# Production of Salinity Tolerant Nile Tilapia, *Oreochromis niloticus* through Introducing Foreign DNA into Fish Gonads

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**Abstract:** This study was conducted to produce a salinity tolerant Nile tilapia, *Oreochromis niloticus* through genetically modified breeding by introducing a fragmented purified DNA isolated from Artemia, *Artemia salina* into the gonads. Two groups of adult fish (16 females and 8 males) were chosen to be injected with the foreign DNA into their gonads using a hypodermic needle with two different concentrations (10 µg and 5 µg /0.1 ml/fish), besides the control group (4 males and 8 females) carried out. Post-hatching fry which produced from each treatment of DNA were collected and weighed then transferred separately to glass. Two salinity levels were used to rear the hatching fry during the present study- 20 ppt (equivalent to half the sea salinity level) and 40 ppt (equivalent to the sea salinity level) - beside the freshwater as a control. The results showed a significant improvement ($P \leq 0.05$) in most of the growth performance parameters of genetically modified *O. niloticus* treated with 10 µg of Artemia DNA compared to the lowest dose of 5 µg of DNA and the control fish reared at 20 ppt of salinity. The results also showed that, the number of amplified bands detected varied, depending on the primers and DNA treatment. Highly genetic polymorphic percentage ranged from (8.00 to 71.79%) with an average of 39.05% using different random primers. The results of the present work suggested that, hyper-saline genetically modified *O. niloticus* with higher growth rate can be produced using a feasible and fast methodology.

***Keywords*:** Nile Tilapia, *Oreochromis niloticus*, genetically modified fish, Salinity tolerance, growth performance.

## INTRODUCTION

The shortage in freshwater in many countries and the competition for it in agriculture and other urban activities has increased the pressure to develop aquaculture in brackish water and sea water (El-Sayed, 2006). Among the species cultured commercially, the Nile tilapia *Oreochromis niloticus* is one of the most important freshwater finfish in aquaculture but is not considered amongst the most saline tolerant species (Kamal and Mair, 2005). Gene transfer relates to the process of introducing foreign DNA/RNA fragments into the nucleus or cytoplasm of gametes, zygotes, embryos or somatic cells using physical or chemical approaches allowing foreign genes to be reproduced and expressed in the host cells. These foreign DNA fragments may be originated from the host genome, related species or totally different species. Such a DNA fragment can be cDNA or genomic DNA but at least it must consist of: (1) The regulatory regions, such as enhancer, repressor, promoter or initiator; (2) the coding region for the production of protein; and (3) the untranslated regions, including terminator. After such a transfer, a gene fragment would then make the protein performs actively inside the cell of the host. When the fish are treated with this transfer technique, they would then feature nature and display the genetic traits encoded by the foreign genes, making it known as genetically or transgenic fish (Tsai, 2003). A commonly used method to introduce foreign DNA, is by microinjection into the nucleus or cytoplasm of fertilized eggs. This method, however, requires some skill and involves some difficulties and is time consuming (Inoue *et al.*, 1990 and Sin *et al.*, 1993). To avoid the difficulties accompanying microinjection, much more convenient methods are required, especially if such techniques are to be applied in aquaculture for fast breeding of commercially important species. The most common potential mass methods are: (1) The use of electroporation of fertilized eggs (Inoue *et al.*, 1990; Inoue, 1992 and Xie *et al.*, 1993); (2) electroporated sperm (Muller *et al.*, 1992; Symonds *et al.*, 1994); (3) the use of sperm cells as vectors to introduce foreign DNA into fish eggs (Khoo *et al.*, 1992); (4) the direct injection of foreign DNA into fish gonads (El-Zaeem *et al.*, 2011; El-Zaeem, 2001 and 2013 and Lu *et al.*, 2002).

Transferring foreign DNA can introduce new traits or improve the original ones in a way that is not possible using conventional breeding methods (Maclean and Laight 2000). This technology has become a popular technique not only for producing desirable traits but also for studying the regulatory functions of various genes and gene promoters in developmental mechanisms such as sterility control, which is one of the most important goals in tilapia breeding (Maclean and Laight 2000; Maclean *et al.*, 2002; Wong and Van Eenennaam 2008; El-Zaeem 2012 and 2013).

Therefore, the present study aims to produce salinity-tolerant Nile tilapia, *O. niloticus* through genetic modification presenting a fragmented, purified DNA isolated from Artemia, *Artemia salina* into the ovaries and testes of *O. niloticus* adult. Also the effects of introducing foreign DNA on growth performance, body composition and feed utilization of the offspring produced under different salinity levels, were employed during the present work.

