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SDS-POLYACRYLAMIDE GEL ELECTROPHORESIS OF MUSCLE ALBUMIN PROTEINS IN THREE SPECIES OF POMFRETS–*PAMPUS ARGENTEUS* (EUPHRASEN, 1788), *PAMPUS CHINENSIS* (EUPHRASEN, 1758) AND *APOLECTIS NIGER* (BLOCH, 1795)

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ABSTRACT
Muscle albumin proteins of three species of pomfrets - *Pampus argenteus, P. chinensis* and *Apolectis niger* were extracted with water and Tris- HCl (0.1M, 0.2M, 0.3M, 0.4M and 0.5M) and resolved using sodium do-decylsulphate polyacrylamide gel electrophoresis (SDS- PAGE). Difference between the sexes of three species and among the three species was observed with respect to protein fraction, relative mobility and molecular weights. Maximum number of activated albumin protein fractions were recorded with water in *A. niger* and at 0.1M Tris-HCl whereas in case of *P. argenteus* and *P. chinensis* they were at 0.1M, 0.2M and 0.3M Tris- HCl. Low values found at 0.4 and 0.5M Tris-Cl.

KEY WORDS: SDS- PAGE, Muscle albumnis, Pomfrets, *Pampus argenteus, Pampus chinensis, Apolectis niger*

INTRODUCTION

Pomfrets are commercially important fishery on the east and west coasts of India belonging to the order: Perciformes and Class: Osteichthyes. Off Visakhapatnam Coast three species – *Pampus argenteus* (Silver pomfret), *P. chinensis* (Chinese pomfret) belonging to the family Stromatidae and *Apolectis niger* (Black pomfret) of the family Apolectidae are recorded and they contribute about 2.4% of total marine catch (Khan, 2000). Protein is a translated phenotypic expression of a genetic code and the variations in the genome usually result in change in the structure of proteins (Bye and Ponnaiah, 1983). These proteins show variation with species, sex and season both quantitatively and qualitatively. Each protein carries a definite electric charge and the molecular mobility of different proteins vary in an electric field with a rate proportional to the magnitude of its charge depending on the amino acid composition and pH of the medium (Ferguson, 1974). Electrophoresis is a method of analysis of biochemical systematics in various taxa. Each species is identified for the number of species-specific proteins by means of high-resolution starch or polyacrylamide and isoelectric focusing (Ferguson, 1974; Basaglia and Marchetti, 1990). According to Mc Laughlin *et al.,* (1982), the molecular mobility of proteins in an electric field depends on their molecular weight, conformation and surface electric charge. Connell (1953) has made a beginning on electrophoretic studies of the skeletal muscles at low ionic strength at 0.05 I, 0.1 I and 0.2 I in cod fish *Gadus morhua*. The biochemical systematic of organisms with reference to proteins has been studied by Alston and turner (1963). The molecular weights of the proteins of cardiac, white and red skeletal muscles of the carp have been found to be different and also in mammals (Deyl and Peloch (1970), Sarkar *et al.* (1971). Hamoir *et al.* (1972) have studied the number and molecular weight of the myosin of skeletal muscles in *Cyprinus carpio* and *Salmo irideus*. The sarcoplasmic proteins of white muscle of an Antarctic hemoglobin- free fish *Champsocephalu gunnari* has been studied by Hamoir *et al* (1979). Several investigators (Hamoir and Gerardin, 1980; Mackie and Ritchie, 1980; Rosenlund *et al,*1983; Basaglia, 1989 & 1989a; Jeroen Van *et al*, 1991; Vander Bank *et al*,1992; Matthiensen and Tellechea, 1993; Houlihan *et al*, 2000; Martin *et al*, 2001; Kowalski *et al*, 2003; Yilman *et al*, 2007; Hongkun *et al,* 2008; Michelis *et al,* 2010; Gaikwad *et al,* 2012) have made electrophoretic studies on the plasma and muscular proteins of different fishes. An attempt is made in the present study to unravel the different types of activated proteins and their molecular weights using SDS-PAGE in three species of pomfrets – *P. argenteus*, *P. chinensis* and *A. niger.*

