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RESEARCH ARTICLE

Electrophoretic studies (PAGE) on the muscle albumin proteins in two species of pomfrets – *Pampus argenteus* (Euphrasen, 1788) and *P. chinensis* (Euphrasen, 1758)

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Abstract

Muscle albumin proteins of males and females of two species of pomfrets – *Pampus argenteus* and *P. chinensis* have been studied electrophoretically using polyacrylamide gel (PAGE) with water and Tris- HCl buffer (0.1M, 0.2M, 0.3M, 0.4M and 0.5 M). Difference between the sexes of the two species and also between the two species was observed with respect to protein fraction, relative mobility and molecular weights. The native protein fractions ranged from 3 to 6 in both sexes of the two species with water and at different concentrations of Tris- HCl. Catalase, Bovine serum albumin and Ovalbumin were used as marker proteins.

Introduction

Fish proteins particularly the albumin proteins differ quantitatively and qualitatively with species, sex, season and physiologically as in other organisms (Nair and Mathew, 2000). The taste of the fish flesh is mainly due to the composition of the albumin proteins, which is a translated phenotypic expression of the genome (Bye and Ponnaiah, 1983). The albumin proteins are extractable with salt solutions of low ionic strength *i.e* < 0.5M (Nair and Mathew, 2000). These proteins carry 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 separation of biochemical constituents. 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). The molecular mobility of proteins in an electric field depends on their molecular weight, conformation and surface electric charge (Mc Laughlin *et al*, 1982). 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 (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 have made electrophoretic studies on the plasma and muscular proteins of different fishes (Carpene *et al*, 1983; Basaglia, 1989 &1989a; Martizen *et al*, 1990; Basaglia and Marchetti, 1991; Deepak and Rao, 1992; Gam *et al*, 2006; Gopalakrishnan *et al*, 2006; Yilman *et al*, 2007; Shagufta and Gayasuddin, 2011; Gaikwad *et al*, 2012). An attempt is made in the present study to unravel the different types of native albumin

proteins of the muscle and their molecular weights using PAGE in two species of pomfrets – *P. argenteus* and *P. chinensis.*

Material and Methods

Fish specimens were collected from the Visakhapatnam Fishing Harbour and were separated as per species. The sex was identified by dissecting the abdomen and observing the gonads. The muscle of both sexes of the two 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 tissue was powdered and was used for electrophoretic studies by PAGE for native albumin proteins following the method of Sambrook and Russell (1988). The albumin proteins of muscles were isolated with water and Tris- HCl buffer (0.1M, 0.2M, 0.3M, 0.4M and 0.5M). The marker proteins (MP) used were MP-1 Catalase (240,000Da), MP-2 Bovine Serum Albumin (67,000Da) and MP-3 Ovalbumin (14,000Da).

RESULTS

Muscle albumin proteins

Water soluble

In *P. argenteus* six proteins with molecular weights from 48.4 kDa to 94.6 kDa were found in males and females, whereas they were four in males and five in females of *P. chinensis* with the molecular weights ranging from 48.4 kDa to 94.6 kDa. The protein fractions of *P. argenteus* with the molecular weights of 59.4 kDa and 73.7 kDa were absent in *P. chinensis.* In *P. chinensis* protein fraction with 67.1 kDa present in female was not found in male (Table 1; Fig's 1 & 2).

At 0.1M Tris- HCl

Five bands in male and three in female were found in *P. argenteus,* whereas in *P. chinensis*, they were four in both male and female. With molecular weights ranging from 48.4 kDa to 94.6 kDa in males whereas they were from 51.7 kDa to 94.6 kDa in *P. argenteus.* The male differed from female in not having the albumin proteins with molecular weight 51.7 kDa and 80.3 kDa. In case of *P. chinensis,* albumin protein with molecular weight 51.7 kDa was absent in female and similarly that with 73.7 kDa of female was not found in male (Table 1; Fig's 1 & 2).

At 0.2M Tris- HCl

P. argenteus and *P. chinensis* showed four bands in male and three bands in female with two similar albumins (69.3 kDa & 82.5 kDa) in both the sexes. In *P. argenteus,* albumin fractions with molecular weight 46.2 kDa and 56.1 kDa were present in male, whereas they were absent in female and albumin with 51.7 kDa was absent in male. In case of *P.chinensis,* the albumin protein with 51.7kDa present in male was absent in female and the other three fractions were similar (Table 1; Fig's 3 & 4).