## MATERIALS AND METHODS

This work was undertaken at the Aquaculture Research Centre, Faculty of Agriculture, Suez Canal

University and biotechnology laboratory, Suez Canal University, Ismailia, Egypt.

**Fish origin:**

The Nile tilapia, *Oreochromis niloticus* used in the present study descended from a randomly mating population at the Bazina Fish Farm, Al Qantara Gharb, El-Ismailia Governorate, Egypt.

**Experimental Design:**

**Preparation of Genomic DNA:**

High molecular weight DNA was extracted according to Bardakci and Skibinski (1994) method. Isolation of DNA was performed reducing whole tissue sample of Artemia, *A.salina*. Each sample was suspended in 500 $\mu$l STE (0.1 *M* NaCl, 0.05 *M* Tris and 0.01 *M* EDTA, pH 8), After adding 30 µl SDS (10%) and 30 $\mu$l 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. DNA was precipitated with ice-cold absolute ethanol and washed with 70% ethanol. The pellet was dried and re-suspended in 200 µl mill Q water. The concentrations of DNA and their purity were measured according to Charles (1970). The extracted DNA was restricted by Eco Rl restriction enzyme type II. It digested DNA between guanine and adenine according to Tsai *et al.* (1993). Then, the concentrations of 10 µg/0.1 ml/fish and 5 µg/0.1 ml/fish were adjusted by extrapolating the dilutions for the DNA extracted using 0.1 x SSC buffer (El-Zaeem, 2001).

**Injection of Genomic DNA into Fish Gonads:**

Adult Nile tilapia (24 females and 12 males) with an average live weight 321.53 ± 15.41 g/male and 163.81 ± 7.90 g/female were chosen. Readiness of females to spawn was determined by examining the degree of swelling of the urogenital papilla (Hussain *et al.*, 1991). Males were examined by stripping sperm (Wester and Foote, 1972). 16 females and 8 males were chosen to be injected with the foreign DNA into their gonads using a hypodermic needle. The chosen males and females were divided into two separated groups, the first one was injected with 10 µg/0.1 ml/fish and the second one was injected with 5 µg/0.1 ml/fish. Besides the control group (4 males and 8 females) carried out.

To inoculate the adult fish, the needle was inserted into the openings of oviduct and sperm duct (El Zaeem, 2001 and Lu *et al.*, 2002). Immediately after DNA treatments were carried out, each group of treated fish was stocked separately in the fiber glass tanks (4m x 1m x 1m) supplied with dechlorinated water with an adequate aeration at a stocking rate of 4 brooders/ $m^3$, and heaters to save water temperature. The sex ratio of the fish was 2 females: 1 male. Fish were fed twice daily on pelleted diets contained 24% crude protein at satiation for 6 days a week.

**Fry, Fingerlings and Adult Fish Rearing:**

Tilapia offspring were produced through about 2-3 weeks after had been stocked to spawn. Post-hatching fry which produced from each treatment of DNA were collected and weighed. Then, transferred separately to glass aquaria (with an area 60 x 30cm x 40cm) at a rate of 1 fish /10 liter, This glass aquaria were provided with a continuous supply of de-chlorinated water and adequate aeration system and heaters, cleaned once a day by siphoning, then one-half to two-third of their water volume was replaced. All water was completely changed once every two weeks during fish sampling. Fish were fed three times daily on pelleted diet containing 38% protein at satiation to the end of the experiment. Fish were weighed bi-weekly for six months.

**Saline water acclimatization:**

There were two salinity levels were used during the present study which were 20 ppt (equivalent to half the sea salinity level) and 40 ppt (equivalent to the sea salinity level) and, beside a third level of freshwater used as control. The first group of fry resulted from each treatment of DNA and their control were transferred gradually acclimated to the corresponding salinities by rising salinity level at the rate of 4 ppt every day (Watanabe and Kuo, 1985). The second group was kept in the freshwater. Water in each glass aquarium was partially changed once daily and totally every three days with the consideration of the salinity degree for each treatment. A refractometer (S/Mill-E, ATAGO Co., LTD) was used to monitor water salinity.

