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:

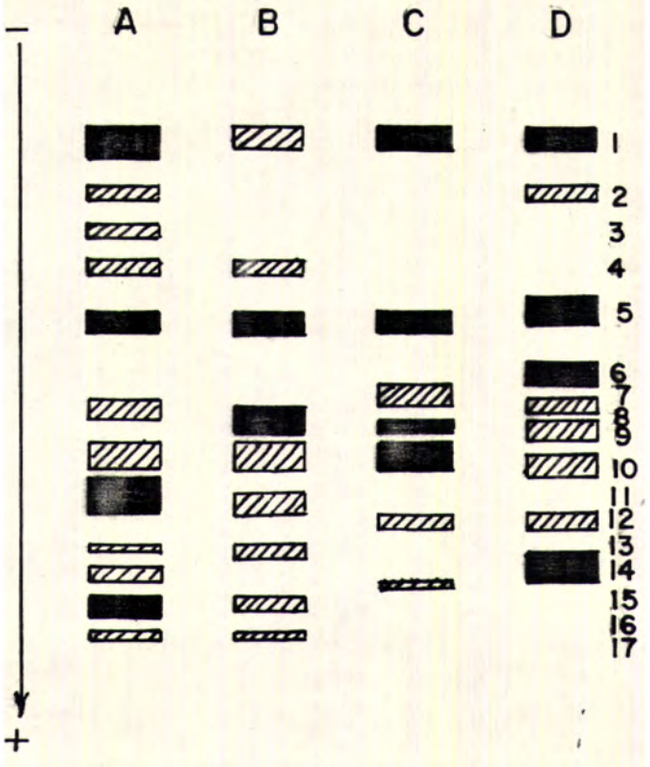
For comparison of each muscle protein band among the four species of Metapenaeus studied here each fraction was alloted a qualifying number obtained according to the electrophoretic mobility position of that particular band, thus the slowest moving and the fastest moving bands receiving the number one 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

