Full Length Research Paper

Phenotype and genotype differentiation between flathead grey mullet [*Mugil cephalus*] and thinlip grey mullet [*Liza ramada* (Pisces: *Mugilidae*)]

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This study aimed to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on random amplified polymorphic DNA (RAPD) fingerprinting between two *Mugilidae*, Flathead grey mullet (*Mugil cephalus*) and Thinlip grey mullet (*Liza ramada*). The results showed that there were significant differences in most of the morphometric character indices but no significant differences were detected in most of the meristic counts between *M. cephalus* and *L. ramada.* In addition, the euclidean distance between *M. cephalus* and *L. ramada* using hierarchical cluster analysis of quantitative phenotype based on morphometric character indices and meristic counts and condition factor was (0.421). Genotype analysis based on RAPD fingerprint showed highly genetic dissimilarity (0.437) between *M. cephalus* and *L. ramada.* These results confirmed that the amount of differences in genotype reflected the same amount of differences in phenotype between *M. cephalus* and *L. ramada.* Therefore, either phenotype analysis can be use based on a large number of morphometric character indices and meristic counts or genotype analysis based on RAPD fingerprinting to discriminate *M. cephalus* and *L. ramada* with the same results.

Key words: Phenotype, genotype, *Mugil cephalus*, *Liza ramada*.

INTRODUCTION

Mullets are euryhaline fish widely distributed in tropical and subtrobical and estuaries. Mullets are catadromous spawning migrating fish, the young life before maturity remains predominantly in the system of rivers and lakes (Lee and Tamaru, 1988; El-Deeb et al., 1996). Five mullet species occur in Egyptian water. According to the different regions, the most commonly-cultured of mullets are *Mugil cephalus* and *Liza ramada* (Oren, 1981; Lee and Tamaru, 1988; El-Sayed, 1991). It is considered an important source of animal protein for the peoples of the Pacific Basin, Southeast Asia, India, the Mediterranean, Eastern Europe and, many parts of central and South America (Lee and Tamaru, 1988). The identification of the fry to be used for stocking purposes is of practical interest and often causes problems for farmers who want to stock the ponds with specific species (Menezes et al.,

1992). Different methods are used for identification but phenotype based on morphometric and meristic is considered as earliest and authentic methods for the identification of fish species in fish biology to measure discreteness and relationships among various taxonomic categories. There are many well documented morphometric studies which provide evidence for stock discreteness (Avsar, 1994; Haddon and Willis, 1995; Bembo et al., 1996; Anene, 1999; Turan, 1999; Zafar et al., 2002; Barriga-Sosa et al., 2004; Doherty and McCarthy, 2004; Naesje et al., 2004). Morphometric is the external measurements of an organism, while meristic counts means serial counts of body elements (Talwar and Jhingran, 1992). Morphological characters including meristic counts and body proportions often vary clinically (that is along a geographic gradient) (Lindsey,

1988).

Ihssen et al. (1981), Allendorf (1988), Swaine et al. (1991) and Turan (1999) reported that phenotypic adaptations do not necessarily result in genetic changes in the population and thus, the detection of such phenotypic differences among populations cannot usually be taken as evidence of genetic differentiation. Studies of morphological character variation are, therefore, vital in order to elucidate patterns observed in phenotypic and genetic character variation among fish populations (Beheregaray and Levy, 2000).

The technique of random amplified polymorphic DNA (RAPD) marker (Welsh and McCelland, 1990; Williams et al., 1990) has been successfully exploited for stock identification and population analysis in fish (Partis and Wells, 1996; Dong and Zhou, 1998; Bartfai et al., 2003; Ahmed et al., 2004; El-Zaeem et al., 2006; El-Zaeem and Ahmed, 2006).

Therefore, the aim of this study was to study the phenotype and genotype differentiation and to compare the amount of differences in phenotype based on morphometric character indices and meristic counts with the amount of differences in genotype based on RAPD fingerprinting between two *Mugilidae*, Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada.*

MATERIALS AND METHODS

This study was carried out at fish breeding and production laboratory, Animal and Fish Production Department, Faculty of Agriculture (Saba-Bacha), Alexandria University and Nucleic Acids Research Department, Genetic Engineering and Biotechnology Research Institute (GEBRI), City for Scientific Research and Technology Applications, Alexandria, Egypt.