**Quantitative traits measurements:**

The growth parameters were initial and final body weight (g), total gain, average daily gain (ADG g/day), and specific growth rate (SGR %/day), these parameters were taken bi-weekly for growth measurements for fry, then fingerlings and adult fish until the end of the experiment. They were calculated as follows:

Total gain = Wt- Wo (Brody, 1945)

*ADG = (Wt – Wo)/n (Brody, 1945)

*SGR %/day = ($Log_e$ Wt - $Log_e$ Wo) 100/n (Castell and Tiews, 1980)

Since [n: number of days; Wo: initial weight at the beginning; Wt: final weight at the end of period].

Some feed utilization parameters such as feed conversion ratio (FCR), and protein efficiency ratio (PER) were estimated as follows:

* FCR = dry matter feed intake/ gain.

* PER = gain/protein intake.

Body fish moisture, crude protein and crude fat contents were estimated according to AOAC (1975) methods by choosing two random samples from each treatment.

Gross energy contents of feed had been calculated by using MacDonald's Tables (MacDonald *et al.*, 1973). Also the fish gross energy was calculated from their chemical composition using the factor of 5.7 and 9.5 for protein and fat, respectively, according to Viola *et al.* (1981). Initial and final body composition analyses were performed for moisture, crude protein and lipid contents according to the standard AOAC (1984) methods.

**Random Amplified Polymorphic DNA (RAPD) analysis:**

DNA was extracted from livers and tissues of genetically modified Nile tilapia and its control group following the method described by Baradakci and Skibinski (1994). Oligonucleotide primers of ten bases and twenty bases long were used to originate PCR amplifications (Table 1). The polymerase chain reaction amplifications were done according to the technique of Williams *et al.,* (1990 and 1993).

The reaction (25 µl) was carried out in a consisted of 0.8 µ of Taq DNA polymerase (Fanzyme), 25 pmol dNTPs and 25 pmol of random primer, 2.5 µl l0_X Taq DNA polymerase buffer and 40ng of genomic DNA. The final reaction mixture was placed in a DNA thermal cycler (ependorff). The PCR programmer included an initial denaturation step at 94°C for 2 mins followed by 45 cycles with 94°C for 30 seconds for DNA denaturation, annealing as mentioned with each primer, extension at 72°C for 30 seconds and final extension at 72 °C for 10 minutes were carried out. The samples were cooled at 4°C. The amplified DNA fragments were separated on 1.5% agarose gel and stained with ethidium bromide. The marker, Φ X 174 DNA marker (bp 1353, 1078, 872, …, 72) was used in this study. The amplified pattern was visualized on an UV trans-illuminator and photographed by Gel Documentation system.

**Table (1):** The sequences and the annealing temperatures of the primers used.

| Primers | Sequence 5'-3' | Annealing temp. (°C/sec.) |
|---|---|---|
| **1** | OPA-11 (CAATCGCCGT) | 37°C |
| **2** | OPA-12 (TCGGCGATAG) | 37°C |
| **3** | OPA-13 (CAGCACCCAC) | 37°C |
| **4** | OPA-14 (TCTGTGCTGG) | 37°C |
| **5** | OPA-19 (CAAACGTCGG) | 37°C |

**Statistical Analysis:-**

Data was analyzed using the following model (CoStat, 1986):

$$Y_{ijk}=\mu +T_i+S_j+ (TS)_{ij} +B_k+e_{ijk}$$

Where: $Y_{ijk}$: Observation of the $ijk^{th}$ parameter measured. µ: Overall mean, $T_i$: Effect of $i^{th}$ concentration of DNA, $S_j$: Effect of $J^{th}$ salinity, $(TS)_{ij}$: Interaction concentration of DNA by salinity, $B_k$: Effect of $K^{th}$ block, $e_{ijk}$: Random error.

For body composition traits at the first analysis, data were analyzed by fitting the following model (CoStat, 1986):

$$Y_{ij}: \mu+T_i+B_j+E_{ij}$$

Where: $Y_{ij}$: Observation of the $ij^{th}$ parameter measured, µ: Overall mean, $T_i$: Effect of $I^{th}$ dose, $B_j$: Effect of $j^{th}$ block, $E_{ij}$: Random error.

Significant differences (P≤0.05) among means were tested by Duncan's multiple range test (Duncan, 1955).

