

## **GENETIC DIFFERENTIATION OF SARCOPLASMIC PROTEIN IN FAMILY MUGILLIDAE AT DIFFERENT HABITATS.**

**BY**

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### **ABSTRACT**

*Electrophoretic analysis of Sarcoplasmic protein were performed for the species of Family mugilidae (**Mugil cephalus**, **Liza ramda** and **Liza aurata**) in marine and fresh water habitats. Genetic polymorphism and coefficients of similarity were investigated in marine and freshwater for the studied groups.*

*This study revealed that sarcoplasmic proteins of the studied groups are markedly conservative, this makes it possible to use them to study the systematic relation between the species. Also this study revealed a close coefficient of similarity between **Mugil cephalus** and **Liza ramada**. Sarcoplasmic protein can be used to differentiate between the juveniles of marine **Mugil cephalus** and marine **Liza ramada**.*

### **INTRODUCTION**

The work in the field of gene protein level is able to differentiate between the very sibling species (Smithies, 1955; Poulik 1957 and Ashton & Braden, 1961). In the term of sarcoplasmic proteins, there are relatively few biochemical genetic studies and knowledge in this regard is still fragmentary (Whitmore, 1986 and Basaglia & Marchetti, 1961).

The Sarcoplasmic proteins polymorphism is controlled by two allelic co-dominant systems in the most of the studied fishes as described by Tsuyuki, *et al.*, 1965 and Tsuyuki & Roberts, 1969. In Mugilidae the sarcoplasmic proteins polymorphism were studied by Carbene, *et al.*, (1983).

The present investigation were carried out on the sarcoplasmic proteins of three mullet species (*Mugil cephalis*, *Liza ramada* inhabiting the marine and fresh water habitats and *Liza aurata* which residing only the marine habitat.

The aim of this investigation is:

1. To study the genetic relationship between the three mullet species.
2. To study genetic structure for the population of each species in each habitat.

### **MATERIALS AND METHODS**

Three dominant species of Mugilidae (*Mugal cephalus*, (Mcm), *Liza ramada* (Lrm) inhabiting the marine and fresh water habitats (mcf, Lrf) and *Liza auratra* which residing only the marine habitat (Lam) were randomly collected 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 33.1‰ 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 & 10-14 for the adult & Juvenile respectively. The average weight ranged from 74.1-366 gm and 5.6-21 gm for the adults and Juveniles respectively.

Sarcoplasmic protein prepared from mixture of fresh red and white muscles from the left side below first dorsal fin of the specimen.

Polyacrylamide gel was used for electrophoretic study according to that Herzeberg and Pasteure (1974).

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

t-test was used for the statistical analysis of results. The coefficient of similarity between pairs of electrophoretic patterns is calculated according to Ferguson (1980).

## RESULTS

The electrophoretic patterns of sarcoplasmic protein of the studied fish showed that all groups have 10 fractions except males of fresh water *Mugil cephalus*, juveniles of marine *Mugil cephalus* and females of *Liza aurata* which they showed only 9 fractions. The first nine ones are major dependent fractions, but the last fraction (number 10 consists of two minor fractions (10 A & 10 B). Fig. (1,2,3,4).

Table (1) illustrates the frequency of individual sarcoplasmic proteins fractions in the studied groups, it ranged from absolute (100%), constant (90% or more), polymorphic appearance (low than 90%) and complete disappearance.

The means and standard errors for each of relative mobility and relative area were studied. Table (2) showed the comparison of the relative mobility and relative area percentage of individual. Sarcoplasmic proteins fractions between different studied species with different similarity coefficient. It worthnoticing that both sex and habitat factors were fixed while the differences in species was only variable. It is clear that the higher protein content was restricted in fraction number 4 for all the studied groups except males of *Liza aurata* fraction (5).

In marine habitat, the similarity was high between males and females either in *Mugil cephalus* or *Liza ramada*. The similarity was rather low in case of males or females of *Mugil cephalus* and *Liza aurata*. But in case of *Liza ramada* and *Liza aurata*, the similarity was obvious between females than males.