Specimen collection

Larvae of Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada* were collected from Mediterranean sea and transferred to culture into earthen ponds at Faculty of agriculture (Saba-Bacha) fish farm for 12 month. From both sex, a total of 216 specimens of Flathead grey mullet, *M. cephalus* and 234 of Thinlip grey mullet, *L. ramada* were randomly collected. Their weight and length ranged from (32.10 to 50.00 g and 16.50 to 19.80 cm) and (28.00 to 45.50 g and 15.60 to 18.60 cm), respectively.

Quantitative phenotype analysis

A total of 34 morphometric characters and 9 meristic counts were recorded within each species as described by Bagenal (1978), Ezzat et al. (1979), Taniguchi et al. (1996), Sahu et al. (2000), Costa et al. (2003) and Doherty and McCarty (2004). Condition factor of each species were measured using the following equation:

$$K= (W/L^3)\ 100\ \text{(Lagler, 1956)},$$

Where, W is the body weight (g) and L is the total length (cm).
All morphometric characters were transformed by dividing the measurement by either total or standard length or head length of each fish to minimize the effect of fish size (Tables 1 and 3).

Phenotype differentiation between Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada* based on morphometric character indices and meristic counts was analysed by means of the hierarchical cluster analysis of the STATISTICA package, version 5.00 (StatSoft, 1995). The cluster analysis using unweighted pair group average method (UPGMA: Sneath and Sokal, 1973) was performed on the matrix of euclidean distance in order to depict hierarchically the shape differences between Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada.*

Statistical analysis

Data significance of the morphometric character indices and meristic counts were analyzed using unpaired Student's t-test (P<0.05) according to Snedecor and Cochran (1980).

Genotype analysis

Genotype analysis was performed based on RAPD fingerprinting. DNA was extracted from liver tissues of each Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada* according to the method described by Bardakci and Skibinski (1994). In this study ten and twenty base long oligonucleiotide primers were used to initiate polymerase chain reaction (PCR) amplifications. Primers were randomly selected on the basis of GC content and annealing temperature for RAPD-PCR amplification (Table 2).

PCR amplifications were performed according to the procedure of Williams et al. (1990, 1993). The reaction (25 µl) was carried out in a mixture consisting of 0.8 U of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs, and 25 pmol of random primer, 2.5 µl 10X Taq DNA polymerase buffer and 40 ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (Eppendorf). The PCR programme included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 30 s for DNA denaturation, annealing as mentioned with each primer (Table 2), extension at 72°C for 30 s and final extension at 72°C for 10 min were carried out. Samples were cooled at 4°C.

The amplified DNA fragments were separated on 2.5% agarose gel and stained with ethidium bromide. ΦX174 DNA Ladder marker (bp 1335, 1078, 872, ………72) was used in this study. The amplified patterns were visualized on an UV transilluminator and photographed by gel documentation system.

RAPD patterns were analyzed and scored from photographs. For the analysis and comparison of the patterns, a set of distinct, well-separated bands were selected. The genotypes were determined by recording the presence (1) or absence (0) in the RAPD profiles. Genetic similarity (GS) between Flathead grey mullet, *M. cephalus* and Thinlip grey mullet, *L. ramada* was calculated according to the formula given by Nei and Li (1979): $B_{ij}=2\ N_{ij}/(N_i + N_j)$, where N_{ij} is the number of common bands observed in individuals i and j, and N_i and N_j are the total number of bands scored in individuals i and j respectively, with regard to all assay units. Thus, GS reflects the proportion of bands shared between two individuals and ranges from zero (no common bands) to one (all bands identical). Genetic dissimilarity (GD) was calculated as: GD = 1- GS (Bartfai et al., 2003).