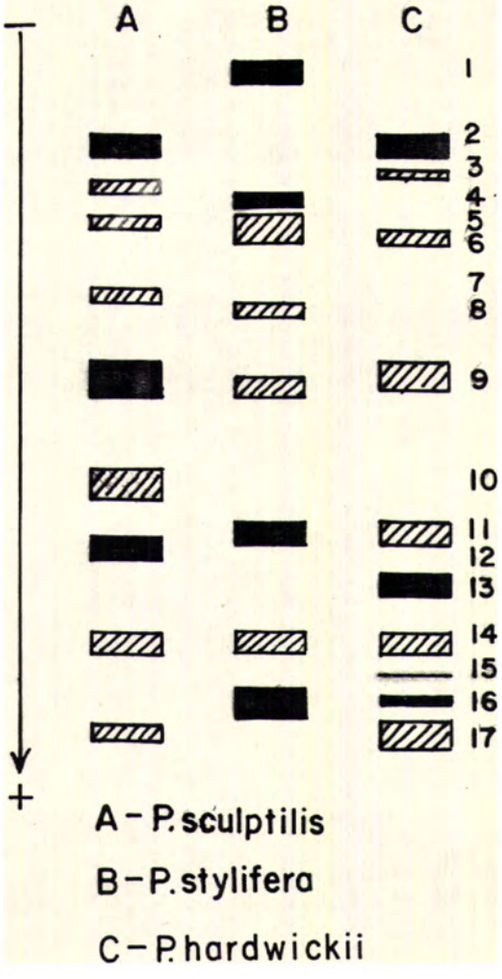


A-M. affinis, B-M. brevicornis

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*

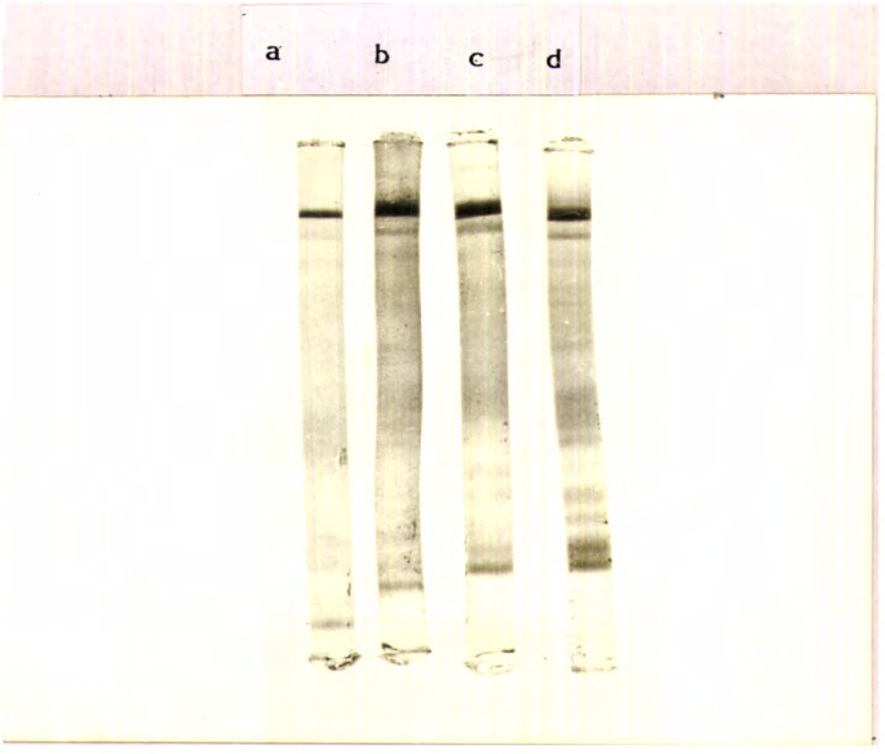
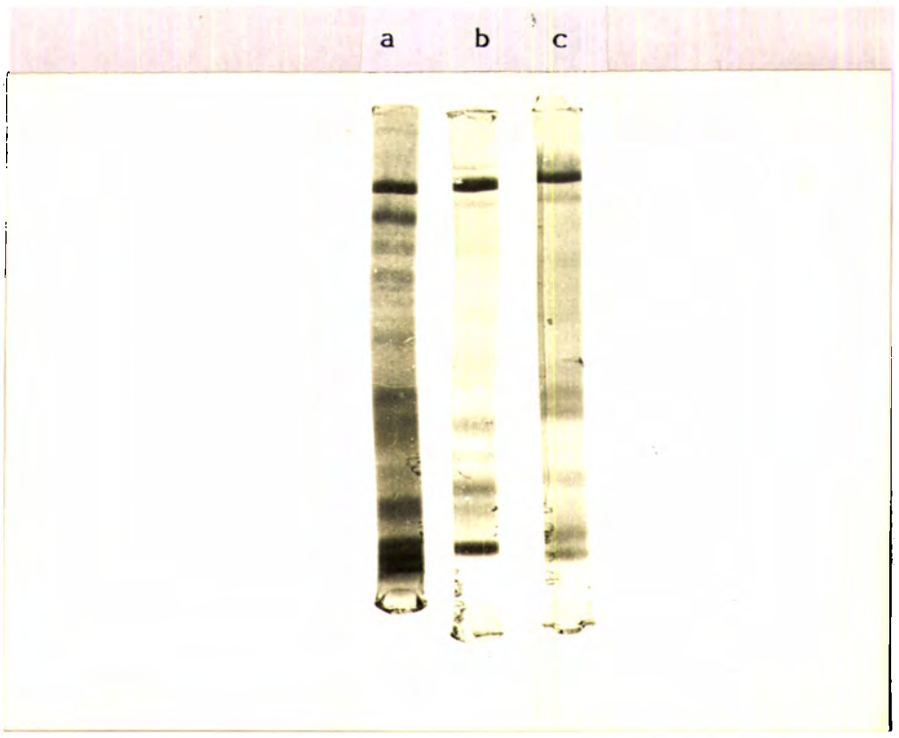