In freshwater habitat the variation was higher between *Mugil cephalus* and *Liza ramda* (either in males or females). Also the variation was observed between marine juveniles of *Mugil cephalus* and those of *Liza ramada*.

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

Group	T. No.		Fraction number										
			1	2	3	4	5	6	7	8	9	10A	10B
Mcm (M)	20	No.	16	20	20	20	20	16	16	18	18	20	9
		%	80	100	100	100	100	80	80	90	90	100	45
Mcm (F)	24	No.	20	24	23	24	21	18	24	24	16	24	10
		%	83.3	100.0	95.8	100.0	87.5	75.0	100.0	100.0	66.7	100.0	41.7
Mcf (M)	18	No.	12	18	18	18	18	18	18	18	18	0	18
		%	66.7	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	100.0
Mcf (F)	20	No.	20	20	20	20	20	15	20	20	20	10	0
		%	100.0	100.0	100.0	100.0	100.0	75.0	100.0	100.0	100.0	50.0	0.0
Lrm (M)	22	No.	20	22	20	22	22	22	22	22	18	18	9
		%	90.9	100.0	90.9	100.0	100.0	100.0	100.0	100.0	100.0	81.8	81.8
Lrm (F)	24	No.	12	24	24	24	24	24	24	24	22	22	10
		%	50.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	91.7	91.7	41.7
Lrf (M)	20	No.	12	20	20	20	16	18	20	20	14	18	9
		%	60.0	100.0	100.0	100.0	80.0	90.0	100.0	100.0	70.0	90.0	45.0
Lrf (F)	20	No.	13	20	19	20	19	16	20	19	16	17	8
		%	65.0	100.0	95.0	100.0	95.0	80.0	100.0	95.0	80.0	85.0	40.0
Lam (M)	24	No.	12	24	24	24	24	18	24	24	24	18	0
		%	50.0	100.0	100.0	100.0	100.0	75.0	100.0	100.0	100.0	75.0	0.0
Lam (F)	18	No.	12	18	18	18	12	12	18	18	0	18	0
		%	66.7	100.0	100.0	100.0	66.7	66.7	100.0	100.0	0.0	100.0	0.0
Mcm (J)	12	No.	12	12	12	12	12	12	12	12	0	9	9
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	0.0	75.0	75.0
Mcf (J)	18	No.	12	18	18	18	18	12	18	15	12	15	12
		%	66.7	100.0	100.0	100.0	100.0	66.7	100.0	83.3	66.7	83.3	66.7
Lrm (J)	12	No.	12	12	12	12	12	12	12	12	8	12	8
		%	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	66.7	100.0	66.7

T.No.: Total number of samples collected  
 No.: Number of samples showing each fraction  
 %: Percentage frequency of appearance.  
 M: Male  
 F: Female

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Table (2): Comparison of the relative mobility and relative area percentage of individual sarcoplasmic proteins fractions between different Mugilidae species.

Group	Relative mobility										coefficient of similarity	Relative area											
	Fraction number											Fraction number											
Mcm M x Lrm M	1	2	3	4	5	6	7	8	9	10A	10B	0.82	1	2	3	4	5	6	7	8	9	10A	10B
Mcm M x Lam M	N	N	N	N	N	N	N	N	4	3	N	0.64	N	1	2	N	N	2	1	1	2	N	N
Lrm M x Lam M	N	1	N	N	N	1	1	N	N	2	*	0.55	N	N	N	2	N	4	N	1	1	1	2
Mcm F x Lrm F	N	N	N	N	N	N	N	N	N	N	N	1.00	N	N	1	N	N	2	N	N	N	N	N
Mcm F x Lam F	N	N	N	N	1	N	N	N	*	1	*	0.64	1	1	1	N	4	N	4	3	4	N	2
Lrm F x Lam F	N	N	1	N	N	N	N	1	*	N	*	0.64	N	N	N	N	4	N	1	4	4	N	2
Mcf M x Lrf M	N	2	4	N	4	4	4	1	*	N	N	0.36	N	N	N	N	2	2	2	2	4	N	4
Mcf F x Lrf F	N	2	N	N	N	N	N	N	N	2	*	0.36	N	1	N	N	2	N	1	1	N	N	2
Mcm J x Lrm J	N	N	2	1	1	1	1	4	*	N	N	0.36	N	1	N	N	1	N	1	1	2	1	N