RESULTS

Quantitative phenotype analysis

Mean values of morphometric character indices were compared between *M. cephalus* and *L. ramada.* The

Table 1: Quantitative phenotype traits based on morphometric characters and meristic counts used for differentiation analysis between *Mugil cephalus* and *Liza ramada.*

Characters	Acronyms
Morphometric analysis	
Total length	SL
Standard length	TL
Head length	HL
Body depth	BD
Body width	BW
Head width	HW
Abdomen length	AL
Caudal peduncle length	CPL
Caudal peduncle depth	CPD
Caudal peduncle width	CPW
Upper jaw length	UJL
Lower jaw length	LJL
Snout length	SnL
Orbit diameter	OD
Pre-orbital length	Pr-OL
Post-orbital length	Po-OL
Inter dorsal distance	IDD
Snout to first dorsal	Sn-FD
Trunk length	TrL
First dorsal fin base length	FDFBL
Second dorsal fin base length	SDFBL
Pelvic fin base length	Pel FBL
Anal fin base length	AFBL
Caudal fin length	CFL
Pectoral fin length	Pec FL
Length of longest first dorsal fin spine	LLoFDFS
Length of last first dorsal fin spine	LLaFDFS
Length of longest second dorsal fin ray	LLoSDFR
Length of last second dorsal fin ray	LLaSDFR
Length of longest anal fin spine	LLoAFS
Length of longest anal fin ray	LLoAFR
Length of last anal fin ray	LLaAFR
Length of longest caudal fin ray	LLoCFR
Length of shortest caudal fin ray	LShCFR
Meristic analysis	
First dorsal fin spines count	FDFSC
Second dorsal fin rays count	SDFRC
Pelvic fin spines count	Pel FSC
Pelvic fin rays count	Pel FRC
Pectoral fin rays count	Pec FRC
Anal fin spines count	AFSC
Anal fin rays count	AFRC
Caudal fin rays count	CFRC
Total number of vertibrae	TNN

Table 2. The sequences, GC% and the annealing temperatures of the primers used.

Primer	Sequence 5`- 3`	GC%	Annealing Tm °C /Sec
1	GAA TGC GAC G	60	34/30
2	ATG ACG TTG A	40	34/30
3	GGA CTG GAG TGT GAT CGC AG	60	58/30
4	GGA CTG GAG TGG TGA CGC AG	65	58/30
5	CAG GCC CTT CCA GCA CCC AC	70	52/30
6	GAA ACG GGT GGT GAT CGC AG	60	52/30
7	TGG TGG ACC A	60	34/30
8	AGC AGG TGG A	60	34/30
9	CTG AGG AGT G	60	34/30
10	GGG CTA GGG T	70	34/30

highest means indices of BD/SL, BW/SL, AL/SL, CPD/SL, CPW/SL, UJL/HL, LJL/HL, HW/HL, SnL/HL, TrL/SL, IDD/SL, LLaSDFR/SL, LLaFDFS/SL, FDFBL/SL, SDFBL/SL, Pel FBL/SL, LLaAFR/SL, AFBL/SL, LLoCFR/TL and Pec FL/SL of *M. cephalus* differ significantly from those of *L. ramad* while, the means indices of HL/SL, Sn-FD/SL, LLoFDFS/SL and LLoAFS/SL, showed significant superiority for *L. ramada* compared with those of *M. cephalus*. The results showed also that no significant differences were detected in the indices of CPL/SL, OD/HL, Pr-OL/HL, Po-OL/HL, LLoSDFR/SL, LLoAFR/SL, LshCFR/TL and CFL/TL, between *M. cephalus* and *L. ramada* (Table 3).

Moreover, the results of meristic counts showed that the highest and lowest mean value of Pec FRC and AFRC were obtained by *M. cephalus* and differ significantly from that of *L. ramada*. While, the other meristic counts records of FDFSC, SDFRC, Pel FSC, Pel FRC, AFSC, CFRC and TNN, showed no significant differences between *M. cephalus* and *L. ramada*. In addition, *M. cephalus* had significant superiority of condition factor (K) compared with *L. ramada* (Table 3). The euclidean distance between *M. cephalus* and *L. ramada* using hierarchical cluster analysis of quantitative phenotype based on morphometric character indices, meristic counts and condition factor was 0.421.