At 0.3M Tris- HCl

Except the female *P. argenteus,* the rest showed four proteins of which three were similar in both sexes with the molecular weights ranging from 51.7 kDa to 82.5 kDa. Albumin protein with molecular weight 46.2 kDa present in male of *P. argenteus* was absent in female whereas in *P. chinensis* male differed from female at one fraction *i.e.,* protein with molecular weight 46.2 kDa was absent in female and that 45.1kDa was absent in male (Table 2; Fig's 3 & 4).

At 0.4M Tris- HCl

In *P. argenteus* five in males and three in females with a range of molecular weights of 48.4 kDa to 86.9 kDa. Proteins of males with molecular weight 52.8 kDa and 56.1kDa were absent in female. Males and females with two albumin proteins were identified in *P. chinensis.* Albumin protein with 50.6kDa of males was absent in female and that with 48.4kDa of female was not present in male (Table 2; Fig's 5 & 6).

At 0.5M Tris- HCl

Males with four protein bands and females with two were observed in *P. argenteus* with the molecular weights ranging from 48.4 kDa to 86.9 kDa. In case of *P. chinensis* three in both the sexes with one dissimilar fraction. The molecular weights of similar bands were 73.7 kDa and 86.9 kDa. The protein with 50.6 kDa present in male was absent in female whereas that with 48.4 kDa was absent in female (Table 2; Fig's 5 & 6).

