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BIOCHEMICAL GENETIC STUDIES OF SERUM PROTEIN OF FAMILY MUGILIDAE IN TWO DIFFERENT HABITATS OF EGYPTIAN WATER.

BY

M.E.M. ZOWAIL*, S. I. El-DEEB; S.S. EL-SERAFY; E.H. RIZKALLA AND H. EL SAIED,

*** Faculty of Science, Zagazig University (Banha Branch).**

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ABSTRACT

Three dominant species of Mugilidae (Mugil cephalus, Liza ramada and Liza aurata) were studied electrophoritically in two different habitats (Marine and Freshwater). The similarity coefficients were studied for the different species in the marine and freshwater, and for the different sexes in each species.

These studies revealed that the pattern of serum proteins for most of the studied species have been eleven fractions. The variation was obvious between protein patterns of juveniles and adults (males and females) of both Mugil cephalus and Liza ramada.

The comparison of serum proteinograms between the adult species indicated that there was similarity between males or females of marine M. cephalus and Liza ramada. On the other hand Similarity was Pronounced only between females of L. ramada and those of L. aurata.

There were clear environmental effects on the patterns of serum protein of juveniles, males of Mugil cephalus and females of Liza ramada. But in the case of females of Mugil cephalus and males of Liza ramada, this effect was small.

INTRODUCTION

Determination of the total number of protein loci and their polymorphic ones was studied by many authors in several fish families (Somer and Soule, 1974). Among fish proteins which have higher degree of genetic variations are serum proteins (Cushing, 1956).

Studies on protein variations have indicated substantial genetic differentiation among populations of freshwater species (Ryman & Stahl, 1981). In contrast, pronounced intraspecific genetic differentiation has only been observed for few marine species (Shaklee, 1983).

Migration of mullets from a freshwater habitat to marine one and vice versa induces variation in the protein character of fish (Grant and Spain, 1975), Serum proteins fluctuations in some mullet species of different sexes in marine and freshwater habitats give a picture about the state of proteins in these fishes in relation to their habitats in the present study.

The aim of the present investigation is to study genetic characters of serum proteins and the genetic similarity between the three mullet species (Mugil cephalus, Liza ramada inhabiting the marine and freshwater habitats and L. Liza aurata which residing only the marine habitat).

MATERIAL AND METHODS

Three dominant species of Mugilidae (Mugil cephalus, M cm), Liza ramada (L rm) inhabiting the marine and freshwater habitats (mcf, Lrf) and Liza aurata which residing only the marine habitat (Lam) were randomly collected in seasons of their existence from two natural different habitats. The first is the east coast of the Mediterranean sea near New Damietta Port which is considerable as the marine habitat (water salinity is not less 18.09%. and not exceeds 33.15%. experiment) during the time of experiment. The second is the freshwater area of Manzala lake near Sirw drain and has water salinity less than 1%. during the time of experiment.

The number of samples are shown in Table (1). The average length ranged from 24-31 cm and 10-14 for the adults and juveniles respectively. The average weight ranged from 74.1-366 gm and 5.6-21 gm for the adults and juveniles respectively.

Blood samples from the three species were collected from severable caudal peduncle of the specimen according to the methods of Dwivedi and Menezes (1975).

Polyacreylamide gel 7.5% was used for electrophoritic study according to that of Herzeberg and Pasteur (1974) with certain modifications.

Both the relative mobility and intensity (relative area percentage) of each protein fraction was measured by a photoelectric densitometer at wave length 525 nm).

Table (1): Frequency of appearence of individual serum proteins fractions in the studied groups of Mugilidae species.

