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Molecular Genetics Survey in *Liza auratus* of Caspian Sea, 2013

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Abstract: To study the biological characteristics and physical appearance, morph metric and electrophoric records and the genetic characteristics of *Liza aurauts*, polymorphism, transferrin, hemoglobin, total number of proteins and the analysis of electrophoresis are performed in Tonekabon and Larim regions across the coastlines of Mazandaran Sea (Globally know as Caspian Sea) in 2013. Based on the random sampling of 200 *Liza auratus* obtained by fishing in these regions (Tonekabon and Larim) and after the observation of the species in the transferrin of *Liza auatus* in these areas, polymorphism transferrin emerged in the forms of 1 or 2 bands by the number of Co dominants. There have been significant differences in the population of fish in both areas (Tonekabon and Larim) and there have been high significant differences observed for *Liza auratus* in Tonekabon than in Larim. Genotypes of the study were AA. AB. BB. AC. CC. BC. and all of the meristic and morph metric factors of the fish in Tonekabon represented larger quantities than those in Larim. In comparison with the samples obtained in Larim, genotype BB has been more frequent in Tonekabon. With regard to these studies, there have been more coefficients of variance for countable factors influenced by genetic factors such as the number of fin rays and the number of up and down scale on the lateral lines of the fish in Tonekabon than those in Larim. In addition, there have been more morph metric factors like the length of head, standard length and the height of body in the fish caught in Tonekabon than those caught in Larim.

Key words: Molecular Genetics · Polymorphism · *Liza auratus* · Mazandaran Sea

INTRODUCTION

Liza auratus or golden grey is one of the most important, economical bony fish in Caspian Sea and also it is very essential for human health. The biological study of the fish in Caspian Sea is an important genetic method in the world. In this respect, the genetic study of *Liza auratus* and the study of differences about the populations of the areas that are not considered as yet are very important in describing the condition of this fish in Caspian Sea.

Liza auratus was the first grafted from Black Sea to Caspian Sea by the Former Soviet Union and , nowadays, this type of fish is found across the Iranian coastlines where there is much fishing done every year. This type of fish has pelagic breeding and unlike other types of the fish from Caspian Sea, they do not migrate for spawning to the rivers near the borders of the sea [1-6].

The largest proportions of the bony fish in Caspian Sea are found in small rivers. Plenty of natural spawning places have disappeared in rivers and shore-ponds. Therefore, the natural breeding of this type of fish is

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affected and it leaves unsatisfying effects that cause serious decreases in the stocks.

Correspondingly, the shortage of stores, the need of site for fishing, overfishing practices and extravagant fish processing have decreased and imposed pressures on all the stocks of the bony fish recently. All of the above-mentioned factors show that fishing has been excessively done across the Iranian borders in Caspian Sea.

Therefore, genes, genotypes and phonotype of this type of fish are often changed as a result of a lack of selection and random confluence [7-9].

On the other hand, there have been problems for this type of fish that have changed the population of the fish in recent years. The application of the biochemical markers and the classic studies of morphology render useful information about the circumstances of the genetic variety of the fish [10-13]. In addition, these studies provide comparative data in examining the changes and possible reasons for numerous studies that are conducted for scientists and some other creatures on the hemoglobin variations and the health levels of creatures. But, these mentioned factors were not considered for this kind of fish in these areas.

Use of biochemicals for this type of fish and differences among species by electrophoreses protein is common in most countries [14-17]. Biochemical specifications contain patterns such as protein and genetic issues and they are used to determine the genetic distinctions among populations [18-20].

However, the need of information for the morphological and biochemical particularities of *Liza auratus* necessitates comprehensive studies to be performed on the biological states of the fish.

Total weight: W
Total length, the fork length, standard length: TB, TC, TS
The form of the dorsal, anal, pectoral and caudal fin: a, p, v, c
The length of dorsal, anal and pectoral fin: dz, gy, pa
The number of rays in soft and hard dorsal fin D, Dó
The number of rays in soft and hard pectoral fin: P. P2
The number of rays in soft and hard caudal fin: V. V2
The number of lateral line, up and down scale: LL, LLa, LLb

MATERIALS AND METHODS

This study has been conducted by random sampling in the populations of 200 *Liza auratus* in Tonekabon and Larim. Biometrical and laboratorial operations continued in the center of coastline (The Tonekabon and Larim) from spring to winter seasons in 2013 and the physical and biochemical conditions of the fish were examined. Biological experiments were performed by electrophoresis on the Gell poly acryl to examine the protein, hemoglobin and Transferrin. After fishing, biometry operations were performed at first. There were some factors viewed in doing the biometry. These factors are the length of body, the length of fins, diameter of eye, the length of head, standard length and the length of snout.