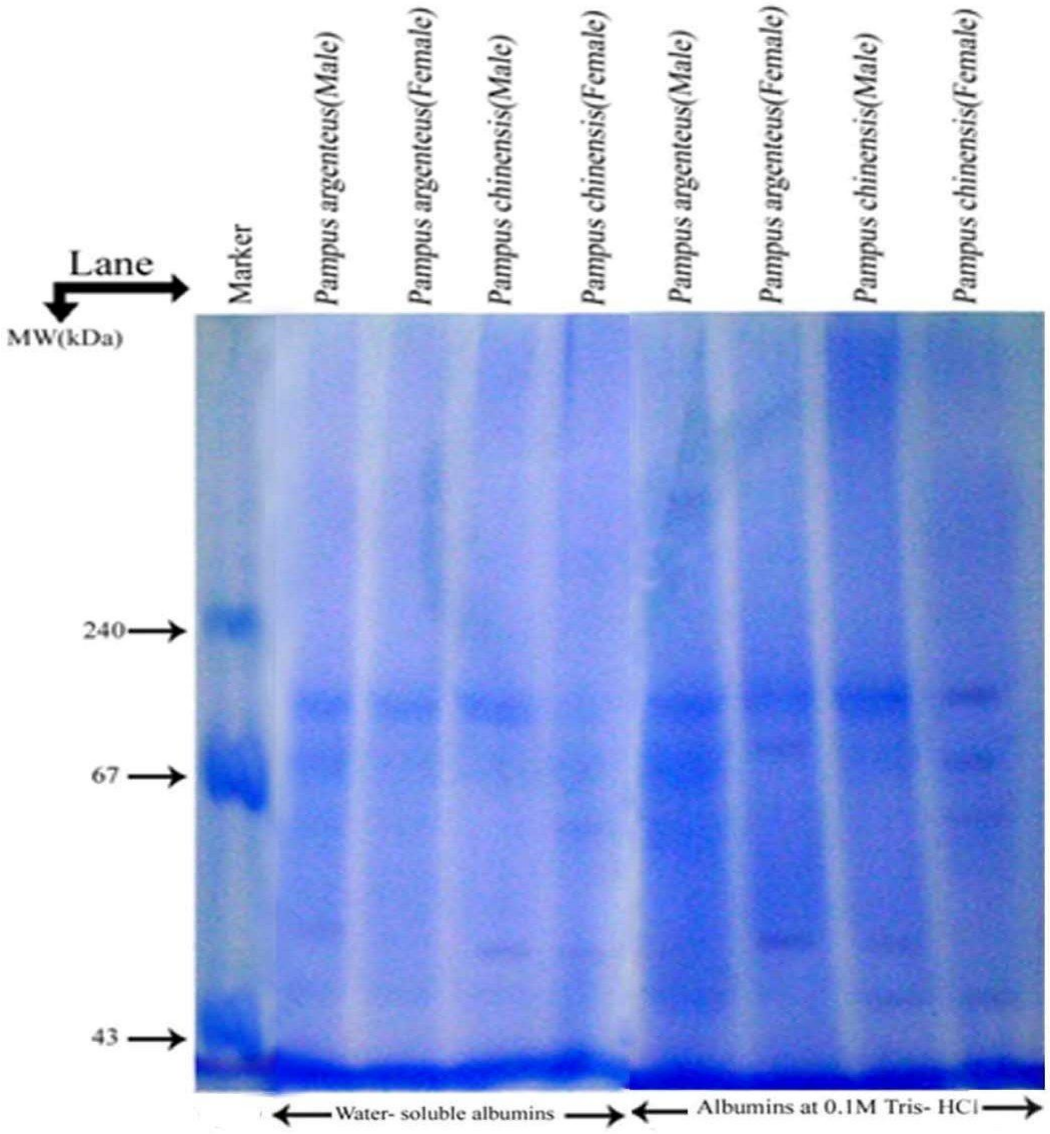

Fig. 1: Electrophoretic pattern (PAGE) of Water-soluble albumins and albumins at 0.1M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

