EJAR

DNA fingerprinting of some tilapia fish in Suez Canal zone

Mohamed E. Megahed[1*], Mahmoud M. Rifaat[2], Mohamed A. EL Danasoury[1], Safaa M. Sharaf[1]

[1]*Department of Animal Production and Fish Resources, Faculty of Agriculture, Suez Canal University, Ismailia, 41522.*
[2]*Department of Plant Genetics, Faculty of Agriculture, Suez Canal University, Ismailia, 41522.*
**Email: melsayedmam@yahoo.com*

Received 9th December 2010, Accepted 3rd March 2010

Abstract

This study was conducted on samples collected from three wild environments with different salinities in the Suez Canal zone involving four species *Oreochromis niloticus*, *Oreochromis aureus*, *Sarotherodon galilaeus* and *Tilapia zilli*. Random Amplified Polymorphic DNA (RAPD PCR) using ten decamers primers was conducted on representative samples from the four species collected from the three different environments in the Suez Canal zone zilli fresh (ZF), zilli brakish (ZB), Zilli saline (ZS), niloticus fresh (NF), niloticus brakish (NB), aureus fresh (AF), aureus brakish (AB), galilaeus fresh (GF), and galilaeus brakish (GB). 340 bands were identified in the nine populations. The RAPD patterns generated by primers (OPA-03) and (OPA-19) were together sufficient to distinguish the nine tilapia populations. The discrimination indices of primers 1 and 6 were 0.89 and 0.83 respectively. The major components of variance for RAPD markers accounted for 72 % of the variance. The use of two ways clustering revealed that certain RAPD markers are associated with adaptation of the genomes to salinity or brackish water conditions. These markers will be useful in future experiments on MAS breeding for tolerance to salinity in Nile Tilapia.

Keywords: DNA Fingerprinting, RAPD-PCR, Tilapia, salinity, Suez Canal.

1. Introduction

Tilapias are the most popular and common cultivated fish in Egypt. Recently, attention has been paid to produce tilapia strains more tolerant to environmental conditions, especially salinity (such as red tilapia). In general, tilapias are mostly fresh water species but the production of tilapia strains in brackish or sea water is needed (Payne, 1983). A simple procedure based on Random Amplified Polymorphic DNA (RAPD) markers has been found useful for revealing the genetic differences within as well as among fish populations (Ali, 2003; Ali *et al*., 2004; Du *et al.,* 2005). RAPD markers have found a wide range of applications in gene mapping, population genetics, molecular evolutionary genetics and plant and animal breeding (Bartish *et al*., 1999 & 2000).

A main objective of management of tilapia species in the Suez Canal zone is the elucidation of the population structure and the preservation of the genetic resources within and between the populations. Management is rather simple to apply if the fish stocks are separated from each other, such as salmon. Salmon populations are separated according to spawning grounds. Many species of fish are separated by occupying separate inland bodies of waters. Stocks in these cases can be managed and harvested separately (Allendorf *et al.,* 1987; and Koeller, 2003). Management becomes complicated if the stocks congregate in mixed populations. In such situations, identification of each contributing population should be accomplished first. Pella and Milner. (1987) and Kaewsanagk *et al.:* (2000) presented the procedure for this analysis, called "The stock composition analysis using genetic markers". By this procedure, the distribution of each contributing population within the mixed stocks can be estimated, and therefore, regulation of harvests to protect weaker populations can be made.

In spite of the commercial importance and increasing demand of tilapia species, little is known about the genetic characteristics of wild and cultured populations of tilapia strains in Egypt. This study involves four tilapia (*Oreochromis niloticus, Oreochromis aureus, Sarotherodon galilaeus and Tilapia zilli*) species collected during geographical survey from wild environments in the Suez Canal zone, namely; Ismailia Fresh Water Canal, Western Lagoon and Lake Timsah. The aim of this study was to characterize at the DNA level, the differences among tilapia species collected from the three different wild environments from Suez Canal zone.

2. Materials and methods

2.1. Sites survey, Fish sampling and Hydrographical Parameters

In this study, four tilapia species; namely; Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), white tilapia (*Sarotherodon galilaeus*), and green tilapia (*Tilapia zilli*) were sampled. These species were collected from three environments in the Suez Canal zone (Table 2; Figure 1) namely; Ismailia freshwater canal, western lagoon, and Lake Timsah (green tilapia only). In order to determine the extent of tilapia production and to select the sampling environments, a general survey of the Suez Canal zone was undertaken. Three different environments were chosen (Ismailia freshwater canal, Western Lagoon and Lake Timsah) in Suez Canal zone which were the main fishing ground of the species concerned and characterized by the presence of fishing activities. The field survey provides basic data and information about the investigated environments, as well as, the distribution and population structure of the species. Water quality parameters; Dissolved oxygen (DO), pH, Water temperature (WT), Salinity (SAL) and chlorophyll a (Chlo a) were recorded for each of the selected sampling sites. All measurements were done according to (Boyd, 1979; AOAC, 1990; and Boyd and Tucker, 1992).

2.2. DNA extraction

DNA was extracted from fin tissue following the method described by Bardacki and Skibinski (1994) with minor modification. Approximately 1 centimeter of a fin tissue was cut into small pieces and suspended in 500 µl STE (0.1 M NaCl, 0.05 M Tris and 0.01 M EDTA. pH 8). After adding 30µl of 10% SDS and 30 µl of proteinase k (10 mg/ml), the mixture was incubated at 50°C for 30 min. DNA was purified by successive extraction with phenol, phenol: chloroform: isoamyl alcohol (25:24:1) and chloroform: isoamyl alcohol (24:1), respectively. The DNA was precipitated with ice-cold absolute ethanol and washed with 70% ethanol. The pellet was dried and resuspended in 200 µl TE buffer (EDTA 1.0 mM; Tris-HCl 10 mM, pH=8).

2.3. RAPD analysis

RAPD PCR analysis was performed on selected samples representing each of the nine tilapia populations. Ten primers (Operon, A Qiagen Company, Qiagen GmbH, Germany) were used to initiate PCR amplification (Table 2). The conditions that were considered optimal for obtaining accurate amplified band profiles with these primers were as follows: the reaction mixture (25 µl) contained 1X Taq polymerase buffer, 50 ng of genomic DNA, 2 mM $MgCl_2$, 0.2 mM of each dNTPs, 5 pmol of a single 10–base primer, and 0.5 unit of Taq DNA polymerase.

Table 1. Sampling environments and site coordinates for the nine populations (four tilapia species) used in this study.

Sampling environments	Site co-ordinates	Species	ID
Ismailia Fresh Water Canal	30° 35' 21.97"N and 32° 16' 59.57"E	*O.niloticus*	NF
		O.aureus	AF
		S.galilaeus	GF
		T.zilli	ZF
Western Lagoon (Brackish)	30°34' 33.47"N and 32° 16' 9.91"E	*O.niloticus*	NB
		O.aureus	AB
		S.galilaeus	GB
		T.zilli	ZB
Lake Timsah (Saline)	30°34'41.13"N and 32° 17'27.86"E	*T.zilli*	ZS

Table 2. Primer code, sequence and GC% of the RAPD primers used.