Group	T. No		Fraction number										
			1	2	3	4	5	6	7	8	9	10	11
Mcm ♂	20	No	18	14	16	20	18	18	14	20	12	20	18
		%	90.0	70.0	80.0	100.0	90.0	90.0	70.0	100.0	60.0	100.0	90.0
Mcm ♀	24	No	20	21	24	24	24	22	18	23	17	24	21
		%	83.3	87.5	100.0	100.0	100.0	91.7	75.0	95.8	70.8	100.0	87.5
Mcf ♂	18	No	18	12	18	18	12	12	12	12	12	12	18
		%	100.0	66.7	100.0	100.0	66.7	66.7	66.7	66.7	66.7	66.7	100.0
Mcf ♀	20	No	20	20	15	20	20	20	15	20	20	20	20
		%	100.0	100.0	75.0	100.0	100.0	100.0	75.0	100.0	100.0	100.0	100.0
Lrm ♂	22	No	16	10	20	22	20	20	20	20	16	22	18
		%	72.7	45.5	90.9	100.0	90.9	90.9	90.9	90.9	72.7	100.0	81.8
Lrm ♀	24	No	20	14	24	24	24	22	20	20	20	22	22
		%	83.3	58.3	100.0	100.0	100.0	91.7	83.3	83.3	83.3	91.7	91.7
Lrf ♂	20	No	16	18	20	20	20	18	14	18	10	20	20
		%	80.0	90.0	100.0	100.0	100.0	90.0	70.0	90.0	50.0	100.0	100.0
Lrf ♀	20	No	14	11	20	20	19	18	12	19	12	17	19
		%	70.0	55.0	100.0	100.0	95.0	90.0	60.0	95.0	60.0	85.0	95.0
Lam ♂	24	No	24	0	24	24	24	0	12	18	18	12	18
		%	100.0	0	100.0	100.0	100.0	0	50.0	75.0	75.0	50.0	75.0
Lam ♀	18	No	0	12	18	18	18	18	18	18	18	18	18
		%	0	66.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Mcm J	12	No	0	12	0	12	12	12	0	12	0	12	12
		%	0	100.0	0	100.0	100.0	100.0	0	100.0	0	100.0	100.0
Mcf J	18	No	15	9	18	18	12	9	12	15	0	12	15
		%	83.3	50.0	100.0	100.0	66.7	50.0	66.7	83.3	0	66.7	83.3
Lrm J	12	No	12	12	12	12	12	12	0	12	0	12	12
		%	100.0	100.0	100.0	100.0	100.0	100.0	0	100.0	0	100.0	100.0

T.No. : Total number of samples collected
No. : Number of samples showing each fraction
% : Percentage frequency of appearence

A student's t test was used for the statistical analysis of results. The coefficient of similarity between pairs of electrophoritic patterns was calculated according to Ferguson (1980).

RESULTS

The electrophoritic blood serum protein patterns of the studied groups are illustrated in Figures (1,2,3, and 4). Most of the electrophoretical patterns clearly showed the presence of eleven major fractions except for females of Liza aurata and juveniles of freshwater Mugil cephalus (10), males of Liza aurata and juveniles of marine Liza ramada (9), while the marine Mugil cephalus juveniles have only 7 fractions.

The frequency of appearance of individual serum proteins fractions of the studied groups of Mugilidae as shown in Table (1) ranged from absolute (100 %), constant (90 % and more), polymorphic (appearance low than 90%) and complete disappearance.

Each fraction was studied from two points, the first was the relative mobility which shows the relative genetic distance, and the second was the relative area percentage which indicates the quantitative percentage of the fraction.

The means and standard errors of relative mobility and relative area of individual serum proteins fractions for the studied groups were calculated.

Table (2) illustrates a comparison of the relative mobility and relative area percentage of individual serum protein fractions between different species. It should be mentioned that both sex and habitat factors were fixed while the differences in species was the only variable.

From Table (2), the relative mobility is clearly different among the serum proteinograms of the three studied species. It was found to be non-uniform within individual species and the distribution of individual serum components (relative area) in all the species analyzed was found to be heterogeneous.

Table (3) shows the comparison of the relative mobility and relative area percentage of individual serum protein fractions between different sexes of the studied fish groups. In this comparison, both species and habitat factors were fixed, while sex was the variable one.