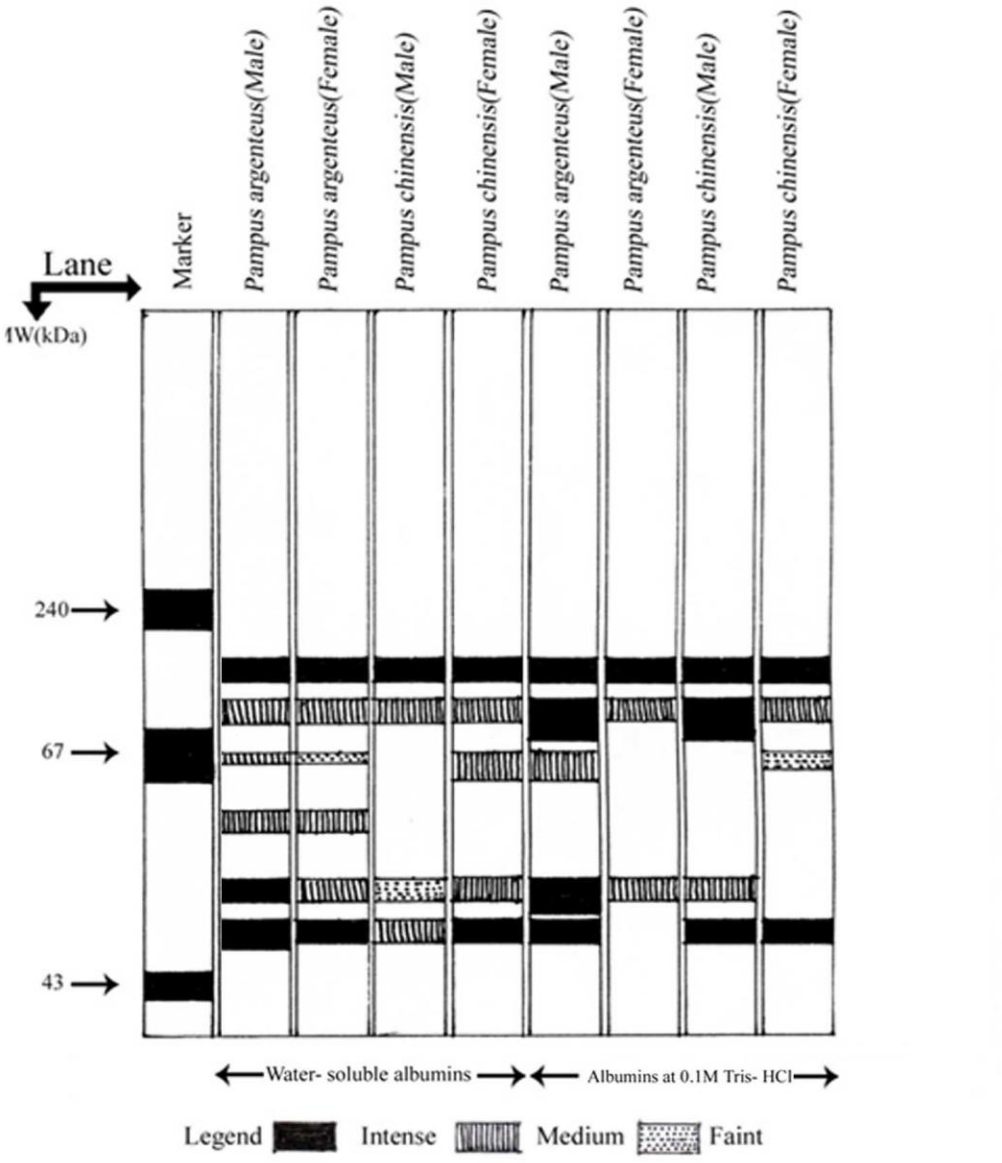

Fig.2: Digrammatic representation of electrophoretic pattern (PAGE) of Water-soluble albumins and albumins at 0.1M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

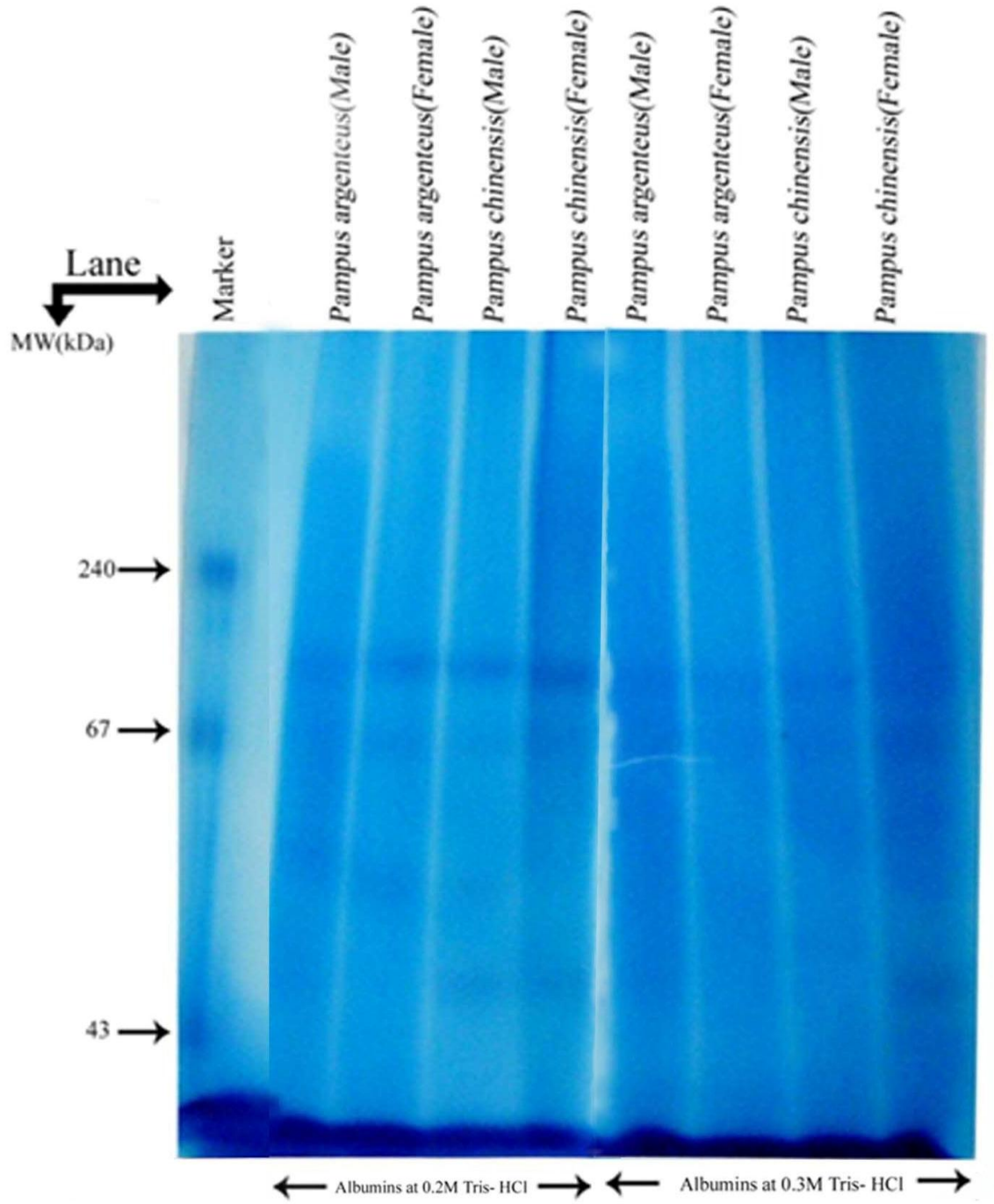

Fig. 3: Electrophoretic pattern (PAGE) of albumins at 0.2M and 0.3M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

