC, heterozygotes

AA AB BB



AA AB BB BC BC CC



viz. Cochin, Tuticorin, Madras and Waltair. Enzymatic expression revealed 3 loci (Fig. 20) viz. 2 loci in hepatopancreas and one in muscle tissue. Ao-1 locus located in slowest migrating zone in hepatopancreas exhibited polymorphism in all the locaties (Fig. 21; Plate 19). Allele frequency was calculated and presented in Table 71. Details of observed and expected phenotypes are tabulated (Table 51).

P. stylifera

Aldehydeoxidase enzyme was examined in eye, hepatopancreas and muscle tissues expressed 3 loci giving rise to three bands of aldehydeoxidase activity in the zymogran (Fig. 22). Hepatopancreas expressed 2 loci whereas muscle one locus. Monomeric subunit structure of this enzyme was revealed by their phenotypic expression. The two diffused bands were scored as heterozygote. Since the allele frequency value exceeded 0.95 it was considered to be monomorphic.

Observed allele frequencies were given in Table 72. There is not much difference noticed in these values in the samples of Bombay and Cochin for this enzyme.

Phenotypic distribution according to Ao-2 and Ao-3 is given Table 52.

Table 51: Observed and expected phenotype frequency of aldehyde oxidase (Ao-1) observed in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	20	2	14	30.16
	Expected	12	18	6	
Tuticorin	Observed	20	2	14	30.2
	Expected	12	18	6	
Madras	Observed	22	1	13	32.5
	Expected	14	17	5	
Waltair	Observed	23	1	12	32.1
	Expected	15.3	16.3	4.3	

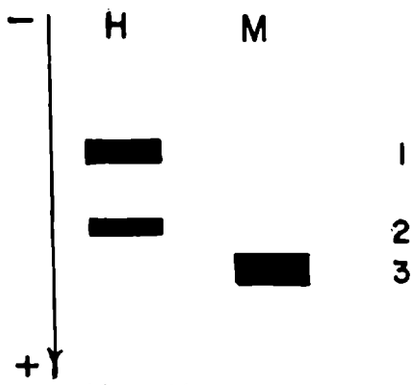
Table 52: Observed and expected phenotype frequency of aldehyde oxidase (Ao-2 and Ao-3) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Ao-2					
Cochin	Observed	34	1	1	16
	Expected	33	2.9	0.06	
Bombay	Observed	34	2	0	0.035
	Expected	34	1.9	0.3	
Ao-3					
Cochin	Observed	35	0	0	3.56
	Expected	34	1.9	0.3	
Bombay	Observed	34	2	9	0.305
	Expected	34	1.9	0.3	

Fig. 20. Expression of aldehyde oxidase in different tissues of Penaeus indicus.

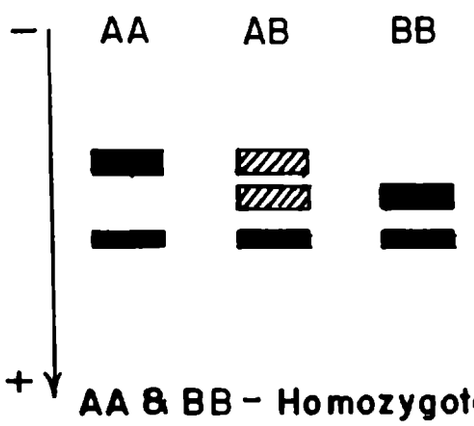
Fig. 21. Expression of different genotypes of aldehydeoxidase (Ao-1) in hepatopancreas tissue of Penaeus indicus.

Fig.20



H − Hepatopancreas
M − Muscle

Fig. 21



AA & BB − Homozygote
AB − Heterozygote

Fig. 22. Expression of aldehyde oxidase in different tissues of *Parapenaeopsis stylifera*.

Fig. 23. Expression of aldolase in muscle tissue of *Penaeus indicus*.

Fig. 24. Expression of different genotypes of aldolase (Ald-1) in muscle tissue of *Penaeus indicus*

Fig.23

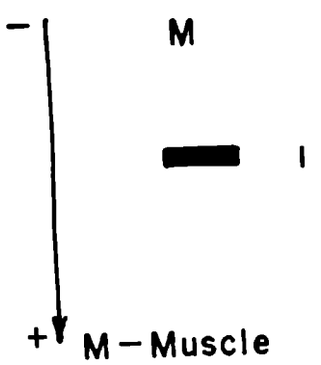


Fig.22

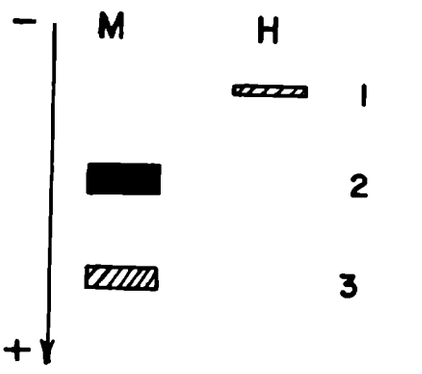


Fig.24

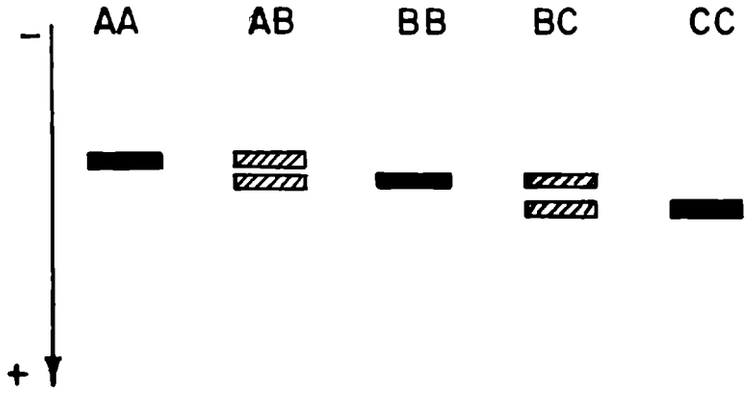
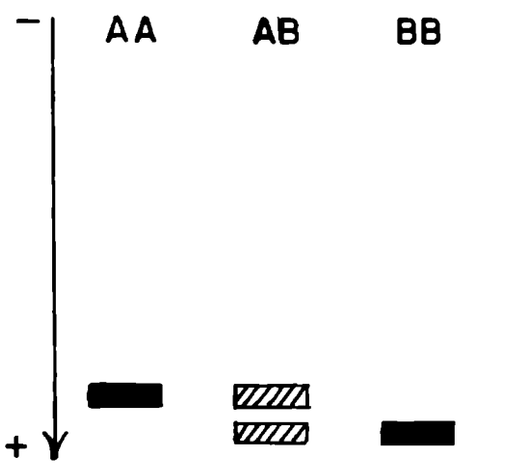


Fig. 25. Expression of different genotype of alkaline phosphatase (Alph) in muscle tissues of *Penaeus indicus*.

Fig.25



AA & BB – Homozygote
AB – Heterozygote

Fig. 26. Expression of alkaline phosphatase in different tissues of Parapenaeopsis stylifera.

Fig. 27. Expression of different genotypes of alkaline phosphatase (Alph-2) in muscle tissue of Parapenaeopsis stylifera.

Fig. 26

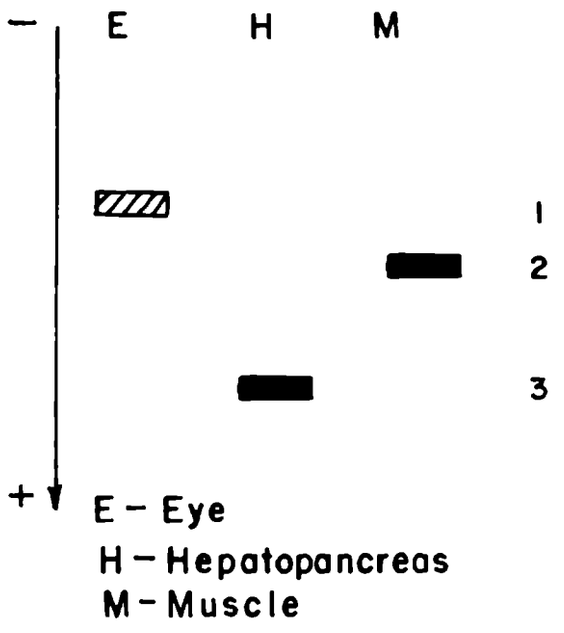

−
E
H
M
1
2
3
+
E – Eye
H – Hepatopancreas
M – Muscle

Fig. 27

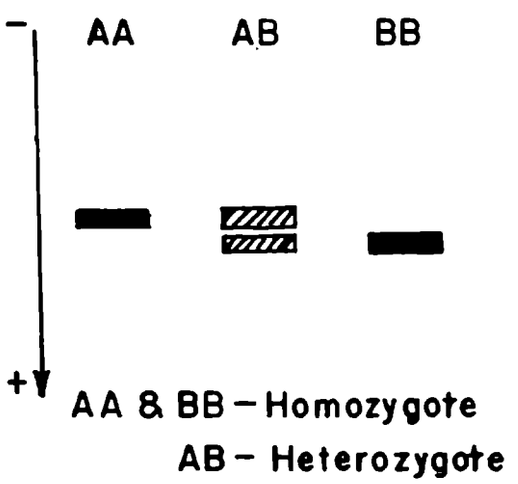
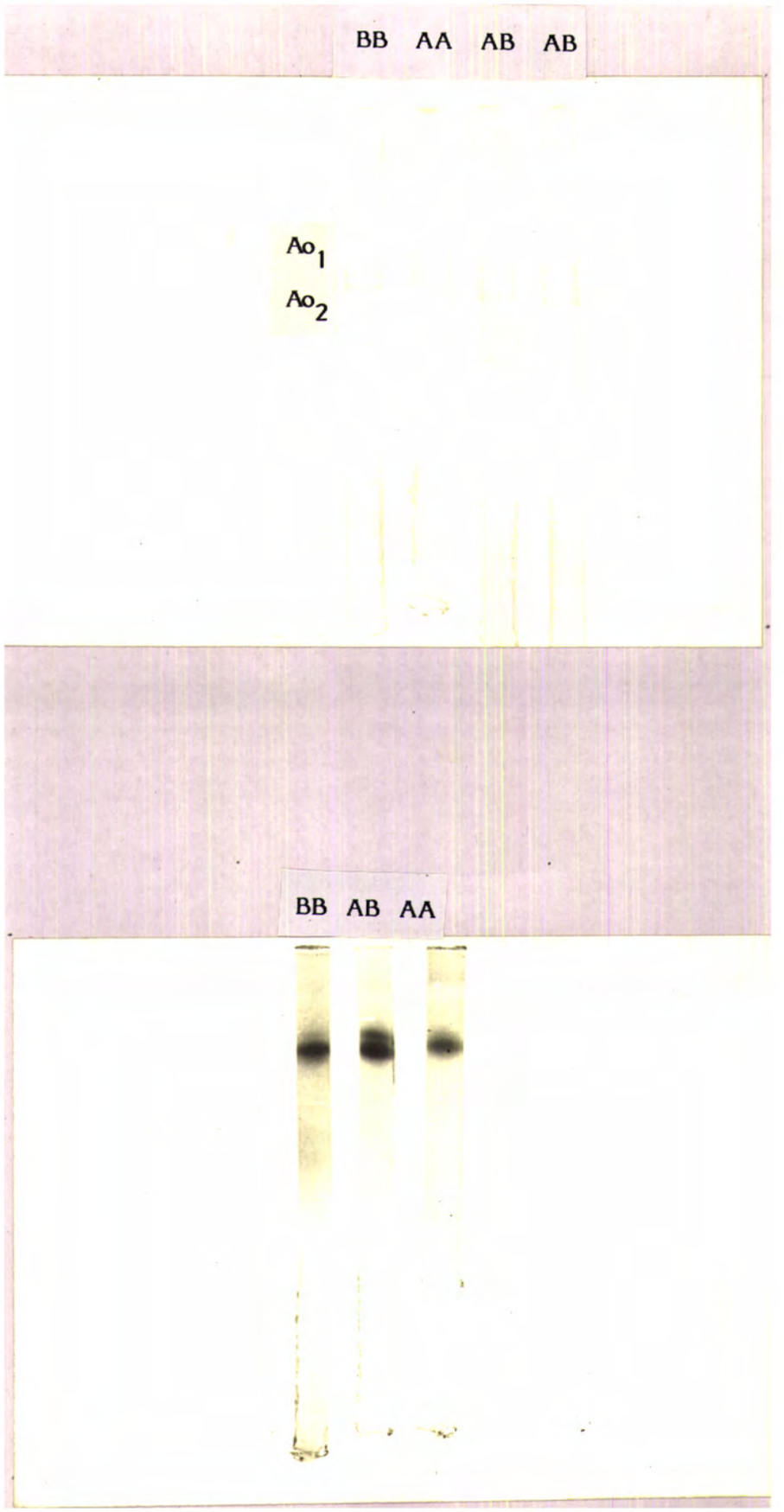

−
AA
AB
BB
+
AA & BB – Homozygote
AB – Heterozygote

Plate 19: Showing the different genotypes of aldehyde oxidase (Ao-1) in hepatopancreas tissue of *Penaeus indicus* AA, BB-homozygotes, BB-heterozygote.

Plate 20: Showing the different genotypes of alkaline phosphatase (Alph-2) in muscle tissues of *Parapenaeopsis stylifera* AA, BB-homozygotes AB-heterozygote.


BB AA AB AB
Ao1
Ao2
BB AB AA

Aldolase:

P. indicus

Aldolase enzyme pattern was analysed in eye, hepatopancreas and muscle of P. indicus collected from different localities like Cochin, Tuticorin, Madras and Waltair. Muscle tissue showed a single zone of enzyme activity (Fig. 23). Polymorphism was observed in all locations with 3 alleles (Fig. 24; Plate 18).

Allele frequency observed is given in Table 71. Details of phenotypes observed is tabulated (Table 53).

Alkaline phosphatase:

P. indicus

This enzyme was analysed for genetic vatiation studies in muscle tissue. Samples were collected only from Waltair. It showed polymorphism in muscle tissue. Phenotypic expression showed a 2 banded heterozygote revealing it to be having monomeric subunit structure (Fig. 25). Number of phenotypes observed is give in table 54.

P. stylifera

Phenotypic expression of alkaline phosphatase enzyme in different tissue were found out. Eye hepatopancreas and muscle showed single zone of enzyme activity in different locations. Thus 3 locus were observed for this enzyme(Fig.26).

Table 53: Observed and expected phenotype frequency of aldolase (Ald-1) observed in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	BC	CC	AC	χ^2
Cochin	Observed	5	1	13	2	15	0	56.59
	Expected	.8	4.3	5.4	12.8	7.6	5	
Tuticorin	Observed	6	2	19	1	8	0	49.994
	Expected	1.4	7.6	10.6	10.3	2.5	3.6	
Madras	Observed	6	1	20	2	7	0	51.08
	Expected	1.2	7.5	12.2	9.9	2	3.1	
Waltair	Observed	4	0	27	2	3	0	60.512
	Expected	0.4	6.22	21.8	6.2	.4	.9	

Table 54: Observed and expected phenotype frequency of alkaline phosphatase (Alph) observed in Penaeus indicus.

Location		AA	AB	BB	χ^2
Waltair	Observed	29	2	5	23.07
	Expected	24.9	10	1	

Table 55: Observed and expected phenotype frequency of alkaline phosphatase (Alph-2) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	32	2	2	14.58
	Expected	30	5.5	.25	
Bombay	Observed	31	2	3	20.84
	Expected	28.4	7.1	.4	

Mu cle was tested for genetic variation. Heterozygote showed 2 bands indicating monomeric structure of the enzyme. Alph-2 locus analysed showed allelic variants (Fig.27; Plate 20) in both location. Phenotypic expressions in this locus is given in Table 55.
Allele frequency observed in given in Table 72.

Esterase:

P. indicus

Tissue expression of esterase was observed in eye hepatopancreas and muscle. The pattern observed for this enzyme was not consistent and therefore genetic variation study couldn't be carried out.

P. stylifera

P. stylifera exhibited polymorphism in esterase enzyme collected from Cochin and Bombay. It expressed two zones of activity in hepatopancreas, one zone of enzyme activity in muscle tissue and three zones of activity in eye tissue (Fig. 28). Genetic analysis was carried out in eye tissue. Phenotypic variation was observed in eye at the Est-2 locus (Fig. 29; Plate 21).

Observed and expected phenotypic frequency was tabulated (Table 56). Allele frequency is given in Table 72.

Table 56: Observed and expected phenotype frequency of esterase (Est-2) seen in Parapenaeopsis stylifera with Chi-square

Location		AA	BB	BB	χ^2
Cochin	Observed	30	3	3	13.8
	Expected	27.6	7.9	.56	
Bombay	Observed	33	1	2	19.2
	Expected	31.2	4.7	.2	

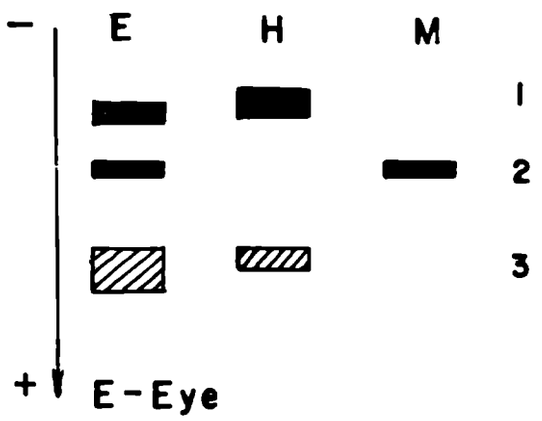
Table 57: Observed and expected phenotype frequency of alpha glycerophosphate (Gpdh-1) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	33	3	0	0.063
	Expected	30	2.9	0.06	
Bombay	Observed	34	1	1	16
	Expected	33	2.9	0.06	

Fig. 28. Expression of esterase in different tissues of Parapenaeopsis stylifera

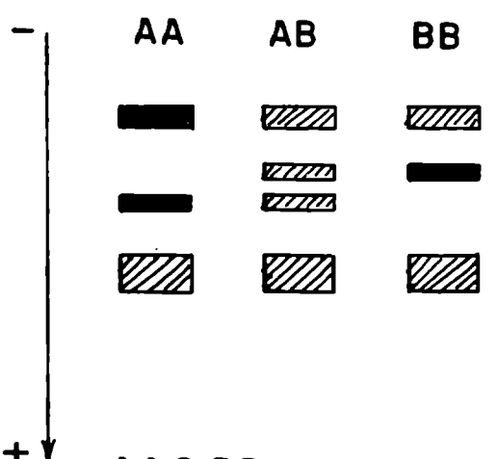
Fig. 29. Expression of different genotypes of esterase (Est-2) in eye tissue of Parapenaeopsis stylifera.

Fig. 28



E - Eye
H - Hepatopancreas
M - Muscle

Fig. 29

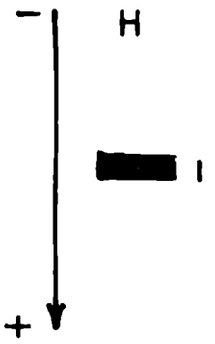


AA & BB – Homozygote
AB – Heterozygote

Fig. 30. Expression of alphaglycerophosphate dehydrogenase in hepatopancreas tissue of *Penaeus indicus* and *Parapenaeopsis stylifera*.

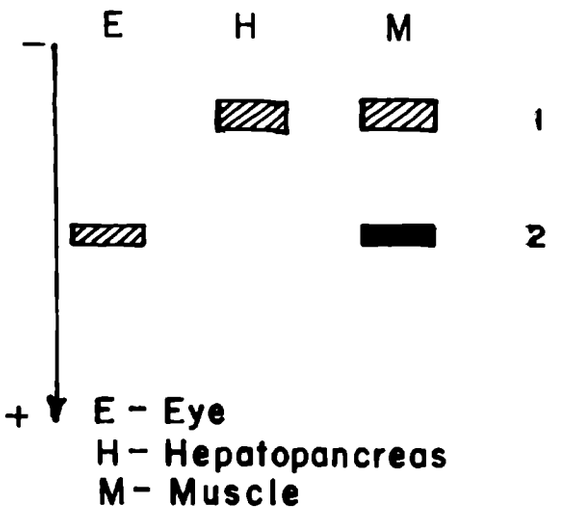
Fig. 31. Expression of lactate dehydrogenase in different tissues of *Penaeus indicus*

Fig. 30



H – Hepatopancreas

Fig. 31

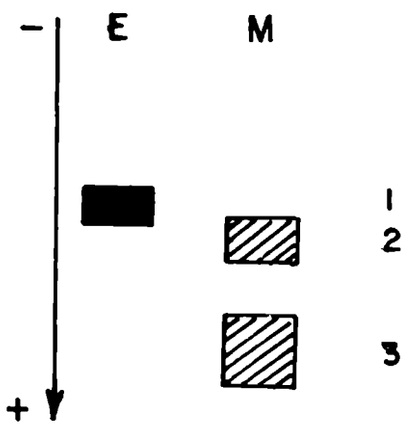


E – Eye
H – Hepatopancreas
M – Muscle

Fig. 32. Expression of malate dehydrogenase in different tissues of *Penaeus indicus*.

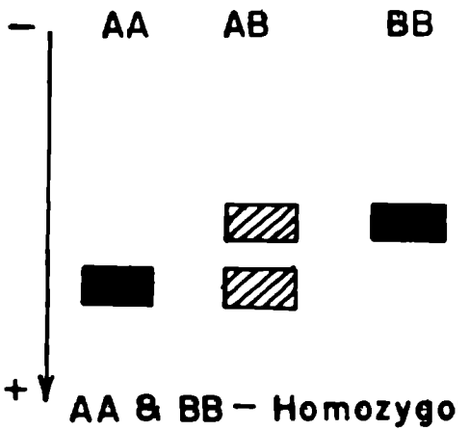
Fig. 33. Expression of different genotypes of malate dehydrogenase (Mdh-1) in eye tissue of *Penaeus indicus*.

Fig.32



E - Eye
M - Muscle

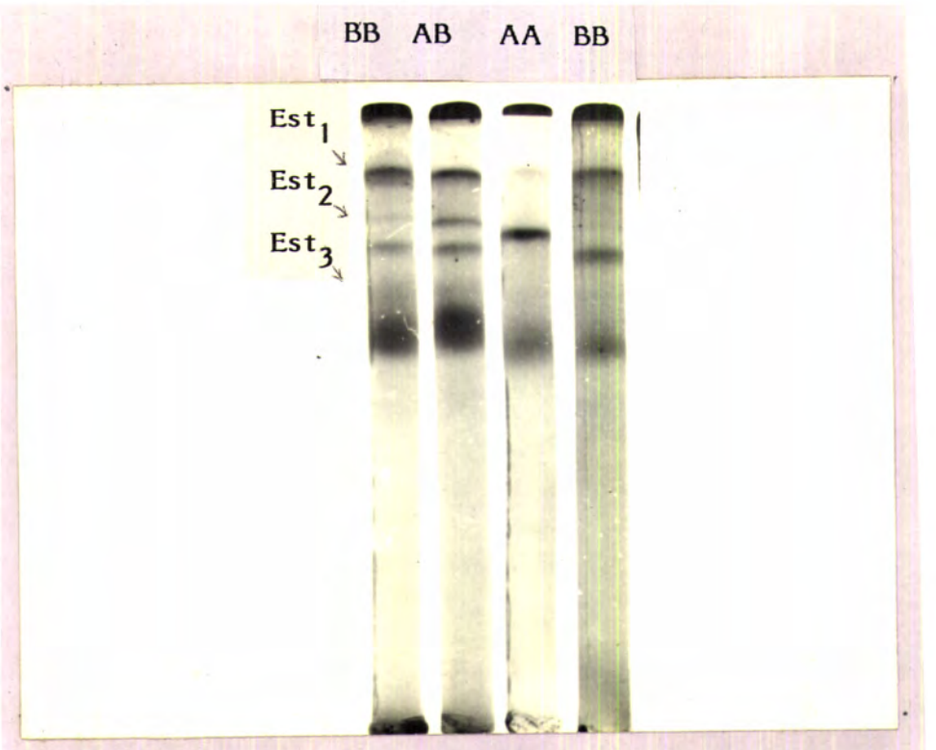
Fig. 33

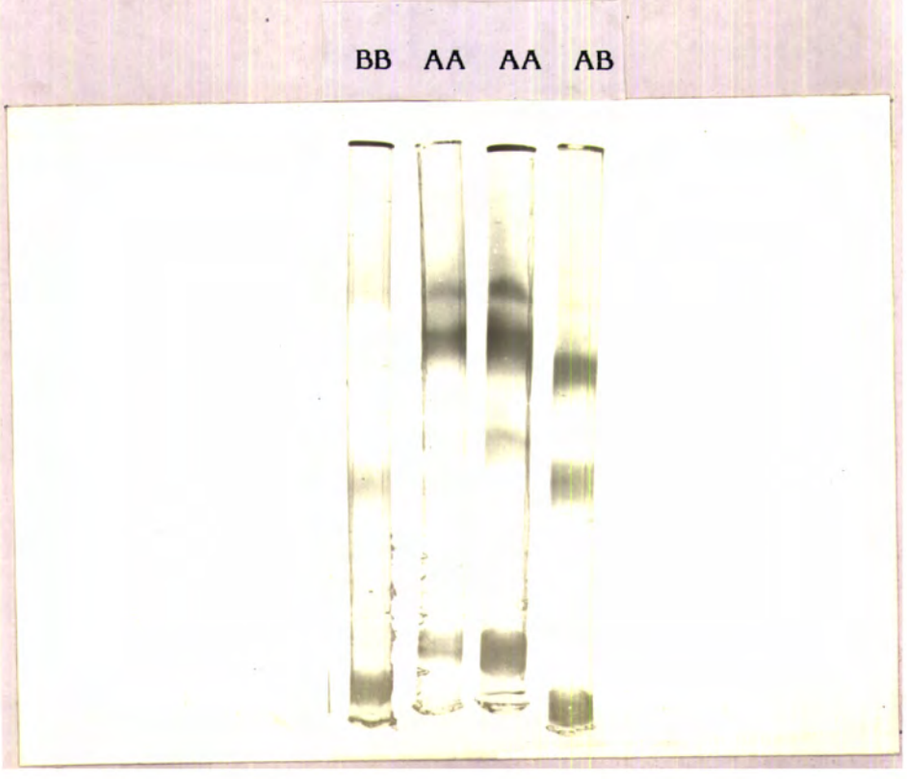


AA & BB − Homozygote
AB − Heterozygote

Plate 21: Showing the different genotypes of esterase (Est-2) in eye tissues of *Parapenaeopsis stylifera* AA, BB-homozygotes, AB-heterozygote

Plate 22: Showing the different genotypes of malate dehydrogenase (Mdh-1) in eye tissue of *Penaeus indicus* AA, BB-homozygotes, AB-heterozygote.