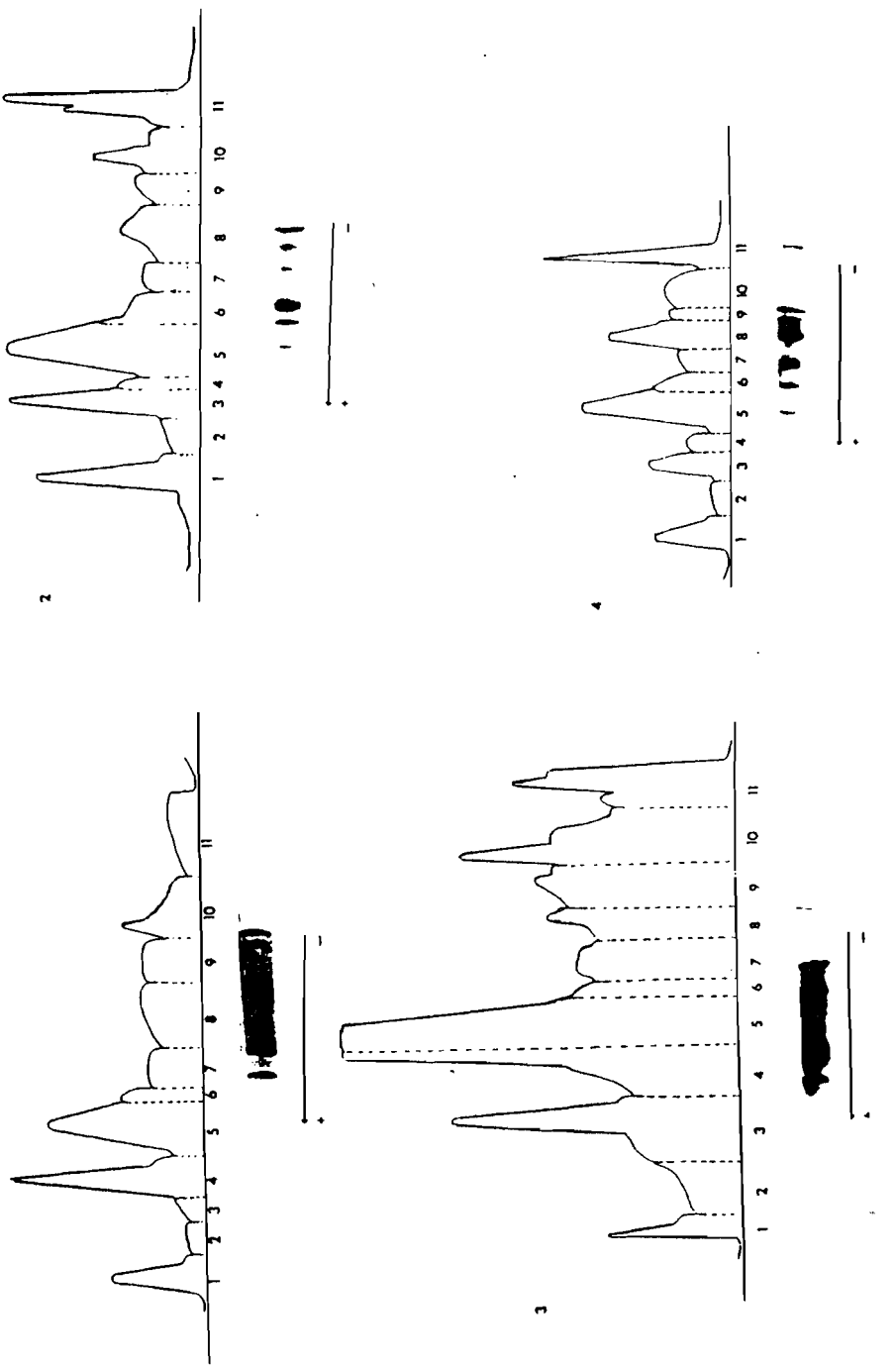

Figure 1: Serum proteins electropherograms for males of Mugil cephal collected from marine (1) freshwater (2) habitats, and for females of Mugil cephalus collected from marine (3) and freshwater (4) habitats.