MATERIALS & METHODS

Three pomfret fish species were collected from the Visakhapatnam Fishing Harbour from the trawl catches. The specimens were separated according to species and the sex was determined by dissecting the abdomen and observing the gonads. The muscle of the both sexes of the three species was separated and blotted. The muscle tissue was dried in hot air oven at 55^0 – 65^0c for about 48 hrs. The dried muscle was powdered and used for electrophoretic studies by SDS- PAGE for activated proteins following the method of Sambrook and Russell (1988). The albumin proteins of muscles were isolated with water and Tris- Cl buffer (0.1M, 0.2M, 0.3M, 0.4M and 0.5M). The marker proteins used were MP-1 Phosphorylase b (97,000 Da), MP-2 Bovine Serum Albumin (66,000 Da), MP-3 Ovalbumin (43,000 Da), MP-

4 Carbonic anhydrase (29,000 Da) and Mp-5 Lactoglobulin (18,400 Da).

RESULTS

Muscle albumin proteins

i) Water soluble **(Table 1 and Fig's 1 & 2)**

In *A. niger* seven protein fractions with molecular weights ranging from 19.2 kDa to 47.2 kDa were found in male and in female they were four with 30.4 kDa to 57.6 kDa. In *P. argenteus* male and female shared two proteins with 40.8 kDa and 44.8 kDa in common and differ in one *i.e.,* 30.4 kDa in male and 31.2 kDa in female. In *P. chinensis,* three similar and one dissimilar proteins were recorded. The similar proteins were of 24 kDa, 40.8 kDa and 44.8 kDa and the dissimilar was of 30.4 kDa in male and 29.6 kDa in female.

TABLE 1: Relative mobility and Molecular weights (kDa) of water soluble albumins of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.14	97.4						
MP 2	0.23	66.0						
MP 3	0.41	43.0						
MP 4	0.63	29.0						
MP 5	0.94	18.4						
1	0.27	57.6	-	+	-	-	-	-
2	0.35	47.2	+	-	-	-	-	-
3	0.38	44.8	-	+	+	+	+	+
4	0.40	43.2	+	-	-	-	-	-
5	0.43	40.8	-	+	+	+	+	+
6	0.55	33.6	+	-	-	-	-	-
7	0.57	31.2	-	-	-	+	-	-
8	0.58	30.4	-	+	+	-	+	-
9	0.59	29.6	-	-	-	-	-	+
10	0.62	28.8	+	-	-	-	-	-
11	0.71	24.8	+	-	-	-	-	-
12	0.72	24.0	-	-	-	-	+	+
13	0.83	20.8	+	-	-	-	-	-
14	0.90	19.2	+	-	-	-	-	-
Total No. of Bands			7	4	3	3	4	4

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
+ Presence - Absence

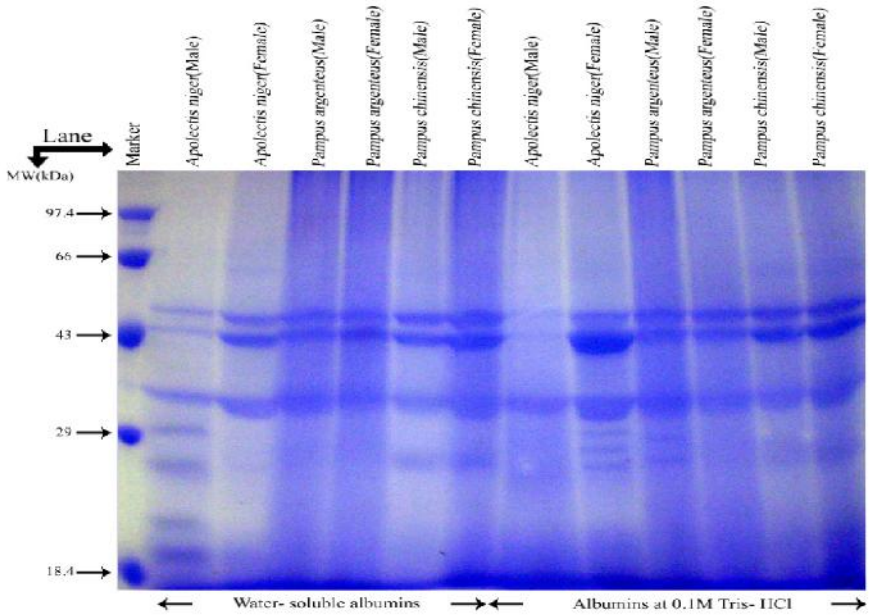

FIGURE 1: Electrophoretic pattern (SDS- PAGE) of Water-soluble albumins and albumins at 0.1M Tris- HCl in males and females of A. *niger, P. argenteus* and *P. chinensis*.