BB AB AA BB
Est1
Est2
Est3


BB AA AA AB

Alpha glycerophosphate dehydrogenase:

P. indicus and P. stylifera showed activity for this enzyme only in hepatopancreas tissue (Fig. 30). Allele frequency was calculated for the observed allelic variants. Since the observed allele frequency of the common allele exceeded 0.95 it was taken as monomorphic loci in both species.

The allele frequency is given in Table 72. The observed phenotypic distribution for P. stylifera is given in Table 57.

Lactate dehydrogenase:

P. indicus

Two zones of lactate dehydrogenase activity were observed. Eye and hepatopancreas expressed one band each having different mobility. These two bands were also observed in muscle tissue (Fig. 31). Since consistent pattern couldn't be observed, these were not utilized for further electrophoretic analysis work.

Malate dehydrogenase:

P. indicus

Malate dehydrogenase resolved as 3 bands of activity in eye and muscle (Fig. 32). Malate dehydrogenase expressed

Table 58: Observed and expected phenotype frequency of malate dehydrogenase (Mdh-1) observed in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	12	1	23	32.1
	Expected	4.3	16.3	15.3	
Tuticorin	Observed	13	1	22	32.4
	Expected	5	16.9	14	
Madras	Observed	10	2	24	27
	Expected	3.4	15.3	17.4	
Waltair	Observed	12	1	23	32.1
	Expected	4.3	16.3	15.3	

Table 59: Observed and expected phenotype frequency of malate dehydrogenase (Mdh-1) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	33	1	2	22.6
	Expected	31	4.6	.17	
Bombay	Observed	30	2	4	21.3
	Expected	26.7	8.6	.69	

monomeric pattern by its 2 banded heterozygote nature in eye. Mdh-1 analysed in eye tissue showed allelic variants (Fig. 33; Plate 22) and showed polymorphism in all the localities sampled. Observed and expected phenotypic frequency is tabulated (Table 58). Allele frequency calculated is given in Table 71.

P. stylifera

P. stylifera expressed two bands of enzyme activity like P. indicus in eyes and muscle tissue. Mdh-1 locus was found to be polymorphic in all the localities (Fig. 34). Two banded heterozygotic nature revealed its monomeric form with 2 alleles (Fig. 35; Plate 23). Phenotypic expression observed in Cochin and Bombay is given in Table 59.

The allele frequency observed is presented in Table 59. Observed and expected phenotype frequency is tabulated (Table 59).

Malic enzyme

P. indicus

Malic enzyme expressed one loci in eyes and another in muscle (Fig. 36). Me-1 found in eyes expressed polymorphism with 2 alleles. Two banded heterozygote was seen (Fig. 37; Plate 24). Phenotypes observed is given in Table 60.

Fig. 34. Expression of malate dehydrogenase in different tissues of *Parapenaeopsis stylifera*.

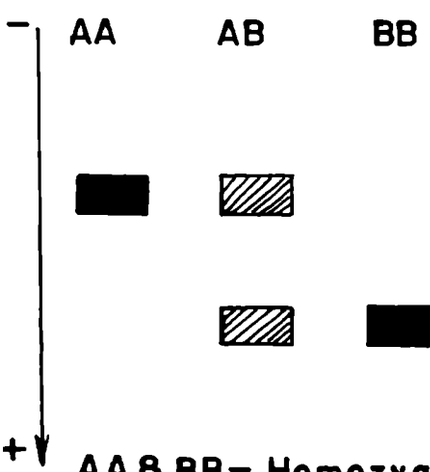
Fig. 35. Expression of different genotypes of malate dehydrogenase (Mdh-1) in eye tissue of *Parapenaeopsis stylifera*.

Fig. 34

E – Eye

M – Muscle

Fig.35



AA & BB – Homozygote

AB – Heterozygote

Fig. 36. Expression of malic enzyme in different tissues of Penaeus indicus

Fig. 37. Expression of different genetypes of malic enzyme (Me-1) in eye tissue of Penaeus indicus.

Fig.36

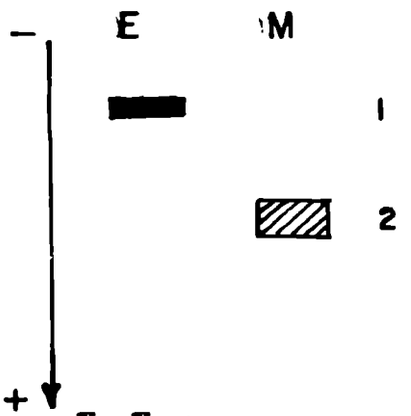


Fig. 37

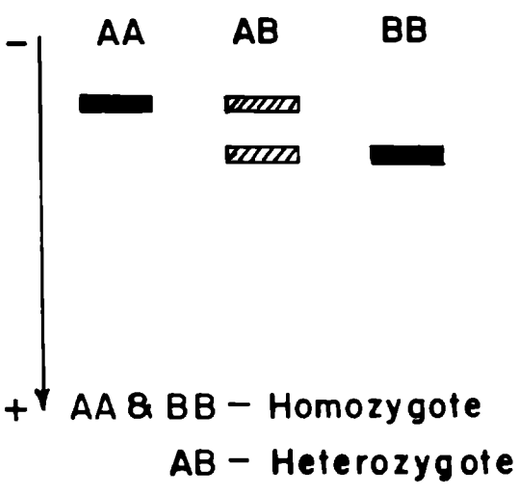
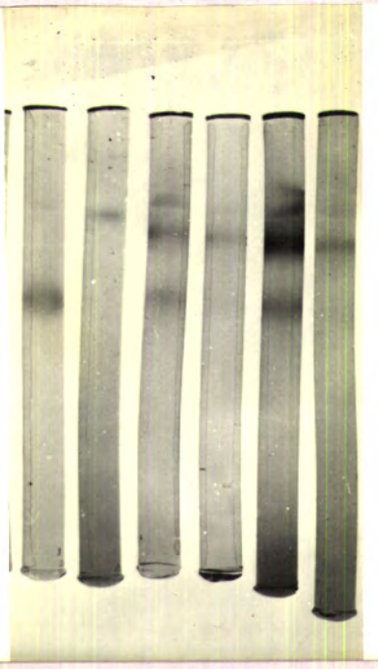


Plate 23: Showing the different genotypes of Malate dehydrogenase (Mdh-1) in eye tissue of *Parapenaeopsis stylifera* AA, BB-homozygotes AB-heterozygote.

Plate 24: Showing the different genotypes of malic enzyme (Me-1) in eye tissues of *Penaeus indicus* AA, BB-homozygotes, AB-heterozygote

BB AA AB AA AB AA



AA AB BB

Allele frequency obtained is given in Table 71. There is slight enhancement in the allele frequency of the allele A from Cochin, Tuticorin, Madras and Waltair.

Observed and expected phenotype frequency is tabulated (Table 60).

P. stylifera

Electrophoretic analysis revealed one locus each in eye and muscle (Fig. 38). Me-1 in eye was found to show polymorphism at Cochin. Phenotypic expression seen is given in Table 61.

Octanol dehydrogenase:

P. indicus

The enzyme expressed zones of enzyme activity in different tissues. The loci observed were Odh-2 in eye, Odh-1 in hepatopancreas and Odh-3 in muscle (Fig.39). The Odh-2 locus showed polymorphism only at Waltair (Fig. 40; Plate 25). Phenotypic expressions with one and 3 banded patterns in homozygous and heterozygous nature, respectively suggested a dimeric polypeptide structure for Octanol dehydrogenase enzyme. Different phenotypic expression observed is given in Table 62.

Table 60: Observed and expected phenotype frequency in malic enzyme (Me-1) seen in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	12	0	6	18
	Expected	8	8	2	
Tuticorin	Observed	13	0	5	17.9
	Expected	9.4	7.2	1.4	
Madras	Observed	14	1	4	17.8
	Expected	10.9	6.2	0.9	
Waltair	Observed	14	1	3	11.8
	Expected	11.7	5.6	.7	

Table 61: Observed and expected phenotype frequency of malic enzyme (Me-1) observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	20	4	12	21.4
	Expected	13.4	17.4	5.4	

Table 62: Observed and expected phenotype frequency of octanol dehydrogenase (Odh-2) observed in Penaeus indicus with Chi-square value.

Location		AA	AB	BB	χ^2
Cochin	Observed	0	0	36	
	Expected	0	0	36	
Tuticorin	Observed	0	0	36	
	Expected	0	0	36	
Madras	Observed	0	0	36	
	Expected	0	0	36	
Waltair	Observed	2	2	32	
	Expected	.24	.549	30.25	14.6

Table 63: Observed and expected phenotype frequency of octanol dehydrogenase (Odh-2) observed in Parapenaeopsis stylifera with Chi-square value

Location		AA	AB	BB	χ^2
Cochin	Observed	34	2	0	
	Expected	34	1.9	0.02	0.0252
Bombay	Observed	35	1	0	
	Expected	35	0.97	0.06	0.0609

Fig. 38. Expression of malic enzyme in different tissues of *Parapenaeopsis stylifera*.

Fig. 39. Expression of Octanol dehydrogenase in different tissues of *Penaeus indicus*.

Fig. 40. Expression of different genotypes of octanol dehydrogenase (Odh-2) in eye tissue of *Penaeus indicus*.

Fig. 38

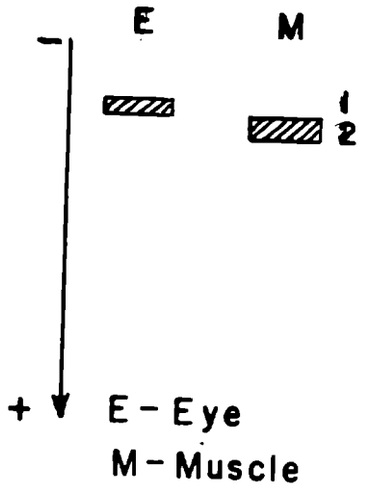


Fig. 39

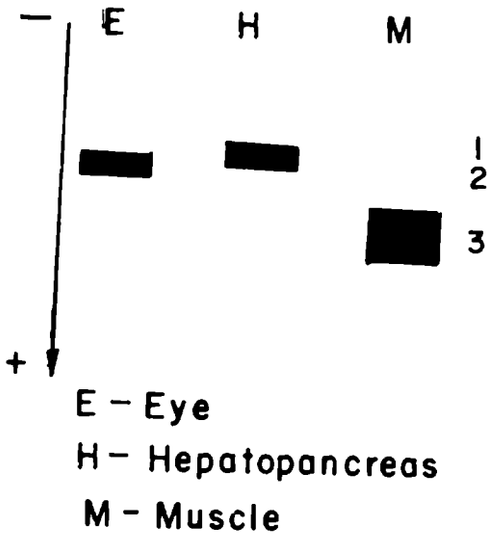


Fig. 40

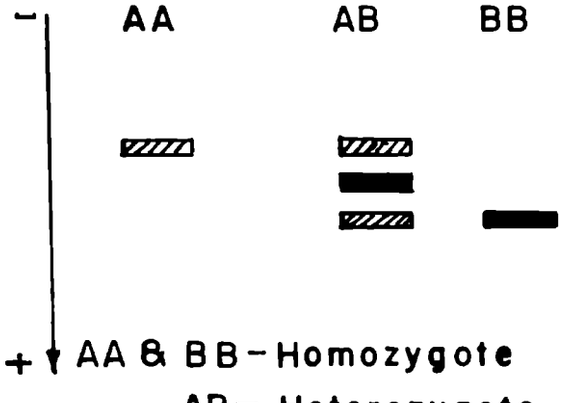


Fig. 41. Expression of octanol dehydrogenase in different tissues of Parapenaeopsis stylifera.

Fig. 42. Expression of 6-Phosphogluconate dehydrogenase observed in difference tissues of Penaeus indicus.

Fig. 41

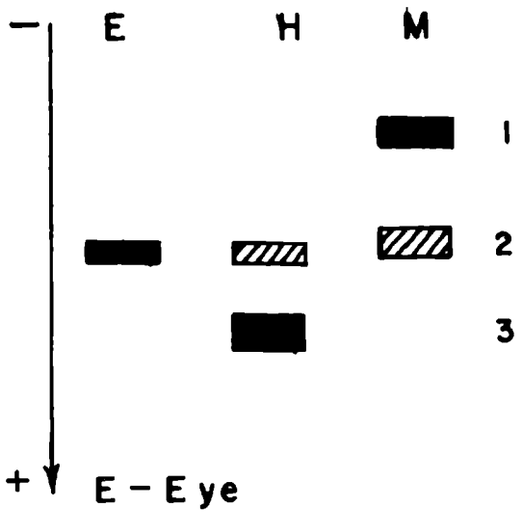

—
E
H
M
1
2
3
+
E – Eye
H – Hepatopancreas
M – Muscle

Fig. 42

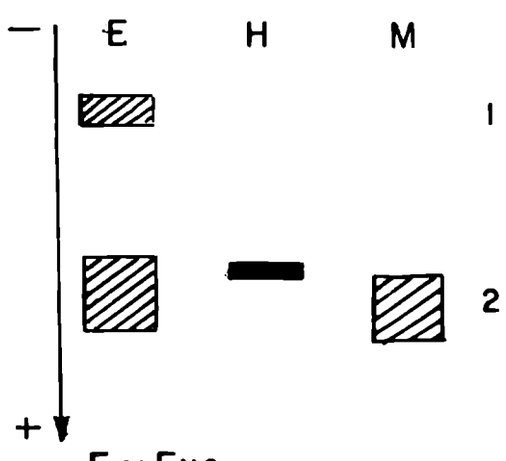

—
E
H
M
1
2
+
E – Eye
H – Hepatopancreas
M – Muscle

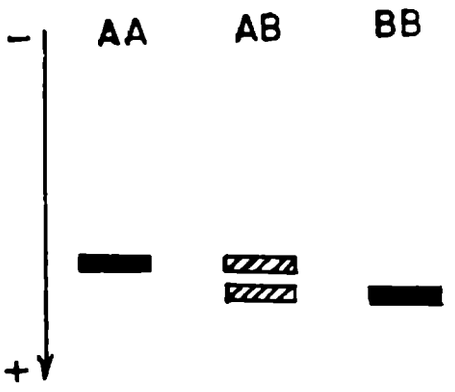
Fig. 43. Expression of different genotypes of 6-phosphogluconate dehydrogenase (6-Pgdh-2) observed in hepatopancreas tissue of *Penaeus indicus*.

Fig. 44. Expression of 1-pyrroline dehydrogenase in different tissues of *Penaeus indicus*.

Fig. 45. Expression of 1-pyrroline dehydrogenase in different tissue of *Parapenaeopsis stylifera*.

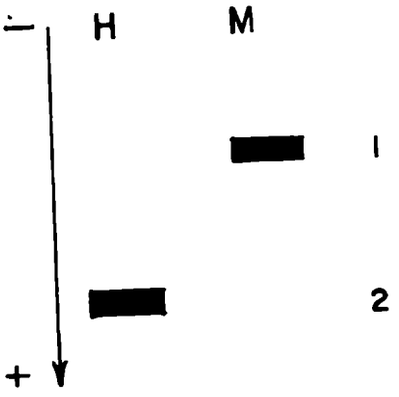
Fig. 46. Expression of sorbitol dehydrogenase in different tissue of *Penaeus indicus*.

Fig. 43



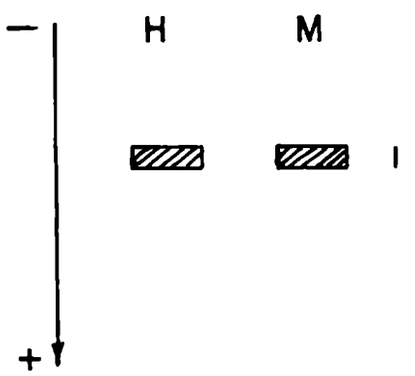
AA & BB – Homozygote
AB – Heterozygote

Fig. 44



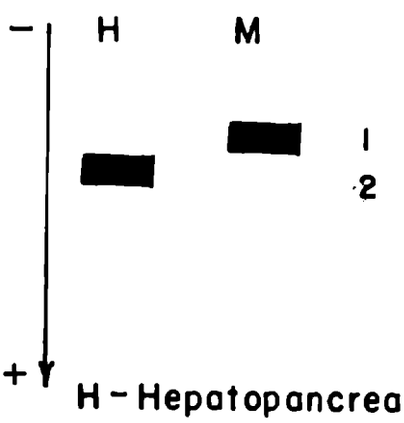
H – Hepatopancreas
M – Muscle

Fig. 45



H - Hepatopancreas
M – Muscle

Fig. 46

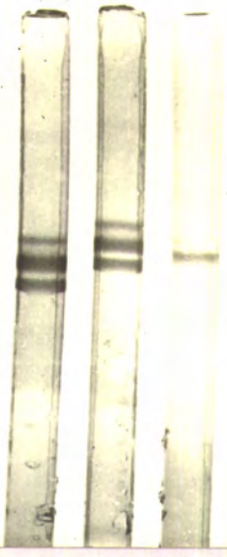


H - Hepatopancreas
M - Muscle

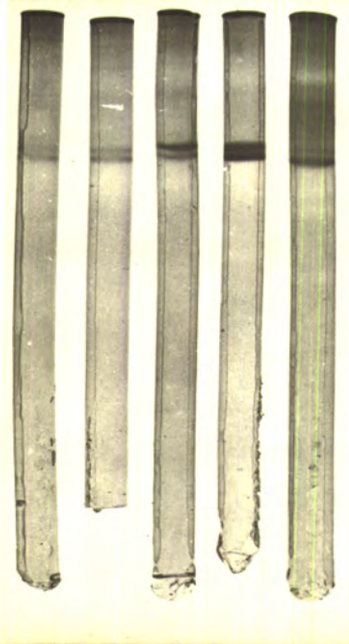
Plate 25: Showing the different genotype of Octanol dehydrogenase (Odh-2) in eye tissue of Penaeus indicus BB-homozygote, AB-heterozygote

Plate 26: Showing the different genotypes of 6-Phosphogluconate dehydrogenase(6-Pgdh-2) in hepatopancreas tissues of Penaeus indicus AA, BB-homozygotes, AB-heterozygote.

AB AB BB



AA AB AA AB BB



This showed 3 phenotypes only in Waltair population. Alleles frequency is given in Table 71.

P. stylifera

P. stylifera expressed 3 loci in its tissue one locus in eye. (odh-2), 2 loci in hepatopancreas (odh-2 and odh-3) and in muscle (Odh-1 and odh-2) (Fig.41). Phenotypic expression observed in odh-2 is given in Table 63. As the allele frequency was above0.95 it was considered as monomorphic.

Allele frequency found is given in Table 72.

6-Phosphogluconate dehydrogenase:

P. indicus

There are two loci for 6-Phosphogluconate dehydrogenase giving rise to a fast 6-Pgdh-1 and slow 6-Pgdh-2 bands of activit Fast locus was observed in eye, hepatopancreas and muscle tissues whereas slow locus was expressed only in eye (Fig.42). This was analysed in hepatopancreas and muscle. Heterozygote showed 2 banded phenotype and homozygote with single banded phenotype in hepatopancreas indicating a typical pattern of monomer (Fig. 43; Plate 26). 6 Pgdh-2 expressed polymorphism with 2 alleles in all the localities. Details of phenotypic expression observed are given in Table 64.

Allele frequencies observed are given in Table 71.

Table 64: Observed and expected phenotype frequency of 6-phospho-gluconate dehydrogenase (6-Pgdh-2) observed in Penaeus indicus with Chi-square value

Location		AA	AB	BB	χ^2
Cochin	Observed	15	1	20	31.9
	Expected	6.7	17.6	1.67	
Tuticorin	Observed	15	1	20	31.9
	Expected	6.7	17.6	11.67	
Madras	Observed	14	1	21	32.3
	Expected	5.8	17.3	12.8	
Waltair	Observed	15	2	19	28.4
	Expected	7.1	17.8	11.1	

Table 65: Observed and expected Phenotype frequency of 1-Pyrroline dehydrogeneus (1-Pydh-1) observed in Parapenaeopsis stylifera with Chi-square value

Location		AA	AB	BB	χ^2
Cochin	Observed	34	1	1	15.93
	Expected	33	2.8	0.06	
Bombay	Observed	35	1	0	0.009
	Expected	35	.95	.006	

1-Pyrroline dehydrogenase:

P. indicus

This enzyme expressed 2 zones of activity, one in hepatopancreas(1-Pydh-2) and another in muscle tissue (1-Pydh-1) (Fig. 44). But it didn't express any allelic variation and hence this was considered as monomorphic locus.

P. stylifera

Hepatopancreas and muscle expressed the activity for this enzyme (Fig. 45). Since the expression was consistent, it was analysed for genetic variation studied in hepatopancreas and the frequency analysis proved it to be monomorphic.

Observed and expected phenotype frequency is tabulated (Table 65).

Sorbitol dehydrogenase

P. indicus

This enzyme showed 2 bands of activity; one in hepatopancreas and another in muscle (Fig. 46). Phenotypic expression studied in muscle tissue revealed only one allele from these loci. So it was suggested to be monomorphic.

Tetrazolium oxidase:

P. indicus

P. indicus exhibited zones of enzyme expression in three

tissues; To-1 and To-2 loci are expressed in eye, To-3 and To-4 loci in hepatopancreas and To-2 locus in muscle tissue (Fig. 47). As alleleic frequencies analysed in muscle was found to be above 0.95 the locus was taken as monomorphic.

P. stylifera

This enzyme exhibited 2 zones of activity in P. stylifera (one locus in eye, 2 loci in hepatopancreas and one in muscle tissue)(Fig.48). To-1 in hepatopancreas showe. allelic variant with a monomer pattern. To-2 (Fig.49; Plate 27) expressed low level of allele frequency so it is discarded from the further calculation. Details of phenotypes observed in To-1 are shown in Table No.66.

Allele frequency calculated is given in Table 72. There is not much variation observed in allele frequencies of these 2 places.

Hardy-Weinberg equilibrium

Details of expected Hardy-weinberg equilibrium distribution of phenotypes analysed are shown (Table Nos.48-66). Difference between observed and expected distribution was tested and found to be significant in Acph-3, Ao-1, Ald-1, Alph, Mdh-1, Me-1, Odh-2 and 6-Pgdh-2 for P. indicus and

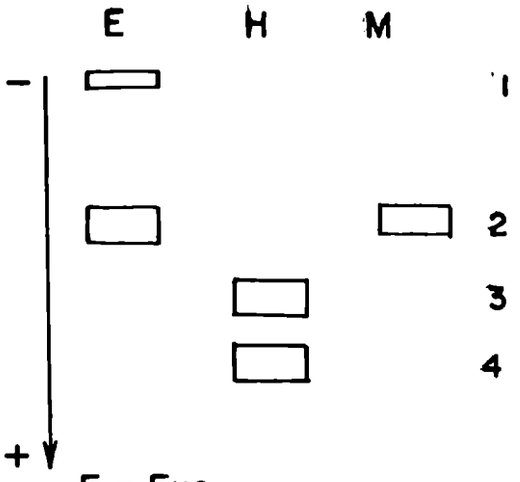
Table 66: Observed and expected phenotype frequency observed in Parapenaeopsis stylifera with Chi-square value.

Location		AA	AB	BB	χ^2
To-1					
Cochin	Observed	20	2	14	28.21
	Expected	12.25	17.5	6.25	
Bombay	Observed	18	1	17	32.1
	Expected	9.15	18	8.5	
		CC	CD	DD	2
To-2					
Cochin	Observed	33	3	0	0.063
	Expected	33	2.9	0.06	
Bombay	Observed	33	3	0	0.063
	Expected	33	2.9	0.06	

Fig. 47. Expression of tetrazolium oxidase in different tissues of *Penaeus indicus*.

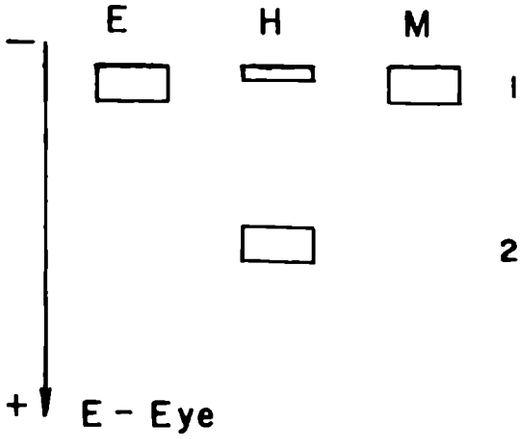
Fig. 48. Expression of tetrazolium oxidase in different tissues of *Parapenaeopsis stylifera*

Fig. 47



E - Eye
H - Hepatopancreas
M - Muscle

Fig. 48



E - Eye
H - Hepatopancreas
M - Muscle

Fig. 49. Expression of different genotypes of tetrazolium oxidase (To-2) in hepato-pancreas tissue of *Parapenaeopsis stylifera*.

