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# Conservation of Some Sea Bream (*Sparus aurata)* Fish Populations

*[1]Y.M. Saad, [2]N.M. Abou Shabana, [3]N.A. El-Ghazaly, [2]M.H. Fawzy and [3]A.M. Mohamed*

[1]Department of Biological Science, Faculty of Science, King Abdulaziz University, KSA
Permanent address is Genetics Lab. NIOF, Egypt
[2]Fish Reproduction, National Institute of Oceanography and Fisheries (NIOF), Egypt
[3]Faculty of Science, Alex. University, Egypt

**Abstract:** Some *S. aurata* (one wild and two farmed fish) populations namely, (D, A & K21) were genetically characterized using some DNA markers. The homogeneity value was high within each estimated fish population. The genetic dissimilarity values were calculated for reconstruction the dendrogram among them. The (D) population was distantly related from (A & K21) populations. The results suggested that, there is human effects on the genetic variations due to fish transportation from locations to another without restricted plan.The total length and weight were calculated for each studied fish population for infringe the growth rate. The (D) population showed a high length and weight than the other studied sources (A & K21). The percentage of (D) hatchability (85%) was higher than the percentage of (K21) hatchability (79.2%). The average of larvae length and weight were calculated for (D) and (K21). The (D) larvae had the best length and weigh relatively. Development of local *S.aurata* breeds is important because many of them will be resilient to climatic stress and represent a unique source of genes for improving *S. aurata* production in the future.

**Key words:** Sea Bream % *Sparus aurata* % Fish Populations % Climatic stress % Genes

## INTRODUCTION

Conservation or good management of aquatic genetic resources should ideally involve a continuum of activities. These activities are documentation, characterization, evaluation and utilization of aquatic genetic resources in sustainable breeding schemes, including the politics of access to germplasm [1].

The Sea bream fish (aquatic genetic resources) are important cultured marine fish in many countries around the world such as in Italy, France, Greece and Egypt [2- 4].

Restocking and stock enhancement programs are now recognized as an important tool for the management of fishery resources. It is important, however, to have an adequate knowledge on the genetic population structure of both the released stock and the wild population before carrying out such programs [5].

Development of local economic fish breeds such as *S. aurata* is important because many of them will be resilient to climatic stress and represent a unique source of genes for improving production. In addition, studying the relative contribution of each fish parent to the next generation is a great concern especially when some of the offspring are intended to be used as parents of the subsequent generation [6, 7].

In fish breeding programs, the genetic variations are needed for good performance especially growth rate and reproduction. In addition, genetic diversity [8] is needed to resist environmental change.

DNA markers (Dominant and/or Co-Dominant markers) generated by various molecular genetic techniques are widely used as marker assisted selection in fish breeding programs such as in Tilapia [7].

The objectives of this study are documentation, characterization, evaluations and utilization of some *S.aurata* populations for conservation of this economic fish.

## MATERIALS AND METHODS

The present work was carried out in National Institute of Oceanography and Fisheries, Kilo 21 marine hatchery and genetics Dept. Fac. of Agric. Ain Shams Univ. Egypt.

**Corresponds Aothur:** Yasser Saad, Department of Biol. Sci., Faculty of Sci., King Abdulaziz, KSA. E-mail: yasser_saad19@yahoo.com.

Table 1: RAPD Primers used in the study and their sequences

| Code | Sequence | Code | Sequence |
|---|---|---|---|
| OPA16 | (3'-AGC CAG CGA A-5') | OPC05 | (3'-GAT GAC CGC C-5') |
| OPA20 | (3'-GTT GCG ATC C-5') | OPC06 | (3'-GAA CGG ACT C-5') |
| OP B08 | (3'-GTC CAC ACG G-5') | OPC08 | (3'-TGG ACC GGT G-5') |
| OPB10 | (3'-CTG CTG GGA C-5') | OPC11 | (3'-AAA GCT GCG G-5') |
| OP B12 | (3'-CCT TGA CGC A-5') | OPC20 | (3'-ACT TCG CCA C-5') |

Some adult *Sparus aurata* individuals were sampled from three Egyptian locations namely (A) Alexandria (wild type), (D) Damietta (farmed) and K21 marine hatchery (K21).

**Molecular Analysis:** From each specimen, small piece of caudal fin tissue was excised, placed in 70% ethanol and held at + 4°C for subsequent DNA extraction. DNA extraction and purification were performed according to Hillis [9].