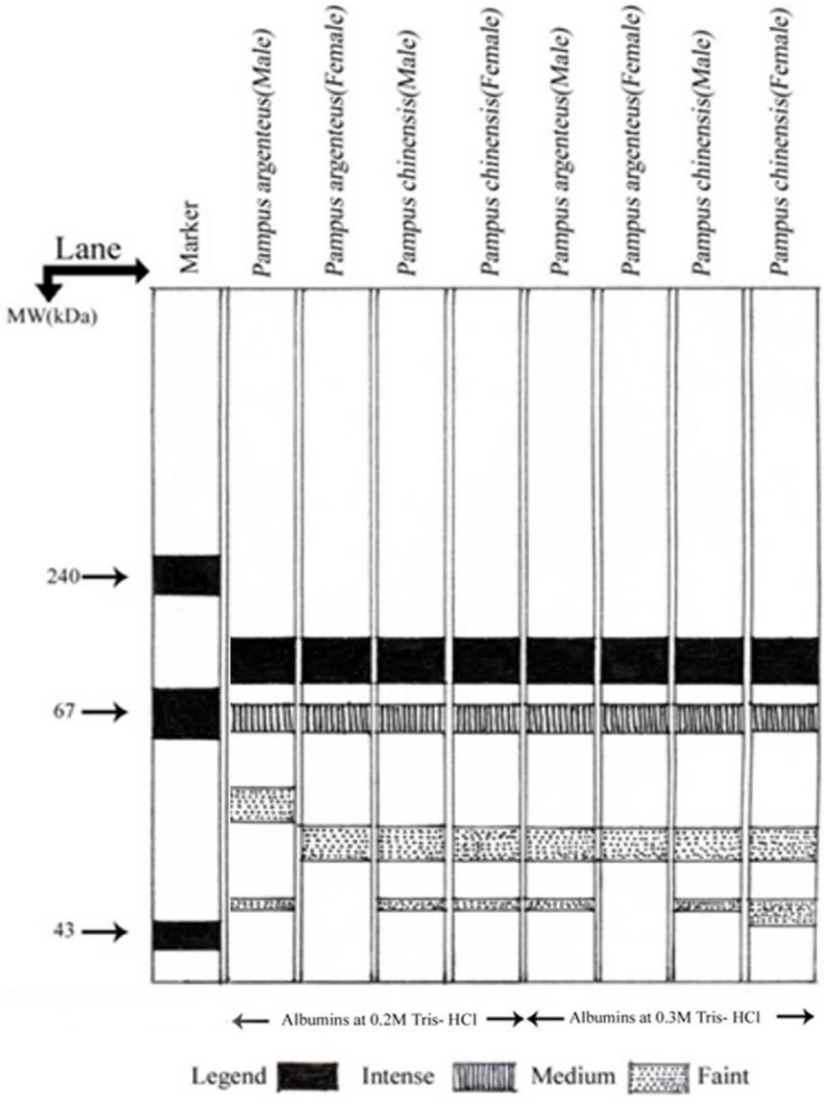

Fig. 4: Digrammatic representation of electrophoretic pattern (PAGE) of albumins at 0.2M and 0.3M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