These factors are parts of morph metric features. In doing meristic biometry, some factors need to be viewed. These factors include the number of fin rays, the number of above and bottom scale lateral line. The changes in the meristic factors stem from genetic sources. The following table shows the biological factors:

The tools and equipment used for the biometry contain sensitive scale, biometry ruler, band ruler, pelt and scissor. After registering the physical specifications of the fish, the habituations are considered for biochemical studies. Habituation is examined from the tail of the fish by the serum carefully so that the water splash and the tissue of the fish do not enter the serum. The level of blood taking from each fish is about 2 ml.

The blood samples in vial are preserved in cold nitrogen and transferred to the laboratory. In laboratory, serum is separated from the serum. The vial that includes blood is circulated in the centrifuge in 3000 (rpm) for 10 minutes after isolating the blood from the serum. It is isolated and placed in the Eppendorf (Centrifuge tube) for scoring the samples and it is then retained for electrophoresis activity in freezer in 20 ^{oc}. The samples should be taken out of the freezer and they can directly be poured in the Gell split-comb about the 20 μl. In another method, samples that are mixed in the cold water or the Buffer Tris solution with Geliserine (14%) can be mixed equally. The proportion of the serum is 1 or 2 in the above-mentioned mixture. This practice causes the samples to become greater and to settle at the bottom of Gell split-comb. Pouring one drop of Brum Fennel in the Eppendorf can highlight the movement of bands in time of electrophoresis practice.

The number of the factors such as the length of snout, the length of head, the length of body, the height of body and standard length are studied to assess the morphometry of *Liza auratus*. The biometry features are counted around the 5% (mm) difference for classifying the data. The morphometry analysis is used according to the [13].and electrophoresis information for the analysis of the heterogenic test is performed by the Chi Square test.

Data collection is done by performing the tests and recording the specifications. The information on the characteristics of the fish are collected and the approach is performed by authorities, like other similar studies done about other types of fish. The sampling of the fish is performed in different coastline areas. The laboratorial experiments are conducted for the recognition of the Genotype. By the ANOVA test, the samples are recorded and the data is compared. The analysis of the genes and the protein is done by Chi Square test. The population of samples in this study contains 200 fish that are selected randomly from fishing in the Tonekabon and Larim area.

RESULTS

To study the polymorphism, transferrin, hemoglobin, protein in Tonekabon and Larim, the results of the research are grouped into two sections (Morphologic and Genotype qualifications) [Table 1]. The genotype is examined for obtaining the results of biochemical investigation that is carried out by the electrophoresis in serum, total protein and transferrin of *Liza auratus* in laboratory.

we can deduce that Genotype (BB) is related to the *Liza auratus* in Tonekabon While the abundance of fish in Larim is less than that in Sari, Gene (BB) is greater in proportion in forming the Genotype [Table 2].

Table 1: The study of abundance in Genotype transferrin of fishes

Place of sampling	The number of sample	AA	AB	BB	AC	CC	BC
Tonekabon	Total(100)						
Station1	23	4	5	9	1	3	1
Station2	32	4	6	8	4	6	4
Station3	22	7	_	12	2	1	_
Station4	23	4	_	12	7	_	_
Larim	Total(100)						
Station1	12	2	_	_	_	6	4
Station2	38	3	4	14	_	7	_
Station3	28	3	_	11	8	6	_
Station4	22	6	_	16	_	_	_

Table 2: The investigation of allelic frequency of Transferrin

The place of sampling	TFA Transferrin Genotype (AA)	TFBTransferrin Genotype (BC)	TFC Transferrin Genotype (CC)
Tonekabon zone	16.7	100	48.2
Larim zone	14.4	100	8.1