Ten primers (Operon Tech.) were initially screened for scoreable amplified bands (Table 1).

The PCR mixture and reaction conditions were carried out as described by Rashed *et al*. [8] with some modifications. These modifications were summarized in RAPD-PCRs. RAPD-PCRs were achieved in thermal cyclers programmed as follows: The temperature degree of first cycle (denaturation) was adjusted to 94°C for 4 min. In the next 30 cycles, the denaturation, annealing and extension temperature degrees were adjusted to 94°C for 45 sec, 37°C for 45 sec and 72°C for 45 sec, respectively. The temperature of final extension was adjusted to 72°C for 5 min. PCR mixture was prepared according to the pamphlet provided with the Sigma (Red Taq Ready mix).

**Data Analysis:** Molecular data were analyzed as described by Bardakci and Skibinski [10] and Saad *et al*. [11] with some modifications. These modifications were summarized in analysis of gels. The gels were analyzed using Total Lab v1. In addition, SPSS 10 software was used to estimate the similarity percentages between the Sea bream populations and reconstructing the phylogenetic relationships using Dice coefficient.

**Biological Analysis:** The brood stock maintenance conditions were temperature (ranged from16°C to 18°C), salinity (35-38 ‰); pH (7-8) and photoperiod (12l: 12D).

Leutinizing Hormone-Releasing Hormone (LH-RH) was used in the present study to induce spawning (as described by Cek and Gokce [12] with some modifications) in the 2 groups (Damietta and kilo 21) as following: the fish were treated with 1000 IU/kg for female and 600 IU/kg for male. The spawning temperature was adjusted to range of 14-16°C less than the above listed rearing medium.

The *S.aurata* eggs were collected, counted and Transferred to the special incubator (for larval rearing). The rearing temperature ranged from18 to 22°C. The larvae growth rate[13] was regularly determined (until 2 months age).

Biological data were analyzed as described by Cek an Gokce [12] and Akyol and Gamsiz [13].

## RESULTS

**Molecular Characterization:** RAPD technique (10 primers) was used for some *S. aurata* populations (one wild and two farmed *S. aurata* populations) characterization. A total of 88 RAPD bands were detected based on all RAPD patterns analysis (through the three studied fish populations). The number of polymorphic bands, population specific markers and average of band frequencies were calculated and presented in table 2. The numbers of polymorphic bands were varied within each studied fish population. It was 17, 20, 19 within A, K21 and D population respectively. The averages of band frequencies were close to each other within each studied population. It was 0.835, 0.831 and 0.837 within A, K21 and D population respectively.

**Population Specific DNA Markers:** A total of seven fish population specific DNA markers were detected Tables (2). One of them was (A) specific DNA marker at MW (0.801 Kb). This marker was generated from primer (B12). Two DNA markers were (K21) specific markers at MW 0.632 Kb (primer C8) and 0.355 Kb (primer C5). The (D) population had four DNA markers generated by primers C8 (0.258 Kb), B10 (1.44 & 0.821 Kb) and C5 (0.781 Kb).

**Homogeneity Within Studied Sea Bream Populations:** The homogeneity value was high within each estimated fish population. The averages of these values were (0.94±0.02), (0.94±0.02) and (0.94±0.02) within A, K21 and D population respectively (Table 3).

Table 2: Number of detected RAPD bands, number of polymorphic bands, population specific markers and average of band frequencies in the three studied Sea bream populations.

| | A | | | | K21 | | | | D | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Code | DB | PB | PSM | ABF | DB | PB | PSM | ABF | DB | PB | PSM | ABF |
| OPA16 | 8 | 5 | 0 | 0.8 | 7 | 5 | 0 | 0.6 | 7 | 4 | 0 | 0.77 |
| OPA20 | 9 | 1 | 0 | 0.95 | 9 | 1 | 0 | 0.95 | 9 | 1 | 0 | 0.91 |
| OPB08 | 8 | 0 | 0 | 1 | 8 | 0 | 0 | 1 | 8 | 2 | 0 | 0.85 |
| OPB10 | 8 | 0 | 0 | 0.8 | 8 | 0 | 0 | 0.8 | 7 | 0 | 2 | 0.7 |
| OPB12 | 7 | 1 | 1 | 0.94 | 6 | 0 | 0 | 0.85 | 6 | 0 | 0 | 0.85 |
| OPC05 | 4 | 1 | 0 | 0.53 | 5 | 1 | 1 | 0.7 | 5 | 1 | 1 | 0.7 |
| OPC06 | 11 | 8 | 0 | 0.7 | 11 | 5 | 0 | 0.76 | 11 | 6 | 0 | 0.81 |
| OPC08 | 8 | 1 | 0 | 0.63 | 11 | 6 | 1 | 0.7 | 11 | 5 | 1 | 0.78 |
| OPC11 | 8 | 0 | 0 | 1 | 8 | 0 | 0 | 1 | 8 | 0 | 0 | 1 |
| OPC20 | 9 | 0 | 0 | 1 | 9 | 2 | 0 | 0.95 | 9 | 0 | 0 | 1 |
| Average | | | | 0.835 | | | | 0.831 | | | | 0.837 |
| SE | | | | ±0.05 | | | | ±0.05 | | | | ±0.05 |

