ELECTROPHORETIC ANALYSIS USING (SDS-PAGE) TECHNIQUES OF MUSCLE AND EYE PROTEINS OF SOME FRESHWATER FISH SPECIES IN EGYPT

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Abstract

A study was made to identify the patterns of finger prints of freshwater fish (*Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio)* were analyzed using muscles and eyes protein electrophoresis. Fish were collected from the fishponds of Central Laboratory for Aquaculture Research (CLAR). Protein fractionation was adapted by Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) was used.

The electrphoretical patterns of the various sera of fishes disclosed the presence of 19 & 26; 18 & 24; 24 &25; 17 & 25; 19 & 27; fractions in muscle and eye protein of *Oreochromis niloticus*; *S. galellius*; *T. zillii*; *Clarias gariepinus and Cyprinus carpio* respectively, while 22 fractions were detected in muscle of *Oreochromis aureus*;. All studied fishes shared in 13 common band numbers 1, 2, 4, 9, 15, 16, 17, 20, 22, 23, 24, 28 & 29 at relative front 0.033, 0.049, 0.103, 0.226, 0.413, 0.43, 0.52, 0.625, 0.688, 0.721, 0.748, 0.872 & 0.897 and molecular weight were 327.2, 303.4, 232.7, 126.1, 89.3, 84.8, 65.04, 47.9, 39.7, 36.1, 33.3, 23.1 & 21.4 (Kda) in two organs (muscle and eye). There were some differences in the protein profiles in sample from muscle than that of the eye of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 3 common band numbers 5, 10 and 19 at relative front 0.124, 0.261 & 0.591 and molecular weight were 209.5, 115.7 & 52.8. (Kda). While, sample from eyes of *Oreochromis niloticus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 4 common band numbers 7, 8, 13 & 26 at relative front 0.163, 0.21, 0.375 & 0.794 and molecular weight were 173.2, 136.8, 98.9 & 29.2 (Kda). Band number 25 at relative front of 0.772 and mobility 6.555 and 10.292 in muscles and eyes of *Oreochromis niloticus* can be used as a marker band for *Oreochromis niloticus*. On the other hand, muscles and eyes of *Tilapia zillii* shared five common band numbers 3,11,12,14 & 18 at relative front of 0.08, 0.313, 0.336, 0.396 & 0.579 and molecular weight were 261.01, 107.9, 104.3, 93.9 & 54.6 (Kda) these bands can be used as marker bands for *Tilapia zillii.*

It concluded that genetic variations has been successfuly detected in muscle and eye of fishes. Using the electrophoretic method Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) of muscles and eyes of fish. Therefore, the genetic polymorphism among and within fish are useful tools in

characterizing most of genetic variations in *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio.*

INTRODUCTION

Fishes are important members of aquatic ecosystem and an important source of food for human. For many reasons, fish often used as test animals in aquatic environmental researches (Katz *et al.*, 1969). Tilapia species have become very important and are cultured in most fish farms throughout the country. Their economic importance is constantly increasing for their fast growth, disease resistance, different feeding habits and palatability (Dagzie, 1982). Also, the African catfish *Clarias gariepinus* is an economically important species, apart from being a model organism in research (Volckaert *et al.*, 1994).

There are many techniques which have been used to identify and characterize different genera, species, individuals and make finger print for different fishes. Recently, isozyme polymorphism and protein banding patterns in addition to the molecular study have been described. Smith (1990) reported that, gel electrophoresis is a powerful technique for fish-stock identification. The protein electrophoresis was successfully employed to study the variations among marine fish populations. The theoretical and empirical problems arising from the use of the technique in stock separation studies are discussed. Also, Oberst *et al.* (1996) studied the general muscle proteins and paralbumins of *Tilapia dageti, Tilapia guineensis* and *Tilapia zilli,* which occur sympatrically in Ghana to identify species-species markers. Polyacrylamide gel electrophoresis and isoelectricfocussing revealed species-species protein markers for the three species. Immunoelectrophoresis and western blot tests of paralbumins produced species-species banding patterns.