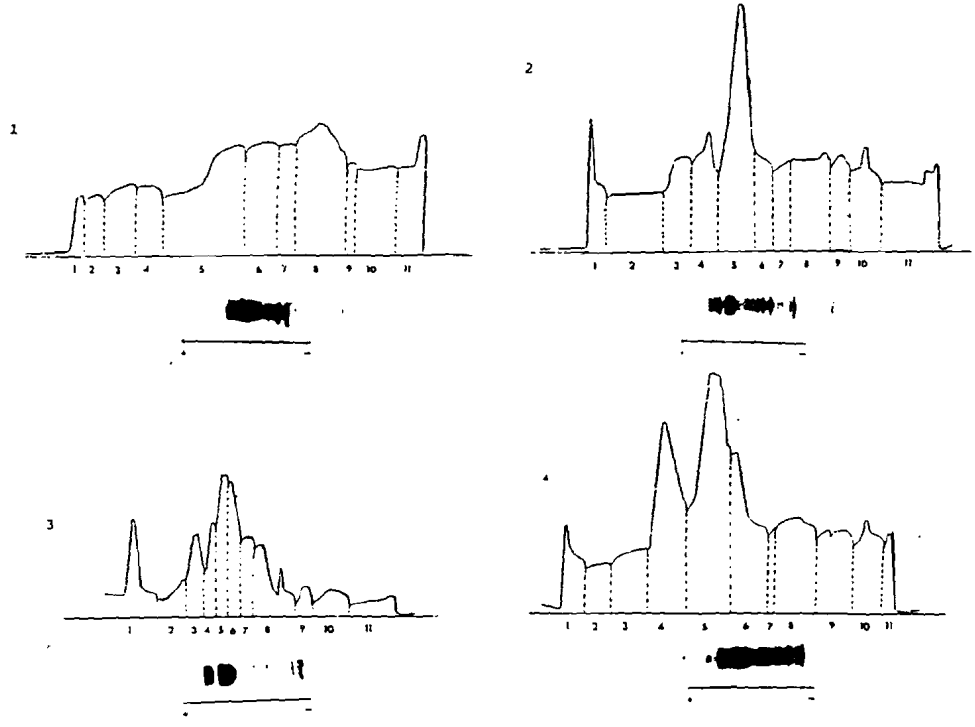

Figure 2: Serum proteins electropherograms for males of Liza ramada collected from marine (1), freshwater (2) habitats and for females collecte from marine (3) and freshwater (4) habitats.

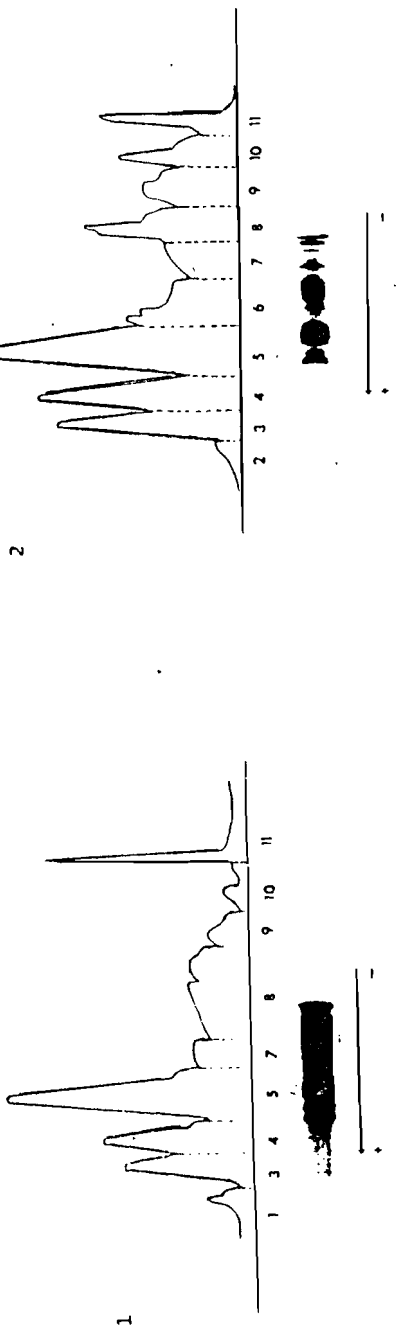

Figure 3: Serum proteins electropherograms for male (1) and female (2) of Liza aurata collected from marine habitat.