Fig. 49

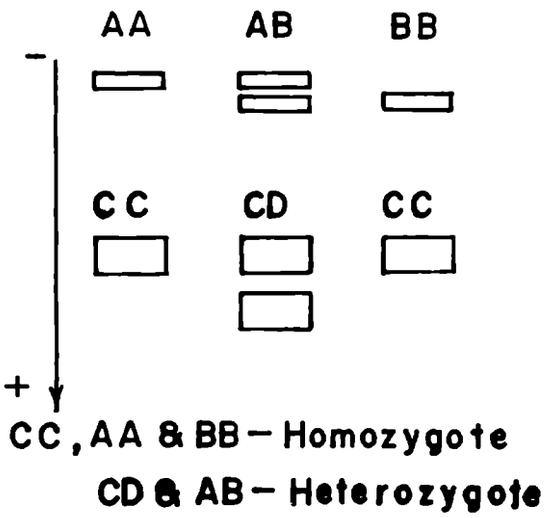
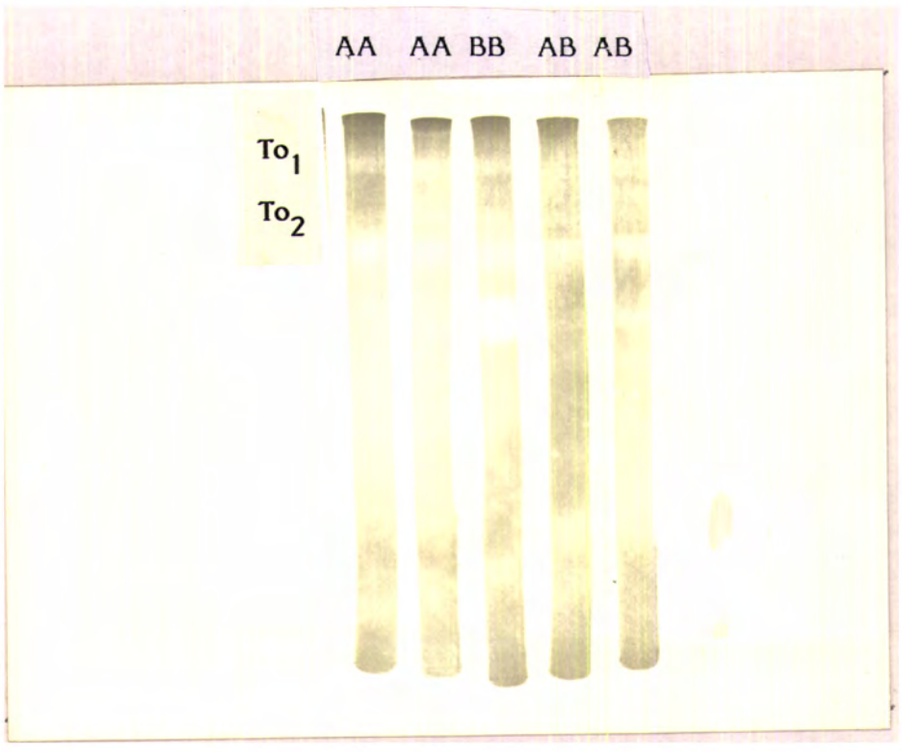


Plate 27: Showing the different genotypes of tetrazolium oxidase (To-2) in hepatopancreas tissue of *Parapenaeopsis stylifera* AA, BB-homozygotes, AB-heterozygote.


AA AA BB AB AB
To1
To2

also for Acph-2, Adh-2, Ao-2 from Cochin, Alph-2, Est-2, Gpdh-1 from Bombay, Mdh-1, Me-1, 1-Pydh-1 in Cochin and To-1 in P. stylifera and insignificant in Ao-2 of Bombay, Gpdh-1 of Cochin, Odh-2, Pydh-1 from Bombay and To-2 in P. stylifera. Significant values were produced by deficiency of heterozygotes/ excess of homozygotes.

Heterozygosity analysis was carried out to find out the amount of genetic variation in the population. So, observed and expected frequency of heterozygotes in these populations was calculated. The difference arose between these two was tabulated for statistical significance as seen in Acph-3, Ald-1, Ao-1, Alph-1, Mdh-1, Me-1, Odh-2 and 6-Pgdh in P. indicus, Mdh-1, Me-1 and To-1 in P. stylifera and insignificant in To-2, Ao-2, Odh-2 and Gpdh and Pydh for P. stylifera . Significant values obtained in polymorphic loci were due to the excess of homozygotes/deficiency of heterozygotes in these two prawn species.

Genetic variation within prawn populations

Overall estimate of average frequency of heterozygotes per locus H in a population was calculated by averaging observed frequency of heterozygote (Ho) overall loci sampled. The average frequency of heterozygotes per locus (H) for P. indicus in Cochin was 0.0114 ± 0.02, in Tuticorin 0.0114±0.02,

in Madras 0.0096 ± 0.02 and in Waltair 0.0142 ± 0.02. Total estimate of average frequency of heterozygotes per locus H is 0.0105 ± 0.02. The same estimate was calculated for P. stylifera collected from Cochin and Bombay. The average frequency of heterozygotes per locus H for P. stylifera collected in Cochin is 0.3 ± 0.03 and for Bombay it is 0.025 ± 0.03. Total average frequency of heterozygote per locus H was found out to be .026 ± 0.03 for P. stylifera.

Proportion of polymorphic loci 'p' was calculated for P. stylifera in Cochin and it was found out to be 0.304 and for P. stylifera in Bombay it was 0.273. For P. indicus, the proportion of polymorphic loci was found out to be 0.261 in Cochin, Tuticorin and Madras and 0.333 in Waltair. Average proportion of polymorphic loci per population Pp is 0.2885 for P. stylifera and 0.279 for P. indicus. (Table 69 & 70).

Mean number of alleles per locus (A) seen in each population was found out. For P. indicus in Cochin, Tuticorin and Madras it was 1.304 and 1.375 in Waltair. For P. stylifera, mean number of alleles per locus was 1.545 in Cochin and 1.575 in Bombay. For these populations in total, the mean number of alleles per locus was found out to be 1.558 for P. stylifera and 1.322 for P. indicus (Table 75 & 76).

Table 67: Nei's D-genetic distance (abode the Diagonal)
I-Genetic identity (below the diagonal)
J(X)-Average homozygozety(on the diagonal)
for P. stylifera and P. indicus

P. stylifera

Location*	Geographic populations*	
	Co	Bom.
Co	(.887)	.009
Bom	.991	(.878)

P. indicus

Location[a]	Geographic populations[a]			
	Co	Tun	Mds	Wal.
Co	(.827)	.002	.003	.055
Tun	.998	(.828)	.001	.049
Mds.	.997	.999	(.836)	.047
Wal	.947	.952	.954	(.84)

* Co-Cochin; Bom- Bombay.

a Co-Cochin; Tun-Tuticorin; Mds-Madras; Wal-Waltair

Table 68: Roger's 'D' (Distance) is above diagonal 'S' (Similarity) is below the diagonal for Penaeus indicus and Parapenæopsis stylifera.

Penaeus indicus

Location[a]	Geographic population[a]			
	Co	Tun	Mds	Wal
Co	-	0.013	0.022	0.071
Tun	.987	-	.014	0.064
Mds	.978	.986	-	0.056
Wal	.929	.936	.944	-

Parapenaeopsis stylifera

Location*	Geographic population*	
	Co	Bom
Co	--	0.03
Bom	.97	-

[a]Co-Cochin; Tun-Tuticorin; Mds-Madras; Wal-Waltair

*Co-Cochin; Bom-Bombay

Table 69: Details of genetic analysis carried out in different enzymes in different tissues of Penaeus indicus.

1.	Acid phosphatase	Acph-1 x Acph-2 x	Acph-1 - 2 - 4 - 5 -	Acph-3 x +
2.	Alcohol dehydrogenase	Adh-2 - 3 -	Adh-1 x 2 x	Adh-2 x
3.	Aldehyde oxidase	-	Ao-1 + 2 x	Ao-3 -
4.	Aldolase	-	-	Ald-1 x +
5.	Alkaline phosphatase	-	-	Alph x +
6.	Glycerophosphate dehydrogenase	-	Gpdh-1 x	
7.	Lactate dehydrogenase	Ldh-2 -	Ldh-1 -	Ldh-1 - 2 -
8.	Malate dehydrogenase	Mdh-1 x +	-	Mdh-2 x 3 x
9.	Malic acid	Me-1 x +	-	Me-2 x
10.	Octanol dehydrogenase	Odh-2 x +	Odh-1 x	Odh-3 x
11.	6-Phosphogluconate dehydrogenase	Pgdh-1 - 2 -	Pgdh-2 x +	Pgdh-2 x
12.	1-Pyrroline dehydrogenase	-	Pydh-2 x	Pydh-1 x
13.	Sorbitol dehydrogenase	-	Sdh-2 -	Sdh-1 x
14.	Tetrasolium oxidase	To-1 - 2 -	To-3 - 4 -	To-2 x

Analysed x
Polymorphic +
Not analysed -

Table 70: Details of genetic analysis carried out in different enzymes in different tissues of *Parapenaeopsis stylifera*.

1.	Acid phosphatase	Acph-1 x	Acph-3 - 4 -	Acph-2 x +
2.	Alcoholdehydrogenase	Adh-1 x	Adh-2 x +	Adh-1 -
3.	Aldehyde oxidase	-	Ao-2 x 3 x	Ao-1 x
4.	Alkaline phosphatase	Alph-1 -	Alph-3 -	Alph-2 x +
5.	Esterase	Est-1 x 2 x + 3 x	Est-1 - 3 -	Est-2 -
6.	Glycerophosphate dehydrogenase	-	Gpdh-1 x	-
7.	Malate dehydrogenase	Mdh-1 x +	-	Mdh-2 x
8.	Malic acid	Me-1 x	-	Me-2
9.	Octanol dehydrogenase	Odh-2 x	Odh-2 x 3 x	Odh-1 2 x
10.	Tetrazolium oxidase	To-1 -	To-1 + 2 x	To-1 x
11.	1-Pyrroline dehydrogenese		Pydh-1 x	Pydh-1 -

Analysed x
Polymorphic +
Not analysed -

Table 71: Allelic frequencies of four natural populations of *P. indicus*

Gene	Alleles	Populations			
		Cochin	Tuticorin	Madras	Waltair
Acph-3	a	0.5278	0.5	0.5972	0.6528
	b	0.4723	0.5	0.4028	0.3472
Ald-1	a	0.1528	0.1944	0.1806	0.1111
	b	0.3889	0.5147	0.5833	0.7778
	c	0.4583	0.2639	0.2361	0.1111
Ao-1	a	0.5833	0.5833	0.6250	0.6528
	b	0.4167	0.4167	0.3750	0.3472
6-Pgdh-2	a	0.4306	0.4306	0.4028	0.4444
	b	0.5694	0.5694	0.5972	0.5556
Alph	a	-	-	-	0.8333
	b	-	-	-	0.1667
Mdh-1	a	0.3472	0.3750	.3056	.3472
	b	0.6528	0.6250	.6944	.6528
Me-1	a	0.6667	0.7222	0.7778	0.8056
	b	0.3333	0.2778	0.2222	0.1944
Odh-2	a	0	0	0	0.0833
	b	1	1	1	0.9167

Table 72: Allelic frequencies of two natural populations of *Parapenaeopsis stylifera*.

Gene	Alleles	Populations	
		Cochin	Bombay
Acph-2	a	0.7361	0.3472
	b	0.2639	0.6528
Adh-2	a	0.9444	0.9028
	b	0.556	0.0972
Ao-2	a	0.9583	0.9722
	b	0.0417	0.0278
Ao-3	a	0.9722	0.9722
	b	0.0278	0.0278
Alph-2	a	0.9167	0.8889
	b	0.0833	0.1111
Est-2	a	0.875	0.9306
	b	0.125	0.0694
Gpdh-1	a	0.9583	0.9583
	b	0.0417	0.0417
Mdh-1	a	0.9305	0.9611
	b	0.0694	0.1389
Me-1	a	0.611	-
	b	0.3889	
Odh-2	a	0.9722	0.9861
	b	0.0278	0.01389
1-Pydh-1	a	0.9583	0.9861
	b	0.0417	0.0134
To-1	a	0.5833	0.5139
	b	0.4167	0.4861
To-2	a	0.9583	0.9583
	b	0.0417	0.0417

Table 73: Average frequency of observed ($\bar{H}o$) and expected ($\bar{H}e$) heterozygotes per locus with 'Z' value for *Penaeus indicus*.

Gene	Population	$\bar{H}o$	$\bar{H}e$	Z
Acph-3	Cochin	0.06	0.498	9.69022
	Tuticorin	0.06	0.5	11.1167
	Madras	0.03	0.48	15.8283
	Waltair	0.03	0.458	15.0545
Ald-1	Cochin	0.08	0.615	11.831
	Tuticorin	0.08	0.6	11.4993
	Madras	0.08	0.571	10.858
	Waltair	0.06	0.37	7.8283
Ao-1	Cochin	0.06	0.486	10.763
	Tuticorin	0.06	0.486	10.763
	Madras	0.03	0.469	15.4414
	Waltair	0.03	0.453	14.8786
6-Pgdh-2	Cochin	0.03	0.49	16.18
	Tuticorin	0.03	0.49	16.18
	Madras	0.03	0.48	15.8283
	Waltair	0.05	0.49	12.114
Alph	Waltair	0.05	0.278	6.2768
Mdh-1	Cochin	0.03	0.453	14.8786
	Tuticorin	0.03	0.469	15.441
	Madras	0.05	0.424	10.297
	Waltair	0.03	0.547	18.2042
Me-1	Cochin	0	0.444	0
	Tuticorin	0	0.401	0
	Madras	0	0.346	0
	Waltair	0.03	0.313	11.127
Odh-2	Cochin	0	0	0
	Tuticorin	0	0	0
	Madras	0	0	0
	Waltair	0.06	0.153	2.349
Total	Cochin	0.0114	0.1357	
	Tuticorin	0.0114	0.1339	
	Madras	0.0096	0.1259	
	Waltair	0.0142	0.1331	

Table 74: Average frequency of observed $\bar{H}o$ and expected $\bar{H}e$ heterozygotes per locus with 'Z' value for *Parapenaeopsis stylifera*.

Gene	Population	Ho	He	Z
Acph-2	Cochin	0.08	0.39	6.858
	Bombay	0.08	0.45	8.186
Adh-2	Cochin	0	0.105	0
	Bombay	0.03	0.176	5.1408
Ao-2	Cochin	0.03	0.08	1.76
	Bombay	0.05	0.054	0.1102
Ao-3	Cochin	0.	0.054	0
	Bombay	0.05	0.054	0.1102
Alph-2	Cochin	0.06	0.153	2.354
	Bombay	0.06	0.198	3.4936
1-Pydh-1	Cochin	0.03	0.054	0.8441
	Bombay	0.03	0.027	0.1056
Est-2	Cochin	0.08	0.219	3.0752
	Bombay	0.03	0.129	3.4859
Gpdh-1	Cochin	0.08	0.08	0
	Bombay	0.03	0.08	1.7605
Mdh-1	Cochin	0.03	0.129	3.4859
	Bombay	0.06	0.239	4.5316
Me-1	Cochin	0.01	0.475	7.5
Odh-2	Cochin	0.06	0	1.51898
	Bombay	0.03	0.08	1.7605
To-1	Cochin	0.06	0.486	10.784
	Bombay	0.03	0.5	16.549
To-2	Cochin	0.08	0.08	0
	Bombay	0.08	0.08	0
Total	Cochin	0.03	0.082	
	Bombay	0.025	0.0984	

Table 75: Summary of genetic variation Data in four geograph: populations of Penaeus indicus.

	Geographic locations[Y]				
	Co	Tun	Mds	Wal	Mean
Sample size	36	36	36	36	36
Number of loci	23	23	23	24	23.25
Proportion of Polymorphic Loci($\underline{P}$)	0.261	0.261	0.261	0.333	0.279
Average frequency of heterozygosity per locus ($\underline{H}o$)	0.0114 ±0.02	0.0114 ±0.02	0.01 ±0.02	0.0142 ±0.02	0.011
Mean number of alleles per loci(A)	1.304	1.304	1.304	1.375	1.322

[Y] Co - Cochin, Tun-Tuticorin, Mds-Madras, Wal-Waltair

Table 76: Summary of genetic variation data in two populations of *Parapenaeopsis stylifera*.

	Geographic locations[z]		
	Co	Bom	Mean
Sample size	36	36	36
Number of loci	23	22	22.5
Proportion of loci polymorphic ($\underline{P}$)	0.304	0.273	0.2885
Average frequency of heterozygosity per locus ($\underline{H}_o$)	0.03 ± 0.03	0.025 ± 0.03	0.028
Mean number of alleles per loci(A)	1.545	1.571	1.558

[z] Co - Cochin; Bom-Bombay

Table 77: Nei's genetic distance (D) and genetic identity (I) analysis in *Penaeus indicus* collected from four different locations.

	Cochin-Tuticorin		Cochin-Madras		Cochin-Waltair		Tuticorin-Madras		Tuticorin-Waltair		Madras-Waltair	
	I	D	I	D	I	D	I	D	I	D	I	D
Acph	1	0	.9903	0.0097	.972	0.0284	1	0	.956	0.0450	.9689	0.0316
Ald	.93502	0.0671	.8933	0.1128	.7525	0.2844	.9973	0.0027	.94	0.0619	.962	0.0387
Ao-1	1	0	.9967	0.0033	.9915	0.0085	.9969	0.0031	.998	0.0020	.999	0.0010
6-Pgdh	1	0	.9984	0.0016	1	0	.9719	0.0285	1	0	.996	0.0040
Mdh	.9987	0.0013	.9973	0.0027	1	0	.9922	0.0078	.998	0.0020	.9973	0.0027
Me	.995	0.0050	.9829	0.0172	.9102	0.0941	.9961	0.0039	1.059	-0.0573	1.068	-0.0658
Odh	1	0	1	0	.9958	0.0042	1	0	.996	0.0440	.996	0.004

Acph-Acidphosphatase;
Ald -Aldolase
Ao-1-Aldehydeoxidose
6-Pgdh-6-Phosphogluconate dehydrogenase
Mdh -Malatedehydrogenase
Me -Malic enzyme
Odh -Octanoldehydrogenase.

Table 78: Nei's genetic distance (D) and genetic identity(I) analysis between Cochin and Bombay samples of *Parapenaeopsis stylifera*.

Enzyme Locus	Cochin-Bombay I	D
Acidphosphatase (Acph)	.74	0.3011
Alcohol dehydrogenase (Adh)	.998	0.0020
Aldehydeoxidase (Ao-1)	.9862	0.0139
Aldehydeoxidase (Ao-2)	.94597	0.0555
Alkalinephosphatase (Alph)	0.996	0.0040
Esterase (Est-2)	.9449	0.0567
Glycerophosphate (Gpdh) dehydrogenase	1	0
Malate dehydrogenase (Mdh)	1	0
Octanol dehydrogenase (Odh-2)	1	0
1-Pyrroline dehydrogenase(Pydh)	1	0
Tetrazoliumoxidase (To-1)	1	0
Tetrazoliumoxidase (To-2)	1	0

Genetic variation between prawn populations:

Genetic divergence among P. indicus and P. stylifera population was quantified by Nei's measure of genetic distance (D) and Genetic identity (I) considered as the average probability per locus of selecting two electrophoretically identical alleles, one from each of 2 different populations. This is calculated directly from gene frequency data with Nei(1972) definition. Genetic distance (D) is defined as the negative natural longarithmic transformation of I and with average number of amino acid substitutions per protein that have diverged from one another (Nei 1973).

Population samples of P. indicus collected from 4 different areas are genetically similar. Genetic identity and genetic distance estimates of Nei & Roger's are given in Table 67 & 68. Likewise, populations of P. stylifera which were sampled from Cochin and Bombay are also genetically identical. It shows genetic identity of 0.991 and genetic distance 0.009. According to Rogers analysis, the distance is 0.03 and similarly 'S' is 0.97. In P. indicus, the genetic similarity and distance for different enzymes of geographic populations like Cochin-Tuticorin, Cochin-Madras, Cochin-Waltair, Tuticorin-Waltair, Madras-Waltair, Tuticorin-Madras were tabulated (Table No. 77). Similarly the genetic variants calculated for different enzymes between Bombay, Cochin population of P. stylifera is given in Table 78.

Discussion:

The present observation of electrophoretic patterns of 15 different enzymes and their loci tested in eye, hepato pancreas and muscle tissues of P. indicus and P. stylifera has not been reported earlier in these two species.

Acid Phosphatase

The buffer system tris-citrate pH 7 used in the present study resolved acid phosphatase enzyme in P. indicus P. stylifera. The search for intra species genetic variation of acid phosphatase enzyme in the prawn species P. indicus and P. stylifera expressed 5 loci in the farmer and 3 loci in the later in selected tissues. Electrophoretic variant forms of the enzyme was tested only in the muscle tissue of both the species. The present observation of electrophoretic variant forms of Acid phosphatase at Acph-3 and Acph-2 locus in muscle tissue of P. indicus and P. stylifera respectively has not been reported earlier. The reports of its allelic forms in eye, hepatopancreas and green glands of Homarus americanus (Tracey et al., 1975), in cephalothorax of penaeid spp. (Mulley and Latter 1980), in muscle of Penaeus kerathurus and P. japonicus (DeMatthaeis et al., 1983), in muscle tissue of crans(Beckwitt 1985) also reveals polymorphic nature of the enzyme in many

other organisms. The present observation of Acid phosphatase loci in many tissues of P. indicus and P. stylifera indicates, as reported in many tissues of other organisms, its important role in the biochemical and physiological functions of the organism.

Classification of electrophoretic position of single banded and double banded variant forms of acid phosphatase in P. indicus and P. stylifera tested here as homozygote and heterozygotes respectively was on the expectation that these two phenotypes are products of Zygotic combination of two alleles observed in the muscle tissues of P. indicus and P. stylifera designated as acid phosphatase A and B (Fig. 14 & 16). On the same assumption allele frequencies and expected and observed genotype distribution in each samples were estimated as required in establishing the genetic nature of the observed enzyme polymorphism according to the law of Hardy-Weinberg equilibrium. Neverthless, significant difference in the values of expected and observed distributions of heterozygotes in the samples suggests that the observed polymorphism is not in Hardy-Weinberg equilibrium as expected in a balanced genetic polymorphism. However, the well established genetic nature of acid phosphatase polymorphism in many organisms (Tracey et al., 1975;

Hedgecock et al., 1977; Berthelemy, 1978; Ayala and Valentine, 1978; Hedgecock et al., 1979; Nelson and Hedgecock, 1980) strengthens the present assumptions that the acid phosphatase variants observed in P. indicus and P. stylifera are of genetic nature. The lack of balanced polymorphism of acid phosphatase in these two species studied here may be due to the certain biological and non-biological factors such as differences in the natural selection of a particular genotype resulting in the differential mortality of such types during its life cycle, or due to ontogenetic effects. Sampling error also might contribute to this result. Breeding experiments which is a direct method for proving the genetic nature of an observed variation, is not practical and was beyond the scope of the present investigations. The lack of Acid phosphatase heterozygotes in P. indicus and P. stylifera obtained here may be also due to either inbreeding effect or higher mortality of the heterozygote individuals in the populations. Problems of inbreeding seen in the species of penaeid prawns (Mulley and Latter 1980) was due to periodic reductions of each species to a very small number of breeding individuals during glacial periods, when the ocean receded from the tidal lakes and river estuaries. Population size bottleneck involves inbreeding effect due to over-exploitation which reduces heterozygosity as in American lobster

Homarus americanus (Tracey et al.,1975). The selection of only intermoult specimens for the present investigation rules out probable role of ontogeny in the pattern of results observed. The important role of errors in the sampling, however, cannot be overlooked. A reasonable sample size for genetic analysis of electrophoretic variants may be that which represents all the possible phenotypes in its nearly natural proportions. Hedgecock et al.,(1976) recommended at least 20-30 specimens to be sampled from a single location. In the present study testing of 36 numbers of P. indicus and P. stylifera specimens in each sample should have shown all the phenotypes in their natural proportions as expected. However, testing of larger sample size in the present case need not improve the result of goodness of fit test conducted in these two species because, the lack of goodness fit was clearly indicated in all the other enzymes tested. Hence the observed deficiency of heterozygotes in acid phosphatase in P. indicus and P. stylifera may be the result of inherent unknown reasons like inbreeding or even shifting of polymorphism towards monomorphism as fixing of certain loci occur due to long sustained inbreeding among the populations and thereby heterozygotes necessary for maintaining normal balanced polymorphism gradually disappear. The phenomenon of low level of heterozygotes as observed in

the present investigation has also been reported in many other decapod species (Nelson and Hedgecock 1980)especially in penaeid species of prawns (Mulley and Latter 1980). Significant, heterozygote deficiency with respect to Hardy-Weinberg expected proportion was consistently observed in Homarus americanus by Hedgecock et al.,(1977) due to Wahlund effect. Trophic environment of prawn species is highly heterogenous (Moriarty 1977). Nevo (1978) found out that marine invertebrates having wide range of physical and trophic environments are characterized by low levels of heterozygosity due to selective elimination of mutational variation. Mulley and Latter (1980)observed low level of genetic variation of prawns due to low mutation rate and selective elimination of mutational variation. The observation of heterozygote deficiency and almost similar gene frequencies for the dominant alleles in population samples collected from different regions such as Cochin, Tuticorin, Madras and Waltair of P. indicus and from Cochin and Bombay of P. stylifera may suggest that all these populations represent a single breeding population. The result of tagging studies conducted by CMFRI (Vijayaraghavan et al.,1982) on P. indicus also corroborate the above conclusion.