SDS-PAGE technique is used to identify the Vitellogenin (large phosphoglycolipoprotein) of *Oreochromis* strains (Buerano *et al.*, 1996). AbdeL-Gawad *et al.* (1997) recorded that the electrophoretic study gives evidence of the great genetic divergence between *Solea vulgaris* and *Solea aegyptiaca* from Abu-Kir Bay. The high value of genetic distance of *Solea aegyptiaca* populations from Abu-Kir Bay and Qarun Lake showed a markedly genetic variability, which may be induced as a result of the environmental differences between these two habitats for many years, which exhibited contrasting selection pressures on fish genome for inducing genetic variations, which seem to reflect adaptation to local conditions. This degree of biogenetic variations indicated that the sole fish from Qarun Lake became a distinct population. Also, Kamel (1999) used SDS-PAGE of soluble muscle proteins to sudy genetic variations among three strains of *Oreochromis niloticus* collected from

different locations in Egypt (Maryout, Abbassa and Kafer El-sheikh). The result indicated that Maryout strain is genetically distinct from both abbassa and Zawia (Kafer El-Sheikh).

The objective of the present investigation is to characterize and identify different genetic resources of O*reochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio* and study the variation of organ protein banding patterns to draw the genetic relationships among them by using muscle and eyes protein electrophoresis patterns.

MATERIALS AND METHODS

1- Fish:

A number of 30 healthy fingerlings (5 fish from each of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio*) were collected from the fishponds of Central Laboratory for Aquaculture Research (CLAR), Abbassa, Abou-Hammad, Sharkia. Fishes were acclimated in laboratory conditions for two weeks in aerated holding tanks with feeding rate of 3% body weight by locally diet containing fish meal, Soybean meal, yellow corn formulation ingredients, bone meal, meat meal, mixture of vitamins, and salts were formulated in a pellted form using glutinative substance from Atmida poultry company, Dakahlia Governorate, Egypt (25% crude protein from fish meal and Soybean meal). The aquaria were supplied with dechlorinated tap water and constant aeration. The dissolved oxygen was adjusted at 5.7 mg/l, the temperature at 26 ± 2 C° and the pH at 7.2 ± 0.2.

Samples were taken from muscles and eyes of fingerlings average size range between 20-30 g of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio.*

2- Water –Soluble Protein (WSP) Electrophoresis:

Sample of 0.5 g of fish muscle or eye was prepared for electerophoretic analysis by Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (10% SDS-PAGE) according to Studier (1973). A sample of 0.5 g of muscle or eye was homogenized with 0.5 ml of sample buffer and centrifuged for 5-10 minutes at 12,000 r.p.m. A volume of 50 µl of sample extraction (0.05 g) was added to the same volume of 2x Laemmli buffer. Mercaptoethanol was added to each tube (10%v/v, for each sample). All samples were then boiled in a water bath for 10 minutes at 100 °C and loaded on the gel after adding one drop of bromophenol blue (0.025%). The run buffer were poured into a running tank (4.2 liters) to be precooled by flowing tap water through cooling tubes. The buffer tank was filled, so that gels were completely covered. 800ml of run buffer were saved for the upper tank. Gels were run at 175V

for 15 minutes and then were raised to 200 V for two hours. Staining gels were placed in 100 ml of Coomassie Brilliant Blue R 250 staining solution, removing the staining solution from tray, 200 ml-destining solution was added. Gel was agitated for one hour. After removing the solution, a new 200 ml distaining solution was added to the gel. Agitation was repeated for three times until gel background was clear. The gel was photographed and diagramatically illustrated.

RESULTS AND DISCUSSION

Many electrophoretic studies have been conducted to identify the differences among Tilapia species over the entire world. In this regard different protein sources (from muscles and eyes) were examined in *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio.* The protein profiles for intra and inter-population identification was successfully applied. Apite and Rao (1992) used the electrophoretic profile of eye lens proteins and muscle proteins that had a sufficient value in determination of species-specific banding pattern and fish taxonomy. Also, Macaranas *et al.* (1996) said that serum and muscle proteins are commonly used to assess the polymorphisms among fish species.