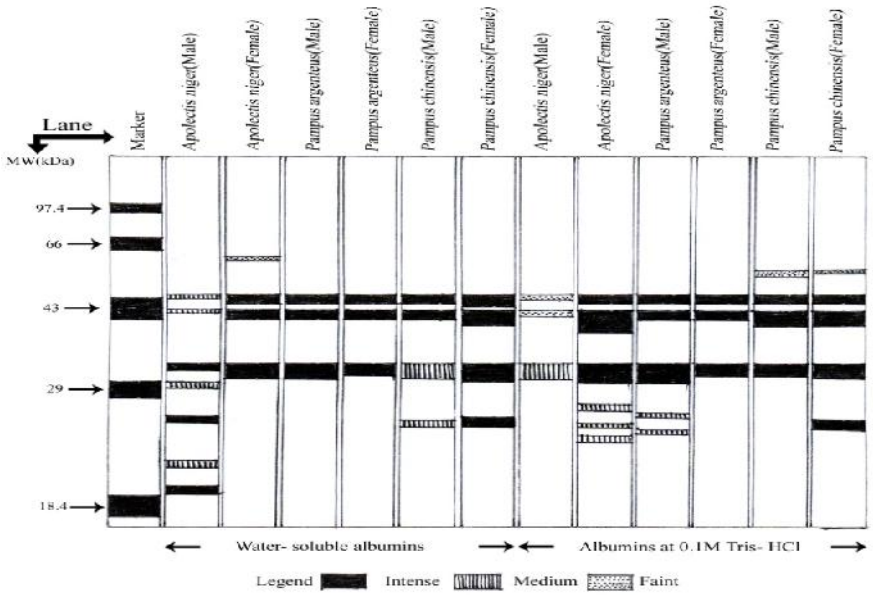

FIGURE 2: Digrammatic representation of electrophoretic pattern (SDS- PAGE) of Water- oluble albumins and albumins at 0.1M Tris- HCl in males and females of *A. niger, P. argenteus* and *P. chinensis*.

ii) At 0.1M Tris- HCl **(Table 2 and Fig's 1 & 2)**

In *A. niger*, three activated proteins in male and six in female were observed. Only a single protein band with 44.0 kDa was similar in both male and female. The other proteins in male were of 30.0 kDa and 40.8 kDa.

P. argenteus showed five protein fractions in male and three in female with two common proteins of 40.0 kDa and 44.0 kDa. The protein present in female and absent in male was of 29.2 kDa. The other proteins present in male were 24.0 kDa, 25.6 kDa and 28.4 kDa. Four protein fractions in male and five in female were found in *P. chinensis*. The common protein fractions were 30.0 kDa, 40.0 kDa and 52.0 kDa. The two proteins which were absent in male and present in male were of 24.8 kDa and 44.8 kDa.

TABLE 2: Relative mobility and molecular weights (kDa) of albumins at 0.1M Tris- Cl of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.14	97.4						
MP 2	0.23	66.0						
MP 3	0.41	43.0						
MP 4	0.63	29.0						
MP 5	0.94	18.4						
1	0.31	52.0	-	-	-	-	+	+
2	0.38	44.8	-	-	-	-	-	+
3	0.39	44.0	+	+	+	+	+	-
4	0.43	40.8	+	-	-	-	-	-
5	0.44	40.0	-	-	+	+	+	+
6	0.45	39.2	-	+	-	-	-	-
7	0.58	30.0	+	-	-	-	+	+
8	0.59	29.2	-	-	-	+	-	-
9	0.60	28.4	-	+	+	-	-	-
10	0.67	26.4	-	+	-	-	-	-
11	0.69	25.6	-	-	+	-	-	-
12	0.72	24.8	-	+	-	-	-	+
13	0.74	24.0	-	-	+	-	-	-
14	0.76	23.2	-	+	-	-	-	-
Total No. of Bands			3	6	5	3	4	5