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

N: Non significant  
 \*: No comparison

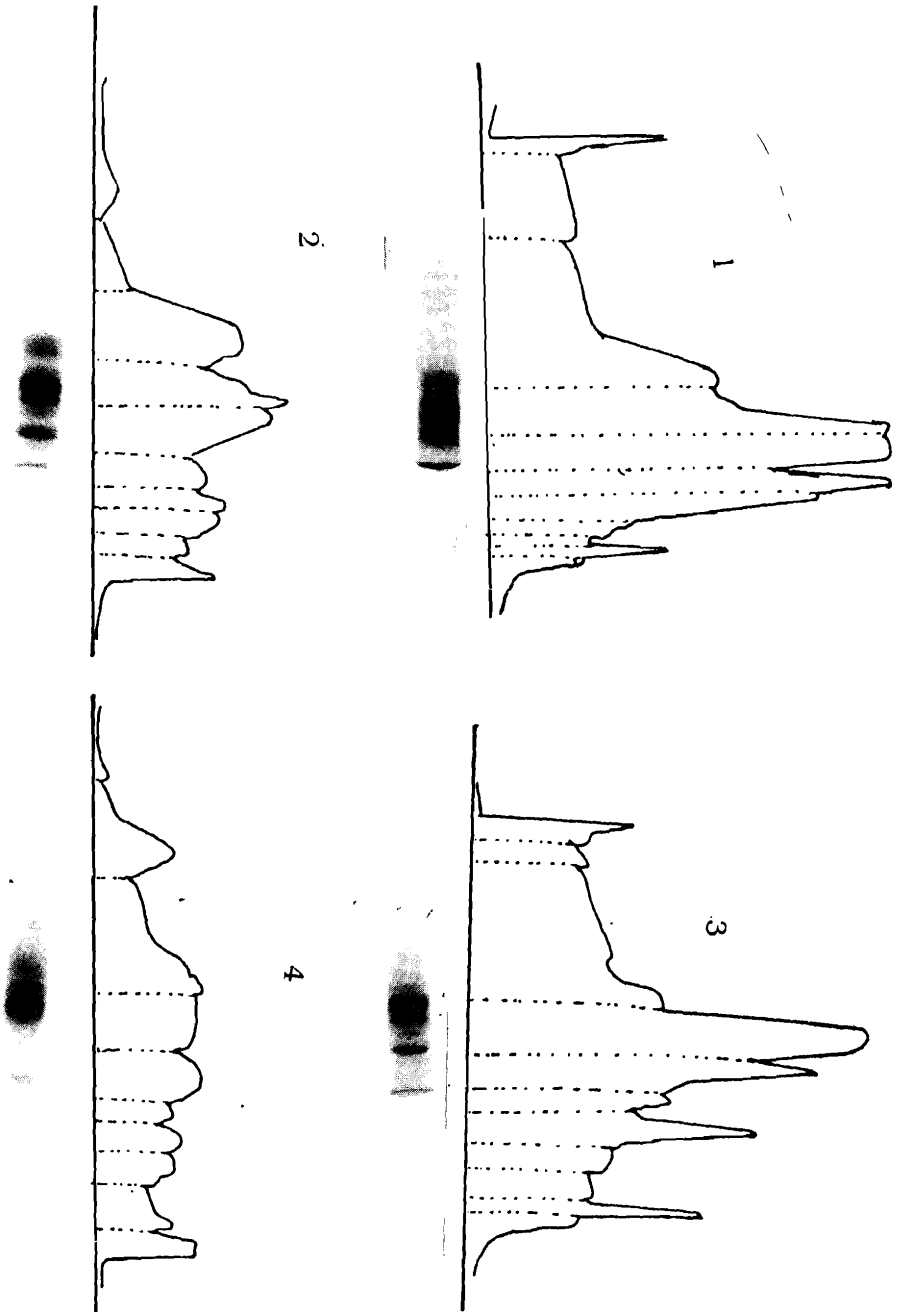


Fig. (1): Sarcoplasmic proteins electropherograms for males of *Mugil cephalus* collected from marine (1) freshwater (2) habitats, and for females of *Mugil