

Effects of *Lates niloticus* Genomic DNA on Growth, Proximate Composition, and Reproductive Indices of *Oreochromis niloticus* (Linnaeus, 1758)

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ABSTRACT

The Nile tilapia, *Oreochromis niloticus*, is a widely cultivated fish throughout the world. The fish is also an important aquaculture species in Nigeria due to its environmental tolerance, high prolificacy and market dominance. The objectives of this study are to investigate the efficacy of *Lates niloticus* DNA on growth, proximate composition, and DNA fingerprint of *O. niloticus*. Various concentrations of DNA (0, 10, 20, 30, and 40 µg/µl) extracted from *Lates niloticus* were injected into the somatic tissue of the *O. niloticus* fingerlings. The injected fish were raised for 120 days in a polyethylene fish pond (2.5 x 1.5 x 1.2 deep). The results show that fingerlings injected with 40 µg/µl have significantly ($P < 0.05$) better final weight, weight gain, average daily weight gain, specific growth rate, crude protein, and metabolizable energy. Random Amplification of Polymorphic DNAs (RAPD) gel electrophoresis revealed distinct banding patterns among the treated fish. The control fish had 5 bands, while fish injected with 10, 20, 30, and 40 µg/µL had 7, 6, 8, and 9 bands, respectively. Out of 5 loci, 80% were polymorphic. This study revealed that fragmented DNA from *L. niloticus* moderately integrated into the *O. niloticus* genome and show the potential to improve growth in *Oreochromis niloticus*. The DNA of *L. niloticus* significantly ($p < 0.05$) altered the proximate composition of the fish, especially by enhancing protein content and metabolizable energy. These changes could offer nutritional, economic, and production benefits in Tilapia aquaculture, though further investigation into safety, long-term performance, and consumer acceptance is essential.

Keywords: Aquaculture, DNA Enhancement, Fish, Nile Perch, Somatic cell

Citation

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INTRODUCTION

Nile tilapia, *Oreochromis niloticus* (plate 1), is one of the world's most widely cultured fish species, particularly in Africa, due to its high adaptability, rapid growth, and ability to thrive in diverse aquatic environments. According to Food and Agriculture Organization (FAO) (2025), global Tilapia production reached about 6.2 million tonnes in 2023, making it the second most farmed freshwater fish after carp. Roderick (2025) reported that the Rabobank Global Seafood Specialist predicted that global Tilapia production will surpass 7 million metric tonnes in 2024.

China remains the largest global producer of Tilapia, followed by Indonesia. Egypt leads Africa, contributing

nearly 1.5 million tonnes (60% of the continent's total production) of Tilapia, followed by Nigeria with about 300,000 tonnes and other sub-Saharan countries like Ghana, Uganda, and Kenya (FAO, 2025). Africa's estimated Tilapia production, therefore, is about 2.0–2.5 million tonnes (FAO, 2025).

According to Steffens (2024), Nile tilapia plays an important global role in supporting food security, by providing affordable, high-quality protein, combat malnutrition in developing countries, and provides income for smallholder farmers and fishers (Wasso *et al.*, 2024). Because of its affordability, the Nile Tilapia is sometimes considered a food for the poor, or the fish for the masses (El-Sayed, 2020).



Plate 1: Representative image of the *Oreochromis niloticus* used for this study

Nile tilapia, *Oreochromis niloticus*, remains a key species in global aquaculture, but its growth performance has declined in many farmed populations due to unstable and unreliable seed supply, and practice of inbreeding by most hatchery operators resulting in inferior seed characterized by low growth rate (Abaho *et al.*, 2025). As demand for faster-growing, more efficient fish increases, there is a growing need for advanced techniques like DNA transfer to enhance growth and other economic traits. However, the potential of DNA transfer in improving the growth performance of Nile tilapia is still underexplored,

particularly about how it may influence other traits such as growth and nutritional composition. This creates a vital research gap that warrants investigation.

The Nile Pike of genus *Lates* belongs to the family Latidae and is of the order Perciform, comprising eleven (11) species that occur in fresh and brackish water (Otero, 2004). The Nile Perch, *Lates niloticus*, belongs to the family Latidae of order Perciformes (Otero, 2004). *Lates niloticus* is a freshwater species indigenous to African rivers and lakes, including the Nile, Chad, Senegal, Niger, and Congo

River basins (Liu, 2008; Paugy, 2003). The fish is found almost everywhere in West Africa, except in Gambia (Paugy, 2003). *Lates niloticus* is one of the largest freshwater fish (Kaufman, 1992) and could serve as valuable genetic materials for enhancing growth and other useful traits in other fish species taking advantage of its size (growth vigour) (Haruna *et al.*, 2025).