Primer code	Sequence (5' to 3')	GC%	MW
OPA-03	AGTCAGCCAC	60	3043
OPA- 04	AATCGGGCTG	60	3098
OPA-07	GAAACGGGTG	60	3163
OPA-10	GTGATCGCAG	60	3098
OPA-13	CAGCACCCAC	70	3003
OPA-19	CAGCACCCAG	70	3043
OPB-10	GGACTGGAGT	60	3138
OPB-03	GTGAGGCGTC	70	3114
OPC-11	AAAGCTGCGG	60	3123
OPC-13	AAGCCTCGTC	60	3017

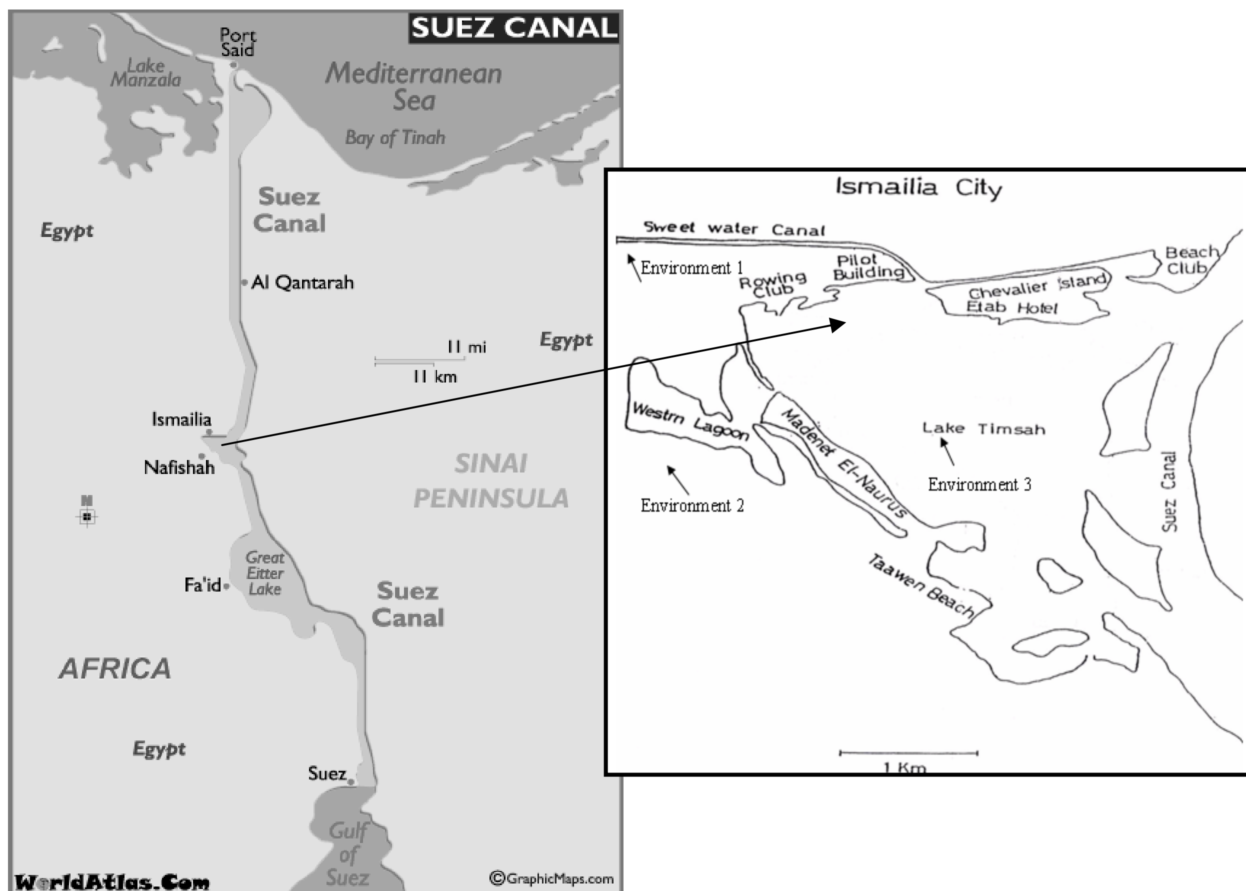

Figure 1. Map of Suez Canal Zone showing the three sampling environments.

Amplification was carried out in Eppendorf Thermocycler for 45 cycles, each consisting of a denaturing step for 1 minute at 94°C, followed by annealing step for 1 minute at 36°C and extension step for 2 minutes at 72°C. The last cycle was followed by 5 minutes of extension at 72°C. The amplification products were separated by gel electrophoresis in 1.5% agarose (Seakem GTG agarose; FMC Biproducts, Rockland, Maine, USA) in 0.5 X TBE (45 mM Tris-borate, 1 mM EDTA) containing ethidium bromide (0.5 µg/ml) at a constant voltage of 5 V/cm. After electrophoresis, the gel was visualized by UV transillumnation, and photographed using a digital camera in a gel documentation system (Alpha Innotech, CA, USA).

2.4. Statistical analysis

RAPD bands were scored as discrete variables, using "1" to indicate presence and "0" to indicate absence of a band at a particular position on the gel. The discrimination index (DI) was calculated for each primer by using Simpson's index of diversity as described by Hunter and Gaston (1988). The similarities between DNA fingerprints were calculated with the band-matching Jaccard's coefficient that ranges from 0 to 1.0, where 1.0 represents 100% identity (presence and position) for all bands in the fingerprints being compared.

A pairwise similarity (or distance) matrix was developed and cluster analysis was performed using this matrix with either the Unweighed Pair Group Method with Arithmatic averages (UPGMA) or the Neighbour Joining (NJ) methods. All computations were performed using the Alphaease FC Stand Alone (v.4.0) software (Alpha Innotech, CA, USA). Principal component analysis was performed on RAPD markers by using the FORTRAN computer program, (NTSYSpc-2.01a program Applied Biostatistcis, Inc., USA), and Cluster and Tree View programs (Eisen *et al.,* 1998).

3. Results

3.1. Hydrographical parameters

The comparison of hydrographical parameters among the three Suez Canal zone environments showed that water temperature (WT) in the Western Lagoon was significantly higher than those of Ismailia fresh water canal and Lake Timsah. The pH did not differ significantly between Ismailia fresh water canal and Western Lagoon, but was higher in Lake Timsah. The dissolved oxygen (DO) and Salinity (SAL) in Lake

Timsah were significantly higher than those of Western Lagoon and Ismailia Canal , Chlorophyll a (Chlo a) in Western Lagoon or Lake Timsah was significantly higher than that of Ismailia freshwater canal (Table 3).

Table 3. Mean and standard error (SE) for four water quality parameters (WT, SAL, DO, pH, and Chlo a) from 108 observations taken from the wild environment in Suez Canal zone.

Environments	WT[1] (C°)	pH	DO[2] (mg/l)	SAL[3] (ppt)	Chlo a[4] Mean (µg/l)
Ismailia Fresh water Canal	24.94[a] ± 0.30	7.59[a] ± 0.04	4.37[b] ± 0.09	0.18[c] ± 0.03	147.44[b] ± 18.39
Western Lagoon	25.88[a] ± 0.35	7.49[a] ± 0.03	3.76[c] ± 0.15	16.18[b] ± 0.30	426.82[a] ± 29.43
Lake Timsah	23.81[b] ± 0.44	8.12[c] ± 0.01	6[a] ± 0.18	34.13[a] ± 0.57	403.58[a] ± 58.10

[1] Water temperature, [2] Dissolved oxygen, [3] Salinity, [4] Chlorophyll a

3.2. Tilapia profiling by all primers

The total number of bands amplified from the nine tilapia samples using the ten primers was 340. The total number of polymorphic bands resolved from all tilapia samples (Figures 2, 3 and 4) was 311 (311/340 = 91.5%). The number of polymorphic bands per primer ranged from 19 bands for primer 3 with 9 (9/19 = 47%) polymorphic bands and primer 8 with 15 (15/19 = 79%) polymorphic bands to 54 bands for primer 10 with 100% polymorphic bands. The discrimination index for RAPD primers varied from 0.23 for primer 3 to 0.89 for primer 1 (Table 4). Table (5) shows the total number of bands and polymorphic bands amplified from each tilapia population by using all RAPD primers. *Tilapia zilli* sampled from Western Lagoon (ZB) showed the highest number of amplified RAPD bands (147 bands), the highest number of polymorphic bands (118 bands or 11.8 polymorphic bands per primer), and the highest percentage of polymorphic bands (118/147 = 80.3%). On the other hand, *Oreochromis niloticus* sampled from Ismailia Fresh Water Canal (NF) showed the lowest number of amplified bands (123 bands), the lowest number of polymorphic bands (94 bands or 9.4 polymorphic bands per primer), and the lowest percentage of polymorphic bands (94/123 = 76.4%).