cephalus* collected from marine (3) and freshwater (4) habitats.

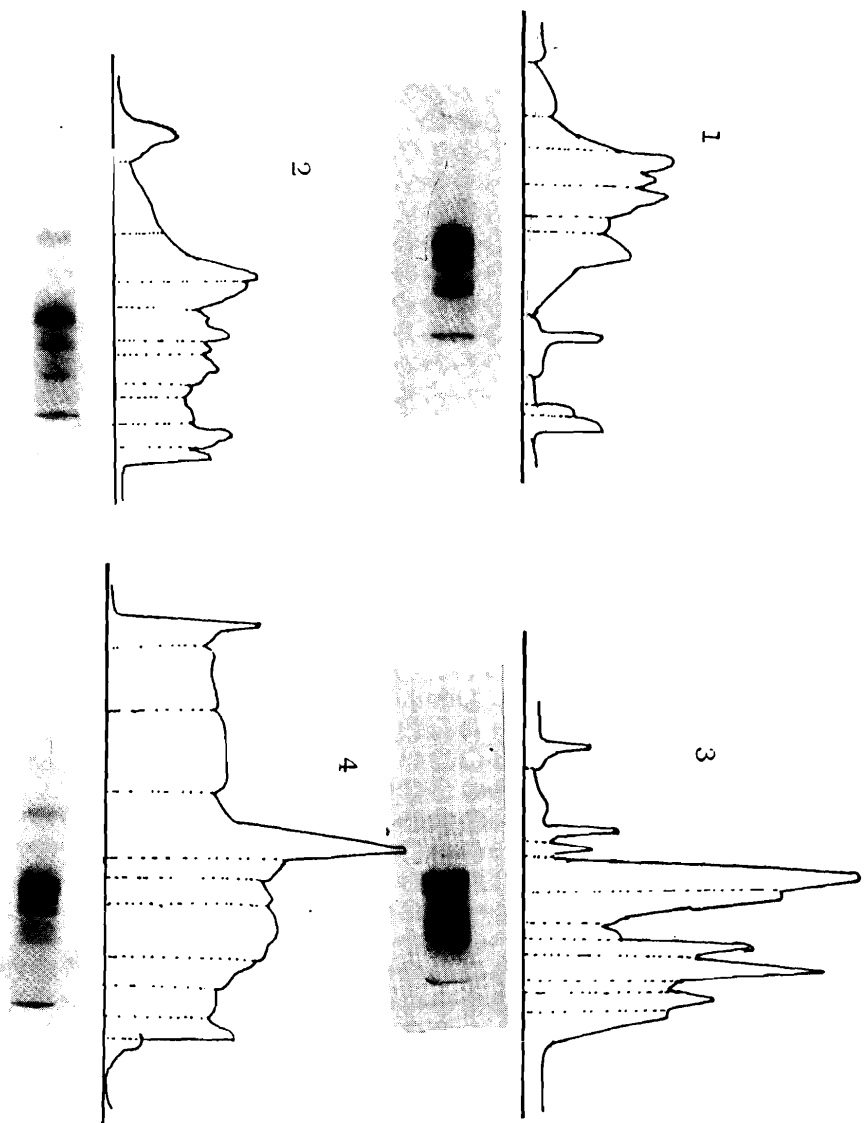


Fig. (2). Sarcoplasmic proteins electropherograms for males of *Liza ramada* collected from marine (1), freshwater (2) habitats and for females collected from marine (3) and freshwater (4) habitats.