Many approaches can be used to improve the growth of fish. One of the methods can be done through transgenesis (gene/DNA transfer). The most common method used is the microinjection of DNA/genes into the pronuclei of zygotes of fish (Shakweer *et al.*, 2023). DNA/gene can be manually injected into the skeletal muscles of fish (Wolff *et al.*, 1990). According to Sudha *et al.* (2001), a foreign gene can be transferred into fish *in-vivo* by introducing DNA into embryos or directly into the somatic tissues of young or adult fish. Assem and El-Zaeem (2005) opined that the direct insertion of DNA into fish muscle is a simple approach that provides faster results and can eliminate the need for screening transgenic individuals and selecting germline carriers. Gene transfer and expression through intramuscular injection of foreign DNA into the skeletal muscles of fish has been achieved by Hansen *et al.* (1991), Rahman and Maclean (1992), Anderson *et al.* (1996), Tan and Chan (1997), Xu *et al.* (1999), Gomez-Chiarri *et al.* (1996), El-Zaeem (2004), El-Zaeem and Assem (2004), and Hemeida *et al.* (2004). Moreover, Sudha *et al.* (2001) stated that the expression of muscular injection of DNA was evident in several non-muscle tissues, such as skin epithelia, pigment cells, blood vessel cells, and neuron-like cells. The major limitation of intramolecular DNA/gene transfer is that expression may be transient, may not be stably inherited, because the injected DNA generally remains episomal and may not integrate efficiently (Lee *et al.*, 2013).

Several genes have now been introduced into various fish species to enhance growth, resistance to disease, tolerance to freezing, etc. (Chatakondi *et al.*, 1995; Dunham *et al.*, 2002; El-Zaeem, 2001, 2004; El-Zaeem and Assem, 2004; Guo *et al.*, 2021; Shears *et al.*, 1991). According to Dunham (2011), DNA transfer techniques have shown promising results in improving the growth

performance of various fish species, including Nile Tilapia. In a study by El-Zaeem and Assem (2004), they found that injecting shark DNA into the skeletal muscles of *O. niloticus* resulted in accelerated growth and improved body composition. Additionally, Martinez *et al.* (2000) reported a 290 % food conversion efficiency in transgenic tilapia compared to the control group. Genetically modified *O. niloticus* showed more than 80 % weight increase compared to non-modified strains (El-Zaeem, 2011). Rahman (2001) reported a mean mass of 653 g in *O. niloticus* containing an exogenous fish growth hormone gene after 7 months of culture, representing a 2.5-fold increase in growth compared with non-transgenic siblings. Rahman *et al.* (2013) reported significant improvements in growth rates by 30 %, feed conversion efficiency, and overall biomass production in *O. niloticus* produced through gene transfer.

Genetically modified or transgenic Nile tilapia (*O. niloticus*) has been developed to enhance growth rates, disease resistance, and feed efficiency. Studies indicate that genetic modifications can influence the proximate composition of the fish, including moisture, protein, lipid, and ash content (Ahmed *et al.*, 2022; Sharma and Sharma, 2021). Studies comparing transgenic and non-transgenic tilapia suggest that transgenic variants may exhibit higher protein content and altered lipid profiles, potentially due to enhanced metabolic activity (Guo *et al.*, 2021). Several authors reported the proximate composition of Nile Tilapia: Job *et al.* (2015), Olopade *et al.* (2016), and Otene *et al.* (2024). The proximate composition of genetically modified Tilapia has been documented by El-Hawarry (2012), El-Zaeem *et al.* (2023) and Suwannatrai *et al.* (2023).

DNA fingerprinting effectively differentiates between closely related species or individuals by examining variations in genomic sequences (Du *et al.*, 2025). Molecular markers used in DNA fingerprinting include Restriction Fragment Length polymorphisms (RFLP), Random Amplification of Polymorphic DNAs (RAPD), microsatellite, and single-nucleotide polymorphism (Khatei *et al.* (2021). Random Amplified Polymorphic DNA has been successfully used to differentiate species and

subspecies of tilapia, such as *O. niloticus*, using polymorphic banding patterns generated by random primers (Bardakci and Skibinski, 1994). The RAPD works by amplifying random segments of genomic DNA using short primers, produced unique banding patterns that reflect genetic variation (Ali *et al.*, 2004; Bardakci and Skibinski, 1994; Dinesh *et al.*, 1993). The RAPD serves as both forward and reverse primers, and is usually able to amplify fragments from 1–10 genomic sites simultaneously. They are quick and easy to assay, require a low quantity of DNA with no sequence data for primer construction (Welsh and McClelland, 1990).

Microinjection of Genomic DNA (gDNA) into another fish could affect the nutritional profile of the recipient fish (Gultom, 2023). Proximate analysis of fish helps to determine the nutritional profile of the fish and is one of the major indicators of fish quality for human consumption (Jannatun *et al.*, 2023). Determining the proximate composition of genetically modified fish ensures that the fish meat meets high standards Otene *et al.* (2024) and ensures that genetic modification does not negatively impact the nutritional profile, making it a healthy protein source.

The constraints for the development and growth of Tilapia farming in Nigeria are slow growth due to poor seed quality (Ahmed and Siriwardena, 2023), early maturity, and high prolificacy. The high prolificacy leads to overpopulating the pond, resulting in stunted fish and poor market. The use of synthetic hormones to control reproduction (sex reversal) to obtain fast-growing all-male Tilapia has been successful. However, the technology is cumbersome, coupled with global concern about the effect of this steroid (hormone) in fish flesh on humans and the environment. The knowledge of the fate of the residuals of the steroids in fish is still low (Mlalila *et al.*, 2015). According to Karaket *et al.* (2023), the use of hormones, especially 17 α -Methyltestosterone in Nile tilapia farming, has been of concern to consumers as it involves potential risks to human health and environmental contamination by its residue. Additionally, the effects of the hormone and its residue on the vital

organs of man are also uncertain. Natural hormonal sex reversal to produce all males Tilapia as an improvement in the growth of Tilapia has been attempted by several authors, Andri *et al.* (2010), Chukwu *et al.* (2012), Diyaware *et al.* (2016). Mohammed *et al.* (2024), but with limited success (less than 100 % male). There is a paucity of information on the effects of *Lates niloticus* DNA on the growth performance, proximate composition, and DNA fingerprint of *Oreochromis niloticus* in Nigeria. The objectives of the study, therefore, are to investigate the growth performance, proximate composition, and DNA fingerprint of *O. niloticus* containing *Lates niloticus* DNA.