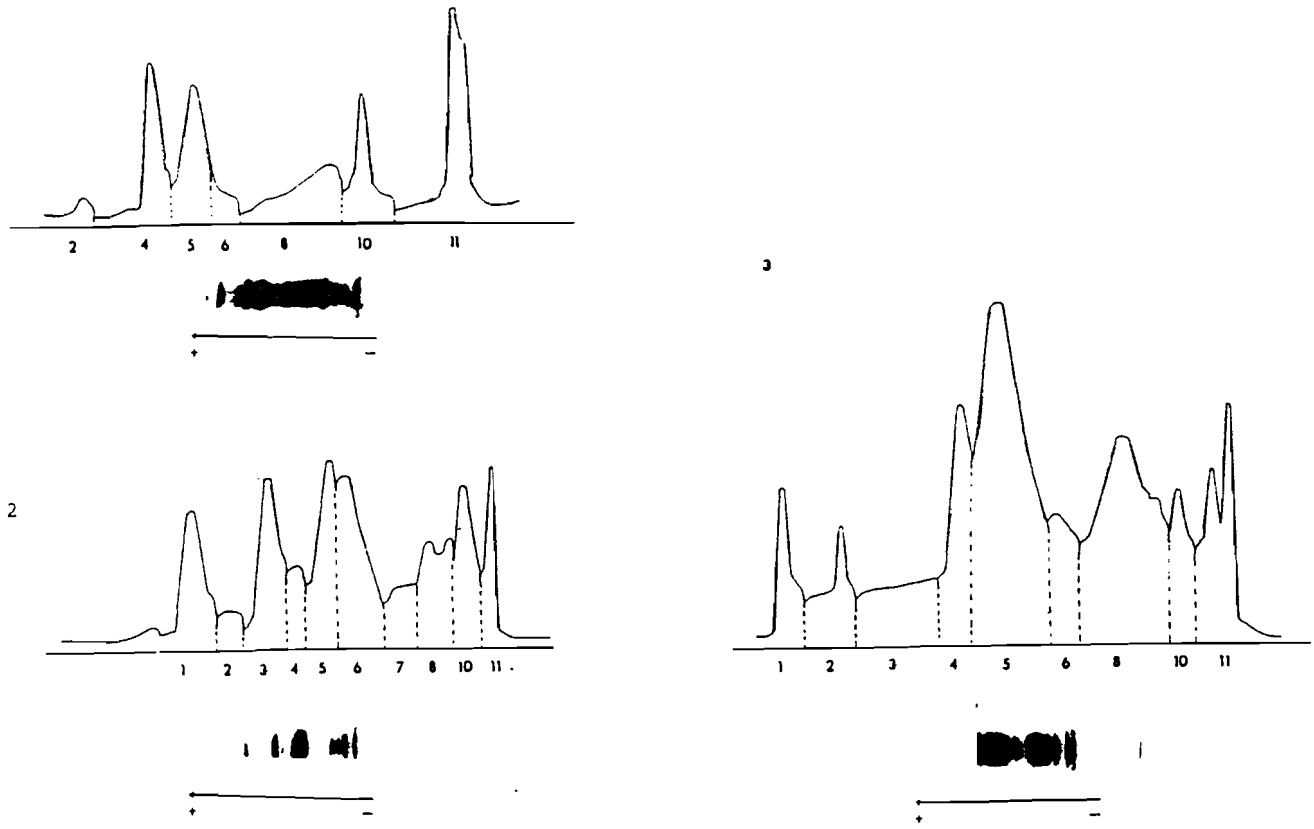

Figure 4: Serum proteins electropherograms for juveniles of Mugil cephalus collected from marine (1) freshwater (2) habitats, and for juvenile of Liza ramada (3) collected from marine habitat.