Figure (5) shows a dendrogram constructed from the pairwise comparisons of Jaccard's similarity coefficients calculated based on the 340 bands (311 bands). *Tilapia zilli* sampled from Western Lagoon (ZB) and Lake Timsah (ZS) were closely clustered (Jaccard's similarity coefficient of 0.72). The two *Tilapia zilli* samples formed a group that displayed a lower similarity (0.65) to *Tilapia zilli* sampled from Ismailia Fresh Water Canal (ZF). *Oreochromis aureus* collected from Ismailia FreshWater Canal (AF) or Western Lagoon (AB) displayed a Jaccard's similarity coefficient of 0.64 and was more closely related to *Sarotherodon galilaeus* collected from Ismailia Fresh Water Canal (GF) than to *Sarotherodon galilaeus* collected from Western Lagoon (GB). *Oreochromis niloticus* (NB) and *Sarotherodon galilaeus* (GB) collected from Western Lagoon were clustered with a Jaccard's similarity coefficient of only 0.44.

Table 4. Discrimination index (D) and number of monomorphic and polymorphic RAPD amplicons produced by each primer for the nine samples of tilapia.

Primer	D	Number of RAPD Markers		Total
		Monomorphic	Polymorphic	
1	0.89	1	47 (98%)	48
2	0.75	0	45 (100%)	45
3	0.23	10	9 (47%)	19
4	0.75	2	36 (95%)	38
5	0.56	9	15 (63%)	24
6	0.83	0	27 (100%)	27
7	0.75	0	26 (100%)	26
8	0.56	4	15 (79%)	19
9	0.64	3	37 (93%)	40
10	0.81	0	54 (100%)	54
Total		29	311 (91.5%)	340

When the PCR patterns generated by the two RAPD primers OPA-03 and OPA-19 were combined, each of the nine tilapia samples had a unique RAPD profile (Table 6). The D values for primers 1 and 6 were 0.89 and 0.83, respectively. However, the combined index of discrimination (D) for RAPD primers 1 and 6 was 1 according to Simpson's index of diversity. Likewise, each of the nine tilapia samples displayed a unique RAPD profile when the PCR patterns generated by RAPD primers OPA-04, OPA-10, OPB-10, and OPC-13 were combined. The D values for primers 2, 4, 7, and 10 were 0.75, 0.75, 0.75, and 0.81, respectively. The combined index of discrimination for RAPD primers 2, 4, 7, and 10 was 1 according to Simpson's index of diversity. The 311 polymorphic bands observed in the nine fish samples (ZF, ZB, ZS, NF, NB, GF, GB, AF, and AB) were subjected to Principal Component Analysis (PCA), using the computer program NTSYS-pc (version 2.0) as described under Materials and Methods. The RAPD marker loci were treated as qualitative variables. The use of PCA is helpful to further resolve the grouping of the various populations of tilapia.

The number of components extracted; i.e. 9 was equal to the number of populations being analyzed (Table 6). The first principal component accounted for a maximal amount of variance (46.94%). The second principal component accounted for 15.32% of the total variability. This component is uncorrelated with the first component and accounts for variance in the data set that was not accounted for by the first component. The second component will be correlated with some of the observed variables that did not display strong

Table 5. Total number of bands and polymorphic bands amplified from each population by using RAPD Primers

	Populations																	
Primer	ZF	ZB	ZS	NF	NB	GF	GB	AF	AB	ZF	ZB	ZS	NF	NB	GF	GB	AF	AB
	Number of bands									Number of polymorphic bands								
1	A_1	A_1	B_1	E_1	F_1	C_1	A_1	D_1	D_1	A_1	A_1	B_1	E_1	F_1	C_1	A_1	D_1	D_1
	19	19	14	7	13	10	19	15	15	18	18	13	6	12	9	18	14	14
2	A_2	A_2	A_2	D_2	C_2	C_2	C_2	B_2	A_2	A_2	A_2	A_2	D_2	C_2	C_2	C_2	B_2	A_2
	9	9	9	15	15	15	15	15	9	9	9	9	15	15	15	15	15	9
3	A_3	A_3	A_3	A_3	A_3	A_3	A_3	A_3	B_3	A_3	A_3	A_3	A_3	A_3	A_3	A_3	A_3	B_3
	16	16	16	16	16	16	16	16	13	6	6	6	6	6	6	6	6	3
4	A_4	A_4	A_4	B_4	C_4	D_4	D_4	D_4	D_4	A_4	A_4	A_4	B_4	C_4	D_4	D_4	D_4	D_4
	19	19	19	9	14	15	15	15	15	17	17	17	7	12	13	13	13	13
5	A_5	A_5	A_5	A_5	A_5	B_5	B_5	B_5	B_5	A_5	A_5	A_5	A_5	A_5	B_5	B_5	B_5	B_5
	16	16	16	16	16	17	17	17	17	7	7	7	7	7	8	8	8	8
6	A_6	B_6	A_6	A_6	C_6	B_6	D_6	C_6	D_6	A_6	B_6	A_6	A_6	C_6	B_6	D_6	C_6	D_6
	7	10	7	7	10	10	9	10	9	7	10	7	7	10	10	9	10	9
7	A_7	A_7	B_7	C_7	D_7	D_7	D_7	A_7	A_7	A_7	A_7	B_7	C_7	D_7	D_7	D_7	A_7	A_7
	12	12	11	7	9	9	9	12	12	12	12	11	7	9	9	9	12	12
8	B_8	B_8	B_8	B_8	A_8	A_8	A_8	A_8	A_8	B_8	B_8	B_8	B_8	A_8	A_8	A_8	A_8	A_8
	14	14	14	14	9	9	9	9	9	10	10	10	10	5	5	5	5	5
9	A_9	C_9	C_9	C_9	C_9	B_9	C_9	B_9	B_9	A_9	C_9	C_9	C_9	C_9	B_9	C_9	B_9	B_9
	14	19	19	19	19	12	19	12	12	11	16	16	16	16	9	16	9	9
10	A_{10}	B_{10}	B_{10}	C_{10}	D_{10}	B_{10}	E_{10}	A_{10}	B_{10}	A_{10}	B_{10}	B_{10}	C_{10}	D_{10}	B_{10}	E_{10}	A_{10}	B_{10}
	17	13	13	13	14	13	13	17	13	17	13	13	13	14	13	13	17	13
	143	147	138	123	135	126	141	138	124	114	118	109	94	106	97	112	109	95
Percentage of Polymorphic Bands (%)										79.7	80.3	79	76.4	78.5	77	79.4	79	76.6
Average Number of Polymorphic Bands / Primer										11.4	11.8	10.9	9.4	10.6	9.7	11.2	10.9	9.5

correlations with component 1. The first three principal components accounted for 72.04% of the total variability based on RAPD polymorphisms in the studied tilapia samples. For the remaining components that were extracted, each component accounted for a maximal amount of variance that was not accounted for by the preceding components, and is uncorrelated with all of the preceding components.