Hypothesis:

H₀ = *Lates niloticus* DNA cannot significantly increase growth and influence proximate composition of *O. niloticus*

H₁ = *Lates niloticus* DNA can significantly increase growth and influence proximate composition of *O. niloticus*

MATERIALS AND METHODS

Study Area

The study was conducted at the Teaching and Research Fish Farm of the Department of Fisheries, Faculty of Agriculture, University of Maiduguri, Nigeria. The study area is situated between latitude 11° 48' 41"N and longitude 13° 12' 33" E, with hot and cool periods as well as rainy (wet) and dry seasons. The wet season has a short duration of erratic rainfall of 3 - 4 months per year, with an approximate 519.34 mm, with annual totals ranging from 292.7 mm to 838.2 mm, with its peak in August, with the highest mean monthly rainfall of approximately 196.66 mm (Abatcha *et al.*, 2024). During the dry season, ambient temperatures are lower in December and January, ranging from 15 - 20 °C (night), and higher in March to June at 33 - 47 °C (day). The relative humidity ranges between 33.5 - 34.5% (Kyari *et al.*, 2026)

Source of Donor Fish

The Nile Perch, *Lates niloticus* (300 - 456.0 g) were obtained from Karabonde landing site (Fig. 1), Kainji Lake, Niger State, Nigeria. Karabonder landing site is situated between latitudes 9.899403° and longitudes 4.564203°.



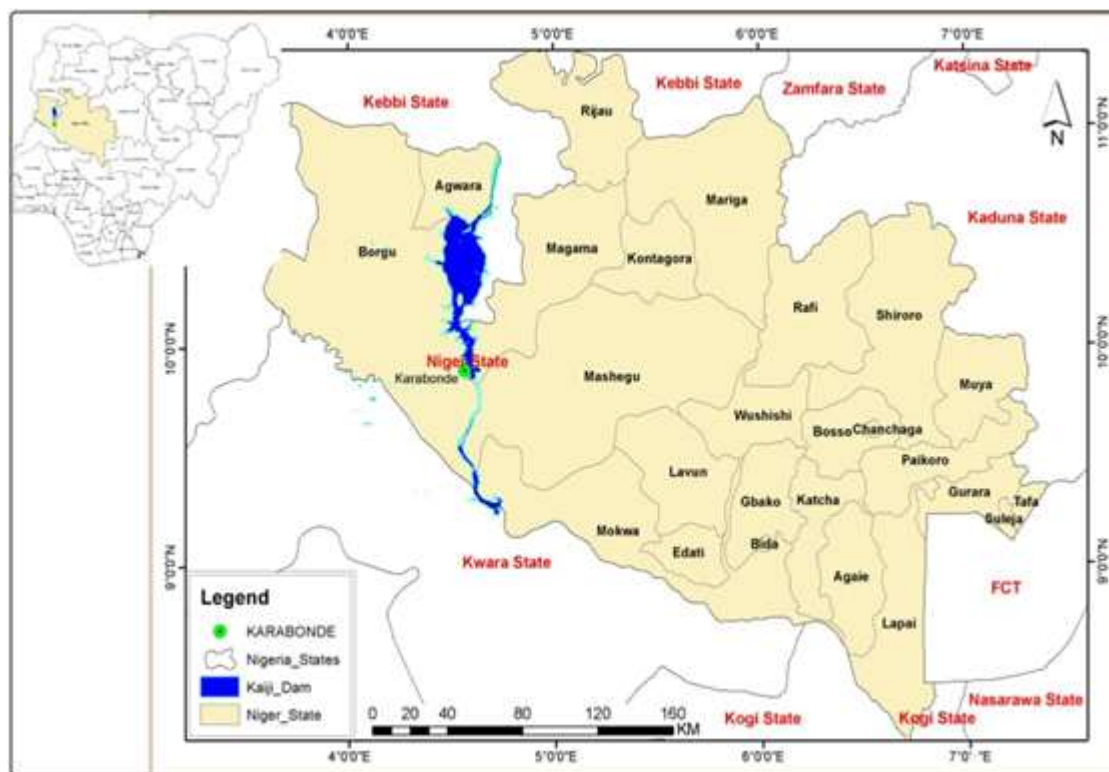


Fig. 1: Map of Niger State showing Karabonde landing site, Kainji Dam, Nigeria.