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
\+ Presence - Absence

iii) At 0.2M Tris- HCl **(Table 3 and Fig's 3 & 4)**
Three fractions were recorded in both male and female at this concentration in *A.niger*, but the molecular weights were different. They were with 32.0 kDa, 40.8 kDa and 46.4 kDa in males and 32.0 kDa, 40.8 kda and 46.4 kDa in females. In *P. argenteus* five fractions in male and six in female were found with a common protein of 37.6 kDa. The other protein fractions in male were with a molecular weight of 25.6 kDa, 30.4 kDa, 32.8 kDa, 37.6 kDa and 43.2 kDa whereas those of female were 24.8 kDa, 31.2 kDa, 32.8 kDa, 37.6 kDa, 42.4 kDa and 56.0 kDa. Four and five in males and female respectively were observed in *P. chinensis* with two common fractions of 37.6 kDa and 42.4 kDa. The molecular weights of other albumin proteins were 30.4 kDa and 51.2 kDa in male and31.2 kDa, 54.4 kDa and 64.8 kDa in female.

TABLE 3: Relative mobility and molecular weights (kDa) of albumins at 0.2M Tris- Cl of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.11	97.4						
MP 2	0.17	66.0						
MP 3	0.30	43.0						
MP 4	0.50	29.0						
MP 5	0.79	18.4						
1	0.17	64.8	-	-	-	-	-	+
2	0.21	56.0	-	-	-	+	-	-
3	0.22	54.4	-	-	-	-	-	+
4	0.24	51.2	-	-	-	-	+	-
5	0.27	46.4	+	-	-	-	-	-
6	0.28	44.8	-	+	-	-	-	-
7	0.30	43.2	-	-	+	-	-	-
8	0.31	42.4	-	-	-	+	+	+
9	0.32	40.8	+	-	-	-	-	-
10	0.33	40.0	-	+	-	-	-	-
11	0.36	37.6	-	-	+	+	+	+
12	0.43	32.8	-	-	-	+	-	-
13	0.44	32.0	+	-	+	-	-	-
14	0.46	31.2	-	+	-	+	-	+
15	0.47	30.4	-	-	+	-	+	-
16	0.56	25.6	-	-	+	-	-	-
17	0.57	24.8	-	-	-	+	-	-
Total No. of Bands			3	3	5	6	4	5

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
+ Presence - Absence

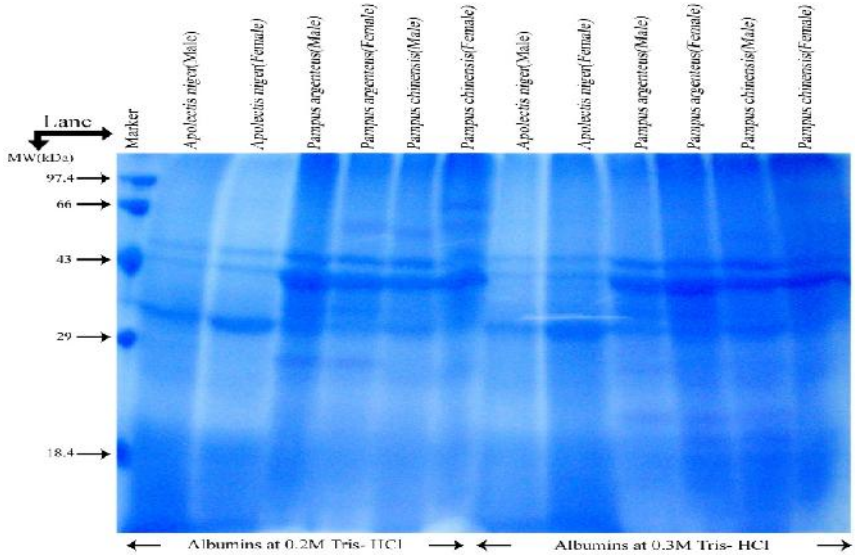

FIGURE 3. Electrophoretic pattern (SDS- PAGE) of albumins at 0.2M and 0.3M Tris- HCl in males and females of *A. niger, P. argenteus* and *P. chinensis*.