Since the first two Principle Components PCs accounted for nearly 62.26% of the variation, the relationships among these tilapia samples can be visualized through two dimensional graphs of these two PC axes (Figures. 6A, B&C and 7). Either the first and second components or the first and third components could clearly separate the Nile tilapia (NF, and NB) and green tilapia (ZF, ZB, and ZS) samples (Figure. 6). The blue (AF, and AB) and white (GF, and GB) tilapia samples were maximally separated by using the second and third components (Figure 6A, B&C). All nine tilapia samples were best resolved by using the first three components as illustrated in Figure (7).

3.3. Two-Way cluster analysis

In addition of clustering a tilapia populations (1 – way), a clustering algorithm was also used to arrange the polymorphic RAPD markers according to the similarity in the pattern of RAPD amplification (2 – way).

Figure (8) shows the results of clustering of PCR amplicons according to their presence or absence in the tilapia genomes. The figure indicated that certain RAPD markers cluster similarly across the genomes studied. RAPD markers unique to ZF sample were 10b31, 10b27, 10b22, 10b03, 10b47, 10b42, 10b39, 10b32, and 10b37 (Figure 8A). RAPD markers unique to NB sample were 1b33, 1b32, 1b20, 1b10, 1b06, 1b01, 10b53, 10b52, 10b45, 10b34, 10b28, 10b25, 10b17, 10b08, 10b05, 10b02, 4b37, 4b30, 4b27, 4b25, 4b23, 4b20, 4b18, 4b11, 4b08, 1b47, and 1b38 (Figure 8B). RAPD markers unique to ZS sample were 1b23, 1b12, 1b09, 1b02, 7b26, 7b24, 7b20, 7b14, 7b12, 7b11, 7b07, 7b04, 1b37, and 1b28 (Figure 8C). Markers found only in GF were 1b27, 1b22, 1b14, and 1b11. Markers found only in GB are 10b10, 10b06, 10b44, 10b41, 10b53, 10b29, 10b24, 10b19, and 10b12. Markers found only in AF are 2b29, 2b26, 2b16, 2b13, 2b08, 2b05, 2b02, 2b01, 2b42, 2b39, 2b37, and 2b35. Markers unique to NF are 2b44, 2b41, 2b36, 2b34, 2b21, 2b15, 2b11, 2b07, 2b04, 1b24, 1b15, 10b54, 10b48, 10b46, 10b40, 10b38, 10b36, 10b33, 10b26, 10b21, 10b18, 10b01, 7b17, 7b09, 7b06, 7b02, 4b13, and 4b06. No RAPD markers were unique

Table 6. PCR patterns generated by each primer in each population.

	Eigenvalues	Percent	Cumulative
PC1	4.22519563	46.9466	46.9466
PC2	1.37882788	15.3203	62.2669
PC3	0.87987908	9.7764	72.0434
PC4	0.59871605	6.6524	78.6958
PC5	0.54394687	6.0439	84.7396
PC6	0.46668482	5.1854	89.9250
PC7	0.38156284	4.2396	94.1646
PC8	0.30762929	3.4181	97.5827
PC9	0.21755752	2.4173	100
Sum	9.0		

Table 7. Eigenvalues, percent of variance, and cumulative percentage of the nine principal components extracted in this study.

Primer	D[1]	ZF	ZB	ZS	NF	NB	GF	GB	AF	AB
1	0.89	A_1	A_1	B_1	E_1	F_1	C_1	A_1	D_1	D_1
6	0.83	A_6	B_6	A_6	A_6	C_6	B_6	D_6	C_6	D_6
10	0.81	A_{10}	B_{10}	B_{10}	C_{10}	D_{10}	B_{10}	E_{10}	A_{10}	B_{10}
2	0.75	A_2	A_2	A_2	D_2	C_2	C_2	C_2	B_2	A_2
4	0.75	A_4	A_4	A_4	B_4	C_4	D_4	D_4	D_4	D_4
7	0.75	A_7	A_7	B_7	C_7	D_7	D_7	D_7	A_7	A_7
9	0.64	A_9	C_9	C_9	C_9	C_9	B_9	C_9	B_9	B_9
5	0.56	A5	A5	A5	A5	A5	B5	B5	B5	B5
8	0.56	B_8	B_8	B_8	B_8	A_8	A_8	A_8	A_8	A_8
3	0.23	A_3	A_3	A_3	A_3	A_3	A_3	A_3	A_3	B_3

[1]D: the discrimination index

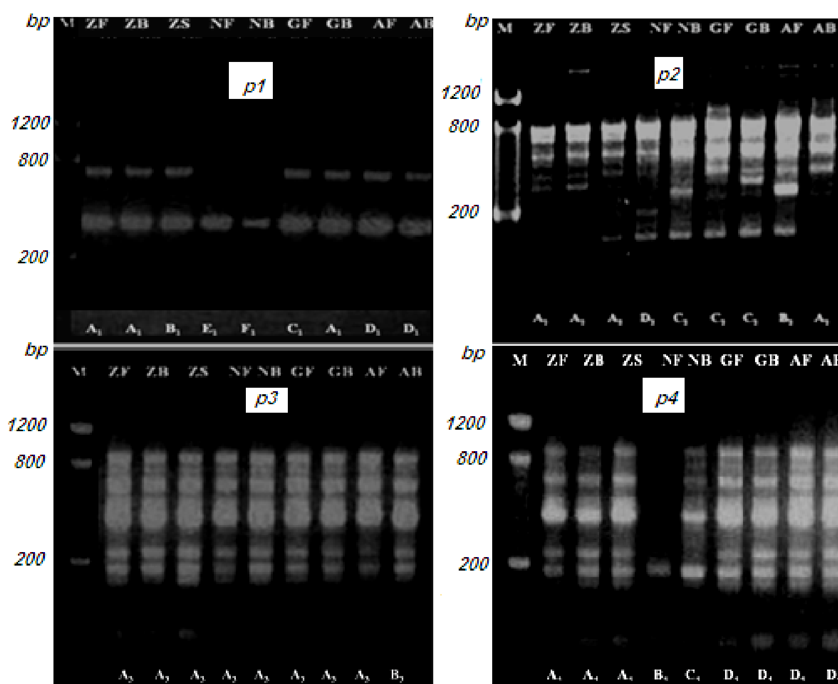

Figure 2. RAPD- PCR analysis of Amplification products of DNA from nine tilapia fish populations using primers P_1 (OPA03), P_2 (OPA04), P_3(OPA-07) and P_4 (OPA10). Lane M: Molecular size (100 bp DNA ladder). Lanes ZF, ZB, ZS, NF, NB, GF, GB, AF and AB represent fish samples from different locations.

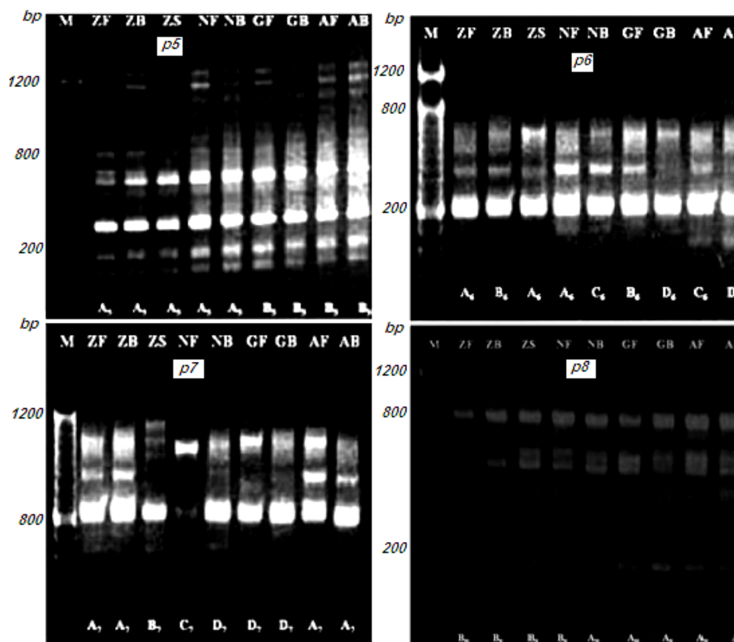

Figure 3. RAPD- PCR analysis of Amplification products of DNA from nine tilapia fish populations using primers P_5 (OPA-13), P_6 (OPA-19), P_7 (OPB-10) and P_8 (OPB-03). Lane M: Molecular size - marker (100 bp DNA ladder). Lanes ZF, ZB, ZS, NF, NB, GF, GB, AF and AB represent fish samples from different locations.