The apparent significant differences in the distribution pattern of acid phosphatase phenotypes and its alleles between Cochin and Bombay suggests, irrespective of similarities at all other loci, that the populations of P. stylifera at Cochin and Bombay may be in the course of genetic differentiation. Neverthless it requires analysis of larger population samples for further confirmation.

Alcohol dehydrogenase:

Alcohol dehydrogenase separated in P. indicus and P. stylifera showed good resolution in the tris boric acid Edta pH 8 buffer as reported in Jackass Morwang (Richardson 1982a) whereas this enzyme also resolved in tris maleic acid in P. latisulcatus (Richardson 1982b).

Analysis of intraspecies variation of alcohol dehydrogenase enzyme in prawn species studied here showed 3 loci in P. indicus and two loci in P. stylifera in different tissues like eye, hepatopancreas and muscle. Alcohol dehydrogenase loci observed in P. indicus was monomorphic in all the locations where as P. stylifera showed a diallelic polymorphism in Aldh-2 locus, expressed in hepatopancreas. The expression of alcohol dehydrogenase was reported in digestive glands of rock lobster (Smith et al 1980). As observed in P. indicus,

alcohol dehydrogenase had monomorphic loci in P. setiferus (Lester 1979). The species P. latisulcatus expressed alcohol dehydrogene activity in eyes, hepatopancreas and muscle with polymorphic loci having 4 alleles in Jackass morwang (Richardson 1982a). Significant divergence from the distribution pattern expected under Hardy-Weinberg equilibrium conditions in the distribution of alcohol dehydrogenase phenotypes was observed in same species by the same authors (Jackass Morwang, Richardson 1982). P. stylifera also showed too many homozygotes. Since no significant variations in alcoholdehydrogenase gene frequencies of P. stylifera are evident, the data do not imply that the samples collected from Cochin and Bombay belonged to different subpopulations. Selection at this locus seems a more likely explanation for the observed heterozygote deficiency. Other probable causes suggested in acid phosphatase may have produced too many homozygotes in alcoholdehydrogenase in P. stylifera studied here.

Aldehyde oxidase:

Aldehyde oxidase is an extensively studied enzyme in Drosophila melanogaster (Ayala et al.,1974). Aldehydeoxidase enzyme of P. indicus and P. stylifera, studied here resolved well in the tris-glycine buffer 8.3 pH Aldehydeoxidase enzyme

of all the 13 penaeid prawn species analysed in Australian waters was resolved in tris citric acid 8.4 pH and that of P. japonicus and P. kerathurus in tris boricacid edta buffer 9.1 pH DeMatthaeis et al(1983).

Aldehyde oxidase in P. indicus tested here exhibited its activity in hepatopancreas and muscle only. Ao-3 locus was seen in muscle whereas Ao-1 and 2 were seen in hepatopancreas. Allele frequency estimate suggested a polymorphic locus Ao-1 and a monomorphic locus Ao-2 in all the localities samples for P. indicus.

In P. stylifera the aldehydeoxidase enzyme expressed 2 loci Ao-2 and Ao-3 in hepatopancreas and Ao-1 in muscle tissue. Genetic variation studies revealed 2 banded heterozygotes indicating monomeric subunit. The allelic frequency being not less than 0.95 implied the monomorphic nature of these loci. Likewise Homarus americanus had a polymorphic locus with 2 alleles (Tracy et al 1975)

Aldehydeoxidase showed monomorphic loci in many marine organisms (Redfield et al,1980; Lester 1979, 1983; Fuller and Lester 1980; DeMatthaeis et al,,1983). In the present study also due to the excess of homozygosity the expected and observed phenotypic distribution showed significant differences in P. indicus (Ao-1) and for Cochin samples of

P. stylifera (Ao-2). The reasons for the observation of excess homozygotes in both the species studied here may be the same as suggested elsewhere.

Aldolase:

Aldolase catalyses a reaction in glycolysis and energy production. P. indicus tested here showed distinct bands in tris boric edta buffer pH 8. The enzyme was also reported to have resolved in tris citrate pH 7 in other penaeid prawns (Mulley and Latter 1980). Muscle tissues of P. indicus showed a monomer subunit with triallellic pattern revealing the polymorphic nature of Aldolase enzyme. But the reports of electrophoretic studies of the enzyme carried out in other crustaceans such as chthamalus montaqui (Dando et al.,1979) Matuta planipes and Uca speciosa (Nelson and Hedgecock 1980) and Uca spinocarpa (Salmon et al.,1979) showed only monomorphic loci.

In other penaeid prawns aldolase was observed in muscle tissue (Mulley and Latter 1980). Red field et al., (1980) reported one monomorphic loci, in P. aztecus Iyes and P. merquiensis. DeMatthaesis et al.,(1983) showed one polymorphic locus with 2 alleles in cephalothorax tissue of P. japonicus and a monomorphic loci in P. kerathurus.

Lester (1983) viewed P. aztecus having monomorphic loci and Redfield et al.,(1980) showed one locus monomorphic in P. merquiensis. In their reports all the other enzymes except aldolase studied showed diallelic pattern.

Nelson and Hedgecock (1980) and Hedgecock et al, (1982) showed that glucose metabolising enzymes, Group I enzymes have correlation with environmental factors affecting their rate of polymorphism as seen in 51 species of decapods. Thus Group I enzymes has showed more alleles and more polymorphic than Group II enzymes.

In aldolase also due to the heterozygote deficiency significant difference with the expected value was observed. The same reasons mentioned in the acid phosphate may be the causative agents for this deviation. The aldolase being group I enzyme its influence on the observed deficiency of heterozygotes is also to be considered.

Alkaline phosphatase:

Alkaline phosphatase resolved well in tris citrate pH 7 buffer in P. indicus and P. stylifera as reported in other penaeid prawns (Mulley and Latter 1980) whereas in Macrobrachium obione (Trudeau 1978) and P. japonicus and P. kerathurus (DeMatthaeis et al.,1983) it has resolved in

tris maleic acid buffer. Electrophoretic variants of alkaline phosphatase were observed in muscle tissue of P. indicus and P. stylifera. Two alleles were detected in the muscle tissue of P. indicus and P. stylifera with monomeric subunit having polymorphism as reported in Australian penaeid prawns with monomer pattern showing 2 bands for heterozygote (Mulley & Latter 1980).

Due to the excess homozygotes, the goodness of fit tested for Hardy-Weinberg law showed significant variation in alkaline phosphatase enzyme. The reasons for the imbalance may be the same as explained in acid phosphatase.

Esterase:

Esterase enzyme was separated using histidine pH 7 and sodium citrate pH 7 buffers. In P. indicus consistant bands couldn't be observed due to lack of uniform pattern. P. stylifera showed 3 loci. One of the anodal zones Est-2 locus which expressed allelic variants showed a monomer structure. Esterase study reports in various other animals expressed the following features. Daphnia magna a monomer form with 3 alleles (Herbert and Ward 1972), in Balanus balanoides with 4 alleles (Flowerdew and Crisp 1975, 1976) in Chthamalus stellatus Est-2 locus with dimer form with 3 alleles and Est-4 locus with monomer form with 6 alleles (Juan 1976).

Esterase polymorphism was reported in prawns like Penaeus aztecus Ives; P. duorarum Burkenroad and P. setiferus Linnaeus (Lester 1979) in P. kerathurus with 2 polymorphic loci and P. japonicus with one polymorphic locus (DeMatthaeis et al 1983) and M. bennettae with one polymorphic locus having 4 alleles and P. plebejus had one polymorphic loci with 5 alleles (Mulley and Latter 1980). Esterases are often found to be weak and unstable and it may change due to nongenetic causes such as ontogenetic or physiological state (Johnson et al.,1974/ Kannupandi 1980). In the present investigation esterases were found to exhibit inconsistent patterns in P. indicus. The observed esterase enzyme phenotypes produced significant variations in their expected frequency due to the excess of homozygotes.

Alpha glycerophosphate dehydrogenase:

Alpha glycerophosphate dehydrogenase plays an important role in the intermediary metabolism. Tissue expression studies carried out in P. indicus showed one region of enzyme activity in hepatopancreas. Since the allelic frequency exceeded 0.95 it was considered as monomorphic loci.

Monomorphic nature of the glycerophosphate dehydrogenase enzyme was also reported in Euphausia superba (Ayala et al.,1975)

Euphausia mucronata, E. distinguienda (Ayala and Valentine 1979) Cthamalus dalli Pilsbry, and C. fissus Darwin (Hedgecock 1979). In rocklobster a single strongly staining band was seen (Smith et al.,1980) and a monomer subunit, was observed in American lobster (Odense and Anand 1978).

Penaeid prawns expressed inadequate resolution in muscle tissue (Mulley and Latter (1980) whereas P. merguiensis showed one monomorphic locus for this enzyme (Redfield et al., 1980) and Macrobrachium rosenbergii also expressed one monomorphic locus in muscle.

Lactate dehydrogenase:

Lactate dehydrogenase plays an important metabolic role and exist in several isozymic forms. Zymogram patterns of this enzyme showed 2 loci in different tissues like eye, hepatopancreas and muscle in P. indicus studied here. Since these didn't exhibit any allelic variants no genetic variation studies could be carried out. In American lobster 2 loci were seen with 3 bands of enzyme activity (Odense and Anand 1978). Rock lobster had a locus in muscle tissue and in J. edwardsii this locus was weakly polymorphic but in J. novaehollandiae it is strongly polymorphic for the same 2 alleles (Smith et al.,1980)

These Lactate dehydrogenase enzyme resolved in muscle of Penaeid prawns showed 2 bands for heterozygous condition.

Lactate dehydrogenase enzymes showed monomorphic loci in surral crustaceans (Berthelemy 1978; Hedgecock et al.,1979; Fuller and Lester 1980; Nelson and Hedgecock 1980; Redfield et al.,1980).

Malate dehydrogenase:

Genetic variation studies for malate dehydrogenase were conducted on P. indicus and P. stylifera. The enzyme was resolved in tris glycine 8.3 pH buffer. Activity of this enzyme was seen in eye and muscle tissues. It gave two banded heterozygote showing a monomer structure. Mdh-1 locus in eye was found to be polymorphic in P. indicus and P. stylifera. Malate dehydrogenase enzyme separated in different animals showed one polymorphic locus in Pandalus jordani (Berthelemy 1978), Homarus americanus (Tracey et al., 1975) and surral penaeid prawns (Lester 1979 and 1983).

Penaeid prawn (Mulley and Latter 1980) and P. japonicus and P. kerathurus (DeMatthaeis et al 1983) Pandalid shrimp (Johnson et al.,1974) expressed malate dehydrogenase activity in muscles, P. latisulcatus resolved in eyes, hepatopancreas and muscle (Richardson 1982 b).

Phenotypic distribution of malate dehydrogenase enzyme in P. indicus and P. stylifera was tested for goodness of fit and found to show significant variation. Probably due to the causes explained in acid phosphatase enzyme.

Malic enzyme:

Electrophoretic separation of this enzyme for genetic variation studies have been done on many animals. In the present study of P. indicus and P. stylifera this enzyme resolved in buffer tris maleic acid edta buffer 7.6 pH. In P. indicus 2 loci were observed one in eye and another in muscle. Me-1 locus showed polymorphism for 2 alleles. Heterozygote with 2 banded nature proved it to be having monomer structure. Eye and muscle of P. stylifera also expressed one locus. Me-1 in eye tissue tested in Cochin expressed polymorphism.

Malic enzyme resolved using tris citric acid 8.4 pH in Penaeud prawns (Mulley and Latter 1980) P. kerathurus and P. japonicus (DeMatthaeis et al.,1983) found to have one monomorphic loci in muscle tissue. Expression in rock lobster was as a diffused band (Smith et al.,1980) whereas in Pandalid shrimp (Johnson et al.,1974) showed it as an anodal band in muscle. Penaeus merguiensis de Man(Hedgecock et al.,1979) Pandalus danae, P. jordani and P. platyceros (Berthelemy 1978) showed one monomorphic loci. Homarus americanus (Tracey et al.,1975) had one polymorphic loci

with 2 allele and H. gammerus (Hedgecock et al.,1977) showed a polymorphic loci with 4 alleles. Expected values of Hardy-Weinberg equilibrium expressed significant variation due to the reasons already mentioned for acid phosphatase enzyme.

Octanol dehydrogenase:

Studies on genetic variation in P. indicus and P. stylifera showed polymorphic locus in P. indicus eye tissue. This enzyme was found out to be showing a dimer pattern with three banded phenotype for heterozygote. Electrophoretic separation was done using tris maleic buffer 7.6 pH. Significant variation in the goodness of fit of phenotype distribution may be due to reasons discussed for acid phosphatase enzyme.

The interesting observation of apparent polymorphism at Octanol dehydrogenase only in the Waltair populations of P. indicus suggests that they may be an isolated population from that of Cochin, Tuticorin and Madras. Further detailed studies alone can confirm the present findings.

Reports of electrophoretic separation of this enzyme carried out in various other prawns like P. esculentus, P. latisulcatus, P. longistylus, M. macrleayi, M. insolitus, M. eboracensis and P. semisulcatus showed one polymorphic

locus with 2 alleles (Mulley and Latter (1980). P. esculentus and M. endeavouri showed 3 alleles in a polymorphic loci while P. merguiensis, M. bennettae (Mulley and Latter 1980)and P. latisulcatus (Richardson 1982b) expressed a polymorphic locus with four alleles.

6-Phosphogluconate dehydrogenase:

The hepatopancrease in P. indicus showed 6 Phospho-glyconate dehydrogenase polymorphism. Gene frequency data on this enzyme was carried out on P. indicus, collected from 4 different localities. It expressed two loci in various tissues like muscle and hepatopancreas of P. indicus. The separation of this enzyme carried out in tris boric acid edta pH 8. Activity of this enzyme was reported in various animal tissues like muscle (Redfield et al., 1980; Mulley and Latter 1980), in digestive gland and muscle of rock Lobsters (Smith et al 1980) and in eyes of P. latisulcatus (Rechardson 1982 b).

In Decapoda P. aztecus, P. duorarum Burkenroad and P. setiferus Linnaeus showed polymorphic loci with 3 alleles each. P. merguiensis deman (Redfield et al.,1980), M. rosenbergii deman (Hedgecock et al.,1979) Homarus americanus (Tracey et al., 1975) and H. gammarus (Hedgecock et al.,1977) showed one monomorphic locus.

Expected phenotypic distribution showed significant differences due to the reasons already discussed for acid Phosphatase enzyme.

1-Pyrroline dehydrogenase:

Genetic variation of Pyrroline dehydrogenase was carried out in P. indicus and P. stylifera species. This enzyme gave good resolution in tris boric edta 8 pH. Hepatopancreas and Muscle showed regions of this enzyme activity. Genetic analysis proved them to be monomorphic. Redfield et al.,(1980) observed this enzyme, resolving in tris-citric and boric lithiumhydroxide, tris boric acid edta and tris hydrochlorit acid buffers in muscle tissue, Mulley and Latter (1980) found this locus to be polymorphic in penaeus species of prawns and monomorphic in metapenaeus species of prawns and viewed the separation using tris citrate discontinuous buffer 8.4 pH.

Tetrazolium oxidase:

This enzyme was used for genetic variation studies in P. indicus and P. stylifera. It resolved in tris boric edta pH 8 buffer and showed a polymorphic enzyme in P. stylifera in hepatopancreas tissue but found put to be monomorphic in P. indicus. Two banded heterozygote pattern observed here was also reported in Penaeid prawns of

Australia (Mulley and Latter 1980). The phenotype distribution of To at locus 2 was found to beiin Hardy-Weinberg equilibrium. Polymorphic loci were reported in Chthamalus stellatus (Dando et al.,1979) Euphauria superba (Ayala et al., 1975), Cambarus bartonii (Nemeth and Tracey 1979) Panulirus interruptus, and Upagebea pugettensis, whereas monomorphic loci were seen in Pandalus dane, P. jordani and P. platyceros (Berthelemy 1978).

Sorbitol dehydrogenase:

Sorbitol dehydrogenase was analysed in P. indicus using tris glycine buffer. Genetic analysis carried out in muscle tissue showed it to be monomorphic. Redfield and Salini(1980) resolved this enzyme in tris boric acid edta pH 9 in muscle tissues of Penaeid spp of prawns. Mulley and Latter (1980) found this enzyme to be polymorphic with dimer structure in muscle tissues.

Like here slight, but significant, heterozygote deficiency with respect to Hardy-Weinberg expected proportion was consistently observed in Homarus americanus (Hedgecock et al., 1977) due to Wahlund effect . The present observation of electrophoretic variant forms of all the enzymes tested in eye, hepatopancreas and muscle tissues of P. indicus and P. stylifera has not been reported earlier.

The present first report of 15 different enzyme systems and their loci in differenti tissues of P. indicus and P. stylifera suggests that the same enzyme can be separated and resolved suitably in different buffer systems ifrespective of the species.

The individual enzyme wise discussion shows clearly that the present observations of monomorphic and polymorphic nature of enzymes in P. indicus and P. stylifera in their different tissues and distribution pattern of different phenotypes in their populations are comparable with that of other prawn species and crustaceans as reported by others. The defeciency of heterozygotes in all the enzymes except in one may be caused by one or more unknown reasons discussed.

The interesting observation of apparent polymorphism at Octanol dehydrogenase only in the Waltair populations of P. indicus suggest that they may be an isolated population from that of Cochin, Tuticorin and Madras. Further detailed studies alone can confirm the present findings.

The results of the present investigation of intra species variation of enzymes such as acid phosphtase alcohol dehydrogenase, aldehydeoxidase, aldolase, alkaline phosphatase, esterase, α-glycerophosphate dehydrogenase,

lactate dehydrogenase, malate dehydrogenase, malic enzyme, octanol dehydrogenase, 6 phosphogluconate dehydrogenase, pyrroline dehydrogenase, sorbitol dehydrogenase, and tetrazolium oxidase in different tissues like eyes, hepatopancreas and muscles and their distribution pattern in different populations of P. indicus and P. stylifera thus become an important contribution in understanding the hitherto unknown population genetics of P. indicus and P. stylifera which are commercially important cultivable prawn species of India.

Electrophoresis gives the basic information upto genotype or allele frequencies at each locus in a given population. Different measures has been utilized to express the amount of genetic variation within a population. The most informative way of expressing this is the occurrance of heterozygosity. The proportion of polymorphic loci in a population is another measure of genetic variation commonly used. A third measure of genetic variation is the average number of alleles per locus.

Genetic differentiation among the populations can be found out using measures of Genetic similarity (I) and genetic distance (D) developed by Nei (1972, 1973). Genetic similarity is the average probability of selecting two electrophoretically identical alleles per locus from 2

different populations. Genetic distance is measured as the mean of electrophoretically detectable amino acid substitutions that have occurred since the 2 populations being compared have diverged from a common ancestor.

Mean number of alleles in a population:

Hydrobiological conditions especially temperature is found to have correlation with differential activity of electrophoretic alleles (Koehn 1969, Smith et al., 1978). This measures is highly dependent on the number of individuals studied since rare alleles are detected in larger samples and also with more number of polymorphic loci. In the present analysis of 23 loci only seven loci were found out to be polymorphic in P. indicus and six polymorphic loci were observed out of twenty two loci analysed in P. stylifera. Since majority of the loci studied in P. stylifera and P. indicus are monomorphic the average number of alleles per locus in low. In P. indicus it was found out to be 1.304 in Cochin and 1.375 in Tuticorin, Madras and Waltair. Overall mean number of alleles per locus in P. indicus was found out to be 1.322. P. stylifera showed mean number of alleles 1.545 in Cochin and 1.571 in Bombay and overall mean number of alleles per locus was 1.558. Similar low values were in H. americanus, (Tracey et al.,1975) where the average number of alleles per population is varied from 1.19 to 1.55, whereas Hedgecock et al(1977) reported

average number of alleles to be 1.2 per locus in H. gammerus. In P. kerathurus and P. japonicus mean number of allele per locus (A) is 1.265 and 1.484 respectively (DeMatthaeis et al., 1983). In Decapoda (Penaeidae and caridea) number of alleles per locus was 1.64 (Hedgecock et al.,1982). In P. indicus and P. stylifera the reason for the observation of lower value of alleles per locus may be due to bottle neck size, that is population size which will curtail the incidence of rare allele in the animal. Average number of alleles per locus increases faster than the heterozygosity when the population is restored (Nei 1975).

In fishes comparisons of allele frequencies suggested significant differentiation among neighbouring populations (Burton and Feldman 1982). In lobsters Tracey et al.(1975) showed Mdh-2^{105} allele at a frequency of 0.2 at Wood Hole whereas the same analysed in Martha's Vineyard samples lacked that allele The same allele has a frequency of 0.11 at the offshore LSA site but was absent from CSE site located 60 km away. Available genetic data indicate substantial differentiation among local H. americanus population despite this species planktonic larval stages. Present study in P. indicus showed Octanol dehydrogenase enzyme, to be polymorphic in Waltair but the same was monomorphic in other locations. Though this may suggest a clear cut differentiation of

Waltair population from that of other areas, a detailed comparative similar investigation is essential for further confirmation.

Polymorphic loci:

Genetic variation measured with proportion of polymorphic loci is highly dependent on the number of individuals studied since the rare alleles are only identified when more and more samples are studied. Average proportion of polymorphic loci per population (P_p) was found out by finding average of proportion of polymorphic loci over all populations. Average proportion of polymorphic loci per population was found out to be 0.279 for P. indicus and 0.289 for P. stylifera. Proportions of polymorphic loci in P. indicus is .261 in Cochin, Tuticorin and Madras and .333 in Waltair. Thus Waltair samples showed more proportion of polymorphic loci. In P. stylifera proportion of polymorphic loci is .304 in Cochin and .273 in Bombay. Meagre variation is noticed in the two populations of P. stylifera. Mulley and Latter (1980) showed 0.14 average number of loci as polymorphic in penaeid species of prawns. Whereas in P. aztecus duorarum and setiferus 0.3 loci were polymorphic (Lester 1979). Hedgecock (1979) noticed average proportion of polymorphic gene enzyme population is 0.14

in M. rosenbergii. In P. americanus proportion of polymorphic loci ranged from 0.17 to 0.4 and from 0.154 to 0.205 in H. gammerus (Tracey et al.,1975). Penaeus kerathurus and P. japonicus showed 0.265 and 0.387 frequency of polymorphic loci (DeMattheis et al.,1983). Lester (1979) reported the proportion of polymorphic loci to be 0.33 in P. aztecus, 0.33 in P. duorarum and 0.29 in P.setiferus. Thus the low amount of polymorphism exhibited by these crustaceans investigated from others supports the present finding of similar low values in P. indicus and P. stylifera.

Hardy-weinberg law:

It is noticed that the observed distribution of phenotypes significantly deviated from the expected Hardy-Weinberg equilibrium. Population which are present nearer will have more similarity. More population of this sort deviated from Hardy-Weinberg equilibrium due to deficiency of heterozygotes and also due to different selection coefficient on particular genotype (Korpelainen 1984). Violent selectively determined oscillations on genetic frequencies marks characteristic deviations of genotypic frequencies at polymorphic loci from Hardy-Weinberg proportions (Herbert 1974). In few cases deviation from Hardy-Weinberg equilibrium is due to heterozycote deficiency

caused by temporal environmental inconsistency tend to lead a relatively lower levels of genetic diversity (Bataglia et al 1978). Significant heterozygote deficiency with respect to Hardy-Weinberg expected proportions is observed in Homarus americanus (Hedgecock et al.,1977).

Significant deviation with reference to Hardy-Weinberg law is noticed in alkaline phosphatase enzyme in Astacus leptodactylis (Romanov et al.,1976), in Esterase in Chthamalus stellatus, C. depressus (Juan 1976) in Mysis relicta (Furst and Nyman 1969) in Phosphoglucomutase enzyme in Pandalus hypsinotus (Johnson et al.,1974) and in protein of Astacus leptodactylus (Brodskii et al.,1976). In eelspout Zoarces viviparus Christiansen et al.,(1977) found a significant deficit of heterozygotes for an esterase polymorphism. Ferguson (1980) suggested deviation from Hardy-Weinberg expectation in fishes is due to deficiency of heterozygote.

Heterozygosity:

The genetic variability distributed within and between populations is generally similar in P. indicus and P. stylifera. The amount of heterozygosity estimated in each population based on observed frequency of heterozygote (Ho) and expected frequency of heterozygote (He) was found out for P. indicus and P. stylifera (Table 73, 74). Average frequency of heterozygote per locus was also found out for each places.

In Penaeus indicus average frequency of heterozygote found per locus is .0114 ± .02 in Cochin, 0.0114 ± 0.02, in Tuticorin, 0.01 ± 0.02 in Madras and 0.0142 ± 0.02 in Waltair. Overall value for P. indicus is 0.011 ± 0.02. In P. stylifera average frequency of heterozygote is .03 ± 0.03 in Cochin, 0.025 ± 0.03 in Bombay and overall for P. stylifera it is 0.026 ± 0.03. Observed and expected frequency of heterozygotes significantly deviates from expected for majority of the loci in P. indicus except for malic enzyme. In P. stylifera also deviation was observed in aldehydeoxidase enzyme, Pyrroline dehydrogenase, Alpha glycerophosphate dehydrogenase, Octanol dehydrogenase and tetrazolium oxidase but not in alcohol dehydrogenase in Cochin. This deviation was due to excess of homozygosity being observed in these samples. Deficiency of heterozygote was observed in Decapod crustaceans (Hedgecock et al.,1982).