In concurrence, were the electrophoretic investigation muscle and eye proteins of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* fingerlings. The electrphoretical patterns of the various sera of fishes disclosed the presence of different number fractions in muscle and eye proteins. Numbers of fractions in muscle and eye proteins were 19 & 26 in *Oreochromis niloticus*; 18 & 24 in *S. galellius*; 24 & 25 in *T. zillii*; 17 & 25 in *Clarias gariepinus*; 19 & 27 *Cyprinus carpio* fractions in muscle and eye proteins respectively, and 22 in muscle of *Oreochromis aureus* (Table 1 and figs. 1-7). This is in accordance with the observation recorded by Elghobashy (2004) who reported that the maximum number of bands of muscle protein patterns of *Oreochromis niloticus* from different populations (Maryout, Manzala, Abbassa and Aswan) were 23, 25, 21 and 20, respectively. While, Rizkalla *et al.* (2005) reported that the electrophoretic patterns of the various plasma proteins of *Oreochromis aureaus* disclosed the presence of 13 main fractions described in terms of their mobility, the faster anode migration ids designated first, followed by the less mobility fraction and so on. Also, Farag (2001) said that the densitometeric analysis of SDS-PAGE of Water Soulble Protein from muscles of *Clarias gariepinus* showed that the total number of bands ranged from 13-15 in fish collected from Abbassa.

Abdel-Tawab *et al.* (1988) reported on the difference among fish population on the same species collected from different locations in Egypt on basis of water soluble muscle protein to the different expermental conditions. However, in this study,

Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio shared in 13 common band numbers 1, 2, 4, 9, 15, 16, 17, 20, 22, 23, 24, 28 & 29 at relative front 0.033, 0.049, 0.103, 0.226, 0.413, 0.43, 0.52, 0.625, 0.688, 0.721, 0.748, 0.872 & 0.897 and molecular weight were 327.2, 303.4, 232.7, 126.1, 89.3, 84.8, 65.04, 47.9, 39.7, 36.1, 33.3, 23.1 & 21.4 (Kda) in two organs (muscle and eyes) as in table 1 and figs. 1-7. This result is in accordance with Farag (2001) he reported that densitometeric analysis of SDS-PAGE of W.S.P. from muscles of *Oreochromis niloticus* collected from the five locations shared three common bands at relative front 0.22, 0.83 & 0.96 and molecular weight were 113.7, 12.99 & 2.06 (Kda). These bands could be used as a marker bands for *Oreochromis niloticus.* Also, he reported that the five locations shared three common bands at relative front 0.21, 0.33 & 0.90 and molecular weight were 113.32, 79.41 & 5.1 (Kda) in *Oreochromis aureaus.* These bands could be used as marker bands for *Oreochromis aureaus.* In *Clarias gariepinus* the four locations shared one common band at relative front 0.26 and molecular weight was 102.2 (Kda). This band could be used as a marker bands for *Clarias gariepinus.*

In the present study, there were some differences in the SDS-PAGE protein profiles of muscles and eyes of fish. SDS-PAGE of Water-soluble protein from muscles of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 3 common band numbers 5, 10 and 19 at relative front 0.124, 0.261 & 0.591 and molecular weight were 209.5, 115.7 & 52.8. (Kda). While, SDS-PAGE of W.S.P. from eyes of *Oreochromis niloticus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 4 common band numbers 7, 8, 13 and 26 at relative front 0.163, 0.21, 0.375 & 0.794 and molecular weight were 173.2, 136.8, 98.9 & 29.2 (Kda) as table 1 and figs (1-7). This result is partially in agreement with Findlay and Tatner (1995) who studied the electrophoretic analysis in rainbow trout *(Onccorhynchs mykiss)* and indicated that there were some clear differences in the SDS-PAGE proteins profiles between thymus gland, spleen, kidney and peripheral blood lymphocyte lysates.

In the present investigation, SDS-PAGE of Water-soluble protein from muscles and eyes of *Oreochromis niloticus* shared one common band number 25 at relative front of 0.772 and mobility 6.555 and 10.292 in muscles and eyes, respectively. This band can be used as a marker band for *Oreochromis niloticus.* On the other hand, Farag (2001) reported that *Oreochromis niloticus* from five locations (Abbassa, Manzalla, Altal-Alkaber, Shader Azàm and Borolos) shared three common bands at relative front 0.22, 0.83 & 0.96 and molecular weight were 113.7, 12.99 & 2.06 (Kda). These bands could be used as marker bands for *Oreochromis niloticus.* Also, SDS-

PAGE of Water-soluble protein from muscles and eyes of *Tilapia zillii* shared five common band numbers 3,11,12,14 and 18 at relative front 0.08, 0.313, 0.336, 0.396 & 0.579 and molecular weight were 261.01, 107.9, 104.3, 93.9 & 54.6 (Kda) these can be bands used as marker bands for *Tilapia zillii* (Table 1 and figs.1 & 5).