## RESULTS AND DISCUSSION

Initial body weight (IBW) was homogenous in control *O. niloticus* and insignificantly differed (P≥0.05) from those of the genetically modified fish treated with 5 or 10µg of Artemia DNA and those reared at different salinity levels (Table 2). El-Zaeem (2004a) reported that, IBW of the first and second generations delivered from fast growing genetically modified *T. zillii* significantly increased (P≤0.05) compared to the control fish. The results of this study are consistent with these findings. The highest mean values of final body weight (FBW), daily gain (DG) and SGR were recorded by genetically modified *O. niloticus* treated with 10µg of Artemia DNA, but did not differ significantly from that of the genetically modified *O. niloticus* treated with 5µg of Artemia DNA.

In all fish groups, the highest mean values of FBW and DG were obtained for the fish reared at 20 ppt and fresh water which differ significantly (P≤0.05) from those of the fish reared at 40 ppt. Moreover, genetically modified *O. niloticus* treated with 10µg of Artemia DNA reared at 20 ppt had significantly higher (P≤0.05) FBW and DG than those of the other *O. niloticus* treated with DNA and the control fish group, and insignificantly differ from that of fish treated with 5µg of DNA reared at fresh water. El-Zaeem *et al.* (2011) reported that genetically modified *O. niloticus* treated with sea bream-DNA reared at different levels of salinities up to 16 ppt had significantly higher (P≤0.05) FBW, DG and SGR than the genetically modified *O. niloticus* treated with Artemia-DNA and the control fish group. The results of the present study are consistent of these findings. Generally, with increasing salinity up to 40 ppt, the growth performance decreased. This may be attributed to the increase in energy cost of osmoregulation at high salinity level. Morgan and Iwama (1996), Toepfer and Barton (1992) and Grau *et al.* (1994) reported that, there is an increasing metabolic rate of osmoregulatory activities at high salinity. Furthermore, Rao (1968) noted that, osmoregulation appears to use a high proportion of the available energy ranging from 20 to 50% of total energy expenditure, depending on the environmental salinity.

Despite the adverse effect of salinity on growth, the genetically modified *O. niloticus* showed higher growth performance than the control. This may be attributed to the effect of growth hormone. Rahman *et al.* (1998) and Meri and Devlin (1999) reported that, growth hormone gene in transgenic fish elevates growth hormone in their plasma from 10 to 13 folds and above 40 fold higher than that of non-transgenic fish. Moreover, Martinez *et al.* (1996, 1999 and 2000), Pitkanen *et al.* (1999), Rahman and Maclean (1999), El-Zaeem (2001) and Mori *et al.* (2007) reported heavier weight of transgenic fish than the non-transgenic fish.

By the end of the experiment, the highest moisture and protein contents (Table 3) were achieved by control fish but did not differ significantly from that of genetically modified fish treated with 5µg of Artemia DNA. While genetically modified fish treated with 5µg of Artemia DNA showed the highest mean of lipid content which significantly higher (P≤0.05) than that of other treatment and control.

Yet, crude protein was significantly low (P≤0.05) in *O. niloticus* reared at freshwater, showing lower mean

than both *O. niloticus* reared at 20 and 40 ppt of salinity. Moreover, the highest mean values of lipids content was achieved by those fish reared in fresh water, which also insignificantly differ from *O. niloticus* reared at 40 ppt of salinity (Table 3). The results of interaction showed that the highest significant mean (P≤0.05) of protein content was obtained by control fish reared at 40 ppt of salinity, which also insignificantly differ from those of genetically modified fish treated with 5 and 10 µg of Artemia DNA reared at 20 ppt of salinity. Moreover, the highest mean value of lipid content was recorded by genetically modified fish treated with 5µg of Artemia DNA reared at freshwater which was significantly higher (P≤0.05) compared to the other treatments reared at different salinity levels.

Fish reared in fresh water had the higher moisture contents compared to the fish reared at 20 and 40 ppt. Chatakondi *et al.* (1995), Dunham *et al.* (2002) and El-Zaeem (2004b) reported that, the moisture and lipids content were lower while the protein content was higher in transgenic common carp and red-belly tilapia muscles than their control. Martinez *et al.* (2000), Lu *et al.* (2002), El-Zaeem (2004a, 2004b), El-Zaeem and Assem (2004) and Assem and El-Zaeem (2005) reported that, anabolic stimulation and average protein synthesis were higher in transgenic than that of non-transgenic fish. The results of the present work are consistent with these findings.