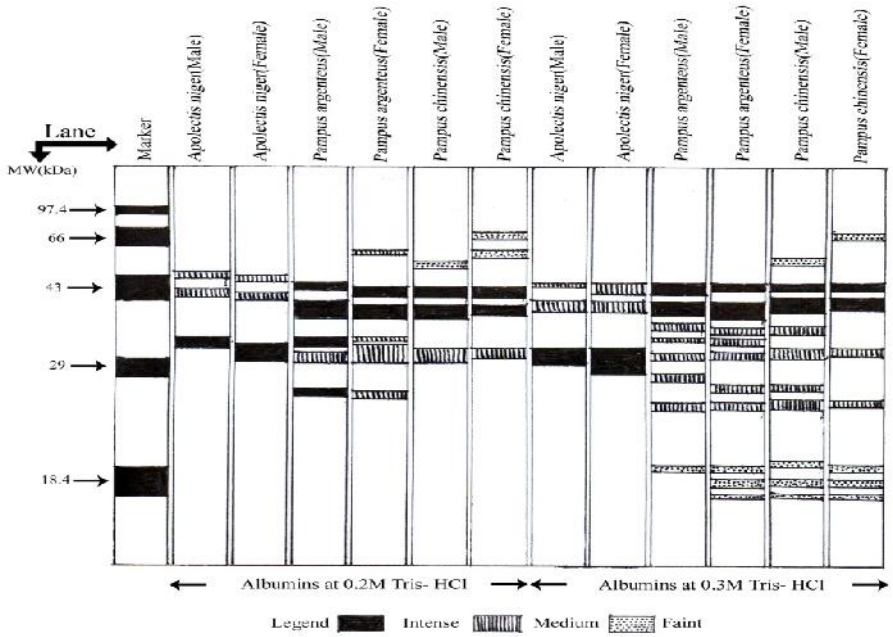

FIGURE 4: Digrammatic representation of electrophoretic pattern (SDS-PAGE) of albumins at 0.2M and 0.3M Tris-HCl in males and females of *A. niger, P. argenteus* and *P. chinensis*.

TABLE 4: Relative mobility and Molecular weights (kDa) of albumins at 0.3M Tris- Cl of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.11	97.4						
MP 2	0.17	66.0						
MP 3	0.30	43.0						
MP 4	0.50	29.0						
MP 5	0.79	18.4						
1	0.17	64.8	-	-	-	-	-	+
2	0.24	51.2	-	-	-	-	+	-
3	0.29	44.0	+	-	-	-	-	-
4	0.30	43.2	-	+	+	+	+	+
5	0.34	40.0	-	-	-	-	+	+
6	0.35	39.2	+	+	+	-	-	-
7	0.36	38.4	-	-	-	+	-	-
8	0.40	35.2	-	-	+	-	-	-
9	0.41	34.4	-	-	-	+	+	-
10	0.43	32.8	-	-	+	-	-	-
11	0.46	31.2	-	-	-	-	-	+
12	0.47	30.4	+	-	+	+	+	-
13	0.48	29.6	-	+	-	-	-	-
14	0.53	26.4	-	-	+	-	-	-
15	0.55	25.6	-	-	-	+	+	-
16	0.59	24.0	-	-	-	+	+	+
17	0.60	23.2	-	-	+	-	-	-
18	0.74	19.8	-	-	-	-	+	-
19	0.75	19.0	-	-	+	+	-	+
20	0.79	18.2	-	-	-	+	+	+
21	0.82	17.4	-	-	-	+	+	+
Total No. of Bands			3	3	8	9	10	8