It can be concluded from the present study that the electrphoretical patterns of the various fishes disclosed the presence of 17-25 fractions in *Oreochromis niloticus*; *S. galellius*; *T. zillii*; *Clarias gariepinus* and *Cyprinus carpio* in muscle and eye proteins and muscle of *Oreochromis aureus* only. The fractions shared in 13 common band numbers 1, 2, 4, 9, 15, 16, 17, 20, 22, 23, 24, 28 & 29 and molecular weight were 327.2, 303.4, 232.7, 126.1, 89.3, 84.8, 65.04, 47.9, 39.7, 36.1, 33.3, 23.1 & 21.4 (Kda) in two organs (muscle and eyes). There were some differences in the SDS-PAGE protein profiles of muscles and eyes of fish. SDS-PAGE of Water-soluble protein from muscles of *Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 3 common band numbers 5, 10 & 19 and molecular weight were 209.5, 115.7 & 52.8. (Kda). While, SDS-PAGE of W.S.P. from eyes of *Oreochromis niloticus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus and Cyprinus carpio* shared in 4 common band numbers 7, 8, 13 & 26 and molecular weight were 173.2, 136.8, 98.9 & 29.2 (Kda). It can be concluded also that electerophoretic analysis by Sodium Dodecyl Sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) from muscles and eyes of fish is a successful method to study the fingers print of fresh waterfish (*Oreochromis niloticus; Oreochromis aureus; Sarotherodon galellius; Tilapia zillii; Clarias gariepinus* and *Cyprinus carpio).*

Table 1. Fingerprint of freshwater fish by muscle and eyes protein fractionation.

Lane	Relative	Mol. Wt.	Average optical density										
			Muscle						Eye				
Number	Front	KDa	*O.n*	*O. a*	*S. g*	*T. z*	*C. g*	*C. c*	*O.n*	*S. g*	*T. z*	*C. g*	*C. c*
1	0.033	327.199	10.446	37.169	7.153	23.746	11.099	51.598	3.895	57.317	3.804	9.448	24.553
2	0.049	303.389	13.439	20.91	9.95	23.938	8.118	45.313	7.796	57.052	19.492	16.024	15.565
3	0.08	261.006				36.583		15.531	15.836	58.785	26.415	14.962	
4	0.103	232.717	3.053	20.599	12.326	26.597	28.518	14.358	33.314	50.069	18.916	8.871	4.287
5	0.124	209.494	10.678	16.02	16.122	11.613	13.871	9.739	16.462	37.145	19.3	8.908	
6	0.145	189.45				27.1		13.521	18.741	43.328		10.68	
7	0.163	173.167		50.117	12.998	9.666		42.964	19.892	37.596	14.839	31.222	20.019
8	0.21	136.798		27.352	14.796	45.574		14.052	26.539	27.964	24.68	16.61	20.311
9	0.226	126.063	84.85	78.863	106.353	37.012	113.645	59.358	17.816	1.65	7.559	4.766	9.752
10	0.261	115.706	60.401	55.58	38.103	89.853	62.55	63.597	5.658	3.522	7.533	13.692	
11	0.313	107.947				53.254		34.542	9.953	7.406	10.828	8.884	
12	0.336	104.319				4.415		2.382	7.55	13.702	11.237	4.286	
13	0.375	98.888		5.242		9.48		5.56	10.683	12.667	17.718	31.893	20.977
14	0.396	93.856				8.608		7.017			18.399		
15	0.413	89.265	12.452	10.933	11.374	15.293	20.764	16.938	12.57	17.494	6.719	46.9	35.981
16	0.43	84.835	45.177	55.576	59.873	40.631	61.957	49.666	34.354	35.831	17.204	61.692	59.566
17	0.52	65.038	42.679	32.284	27.818	54.148	17.656	29.148	26.719	32.698	33.157	46.579	49.397
18	0.579	54.556	34.58			50.862		53.906			25.21		
19	0.591	52.828	13.931	37.367	14.523	18.141	32.669	39.317	20.006	12.175	16.873	23.74	
20	0.625	47.899	11.769	31.582	19.201	26.499	37.673	12.885	41.804	8.995	23.149	12.518	64.615
21	0.68	40.55		96.1		75		61.343					
22	0.688	39.685	43.284	91.769	68.053	87.986	67.508	74.997	70.755	49.305	64.462	59.814	86.423
23	0.721	36.095	58.661	92.986	36.14	80.145	79.232	30.595	53.573	71.073	68.605	83.378	40.83
24	0.748	33.274	42.037	34.023	59.261	93.693	38.959	6.998	5.322	17.892	18.018	12.675	9.781
25	0.772	30.929	6.555	11.387				29.454	10.292			5.983	
26	0.794	29.172	5.801	14.694		101.63	48.726	38.142	12.842	17.926	13.472	8.787	24.384
27	0.862	23.746						74.977	34.509				
28	0.872	23.067	62.386	51.402	82.859	49.922	129.402	57.155	90.15	73.185	93.327	75.003	52.73
29	0.897	21.393	86.555	83.501	108.8	83.297	87.922	73.562	84.647	83.703	85.336	34.56	39.657