Table(2):Sexual variability of the relative mobility and relative area percentage of of individual serum proteins fractions of Mugilidae species.

Groups	Relative mobility												Relative area percentage										
	Fraction number											Coefficient of similarity	Fraction number										
	1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11
Mcm ♂ X Mcm ♀	N	N	N	N	N	N	N	N	N	N	N	1.00	1	2	N	1	4	N	N	1	N	N	N
Mcm ♂ X Mcm J	*	N	*	N	1	2	*	2	*	N	1	0.27	4	1	4	1	N	N	4	4	2	4	4
Mcm ♀ X Mcm J	*	N	*	1	2	4	*	4	*	N	N	0.27	4	N	4	N	3	N	2	4	3	4	4
Mcf ♂ X Mcf ♀	N	N	3	1	1	2	4	1	2	1	N	0.27	1	1	2	N	N	4	N	4	N	4	2
Mcf ♂ X Mcf J	N	N	1	4	4	4	4	4	*	4	N	0.27	N	N	N	N	N	N	1	2	4	N	N
Mcf ♀ X Mcf J	N	N	N	N	1	N	N	4	*	1	N	0.64	1	2	4	N	2	1	1	N	4	2	N
Lrm ♂ X Lrm ♀	N	N	N	1	N	N	N	N	N	N	N	0.91	2	1	N	N	N	N	N	N	N	1	N
Lrm ♂ X Lrm J	N	1	N	4	4	4	*	2	*	1	N	0.27	4	4	N	N	N	1	4	1	2	2	N
Lrm ♀ X Lrm J	N	4	N	1	2	4	*	2	*	2	N	0.27	N	4	N	N	N	1	4	2	4	N	N
Lrf ♂ X Lrf ♀	N	N	N	N	N	2	1	N	1	N	N	0.73	N	4	N	N	1	N	1	N	N	N	N
Lam ♂ X Lam ♀	*	*	1	N	1	*	2	N	N	N	4	0.36	4	4	3	1	4	4	4	2	3	4	2

1: P.< 0.05
2: P.< 0.01
3: P.< 0.001
4: P.< 0.0005

N: Non significant
*: No comparison

Table(3):Comparison of the relative mobility and relative area percentage of individual serum proteins fractions between different Mugilidae species.

Groups	Relative mobility												Relative area percentage										
	Fraction number											Coeffici-ent of similarity	Fraction number										
	1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11
Mcm ♂ X Lrm ♂	N	N	N	1	2	2	N	N	N	N	N	0.73	4	N	N	2	2	1	N	N	N	N	N
Mcm ♂ X Lam ♂	N	*	N	N	1	*	N	2	N	4	4	0.45	4	4	N	2	4	4	N	N	N	4	N
Lrm ♂ X Lam ♂	N	*	N	1	N	*	N	1	N	2	4	0.45	N	4	N	N	4	4	1	N	N	4	N
Mcm ♀ X Lrm ♀	N	N	N	N	1	2	N	1	N	N	N	0.73	N	N	N	3	N	N	N	N	N	1	N
Mcm ♀ X Lam ♀	*	N	N	2	4	4	1	4	2	2	4	0.18	4	N	N	3	N	N	2	4	2	N	4
Lrm ♀ X Lam ♀	*	1	N	N	N	N	N	N	N	N	1	0.73	4	N	N	N	N	1	N	4	1	N	2
Mcf ♂ X Lrf ♂	N	4	N	1	1	N	N	N	1	2	1	0.45	4	4	2	1	N	2	1	4	N	N	2
Mcf ♀ X Lrf ♀	N	N	N	N	N	N	N	1	1	N	N	0.82	2	N	2	1	1	N	N	N	1	4	N
Mcm J X Lrm J	*	N	*	2	2	4	*	4	*	4	N	0.22	4	4	4	N	2	N	N	N	N	4	4

1: P.< 0.05
2: P.< 0.01
3: P.< 0.001
4: P.< 0.0005

N: Non significant
*: No comparison

The present results show that the content of individual protein fractions varied among different sexes and is subjected to considerable modification toward maturation. The most appreciable results occur in fraction 2 in which females have significantly higher protein percentage than males in all of studied groups except freshwater Liza ramada females and males.