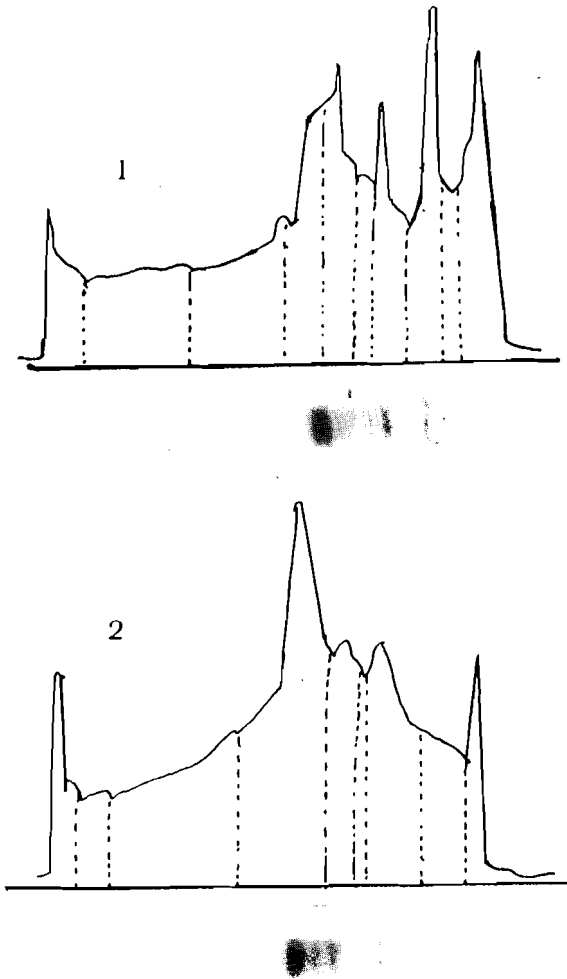


Fig. (3): Sacroplasmic proteins electropherograms for male (1) and female (2) of *Liza aurata* collected from marine habitat.