Homozygosity evolves in environments with temporally seasonally fluctuating trophic resources (Valentine 1976). When the feeding habit was taken, prawns have onnivorous habits. Gopalakrishnan (1952) and Panikkar (1952) stated that food of young penaeids consist of organic detritus found on the mud algal material and other small organisms in the mud. Vegetable matters included diatoms like ¢oscinodiscus, Pleurosigma, Rhizosolenia, the pelagic alga

Trichodesmium and cutting of sea weeds. The crustacean included copepods, ostracods, amphipods, tiny decapods and their larval stages, Molluscan shell pieces, polychaetes, echinoderm larvae hydroids, trematodes and foraminifera were occasionally consumed. Panikkar and Menon (1956) stated that P. indicus food consists of detritus, both animal and plant that accumulate at the bottom of their habitates which are usually areas with muddy bottom. When algal matter is available they consume it in large quantity.

Studies on phytoplankton availability in Menon 1945 proved peak season during May in Trivandrum coast. Gonzalves (1947) observed peak season during January-February in Bombay George (1953) found out a peak season in abundance during southwest monsoon. In Madras peak season is during April and May (Menon 1931), Prasad (1956) worked on the phyto-plankton availability in east coast of India. Subramanyan (1959) pointed out the seasonal variation in abundance of phytoplankton. Availability of Zooplankton was seen to be showing peak season in Madras during November to February (Menon 1931) in Trivandrum the peak season is during December to February (Menon 1945). A bimodal cycle was proposed by Prasad (1954) on the availability of zooplankton during February to April and October. Prasad (1956) found a bimodal in the zooplankton distribution in Munnar and Palk Bay.

5 years study on zooplankton availability conducted by Menon and George (1977) showed the zooplankton peak during July-September and a Secondary peak in November along the south-west coast of India. All these studies proves that a variety of phytoplankton and zooplankton is available for consumption. But the availability is restricted to particular season only. Phytoplankton bloom is there during southwest monsoon in West coast and North east monsoon in East coast of India. Thus a bimodal oscillation of phytoplankton is noticed in Indian waters (Chenabhotla 1981). After this monsoon the Zooplankton increases in its quantity. So the availability of phytoplankton and zooplankton fluctuates according to season. Thus prawn is restricted with one diet for that particular season. Moriarty (1977) explains the trophic environment of prawn species to be highly heterogeneous. In this type of seasonally fluctuating trophic resource environment homozygosity evolves to a great extent (Valentine 1976). Again homozygosity excess observed in enzyme locus suggests species in inbreeding condition in natural environment (Nakajima and Masuda 1985).

Usually in large samples from random population observed and expected frequency of heterozygotes ar Difference exist in observed frequency of heterozygote is due to natural selection or other factors (Ayala and Valentine 1977). Tracey et al (1975) noticed consistent deficiency of

observed heterozygote. Every population sample appears deficient of heterozygotes and thus in total H. americanus showed a slight but significant excess of homozygotes. In the 50 decapod species average heterozygosity has a mean value of nearly 5.5%. It showed low heterozygosity of 0.05-0.06 (Nelson and Hedgecock 1980) in H. americanus average number of heterozygotes per locus is 0.045. As a whole decapods are characterized by low levels of genetic variation (Hedgecock et al. 1976, Gooch 1977, Cole and Morgan 1978). Average on frequency of heterozygote seen in different prawn species is given below in the table.

Details of average frequency of heterozygotes individual per population expected at Hardy-Weinberg equilibrium ($\bar{H}e$) and proportion of polymorphic loci (P).

Species	$\bar{H}e$	p
P. setiferus[1]	0.089	-
P. aztecus[1]	0.076	-
P. duorarum[1]	0.092	-
P. kerathurus[2]	0.055	0.26
P. japonicus[2]	0.121	0.39
P. merguiensis[3]	0.008	0.16
P. semisulcatus[3]	0.017	0.22
P. monodon[3]	0.009	0.09
P. esculentus[3]	0.033	0.24
P. plebejus[3]	0.022	0.24
P. latisulcatus[3]	0.032	0.13
P. longistylus[3]	0.006	0.08
M. macleayi[3]	0.026	0.17
M. bennettae[3]	0.020	0.20
M. endeavouri[3]	0.030	0.20
M. ensis[3]	0.013	0.20
M. insolitus[3]	0.010	0.10
M. eboracensis[3]	0.019	0.17
P. indicus[4]	0.011	.28
P. stylifera[4]	0.026	.29

1. Lester (1979)
2. DeMatthaeis et al., 1983.
3. Mulley and Latter, 1980.
4. Present study.

Even though samples of P. indicus and P. stylifera the present studies were collected from extensive geographic area it showed low heterozygosity as seen in P. monodon and P. latisulcatus found out by Mulley and Latter (1980). Higher amount of heterozygosity is seen in P. japonicus was considered to be due to large effective population and the admixture of different geographical population (DeMatthaeis et al.,1983). Successful and widespread marine invertebrates occupy wide range of physical and trophic environment and characterized by extremely low levels of heterozygosity (Mulley and Latter 1980) since mutational variants are selectively eliminated as seen in Australian prawn (Mulley and Latter 1980). Thus all the above said factors are main causative agents which reduces the amount of heterozygosity in P. indicus and P. stylifera population.

A significant excess of homozygote over Hardy-Weinberg expectations is commonly encountered in electrophoretic phenotypes of marine invertebrates. This might be due to preferential selection of homozygotes over the less fit heterozygotes occupying different niches. Another factor which gives rise to apparent homozygote excess is Wahlund effect. This is due to mixing of populations containing same pair of alleles at different frequencies (Crisp 1977). Hedgecock et al.,(1982) observed that proportional difference between observed and expected heterozygosity is seen over all

two allele cases of polymorphism in each of 38 species of decapods. The mean differences over all species was D = -0.009 ± 0.010 not significantly different from zero. So at this level no evidence for widespread subdivision of decapod population (Hedgecock et al., 1982). Mixing of prawn populations has been proved by tagging studies. New light on the migration of the P. indicus using Tagging studies revealed that Tirunelveli coast is replenished by prawns migrated from the backwaters of Cochin (Vijayaraghavan et al., 1982). Suggesting that lack of heterozygosity in P. indicus and P. stylifera as observed in the present study need not be due to subdivision and separation of these species with genetically different populations.

Enzyme function and structure have influence on mean heterozygosites (Selander, 1976). Three different classifications of enzymes into two groups have been proposed on the above basis. Glucose and non-glucose metabolising enzymes (Gillespie and Kojima, 1968). Enzymes involved in processing substrates derived from external environment and those enzymes handling substrates from internal house keeping transactions. (Johnson et al., 1973; Ayala et al., 1972). Enzymes having single and multiple specificing substrates (Gillespie and Langley 1974 Under each of the above classification mean heterozygosity

is greater in the second group of enzymes rather than first group of enzymes (Selander 1976). Vertebrates didn't show any significant differences between glucose and non-glucose metabolising enzyme heterozygosities, but invertebrates show differences.

Mean heterozygosites in glucose-metabolising (Group I) and non-glucose metabolising enzymes (Group II) found in *P. indicus* and *P. stylifera* is given below:

Protein	Heterozygosity	
	P. indicus	*P. stylifera*
Glucose-metabolising enzyme (Group I)		
Aldolase	0.08	
Alpha Glycerophosphate dehydrogenase		0.06
Malate dehydrogenase	0.03	0.04
Malic enzyme	0.0069	0.1
6-Phosphogluconate dehydrogenase	0.03	
Non-glucose metabolising enzymes (Group II)		
Alcohol dehydrogenase		0.014
Acid phosphatase	0.04	.08
Esterase		0.06
Tetrazoliumoxidase		To_1- 0.08, To_2- 0.04
Octanol dehydrogenase	0.014	0.04
Pyrroline dehydrogenase		0.054
Alkaline phosphatase	0.05	0.06
Aldehyde oxidase	Ao-1 0.04	Ao-1 0.04, Ao_2- 0.03

From the above results it is revealed that P. indicus showed lesser heterozygosity in aldolase, malate dehydrogenase, malic enzyme and 6-phosphogluconate dehydrogenase which are glucose metabolising enzymes and more heterozygosity in acid phosphatase, octanol dehydrogenase, alkaline phosphatase and aldehyde oxidase which are non-glucose metabolising enzymes. P. stylifera shows more heterozygosity in many of the non-glucose metabolising enzymes like alcohol dehydrogenase, acid phosphatase, esterase, tetrazolium oxidase, octanol dehydrogenase, 1-Pyrroline dehydrogenase, alkaline phosphatase and aldehyde oxidase and little heterozygosity in some of the glucose metabolising enzymes like glycerophosphate dehydrogenase, malate dehydrogenase and malic enzyme. Thus the above results reveals the difference between glucose and non-glucose metablising enzyme heterozygosities. Thus shows the more variable enzyme in Group II enzymes and less variable enzymes in Group I enzyme.

Nei's identity is usually less than 0.75 between species. Between population of a single species the level of genetic identity is usually 0.90 or greater (Nei, 1973)

Ayala (1975) found out Limulus polyphemus, Phoronopsis viridis and Tridacna mixima to be having identity 0.99, 0.96 and 0.968 and deviation to be 0.01, 0.004 and 0.032 for their populations. The values obtained in the present

study (Tables 67 & 68) reveal that they belong to one and the same species. Population collected have more or less similar allele frequencies for polymorphic loci.

Genetic identity and deviation analysis reveal that prawns P. indicus and P. stylifera collected from different populations belong to the one and the same respective species. Only a small proportion of loci were found to have alleles at significantly different frequencies. Populations regarded as subspecies generally show four times as much genetic divergence as geographically separated but morphologically similar one.

Population size has been postulated as influencing heterozygosity (Soule 1976). Small population size associated with specialised local or isolated population may theoretically limit heterozygosity (Kimura and Ohta, 1971; Nei, 1975). P. japonicus showed high heterozygosity due to large effective population size (DeMatthaesis et al., 1983).

In the present study 36 numbers of specimens were collected in each location for electrophoretic analysis where as Hedgecock et al., (1976) suggested atleast 20-30 individuals to be sampled from a single location. Hence, the sample size of present investigation might not have effect on the expected results.

Several models of genetic variability and heterozygosity and their predictions for environments or species with low and high levels of genetic variation has been proposed by many authors. According to the size and mobility Selander and Kaufman (1973) proposed that small and sessile organism to have more genetic variation than large and mobile organisms. By taking into consideration of trophic resources stability Ayala and Valentine (1977) explained that high seasonality to exhibit low genetic variation whereas low seasonality to show high genetic variation.

By environmental heterogenity Levins (1968) characterised habitat specialists to show high levels and habitat generalists by low levels of genetic variation. Difference in heterozygosity between specialists and generalists was explained through environmental heterogenity. A specialists species perceives its environment as coarse grained or heterogeneous having common, widespread, broad niched, main land species and will be affected by the environment. A generalist species perceives its environment as fine grained or homogenous (Smith and Fujio 1982). Any difference in this environment is of minor importance to the animal. Thus a fine grained environment for one species may be a course-grained environment for the other species (Valentine 1976

Most of the prawn species occupy an extensive geographic area like P. indicus found in Indo-Pacific, S. Africa to China, New Guinea and Australia and P. stylifera seen in Indo-West Pacific. Besides this they have mixed life cycle, to include a broad-niched species. Long larval period faced by these animals result in unpredictable environment with consequent selection of few alleles.

Life cycle of P. indicus is completed after passing through two distinct environments the sea and the estuary. The larval development takes place in the sea and the migration into the estuaries, lakes and backwaters commences when they are in late mysis or early post-larval stages (Mohamed 1970

Berger's (1973) findings in genus Littorina supports the inverse relationship between the capacity of larval disparsal and the extent of population. Genetic differentiation proposed by Gooch and Schopf(1972) and Snyder and Gooch (1973) compared L. saxatilis (no planktonic larvae) to Nassarius obsoletus (with long lived planktonic larvae) and found significantly greater differentiation in the former species. Hence Barton (1983) expected that adults which are sedentary and lack planktonic larvae always found to show significant differentiation of population on a relatively small geographical scale. Burton (1983) found limited geneflow among geographically separated invertebrates.

Smith et al, (1980) showed that long larval phase is apotential for extensive gene flow, suggesting that stock differences are unlikely. Turner and Lyerla (1980) proved that exchange of pelagic larvae is found to be the reason for the genetic similarity whereas New Zealand Shapper which has a short larval stage is divided into a number of discrete stocks (Smith 1979). Tracey et al.,(1975) showed large, mobile, generalised lobsters adapted to temporarly and spatially varying environment through phenotypic variability rather than genetic variability. As the larval stages of the two species P. indicus and P. stylifera studied here are of greatly mobile in nature lack of significant genetic variability presently observed shows their different populations comparable to that of lobsters.

Mixed life cycle in decapods shows a change during the life cycle from a pelagic and planktonic larva to a free swimming adult. This life cycle heterogeneity might force the animal to perceive its environment as coarse-grained and select a few generalised rather than many specialised alleles. Because of this a low level of genetic variation have been found in decapods with a more heterogeneous life cycle. The tropic environment of prawn is shown to be highly heterogeneous (Moriarty 1977) Marine invertebrate species such a thirteen penaeid prawn species

subject to wide range of physical and trophic environments were characterized by low levels of heterozygosity (Mulley and Latter 1980).

According to the environment the specialist species, select several narrow-range alleles and is characterized by high heterozygosities. In the generalists the individuals bear "flexible" alleles which are few and wide ranged alleles characterized by low heterozygosities (Smith and Fugio 1982) This same observation was seen in P. indicus and P. stylifera. Specialists species feed on narrow range of food, whereas generalists consume a wider range of food. In this way high heterozygosity is seen in specialists such as Aulorhynchus flavidus (Hart 1973) and low heterozygosity such as Enophrys bison feeding on algae and hard and soft invertebrates (Hart 1973). Penaeid prawns can be included in generalists which occupy wide range environment and feed and exhibit low levels of heterozygosity.

A hybrid environmental heterogeneity trophic diversity model has been proposed to explain genetic variability in decapod crustacea (Nelson and Hedgecock 1980). In 51 species of coastal, intertidal, temperate and tropical decapods, the specialist species which are characterized by small, less mobile animals occupying a number of sub niches has high variability in Group I enzymes and low variability in Group II

enzyme and thus act as trophic specialists with narrow range of substrates promoting low variability. In the same way the generalist decapods, species with a fine-grained are characterized by low genetic variation in Group I and high genetic variation, in Group II enzymes. These include large, mobile crustaceans like prawns. As trophic generalists they face a wide variety of food species and heterogeneous tropic environment (Moriarty 1977) showing high variability in the external substrate (Group II) enzymes. This habitat specialist-generalist model would classify the decapods as generalists and observed low variability (Valentine 1976) which is observed in the case of P. indicus and P. stylifera analysed in the present study.

A relationship between heterozygosity and the level of variation of environmental factors as well as the variation in morphological traits was established (Johnson and Mickevich 1977). Several studies have shown correlations between water temperature or latitude and frequencies of electromorphs in marine animals (Johnson, 1971, 1974, 1977; Mitton and Koehn, 1975; Powers and Powers, 1975;) corresponding biochemical differences have been demonstrated between genotypes (Koehn 1969, Powers and Powers 1975). Allele frequencies at enzymes were related to hydrobiological conditions (Koehn 1969 Smith et al.,1978; Smith 1979).

Under extreme conditions such as high temperature oxygen deficiency different genotypes possessing varying viability (Kirpichnickov 1981). Relationships between biochemical variability and environment appear to be very complex in nature. This is supported by evidence shown that variation in Lactate dehydrogenase and haemoglobin may depend on fluctuations in many environmental factors - water temperature, oxygen content, pH, salinity and others (Powers, 1980). For polymorphism constantly fluctuating conditions of intra-cellular metabolism may also play an important part (Johnson 1976).

The temperature conditions of life for most species of animals, plants and micro-organism correlate with the heat stability of proteins (Alexandrove 1975); speciation appear to be accompanied by heredity changes in the heat stability of protein molecules. Snith et al., 1980; showed that in an open loop system gene flow tend to erode the different selective pressure which exerts on the animal and the selection will be for few generalised alleles rather than many specific alleles observed herein P. indicus and P. stylifera which showed 2 alleles in majority of the enzymes except Aldolase in P. indicus.

The following important conclusions are derived from the intra species genetic variation studies conducted on the prawn species P. indicus and P. stylifera.

Electrophoretic investigations of different isozymes in these two species, have enabled to detect seven polymorphic loci in P. indicus and six polymorphic loci in P. stylifera out of 23 and 22 loci analysed in the respective species, resulting in low average number of alleles per locus in both species. The lack of goodness fit as per Hardy-Weinberg equilibrium, in the distribution of different phenotypes in all the population of P. indicus and P. stylifera tested in the present study was due to deficiency of heterozygotes and excess of homozygotes.

One or more unknown factors or models such as small sample size, preferential selection of phenotypes, temporal and seasonal fluctuations of feed, nature of function and structure of enzymes tested, environmental heterogenity etc. might have produced the observed significant deficiency of heterozygotes and excess of hemozygotes in P. indicus and P. stylifera tested in the present investigation. The values of gene frequencies, average number of alleles per locus, average proportion of polymorphic loci per populations, average frequency of heterozygotes per locus and the values of genetic distance of populations being non-significant in different populations of P. indicus and P. stylifera collected from different regions indicate that the populations

tested are not genetically different. The present findings thus support the tagging results that P. indicus populations Tirunelveli coast is actually replenished by migrated prawns from Cochin.

Thus the present study shows little evidence that the prawns P. indicus and P. stylifera are subdivided into two or more genetic stocks. For management purpose all the population of Cochin of these species of prawns can be treated as one biological unit.

MORPHOMETRY IN RELATION TO GENETIC VARIATION

Resume of literature:

Organisms occur more or less in distinct or discrete population or stocks. Stock has to be defined as a group or population of a species maintaining one or more common characteristics depending on the type of environment of domicile. (Kutkuhn 1981). Two or more unit stocks can be satisfactorily demonstrated by applying any of the following techniques such as biochemical, immunological, serological, behavioural, morphometric, meristic, mark recapture, electrophoretic etc.

Morphological variation in Zoarces viviparus was observed by Schmidt (1917a, 1918) from 61 different locations. The characters are heritable and are reported to have direct environmental influence (Schmidt 1917a, 1917b; 1918, 1920 1921a; 1921b). Morphological variation was analysed in three races of kokanee Oncorhyncus nerka (Vernon 1957), in mountain white fish Prosopium williamsoni (Holt 1960), in three sympatric Arctic cod fishes of the genera Arctogadus and Gadus (Boulva 1972) in Upeneus sulphureus (Cuvier) from Maharashtra coast (Musharraf Ali 1978) in Sardinella sirmwal from Andaman Sea (Abidi et al.,1978-79), in Lactarius lactarius

(Choudhary and Dwivedi 1980-81), and in pink salmon Oncorhynchus gorbuscha (Beacham 1985). Morphometric technique has been used for stock separation in several species of fishes including Salmo salar (Lear and Misra 1978; Riddell and Leggett 1981), Mallotus villosus (Sharp et al.,1978), Salvelinus malma (Morrow 1980) and Coregonus spp. (Casselman et al.,1981, Ihssen et al.,1981; Todd et al.,1981). The main advantage of this method is that it effectively isolate shape differences than most of the other traditional methods (Ihssen et al.,1981).

In crustaceans also some work has been done using morphological characters for stock delineation. Metric variation in populations of Carcinus maenas (William and Needham 1941) and geographic morphometric variation in American lobster Homarus americanus (Templetson 1935; Saila and Flowers 1969) were noticed.

Geographical variation using morphological characters was seen in the genus Nematocelis (Crustacea:Euphausidae) by Gopalakrishnan (1974), in isopod Sphaeroma rugicauda by Heath (1975), in Western Atlantic population of Gammarus oceanicus segerstrale (Amphipoda) by Croker and Gable (1977), in the dwarf cray fish by Chambers et al.,(1979), in Sphaeroma serratum (Isopoda) by Consiglio and Argano (1968)

and in Pontinella dara (Copepoda) by Fleminger and Hulsemann (1974).

Morphological variance was used in prawns to find intra species variation in Macrobrachium rosenbergii de man by Lindenfelser (1980), to analyse specific variation in fresh water prawn M. niloticum in lake chad and Lake Rudolf by Williamson (1972) in Penaeus semisulcatus by Morgan (1982) and P. vannamei and P. stylirostris by Lester (1983). Lui (1979) after measuring and statistically testing the morphological variables in Macrobrachium australiense disapproved Rick's (1951) suggestion of possible subdivisions.

Morphological relationship of Penaeus semisulcatus Metapenaeus affinis and Parapenaeopsis stylifera was found out by Farmer (1986). Besides morphometry in some organisms electrophoresis also gained momentum to identify different stocks as seen in Cynoglossus bilineatus from Bombay waters (Kasinathan et al.,1972), in rainbow trout Salmo gairdneri (Gjedrem and Skjesvold 1972), in four population of Menidia (Mickevich and Johnson 1976) in Gasterosteus aculeatus (Bell 1976), in fiddler crab Uca (Selander et al.,1971; Salmon et al. 1979) in Chthamalus montagui (Crustacea: Cirripedia) in the Adiatric (Dando et al.,1979) and in Atlantic show crab (Davidson et al.,1985).

Several causative agents like environmental factors play crucial role in the speciation process as seen in Malaysian prawn Macrobrachium rosenbergii (de man) reared in earthern ponds in South Carolina (Smith et al.,1978). Particularly salinity variation and diet cause differences in M. carcinus from Barbados and Jamaica (Choudhury 1971) and in species of M. rosenbergii, M. javanicum and M. pilimanum (Johnson 1960) and in laboratory population of brown shrimp Penaeus aztecus (Venkataramiah et al.,1975). Maturation and spawning was found to exhibit difference between costa Rican and Maxican P. stylirostris (Brown et al.,1980).

Results:

Univariate and Multivariate analysis were carried out for the 3 geographical population (Cochin, Tuticorin and Madras) of P. indicus (Table No.79,- 81 & 83-85) and 2 geographical population (Cochin and Bombay)of P. stylifera (Table No. 82, 86 & 87).

Table 79: Comparison of morphometric variables of *Penaeus indicus* samples from Cochin and Tuticorin

Character	Sampling Location	Sample size range	Mean value	S.D.	't' value
SSL	Cochin	9-15 mm	11.2639	1.3065	3.3498*
	Tuticorin	10-15 mm	12.278	1.262	
FSL	Cochin	6-14 mm	8.3889	1.4595	2.4501*
	Tuticorin	6-14 mm	9.361	1.881	
PCL	Cochin	19-32 mm	22.1667	2.3115	1.4761
	Tuticorin	18-29 mm	23.056	2.779	
CW	Cochin	10-18 mm	11.4028	1.553	0.4303
	Tuticorin	8-15 mm	11.569	1.72	
FLF	Cochin	3.5-7 mm	5.0556	0.8348	1.3871
	Tuticorin	6-11 mm	5.333	0.862	
SSD	Cochin	7-14 mm	9.0139	1.2506	0.4705
	Tuticorin	6-11 mm	9.153	1.258	
SAD	Cochin	10-16 mm	11.3611	1.3764	0.1526
	Tuticorin	8-15 mm	11.417	1.713	
AAC	Cochin	34-54 mm	39.5833	3.9668	0.3451
	Tuticorin	31-49 mm	39.944	4.858	
PAC	Cochin	25-41 mm	30.0556	2.8977	1.439
	Tuticorin	20-36 mm	28.917	3.76	
TW	Cochin	7-28 gm	13.1111	11.2282	0.0168
	Tuticorin	7-19 gm	13.078	3.656	
TL	Cochin	101-150mm	115.0000	9.2921	0.6397
	Tuticorin	90-140mm	116.667	12.574	

* Significant at 5% level.

Table 8.0: Comparison of morphometric variables of *Penaeus indicus* samples from Cochin and Madras

Character	Sampling Location	Sample size range	Mean Value	S.D.	't' Value
SSL	Cochin	9-15 mm	11.2639	1.3065	4.8557*
	Madras	10-15 mm	12.681	1.166	
FSL	Cochin	6-14 mm	8.3889	1.4595	6.216*
	Madras	8-14 mm	10.472	1.383	
PCL	Cochin	19-32 mm	22.1667	2.3115	4.133*
	Madras	17-29 mm	24.458	2.392	
CW	Cochin	10-18 mm	11.4028	1.5530	3.6058*
	Madras	8-15 mm	12.667	1.419	
FLF	Cochin	3.5-7mm	5.0556	0.8348	2.8325*
	Madras	4-7 mm	5.611	0.829	
SSD	Cochin	7-14 mm	9.0139	1.2506	2.8624*
	Madras	12-18 mm	9.889	1.342	
SAD	Cochin	10-16mm	11.3611	1.3764	4.4192*
	Madras	9-14.5mm	12.778	1.344	
AAC	Cochin	34-54 mm	39.5833	3.9668	3.8073*
	Madras	32-52 mm	43.0000	3.641	
PAC	Cochin	25-41 mm	30.0556	2.8977	1.4759
	Madras	20-39 mm	31.083	3.008	
TW	Cochin	7-28 gm	13.1111	11.2282	1.16
	Madras	5.3-20.5gm	15.375	3.326	
TL	Cochin	101-150mm	115.0000	9.2921	1.4752
	Madras	110-142mm	120.683	21.165	

* Significant at 5% level.

Table 81: Comparison of Morphometric variables of Penaeus indicus samples from Tuticorin and Madras.