The highest mean values of feed intake (Table 3) was recorded for genetically modified *O. niloticus* treated with 10µg of Artemia DNA, with insignificant differences (P≥0.05) compared to genetically modified *O. niloticus* treated with 5µg of Artemia DNA. The highest feed intake was obtained by fish reared at 20 ppt of salinity but did not differ significantly from that of fish reared at freshwater. Moreover, the highest feed intake was recorded by genetically fish treated with 10µg of Artemia DNA reared at 20 ppt of salinity. The highest mean values of PR% was recorded by control *O. niloticus*, but did not differ significantly (P≥0.05) from that of fish treated with 5µg of Artemia DNA. Moreover, the highest mean values of PR% was recorded by *O. niloticus* reared at 20 ppt of salinity but did not differ significantly (P≥0.05) from that of fish reared at freshwater.

The best means of feed conversion ratio (FCR), protein efficiency ratio (PER), and energy retention (ER) percentage were achieved by fish reared at freshwater with insignificant differences (P≥0.05) from that of *O. niloticus* reared at 20 ppt.

El-Zaeem *et al.* (2011) reported that feed consumption of genetically modified *O. niloticus* treated by sea bream-DNA at different salinity up to 16 ppt was improved. This may be attributed to the effect of elevated growth hormone in fish plasma that resulted from those treated by sea bream-DNA. Rahman *et al.* (1998) reported that, growth hormone binds to specific cell receptors, which induces synthesis and secretion of insulin-like growth factors (IGF-1 and IGF-II), resulting in the promotion of somatic growth through improved appetite, feeding efficiency and growth rate (De la Fuente and Castro, 1998). Also, Oakes *et al.* (2007) reported that, the enhancement performance of transgenic Coho salmon was due to enhanced dietary intake. Many authors (Cook *et al.*, 2000; Wu *et al.*, 2003 and Kapuscinski *et al.*, 2007) noted that, growth hormone transgenic fish had feed efficiency better than non-transgenic. Besides, Ron *et al.* (1995) and Haroun (1999) reported that, tilapia in sea water utilize the feed more efficiently than in fresh water. Clark *et al.* (1990) noted that, maximum growth rate of Florida red tilapia in sea water occurred at satiation feed rate, on the other hand the feed conversion improved at lower feeding rate.

**Table (2):** Effect of different types of foreign DNA, and salinity levels on growth performance of *O. niloticus*.

| Treatments | IBW (g) | FBW (g) | DG (g/day) | SGR%/ day |
|---|---|---|---|---|
| **DNA source** | | | | |
| Control | 0.451±0.0 | $66.22^{b}$ ±11.0 | $0.59^{b}$ ±0.1 | $4.45^{b}$ ±0.2 |
| 5µg | 0.447±0.0 | $87.31^{a}$ ±22.3 | $0.78^{a}$ ±0.2 | $4.69^{a}$ ±0.2 |
| 10 µg | 0.441±0.0 | $91.42^{a}$ ±22.4 | $0.81^{a}$ ±0.2 | $4.74^{a}$ ±0.2 |
| **Salinity ppt** | | | | |
| Fresh water (FW) | 0.444±0.0 | $86.56^{a}$ ±26.0 | $0.77^{a}$ ±0.2 | 4.67±0.3 |
| 20 ppt | 0.448±0.0 | $88.74^{a}$ ±23.2 | $0.79^{a}$ ±0.2 | 4.70±0.2 |
| 40 ppt | 0.446±0.0 | $69.65^{b}$ ±9.0 | $0.62^{b}$ ±0.1 | 4.50±0.1 |
| **DNA X Sal.** | | | | |
| Control at FW | 0.448±0.0 | $61.56^{c}$ ±16.4 | $0.55^{c}$ ±0.2 | 4.38±0.2 |
| 5µg at FW | 0.445±0.0 | $112.32^{ab}$ ±15.6 | $1.00^{ab}$ ±0.1 | 4.94±0.1 |
| 10 µg at FW | 0.440±0.0 | $85.80^{bc}$ ±16.6 | $0.76^{bc}$ ±0.2 | 4.70±0.2 |
| Control at 20 ppt | 0.454±0.0 | $66.46^{c}$ ±3.7 | $0.59^{c}$ ±0.0 | 4.45±0.0 |
| 5µg at 20 ppt | 0.444±0.0 | $83.31^{c}$ ±6.3 | $0.74^{c}$ ±0.1 | 4.67±0.1 |
| 10 µg 20 ppt | 0.445±0.0 | $116.46^{a}$ ±6.6 | $1.04^{a}$ ±0.1 | 4.97±0.1 |
| Control at 40 ppt | 0.450±0.0 | $70.64^{c}$ ±15.5 | $0.63^{c}$ ±0.1 | 4.50±0.2 |
| 5µg at 40 ppt | 0.451±0.0 | $66.30^{c}$ ±5.3 | $0.59^{c}$ ±0.1 | 4.45±0.1 |
| 10 µg at 40 ppt | 0.438±0.0 | $72.00^{c}$ ±10.3 | $0.64^{c}$ ±0.1 | 4.55±0.1 |