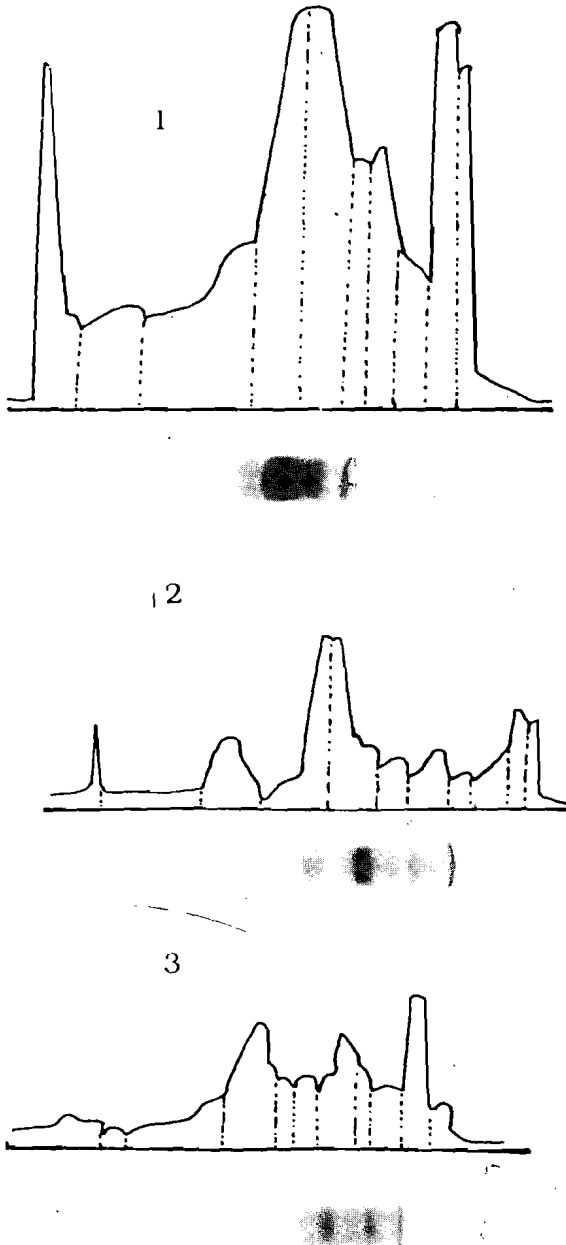


Fig. (4): Sarcoplasmic proteins electropherograms for juveniles of *Mugil cephalus* collected from marine (1), freshwater (2) habitats, and for juvenile of *Liza ramada* (3) collected from marine habitat.

Table (3) illustrates the comparison of the relative mobility and relative area percentage of individuals Sarcoplasmic protein fractions between different sexes of the studied fish groups. Both species and habitat factors were fixed, while sex was variable one. It is obvious that in the case of marine *Mugil cephalus* higher Sc (0.91) was observed in comparison between (male Mcm) and (Female Mcm). On the other hand, the variation was higher between the juveniles groups (Mcm J) and both males and females.

In fresh water males *Mugil cephalus* (mcf) were different from females Sc= 0.45. Also, the juveniles group of the same species (Mce J) were differed from males than females.

The comparison between sarcoplasmic proteins patterns of males and females of freshwater *Liza ramada* (Lrf M, Lrf F) revealed higher similarity in either relative mobility or relative area. But in marine *Liza aurata* (Lam M & Lam F) variation was observed in relative mobility and relative area.

Table (4) shows the comparison between the sarcoplasmic protein of the studied species in different habitats. It is clear that habitat affects clearly on the similarity of sarcoplasmic proteins in case of juveniles, males and females of *Mugil cepahlus*. But in *Liza ramada* no environmental effect was noticed on similarity especially in females S= 1 than males Sc= 0.73.

## **DISCUSSION**

From Table (1) it is clear that the highly polymorphism among the sarcoplasmic protein fractions of the studied groups was represented in fractions 9 and 10 B, and the other fractions showed different degrees of frequency of appearance, ranged from absolute appearance to polymorphic one. Sarcoplasmic proteins of the studied groups are markedly conservative, this makes it possible to use them to study the systematic relationship of organisms. This is in accordance with that observed by Tsventnenko (1991) in which no polymorphism of the muscle. proteins in the mullet species was found.

Kirpichnikov (1981) stated that the electrophoresis of fish muscle proteins are monomorphic and species specific within a species or population.

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

Groups	Relative mobility										coefficient of similarity	Relative area											
	Fraction number											Fraction number											
Mcm M x Mem F	1	2	3	4	5	6	7	8	9	10A	10B	0.91	1	2	3	4	5	6	7	8	9	10A	10B
Mcm M x Mem J	N	N	N	N	N	N	N	N	N	3	N	0.18	1	4	N	N	N	N	1	N	N	1	N
Mcm F x Mem J	N	1	1	4	4	4	4	4	*	2	N	0.27	4	4	N	1	N	N	N	4	1	1	N
Mcf M x Mcf F	N	4	3	2	2	3	2	1	*	N	N	0.45	2	N	N	1	N	N	N	2	N	2	4
Mcf M x Mcf J	N	N	4	4	2	1	2	4	*	4	2	0.18	N	2	1	2	N	4	N	1	3	N	1
Mcf F x Mcf J	N	2	N	N	N	N	1	2	N	N	*	0.64	2	N	N	N	1	1	N	1	N	1	4
Lrm M x Lrm F	N	2	N	N	N	N	N	N	N	N	N	0.91	N	N	N	N	1	1	1	2	N	N	N
Lrm M x Lrm J	N	1	N	2	1	N	N	N	4	1	N	0.55	2	N	1	2	4	1	N	2	N	3	N
Lrm F x Lrm J	N	4	N	2	1	N	1	N	2	1	N	0.45	N	N	N	N	2	4	N	N	N	1	N
Lrm M x Lrf F	N	1	N	N	N	1	N	N	N	N	4	0.73	N	N	N	1	N	1	N	N	N	N	N
Lam F x Lam M	N	N	4	N	2	2	4	N	*	N	*	0.50	2	1	1	1	4	N	3	N	4	N	N