Genotype analysis

All the ten different primers used in this study produced different RAPD band patterns (Table 4). The number of amplified bands detected varied, depending on the primers and species; in addition to ensure that the amplified DNA bands originated from genomic DNA and not from primer artifacts. Also, negative control was done for each primer/species combination. No amplification was detected in the control reactions. All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Table

4 and Figure 1). The RAPD fingerprint was used for the detection of the genetic diversity between *M. cephalus* and *L. ramada*. The results showed highly genetic dissimilarity range (0.00 to 1.00) with an average of 0.437 using different random primers (Figure 1 and Table 4).

DISCUSSION

Morphometric characters and meristic counts have been the most widely used tool in racial studies in fish taxonomy (Anene, 1999). Meristic counts were much easier to evaluate and seem to be advantageous because most counts can be collected from live fish. However, meristic data alone may not provide the detail necessary to discern dissimilarities between different populations at the same species and sometimes at the same genus (North et al., 2002). According to Katselis et al. (2006), discriminate analysis in eight morphometric characters of the fry of four Grey mullets species in Western Greece showed that *M. cephalus* and *Chelon labrosus* were quite similar in morphology, while *L. aurata* and *Liza saliens* were rather different. The study also stated that the analysis of the morphometric variation of mullet fry could be used for their discrimination.

Furthermore, Akyol and Kinacigil (2001) found that the discriminate analysis in seven morphometric characters in adult specimens of Grey mullets showed that *L. saliens* and *L. aurata* were similar in forms, while *M. cephalus* and *C. labrosus* were rather different. Also, the results of morphological measurements on the *L. saliens* and *L. ramada*, obtained by Minos et al. (1994, 1995) supported these findings. The results of this study based on 34 morphometric character indices and 9 meristic counts are consistent with these findings as most of the morphometric character indices showed significant differences between *M. cephalus* and *L. ramada*, while most of the meristic counts showed insignificant differences between *M. cephalus* and *L. ramada.*

The main advantages of RAPD markers are the

Table (3): Means and standard error of quantitative phenotype traits based on morphometric character indices and meristic counts used for differentiation analysis between *Mugil cephalus* and *Liza ramada*.

Characters	*Mugil cephalus* Mean±S.E.	*Liza ramada* Mean±S.E.	*t*-test
Morphometric analysis			
BD/SL	23.87±0.21	21.55±0.21	*
BW/SL	14.98±0.13	11.75±0.09	*
HL/SL	24.68±0.21	27.60±0.29	*
HW/HL	67.55±0.57	54.68±0.60	*
AL/SL	45.55±0.24	43.37±0.26	*
CPL/SL	16.91±0.24	17.36±0.18	NS
CPD/SL	11.45±0.12	10.37±0.14	*
CPW/SL	6.56±0.09	5.51±0.10	*
UJL/HL	21.87±0.42	16.94±0.31	*
LJL/HL	19.70±0.38	15.46±0.27	*
SnL/HL	21.97±0.39	16.90±0.35	*
OD/HL	22.59±0.48	23.02±0.34	NS
Pr-OL/HL	21.89±0.45	22.56±0.30	NS
Po-OL/HL	58.99±0.52	57.96±0.58	NS
IDD/SL	15.23±0.35	13.00±0.23	*
Sn-FD/SL	47.98±0.30	51.56±0.23	*
TrL/SL	58.84±0.23	56.55±0.27	*
FDFBL/SL	11.06±0.29	10.19±0.18	*
SDFBL/SL	10.80±0.19	8.33±0.08	*
Pel FBL/SL	11.00±0.22	9.81±0.08	*
AFBL/SL	12.38±0.22	11.85±0.11	*
CFL/TL	20.79±0.16	20.68±0.16	NS
Pec FL/SL	17.69±0.20	17.05±0.22	*
LLoFDFS/SL	13.23±0.23	15.48±0.16	*
LLaFDFS/SL	4.92±0.14	4.79±0.12	*
LLoSDFR/SL	13.43±0.26	13.39±0.16	NS
LLaSDFR/SL	6.67±0.10	5.62±0.12	*
LLoAFS/SL	7.76±0.14	10.28±0.23	*
LLoAFR/SL	13.26±0.26	13.76±0.19	NS
LLaAFR/SL	6.65±0.12	5.96±0.12	*
LLoCFR/TL	19.66±0.19	18.95±0.17	*
LShCFR/TL	8.82±0.12	8.79±0.16	NS
Meristic analysis			
FDFSC	4.00±0.00	4.00±0.00	NS
SDFRC	9.00±0.00	9.00±0.00	NS
Pel FSC	1.00±0.00	1.00±0.00	NS
Pel FRC	5.00±0.00	5.00±0.00	NS
Pec FRC	16.28±0.11	16.00±0.00	*
AFSC	3.00±0.00	3.00±0.00	NS
AFRC	7.94±0.06	9.04±0.04	*
CFRC	16.00±0.00	16.00±0.00	NS
TNN	24.00±0.00	24.00±0.00	NS
Length-weight relationship			
K	0.92±0.01	0.72±0.01	*