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
\+ Presence - Absence

iv) At 0.3M Tris- HCl **(Table 4 and Fig's 3 & 4)**In *A. niger* three fractions were found in both male and female with one similar protein of 39.2 kDa. The remaining proteins were 30.4 kDa and 44.0 kDa in male and 29.6

kDa and 43.2 kDa in female. *P. argenteus* showed eight protein bands in male and nine in female with three common fractions and the molecular weights ranged from 19.0 kDa to 43.2 kDa in male, whereas they were from 17.4 kDa to 43.2 kDa in female. The molecular weights of the common fractions were 19.0 kDa, 30.4 kDa and 43.2 kDa. Ten protein bands in male and eight in female were observed in *P. chinensis* with the molecular weights ranging from 17.4 kDa to 51.2 kDa and from 17.4 kDa to 64.8 kDa in male and female respectively. The molecular weights of the common fractions were 17.4 kDa,18.2 kDa,24.0kDa, 40.0 kDa and 43.2 kDa. The protein fractions present in female and absent in male were of 19.0kDa, 31.2kDa and 64.8 kDa.

***v) At 0.4M Tris- HCl* (Table 5 and Fig's 5 & 6)**

Two fractions were found in both male and female in *A. niger* with the molecular weights of 30.4 kDa and 44.8 kDa. In *P. argenteus* four and two in male and female respectively were observed with one similar protein (44.8kDa) in both the sexes. The molecular weights of other muscle albumin fractions in male were 24.8 kDa, 34.4 kDa, 37.6 kDa and 44.8 kDa and that in female were 39.2 kDa and 44.8 kDa. *P. chinensis* showed two in male and three in female. Two fractions with 37.6 kDa and 44.8 kDa were similar in both sexes and 30.4 kDa was present only in female.

TABLE 5: Relative mobility and molecular weights (kDa) of albumins at 0.4M Tris- Cl of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.17	97.4						
MP 2	0.28	66.0						
MP 3	0.47	43.0						
MP 4	0.72	29.0						
MP 5	0.96	18.4						
1	0.44	44.8	+	+	+	+	+	+
2	0.50	39.2	-	-	-	+	-	-
3	0.52	37.6	-	-	+	-	+	+
4	0.58	34.4	-	-	+	-	-	-
5	0.65	30.4	+	+	-	-	-	+
6	0.78	24.8	-	-	+	-	-	-
Total No. of Bands			2	2	4	2	2	3

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
+ Presence - Absence

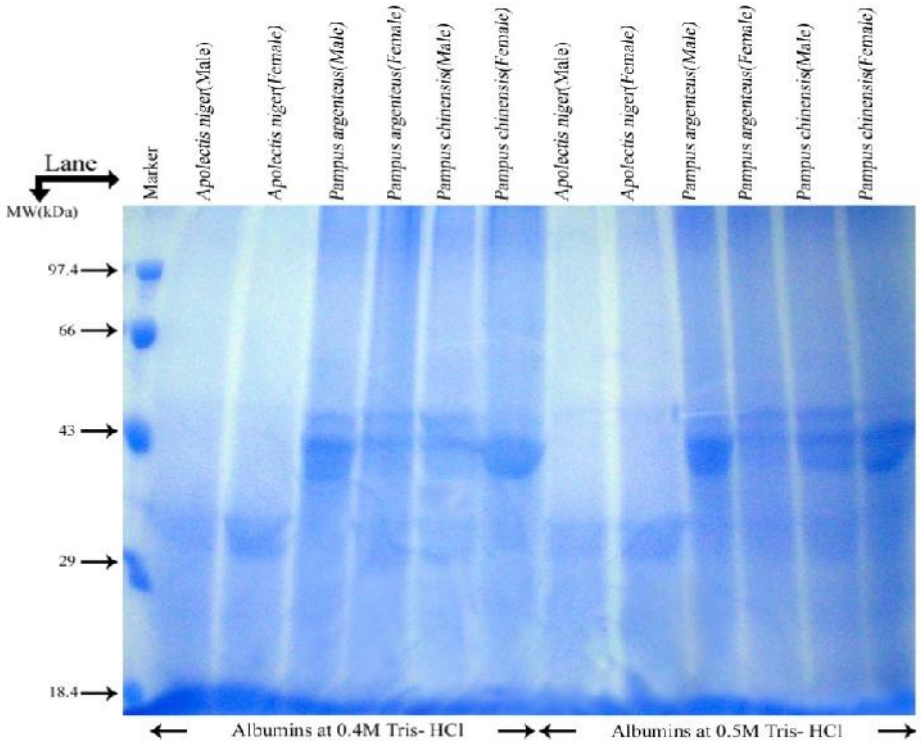

FIGURE 5: Electrophoretic pattern (SDS-PAGE) of albumins at 0.4M and 0.5M Tris-HCl in males and females of *A. niger,*