Table 3: The meristic data of fishes in Tonekabon and Larim zones

Average mean	44.2A	A′	P	P′	V	V′	D	D′	D′′	C	C′	LL	Lla	Llb
Tonekabon	3.8	9.1	1.2	14	4	9.1	6	9.5	1.2	8.1	6.4	45	66	8.9
Larim	0.1A	8.7	1.2	15	4	8.3	4	9.1	1.2	9	7	44	8	8.1
Variance	0.3	A′	1.2P	P′	0.6V	V′	0.4D	D′	D′′	0.6C	C′	LL	Lla	LLb
Tonekabon	0A	0.9	0.16	1.2	0.2	0.1	0.3	3.3	0.1	0.4	0.4	4.3	0.6	0.6
Larim	0.5	0.3	P	1.2	0.4V	0.1	0.6D	0.8	0.1	0.8C	0.3	3.1	0.5	0.5
Standard deviation	0A	A′	0.1	P′	0.6	V′	0.5	D′	D′′	0.6	C′	LL	Lla	LLb
Tonekabon	1.9	0.7	0.5	1.1	11V	0.4	D	1.6	1.3	7.3C	0.5	2.1	0.6	0.9
Larim		0.5	P	1.1	9.4	0.4	14.2	1.3	1.4	6.2	0.3	2	0.5	0.7
Coefficient of changes		A′	0	P′		V′	13	D′	D′′		C′	LL	Lla	LLb
Tonekabon		1.4	1.2	5.9		4		6	0		5.7	4.2	4.2	7
Larim		8.6		5.8		3.7		5.8	0		6.8	4	5	6.1

A= The number of hard rays of anal fin A' = The number of soft rays of anal fin

P = The number of hard rays of pectoral fin P' = The number of soft rays of pectoral fin

V = The number of hard rays of ventral fin V' = The number of soft rays of ventral fin

C= The number of hard rays of caudal fin C' = The number of soft rays of caudal fin

LL = The number of scale on the lateral line LLa = The number of scale upon on the lateral fin

LLb = The number of scale under the lateral fin D = The number of hard rays of first dorsal fin

Table 4: The comparison of some proportions between morphologic particulars about fishes

Some of the morphologic qualifications	Tonekabon	Larim
HDS	%27	%21
HEH	%10	%7
SSD	%97	%92
VSD	%51	%45
DSD	%53	%48

HDS= The proportion of the length of the head to standard length.
HEH= The proportion of the length of the head to height of body.
SSD= The proportion of the length of snout to standard length.
VSD= Distance from the beginning of snout to ventral fin to standard length.
DSD= Distance from the beginning of snout to dorsal fin to standard length.

Also the table indicates that co dominant (B) is more frequent in Tonekabon. The second more frequent item is co dominant (C). However, in the Sari, co dominant (B) is entirely more abundant than it is in Larim.

Remarkably the fish in Tonekabon represent better growths and this is very significant in terms of morphological factors[Table 3,4].

DISCUSSION

The study of the construction of polymorphism transfer in the total number of proteins and hemoglobin and the analysis of the parts of cells are performed for a great population of animals [2, 21, 22].

This method of study can be applied for the study of the population of fish. In this investigation, two factors are taken for examining the possible differences in the population of *Liza auratus* in both areas (Tonekabon and Larim).

The protein variety is employed via electrophoresis just as this variety bears a genetic origin. Furthermore, electrophoresis variety is one of the most important tools in studying the animals. Also, it is a practical tool in studying the genetic subjects.

If there are two great proportions of populations that represent distinctive electrophoresis characteristics, the interchange of genes among the populations will be very low or nothing.

There have been no contradictory results as yet, because one of these groups is created by increasing the generator of the other group, or both of these groups are broken down and divided recently.

In another way, eiher groups or populations with the same alleles may separate from each other but they can be affected by a selection mechanism [13, 14]. However, to identify different populations and to obtain more information, the records of groups should be studied.

The important point considered in this study is the distinction between *Liza auratus* in Larim and Tonekabon. Attaining authentic and reliable information is very important in performing the breeding activity [15].

Another quality observed in this study is morphologic characteristics, but none of the morphologic specification is influenced by genetic factors. Therefore, it is not an appropriate method to recognize the populations since the distinction between the species is not very significant. However, the characteristics that are not affected by genetic factors are useful in studying the concept of performance in the populations. These traits can represent the genetic concept of population more clearly. Electrophoresis and morphologic characteristics demonstrate a large body of information on the structure of the populations and both of these groups of information should be taken in doing this type of research.

Concerning the physical and biochemical distinctions observed among the types of the fish in Tonekabon and Larim in this research, we couldn't acknowledge that the fish have various populations. However, we can compare the genetic and morphologic specifications in these areas.

Regarding the aforementioned arguments in this study, the distinction and recognition of some populations in *Liza auratus* are the main purposes.

CONCLUSION

It could be concluded that the population of this fish in different parts were not the same and also in both groups the same alleles may separate from each other but they can be affected by a selection mechanism.

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