DB=Number of detected bands PB=Number of polymorphic bands, PMS=Population specific markers ABF= Average of band frequencies.
A= Alexandria D= Damietta K21=marine hatchery

Table 3: Similarity (bottom diagonal), dissimilarity (upper diagonal) among the studied Sea bream populations and similarity values within (diagonal) these populations

| | A | K21 | D |
|---|---|---|---|
| A | 0.94±0.02 | 0.09 | 0.11 |
| K | 0.91 | 0.94±0.02 | 0.12 |
| D | 0.89 | 0.88 | 0.94±0.02 |

A= Alexandria, D= Damietta and K21=marine hatchery.

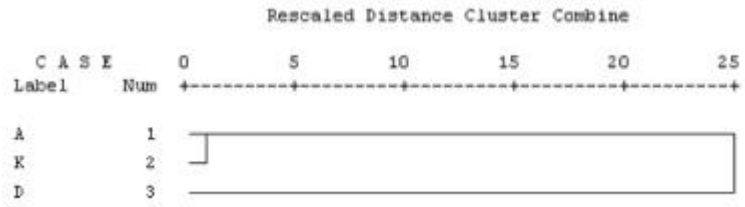


Fig. 1: Dendrogram revealed genetic relationships among the applied *S. aurata* populations based on RAPD polymorphism.
A= Alexandria, D= Damietta and K21=marine hatchery.

**Dendrogram Analysis:** The genetic distance values were calculated for reconstruction the dendrogram (Figure 1) among them (D, A & K21). The genetic dissimilarity values were (0.09), (0.11) and (0.12) between (K21 &A), (D&A) and (D& K21) respectively. The (D) population was distantly related from the other two estimated fish populations (Table 3 and Figure 1).

**Biological Performance of (A, K21 & D) Fish Populations:**

**A, K21 and D Population Growth Performance:** The total length and weight were calculated for each studied fish population (Table 4 and Figure 2 &3) for infringe the growth rate. The Averages of parent (A, K21 & D) initial lengths (23cm), final lengths (33cm) initial weights (130.8g) and final weights (550.8g) were presented in (Table 4). As presented in Figures (2 &3), the (D) population had the highest length and weight values relatively. On the other hand, (K21) showed the lowest performance relatively.

**Hatchability, Egg Characterization and Larvae Survival Performance:** The percentage of both hatchability and survival were calculated (Table 4) for both (D) and (K21) fish populations.

Table 4: Average (± SD) for initial length, initial weight, final length final weight egg diameter oil globule diameter and daily gain within the (A, K21 and D).

| Prameters | A | K21 | D |
|---|---|---|---|
| Average of parent initial lengths (cm) | 21.9 ± 1.43 | 21 ± 2.56 | 23 ± 1.53 |
| Average of parent initial weights (g ) | 120.4 ± 10.38 | 112.6 ± 12.40 | 130.8 ± 8.58 |
| Average of parent final lengths (cm) | 31.4 ± 1.81 | 31 ± 0.77 | 33 ± 0.42 |
| Average of parent final weights(g ) | 537.6 ± 26.76 | 430.2 ± 18.47 | 550.8 ± 34.54 |
| Daily length gain(cm/day) | 0.045 | 0.048 | 0.048 |
| Daily weight gain(gm/day) | 1.9 | 1.5 | 2 |
| Total eggs | - | 867320 | 1032110 |
| Hatchability % | - | 79.2 % | 85 % |
| Egg Diameter | - | 0.884 ± 0.01 | 0.899 ± 0.01 |
| Oil globule diameter | - | 0.349 ± 0.08 | 0. 357 ± 0.09 |
| Average larvae initial lengths (mm) | - | 3.8 ± 0.16 | 3.9 ± 0.19 |
| Average larvae initial weights (mg) | - | 4.47 ± 0.646 | 4.45 ± 0.546 |
| Average larvae final lengths (mm) | - | 7.08 ± 0.21 | 19 ± 1.19 |
| Average larvae final weights (mg) | - | 47.95 ± 4.161 | 59.25 ± 4.38 |
| Daily larvae length gain(cm/day) | - | 0.015 | 0.071 |
| Daily larvae weight gain(gm/day) | - | 0.20 | 0.26 |