t-test: * $P<0.05$ NS: not significant.

Table 4. Total number of band, polymorphic bands and genetic dissimilarity between *Mugil cephalus* and *Liza ramada* using different random sequence of primers.

Primer number	Total band	Polymorphic band	Genetic dissimilarity
1	8.00	2.00	0.25
2	7.00	5.00	0.71
3	8.00	4.00	0.50
4	6.00	2.00	0.33
5	8.00	0.00	0.00
6	5.00	3.00	0.60
7	11.00	5.00	0.45
8	6.00	2.00	0.33
9	5.00	1.00	0.20
10	2.00	2.00	1.00
Average	-	-	0.437
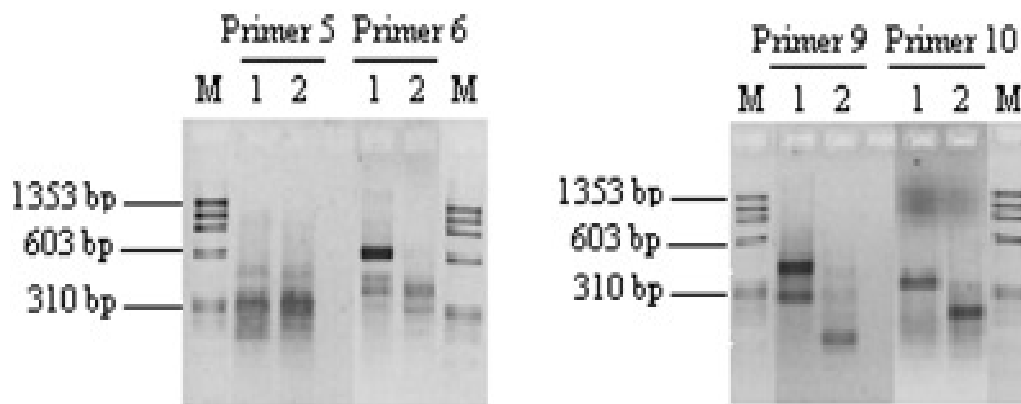
Figure 1. Example of RAPD amplification products. Lane M: ΦX174 DNA marker, the lanes 1 and 2 of each primer are *M. cephalus* and *L. ramada*, respectively.

possibility of working with anonymous DNA and the relatively low expense, also fast and simple to produce RAPD marker (Hadrys et al., 1992; Elo et al., 1997; Ali et al., 2004). Moreover, RAPD analysis might be useful for systematic investigation at the level of species and subspecies (Bardakci and Skibinski, 1994), and more sensitive and technically easier to perform and produced results with low statistical error, whereas DNA fingerprinting detected greater genetic differentiation between Nile tilapia stains than other molecular techniques such as multilocus minisatellite marker (Naish et al., 1995).

The results of this study are consistent with the findings reported by (Papasotiropoulos et al., 2001, 2002, 2007; Blel et al., 2008; Semina et al., 2007), they applied several techniques for genetic analysis among *Mugilidae* family and reported that *M. cephalus* is the most distinct species compared with *Liza* genus. Also, the results confirmed that the amount of differences in genotype reflected the same amount of differences in phenotype between *M. cephalus* and *L. ramada*. In addition, these results stated that genetic analysis based on RAPD fingerprinting confirm the existing taxonomic system based on morphometric character indices and meristic counts and confirmed the results reported by Li et al. (1993) and Louis and Barlow (1987).