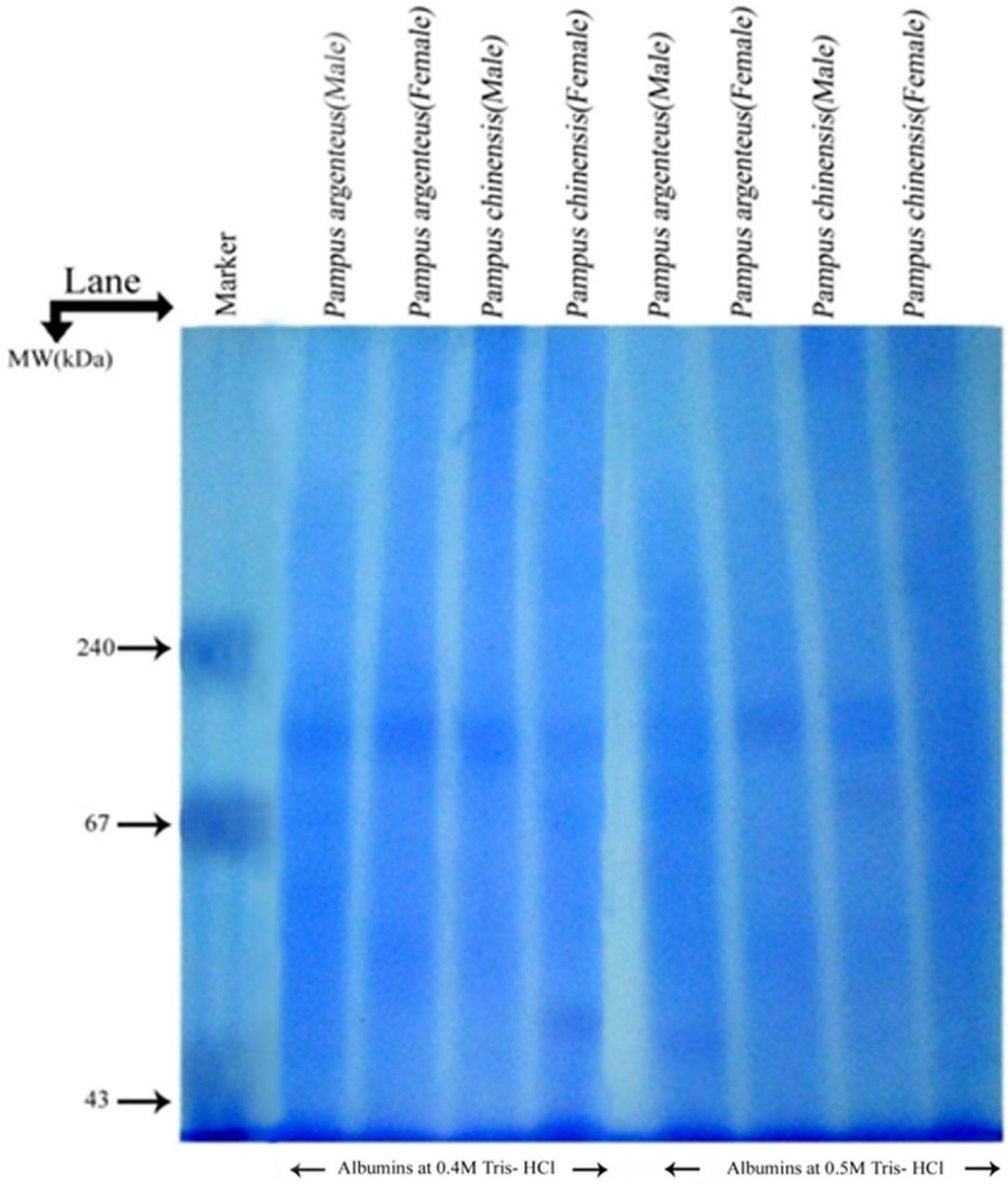

Fig. 5: Electrophoretic pattern (PAGE) of albumins at 0.4M and 0.5M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