DNA Extraction and Quantification

Fifty (50) mg tissue of the Nile Perch, *Lates niloticus*, were collected, preserved in DNA-grade ethanol, and transported to the Northeast Biotechnology Centre, University of Maiduguri, Nigeria. Genomic DNA was extracted from the tissue of *L. niloticus* following the protocol described by Baradakci and Skibinski (1994). The tissues were crushed into smaller pieces using a laboratory pestle and mortar and homogenized using a tissue homogenizer. The crushed tissues were then transferred into a microcentrifuge tube containing 50 mM Tris Bis buffer, 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.1 % SDS, and 0.5 mg/ml proteinase K, and incubated overnight for the samples to digest. After incubation, DNA was extracted twice for 15 - 20 minutes with a 1:1 ratio of phenol and chloroform and again twice for 15 minutes with 24:1 volume of chloroform and isoamyl alcohol. The aqueous phase was then precipitated with 2.5 volumes of 100% DNA grade ethanol in the presence of one-tenth volume of 3.0 M sodium acetate (pH 6.0). The DNA pellets were

washed with 70 % DNA-grade ethanol and dissolved in 0.1 ml saline sodium citrate (SSC) buffer. The DNA concentration was measured at 260nm wavelength while purity was estimated at a ratio of A_{260}/A_{280} using an Ultraviolet (UV) Spectrophotometer (Model: Nanodrop 2000/2000c, Thermo-Scientific, USA).

Experimental Design

A total of 675 juveniles of *Oreochromis niloticus* (mean weight of 104.47 g and 5.47 cm length) were divided into five groups, with 135 juveniles per group. Different concentrations of SSC buffer-based DNA: 0 as control (injected with buffer solution only), 10, 20, 30, and 40 $\mu\text{g}/\mu\text{l}$ with a final volume of 0.1ml were injected into the dorsal somatic tissue of the fingerlings, above the lateral line towards the head using an insulin syringe and needle (29 G x 1/2) in three replicates. The fingerlings were then reared in a 3 x 2 x 1.2 m deep polyethylene fish pond at 7.5 fish/ m^2 in three replicates (45 x 3 =135) arranged randomly. The fingerlings were fed with a 35 % crude

protein commercial diet at 5% body weight twice daily at 9:00 am and 3:00 pm for 120 days.

Growth Performance of *O. niloticus* Containing Exogenous DNA

At the end of the three months rearing period, final weight (g), final length (cm), fish mortality, and quantity of feed applied were recorded. The following growth indices were estimated for each treatment:

- i) Weight gain (g) = $W_2 - W_1$, where W_2 and W_1 are the final and initial weights of the fish, respectively.
- ii) Percentage weight gain = $\frac{W_2 - W_1 \times 100}{W_1}$
- iii) Mean Daily Weight Gain (MDWG) in grams = $\frac{W_2 - W_1}{t}$, t = the culture period (days).
- iv) Specific Growth Rate (SGR % per day) = $\frac{\ln \log W_2 - \ln \log W_1}{t} \times 100$, where $\ln \log W_2$ = natural log of final weight, $\ln \log W_1$ = natural log of initial weight, and t = culture period (Busacker *et al.*, 2012).
- v) Feed conversion Ratio (FCR) = $\frac{\text{feed intake (g)}}{\text{fish weight gain (g)}}$
- vi) Survival (%) = $\frac{\text{No. of fish survived}}{\text{No. of fish stocked}} \times 100$.

Monitoring of Water Quality

Time series water quality was monitored during the study. The temperature (°C), pH, electrical conductivity (µS/cm), and total dissolved solids (ppm) were measured using a Multifunction 4-in-1 Temp./EC/ TDS/pH Meter (Model: 9908, China), and dissolved oxygen were recorded using a portable digital DO meter (Model: BLE-9100).

Proximate Composition of *O. niloticus* Improves Through DNA Transfer.

One hundred and fifty (150) g of fish muscle from each treatment were collected and placed in an icepack containing ice flakes and shipped to the Animal Care quality control laboratory, Kano State, Nigeria, for proximate composition. Before the proximate analysis, wet chemistry calibration against the Association of Official Analytical Chemists (AOAC) standard was done. To calibrate against standard methods, 50 g representative of

fish samples containing *L. niloticus* DNA were collected and subjected to proximate composition analysis using standard wet chemistry methods as described in the Association of Official Analytical Chemists (AOAC) (2023). Moisture content was determined by oven drying at 105 °C until constant weight. Crude protein was analysed using the Kjeldahl method, Crude lipid determined using Soxhlet extraction with petroleum ether, Ash content was determined by incineration in a muffle furnace at 550 °C, and crude fibre was analysed using the enzymatic gravimetric method. The same fish samples were scanned using the Near-Infrared Reflectance (NIR) multi-checker across the spectral range of 780 –2500nm. Spectral data were processed using Partial Least Squares (PLS) regression to establish calibration equations by correlating NIR absorbance patterns with the AOAC-verified wet chemistry values. Calibration models were validated with independent fish samples, and prediction accuracy was assessed using the Root Mean Squared Error of Prediction (RMSEP). The proximate composition was conducted in triplicate using a digital Near Infrared (NIR) Multi-Checker (Model: 21MC11309A06, China) according to AOAC (2023). While metabolizable energy was estimated using Atwater conversion factors, Sanchez-Pena, *et al.* (2017) formula:

$ME \text{ (kcal/kg)} = (CP \times 5.65) + (EE \times 9.45) + (NFE \times 4.15)$, where CP = Crude Protein (%), EE = Ether Extract / Crude Fat (%), and NFE = Nitrogen-Free Extract (%)