The major limitation of morphological characters at the intra-specific level is that phenotypic variation is not directly under genetic control but subjected to environmental modification (Clayton, 1981). Therefore, it can be use either phenotype analysis based on a large number of morphometric character indices and meristic counts or genotype analysis based on RAPD finger-printing to discriminate *M. cephalus* and *L. ramada* with the same results.

RGP-VPP-010.

REFERENCES

Ahmed MMM, Ali BA, El-Zaeem SY (2004). Application of RAPD markers in fish: Part I- Some genera (*Tilapia, Sarotherodon* and *Oreochromis*) and species (*Oreochromis aureus* and *Oreochromis niloticus*) of Tilapia. Int. J. Biotechnol. 6(1): 86-93.

Akyol O, Kinacigil T (2001). Comparative body and otolith morphometrics of Mugilidae in Homa Lagoon (Izmir Bay, Aegean Sea). Acta. Adriatica, 42(2): 3-13.

Ali BA, Huang T, Qin D, Wang X (2004). A review of random amplified polymorphic DNA (RAPD) markers in fish research. Reviews Fish Biol. Fisheries, 14: 443-453.

Allendorf FW (1988). Conservation biology of fishes. Conserv. Biol. 2: 145-148.

Anene A (1999). Morphometric and meristic description of *Tilapia mariae* (Boulenger, 1901) and *Tilapia zillii* (Gervais, 1848) from the Umuoseriche Lake in the freshwater reaches of the Niger delta floodplains. Acta. Hydrobiol. 41: 211-218.

Avsar D (1994). Stock differentiation study of the sprat off the southern coast of the Black sea. Fish. Res. 19: 363-378.

Bagenal TB (1978). Methods for assessment of fish production in fresh waters (3rd ed). Oxford- London, Blackwell Sci. Publ. p. 365.

Bardakci F, Skibinski DOF (1994). Application of the RAPD technique in tilapia fish: species and subspecies identification. Heredity, 73: 117-123.

Barriga-Sosa DLA, Jimenez-Badillo MDL, Ibanez AL, Arredondo-Figueroa JL (2004). Variability of Tilapias (*Oreochromis* Spp.) introduced in Mexico: morphometric, meristic and genetic characters. J. Appl. Ichthyol, 20: 7-14.

Bartfai R, Egedi S, Yue GH, Kovacs B, Urbanyi B, Tamas G, Horvath L, Orban L (2003). Genetic analysis of two common carp broodstocks by RAPD and microsatellite markers. Aquaculture, 219: 157-167.

Bembo DG, Carvalho GR, Cingolani N, Ameri E, Gianetti G, Pitcher TJ (1996). Allozymic and morphometric evidence for two stocks of the European anchovy *Engraulis encrasicolus* in Adriatic waters. Mar. Biol. 126: 529-538.

Beheregaray LB, Levy JA (2000). Population genetics of the silverside *Odontesthes argentinensis* (Teleostei, *Atherinopsidae*): evidence for speciation in an estuary of southern Brazil. Copeia, pp. 444-447.

Blel H, Chatti N, Besbes R, Farjallah S, Elouaer A, Guerbej H, Said K (2008). Phylogenetic relationships in grey mullets (*Mugilidae*) in a Tunisian lagoon. Aquacult. Res. 39: 268-275.

Costa JL, De Almeida PR, Costa MJ (2003). A morphometric and meristic investigation of Lusitanian toadfish *Halobatrachus didactylus* (Bloch and Schneider, 1801): evidence of population fragmentation on Portuguese coast. Scientia Marina, 67: 219-231.

Clayton JW (1981). The stock concept and the uncoupling of organismal and molecular evolution. Can. J. Fish. Aquatic Sci., 38: 1515-1522.

Doherty D, McCarty TK (2004). Morphometric and meristic characteristics analysis of two western Irish populations of Arctic char, *Salvelinus alpinus* (L.). Biology Environment: Proceedings Royal Irish Academy, 104: 75-85.