O. n = Oreochromis niloticus *O.a = Oreochromis aureus* *S. g = Sarotherodon galellius*

T. z = Tilapia zillii *C. g = Clarias gariepinus* *C. c = Cyprinus carpio*

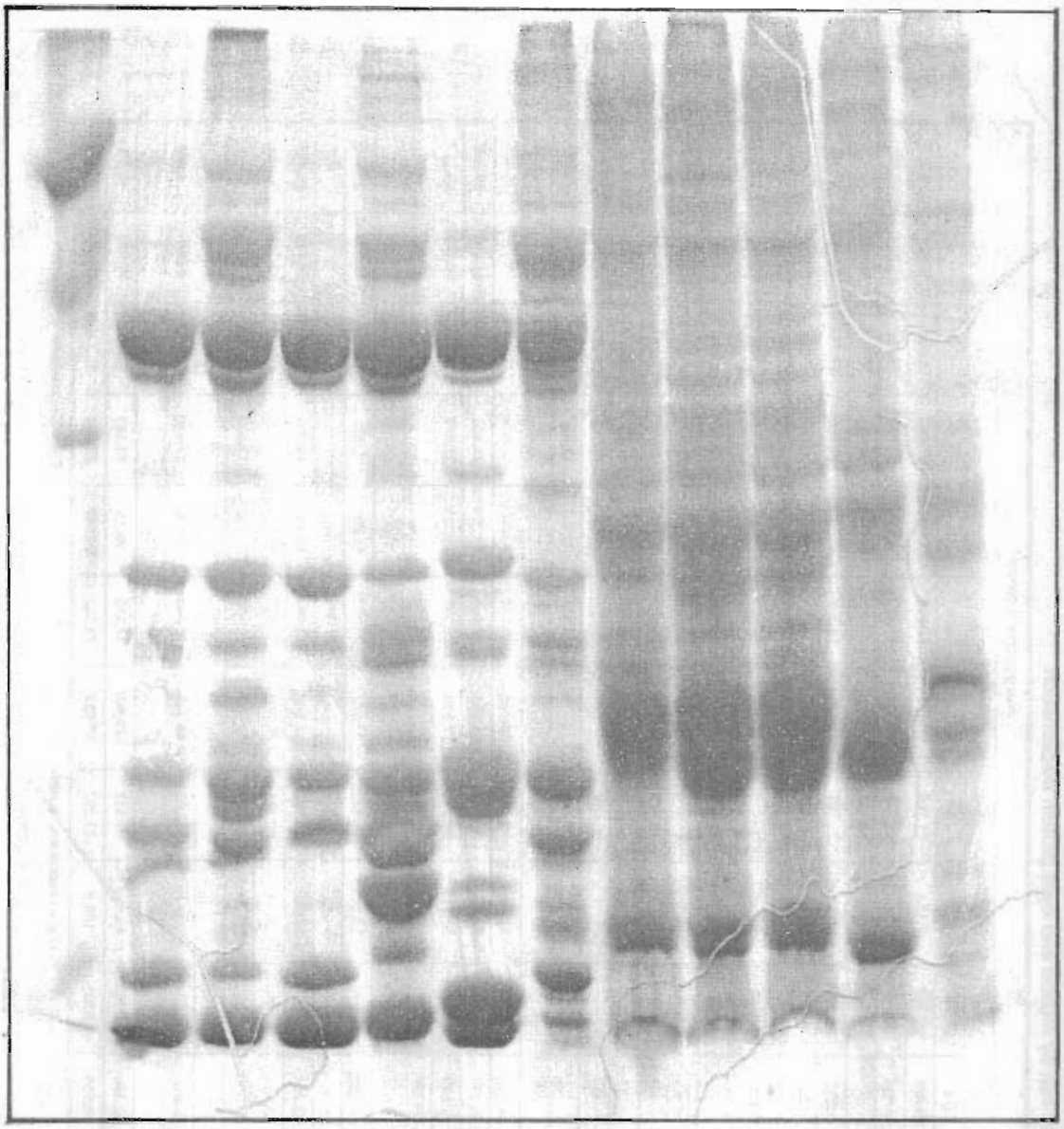

Fig. (1): Variation in electrophoretic patterns for muscle and eye protein of fish.