P. argenteus and *P. chinensis*.

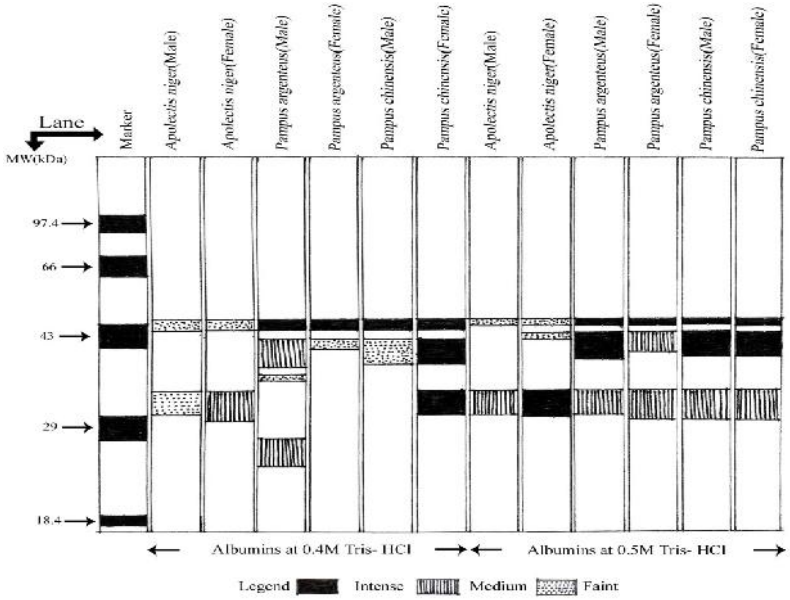

FIGURE 6: Digrammatic representation of electrophoretic pattern (SDS-PAGE) of albumins at 0.4M and 0.5M Tris-HCl in males and females of *A. niger, P. argenteus* and *P. chinensis*.

vi) At 0.5M Tris- HCl **(Table 6; Fig's 5 & 6)**

Male with two albumin fractions and female with three were present in *A. niger* with one common fraction (44.8 kDa). The molecular weight of the remaining fraction in male was 30.4 kDa and those of female were 21.6 kDa, 42.4 kDa and 44.8 kDa. In *P. argenteus* three protein fractions were recorded in male and female with two common in both sexes. The molecular weights of the common fractions were 29.6 kDa and 44.8 kDa. The protein present in male and absent in female was with 30.4 kDa and that present in female and absent in male was 29.6 kDa. Males and females of *P. chinensis* showed three with two common protein fractions in both sexes (29.6 kDa and 44.8 kDa). The protein fraction with 35.2 kDa was absent in male and present in female whereas that with 39.2 kDa was absent in female and present in male.

TABLE 6: Relative mobility and molecular weights (kDa) of albumins at 0.5M Tris- Cl of males and females of *A. niger, P. argenteus* and *P. chinensis*.

S.No.	Rm values	Molecular weight(kDa)	*A. niger*		*P. argenteus*		*P.chinensis*	
			Male	Female	Male	Female	Male	Female
MP 1	0.17	97.4						
MP 2	0.28	66.0						
MP 3	0.47	43.0						
MP 4	0.72	29.0						
MP 5	0.96	18.4						
1	0.44	44.8	+	+	+	+	+	+
2	0.47	42.4	-	+	-	-	-	-
3	0.50	39.2	-	-	+	+	+	-
4	0.55	35.2	-	-	-	-	-	+
5	0.65	30.4	+	-	+	-	-	-
6	0.66	29.6	-	+	-	+	+	+
Total No. of Bands			2	3	3	2	3	3