RAPD Protocol/Fingerprint Analysis

At the end of the experiment, 50 mg tissue sample from each treatment (including the control) in three replicates was collected after sedation according to the method described by Diyaware *et al.* (2017). Three tissues were also collected from the donor fish (*L. niloticus*). The samples were stored at –20°C until DNA extraction. Genomic DNA was extracted from the tissues following the protocol described by Baradakci & Skibinski (1994). Each tissue was crushed individually into smaller pieces using a laboratory pestle and mortar and homogenized using a tissue homogenizer. The crushed tissues were then transferred into separate microcentrifuge tubes containing 50 mM Tris Bis buffer, 100 mM EDTA (pH 8.0), 100 mM NaCl, 0.1 %



SDS, and 0.5 mg/ml proteinase K. The mixture was incubated overnight for the samples to digest. After incubation, DNA was extracted twice for 15 - 20 minutes with a 1:1 ratio of phenol and chloroform and again twice for 15 minutes with 24:1 volume of chloroform and isoamyl alcohol. The aqueous phase was then precipitated with 2.5 volumes of 100 % DNA grade ethanol in the presence of one-tenth volume of 3.0 M sodium acetate (pH 6.0). DNA pellets were washed with 70 % DNA-grade ethanol and dissolved in 0.1 ml saline sodium citrate (SSC) buffer. The DNA concentration was measured at 260nm wavelength, while purity was estimated at a ratio of A_{260}/A_{280} using an Ultraviolet (UV) Spectrophotometer (Model: Nanodrop 2000/2000c, Thermo-Scientific, USA).

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) reactions were performed in 25 μ L volumes containing 10 $\times$ PCR buffer, 2.0 mM $MgCl_2$, 0.2 mM dNTPs, 1.0 μ M primer, 1 U Taq DNA polymerase, and 20 – 50 ng template DNA. Three RAPD primers modified from Ogbuebunu and Awodiran (2017) were used for the PCR (Table 1). All primers were amplified at an initial denaturation of 94 $^{\circ}C$ for 3 minutes, 40 cycles, denaturation temperature of 94 $^{\circ}C$ for 30 seconds, annealing temperature of 36 $^{\circ}C$ for 1 minute, extension at 72 $^{\circ}C$ for 1 minute, final extension at 72 $^{\circ}C$ for 10 minutes, and holding temperature of 4 $^{\circ}C$.

Table 1: Random Amplified Polymorphic DNA (RAPD) Primer Sequences

Primers	Primer Sequence
OPH-05	AGTCGTCCCC
OPT-06	CAAGGGCAGA
OPT-20	GACCAATGCC

Gel Electrophoresis

Polymerase chain reaction products were resolved on 1.5 % agarose gels at a voltage of 70V for 40-50 minutes stained with ethidium bromide. The gels were visualized under gel documentation Ultraviolet (UV) light plate and photographed. Banding patterns were compared between the fish that received the DNA and the control fish. The presence of unique bands in injected fish was interpreted as evidence of foreign DNA presence or integration.

Scoring, Analysis of RAPDs, and Estimation of the Molecular Weight

The genotypes were determined by recording the presence (1) or absence (0) of bands in the RAPD profiles. The dendrogram was then constructed using Euclidean cluster analysis. The molecular weights of RAPD fragments were estimated according to the method described by Sambrook & Russell (2001) by comparing their electrophoretic mobility with that of a standard DNA ladder. Migration distances (cm) of the marker bands were measured from the wells to the center of each band, and a standard curve was constructed by plotting the logarithm ($\log_{10}$) of the known fragment sizes (bp) against

their migration distances. The sizes of RAPD bands were then interpolated from this curve.

Data Analysis

Data obtained on growth indices, proximate composition, and time series water quality parameters were subjected to a One-way Analysis of Variance (ANOVA). The differences between the means were determined using Fisher's Least Significant Difference (LSD) at 95 % confidence level ($p < 0.05$) with the aid of Statistix 8.0. The RAPD fingerprints generated for all treatments were scored using Gel analyzer. The genetic diversity was determined using GenAlex (Peakall and Smouse, 2012). Phylogenetic trees to check genetic distance were constructed using Nei (1972). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) Euclidean Cluster analysis with the aid of PAST 4.03.

RESULTS AND DISCUSSION

Water Quality Parameters

Table 2 shows the time series water quality parameters observed during the study. Dissolved Oxygen (DO) ranged



from 6.20 to 6.48 mg/L in May, 6.41- 6.66 mg/L in June, 6.40-6.80 mg/L in July, and 6.15-6.80 mg/L in August. While the temperature ranged between 30.80-32.20, 28.70-29.80, 28.30-29.80, and 28.60-29.33°C in May, June, July, and August, respectively. No significant variation ($p>0.05$) was observed between the DO in May, June, and July, except in August, where significant differences ($p>0.05$) were observed between the DO values among the rearing tanks. The pH values in the culture media during May were between 7.48 and 8.34, in May, 8-8.62 in June, 8-8.90 in July, and 7-8.01 in August. The TDS were between 93.00 - 125.33 (ppm) in May, 150.33 – 162.33 (ppm) in June, 150 – 152 in July, while in August the TDS

were between 107 - 127.67 (ppm). The Electric Conductivity (EC) ranged between 186-251.33 ($\mu\text{S}/\text{cm}$) in May 301-308. ($\mu\text{S}/\text{cm}$) June, while in July and August the EC were between 255-305 and 212 - 257.33 $\mu\text{S}/\text{cm}$, respectively.