a
b
c
d

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

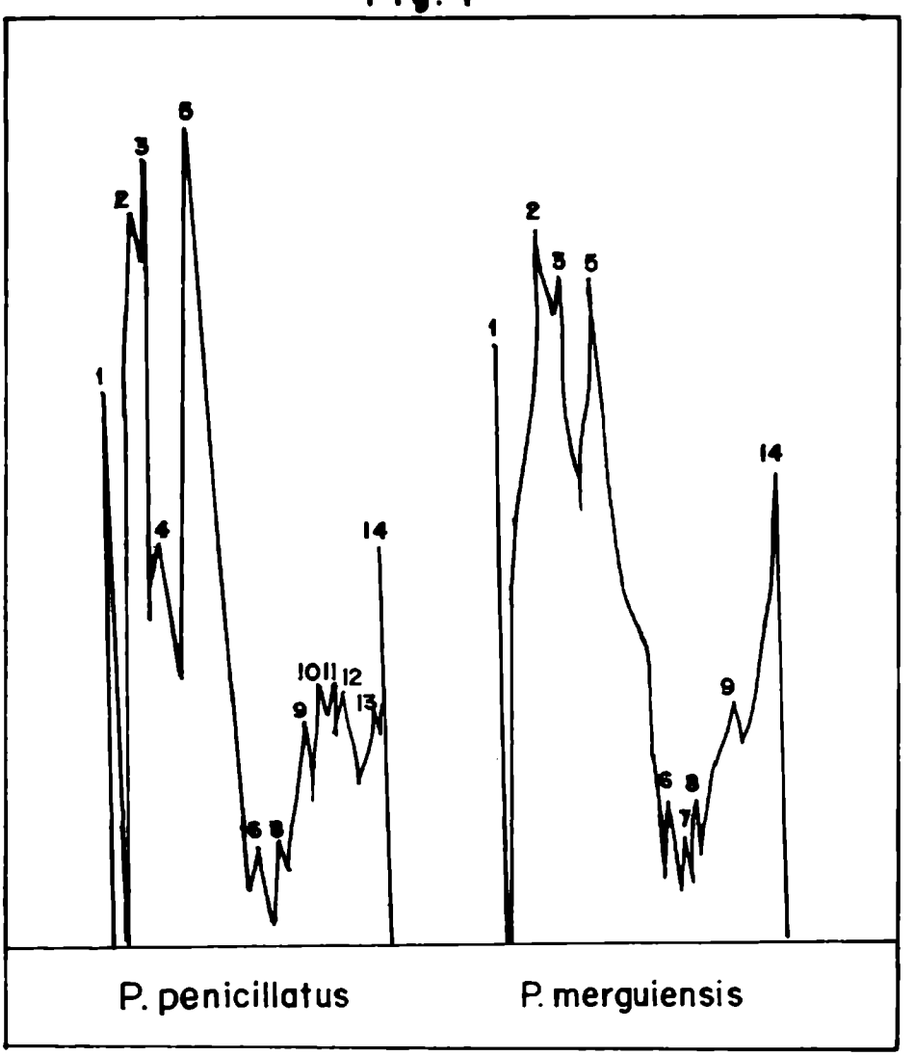
c-smaller fractions

'+' 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



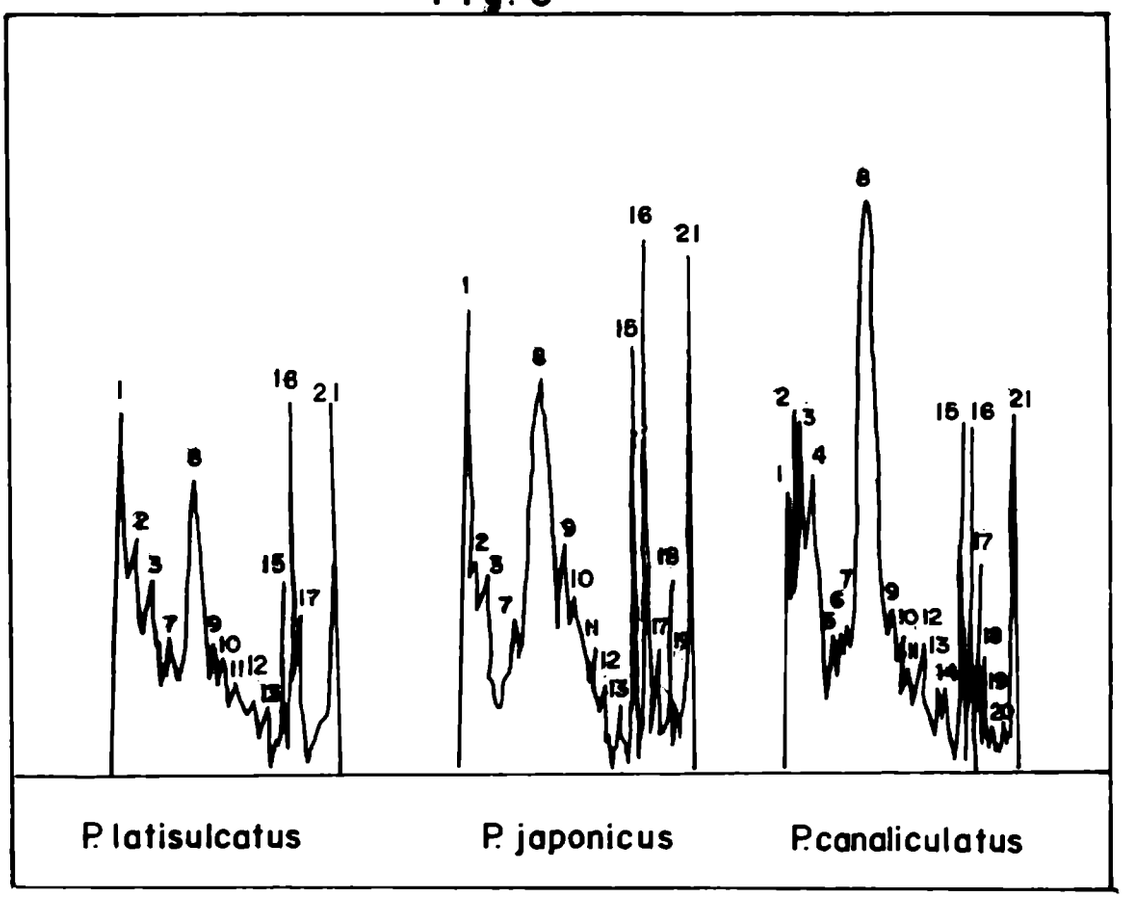
(Band No. 6, 7 and 8). When the second type of thinner bands are compared P. penicillatus showed band Nos. 4, 9, 10, 11, 12 and 13 and P. merguiensis showed band No. 9.

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)