Character	Sampling Location	Sample size range	Mean Value	S.D.	't' value
SSL	Tuticorin	10-15 mm	12.278	1.262	1.4074
	Madras	10-15 mm	12.681	1.166	
FSL	Tuticorin	6-14 mm	9.361	1.881	2.8552*
	Madras	8-14 mm	10.472	1.383	
PCL	Tuticorin	18-29 mm	23.056	2.779	2.2942*
	Madras	17-29 mm	24.458	2.392	
CW	Tuticorin	8-15 mm	11.569	1.72	2.9546*
	Madras	8-15 mm	12.667	1.419	
FLF	Tuticorin	6-11 mm	5.333	0.862	1.3947
	Madras	4-77 mm	6.611	0.829	
SSD	Tuticorin	6-11 mm	9.153	1.258	2.4007*
	Madras	12-18 mm	9.889	1.342	
SAD	Tuticorin	8-15 mm	11.417	1.713	3.7505*
	Madras	9-14.5mm	12.778	1.344	
AAC	Tuticorin	31-49 mm	39.944	4.858	3.0203*
	Madras	32-52 mm	43.0000	3.641	
PAC	Tuticorin	20-36 mm	28.917	3.760	2.699*
	Madras	20-39 mm	31.083	3.008	
TW	Tuticorin	7-19 gm	13.078	3.656	2.7885*
	Madras	5.3-20.5gm	15.375	3.326	
TL	Tuticorin	90-140mm	116.667	12.574	0.9788
	Madras	110-142mm	120.683	21.165	

* Significant at 5% level.

Table 82: Comparisons of morphometric variables of *Parapenaeopsis stylifera* samples from Cochin and Bombay.

Character	Sampling location	Sample size range	Mean value	S.D.	't' value
SSL	Cochin	8-11 mm	9.7778	0.7215	1.4461
	Bombay	9-11 mm	9.5139	0.8236	
FSL	Cochin	4-9 mm	6.5000	0.94111	5.8919*
	Bombay	6-9 mm	7.7222	0.8145	
PCL	Cochin	20-29 mm	23.8611	2.8601	1.2023
	Bombay	18-29 mm	23.0278	3.0189	
CW	Cochin	9-15 mm	11.5556	1.5389	0.6055
	Bombay	10-16 mm	11.7639	1.3757	
FLF	Cochin	4-6 mm	5.1667	0.5606	0.5247
	Bombay	3-7 mm	5.2500	0.7700	
SSD	Cochin	7-11 mm	9.3611	1.0185	3.3655*
	Bombay	7-10 mm	8.5278	1.0820	
SAD	Cochin	8-12 mm	10.3889	0.9344	1.3219
	Bombay	8-12 mm	10.0694	1.1094	
AAC	Cochin	23-40 mm	31.8611	3.4654	0.7885
	Bombay	26-37 mm	31.2778	2.7735	
AAC	Cochin	22-30 mm	26.1667	2.3845	1.9530*
	Bombay	21-30 mm	25.0833	2.3223	
TW	Cochin	4.1-10gm	6.1111	1.6039	0.5892
	Bombay	4-9 gm	6.3333	1.5959	
TL	Cochin	86-116mm	97.75	8.1744	0.0129
	Bombay	82-117mm	97.7222	10.0046	

* Significant at 5% level.

Table 83: Matrix of correlation coefficient among eleven morphological variables in *Penaeus indicus* collected at Cochin.

	SSL	FSL	PCL	CW	FLF	SSD	SAD	AAC	PAC	TW	TL
SSL	1.0000										
FSL	0.5365	1.0000									
PCL	0.7608	0.6916	1.0000								
CW	0.7666	0.7861	0.8981	1.0000							
FLF	0.6411	0.5680	0.6910	0.6324	1.0000						
SSD	0.8589	0.7248	0.8665	0.9054	0.6697	1.0000					
SAD	0.7240	0.7103	0.7529	0.8456	0.6783	0.8352	1.0000				
AAC	0.7881	0.6851	0.7946	0.8536	0.6327	0.8996	0.8185	1.0000			
PAC	0.7092	0.6568	0.7685	0.8552	0.5243	0.8591	0.7757	0.8521	1.0000		
TW	-0.0298	0.2222	0.1371	0.1633	-0.0037	0.0380	0.1230	0.1066	0.1746	1.0000	
TL	0.7849	0.7163	0.8327	0.8811	0.6704	0.9220	0.8511	0.9054	0.8425	0.1235	1.0000

Table 84 : Matrix of correlation coefficient among eleven morphological variables in *Penaeus indicus* collected at Tuticorin.

	SSL	FSL	PCL	CW	FLF	SSD	SAD	AAC	PAC	TW	TL
SSL	1.000										
FSL	0.751	1.000									
PCL	0.828	0.776	1.000								
CW	0.692	0.774	0.924	1.000							
FLF	0.779	0.726	0.803	0.745	1.000						
SSD	0.755	0.743	0.864	0.902	0.676	1.000					
SAD	0.711	0.701	0.835	0.877	0.619	0.884	1.000				
AAC	0.846	0.793	0.893	0.848	0.769	0.876	0.792	1.000			
PAC	0.806	0.760	0.881	0.845	0.820	0.882	0.746	0.895	1.000		
TW	0.732	0.732	0.930	0.958	0.776	0.878	0.892	0.823	0.847	1.000	
TL	0.813	0.771	0.896	0.899	0.778	0.898	0.871	0.895	0.856	0.909	1.000

Table 85: Matrix of correlation coefficient among eleven morphological variables in Penaeus indicus collected at Madras.

	SSL	FSL	PCL	CW	FLF	SSD	SAD	AAC	PAC	TW	TL
SSL	1.000										
FSL	0.645	1.000									
PCL	0.833	0.736	1.000								
CW	0.650	0.679	0.909	1.000							
FLF	0.518	0.626	0.709	0.609	1.000						
SSD	0.561	0.483	0.699	0.734	0.435	1.000					
SAD	0.450	0.473	0.686	0.716	0.446	0.421	1.000				
AAC	0.872	0.743	0.879	0.755	0.573	0.623	0.593	1.000			
PAC	0.765	0.656	0.842	0.753	0.655	0.692	0.535	0.814	1.000		
TW	0.709	0.676	0.912	0.943	0.658	0.779	0.721	0.755	0.769	1.000	
TL	0.495	0.270	0.425	0.315	0.186	0.325	0.293	0.544	0.378	0.315	1.000

Table 86: Matrix of correlation coefficient among eleven morphological variables in *Parapenaeopsis stylifera* collected at Cochin.

	SSC	FSL	PCL	CW	FLF	SSD	SAD	AAC	PAC	TW	TL
SSL	1.0000										
FSL	0.1683	1.0000									
PCL	0.3861	0.5679	1.0000								
CW	0.3459	0.5721	0.8879	1.0000							
FLF	0.4473	0.4332	0.4782	0.4857	1.0000						
SSD	0.6177	0.4918	0.7828	0.7980	0.5421	1.0000					
SAD	0.6828	0.4224	0.6623	0.6403	0.6364	0.8990	1.0000				
AAC	0.1587	0.4775	0.7533	0.7757	0.4535	0.6056	0.5378	1.0000			
PAC	0.2546	0.4711	0.6906	0.6592	0.5985	0.5392	0.4959	0.7394	1.0000		
TW	0.4441	0.5981	0.7521	0.8193	0.5444	0.7163	0.7101	0.7791	0.7443	1.0000	
TL	0.4844	0.5589	0.8295	0.8472	0.4645	0.6975	0.6715	0.7300	0.6486	0.8436	1.0000

Table 87: Matrix of correlation coefficient among eleven morphological variables in *Parapenaeopsis stylifera* collected at Bombay.

	SSL	FSL	PCL	CW	FLF	SSD	SAD	AAC	PAC	TW	TL
SSL	1.0000										
FSL	0.6447	1.0000									
PCL	0.7065	0.5028	1.0000								
CW	0.6460	0.3732	0.8960	1.0000							
FLF	0.5125	0.4783	0.5009	0.5428	1.0000						
SSD	0.6809	0.4953	0.7914	0.7003	0.5573	1.0000					
SAD	0.7259	0.5278	0.8184	0.7411	0.4808	0.8969	1.0000				
AAC	0.6487	0.5790	0.8419	0.8264	0.5954	0.7876	0.8107	1.0000			
PAC	0.6641	0.5563	0.7251	0.7665	0.5473	0.6643	0.7795	0.8347	1.0000		
TW	0.7256	0.4799	0.8852	0.8765	0.5743	0.7871	0.8257	0.8635	0.7933	1.0000	
TL	0.6628	0.4811	0.8810	0.8462	0.4952	0.7451	0.7998	0.8647	0.8360	0.8588	1.0000

Discussion:

Various population parameters and physiological, behavioral, morphometric, meristic, calcareous, biochemical and cytogenetic characters have been used to identify fish stocks. Population measures are useful primarily for the recognition of punitive stocks at the practical management level. Application of morphometric and meristic characters in stock identification is complicated by the fact that phenotypic variation in these characters has not been directly related to particular differences in the genome (Clayton, 1981). Effects of physiological and epigenetic constraints on morphology is directly related to certain environmental parameters such as temperature and oxygen (Martin 1949, Gould 1977, Stanley 1979 and Todd et al, 1981). The number of serially repeated characters alters with the environmental changes associated with attitude (Taning 1952; McGlade 1981).

Morphological (morphometric) characters represent a series of measured variables and represent the synergism between shape and size. Using these morphological characters differenciation of stocks is likely to be subtle as seen in most fish species since it is affected by allometry (Gould 1966, Sweet 1980).

Fish stocks appear to develop as a result of complex interaction between genetic (biochemical level), organismic (level of morphology, physiology and behaviour) and ecological factors (Lindsey 1981; Clayton 1981).

Multivariate comparisons of morphological measurement among closely related group of organism reflect some biological variables such as growth rate or sexual dimorphism (e g. Eyles and Blackith 1965) According to the present study a large portion of morphometric variation among stocks is probably due to difference in growth rates in the stocks. As per the present results, morphometric data of Penaeus indicus differentiates Tuticorin-Madras, Cochin-Madras stocks completely and the resulting relationships among the stocks did not appear to resemble the relationships obtained from the biochemical electrophoretic data. But Cochin-Tuticorin geographical populations of P. indicus and Cochin-Bombay geographical populations of P. stylifera showed significant variation only for few morphological variables. Kirkpatrick and Selander (1979) found out speciation occurs in sympatric stocks of white fish with only minor changes in the allelic frequencies measured by electrophoresis.

Lester (1983) found out that different variables analysed in each species data set have quite distinct

correlations despite their appearance of morphological similarity among penaeid species. In the commercial mariculture operation of prawns any reduction of the size of the maturation tanks, hatchery tanks and growout ponds will affect behaviour and survival (Lester 1983), and consequently size.

Phenotypic differences observed between prawn species P. indicus samples of Tuticorin-Madras, Cochin-Madras, may be due to morphological differentiation in response to environmental factors during the ontogeny when these prawns enter the back waters and lakes for larval development as seen in snow crabs (Davidson et al.,1985). P. indicus can withstand wide range of salinity, especially in younger stages. To some extent the species is eurythermal, as seen from wide gradient of temperature of its natural habitats. Thus they are faced with relatively heterogeneous environments. The magnitude of larval exchange between areas would be affected by oceanographic patterns and proportional to the distance between areas and velosity of surface currents (Davidson et al.,1985).

As they grow larger they move to the sea and thus have a relatively homogeneous adult environments. Then tend to converge in their morphological attributes as seen in snow crab (Davidson et al.,1985).

Invertebrates such as crustacean generally exhibit little morphological change in relation to short-term changes in their environments. Their rigid exoskeleton often allows for more precise morphometric measurements compared with soft bodied vertebrates (Davidson et al.,1985). This proves that morphological comparison of crustacean population may provide useful evidence for delineating stocks. But it is often difficult to distinguish between genetic and environmental effects on phenotypic characters (Booke 1981). But majority of the phenotypic variability (Morphovariance) observed between areas would be due to the notion that structural genes evolution (measured by electrophoresis) proceeded independently at a different rate from evolution at more complex phenotypic levels (King and Wilson 1975, Wilson, Maxson and Sarich 1974; Wilson, Sarich and Maxson 1974). Besides these rate of protein evolution appears to be proportional to time (Ayala 1976; Carson 1976). This is probably the reason why some morpho variance is noticed in specimen from different areas, although biochemically they appear to belong to the same population.

SUMMARY

1. For the detailed analytical work different procedures were modified and methods standardised in order to get better results. For optimum resolution of different enzymes standardisation of methods indicated 10% acrylamide concentration giving best resolution for protein extracted from different tissues of the species of prawns under study.

2. The buffers employed for the separation of the enzymes Acid phosphatase, Alkaline phosphatase, Alcohol dehydrogenase, Aldehydeoxidase, Esterase, Alpha glycerophosphate dehydrogenase, Lactate dehydrogenase, Malate dehydrogenase, Malic enzyme, Octanol dehydrogenase, Peroxidase, 6-Phosphogluconate dehydrogenase, 1-Pyrroline dehydrogenase, Tetrazolium oxidase and Sorbitol dehydrogenase were Tris citrate Buffer pH 7; Tris citrate Buffer pH 7, Tris versene Borate Buffer pH 8, Tris glycine Buffer pH 8.3, Histidine pH 7 & Sodium citrate pH 7, Tris versene Borate pH 8, Tris citric acid pH 8.3 and Lithium hydroxide pH 8.26, Tris glycine buffer pH 8.3, Tris maleic acid Edta pH 7.6, Tris Maleic acid Buffer pH 7.6, Histidine pH 7 and Sodium citrate pH 7, Tris versene Borate pH 8, Tris versene Borate pH 8, Tris versene Borate pH 8 and Tris glycine Buffer pH 8.3 respectively.

3. The electrophoretic patterns of 15 different enzymes and their loci tested in different tissues, namely eye, hepatopancreas and muscle of two species of prawns P. indicus and P. stylifera has been studied for the first time.

4. Muscle myogen pattern of closely allied species of prawns were anlysed to find out the interspecies genetic variation. Species of genus Metapenaeus, namely M. kutchenensis, M. affinis, M. monoceros and M. brevicornis collected from Bombay waters showed characteristic bands of 7, 12, 9 & 9 respectively. Similarly species of genus Parapenaeopsis such as P. sculptilis P. stylifera and P. hardwickii from Bombay had 9, 8 & 10 bands respectively.

5. Very closely allied species like Penaeus penicillatus and P. merguiensis as well as P. japonicus, P. latisulcatus and P. canaliculatus. were further subjected to ultra scanning and photography of the gels which showed distinct band nature useful for identifying the species.

6. The present ontogenetic observation in P. indicus shows that each larval stage of a species can be clearly identified on the basis of species-specific number of enzymatic protein bands. In cases where same number of bands exist in different stages the characteristic pattern of the bands would be useful in differentiating these stages.

7. Electrophoretic investigations of different isozymes in Penaeus indicus and Parapenaeopsis stylifera have enabled to detect seven polymorphic loci in P. indicus and six in P. stylifera, out of 23 and 22 loci analysed in the respective species.

8. The lack of goodness of fit as per Hardy-Weinberg equilibrium, in the distribution of different phenotypes in all the population of P. indicus and P. stylifera tested from samples from different places may be due to deficiency of heterozygotes and excess of homozygotes.

9. Genetic identity and genetic distance estimates following the analysis of Nei as well as Roger suggests that the population samples from four locations in the case of Penaeus indicus and the population samples from two location in the case of Parapenaeopsis stylifera are genetically similar.

10. There is little evidence to show that the prawns P. indicus and P. stylifera are subdivided into two or more genetic stocks. For management purpose all the population of these two species of prawns sampled from different locations, namely P. indicus from Cochin, Tuticorin, Madras and Waltair and P. stylifera from Cochin and Bombay appear to belong to a single unit biochemically.

11. The observation of apparent polymorphism in the enzyme octanol dehydrogenase alone in the Waltair samples of P. indicus would suggest the probable existence of an isolated population of the species there.

12. In P. stylifera out of the various enzymes analysed acid phosphatase alone showed some difference in the phenotypic distribution and allele frequency between Cochin and Bombay samples.

13. Statistical analysis of certain selected morphometric characters of sample specimen collected from Cochin and Tuticorin in the case of P. indicus and Cochin and Bombay in the case of P. stylifera exhibited very little significant variation, in confirmity with the biochemical results.

14. Thus, as far as P. stylifera is concerned the populations at both Cochin and Bombay appear to be the same both biochemically and morphologically. Similar is the case with P. indicus of Cochin and Tuticorin. However, in the case of P. indicus some significant variation has been noticed in certain morphological features between Madras-Cochin and Madras - Tuticorin specimens, probably brought about by the differential growth due to different environmental features in relation to geographical situation.

REFERENCE

Abidi, S.A.H., B.N. Desai, and O.S. Gautam, 1978. The lengthweight relationship in Sardinella, Sirmwal from the Andaman sea. J. Ind. Fish. Assoc. Vol 8 & 9 15-19.

Alexandrov, V. Ya., 1975. Cell, Molecules and temperature. Springer, Heidelberg/ Berlin New York.

Alikhan, M.A., and S. Akthar, 1980. The phylogenetic and ecological implications of the haemolymph proteins in crustacea. Curr. Sci. (Bangalore), 49(2): 53-56.

Allendrof, F., N. Ryman, A. Stennek, and G. Stahl, 1976. Genetic variation in scandinavian brown trout (Salmo Trutta L.) evidence of distinct sympatric populations. Hereditas. 83(1): 73-82.

Allendrof, F.W., and F.M. Utter, 1979. Population genetic of fish. In: Fish Physiology. Vol. 8. Academic Press, London, New York, pp. 136-187.

Altaknov, J.P., and G.N. Nejyodov, 1968. Res. Bull. Int. Comm. Northw. Atlant. Fish 5, 86-90.

Arias, E., and E. Morales, 1977. Comparative electrophorograms of muscle proteins of Mullus surmuletus and Mulles barbatus. Invert. Pesq. 41(2): 323-330.

Arias, E., and E. Moralas, 1980. Comparative electrophoregrams of muscle porteins Sarpa salpa and Boops boops. Invest. Pesq. (Barc) 44(1): 35-41.

Avise, J.C., J. Smith and F. Ayala, 1975. Adaptive differentiation with little genic change between two native california minnows. Evolution 29(3): 411-426.

Avise, J.C., and J. Felley, 1979. Population structure of freshwater fishes. I. Genetic variation of blue gill (Lepomis macrochirus) populations in man-made reservoirs. Evolution 33(1): 15-26.

Ayala, F.J., 1975. Genetic differentiation during specification process: In: Evolutionary biology Vol. 8. Plenum press. New York. London: 1-78.

Ayala, F.J., 1976. Genetic differentiation during the specification process. Pages 78 in the Dobzhansky, M.K.Hecht and W.C. Steere, Eds. Evolutionary biology volume 8. Appleton - Century - Crofts, New York.

Ayala, F.J., and J.W. Valentine, 1977. Genetic variation and resources stability in marine invertebrates in NATO conference series. Series IV: Marine Sciences, Volume 2. Marine organisms Genetics: Ecology and Evolution Ed: B. Battaglia and J.A. Beardomore.

Ayala, F.J., and J.W. Valentine, 1978. Genetic variation and resource stability in Marine Invertebrates. In: Marine Organisms: Genetics, Ecology and Evolution, pp. 23-51. Ed. by B. Battaglia and J.A. Beardmore. New York: Plenum Press 1978.

Ayala, F.J., and J.W. Valentine, 1979. Genetic variability in the pelagic environment: A paradox. Ecology 60: 24-29.

Ayala, F.J., T.R. Powell, M.C. Tracey, C.A. Mourao and S. Perez-Salas, 1972. Enzyme variability in the Drosophilla willistoni group IV. Genetic variation in natural population of Drosphila Willistoni. Genetics Vol. 70: 113-139.

Ayala, F.J., M.L. Tracey, L.G. Barr, J.F. McDonald and S. Perez-Solar, 1974. Genetic variation in natural Populations of five Drosophila species and the hypothesis of the selective neutrality of protein polymorphism. Genetics 77, 343-384.

Ayala, F.J., J.W. Valentine, and G.S. Zumwalt, 1975. An Electrophoretic study of the Antarctic zooplankter Euphausia superba. Limnol. Oceanogr. 20(4): 635-640.

Bagshaw, J.C., and A.H. Warner, 1979. Biochemistry of Artemia Development. University Microfilms Intl., Ann. Arbor, MI. pp7.

Barton, R.S., 1983. Protein Polymorphism and genetic differentiation of Marine invertbrate populations. Mar. Biol. Lett. 4: 193-206.

Bactacharya, A., and J.R.B. Alfres, 1982. Electrophoretic variation of soluble protein fractions in tissues of different size groups of Channa stewartii and Danio dangila, Matsya 8: 23-32

Battaglia, B., P.M. Bisol, and G. Fava, 1978a. Genetic variability in relation to the environment in some marine invertebrates. In "Marine Organisms, Genetics, Ecology and Evolution" (B. Battaglia and J.A. Beardmore eds.), pp. 53-70. Plenum New York.

Beachem, T.D., 1985. Variation and Morphometric variation in pink salmon. Oncorhynchus gorbuscha in southern British columbia and Puget sound. Can. J. Zool. 63: (2): 366-372.

Beckam, L., and F.M. Johnson, 1964. Variations in larval Alkaline phosphatase controlled by Aph alleles in Drosophilla melanogaster. Hereditas. 51, 221.

Beckwitt, R., 1985. Population genetics of the sand crab Emerita analoga Stimpson in S. california J. Exp. Mar. Biol. Ecol. 91 (1-2): 45-52.

Bell, M.A., 1976. Evolution of phenotypic diversity in Gasterosteus aculeatus superspecies on the pacific coast of North America/ Syst. Zool 25(3): 211-227.

Berger, E., 1973. Gene-enzyme variation in three sympatric species of ittorina. Biol. Bull. (Woods Hole, mass.) 145: 83-90.

Berger, E., and J. Sutherland, 1979. Allozyme variation in two natural populations of Daphnia pulex. Heredity 41(1): 13-23.

Berthelemy, N.J., 1978. Contribution a l etude de la genetique des populations de Pandalus danae, P. jordani, et P. platyceros (Crustaces, Decapodes) des cotes de Californie. These de doctorat de eme cycle. Universite d' Axis-Marseille, Marseille.

*Blanco, A., and W.H. Zinkham, 1966. Bull. Johns. Hopkins Hosp. 118,27.

*Boulva, J., 1972. Morphometrics of 3 Sympatric Arctic Cod fishes of the Genera Aretogadus and Godus/J. Fish. Res. Board. Can. 29: 243-249.

Brewer, G.J., 1979. An introduction to isozyme techniques. Academic Press, New York. pp.186

Brodskii, S.Y., A. Blakhnin, and L.I. Romanov, 1976. Genetic analysis of the population structure of crayfish in the waters of the Ukrainian SSR. Electrophoretic investigation of the haemolymph serum components of Astacus leptodactylus/Sov. Genet. 11: 462-467.

Brown, A., Mc Vey, J.P. Scott, B.M. Williams, T.D. Middleditch, B.S. and Lawrence, A.L., 1980. The maturation and spawning of Penaeus stylirostris under laboratory conditions. Proc. World Maricult Soc., 11: 488-499.

Burton, R.S., 1983. Protein polymorphism and genetic differentiation of marine invertebrate population Review. Mar. Biol. Lett. 4: 193-206.

Burton, R.S., and M.W. Feldman, 1982b. Changes in free amino acid concentrations during osmotic response in the interidal copepod Tigriopus californicus. Comp. Biochem. Physiol. 73A: 441-445.

*Buzzati-Traverso, A.A., and A.B. Rechnitzer, 1953. Science N.Y. 117, 58.

Carson, H.C., 1976. Inference of the time of some Drosophila species. Nature 259: 395-396.

Casselman, J.M., J.J. Collins, E.J. Crossman, P.E. Ihssen, and G.R. Spangler, 1981. Lake Whitefish (Coregonus clupeaformis) stocks of the Ontario waters of Lake Huron. Can. J. Fish. Aquat. Sci. 38: 1772-1789.

Chambers, C.C., J.F. Payne, M.C. Kennedy, 1979. Geographic variation in the Dwarf Crayfish. Cambarellus puer Hobbs Decapoda combaridae. Crustaceana 36(1): 39-55.

Choudhury, P.C., 1971. Responses of larval Macrobrachium carcinus to variation in salinity and diet crustaceana 20(2): 113-120.

Choudhary, P.S., N.S. Dwivedi, 1980. Meristic and morphometric studies of Lactarius lactarius (Schneider), off Bombay Coast. J. Ind. Fish Assoc. 10 & 11 1-4.

Christiansen, F.B., and V. Simon Sen, 1978. Georgraphic variation in protein polymorphism in the eel pout Zoarces vivparus (L) In: Marine organisms, Pleum Publ. Corp, New York, pp. 1701-194.

Christeansen, F.B., Frydenberg, and Simonsen, 1977. Genetics of Zoarces populations X. Selection component analysis of Est. III. Polymorphism using samples of successive Cohort. Hereditas 87 (2): 129-150.

Christiansen, F.B., O. Frydenberg, A.O. Clydenholm, V. Simonsen, 1974. Genetics of Zoarces population. VI. Further evidence based on age group samples of a heterozygote deficit in the Est. II polymorphism. Hereditas 77(2): 225-236.

Clayton, J.W., 1981. The stock concept and the uncoupling of organismal and molecular evolution. Can. J. Fish. Aquat. Sci. 38: 1515-1522.

Clegg, J.S., and F.P. Conte, 1980. A review of the cellular and developmental biology of Artemia. In: Biochemistry and Molecular Biology (Edited by Personne G., Sorgeloos P., Roels O. and Jospen E.) Vol.2. Universea Press, Wetteren, Belgium.

CMFRI., 1985. Hatchery production of Penaeid seed Penaeus indicus. CMFRI Special Publication, No. 23.

Cole, M.A., and R.P. Morgan, 1978a. Genetic variation in two populations of blue crab Callinectus sapidus. Estuaries. (3): 202-205.