Table (4) shows the comparison between the serum proteinograms of studied species in different habitats. It is obvious that the marine males of Mugil cephalus (Mcm-male) and freshwater ones (Mcf-female) were differed significantly either in the relative mobility or relative area percentage, but the opposite is appeared in the female of the same species. In case of Liza ramada the marine males (Lrm-male) and freshwater ones (Lrf-male) have higher SC=(0.82), while in the females of the same species the difference indicated by wide range in the term of relative mobility.

No similarity was observed in the case of marine and freshwater juviniles of Mugil cephalus (Mcmj & Mcfj).

DISCUSSION

It is clear from Table (1) that fraction (7) showed clear polymorphism except in the females of marine Liza aurata. Fractions 2 & 9 were similar to fraction 7 in its polymorphism except for females of both freshwater Mugil cephalus and marine Liza aurata. In freshwater juveniles all fractions were polymorphic except fraction 3 and 4 that absolutely appeared. Since proteins are only one or two chemical steps removed from the gene, analysis of frequencies of individual protein of polymorphic series reflect gene representation within and between populations (Ingram, 1960 and Dobzhansky, 1962). Polymorphism within a population of Mugil cephalus was found by Hongskul, 1968). He detected ten protein components by electrophoritic analysis, but only one band identified by him as component III displayed genetic variability.

The absolute disappearance of some fractions were observed only in both sexes of Liza aurata (fractions 2 and 6 in males and fraction 1 in females). Thus the disappearance of these fractions can be used to differentiate males from females of the species. Fraction 9 completely disappeared in the three studied juvenile groups. This could be also used as a tool to differentiate juveniles from adults (in which this fraction appeared) of both Mugil cephalus and Liza ramada.

Table(4): Comparison of the relative mobility and relative area percentage of individual serum proteins fractions for Mugilidae species in different habitats.

Groups	Relative mobility												Relative area percentage										
	Fraction number											Coefficient of similarity	Fraction number										
	1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11
Mcm ♂ X Mcf ♂	N	N	2	N	2	2	1	1	N	2	1	0.36	1	1	1	3	N	1	1	4	N	1	3
Mcm ♀ X Mcf ♀	N	1	1	N	N	N	N	N	N	N	N	0.82	1	N	2	3	N	N	N	2	1	N	N
Lrm ♂ X Lrf ♂	N	N	N	2	1	N	N	N	N	N	N	0.82	N	4	N	N	N	N	N	1	N	N	N
Lrm ♀ X Lrf ♀	N	N	N	N	1	2	1	1	1	1	N	0.45	N	N	N	1	N	N	2	N	N	N	N
Mcm J X Mcf J	*	1	*	2	2	2	*	4	*	4	2	0.00	4	N	4	1	N	N	4	2	N	4	1

1: P.< 0.05
2: P.< 0.01
3: P.< 0.001
4: P.< 0.0005

N: Non significant
*: No comparison

It is clear from figures 1,2,3 and 4, that fraction (5) contains the most protein content in area of both sexes of the two Liza species and in females of Mugil cephalus at different studied habitats. Also fractions 3 and 4 in males of Mugil cephalus sera at fresh and marine water respectively. In the juvenile stage, fractions 3,10 and 5 in freshwater, marine Mugil cephalus and Liza ramada, respectively was formed to have the highest percentage of sera protein. Low sera protein were detected mainly in fraction 2 except freshwater males of Liza ramada (Fraction 9), marine juveniles Liza ramada (fraction 1) and marine males Liza aurata (fractions 7 & 9).