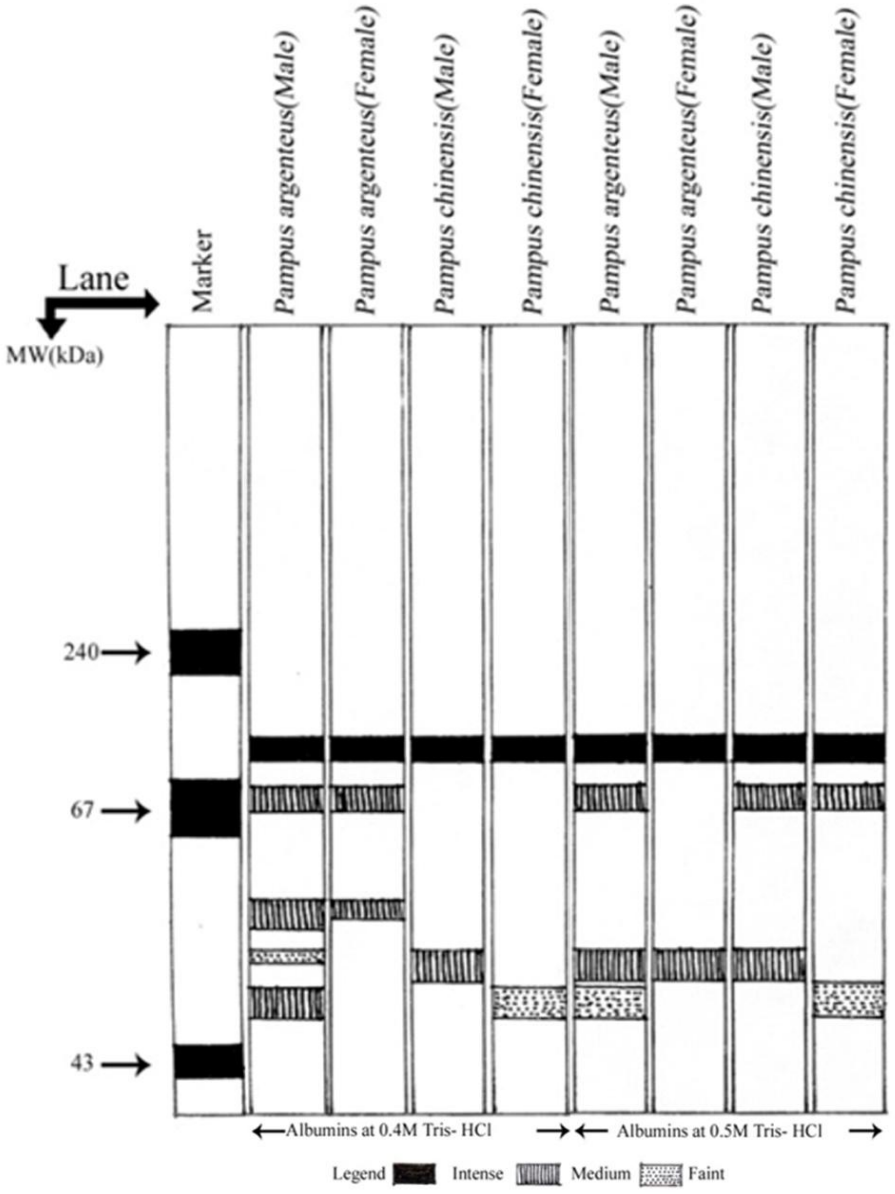

Fig. 6: Digrammatic representation of electrophoretic pattern (PAGE) of albumins at 0.4M and 0.5M Tris-HCl in males and females of *P. argenteus* and *P. chinensis*.

Table 1: Relative mobility and molecular weights (kDa) of water soluble, 0.1M & 0.2 M Tris- HCl albumins of males and females of *P. argenteus* and *P. chinensis*.

			Water soluble albumins				0.1 M Tris- HCl				0.2 M Tris- HCl			
S.No.	Rm values	Molecular weight (kDa)	*P. argenteus*		*P. chinensis*		*P. argenteus*		*P. chinensis*		*P. argenteus*		*P. chinensis*	
			Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
MP 1	0.44	240												
MP 2	0.65	67												
MP 3	0.95	43												
1	0.51	94.6	+	+	+	+	+	+	+	+	-	-	-	-
2	0.55	86.9	-	-	-	-	-	-	-	-	-	-	-	-
3	0.57	82.5	-	-	-	-	-	-	-	-	+	+	+	+
4	0.58	80.3	+	+	+	+	-	+	+	+	-	-	-	-
5	0.60	77.0	-	-	-	-	+	-	-	-	-	-	-	-
6	0.62	73.7	+	+	-	-	-	-	-	+	-	-	-	-
7	0.64	69.3	-	-	-	-	-	-	-	-	+	+	+	+
8	0.65	67.1	-	-	-	+	+	-	-	-	-	-	-	-
9	0.72	59.4	+	+	-	-	-	-	-	-	-	-	-	-
10	0.76	56.1	-	-	-	-	-	-	-	-	+	-	-	-
11	0.81	52.8	-	-	-	-	-	-	-	-	-	-	-	-
12	0.82	51.7	+	+	+	+	-	+	+	-	-	+	+	-
13	0.83	50.6	-	-	-	-	+	-	-	-	-	-	-	-
14	0.87	48.4	+	+	+	+	+	-	+	+	-	-	-	-
15	0.89	46.2	-	-	-	-	-	-	-	-	+	-	+	+
16	0.92	45.1	-	-	-	-	-	-	-	-	-	-	-	-
Total No. of Bands			6	6	4	5	5	3	4	4	4	3	4	3

MP 1: Catalase MP 2: Bovine serum albumin MP 3: Ovalbumin + Presence - Absence

Table 2: Relative mobility and molecular weights (kDa) of 0.3M, 0.4 & 0.5M Tris- HCl albumins of males and females of *P. argenteus* and *P. chinensis*.