Means within each comparison in the same column with the different superscripts differ significantly (P≤0.05), Initial and final body weight (IBW and FBW) = body weight at start and end of experiment, Daily gain (DG) = (final weight - initial weight)/ number of days, Specific growth rate (SGR%/day)=(Ln final weight–Ln initial weight) 100/number of days.

**Table (3):** Effect of different types of foreign DNA and salinity levels on body composition and feed utilization of *O. niloticus.*

| Treatments | Moisture | % on dry matter basis | | Feed intake | FCR | PER | PR% | ER% |
|---|---|---|---|---|---|---|---|---|
| | | Protein | Lipid | | | | | |
| **DNA source** | | | | | | | | |
| Control | 72.68$^{a}$ ±2.3 | 64.27$^{a}$ ±3.0 | 20.25$^{c}$ ±1.3 | 198.73$^{b}$ ±30.2 | 3.03±0.2 | 0.87±0.1 | 55.91$^{a}$ ±3.a | 50.92±3.7 |
| 5µg | 72.44$^{a}$ ±1.4 | 61.58$^{a}$ ±4.8 | 25.14$^{a}$ ±5.0 | 255.33$^{a}$ ±32.5 | 3.02±0.4 | 0.88±0.1 | 54.12$^{a}$ ±4.a | 54.74±8.9 |
| 10 µg | 67.01$^{b}$ ±3.4 | 58.19$^{b}$ ±5.7 | 22.49$^{b}$ ±1.4 | 279.20$^{a}$ ±51.0 | 3.12±0.3 | 0.85±0.1 | 48.55$^{b}$ ±6.b | 48.26±5.9 |
| **Salinity ppt** | | | | | | | | |
| Fresh water (FW) | 71.65±3.5 | 57.81$^{c}$ ±4.6 | 24.03$^{a}$ ±5.3a | 240.54$^{ab}$ ±53.4 | 2.86$^{b}$ ±0.3 | 0.93$^{a}$ ±0.1 | 53.76$^{ab}$ ±6.4 | 54.67$^{a}$ ±10.0 |
| 20 ppt | 70.59±3.0 | 64.58$^{a}$ ±1.9 | 21.29$^{b}$ ±2.1b | 266.56$^{a}$ ±66.5 | 3.03$^{ab}$ ±0.2 | 0.87$^{ab}$ ±0.1 | 55.20$^{a}$ ±2.5 | 51.64$^{ab}$ ±2.8 |
| 40 ppt | 69.88±4.5 | 61.66$^{b}$ ±5.9 | 22.56$^{ab}$ ±2.6 | 226.16$^{b}$ ±19.8 | 3.29$^{a}$ ±0.2 | 0.80$^{b}$ ±0.1 | 49.62$^{b}$ ±6.2 | 47.60$^{b}$ ±3.9 |
| **DNA X Sal.** | | | | | | | | |
| Control at FW | 74.39±0.6 | 62.50$^{b}$ ±0.9 | 19.75$^{c}$ ±1.8 | 178.08$^{e}$ ±34.3 | 2.94±0.2 | 0.90±0.1 | 56.05±3.6 | 51.08±5.1 |
| 5µg at at FW | 73.07±2.0 | 56.86$^{c}$ ±5.3 | 30.34$^{a}$ ±3.0 | 287.25$^{b}$ ±17.5 | 2.58±0.2 | 1.02±0.1 | 57.93±0.9 | 65.54±4.9 |
| 10 µg at FW | 67.49±1.2 | 54.06$^{c}$ ±0.8 | 22.00$^{bc}$ ±1.3 | 256.30$^{bc}$ ±10.1 | 3.05±0.5 | 0.87±0.1 | 47.29±8.1 | 47.40±8.9 |
| Control at 20 ppt | 70.33±1.9 | 62.29$^{b}$ ±0.5 | 21.10$^{c}$ ±1.7 | 195.40$^{de}$ ±3.4 | 2.97±0.2 | 0.89±0.1 | 55.44±3.6 | 51.78±5.0 |
| 5µg at 20 ppt | 72.27±1.7 | 66.11$^{ab}$ ±1.0 | 20.44$^{c}$ ±3.1 | 260.68$^{bc}$ ±3.5 | 3.16±0.3 | 0.84±0.1 | 55.35±4.18 | 49.90±1.5 |
| 10 µg 20 ppt | 69.18±5.4 | 65.33$^{ab}$ ±0.9 | 22.33$^{bc}$ ±2.4 | 343.59$^{a}$±5.0 | 2.97±0.1 | 0.89±0.0 | 54.81±0.1 | 53.24±0.7 |
| Control at 40 ppt | 73.31±2.1 | 68.02$^{a}$ ±1.3 | 19.91$^{c}$ ±0.3 | 222.72$^{cde}$ ±36.7 | 3.19±0.2 | 0.83±0.1 | 56.25±4.2 | 49.89±3.7 |
| 5µg at 40 ppt | 71.97±1.3 | 61.78$^{b}$ ±0.8 | 24.64$^{b}$ ±3.1 | 218.07$^{cde}$ ±9.4 | 3.32±0.1 | 0.79±0.0 | 49.08±1.2 | 48.79±3.9 |
| 10 µg at 40 ppt | 64.37±1.3 | 55.18$^{c}$ ±2.1 | 23.14$^{bc}$ ±0.8 | 237.69$^{cd}$ ±10.2 | 3.35±0.3 | 0.79±0.1 | 43.54±2.8 | 44.14±2.8 |