Cole, A.M., and R.P. Morgan, 1978b. Muscle, eye and serum proteins in the blue crab, Callinectus sapidus Rathben. Comp. Biochem. Physiol., 59B: 25-26.

*Connell, J.J. 1953a. Biochem. J. 54, 119-126.

*Connell, J.J. 1953b. Biochem. J. 55, 378-388.

Consiglio, C., and R. Argano 1968. The distribution of monomorphic population in Sphaesoma serratum (Isopoda) crustaceana 14: 137-742.

Cook, H.L., and M.A. Murphy, 1966. Rearing of Penaeid shrimp from eggs to post-larvae. Proc. 19th Annu. Conf. Southeast. Ass. Game Fish. Comm. 283-288.

Cook, H.L., and M.A. Murphy, 1969. The culture of larval Penaeid shrimp. Trans. Amer. Fish. Soc. 98(4): 751-754.

Cook, H.L., and M.A. Murphy, 1971. Early developemental stages of the brown shrimp Penaeus aztecus Ives, reared in the laboratory. Fish. Bull. U.S. Dept. Comm. 69(1): 223-239.

Costa, R., and P.M. Bisol, 1978. Genetic variability in deep-sea organisms. Biol. Bull. (Woods Hole, Mass) 155: 125-133.

Costlow, J.D. Jr., 1968 Metamorphosis in crustaceans. In: Metamorphosis: a problem in developmental biology, pp. 3-42 Ed. by W. Etkins and L.I. Gilbert. New York.

Cowden, R.R., and J.R. Coleman, 1962. A starch gel electrophoretic study of haemolymph proteins of some Bermuda Crustacea. Experimentia, Bascl, 18: 265-266.

*Crick, F.H.C., 1963. On the genetic code. Science, N.Y. 139, 461-464.

Crisp, D.J., 1977. Genetic consequences of different reproductive strategies in marine invertebrates: 257-273.

Crisp, D.J. 1977. Genetic consequences of different reproductive strategies in marine invertebrates: 257-273. NATO conference Series IV Marine Sciences Vol. 2 Marine organism Genetics, Ecology and Evolution Ed. Battaglia and J.A. Beardmore.

Croker, R.A., M.F. Gable, 1977. Georgraphic variation in Western Atlantic populations of Gammarus oceanicus segerstrale Amphipoda. Crustacena 32(1): 55-73.

Crozier, N.W., 1987. Biochemical genetic variation and population structure in angler-fish Tophius piscatorius-1. from the Irish sea and West of Scotland. J. Exp. Mar. Biol. Ecol. Vol. 106: 125-136.

Dall, W., 1964. Studies on physiology of shrimp, Metapenaeus mastersii (Haswell) (Crustacea: Decapoda: Penaeidae) I. Blood constituents. Aust. J. Mar. Freshw. Res., 15(2): 145-161.

*Dando, P.R., A.J. Southward and D.J. Crisp., 1979. Enzyme variation in Chthamalus stellatus and Chthamalus montagui (Crustacea: Cirripedia). Evidence for the presence of the C. montagui in the Adriatic. J. Mar. Biol. Asso. U.K. 59; 307-320.

Davidson, K., J.C. Roff, and L.W. Elner, 1985. Morphological electrophoretic, and fecundity characteristics of Atlantic snow crab, Chionoecetes opilio, and implications for fisheries management. Can. J. Fish. Aquat. Sci. 42: 474-482.

Davis, B.J., 1964. Disc Electrophoresis - II method and application to human serum proteins. Annals. New York. Academy Science: 404-427.

Decleir, W., 1961. The localisation of copper in agar gel electrophoretic pattern of crustaceans. Naturwissenshafte 48: 102-103.

DeMatthaeis, E.G. Allegrucci, A. Cocoone, D. Cesaroni, M. cobabli Sbordoni and V. Sbordoni, 1983. Genetic differentiation between Penaeus kerathurus and P. japonicus (Crustacea, Decapoda. Mar. Ecol. Prog. Ser. Vol. 12: 191-197.

*Dingle, J.R., D.E. Eagles and J.M. Neelin, 1955. J. Fish. Res. Bd. Can 12.

Dobkin, S., 1961. Early developmental stages of pink shrimp, Penaeus duorarum, from Florida waters. Fish. Bull Fish., Wildl. Serv. U.S. 61(190): 321-349.

Doyle, R.W., and W. Hunte, 1981. Genetic changes in fitness and field of a crustacean population in a controlled environment. J. Exp. Mar. Biol. 52: 147-156.

Eyles, A.C. and R.E. Blackfth, 1965. Studies on hybridization in Scolopostethus Fieber (Heteroptera Lygaeidae) Evolution 19: 465-479.

Farmer A.S.D., 1986. Morphometric relationship of commercially important species of Penaeus shrimp from the Arabian Gulf. 1-21, Kuwait. Bull. of Mar. Sci. No. 7.

*Ferguson, A., 1980. Biochemical systematics and evolution Blackie, Glasgow/London.

Ferguson, K.A. and A.L.C. Wallace, 1961. Starch-gel electrophoresis of anterior pituitory hormones. Nature (Lond) 190, 629-630.

Fischer and Bianchi, 1984. Fao species Identification sheets for fishery purpose. Western Indian Ocean. (Fishery Area 51) Volume 5 FAO of United Nations Rome, 1984.

Fleminger, A., K. Husemann, 1974. Systematics of distribution of the four sibling species comprising the genus Pontinella Dara (Copepoda, Calanoida). Fish Bull. 72(1): 63-120.

Flowerdew, M.W., 1976. Allelic esterase isozymes their variation with season position on the shore and stage of development in the cirripede Balanus balanoides. Mar. Biol. 35: 319-325.

*Flowerdew, M.W. and D.J. Crisp, 1975. Esterase heterogeneity and an investigation into racial differences in the cirripede Balanus balanoides using acrylamide gel electrophoresis Mar. Biol. 33: 33-39.

*Flowerdew, M.W. and D.J. Crisp, 1976. Allelic esterase isozymes their variation with season, position on the show and stage of development in the cirripede Balanus balanoides. Mar. Biol. 35: 319-325.

Frank, J.R., S.D. Sulkin and R.P. Morgan, II 1975. Biochemical changes during larval development of the Xanthid crab. Rhithropan opeus harrisic I. Protein, total lipid, alkaline phosphatase and glutamic oxalo acetic trans aminase. Mar. Biol. 32: 105-111.

Frankel, J.S., 1981. Alcohol dehydrogenase ontogeny and liver maturation in the leopard danio. Brachydanio nigro faciatus. Comp. Biochem. Physiol. 70B, 643-644.

Fuller, B., and L.J. Lester, 1980. Correlations of allozymic variation with habitat parameters using the grass shrimp. Palaemonetes pugio. Evolution 34, 1099-1104.

*Furst, M. and L. Nyman, 1969. Isoenzyme polymorphism in Mysis relicta, Loven. Rep. Inst. Freshwater Res., Drottningholm.

Garcia, T., 1980. Electrophorogram of the muscle proteins of 4 species of Sciaenidae pisces from cuban waters. Rev. Rown. Biol. Sev. Biol. Anim. 25(1): 23-25.

Garry, A.W., 1980. Geographic variation in the milkfish Chanos chanos I. Biochemical evidence. Evolution 34 (3) 558-574.

George, P.C., 1953. The marine plankton of the coastal waters of Calicut with observations in the hydro-biological conditions. J. Zool Sci., India, 51: 67-68.

George, M.J., 1970a. Synopsis of biological data on penaeid prawn Metapenaeus dobsoni (Miers) 1878. FAC Fish. Rep., (57) Vol. 4: 1335-1357.

George M.J., 1970b. Synopsis of biological data on penaeid prawn metapenaeus affinis (H. Milne Edwards) 1387. FAO Fish Rep., (57) Vol. 4: 1359-1375.

George, M.J., 1970c. Synopsis of biological data on penaeid prawn Metapenaeus monoceros (Fabricius) FAO Fish Rep., (57) Vol. 4; 1539-1557.

George, M.J., 1970d. Synopsis of biological data on penaeid prawn Metapenaeus brevicornis (H. Milne Edwards) 1837. FAO Fish. Rep., (57) Vol. 4: 1559-1573.

George, M.J., 1972. On the zoogeorgraphic distribution of Indian penaeidae. Indian J. Mar. Sci., 1: 89-92.

George, M.J., 1975. On the penaeid prawn Parapenaeopsis stylifera and new variety of the species from Cochin. J. Mar. Biol. Ass. India 15: 420-423.

George, M.J., 1978. Trends in marine prawn production in India. Indian Seafoods, 13(4) and 14(1): 19-24.

George, M.J., 1979. Taxonomy of Indian Prawns Penaeidae Crustacea Decapoda. Contributions to Marine Sciences Dedicated to St. C.V. Kurian 1975: 25-59.

Gillespie, J., and K. Kojma, 1968. The degree of polymorphism in enzymes involved in energy production compared to that in non-specific enzymes in two D. ananassae populations. Proc. Natl. Acad. Sci. U.S.A. 61 (3) 582-585.

Gillespie, J.H., and C.H. Langley, 1974. A general model to account for enzyme variation in natural populations, Genetics 76 (4): 837-848.

Gjedrem, A.D., and T.H. Skjesvold, 1972. Genetic and environmental sources of variation in length and weight of rainbow trout Salmo gairdneri. J. Fish. Res. Board Can. 29: 237-241.

Gonzalves, E.A., 1947. Variations in the seasonal composition of the Phytoplankton of Bombay harbour. Curr. Sci., 16: 304-305.

Gooch, J.I., 1977. Allozyme genetics of the life cycle stages of brachyurans. Chesapeake Sci. 18, 284-289

Gooch, J.L., B.S. Smith and P. Knupp, 1972. Regional Survey of gene frequencies in the mind snail Nassarius obsoletus. Biol. Bull 142; 36-48.

Gooch, J.L., and T.J.M. Schopf, 1972. Genetic variability in the deep sea; relation to environmental variability. Evolution 26, 545-552.

Gooch, J.L., and T.J.M. Schopf, 1973. Genetic variability in deep sea: Relation to environmental variability. Evolution 26: 545-552.

Gooch, J.L., and S.T. Hetrick, 1979. The relation of genetic structure to environmental structure: Gammarus minus in a karst area. Evolution 33(1): 192-206.

Gopalakrishnan, V., 1952. Food and feeding habits of Penaeus indicus. J. Madras Univ. (B) 22(1): 69-75.

Gopalakrishnan, K., 1974. Zoogeography of the genus Nematoscelis (Crustacea Euphausidae) Fish. Bull 72 (4) 1039-1076.

Gordon, 1978. Electrophoresis of protein in polyacrylamide and starch gels. In: Laboratory Techniques in Biochemistry and Molecular Biology. Ed. T.S. work and E. Work.

Gould, S.J., 1966. Allometry and size in ontogency and phylogeny. Biol. Rev. Cambridge Philos. Soc. 41: 587-640.

Gould, S.J., 1977. Ontogeny and phylogency. Belknap Press, Harvard University, Cambridge. 510 p.

Grant, W.S., 1984. Biochemical population genetics of Atlantic Herring Clupea harrengus. Copeia (2): 364-369.

Grant, W.S., and F.W. Utter, 1984. Biochemical population genetic/ of pacific Herring (Clupea pallasi) Can. J. Fish Agri. Sci. Vol. 41 (6) pp.

Green, D.M., 1979. A basic computer program for calculation indices of genetic distance and similarity J. Herld 70: 429-430.

Greenberg, S.S., and M.J. Kopac, 1965. Physiol. Zool. 39(2): 149-157.

Guyomard , R., 1981. Electrophoretic variation in four french populations of domesticated rainbow trout (Salmo gairdneri)/Can. J. Genet. Cytol. 23: 33-47.

*Haen and F.J.O'Rourke, 1969. Proc. R. Ir. Acad. 68B.(7) 101-108.

Hart J.C., 1973. Pacific fishes of Canada/Bull. Fish Res. Bd. Can. 180. 1-740.

*Heath D.J., 1975. Geographical variation in populations of the polymorphic isopod. Sphaeroma rugicauda. Heredity 35(1): 99-107.

Hedgecock, D., 1979. Biochemical genetic variation and evidence of specification in Chthamalus barnacles of the tropical eastern Pacific. Mar. Biol. 54:207-214.

Hedgecock, D., K. Nelson and R.A. Shleser, 1976. Application of biochemical genetics of Aquaculture. J. Fish. Res. Board Can., 33: 1108-1119.

Hedgecock, D., K. Nelson, R.A. Shleser and M.L. Tracey, 1975. Biochemical genetics of lobsters (Homarus). II. Inheritance of allozymes in H. americanus. J. Hered., 66: 114-118.

Hedgecock, D., M.L. Tracey and K. Nelson, 1982. Genetics. In: D.E. Bliss (Editor), The Biology of Crustacea, Vol. 2 Embrology, Morphology and Genetics. Academic Press, New York.

Hedgecock, D., K. Nelson, J. Simons and R. Sheleser, 1977. Genetic similarity of American and European species of the lobster Homarus. Biol. Bull. 152: 41-50.

Hedgecock,D., D.H.Stelmach, K.Nelson, M.E. Linder gelser and S.R.Malecha, 1979. Genetic divergence and biogeography of natural population of Machrobrachium rosenbergii/Proc. Annv. Meet-World. Maric. Soc. 10, 873-879.

Heegaard, P.E., 1953. Observations on spawning and larval history of the shrimp, Penaeus setiferus (L.) Publ. Inst. mar. Sci. Univ. Tex., 3(1): 73-105.

Hentschel C.C. and K.R. Tata, 1976. The molecular embryology of the brine shrimp. Trends Biochem. Sci. 1, 97-100.

Hines R. and A. Yashouv, 1970. Preliminary studies on muscle protein polymorphism occurring within the genus Tilapia! Bamidgeh 22: 69-71.

Hjorth A.P., 1974. Genetics of Zoarces populations. VII. Foetal and adults haemoglobin and a polymorphism common to both. Hereditas 78(1): 69-72.

Herbert P.D.N. and R.D. Ward, 1972. Inheritance during parthenogenesis in Daphnia magna. Genetics 71, 639-642.

Herbert P.D.N., 1974. Enzyme variability in natural populations of Daphnia magna. Population structure in East Anglia. Evolution 28: 546-556.

Herzberg A. and R. Pasteur, 1975. The identification of grey mullet by disc electrophoresis, Aquaculture 5: 99-106.

Hewitt, R.E. et al., 1963. Copeia 2, 296-303.

Holmes, R.S. and C.J. Masters, 1967. Biochem. Biophys. Acta 132, 379.

Holt V., 1960. Comparative morphometry of the mountain whitefish Prosopium williamsoni copeia (3) 192-200.

Homoir, G., 1955. Adv. Protein Chem. 10, 227-288.

Hudinaga, M., 1935. The study of penaeus 1. The development of Penaeus japonicus Bate. Rep. Hayatoma Fish. Res. Lab., 1(1): 1-51.

Hughes, L.D. and L.R. Winkler, 1966. Paper electrophoresis patterns of sera from seven generae of decapod crustaceans. Pac. Sci., 20: 521-524.

Hunter, R.L. and C.L. Markert, 1957. Histochemical demonstration of enzymes separated by zone electrophoresis in starch gels, "Science" 125, 1294-1295.

*Hunter, R.L., J.T. Rocha, A.R. Pfrender and D.C. Dejong, 1964. Ann. N.Y. Acad. Sci. 121, 532.

Inhof, M., R. Leeary, and H.E. Booke, 1980. Population or stock structure of lake white fish Coregonus clupea formics in Northern lake Michégan as assessed by isozyme electrophoresis. Can. J. Fish. Aquat. Sci. 38(5): 783-793.

Inssen, P.E., D.O. Evans, W.J.Christic, J.A.Reckahn and R.L.Desfardine, 1981. Life history, morphology and electrophoretic characters of five allopatric stocks of lake white fish (Coregonus clupeaformis) in the great lake region. J. Fish. Res. Bd. Can. 38: 1790-1807.

Ihssen, P.E., H.E.Booke, J.M.Casselman, J.M.McGlade, N.R. Payne, and F.M.Utter, 1981. Stock identification: materials and methods. Can. J. Fish. Aquat. Sci. 38: 1838-1855.

Johnson, D.S., 1960. Subspecific and intraspecific variation in some freshwater prawns of the Indopacific Region, in Proc. of the centenary and bicentenary congress of Biology, Singapore Dec. 2-9 1958 ed. RD. Purchon, University of Malaya Press, Singapore. pp. 259-267.

Johnson, M.S., 1971. Adaptive lactate dehydrogenase variation in the crested blenny, Anoplarchus. Heredity, Lond. 27: 205-226.

Johnson, M.S., 1974. Comparative geographic variation in Menidia. Evolution, Lancaster, 28: 607-618.

Johnson, M.S., 1976. Biochemical systematics of the atherinid genus Menidia. Copiea 4: 662-691.

Johnson, M.S., 1977. Association of allozymes and temperature in the crested blenny Anoplarchus purpurescens. Mar. Biol. 41: 147-152.

Johnson, A.G., 1981. Electrophoretic patterns of protein in Spanish mackeral (Scomberomorus maculatus) NOAA Technical memorandum NMFS-SEFC-76. May.

Johnson, M.S., and M.F.Mickevich, 1977. Variability and evolutionary rates of characters. Evolution 31(3): 642-648.

Johnson, A.G., F.M.Utter, and H.O.Hodgins, 1972. Electrophoretic investigation of the family scorpeanidae. Fish. Bull. 70: 403-413.

Johnson, A.G., F.M.Utter, and H.O. Hodgins, 1973. Estimate of genetic polymorphism and heterozygosity in three species of rockfish (Genus Sebastes). Comp. Biochem. Physiol. 44B: 397-406.

Johnson,A.G., F.M. Utter, and H.O. Hodgins, 1974. Electrophoretic comparison of five species of pandalid shrimp from the north-eastern Pacific ocean. Fish. Bull., 72: 799-803.

Johns,B.W. and I.M.Mackie, 1970. An application of electrophoretic analysis of muscle myogen to taxonomic studies in the genus Merluccins. Comp. Biochem. Physiol. 32: 267-273.

Juan,E., 1976. Polimorfismo enzimatico en poblacines de Chthamalus stellatus YC. depress us (Crustacea, cirripedia) Oecol. Aquat. 2: 111-119.

Kannupandi,T., 1980. Protein patterns during ontogeny of the Xanthid crab, Rhithropanopeus harrisi, Indian J. Mar. Sci. 9: 127-131.

Kannupandi and P. Paulpandian, 1975. Studies on the blood and muscle proteins of crabs of Porto Novo. Bull. Dep. Marine Sci. Univ. Cochin 7(3).

Kasinathan,R., B.N.Desai and S.N.Durivedi, 1972. Morphometry and electrophoretic studies of Cynoglossus bilineatus (Lac) from Bombay waters J. Indian Fish. Ass. 2: 110-115.

Keese,A., and H.J. Langhodoz, 1974. Electrophoretische studies ozur population sanalyse bei der Regenbogenfor elle. Z. Tierzucht Zucht Biol. 91(1-2): 109-124.

Kimura, M. and T. Ohta, 1971. Theoretical aspects of population genetics, 219 pp. Princeton, New Jersey: Princeton University Press.

King, M.C., and A. Wilson, 1975. Evolution at two levels in humans and Chimpanzees/ Science 188: 107-116.

Kirpichnick, M., and R.K. Selander, 1979. Genetics of speciation in lake whitefish in the Allegash Basin Evolution 33: 478-485.

Kirpicknikov, V.S., 1981. Genetic bases of fish selection translated by G.G. Gause. Spriyer-Verlag Berelin Heidelberg New York. 400 pp.

Koehn, R.K. 1969. Esterase. Heterogeneity dynamic of a population. Science 163: (3870): 943-944.

Koehn, R.K. and G.C. William, 1978. Genetic differentiation without isolation in the American eel, Anguilla rostrata. II. Temperature stability of geographic patterns Evolution 32: 624-639.

Korpelainen, H., 1984. Genetic differentiation of Daphnia magna population. Hereditas 101(2): 209-216.

Krieg, F. and R. Guymard, 1984. Electrophoretic evidence for a large genetic differentiation between brown trout populations of corsica. C.R. Hebd. Seances. Acad. Sci. III Paris.

Kulkarni, G.K., R.N.Nagabhushanam and P.K.Joshi, 1980. Electrophoretic separation of protein patterns of different tissues of 4 marine penaeid prawns in relation to sex Hydrobiologica, 69(1x2): 25-28.

Kunju, M.M., 1970. Synposis of biological data on the penaeid prawn Solenocera indica Nataraj 1945. FAO Fish Rep. (57) Vol. 4: 1317-33.

Kunz,Y.W., 1975. Ontogenesis of lactate dehydrogenase isozyme patterns in 2 Salmonoids. Salmo salar and S. trutta. Experimenta 31(2): 152-153.

Kurien,C.V. and V.O.Sebastian, 1975. Prawns and prawn fisheries of India, Hindustan Publishing Corporation (India): 280 pp.

Kutkuhn, J.H., 1981. Stock definition as a necessary basis for co-operative management of Great Lakes fish resources. Can. J. Fish. Aquat. Sci. 1476-1478.

Laemeli,U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage Nature. 227: 680-685.

Larkin, P.A., 1981. A perspective on population genetics and salmon management. Can. J. Fish. Aq. Sci. 38, (12): 1469-1475.

Lear,W.H. and R.K.Misra, 1978. Clinal variation in scale characters of Atlantic salmon (Salmo salar) based on discriminant function analysis. J. Fish Res. Board Can. 35: 43-47.

Lee,B.D., and T.Y. Lee, 1968. Larval development of the Penaedean shrimp Metapenaeus joyneri (Miers). Publ. Haewundae Marine Lab. Pusan. Fish Coll. 1:1-18.

Lee, B.D. and T.Y. Lee, 1969. Studies on the larval development of Metapenaeus joyneri (Miers) Publ. Haewundae Marine Lab. Pusan. Fish Coll. 3: 27-56.

Lee,S.S., and T.K.Lim, 1973. Electrophoretic serum protein patterns in some penaeid prawns (Crustacea: Decapoda), Malaysian Agric. J., 49(1): 11-15.

Lester, L.J., 1979. Population genetics of penaeid shrimp from the Gulf of Mexico. J. Hered. 70, 175-180.

Lester, L.J., 1980. Subspecific stock identification of Northeastern south America shrimp fishery. Proceedings of the working group on Shrimp Fisheries of the North eastern South America Panamacity, Panama 23-27 April Wecaf Reports 28: 131-143.

Lester, L.J., 1983. Developing a selective breeding program for penaeid shrimp mariculture. Aquaculture, 33: 41-50.

Lester, L.J., and J.P. Coc , 1987. Ontogenetic changes in Isozyme patterns of Penaeus species Comp. Biochem. Physiol. Vol. 86B, No.2, 253-258.

Levins, R., 1968. Evolution in changing environments. 120 pp. Princeton, N.J. Princeton University Press.

Lim, T.K., and S.S.Lee, 1970. Electrophoretic studies on the muscle myogens of some penaeid prawns. Mar. Bio. 5: 83-88.

Lindelfelser, M.E., 1980. Subspecific variation and evolution in the freshwater prawn Macrobrachium rosenbergii (de Man) (Decapoda:Palaemonidae) as Reflected in its Morphometric and Allozymic variation. Ph.D. Thesis, Univ. Hawaii.

Lindsey, C.C., 1981. Stocks are chameleons: Character displacement in temperate fishwater fishes. Can. J. Fish. Aquat. Sci. 38: 1497-1506.

Loudenslager, E.J. and G.A.E.Gall, 1980. Geographic pattern of protein variation and sub specification in cutt trout, Salmo clarki. Syst. Zool. 29(2): 27-42.

Lowry,O.H., N.J.Rosenrough,A.L. Farr and R.J.Randall, 1951. Prote determination with folin phenol reagent. J. Bio. Chem: 193-265.

Lui,L.C., 1979. Some aspects of the taxonomy behaviour and biology of the freshwater prawns, Macrobrachium australiense Holthuis 1950. (Crustacea:Decapoda: Palemonidae) Ph.D. Thesis, Univ. Queensland. Australia).

Mangaly,G. and A. Jamieson, 1978. Genetic tags applied to the European lake, Merluccius marluccius (L). Anim. Blood. Grps. Biochem. Genet. 9: 39-48.

Maraques,E.D. 1978. A comparison of lactate dehydrogenase in tissue of immature and mature coho salmon Oncorhynchus kisutch. J. Exp. Zool. 203(3): 475-481.

Maria, R.M., 1979. Serum pattern of flat tissues. Mahasagr, Bulletin. of National Institute of Oceanography,12(1): 45-48.

Mark Stoneking, Daniel J. Wagner and Ann. C. Hildebrand, 1981. Genetic evidence suggesting subspecific differences between northern and southern population of Brook Trout (Salvenlimus fontinalis). Copeia (4) pp.810-819.

Markert, C.L., 1983. Isozymes: conceptual history and Biological significance Isozymes: current Topics in Biological and Medical Research Vo. 7: Molecular structure and Regulation 1-17.

Martin,W.R., 1948. The machines of environmental control of body from in fishes. Publ. Ont. Fish. Res. Lab.70:5-76.

Mary, M. 1985. Electrophoretic studies on Mugil cephalus and Liza parsia. M.Sc. dissertation, Univ. Cochin.

May, B, F.M. Utter and Allendorf, 1975. Biochemical genetic variation in pink and chum salmon. Inheritance of introspecies variation and apparent absence of interspecies introgression following massive hybridization of hatchery stocks. J. Hered 66(4): 227-232.

McGlade, J.M. 1981. Genotypic and phenotypic variation in the brook trout Salvelinus fontinalis (Mitchill). Ph.D. Thesis, Univ. Guelph, Guelph, Ont. 297-p.