			0.3 M Tris- HCl				0.4 M Tris- HCl				0.5 M Tris- HCl			
S.No.	Rm values	Molecular weight (kDa)	*P. argenteus* Male	*P. argenteus* Female	*P. chinensis* Male	*P. chinensis* Female	*P. argenteus* Male	*P. argenteus* Female	*P. chinensis* Male	*P. chinensis* Female	*P. argenteus* Male	*P. argenteus* Female	*P. chinensis* Male	*P. chinensis* Female
MP 1	0.44	240												
MP 2	0.65	67												
MP 3	0.95	43												
1	0.51	94.6	-	-	-	-	-	-	-	-				
2	0.55	86.9	-	-	-	-	+	+	+	+	+	+	+	+
3	0.57	82.5	+	+	+	+	-	-	-	-	-	-	-	-
4	0.58	80.3	-	-	-	-	-	-	-	-	-	-	-	-
5	0.60	77.0	-	-	-	-	-	-	-	-	-	-	-	-
6	0.62	73.7	-	-	-	-	+	+	-	-	+		+	+
7	0.64	69.3	+	+	+	+	-	-	-	-	-	-	-	-
8	0.65	67.1	-	-	-	-	-	-	-	-	-	-	-	-
9	0.72	59.4	-	-	-	-	-	-	-	-	-	-	-	-
10	0.76	56.1	-	-	-	-	+	+	-	-	-	-	-	-
11	0.77	56.1	-	-	-	-	-	-	-	-	-	-	-	-
12	0.81	52.8	-	-	-	-	+	-	-	-	-	-	-	-
13	0.82	51.7	+	+	+	+	-	-	-	-	-	-	-	-
14	0.83	50.6	-	-	-	-	-	-	+	-	+	+	+	
15	0.87	48.4	-	-	-	-	+	-	-	+	+			+
16	0.89	46.2	+	-	+	-	-	-	-	-	-	-	-	-
17	0.92	45.1	-	-	-	+	-	-	-	-	-	-	-	-
Total			4	3	4	4	5	3	2	2	4	2	3	3

MP 1: Catalase MP 2: Bovine serum albumin MP 3: Ovalbumin + Presence - Absence

DISCUSSION

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 PAGE muscle albumin proteins with water and salt solutions of low ionic strengths (0.1M to 0.5M Tris-HCl) reveal similarity and dissimilarity of the respective proteins among two 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. A total of sixteen protein bands with molecular weights ranging from 45.1 kDa to 94.6 kDa with varying mobility were observed in both the species.

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 (1977) have shown molecular weight of white skeletal muscle of myosin of two species of fish ranging from 12,270D to 68,000D. Perzanowska and Smialowska (1980) have observed same molecular weight of 43,000 D in M line and regulatory proteins in three species of white- and red- blooded fish. 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 (Martinez *et al,* 1990).

Basaglia and Marchetti (1991) have noticed similar mobility at genus or at sub family level and significant difference in others in the soluble proteins of white skeletal muscle tissue of fifteen Sparidae species. Varghese and Shankar (1999) have studied the electrophoretic patterns of sarcoplasmic proteins in Carngid fishes and observed Rf values ranging from 21 to 84 in *Selar crumenopthalmus,* 2 to 72 in *M. cordyla*, 15 to 89 in *Decapterus macrosoma* and 18 to 96 in *D. russelli*. Yilman *et al* (2007) have indicated similarities and differences in the molecular weights of serum proteins among *Acanthobrama marmia, Leuciseus cephalous* and *Chondrostoma regium*. A total of eleven protein bands with varying mobility and molecular weights ranging from 14.79 kDa to 154.20 kDa have been reported by Gaikwad *et al* (2012) in the blood serum of *Channa gachu*.

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