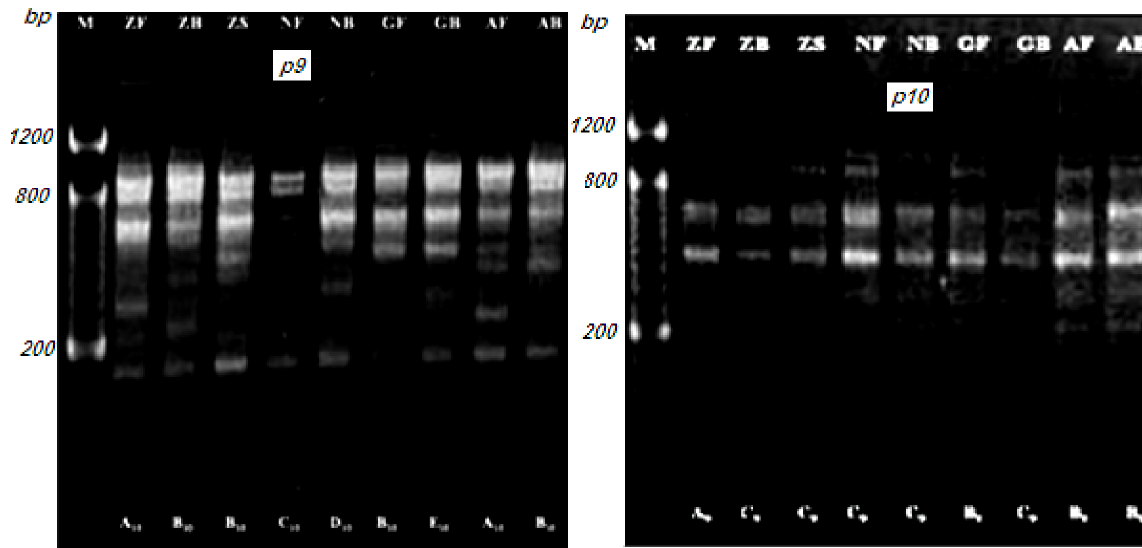

Figure 4. RAPD- PCR analysis of Amplification products of DNA from nine tilapia fish populations using primers P_9 (OPC-11), P_{10}(OPC-13). Lane M: Molecular size - marker (100 bp DNA ladder). Lanes ZF, ZB, ZS, NF, NB, GF, GB, AF and AB represent fish samples from different locations.

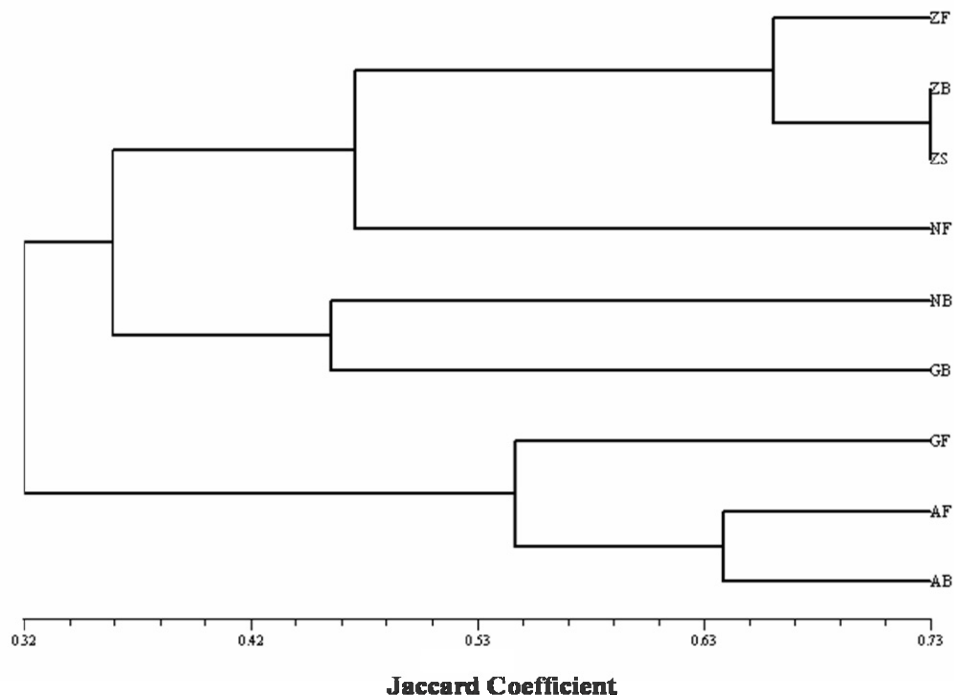

Figure 5. A dendrogram constructed from the Pair wise comparisons of Jaccard's similarity coefficients calculated based on 340 RAPD bands (311 polymorphic bands) using ten RAPD primers on each fish sample of all fish samples.