M – Marker
1 – Muscle of *Oreochromis niloticus*
2 – Muscle of *Oreochromius aureus*
3 - Muscle of *Sarotherodon galellius*
4 - Muscle of *Tilapia zillii*
5 - Muscle of *Claries gariepinus*
6 - Muscle of *Cyprinus carpio*
7 - Eye of *Oreochromis niloticus*
8 - Eye of *Sarotherodon galellius*
9 – Eye of *Tilapia zillii*
10- Eye of *Claries gariepinus*
11- Eye of Cyprinus carpio.

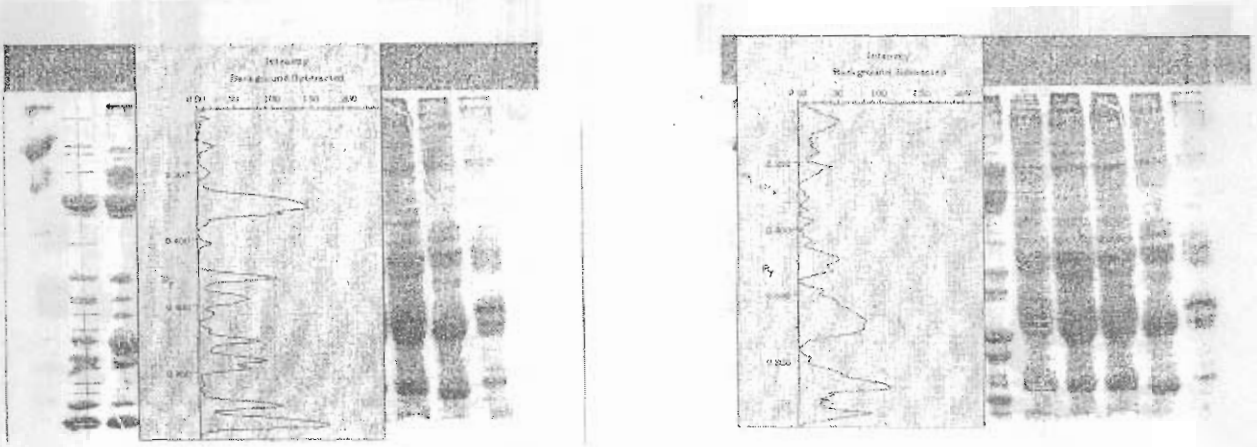

Fig. (2): SDS-PAGE patterns for muscle and eye of *Oreochromis niloticus*

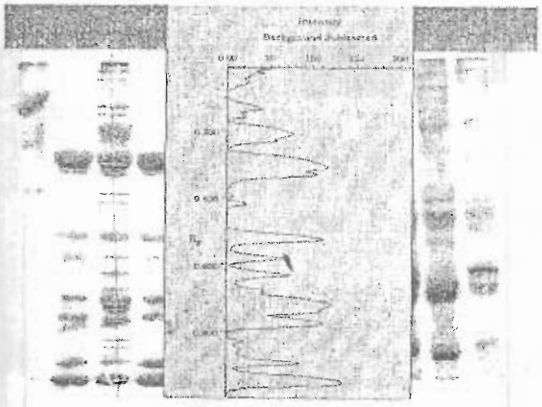

Fig. (3): SDS-PAGE patterns of muscle of *Oreochromius aureus*

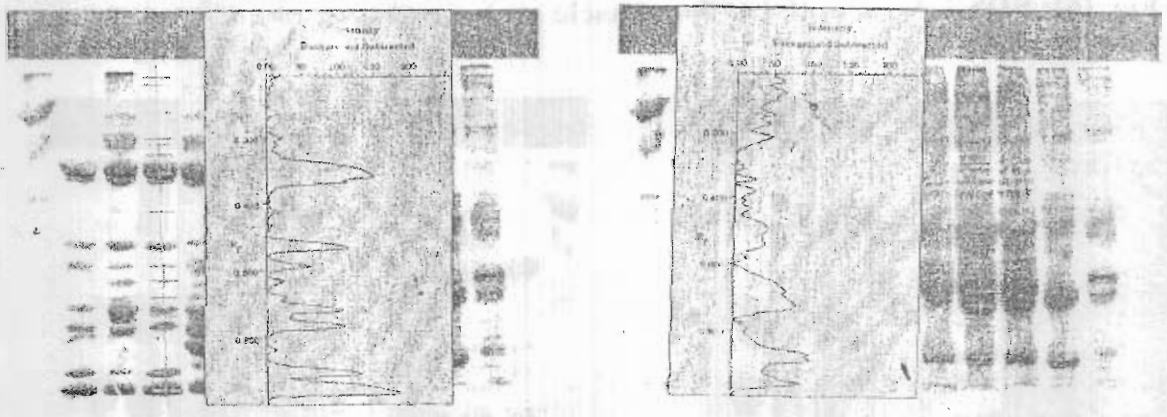

Fig. (4): SDS-PAGE patterns for muscle and eye of *Sarotherodon galellius*

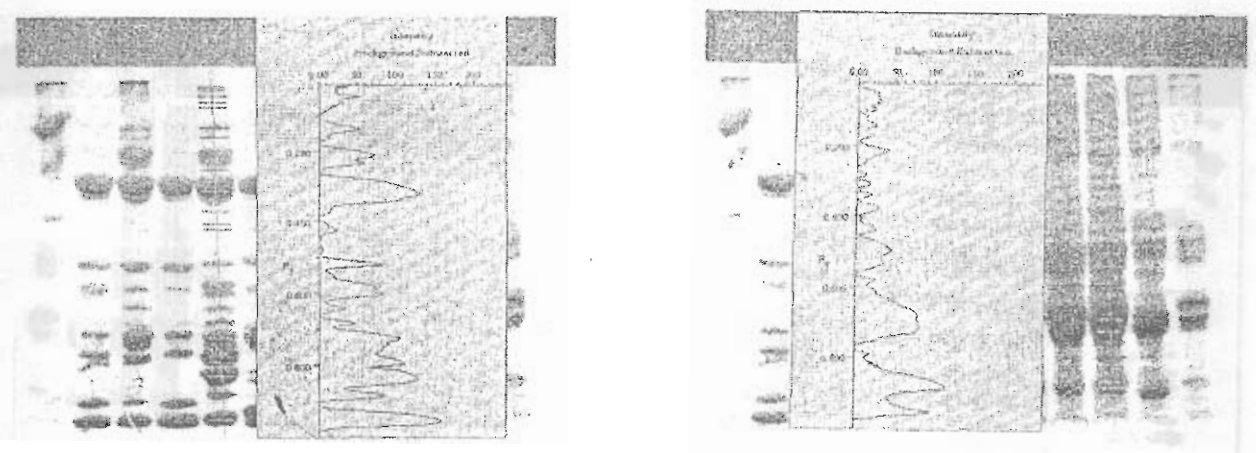

Fig. (5): SDS-PAGE patterns for muscle and eye of *Tilapia zillii*

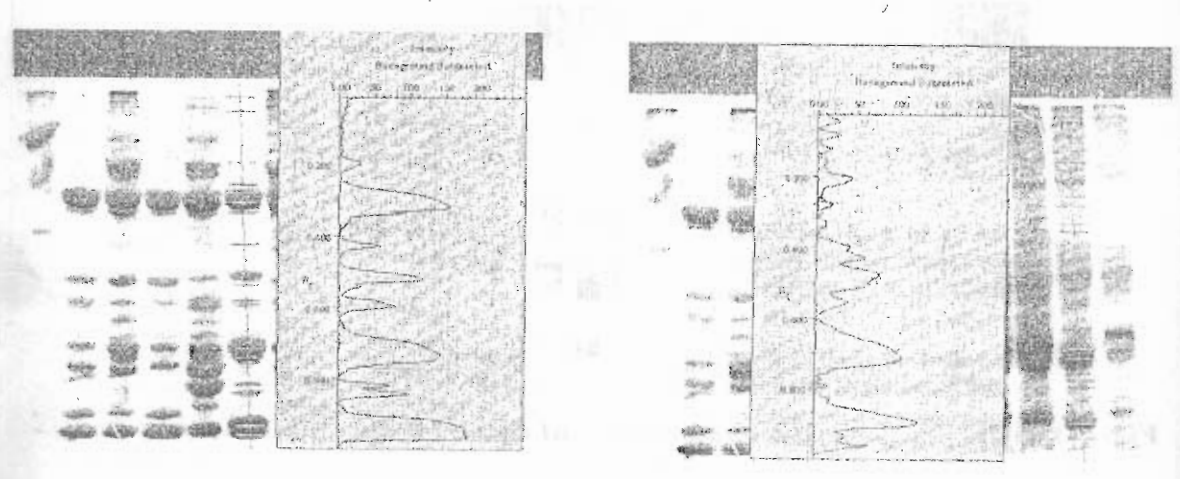

Fig. (6): SDS-PAGE patterns for muscle and eye of *Claries gariepinus*