Dong Z, Zhou E (1998). Application of the random amplified polymorphic DNA technique in a study of hetrosis in commen carp, *Cyprinus carpio* L. Aquacult. Res. 29: 595-600.

El-Deeb SI, Zowail MEM, El-Serafy SS, El-Sayed HE (1996). Genetic variability of Mugil cephalus in freshwater and marine habitate. J. Agric. Sci. Mansura Univ. 21: 2093-2101.

Elo K, Ivanoff S, Vuorinen JA, Piironen J (1997). Inheritance of RAPD markers and detection of interspecific hybridization with brown trout and Atlantic salmon. Aquaculture, 152: 55-65.

El-Sayed AEM (1991). Protein requirements for optimum growth of *Liza ramada* fry (*Mugilidae*) at different water salinities. Aquat. Living Resour. 4: 117-123.

El-Zaeem SY, Ahmed MMM (2006). Genetic differentiation between sex reversal and normal of full-sib Nile Tilapia (*Oreochromis niloticus*) based on DNA finger printing. Res. J. Fish. Hydrobiol. 1: 1-5.

El-Zaeem SY, Ali BA, Ahmed MMM (2006). Random amplified polymorphic DNA fingerprint and genetic similarity among four genera and between two phenotypes of cultured carps in Egypt. Int. J. Agric. Biol. 8: 111-115.

Ezzat AA, Hashem MJ, El Gharabawy MM (1979). Biometric variations in (*Solea vulgaris*) acclimatized in Lake Quarum, Upper Egypt. J. Fish Biol. 14: 39-46.

Haddon M, Willis TJ (1995). Morphometric and meristic comparison of Orange Roughy (*Hoplostethus attanticus*, *Trachichthyidae*) from the puysegur Bank and Lord-Howe-Rise, New-Zealand, and its implications for stock structure. Mar. Biol. 123: 19-27.

Hadrys H, Balick M, Schierwater B (1992). Applications of random amplified polymorphic DNA (RAPD) in molecular ecology. Mol. Ecol. 1: 55-63.

Ihssen PE, Booke HE, Casselman JM, McGlade JM, Payne NR, Utter FM (1981). Stock identification: Materials and methods. Can. J. Fish. Aquat. Sci. 38: 1838-1855.

Katselis G, Hotos G, Minos G, Vidalis K (2006). Phenotypic affinities on fry of four Mediterranean Grey Mullet species. Turk. J. Fish. Aquat. Sci. 6: 49-55.

Lagler KF (1956). Fresh water fishery biology. W.M.C. Brown Company Publishers, Dubuque, Iowa, p. 421.

Lee CS, Tamaru CS (1988). Advances and future prospects of controlled maturation and spawning of grey mullet (*Mugil cephalus* L.) in captivity. Aquaculture, 74: 63-73.

Li S, Cai W, Zhou B (1993). Variation in morphology and biochemical genetic markers among populations of blunt snout bream (*Megalobrama amblycephala*). Aquaculture, 111: 117-127.

Lindsey CC (1988). Factors controlling meristic variation. In Fish physiology, Vol. XI-B (Hoar,W.S. & Randall, D.J., eds), San Diego, CA: Academic Press. pp. 197-274.

Louis VLST, Barlow JC (1987). Comparisons between morphometric and genetic differentiation among populations of the Eurasian tree sparrow (*Passer montanus*). Wilson Bull. 4: 628-641.

Menezes MR, Martins M, Naik S (1992). Interspecific genetic divergence in grey mullets from the Gao region. Aquaculture, 105: 117-129.

Minos G, Katselis G, Kaspiris P, Ondrias I (1994). The differential increase of the morphometrical characters during the growth of the Grey mullets, *Liza ramada* (Risso) and *Liza saliens* (Risso) in Messolongi-Etoliko lagoon. Bios (Macedonia, Greece), 2: 149-154.

Minos G, Katselis G, Kaspiris P, Ondrias I (1995). Comparison of the change in morphological pattern during the growth in length of the Grey mullets, *Liza ramada* and *Liza saliens* from Western Greece. Fish. Res. 23: 143-155.