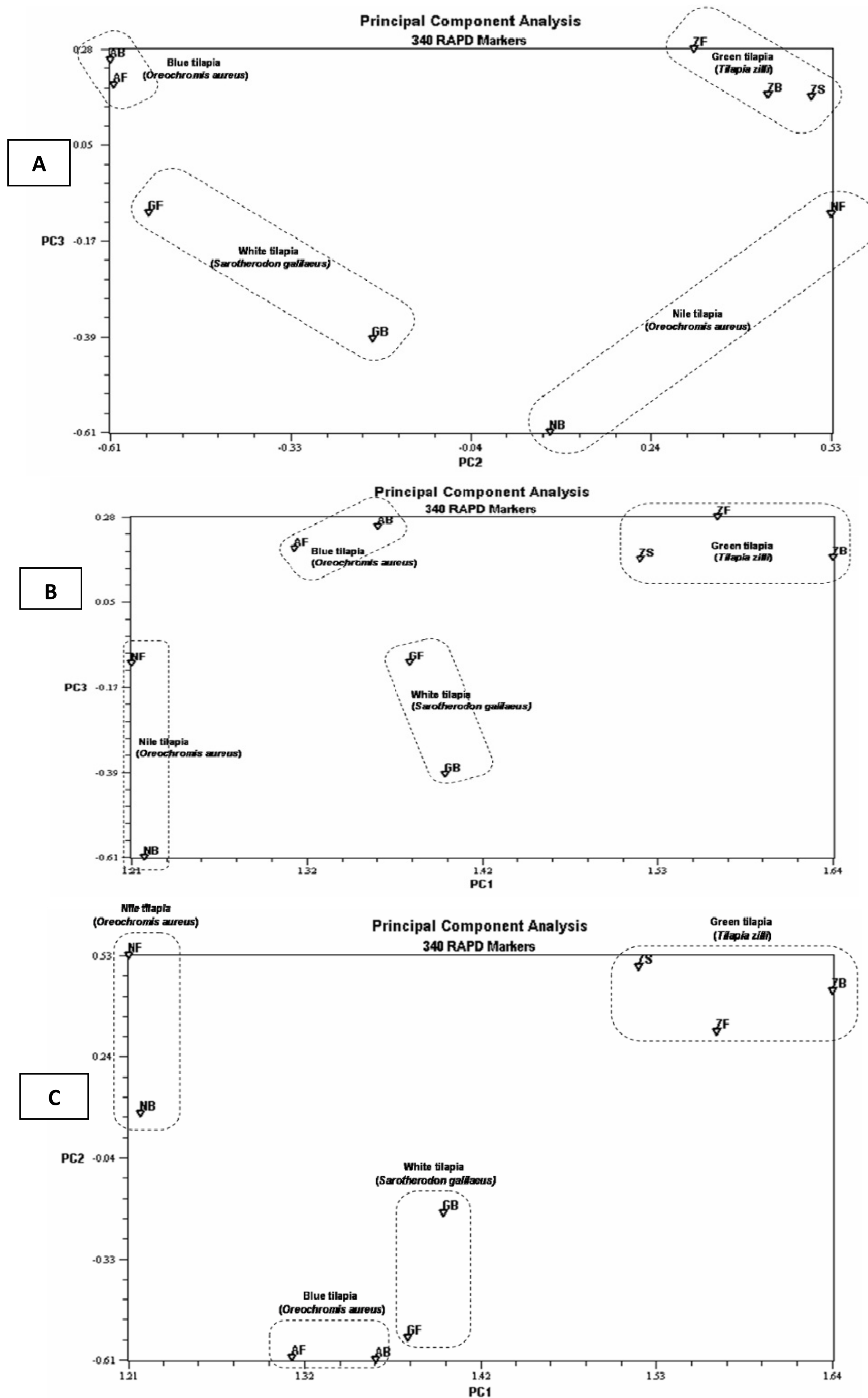

Figure 6. Principal Component Analysis (A) PCA 1&2 (B) PCA 1&3 (C) PCA 2&3

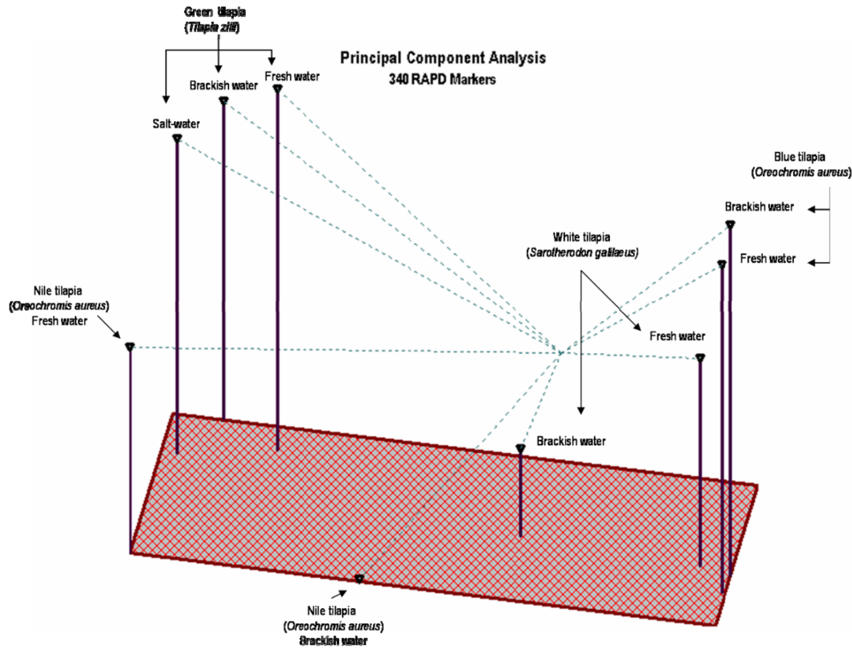

Figure 7. Principal Component Analysis of PCA 1, 2 & 3

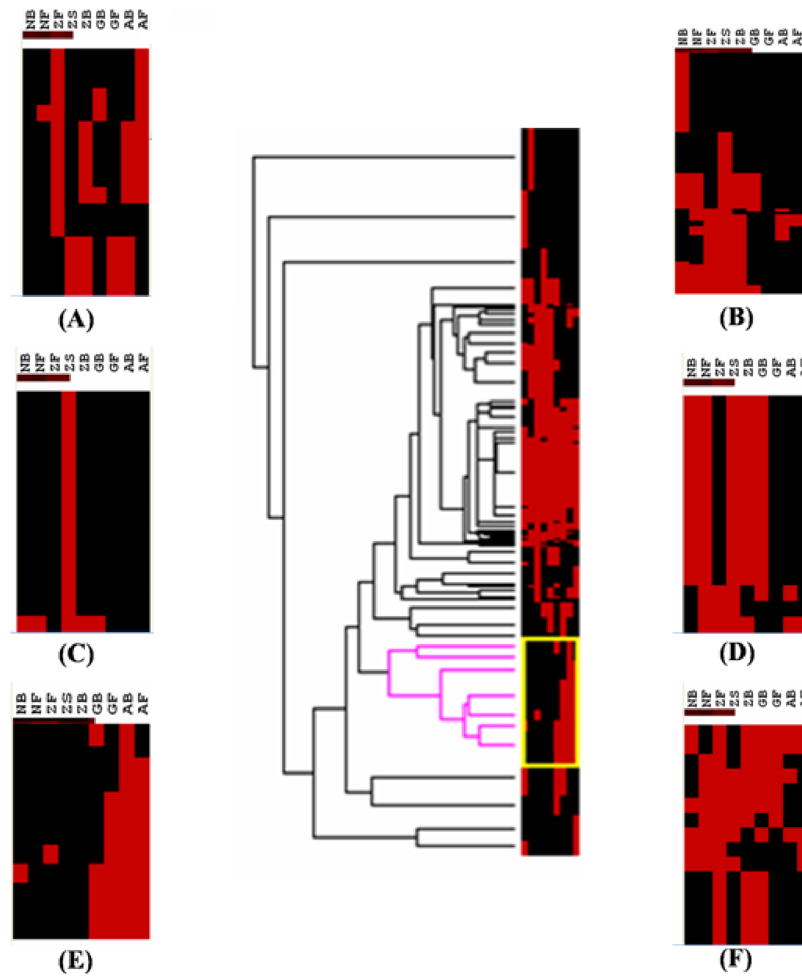

Figure 8. RAPD amplicons were clustered according to their presence or absence in the tilapia genomes. Clustering was performed by using cluster as Tree View software, (Stanford University, USA), as described under Materials and methods.

to either ZB or AB. On the other hand, RAPD markers found in both ZF and AF only were 10b31, 10b27, 10b22, 10b03, 10b47, 10b42, 10b39, and 10b32.
RAPD markers present in ZS and ZB but absent from ZF are 9b39, 9b38, 9b36, 9b32, 9b28, 9b25, 9b23, 9b20, 9b15, 9b12, 9b07, and 9b05 (Figure. 9D). RAPD markers present in GB but absent in GF are 6b24, 6b12, 6b10, 6b08, 6b04 and 6b01, while RAPD markers present in GF but absent in GB are 9b27, 9b21, 9b18, 9b17, 9b16, 9b13, 9b11, 9b08, 9b06, 9b03, 9b02, 9b01, 9b33, 9b31, 9b24, 9b04, 9b40, 9b35, and 9b30 (Figure 8E). RAPD markers absent from ZS but present in ZF and ZB are 7b18, 7b03, 7b21, 1b39, 1b05, 1b04, 1b48, 1b45, 1b44, 1b21, 1b07, 1b46, and 1b03 (Figure 8F).