The variations in the dissolved oxygen contents in the culture medium might have affected the growth performance and survival among treatment groups, despite the fact that all the water quality parameters observed were within the recommended range for Tilapia culture (Boyd and Tucker, 1998).

Table 2: Time Series water quality parameters observed during the study

Parameters	Deoxyribonucleic Acid (DNA) Concentration (µg/µl)					SE (p≤0.05)
	0	10	20	30	40	
May						
DO (mg/L)	6.20	6.22	6.42	6.28	6.20	0.46
Temp. (°C)	31.77	32.20	31.10	32.17	30.80	0.67
pH	7.87	8.24	8.34	8.12	7.95	0.54
TDS (ppm)	109.33 ^{ab}	111.33 ^{ab}	105.33 ^{ab}	125.33 ^a	93.00 ^b	10.37
EC (µS/cm)	219.33 ^{ab}	223.67 ^{ab}	211.00 ^{ab}	251.33 ^a	186.67 ^b	20.97
June						
DO (mg/L)	6.52	6.58	6.49	6.66	6.41	0.26
Temp. (°C)	28.70	29.80	29.47	29.63	29.47	0.60
pH	8.47	8.45	8.62	8.50	8.43	0.17
TDS (ppm)	162.33	150.33	152.67	152.67	151.67	10.99
EC (µS/cm)	305.33	301.00	308.00	305.67	303.67	23.16
July						
DO (mg/L)	6.80	6.58	6.49	6.49	6.40	0.37
Temp. (°C)	28.30 ^a	29.80	29.23 ^{ab}	29.63	29.47	0.52
pH	8.90	8.45	8.90	8.50	8.05	0.28
TDS (ppm)	128.67	150.33	127.33	152.67	151.67	18.33
EC (µS/cm)	257.67	301.00	255.67	305.67	303.67	36.70
August						
DO (mg/L)	6.80 ^a	6.45 ^{ab}	6.15 ^b	6.48 ^{ab}	6.67 ^{ab}	0.26
Temp. (°C)	28.87 ^{ab}	29.17 ^{ab}	28.77 ^{ab}	29.33 ^a	28.60 ^b	0.32
pH	7.27	7.47	8.01	7.90	7.38	0.44
TDS (ppm)	112.00 ^{ab}	113.00 ^{ab}	110.33 ^b	127.67 ^a	107.33 ^b	7.62
EC (µS/cm)	215.00 ^b	227.00 ^{ab}	212.00 ^b	257.33 ^a	202.33 ^b	18.35

Growth Performance of *O. niloticus* Containing Exogenous Fish DNA

Table 3 shows growth performance of *O. niloticus* injected with *L. niloticus* DNA. Fish injected with 40 µg/µL *L. niloticus* DNA had significantly ($p < 0.05$) higher final weight (751.90 ± 18.50 g), weight gain (650.02 ± 19.25 g), average daily weight gain (5.41 ± 0.16 g/day), percentage weight gain (432.96 ± 10.34 %), and specific growth rate (2.86 ± 0.01 %/day) compared to other treatments. The results are in agreement with El-Zaeem (2004), El-Zaeem and Assem (2004), Hemeida *et al.* (2004), and Assem and El-Zaeem (2005), who found enhanced growth performance in *O.*

niloticus and *T. zillii* (*Coptodon zilli*) injected with 40 µg/µL shark DNA. Similarly, El-Zaeem *et al.* (2011, 2012) observed higher body weight and specific growth rate in *O. niloticus* injected with Seabream and *Artemia salina* DNA. El-Zaeem (2012) also reported improvements in growth performance of grey mullet fingerlings injected with shark DNA. Kusa *et al.* (2025) and Thabet *et al.* (2025) reported improved final weight, mean daily weight gain, and specific growth rate in *O. niloticus* injected with 40 µg/µL *Heterotis niloticus* DNA and Salmon DNA, respectively. No significant variation ($p > 0.05$) was observed among the final length cross all the treatments.

Table 3: Growth Performance of *O. niloticus* Containing Exogenous Fish DNA

Parameters	DNA Concentrations (µg/µL)				
	0	10	20	30	40
IW (g)	103.29±0.33	103.03±0.73	103.18±0.75	102.21±0.70	101.88±0.84
FW(g)	653.35±12.55 ^b	587.01±6.11 ^c	573.53±34.50 ^c	658.91±5.20 ^b	751.90±18.50 ^a
IL (cm)	5.21±0.14	5.28±0.064	5.80±0.42	5.70±0.12	5.34±0.09
FL (cm)	11.37±0.34	11.38±0.59	12.28±0.70	12.35±0.38	13.51±0.26
WG (g)	550.06±12.34 ^b	483.97±6.54 ^c	470.35±35.24 ^c	556.69±4.62 ^b	650.02±19.25 ^a
ADWG (g/day)	4.58±0.10 ^b	4.03±0.05 ^c	3.92±0.29 ^c	4.64±0.04 ^b	5.41±0.16 ^a
% WG	375.40±23.57 ^b	388.67±53.78 ^a	366.42±45.02 ^a	397.39±2.99 ^b	432.96±10.34 ^a
SGR (%/Day)	2.79±0.01 ^b	2.74±0.02 ^c	2.74±0.02 ^c	2.80±0.01 ^b	2.86±0.01 ^a
FCR	1.19±0.02 ^c	1.38±0.06 ^{ab}	1.46±0.07 ^a	1.37±0.01 ^{ab}	1.31±0.03 ^{bc}
Survival (%)	27.00±0.56	24.33±1.45	23.33±2.19	25.33±0.88	27.67±05.46

Means with the same superscript in the same row are not significantly different ($p > 0.05$).