Naesje TF, Vuorinen JA, Sandlund OT (2004). Genetic and morphometric differentiation among sympatric spawning stocks of white fish (*Coregonus lavaretus* L.) in Lake Femund, Norway. J. Limnol. 63: 233-243.

Naish KA, Warren M, Bardakci F, Skibinski DO, Carvalho GR, Mair GC (1995). Multilocus DNA fingerprinting and RAPD reveal similar genetic relationships between strains of *Oreochromis niloticus* (Pisces: *Cichlidae*). Molecular Ecol. 4: 271-274.

Nei M, Li WH (1979). Mathematical modeling for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 76: 5269-5273.

North JA, Farr RA, Vescei P (2002). A comparison of meristic and morphometric characters of green sturgeon, *Acipencer medirostris*. J. Appl. Ichthyol., 18: 234-239.

Oren OH (1981). Aquaculture of Grey Mullets. Cambridge University Press, Cambridge, p. 507.

Papasotiropoulos V, Klossa-Kilia E, Kilias G, Alahiotis S (2001). Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: *Mugilidae*) using allozyme data. Biochem. Genet., 39: 155-168.

Papasotiropoulos V, Klossa-Kilia E, Kilias G, Alahiotis S (2002). Genetic divergence and phylogenetic relationships in grey mullets (Teleostei: *Mugilidae*) based on PCR-RFLP analysis of mtDNA segments. Biochem. Genet., 40: 71-86.

Papasotiropoulos V, Klossa-Kilia E, Alahiotis S, Kilias G (2007). Molecular phylogeny of grey mullets (Teleostei: Mugilidae) in Greece: evidence from sequence analysis of mtDNA segments. Biochem. Genet., 45: 623-636.

Partis L, Wells RJ (1996). Identification of fish species using random amplified polymorphic DNA (RAPD). Mol. Cell. Probes, 10: 435-441.

Sahu BB, Meher PK, Mohanty S, Reddy PVGK, Ayyappan S (2000). Evaluation of the Carcass and Commercial Characteristics of Carps. Naga, The ICLARM Quarterly, 23(2): 10-14.

Semina AV, Polyakova NE, Brykov VA (2007). Analysis of mitochondrial DNA: taxonomic and phylogenetic relationships in two fish taxa (Pisces: *Mugilidae* and *Cyprinidae*). Biochemistry-Russia, 72: 1349-1355.

Sneath PHA, Sokal RR (1973). Numerical taxonomy: the principles and practice of numerical classification. San Francisco: WH Freeman, p. 573.

Snedecor GW, Cochran WG (1980). Statistical methods, 7th edition, Iowa state Univ. Press, Ames, IA, USA. p. 507.

StatSoft (1995). STATISTICA/w User's Guide-Tulsa, OK: StatSoft, Inc.

Swaine DP, Ridell BE, Murray CB (1991). Morphological differences between hatchery and wild populations of coho salmon (*Oncorhynchus kisutch*): environmental versus genetic origin. Can. J. Fish. Aquat. Sci. 48: 1783-1791.

Talwar PK, Jhingran AG (1992). Inland fishes of India. Rec. Ind. J. 3: 19-24.

Taniguchi N, Yamasaki M, Takagi M, Tsujimura A (1996). Genetic and environmental variances of body size and morphological traits in communally reared clonal lines from gynogenetic diploid ayu, *Plecoglossus altivelis*. Aquaculture, 140: 333-341.

Turan C (1999). A note on the examination of morphometric differentiation among fish population: The truss system. Trend J. Zool., 23: 259-263.

Welsh J, McCelland M (1990). Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acid Res. 18: 7213-7218.

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18: 6531-6535.

Williams JGK, Hanafey MK, Rafalaski JA, Tingey SV (1993). Genetic analysis using RAPD markers. Methods Enzymol. 218: 704-740.

Zafar M, Nazir A, Akhtar N, Naqvi SMHM, Zia-ur-Rehman M (2002). Studies on meristic counts and morphometric measurments of Mahseer (Tor putitora) from a spawning ground of himalayan Foot-hill River korang Islamabad, Pakistan. Pakistan J. Biological Sci. 5(6): 733-735.