Fig. (7): SDS-PAGE patterns for muscle and eye of *Cyprinus carpio*

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التفريد الكهربى لبروتين العضلات وأعين بعض أسماك المياه العذبه فى مصر

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المعمل المركزي لبحوث الثروة السمكية بالعباسة- مركزالبحوث الزراعية – وزارة الزراعة – الدقى الجيزة.

أجريت هذه الدراسة بمعمل قسم الوراثة- المعمل المركزى لبحوث الاسماك – العباسة ابوحماد شرقية. تهدف هذه الدراسة الى تحديد درجات التشابه والاختلاف بين البلطى النيلى والاوريا والجاليلى والزيلى والقرموط الافريقى والمبروك العادى باستخدام التفريد الكهربى لبروتينات العضلات والعين للاسماك المجمعه من مزرعة العباسة.

أوضحت الدراسة أن كل نوع قد تميز بطرز محدد من الحزم البروتينيه فمثلا وجود ١٩ ، ٢٦ طرز جينيه فى بروتين العضلات والعين للبلطى النيلى و٢٢ لعضلات البلطى الاوريا و ١٨ ، ٢٤ للبطى الجاليلى و ٢٤ ، ٢٥ للبلطي الزيلى و ١٧ ، ٢٥ للقرموط الافريقى و ١٩ ، ٢٧فى بروتين