A= Alexandria, D= Damietta and K21=marine hatchery, -=not detected.

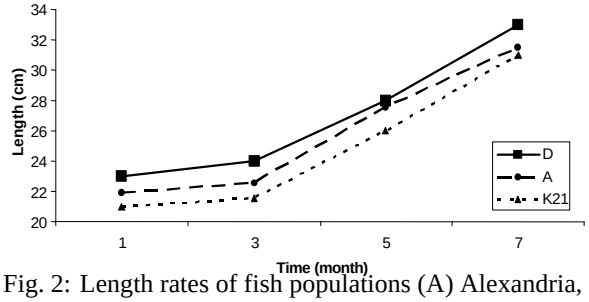


Fig. 2: Length rates of fish populations (A) Alexandria, (D) Damietta and marine hatchery (K21).

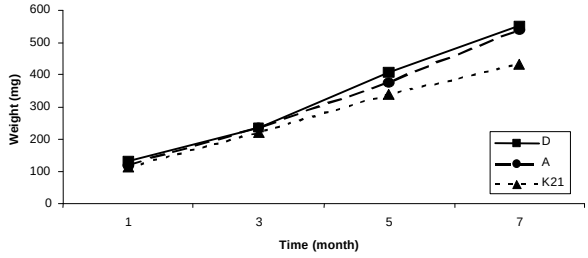


Fig. 3: Weight rates of fish populations (A) Alexandria, (D) Damietta and marine hatchery (K21).

The percentage of (D) hatchability (85%) was higher than the percentage of (K21) hatchability (79.2%). Concerning survival performance, the percentage of (K21) survival (3.6%) was lower than (D) survival (4.6%).The total (D) eggs (1032110) was higher than the total (K21) Eggs (867320). The egg diameters and oil globule diameters for both (D and K21) populations were calculated. The averages of these values presented in Table (4).

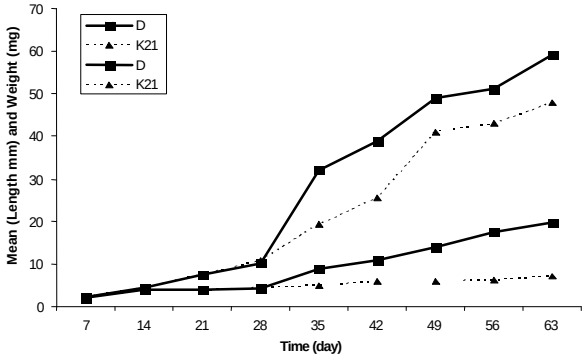


Fig. 4: Weight and length rates of fish larvae (D) Damietta and marine hatchery (K21).

**K21 and D Larvae Growth Performance:** The averages of (D) and (K21) larvae length and weight values were calculated. The (D) larvae had the highest length and weigh values relatively (Figure 4). The measured values of average length and weight of the larvae show higher values for average length and weight of (D) larvae over (K21) ones where no observed difference in total length and total weight at age of 14 days. The differences began to appear from the age of 30 days in length and weight for (D) larvae over (K21) larvae and this difference continuo over 41, 48 and 60 days for also (D) larvae.

Daily length gain was 0.071 mm/day and 0.015 mm/day for (D) and (K21) larvae respectively and daily weight gain was 0.26 mg/day and 0.20 mg/day for (D) and (k21) larvae respectively.

## DISCUSSION

Generally, fish fragmentation into subpopulations revealing groups that is genetically different from main source. The genetic structure of sea bream fish populations [5, 14, 15] is not fully maximized and unclear. Generally, genetic diversity which is required for populations to be more adaptive with the environmental changes can be measured using an array of molecular and quantitative methods [8].