Key: IW = Initial weight, FW = Final weight, IL=initial length, final length, WG = Weight gain, ADWG = Average daily weight gain, SGR = Specific growth rate, FCR = Feed conversion ratio.

Abd El Hamied (2009) and Elwan (2009) observed improved growth performance, feed utilization, and other quantitative traits in *O. niloticus* injected with foreign DNA. Higher growth indices in fish injected with higher DNA may indicate transient expression or epigenetic effects, as suggested by Konstantinidis *et al.* (2020).

Feed Conversion Ratio (FCR) was significantly ($p < 0.05$) higher (1.46 ± 0.07), in fish injected with 20 (µg/µL) DNA. There were no significant variations ($p < 0.05$) observed in the FCR of fish injected with 10 and 30 µg/µL DNA compared to those injected with 20 µg/µL. Significant variations ($p > 0.05$) were observed in FCR of fish injected with 40 (µg/µL) DNA compared to those injected with 10-

30 (µg/µL) *L. niloticus* DNA and control fish. The FCR of *O. niloticus* injected with *L. niloticus* DNA in this study was lower than 1.88 and 1.89 reported by El-Zaeem (2012) for grey mullets (*Mugil cephalus*) injected with Shark DNA. The FCR was also lower than 1.83 reported by El-Zaeem *et al.* (2023) in *Coptodon zilli* following injection with shark DNA, and 1.5 reported by Krivins (2024) for *O. niloticus* fed a commercial diet. The variation may be due to differences in the fish species, rearing conditions, the sources of the DNA, and the degree of domestication selection the species might have undergone. The lower FCR observed in this study may also be due to the trade-off between growth acceleration and nutrient utilization. The trade-off

between growth acceleration and nutrient utilization efficiency has been reported in several studies in transgenic and genetically improved fish. El-Zaeem *et al.* (2009) observed that shark DNA injected into red tilapia improved growth and feed utilization, but optimal efficiency was achieved under moderate dietary protein levels, suggesting that excessive stimulation might have compromise metabolic balance. Rahman *et al.* (2001) confirmed that transgenic tilapia are also more efficient in utilizing protein and energy.

The survival of *O. niloticus* exposed to varying concentrations of *L. niloticus* DNA (0–40 µg/µL) ranged between 23.33% and 27.67%, with no significant differences ($p > 0.05$) among treatments. This indicates that DNA concentration did not affect survival, which is consistent with earlier reports that tilapia tolerates foreign DNA exposure without major mortality effects (Rahman *et al.*, 1998; El-Zaeem, 2012; Konstantinidis *et al.*, 2020). The

relatively low overall survival likely reflects environmental or handling stress, which has been shown to exert a stronger influence on tilapia survival than genetic manipulation (Mwaura *et al.* 2023).

The Growth Pattern of *O. niloticus*, Containing Exogenous Fish DNA

Fig 2 shows the growth pattern of *O. niloticus*, containing exogenous fish DNA. A significant ($p < 0.05$) progressive growth increase was observed with the increase in DNA concentration throughout the study. The progressive growth pattern may indicate that fish containing high concentrations of DNA might have efficiently utilized the diet given to them. Fu *et al.* (1998) reported that transgenics were more efficient in utilizing dietary protein compared to their non-transgenic siblings. Rahman *et al.* (2001) confirmed that transgenic tilapia are also more efficient in utilizing protein and energy.