Means within each comparison in the same column with the different superscripts differ significantly (P≤0.05).
Feed conversion ratio (FCR) = dry feed intake/ gain.
Protein efficiency ratio (PER) = gain/ protein intake.
Protein retention percent (PR%) = protein increment (100)/ protein intake.
Energy retention percent (ER %) = energy increment (100)/ energy intake.

**Random Amplified Polymorphic DNA (RAPD) Fingerprinting:**

All DNA samples from genetically modified *O. niloticus* treated with different types of DNA and their control were examined using RAPD marker. Five random primers were used to determine DNA fingerprinting in genetically modified *O. niloticus* treated with two concentration of Artemia-DNA and their control fish. The results showed that, no amplification was detected in the control reactions (without DNA source). All amplification products were found to be reproducible when reactions were repeated using the same reaction conditions (Figure 1). The results also showed that, the number of amplified bands detected varied, depending on the primers and DNA treatment. Highly genetic polymorphic percentage ranged from (8.00 to 71.79%) with an average of 39.05% using different random primers (Figure 1). It may be due to the differences in DNA molecule among normal and modified fish as a result of direct injection of two concentration of Artemia DNA. Moreover, some fragments of foreign DNA may be randomly integrated into *O. niloticus* genomes. This integration could be functional or silent (Yaping *et al.*, 2001). The results of the present work are consistent with the findings obtained from previous studies (El- Zaeem, 2001; Hemeida *et al.*, 2004; Ali, 2002; Assem and El-Zaeem, 2005, El-Zaeem and Assem, 2006). Also, the sensivity of the RAPD marker played an important role in the detection of these differences (Ahmed *et al.*, 2004; Ali *et al.*, 2004; El-Zaeem *et al*, 2006; El-Zaeem and Ahmed, 2006). The specific characterization of the RAPD method (random, uncharacterized multiple genome loci; dominant nature of markers; and possibility of migration of no-homologous bands) result in limitations based on RAPD analysis alone. Despite these limitations, the RAPD analysis can be used effectively for initial assessment of genetic variation among fish species (Barman *et al.*, 2003). The main advantages of RAPD markers are the possibility of working with anonymous DNA, relatively low expense, fast and simple to produce (Hadrys *et al.*, 1992; Elo *et al.*, 1997 and Ali *et al.*, 2004).

The results of the present work suggested that, hypersaline genetically modified *O. niloticus* which can be produced by the transfer of a foreign DNA isolated from Artemia represented higher growth rate which could be used as a feasible and fast methodology compared to interspecific hybridization which is one classical breeding methods (El- Zaeem *et al.*, 2010 and 2011).