Menzes, M.K., 1979. Serum Pattern of flat fishes. Mahasagar Bulletin of Natl. Inst. Ocean. 12(1): 45-48.

Menon, K.S. 1983. A preliminary account of the Madras Plankton Rec. Indian Mus., 33: 409-516.

Menon, M.A.S., 1945. Observations on the seasonal distribution of the plankton of the Trivandrum coat. Proc. Indian. Acad. Sci., 22(2): 31-62.

Menon, M.K., 1951. The life history and bionomics of the Indian penaeid prawn Metapenaeus dobsoni Miers Proc. Indo-Pacif. Fish. Counc., 3rd meet Sec. 11: 80-93.

Menon, M.D. and K.C. George, 1977. On the abundance of zooplankton along the southwest coast of India during the years 1971-75. Proc. Symp. Warm. Water. Zooplank, sp. Publ. N10: 205-213.

Messieh, S.N. and S.N. Tibbo, 1971. Discreteness of Atlantic herring (Clupea harengus harengus) populations in spring and autumn fisheries in the Southern gulf of St. Lawrence. J. Fish. Res. Board. Can., 28: 1009-1014.

Messieh,S.N., 1975. Delineating spring and autumn herring populations in the southern Gulf of St. Lawrence by discriminant function analysis. J. Fish. Res. Board Can., 32: 471-477.

Mickevich, M., and M.S.Johnson, 1976. Congruence between morphological and allozyme data in evolutionary inference and character evolution. Syst. Zool. 25: 260-270.

Milner,G.B., P.J.Teel, F.M.Utter and A. Gary, 1986. A genetic model of stock identification in mixed population of Pacific salmon. Oncorhynchus spp. Mar. Fish Re. 48(1) 1985.

Mitton,J.B., and R.K.Kochn 1975. Genetic organisation and adaptive response of allozymes to ecological variables in Fundulus heteroclitus. Genetics, Princeton 79:97-111

Mohamed, K.H., 1979. Synopsis of biological data on the jumbo tiger prawn Penaeus monodon fabricius. 1798. FAO Fish Rep.,(57) Vol. 4: 1251-1256.

Mohammed,K.H., 1970b. Synopsis of biological data on the Indian prawn Penaeus indicus H.Milne Edwards, 1837. FAO Fish Rep., (57) Vol. 4: 1267-1288.

Mohammed,K.H., 1973. Penaeud prawn resolurces of India.Proc. Symp. Living Resources of the seas around India Spl. Publ. CMFRI: 548-556.

Mc ,K.H., T. Rao and M.J.George, 1967. Post-larvae of penaeid prawns of south east coast of India with a key to their identification. FAO Fish Rep. No.57 Vol. 2:487-503.

Morgan,G.R., 1982. Stock and recruitment relationship in the shrimp Penaeus semisulcatus. KISR Safat (Kuwait) Jul. 228-241.

Morgan,.P., E. Kramarsky and S.D. Sulkin, 1978. Biochemical changes during larval development of the Xanthid Crab Richropanopeus harrisii. III. Isozyme changes during ontogeny. Mar. Biol. 48: 223-226.

Moriarty,D.J.M., 1977. Quantification of carbon, nitrogen, and bacterial biomass in the food of some penaeid prawns. Austral. J. Mar. Freshwater Res. 28: 113-118.

Morrow,J.E., 1980. Analysis of the dolly varden char Salvelinus malma of northwestern North American and northeastern Siberia. p.323-338. In E.K. Balon (Ed) Charrs: salmonid fishes of the genus Salvelinus. Perspectives in vertebrate science. Vol. I Dr.W.Junk, Publ., The Hague, Netherlands.

Muriel,C.M. and I. Bennett, 1951. The life history of a penaeid prawn (Metapenaeus) breeding in a coastal lake (Tuggerah New South Wales) Proc. Linn: Soc. N.S.W., 76(5-6):164-18

Mulley,J.C. and R.D.H. Latter, 1980. Genetic variation and evolutionary relationships within a group of thirteen species of Penaeid prawns. Evolution 34(5): 904-916.

Mulley,J.C. and B.D.H.Latter, 1981a. Geographic differentiatio of eastern Australian penaeid prawn population. Aust. J. Mar. Freshwater Res. 32: 889-895.

Mulley, J.C. and B.D.H. Latter, 1981b. Geographic differentiation of tropical Australian penaeid prawn population. Aust. J. Mar. Freshwater Res. 32: 897-906.

Mazharraf Ali, 1978. Length weight relationship of Upeneus sulphureus (Cuvier) collected off Maharashtra coast J. Indian Fish Assoc. 8 & 9. 32-36.

Nakajima, K. and T. Masuda, 1985. Identification of local population of fresh water crab Geothelphusa dehaani in Sawagani Mochiho shuzuku hanbetsu, Bull. Jap. Soci. Sci. Fish/Nissuishi Vol. 51 No.2: 175-171.

Natarajan V., T. Kannupandi, A.L. Paulpandian, and R. Natarajan, 1975. Electrophoretic studies on muscle proteins of some Gobioids of Porto Novo. Bull. Dept. Mar. Sci. Univ. Cochin 7(3): 583-589.

Nei, M., 1972. Genetic distance between populations. Am. Nat. 106: 283-292.

Nei, M., 1973. Analysis of gene diversity in subdivided populations (population structure/genetic variability/ heterozygosity/gene differentiation). Proc. Nati. Acad. Sci. U.S.A. 70:3221-3323.

Nei, M., 1975. Molecular population genetics and evolution, pp. Amsterdam North Hollandi.

Nelson K. and D. Nedgecock, 1980. Enzyme polymorphism and adaptive strategy in the decapod crustacea/ Am. Nat. 116, 238-280.

Nemeth, S.T. and M.L. Tracey, 1979. Allozyme variability and relatedness in six crayfish species. J. Hered. 70, 37-43.

Nirenberg, M.W., J.H. Mathaei, O.W. Jones, R.C. Martins and S.H. Barondes, 1963. Approximation of genetic coda via cell - free protein synthesis directed by template RNA Fedn. Proc. Fed. Am. Socs. Exp. Biol. 22, 55-61.

Novo. E., 1978. Genetic variation in natural populations: Patterns and theory. Theror. Pop. Biol. 13: 121-177.

*Nyman, 1967. Rep. Inst. Fresh. Wat. Res. Drottningholm 47: 5-3

*Ochoa, S., 1963. Synthetic polynucleotides and the genetic code Fedn. Proc. Fedn. Am. Socs. Exp. Biol. 22, 62-74.

O'Connor, J.D. and L.I.Gilbert, Aspect of lipid metabolism i crustaceans. Am. Zool. 8: 529-540.

Odense P.H., and C. Anand, 1978. Isoenzyme systems of an in shore and off-shore lobster populations. International Council for the exploration of the sea C.M. 1978/K:15 shellfish committee.

Panikkar N.K., 1952. Possibilities of further expansion of fish and prawn cultural practices in India. Curr. Sci. 1: 29-33.

Panikker, N.K. and M.K. Menon, 1956. Prawn fisheries of India Proc. Indo. Pacif. Fish Coun., 6(3): 328-344.

Parag, 1984. Genetic variation in the fish Liza parsia (Hamilton Buchanan). M.Sc. Dissertation, Univ. Cochin.

Parsons, L.S. and V.M. Hodder, 1971. Meristic differences between spring and autumn-spawning Atlantic herring (Clupea harengus harengus) from south western New found land. J. Fish. Res. Board Can., 28: 553-558.

Paulinose V.T., 1982. Key to the identification of the larvae and post larvae of the penaeid prawns (Decapoda: Penaeidae) of the Indian Ocean Mahasagar. Bull. of Nat. Inst. Oceanogr. 15:223-229.

*Paul J. and Footrell. P., 1961. Biochem. J. 78, 418.

Pres Parfante, I., 1978. In: FAO species Identification sheets for fishery purposes. Western central Atnaltic (Fishing area 31) Vol. 7(Shrimps prawns). FAO of the United Nations, Rome.

Philip D.P., W.K. Childers and G.J. Whitt, 1981. Management Implications for different genetic stocks of large mouth bads (Micropterus salmonides) in the United states) J. Fish. Res. Board. Can. 38: 1715-1723.

Powers, D.A., 1980. Molecular ecology of teleost fish hemoglobi Strategies for adapting to changing environments, Zool. 20(1): 139-162.

Powers, D.A. and D.Powers 1975. Predicting gene frequencies in natural populations: a testable hypothesis. In: Isozyme IV. Genetics and evolution, pp. 68-84. Ed. by C.L. Markert. New York: Academic Press.

Prasad, R.R., 1954. The characteristics of Marine plankton at an inshore station in the Gulf of Mannar near Mandapam. Indian J. Fish. 1: 1-36.

Prasad, R.R., 1956. Further studies on the plankton of the inshore waters off Mandapam. Indian J. Fish, 3: 1-42.

Prathibha P., 1984. Electrophoretic studies on Penaeus monodon Fabricius. M.Sc. Dissertation, Univ. Cochin.

Rabacy, M., 1964. Protides Biol. Fluids. 12, 273-277.

Rao, P. Vedavyasa, 1970. A synposis of biological data on Penaeid prawn Parapenaeopsis stylifera (H. Milne Edwards) 1837. FAO Fish. Resp. '57) Vol. 4: 1575-1606.

Rao, K. Virabhadra, 1973. Distribution pattern of the major exploited marine fishery resources in India. Proc. Symp Living Resources of the Seas around India, Spl. Publ. CMFRI: 18: 101.

Rao, P.V., 1973. Studies on the larval development of the commercially important penaeid prawns of India. J. Mar. Biol. Ass. India 15(1): 95-124.

Rao, P.V. 1987. Present status of crustacean culture in India. Summer Institute in "Recent Advances in Finfish and Shell fish Nutrition" CMFRI, Cochin 31 pp.

Redfield, J.A., D. Hedgecock, K. Nelson and J.P.Salini, 1980. Low heterozygosity in tropical marine crustaceans of Australia and the trophic stability hypothesis Mar. Biol. Lett. 1: 303-313.

Redfield, J.A. and J.P. Salini, 1980. Techniques of starch gel electrophoresis of penaeid prawn enzyme (Penaeus spp. Metapenaeus spp.) CSIRO Division of Fisheries and Oceanography. Report, Reprint 1086.

Richardson, B.J., 1982. Geographical ditribution of electrophoretically detected protein variation in Australian commercial fishes.II.Jackass Morwong Cheilodactylus macropterus Bloch & Schneider. Aust. J. Mar. Freshw. Res. 1982 33: 927-31.

Richardson B.J., 1982b. Geographical distribution of Electrophoretically detected protein variation in Australian Commercial fishes III. Western King prawn Penaeus latisulcatus Kishinouye. Aust. J. Mar. Fishw. Res. 33: 933-7.

Richle, G. 1981. The origin to compare Rainbow trout population/ Fisch. Teich. Writ. 32(1): 9-13.

Rick,E.F., 1951. The Australian freshwater prawn of the famil Paleomonidae. Rec. Aust. Museum 22: 358-367.

Rider, C.C., 1980. Isoenzymes ISBN, 0412156407.

Riddell, B.E., and W.C.Leggett, 1981. Evidence of an adaptive basis for geographic variation in body morphology and time of downstream migration of juvenile Atlantic salmon (Salmo salar). Can. J. Fish. Aquat. Sci. 38: 3

Rizzotti, M.,A.Comparini, and E. Rodino., 1977. The haemoglobi of Anguilla anguilla (L) Ontogenetic variation. Comp. Biochem. Physiol. 58A(2): 173-176.

Rodino,F.,and A. Camparini, 1978. Genetic variability in the European eel Anguilla anguilla L. In: Marine organisms Plenum Press, New York, pp. 389-425.

Romanov, L.M., I.A. Balakhnin and S.Y. Brodsky, 1976. Genetic analysis of crayfish population structure in the Ukrar SSR. II. Electrophoretic studies on isoenzymes systems hemolymph serum components in Astacus (Pontastacus) leptodactylis Eschottz, 1823. Genetika (Moscow) 12: 81

Sage R.D. and R.K. Selander, 1975. Trophic radiation through polymorphism in cichl d tissues. Proc. Natl. Acad. Sci. U.S.A. 72:4569-4673.

Saila, S.B. and J.M. Flowers, 1969. Geographic morphometric variation in the American lobster. Syst. Zool. 18(3): 330-338.

Salauddin A.S.M., 1969. Isoenzyme of Alkaline phosphatase in Anodonta grandis (Bivalvia Unionidae) during shell regeneration. Malacologis 9(2): 501-508.

Salmon, M., S.D. Ferris, D. Johnston, G. Hyatt, and G.S. Whitt 1979. Behavioral and biochemical evidence for species distinctiveness in the fiddler crabs, Uca speciosa and U. spincicarpa. Evolution 33(1): 182-191.

Schmidt, J., 1917a. Racial investigations. I. Zoarces vivipar L. and the local races of the same. C.R. Trav. Lab. Carlsberg 13(3): 277-397.

Schmidt, J., 1971b. Racial investigations. II. Constancy investigations continued. C.R. Trav. Lab Carlsberg, 14(1): 1-19.

Schmidt, J., 1918. Racial studies in fishes. I. Statistical investigations with Zoarces viviparus L. J. Gent. 7: 10!

Schmidt, J., 1920. Racial investigations. V. Experimental investigations with Zoarces viviparus L. C.R. Trav. Lab Carlsberg 14(9): 1-14.

Schmidt, J., 1921a. Racial investigations VII. Annual fluct uations of racial characters in Zoarces viviparus. L.C. Trav. Lab. Carlsberg 14(15): 1-24.

Schmidt. J. 1921b. Racial investigations. VIII The numerical signification of fused vertebrae. C.R. Trav. Lab. Carlsberg. 14(16): 1-15.

Selander R.H., 1976. Genic variation in natural populations: In: Molecular evolution. Sineaeur. Assoc. Sunderland, pp. 21-45.

Selander R.K. and D.W. Kaufman, 1973. Genic variability and strategies of adaptation in animals. Proc. Natn. Acad. Sci. USA. 70: 1875-1877.

Selander, R.K., U.E. Johnson and J.C. 1971. Avise Biochemical population genetics of fiddler crabs Uca. Biol. Bull. 141: 402.

Shakelee J.B., M. Champion and G.S. Whitt, 1974. Developmental genetics of teleosts a biochemical analysis of the lake chub sucker ontogeny. Dev. Biol. 38: 356-382.

Shakele, J.B. and J.P. Salini, 1983. Studies suggest multiple stocks of Australian barrumundi. Aust. Fish. 42(2):

Sharp, J.C.K., W. Able, W.C. Leggett, and J.E. Carscadden, 197 Utility of meristic and morphometric characters for identification of capelin (Mallotus villosus) Stocks in Canadian atlantic waters. J. Fish. Res. Board Can., 35: 124-130.

Shaw, C.R. and Prasad R., 1970. Starch gel electrophoresis of enzymes " a complication of recipes". Biochem. Genet. 4: 294-320.

Scilliano, M.J. and R. Shaw, 1976. Separation and visualization of enzymes of gels pp. 185-209. In: I Smith (ed) Chromatographic and electrophoretic techniques, Vol. II. 4th ed. Heinemann medical Books London.

Silas E.G., M.J. George, T. Jacob, 1984. A review of the shrimp fisheries of India:A Scientific basis for the management of the resources. In: J.A. Gulland and B.J.Rothschild (eds.), Penaeid shrimps their biology and management. Finshing News, Books Ltd, Farnham, Surrey, England 83-1

Smith, I. 1968. Acrylamide gel disc electrophoresis. Sec. I. Techniques of disc electrophoresis In: 'Chromatographic and electrophoretic techniques'. (Ed. Smith, I. Vol. 1, pp. 104-147.

Smith, P.J., 1979. Esterase gene frequency and temperate relationships in the new Zealand snapper Chrysophrys auratus Mar. Biol. 53: 305-310.

Smith, M.Y. and A. Fraser, 1976. Polymorphism in a cyclic partheno genetic species: Simocephalus serrulatus. Genetics 84: 631-637.

Smith and A. Jamison, 1978. Enzyme polymorphisms in the Atlant Mackerel, Scomber scombrus L. Com. Biochem. Physiol. 60(B) (3): 487-489.

Smith P.J. and Y. Fujio, 1982. Genetic variation in Marine Teleosts. High variability in Habitat Specialists and Low variability in Habitat generalists. Mar. Biol., 69: 7-20.

Smith P.F. and A. Jamilson, 1980. Protein variation in the Atlantic Mackeral Scomber scombrus/ Ann. Blood. Grps. Biochem. Genet. 11(207-214).

Smith P.J., R.I.C.C. Francis and L.F. Paul, 1978. Genetic variation and population structure in the New Zealand Snapper. NZ. Jl. Mar. Freshwat. Res. 12(4): 343-350.

Smith P.J., J.L. McKoy and P.J. Machin, 1980. Genetic variati in the rock lobsters, Jasus edwardsii and Jasus novaehollandiae N.Z. Jl. Mar. Freshwat. Res. 4(1):55-63

Smith, T.I.J., P.A. Sanifer, M.H. Smith, 1978. Population structure of malaysian prawns M. rosenbergii (de man) reared in earthern ponds in South Carolina Proc. Annu . Meet. World. Maric. Soc. 9: 21-28.

Smith P.J., G.J. Patchell and P.G. Benson 1981. Genetic tags in the New Zealand Loki, Macrusronus noraezelandiae Ann. Blood. Grps. Biochem. Genet. 12, 37-45.

Smithies. O., 1955. Zone electrophoresis in starch gels. Grou Variation in the serum proteins of normal human adults. Biochem. J. 61: 629,

Snyder, T.P. and J.L. Gooch, 1973. Genetic differentiation in Littorina saxatilis (Gastropoda). Mar. Biol. 22: 177-1

*Soule, M., 1976. Allozyme variation its determination in spac and time: In: Molecular evolution pp. 6677 Ed. by F.J. Ayala Massachusetts: Sinauer Associates 1976.

Subramanyan, R., 1959. Studies on the Plankton of West coast of India. Parts I & II. Proc. Indian Acad. Sci., 50B:(4):133-252.

Sriraman K. and P.S. Reddy, 1977. Biochemical studies in planktonic juveniles and adults of Penaeus indicus (Milne-Edwards) and Penaeus monodon (Fabricius). Proc. Symp. Warm Water Zooplankton, NIO. Goa.

Stanley, S.M., 1979. Macroevolution. Pattern and Process, W.H. Freeman and Co., San Francisco. CA 382. p.

Sweet S.S. 1980. Allometric inference in morphology Am. Zool. 20: 643-652.

Taggert, J.W. and A. Ferguson, 1986. Electrophoretic evaluation of a supplemental stocking programme for brown trout Salmo trutta L. Aquaculture Fish. Magazine 17: 155-162.

Taniguichi, M. and T. Isiwatwari, 1972. Inter and intraspecif variation of muscle proteins in the Japanese crucian carp-I cellulose acetate electrophoretic pattern. Jap. J. Ichthyl 19(4): 217-222.

Taniguchi N. and Sakata, K. 1977. Interspecific and intra specific variations of muscle protein in the Panamese crucian carp. 2. Starch gel electrophoresis. Jap. J. Ichthyol. 24(1): 1-11.

Taning, A.V. 1952. Experimental study of meristic characters in fishes. Biol. Rev. Cambridge Philos. Soc. 27: 169-193.

Templetson W., 1935. Local differences with body proportion of the lobster Homarus americanus. J. Biol. Board. Can. (1)(B): 213-226.

Thebault M.J. and A. Bernicard, 1978. Variations in the Lactate dehydrogenase activity during the development of the shrimp Palaemon serratus. Comp. Biochem. Physiol Vol. 61 B: 379-384.

Thomas M.M., 1981. Preliminary results of electrophoretic studies on marine prawns. Indian J. Fish 28(1&2): 292-294.

Todd, T.N., G.R. Smith and L.E.Cable 1981. Environmental and genetic contributions to morphological differentiation in ciscoes (Coregoninae) of the Great Lakes. Can. J. Fish. Aquat. Sci. 38: 59-67

Tracey, M.L., K. Nelson, D. Hedgecock, R.A. Shleser and M.L. Pressick, 1975. Biochemical genetics of lobsters: Genetic variation and the structure of American Lobster (Homarus americanus) Populations J. Fish. Res. Board, Can., 32: 2091-2101.

Trudeau T.N., 1978. Electrophoretic protein variation in Penaeus brachyurus chiono and its implications in genetic studies Proc. Annu. Meet. World Marc. Soc. 9,139-145.

Tsuyuki, H.E. Roberts, R.H. Lower and W. Hadaway, S.J. Westreheim, 1968. Contribution of protein electrophoresis to Rock Fish scorpaenidae systematics. J. Fish. Res. Bd. Can. 25(11); 2472-2501.

Tsuyuki, H., E. Roberts, W.E. Vanstone, and R.J. Markert, 1965. The species specificity and constancy of muscle myogen and haemoglobin electrophorograms of oncorhynchus. J. Fish. Res. Bd. Can., 22(1): 215-217.

Tsuyuki, H., J.F. Uthe, E. Roberts and L.W. Clarke, 1966. Comparative electrophorograms of coregonus clupea/ formis, Salvelinus namayarsh, S. alpinus, S. malma and S. fontinalis from the family salmonidae. J. Fish. Res. Bd. Can 23(10): 1599-1606.

*Turner, K., and T. Lyerla, 1980. Electrophoretic variation in sympatric and crabs from North Inlet, South Carolina USA Biol. Bull. (Woods Hole) 159(2): 418-427.

Uthe, J.F., and H. Tsuyuki, 1966. Comparative zone electrophoresis of muscle myogens and blood proteins of adult Ammocetus lamprey J. Fish. Res. Ed. Can., 24: 1269-1273.

Utter, F.M., H.O. Hodgins, F.W. Allendrof, A.G. Johnson, J.L. Mighell, 1973. Biochemical varkants in Pacific salmon and rainbow trout. Their inheritance and application in population studies In: Genetics and murtgemen of fish, springer, Berlin Heidelberg New York, pp. 329-339.

Valentine, J.W., 1976. Genetic strategis of adaptation. In: Molecular evolution pp. 78-94. Ed. by F.J. Ayala. Massachusetts - Sinauer Associates 1976.

Venkataramiah, A., G.J. Lakshmi and G. Gunter 1975. A review of environmental and nutritional factors on brown shrimp. Penaeus aztecus Ives in laboratory cultures. 10th Europ. Symp. Mar. Biol. 1: 523-547.

Vernon, E.H. 1957 Morphometric comparision of 3 races of Koanee (oncorhyncys nerks) Within a large British columbia lake. J. Fish. Res. Board Can. 14: 573-598.

Vijayaraghavan and others, 1982. New light of migration of Indian white prawn Penaeus indicus (Marine, Fish. Inform. Bull. 45.

Ward, R.D., 1977. Relationship between enzyme heterozygosity and quartnary structure. Biochem. Genet. 15(1-2): 123-135.

Whittaker, J.R., 1959. Localisation of hemocyanin on starch gel electrophoretic patterns. Nature (Lond), 184: 193-194.

*Wilkins, N.P., 1967. J. Cons. Perm. Int. Explor. Mer 31(1), 77-88.

Wilkins, N.P., 1981. The rationale and relevance of genetics in Aquaculture an overview Aquaculture 22: 209-228.

Williams, A.M., 1953. Identification of juvenile shrimp (Penaeidae) in North Cardina. J. Elisha Mitchell Sci. Soc., 69(2): 156-160.

Williams, A.M., 1955. A contribution to the life histories of the commercial shrimps (Penaeidae) in North carolina. Bull. Mar. Sci. Gulf Caribb, 5(2): 116-146.

Williams, A.M., 1959. Spotted and brown shrimps postlarvae (Penaeus) in North cardina. J. Elisha mitchell 9 (3) Sci. Soc. 281-290.

Williams, G., R.K. Koehn, and J.B. Mitton, 1973. Genetic differentation without isolation in the American old Anguilla rostrata. Evolution 27(2): 192-201.

William, G., and A.E. Needham, 1941. Metric variation in populations of carcinus menas. J. Mar. Biol. Assoc. U.K. 24: 261-281.

Williamson, D.I., 1972. Larval development in a marine and freshwater species of Macrobrachium (Decapoda, Palaemonidae) Crustaceana 23(3): 282-298.

Wilson, A.C., V.M. Sarich, and L.R. Maxon, 1974. The importance of gene rearrangement in evolution evidence from studies on rates of Chromosomal, protein and anatamical evolution. Proc. Nat. Acad. Sci. U.S.A. 71: 3028-3030.

Wilson, A.C., L.R. Maxon, and V.M. Sarich, 1974. Two types of molecular evolution. Evidence from studies of interspecific hybridization. Proc. Nat. Acad. Sci. U.S.A. 71: 2843-2847.

Wishard, L.N., F.M. Utter, and Dr. Gunderson, 1980. Stock separation of five rockfish species using naturally occuring Biochemical genetic markets Mar. Fish. Res. Mar-Apr. pp. 64-73.

Wooluff, D.S., 1978. Evolution and adaptive radiation of cerion: A remarkably diverse group of West Indian land snails: Malacologia 17: 223-39.

*Wright, C.A., 1966. Int. Rev. Gen. Exp. Zool. 2, 1-42

Yamaka, L.H., and B.T. Scheer, 1970. Chemistry of growth and development in crustaceans. In: Zoology Vol. V. Arthropoda pp. 321-338 Ed. by M. Florkin and B.T. Scheer. New York Academic Press.

Yesh, S.A., and R.T. Yang, 1977. Population study of frigate tuna Auxis thamzard in the waters around Taiwan Part I. Electrophoresis of the muscle protein extracts. Acta. Oceanogr. Taiwan No.7: 125-133.

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* Originals not referred.