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

N: Non significant  
 \*: No comparison

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

Groups	Relative mobility										coefficient of similarity	Relative area											
	Fraction number											Fraction number											
	1	2	3	4	5	6	7	8	9	10A	10B		1	2	3	4	5	6	7	8	9	10A	10B
Mcm M X Mcf F	N	N	1	4	4	4	4	4	*	4	2	0.18	1	4	N	N	1	N	N	N	4	N	1
Mcm F X Mcf F	N	4	N	N	1	1	1	N	N	2	*	0.45	N	2	N	2	1	N	N	1	N	N	2
Lrm M X Lrf M	N	4	N	N	N	2	N	N	N	N	1	0.73	N	N	N	N	3	N	N	N	N	N	N
Lrm F X Lrf F	N	N	N	N	N	N	N	N	N	N	N	1.00	N	N	N	N	1	N	N	N	N	N	N
Mcm J X Mcf J	N	4	4	4	4	2	1	2	3	*	1	0.18	4	4	N	4	N	4	N	N	2	N	N

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

N: Non significant  
 \*: No comparison

The greatest coefficient of similarity was found in the pairs of species most morphologically, taxonomically close (Marine *Mugil cephalus* & marine *Liza ramada*) with  $Sc=0.82$  for males and  $Sc=1$  for females, while the lowest similarity was found between the most taxonomically distant pairs of species (Marine *Mugil cephalus* & marine *Liza aurata*) with  $Sc=0.64$  for both sexes and this agreed with the results obtained by El-Serafy *et al.* (1993) and Zowil *et al.* (1994). The same observation was supported by Tsventnenko (1991) in which the similarity coefficient between *Mugil cephalus* and *Liza aurata* was (0.44). On the other hand, the present observations show lowest similarity (0.36) between either males or females of freshwater *Mugil cephalus* and *Liza ramada* and this is supported by the findings in cytogenetic studies of El Serafy *et al.* (1993).

It is difficult to differentiate between males and females of both marine *Mugil cephalus* and *Liza ramada* by using Sarcoplasmic proteins patterns. Both sexes of each species showed a similarity coefficient  $Sc=0.91\%$ . On the other hand, in the case of *Liza aurata* the sarcoplasmic proteins were most sex-specific ( $Sc=0.50$ ). The similarity between sexes in the sarcoplasmic proteins patterns of both *Mugil cephalus* and *Liza ramada* was decreased obviously when compared with the juveniles of the same species. This is supported by the findings of Zowil *et al.* (1994). Herzeberg and Pasteur (1974) mentioned that juveniles Mugilidae differ from the adults of their species in fast moving region by addition or strengthening of band.

The sarcoplasmic proteins patterns of the investigated Mugilidae in the different studied habitats were characteristic for each species. The obtained patterns of both sexes of *Liza ramada* are constant in marine and freshwater habitats. The similarity coefficient of the mobility of fractions (1.00 for females and 0.73 for males) are high. Whereas, there is a low similarity for females *Mugil cephalus* (0.45) and very low similarity coefficient either for males or juveniles stage of the same species (0.18) and this is supported by the results obtained from serum protein patterns (Zowil *et al.*, 1994).

**Finally, it can be concluded that:**

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

2. The sacroplasmic proteins patterns are species specific. This study helps to identify the types of available mullet fryes in their natural habitats by an accurate manner, where the morphological identifications of these fryes are time consuming. Hence, it can be selected for aquaculture in either marine or fresh water ponds.

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