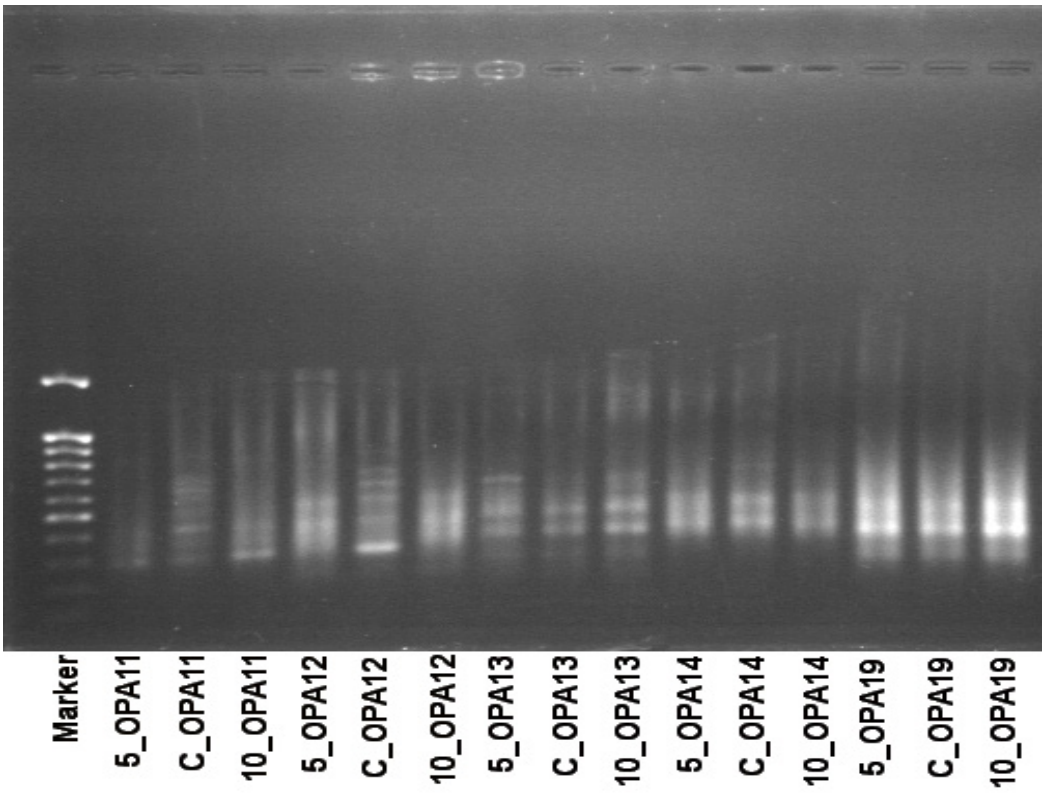


**Figure (1):** RAPD amplification products generated from genetically modified *O. niloticus* treated with two concentrations of Artemia-DNA and their control fish, using five random primers. Lane Marker: ΦX174 DNA marker, lanes 5, C and 10: *O.niloticus* treated with 5µg of Artemia-DNA, control fish and, *O. niloticus* treated with 10µg of Artemia-DNA respectively.

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# إنتاج أسماك البلطى النيلى (*Oreochromis niloticus*) المقاومة للملوحة عن طريق حقن الحمض النووي فى المناسل

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أجريت هذه الدراسة بهدف انتاج أسماك بلطى نيلى مقاومة لملوحة المياه من خلال تطبيق طريقة التربية بالتعديل الوراثى عن طريق إدخال قطع الحمض النووي (DNA) بعد عزلها وتنقيتها من الأرتيميا في الغدد التناسلية للأسماك. وقد أظهرت النتائج المتحصل عليها من تلك الدراسة وجود تحسن ذو دلالة احصائية فى معظم صفات النمو لأسماك البلطى النيلى المعدلة وراثيا والتى عوملت بتركيز ١٠ ميكروجرام من المادة الوراثية المستخلصة من الاتيميا مقارنة بكل من الأسماك التى عوملت بتركيز ٥ ميكروجرام وأسماك مجموعة المقارنة عن التربية فى تركيز ملوحة ٢٠جزء فى الألف. وقد أظهرت النتائج تأثير كل من نوع البرايمر وتركيز المادة الوراثية المحقونة على تنوع الـ Bands. وأيضاً اظهرت نتائج تحليل البصمة الوراثية وجود اختلافات بين المعاملات المختلفة بمعدلات تراوحت من ٨-٧١,٧٩% بمتوسط ٣٩,٠٥%. يستخلص من تلك النتائج انه يمكن انتاج أسماك بلطى نيلى ذى تحمل عالى للملوحة ونمو مرتفع باستخدام طريقة التربية بالتعديل الوراثى كطريقة سريعة ومجدية.