DNA polymorphism detected by RAPD can be seen from two viewpoints. First, the presence (or absence) of one or more RAPD fragment which possess particular size from the RAPD patterns. Second, changes in the intensity of fragments having the same size. RAPD technique widely used to estimate genetic variations within and among many fish populations such as in Sea bream *Diplodus sargus* and *D. vulgaris* [5] and in *O. niloticus* [8].

In the present study, a total of 88 storable bands were produced with molecular size ranged from 0.159 to 2.35 Kb. The number of bands was variable among studied fish populations and ranged from 6 to 11. Results of the analyzed data showed that the average of band frequencies ranged from 0.5 to 1 (within each studied fish population). Number of bands, number of polymorphic bands and average of band frequencies were calculated in the present study because they widely used to reflect the genetic polymorphism within and among biological samples especially in fish characterization and studying fish population structure [8,11]. In the present study, the number of polymorphic bands was relatively high within each studied population and among all of them but because of dominance; RAPD cannot provide totally reliable estimates of heterozygosity.

In the present study, seven fish population specific DNA markers were detected. One of them was (A) specific DNA marker (generated by primer B12). Two DNA markers were (K) specific (primer C8 and C5). The (D) population had four DNA markers (primers C8, B10 and C5). These markers are useful especially as marker assisted selection to develop of local sea bream breeds in the future. These breeds are important because many of them will utilize lower quality feed, are more resilient to climatic stress and represent a unique source of genes for improving health. In addition, studying the relative contribution of each fish parent to the next generation is a great concern especially when some of the offspring are intended to be used as parents of the subsequent generation [6, 7].

The similarity values in the present study were (0.91), (0.89) and (0.88) between (A&K), (A&D) and (K&D) respectively. Generally, these high values reflect low genetic distances among studied fish populations. These low genetic distance values may be due to high gene flow among the origins of these populations. In addition, this may be due to recent isolation of studied fish populations from the main origin Mediterranean Sea.

The reconstructed dendrogram showed that, (D) population is distantly related from the other two studied fish populations (A and K21). The A and K21populations were close to each other. This may be due to a high gene flow values among the origins of studied fish populations. The estimated genetic diversity was clearly different from zero and reflects presence of relatively genetic polymorphism levels. The high genetic similarity level within each studied fish population is reflect the levels of homogeneity and inbreeding in the studied fish populations.

The biological data showed that, the (D) population gave a high length and weight than the other studied sources (A & K21). So (D) population was chosen to be the source parent for the next generation in our institute (NIOF). For comparison with the lowest one (in length and weight), the (K21) total length and total weight were calculated. The average total length and total weight of the mothers showed a high value for (D) population over (A) and (K21) and this dominancy is consistent to the increase of total weight of (D) over (A) and (K21).

The measured length and weight of the larvae showed high values for average length and weight of (D) larvae over (K21).

In the present study, the percentage of (D) Hatchability (85%) was higher than the percentage of (K) Hatchability (79.2%).

Some specific hormones have been employed to accelerate the induction of spawning in a variety of fish in captivity [16]. In the present study female *S. aurata* were injected with a single intramuscular injection dose of LHRH as female fish was injected with 1000 IU/kg and male fish was injected with 600 IU/kg as described by Cek and Gokce [12]. This was sufficient to induce fully mature gametes and spawning. Zaki *et al*. [17] induced spawning of *S. aurata* using hormonal doses of HCG, CPE and mix of HCG+CPE. In the present study, the hatchability results were close to Zaki *et al*. [17] work relatively.

LHRH hormone represent a successful, cheap and available agent in induce spawning in *S.aurata* fish and other fish species.

Restocking using a good fish performance such as (D) population and stock enhancement programs are now recognized as an important tool for good management of fishery resources.

In fish breeding programs [7], the genetic variations are needed for good performance especially growth rate and reproduction. The loss of genetic diversity is often associated with reduced reproductive fitness [18].

## CONCLUSION

In Egypt, there is a human effect on the genetic variations within and among studied *S. aurata* populations due to fish transportation from location to another without restricted plan. So, the genetic markers should be conducted to provide the information needed for a sound management of this economic fish in farms and/or wild fish stocks. This way will be useful, especially in fish breeding programs which use genetic markers as a marker assisted selection to improve the fish economic traits. In addition, development of local *S. aurata* breeds is important because many of them will be resilient to climatic stress and represent a unique source of genes for improving *S. aurata* production and conservation in the future.

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