العضلات والعين للمبروك العادى على التوالى. وكذلك جميع اسماك الدراسة تتفق فى ١٣ طراز جينى رقم ١،٢،٤،٩،١٥،١٦،١٧،٢٠،٢٢،٢٣،٢٤،٢٨،٢٩ ووزن جزيئى ٣٢٧,٢ ، ٣٠٣,٤ ١٢٦,١،٢٣٢,٧ ، ٨٩,٣، ٨٤,٨، ٦٥,٠٤، ٤٧,٩، ٣٩,٧، ٣٦,١، ٣٣,٣، ٢٣,١، ٢١,٤ كيلو دالتون وكذلك وجود اختلاف فى بروتينات العضلات والعين فمثلا تتفق بروتينات العضلات لجميع الاسماك فى ٣ طرز جينيه رقم ٥، ١٠، ١٩ ووزن جزيئى ٢٠٩,٥، ١١٥,٧، ٥٢,٨ كيلو دالتون بينما بروتينات العين لجميع الاسماك تتفق فى ٤ طرز جينيه رقم ٧، ٨، ١٣. ووزن جزيئى ١٧٣,٢، ١٣٦,٨، ٩٨,٩، ٢٩,٢ كيلو دالتون. وكذلك يمكن استخام الطراز الجينى رقم ٢٥ مميز للبلطى النيلى حيث تتفق عينات العضلات والعين فى هذا الطراز عن باقى الاسماك وكذلك الطرز الجينيه رقم ٣، ١١، ١٢، ١٤، ١٨ ووزن جزيئى ٢٦١,٠١، ١٠٧,٩، ١٠٤,٣، ٩٣,٩، ٥٤,٦ كيلو دالتون كطرز جينيه مميزه للبلطى الزيلى.

مما سبق يتضح امكان الاعتماد على البصمة الوراثية للبروتينات خاصة بروتين العضلات كما سبق للتفريق بين الانواع المختلفة بالاضافة الى دراسة التركيب الوراثى للاسماك المختلفة.