A genetic analysis of alloforms with identical mobility in the electric field, but differing in the intensity of bands staining makes it possible to detect a variation in the regulatory genes stimulating or weaking the action of one structural locus or another (Kirpchnikov, 1981).

The variation within species, sexes and habitats can be correlated by carrying out multiple comparisons and determining the variance of the similarity coefficients (Ferguson, 1980). The difference in values of similarity imply greater structural differences. The species with the greatest similarity coefficient are the most related to each other and vise versa.

The similarity coefficient between species reveals that both Mugil cephalus and Liza ramada are closely related to each other as indicated in both sexes (males or females) of marine fishes (SC=0.73). In the freshwater habitat the females of the same species are highly related to each other (SC=0.82), while the males are relatively related (SC= 0.45). There is a similarity between females of marine Liza ramada and those of Liza aurata (SC=0.73). In contrast, low similarity coefficient between females of marine Mugil cephalus and those of Liza aurata was detected (SC=0.18) this is supported by the findings of El-Serafy et al. (1993) in cytogenetic studies. The low similarity value (0.48) between Mugil cephalus and Liza aurata detected by Tsvetnenko (1991) supports the present findings (SC=0.45) between males of the same species and the opinion of Svetovidov (1964), (c.f Tsvetnenko, 1991) on the possibility of separate generic (or subgeneric) status for the Mugil cephalus.

The closest similarity coefficient was observed between Mugil cephalus and Liza ramada in the mature stage was decreased in the juvenile stage (SC=0.22) and this is in agreement with the results obtained by El-Serafy et al. (1993). This change in similarity coefficient between the two stages could be due to maturation. There was a weak coefficient of similarities between mature males or females and juvenile stages in marine case of both species (SC=0.27). In freshwater Mugil cephalus there were weak and moderate similarity coefficients between juvenile stage and both mature males (SC=0.27) and females (SC=0.64). Since perceptible changes take place in the protein picture of the blood in connection with maturation. Vansten & Chumg- Wai,

1961., It was important to investigate the intra specific variability of mullet serum proteins at different stages. In the present study, fractions numbers 7 and 9 were affected with maturation (completely disappeared in the juveniles stage). Senkevich and Kulikova (1970) showed with the serum of striped mullet and golden grey mullet, that some changes in the degree of separation into fractions and the percentage ratio of individual protein component were detected during the period of maturation.

The effect of sex on the proteinogram of Mugilidae species demonstrated that there are very highly close similarity coefficient between both sexes in marine Mugil cephalus (SC=1.00) and Liza ramada (SC=0.91).In contrast marine Liza aurata showed low similarity coefficient (SC=0.36) between males and females. In the freshwater habitat low and high similarity coefficients were detected between both sexes in Mugil cephalus (SC= 0.27) and Liza ramada (SC =0.73), respectively.

Comparing between the serum proteinograms of the studied species in different habitats (Table 4). It is obvious that the habitat affects strongly the mobility of fractions (especially fraction 5), but on the other hand, the intensities (relative area) of fraction 4 and 8 were clearly affected by the habitat. On the other hand, it is obvious that in Liza ramada, while high similarity coefficient in sera proteins of males (SC= 0.82) existed between the different studied habitats this was decreased in case of females (SC= 0.45). The same decrease was observed in case of Mugil cephalus but in a reverse manner, where females have a higher similarity coefficient (SC=0.82) than males (SC=0.36). In juveniles Mugil cephalus no similarity coefficient was observed (SC=0.0).

Finally from this study, it can be concluded that:-

1. Mugil cephalus and Liza ramada are closely related to each other and Liza ramada related to Liza aurata. Therefore, it is probable to make hybridization between Mugil cephalus and Liza ramada.

2. Serum protein can be used to differentiate juveniles from adults in Mugil cephalus and Liza ramda, and to differentiate between males and females of Liza aurata.

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