4. Discussion

In this study, environments were chosen because they differ in several major abiotic and biotic variables. In order to demonstrate the differences between the three environments, water quality parameters and chlorophyll *a* content were measured in each environment. There were significant differences among the three investigated environments (Table 3). Water temperature in Western Lagoon was significantly higher than that of Ismailia freshwater canal or Lake Timsah. This result is expected since Western Lagoon represents a semi-closed water ecosystem. The timing of reproduction of annually spawning temperate fishes is thought to be controlled by an endogenous cycle, which in turn entrained by environmental cues (Barbieri, 1989), such as temperature (Torrans and Lowell, 1985).

The high temperature might be the spawning cue for most of tilapia species, and the low temperature is not favorable to spawning. However, Adeogun *et al* (2005) reported that water temperature exerted a lesser influence on fish production than other water quality parameters. Environmental factors have been proven to play an important role in the occurrence, density and growth of fishes in the early life stage (Porath *et al.,* 2005 and Person and Stenberg, 2006). Lagoons, which receive drain water containing high concentration of nutrients from land run-off support high productivity than in the sea water (Lopes *et al.,* 2007). The use of Lagoon's water as a nursery grounds has been reported for many fish organisms including commercially available tilapia fish (Niencheski and Johanke, 2002). The Lake Timsah water receives little fresh water, and is utilized as shellfish (Clams) nursery grounds. The River Nile water is poor in nutrients and support few or less productivity than Western Lagoon and Lake Timsah.

Salinity is one of the environmental factors that differs among the three investigated environments. The salinity and dissolved oxygen in Lake Timsah were significantly higher than those of Western Lagoon and Ismailia fresh water canal. The distribution of tilapia species in the Suez Canal zone was positively correlated with salinity and salinity associated factors (Jana *et al.,* 2006 and Sipauba–Tavares *et al.,* 2006), and the salinity of the surface water was one of the primary factors that were positively correlated to the aggregation, recruitment and commercial catches of tilapia species in the Suez Canal zone. Chlorophyll *a* in Western Lagoon or Lake Timsah was significantly higher than that of Ismailia freshwater canal. Therefore, it is conceivable the duration and abundance of the tilapia species would be relatively short in Ismailia freshwater canal. The Western Lagoon and Lake Timsah receives high loads of domestic sewage and industrial wastes, both are known to cause adverse effects on growth and survival of fish larvae (Kumar, 2006; Sipauba-Tavares *et al.,* 2006; Uddin, *et al.,* 2006).

RAPD analysis has been used effectively for initial assessment of genetic variation among fish species (Dinesh *et al.,* 1993; Johnson *et al.,* 1994; Foo *et al.,* 1995; Bielawski and Pumo, 1997; Caccone *et al.,* 1997; Barman *et al.,* 2002; Ali, 2003; Ali *et al*., 2004). In this study, the total number of sites amplified from the nine tilapia samples by RAPD PCR using the ten primers was 340.

RAPD analysis, therefore, proved to be a reliable marker in small low-tech laboratories.For genome mapping, AFLPs were highly superior to RAPDs because of their better reproducibility (Kjolner *et al.,* 2004). Interestingly, only two RAPD primers (OPA-03 and OPA-19) were adequate to distinguish the nine tilapia samples in this study.

The ability of the RAPD technique to reveal intra-specific variation can be very useful. Evidence was presented by Bardacki and Sibrinski (1994) that RAPD markers might be useful for systematic investigation at the level of species and subspecies. RAPD analysis was applied to three species of the tilapia genus *Oreochromis* and four subspecies of *O. niloticus* and different RAPD fragment patterns were observed for different species, although not always for different subspecies. Hassanien *et al.* (2004) showed by using a molecular phylogenetic tree that Manzallah and Burullus populations of *O. niloticus* populations in Egypt were strongly linked and separated from the population of Assuit and Cairo populations, with Qena population as an outgroup. Using of PCA to analyze RAPD polymorphisms, the first three principal components accounted for 72.04% of the total variability. All nine tilapia samples were best resolved by using the first three components.

The genetic documentation in this study covers only a part of the data needed for better management of tilapia species in Suez Canal zone. Further documentation, both of natural and farm populations need to be completed. For this purpose, collection of samples from many additional locations should be done. New populations, sampled must include tilapia from fish farms and waters adjacent to the aquaculture

farms. The results of this study will give a more complete picture of the genetic resources of the four tilapia species in Suez Canal zone, including the degree of hybridization and introgression between natural and farm population that have occurred. Recommendations arising from these studies may include the maintenance of populations in natural waters, reproduction and population control, genetic improvement programs, and better control of hybrid populations in the farms as well as in the natural surroundings.

Acknowledgment

The authors wish to thank technical staff of Biotechnology Research Institute, Suez Canal University for their assistance.

References

Adeogun, O.A., Fafioye,O.O., Olaleye, B.A. and Ngobili,G.O.: 2005, The relationship between some physiological parameters and plankton composition on fish production in ponds. Procceedings of the 19th annual conference of the Fisheries society of Nigeria (FISON). pp. 424-428.

Ali, B.A.: 2003, Genetics similarity among four breeds of sheep in Egypt detected by random amplified polymorphic DNA markers. *African Journal of Biotechnology*, 2 (7): 194-197.

Ali, B.A., Ahmed, M.M.M. and El-Zaeem, S.Y.: 2004, Application of RAPD markers in fish: Part II- Among and within families; Cichlidae (Freshwater), Mugilidae (Catadromous), Sparidae and Serranidae (Marine). *International Journal of Biotechnology*, 6 (4): 393 -401.

Allendorf, F., Ryman N and Utter F.: 1987, Genetics and Fishery Management: Past, Present, and Future. In: N. Ryman (ed.). Population Genetics and Fishery Management. The University of Washington, USA. pp. 1-20.

AOAC.: 1990, Official Methods of Analysis (Helrich, K. (ed.)). Association of Official Analytical Chemists, Arlington.

Barbieri, G.: 1989, Spawning type and fecundity of three sympatric species of tropical fishes in Brazil. *Journal of Fish Biology,* 35 (2): 311-315.

Bardacki, F. and Skibinski, D.O.F.: 1994, Application of the RAPD technique in tilapia Fish: Species and subspecies identification. *Heredity*, 73: 117-123.

Barman, H.K., Barat, A., Yadav, B.M. ,Banerjee, S., Meher, P.K., Reddy, P.V.G.K and Jana, R.K.: 2002, Genetic variation between four species of Indian major carps as revealed by random amplified polymorphic DNA assay. *Aquaculture*, 217:115-123.

Bartish I. V., Jeppsson N. and Nybom H.:1999, Population genetic structure in the dioecious pioneer plant species *Hippophae rhamnoides* investigated by RAPD markers. *Molecular Ecology,* 8:791-802

Bartish I. V., Jeppsson N., Bartish G. I., Lu R. and Nybom H.: 2000, Inter- and intraspecific genetic variation in Hippophae (Elaeagnaceae) investigated by RAPD markers. *Plant Systematics and Evolution*, 225: 85-101

Bielawski, J.P, and Pumo, D.E.:1997, Randomly amplified polymorphic DNA (RAPD) analysis of Atlantic Coast striped bass. *Heredity*, 78: 32-40.

Boyd, C.E.:1979, Water quality in warm water fish ponds. Agricultural Experiment Station, Auburn University, Alabama, USA. 359 pp.

Boyd, C.E., and Tucker, C.S.:1992, Water quality and pond soil analyses for aquaculture. Alabama Agricultural Experiment Station, Auburn University, pp. 148-149.