MP 1: Phosphorylase b MP 2: Bovine serum albumin MP 3: Ovalbumin
MP 4: Carbonic anhydrase MP 5: Lactoglobulin
+ Presence - Absence

DISCUSSION

Proteins are the chief source of energy in fishes, since they live in an environment, which is carbohydrate free (Lovell, 1989) and muscular proteins are important from the point of view of the edibility. Muscle albumins are one of the most important of the fish proximate principles found in the aqueous system of the muscle *i.e.,* sarcoplasm and myoplasm extractable with water or dilute salt solutions (Lovell, 1989). Electrophoretic separation by SDS-PAGE muscle albumin proteins with water and salt solutions of

low ionic strength (0.1M to 0.5M Tris-HCl) reveal similarity and dissimilarity of the respective proteins among three species of pomfrets and also between both sexes of a species in terms of presence or absence of a particular protein fraction, relative mobility, staining intensity and molecular weights. Maximum number of protein fractions has been observed with water and at 0.1M Tris-HCl buffer in all three species. Similarity has been found at one albumin fraction with molecular weight 44.8kDa at 0.4M Tris-HCl buffer. The *P. chinensis* has exhibited similarity between male and female at two loci with molecular weights with 37.6kDa and 44.8kDa. The number of fractions resolved are also low. At 0.5m Tris-HCl also, the number of fractions resolved are also low in number with only one common fraction having a molecular weight of 44.8kDa. *P. argeneteus* and *P. chinensis* have exhibited another common fraction between male and female *i.e.,* 29.6kDa in *P. chinensis* and 39.2kDa in *P. argenteus*.

Most of the electrophoretic studies carried out are related to either total proteins or myofibrillar protein or corneal proteins except those of Connell (1953) on low ionic strength proteins *i.e*., 0.5M in fishes. Perzanowska and Smialowska (1980) have observed same molecular weight of 43,000D in M line and regulatory proteins in three species of white- and red- blooded fish. Difference between two species in electrophoresis and iso-electric focusing of the water- soluble proteins of the skeletal muscles of Atlantic cod *G. morhua morhua* and Pacific cod *G. maruhua macrocephalus* has been observed by Mackie and Ritchie, 1980. Hamoir and Gerardin (1980) have differentiated the sarcoplasmic proteins of white, yellow and cardiac muscles of an Antarctic haemoglobin-free fish *C. gunnari*. Difference in the distribution of β- and γ- crystalline soluble eye lens proteins of Sparidae family has been observed by Basaglia (1989). The white skeletal muscle myosins of four marine teleost fish species- cod, blue whitting, haddock and spotted wolf- fish have shown similarity electrophoretically in four types native proteins in three species and cod has shown an extra band of higher mobility than the main one (Martizen *et al,* 1990). Matthiensen and Tellechea (1993) have studied the esterase isozyme by electrophoresis and reported the absence of arylesterase in the skeletal muscle of the white-mouthed Croaker. Oberst *et al,* (1996) has studied the immunological markers for the discrimination of three tilapia species and found SDS-PAGE and isoelectro-focussing of parvalbumins and found species- specific protein profiles in all the three species of *Tilapia*. Chummar *et al,* (2004) have studied electrophoretic patterns of protein in frozen stored fish (*M. cordyla* and *L. rohita*) and found that the water soluble proteins remain almost the same through the frozen storage period with some minor bands becoming less intense with storage period. Yilman *et al,* (2007) have indicated that there are similarities and differences in the molecular weights of serum proteins among *A. marmia, L. cephalous* and *C. regium*. Michelis *et al,* (2010) have observed the difference of albumin mobility in *N. melanostomus* from Black Sea and Azov sea which may be due to result of gene mutation or specificity of transport function and binding properties. Osman *et al,* (2010) have documented the changes of electrophoretic spectra of serum proteins in fish from chemical polluted areas due to the consumption of xenobiotics by fish with food and water bind with albumin and change its electrophoretic mobility. Gaikwad *et al,* (2012) have observed a total of eleven protein bands with varying migration having molecular weights ranging from 14.79 kDa to 154.20 k Da on 10% polyacrylamide gel and observed no alterations of protein profiles on 12 % SDS- Page suggesting that these proteins consists of single polypeptide chains.

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