Caccone, A., Allegrucci, G., Fortunato, C. and Sbordoni, V.:1997, Genetic differentiation within the European sea bass *D. labrax* as revealed by 14 RAPD-PCR assays. *J. Hered*., 88: 316-324.

Dinesh, K.R., Lim, T.M., Chua, K.L., Chan, W.K. and Phang V.P.E.:1993, RAPD analysis: an efficient method of DNA fingerprinting in fishes. *Zoological science*, 10 (5): 849-854.

Du, Cheng; Lu, Maixin; Ye, Xing; Lao, Haihua, Huang, Zhanghan and Bai, Junjie.: 2005, Genetic analysis and molecular markers of five tilapia populations. *Journal of Dalian Fisheries University,* 20 (1): 25-28.

Eisen, M.B., Spellman, P.T., Brown, P.O., and Botestein, D.:1998, Cluster analysis and display of genomic–wide expression patterns. *The Proceedings of the National Academy of Sciences of the United States of America,* 95: 14862-14868.

Foo, C.L., Dinesh, K.R., Lim, T.M., Chan, W.K. and Phang, V.P.:1995, Inheritance of RAPD markers in the guppy fish, *Poecilia reticulata. Zoological science*, 12: 535-541.

Hassanien, H.A. Elnady, M. Obeida, A. and Itriby, H.: 2004, Genetic diversity of Nile tilapia populations revealed by randomly amplified polymorphic DNA (RAPD). *Aquaculture Research*, 35: 587-593.

Hunter, P.R., and Gaston, M.A.:1988, Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology*, 26(11): 2465-2466.

Jana, S.N., Garg, S.K. and Patra, B.C.:2006, Effects of inland water salinity on growth performance and nutritional physiology in growing milkfish, *Chanos Chanos* (Forskal): and Laboratory studies. *Journal of Applied Ichthyology,* 22(1): 25-34.

Johnson, S.L., Midson, C., Ballinger, E.W., and Postlethwait, J.H.:1994, Identification of RAPD primers that reveal extensive polymorphisms between laboratory strains of zebrafish. *Genomics*, 19:152-156.

Kaewsanagk, K., Hayashi, Z., Ken- Ichi, H., Asahida, T. and Ida, H.:2000, An evaluation of contribution of stocks in the supplementation of ayu

Plecoglossus altivelis in the Tohokuarea, using Allozyme markers. *Fisheries Science,* 66(5): 915-923.

Kjolner, S.; Sastad, S.M.; Taberlet, P.; and Brochmann, C.:2004, Amplified fragment length polymorphism versus random amplified polymorphic DNA markers: clonal diversity in *Saxifraga cernua. Molecular Ecology*, 13(1):81-86.

Koeller, P.:2003, The lighter side of reference points. *Fisheries Research*, 62 (1): 1-6.

Kumar, J.A.: 2006, Effect of sewage sludges on growth of freshwater fish *Catla catla. Journal of Ecotoxicology and Environmental Monitoring,* 6 (6): 595-597.

Lopes, C.B., Pereira, M.E., Vale,C; Lillebora, A.l., Pardal, M.A. and Duarte, A.C.:2007, Assessment of spatial environmental quality status in Ria de Aveiro (Portugal). *Scientia Marina,* 71: 293-304.

Niencheski, L.F., and Johanke, R.A.:2002, Benthic respiration and Inorganic nutrient fluxes in the estuarine region of Patos Lagoon (Brazil). *Aquatic Geochemistry*, 8: 135-152.

Payne, A.J.:1983, Estuarine and salt tolerant tilapias. In: Fishelson, L. and Yaron, Z. (eds) Proccedings of the International Symposium on Tilapia in Aquaculture. Tel Aviv University, Israel, pp. 534-543.

Pella, J. J. and G. B. Milner.: 1987, Use of genetic marks in stock composition analysis. Pages 247–276. in Ryman, N. and F. Utter, editors. Population genetics and fishery management. University of Washington Press. Seattle, USA.

Person, A; and Stenberg, M.:2006, Linking patch-use behaviour, resource, density, and growth expectations in fish. *Ecology*, 87: 1953-1959.

Porath, M. T., and Hurley, K. L.:2005, Effects of waterbody type and Management actions on Bluegill growth rates. *North American Journal of Fisheries Management*. 25: 1041-1050.

Sipauba –Tavares, L.H., Baccarin, A.E., and de Barga, F.M.S.:2006, Limnological parameters and Plankton community responses in Nile tilapia ponds under chicken dung and NPK (4-14-8) fertilizers. *Acta Limnology Brasiliensia,* 18(3): 335-346

Torrans, E. L. and F. Lowell .:1985, Spawning season of blue tilapia in Arkansas. *Arkansas Farm Research Journal*, 34 (1): 3.

Uddin, S., Azim, M.E., Wahab, M.A., Verdegem, M.C.J.:2006, The potential of mixed cultural genetically improved farmed tilapia (*Oreochromis niloticus*) and Fresh water giant prawn (*Macrobrachium rosenbergii*) in periphyton-based systems. *Aquaculture Research*, 37: 241-247.

تحديد البصمة الوراثية لبعض أسماك البلطى فى منطقة قناة السويس

محمد السيد مجاهد[1] - محمود رفعت[2] - محمد عبد الحميد الدناصورى[1] - صفاء محمود شرف[1]

[1] قسم الانتاج الحيوانى والثروة السمكية، كلية الزراعة - جامعة قناة السويس بالإسماعيلية
[2] قسم وراثة النبات، كلية الزراعة - جامعة قناة السويس بالإسماعيلية

أجريت الدراسة الحالية على عينات مجمعة من ثلاث بيئات طبيعية ذات تركيزات ملوحة مختلفة فى منطقة قناة السويس وتشمل أربعة أنواع من أسماك البلطي وهى البلطي النيلي ، البلطي الازرق ، البلطي الأبيض ، والبلطي الأخضر. تم إجراء تحليلات البصمة الوراثية بواسطة طريقة الرابيد (RAPD - PCR) باستخدام بوادئ طول كلا منها عشرة قواعد نيتروجينية وذلك على عينات ممثلة للأربعة أنواع السمكية من الثلاث بيئات المختلفة فى منطقة قناة السويس وهى البلطي الأخضر فى المياه العذبة (ZF)، البلطى الأخضر فى المياه الشروب (ZB) ، البلطي الأخضر فى المياه المالحة (ZS) ، البلطي النيلي فى المياه العذبة (NF) ، البلطى النيلى فى المياه الشروب (NB)، ، البلطي الازرق فى المياه العذبة (AF)، البلطي الازرق فى المياه الشروب (AB)،البلطى الأبيض فى المياه العذبة (GF)، البلطي الأبيض فى المياه الشروب (GB). تم تحديد 340 دليل جزيئي من التسعة عشائر من أسماك البلطي من البيئات الثلاث المختلفة. و قد وجد ان البادئين رقم 1 ورقم 6 سويا كانوا قادرين على ال

تمييز بين التسعة عشائر من أسماك البلطي فى هذه الدراسة. كانت دلائل التمييز لكل من البوادئ 1 و6 هى 0.98 و 0.83 على التوالى . إن اكبر مكونات التباين لدلائل الرابيد كانت تمثل 72% من التباين الكلى. إن استخدام الطريقة ذات الاتجاهين لرسم علاقات التشابه والاختلاف قد أظهرت أن بعض دلائل الرابيد قد تكون مرتبطة بأقلمة التركيب الوراثي المحدد للمعيشة فى المياه المالحة او المياه الشروب . أن هذه الدلائل الجزيئية سوف تكون مفيدة فى التجارب المستقبلية لاستخدامها فى برامج الانتخاب الوراثي لمقاومة الملوحة فى أسماك البلطي اعتمادا على الدلائل الجزيئية.