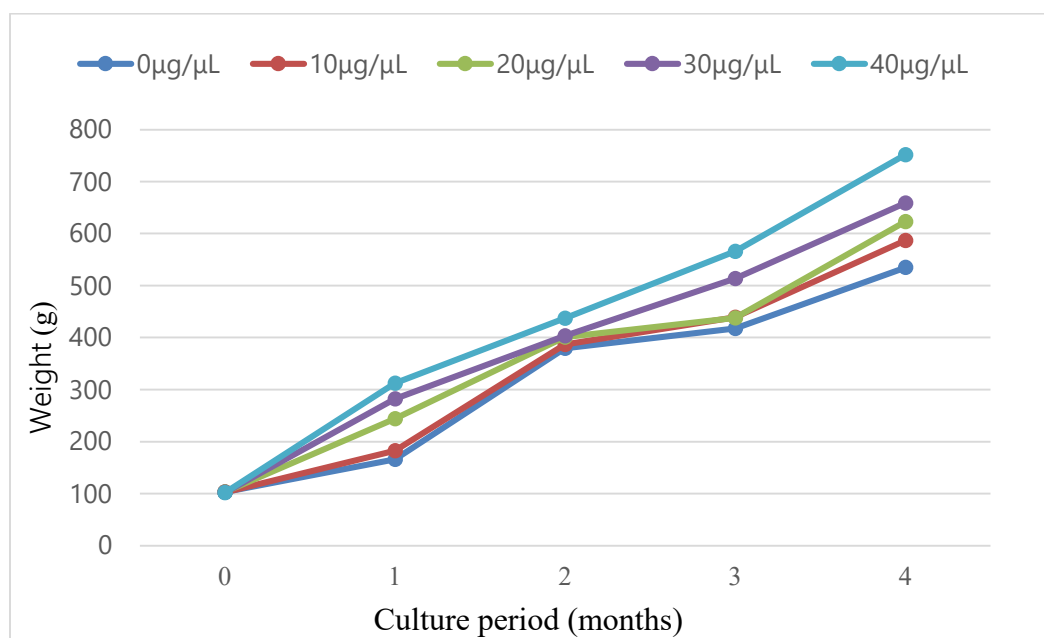


Fig. 2: Growth pattern of *O. niloticus* containing exogenous fish DNA

Proximate Composition of *O. niloticus* Containing Exogenous Fish DNA

Table 4 shows the proximate composition of *O. niloticus* containing exogenous *L. niloticus* DNA. Crude protein (CP)

content increased significantly ($p < 0.05$) with an increase in the DNA concentration. A higher CP of 39.05 % was observed in fish injected with 40 µg/µl of DNA, followed by 36.36 % CP in fish injected with 30 µg/µl of DNA. A

lower CP of 27.90 % was observed in the control group, suggesting that the foreign DNA might have enhanced muscle protein synthesis in the Nile Tilapia.

The protein contents observed in this study were higher than 14.62 % CP observed in red belly tilapia (*Coptodon zilli*) injected with Shark DNA, as observed by El-Zaeem *et al.* (2023), and 11.32, 13.10, and 9.80 % in *O. niloticus*, *O. aureus*, and their hybrids respectively, reported by El-Hawarry (2012). Suwannatrai *et al.* (2023) reported 14.27 % crude protein in *O. niloticus* from Sakon Nakhon province, Thailand, while Premarathna *et al.* (2020) documented 7.85 % crude protein in *O. niloticus* from Sri Lanka. The variation in the crude protein levels may be due to the differences in the fish species, the source of the

DNA, and the age of the fish. However, Mukti *et al.* (2020) reported 85.70 and 87.00 % crude protein in triploid *O. niloticus*. The greater variation observed in the crude protein levels of the triploid (sterile) Tilapia compared to the tilapia containing *L. nilotiucs* DNA could be due to the complete utilization of feed for growth only by the triploid fish, rather than growth and reproduction as occurs in the fertile Tilapia containing foreign DNA. Martinez *et al.* (2000) reported an increased protein synthesis and growth rate together with enhanced glycolysis and oxidation of amino acids in juvenile *O. niloticus* containing the DNA. The increase in crude protein level observed in the fish injected with 40 µg/µl *L. niloticus* DNA may be due to the effective conversion of sparing nutrients from fish feed by the fish.

Table 4: Proximate Composition of *O. niloticus* Containing Exogenous Fish DNA.

Nutrients (%)	DNA Concentration (µg/µl)				
	0	10	20	30	40
CP	27.90±0.33 ^e	33.28±0.39 ^d	34.76±0.21 ^c	36.36±0.33 ^b	39.05±0.49 ^a
Ash	0.89±0.05	0.86±0.08	0.94±0.03	0.63±0.32	0.88±0.06
EE	10.19±0.4 ^c	13.12±0.03 ^a	11.13±0.02 ^c	9.16±0.03 ^e	12.47±0.02 ^a
CF	1.34±0.00 ^b	1.64±0.03 ^a	0.98±0.01 ^d	1.06±0.00 ^c	0.92±0.03 ^e
Moisture	60.83±0.17 ^b	58.63±0.25 ^c	60.44±0.96 ^b	61.40±0.00 ^b	63.94±0.02 ^a
ME(Kcal)	2560.40±3.73 ^d	3000.60±13.68 ^b	2754.40±100.60 ^c	2970.20±3.09 ^b	3474.10±91.36 ^a

Means in the same column having similar superscripts are not significantly different ($p>0.05$).

Key: CP = Crude protein, EE = Ether extract, CF = Crude fiber, ME = Metabolizable energy.

A similar trend was also reported by Yuvarajan *et al.* (2019) in Genetically Improved Farmed Tilapia (GIFT), which recorded a remarkable increment in protein and lipid contents. Such an increase implies improved nutritional quality, which is important for Tilapia aquaculture Cebeci *et al.*, 2020). Abd El Hamied (2009) and Elwan (2009) reported higher body composition, in *O. niloticus* injected with foreign DNA.

Ash

The Ash levels showed no significant variation ($p>0.05$) among the entire treatments, suggesting that foreign DNA may had minimal effect on mineral content. The insignificant ash contents among the entire treatments aligns with report of Abdel-Tawwab *et al.* (2010) who opined that ash is less sensitive to genetic or dietary

manipulation. The ash contents observed in this study are lower than 1.36 % in *O. niloticus* and hybrid red tilapia reported by Olopade *et al.* (2016) in Nigeria and 2.27±0.34 documented by Kamruzzaman *et al.* (2018) for *O. niloticus* collected from Mymensingh Messua Baza of Bangladesh.

Ether Extract

Ether Extract (EE) was significantly ($p<0.05$) higher (13.12±0.3 and 12.47±0.02 %) in fish treated with 10 and 40 µg/µl DNA, respectively. The lowest EE (9.16±0.03 %) value was observed in fish containing 30 µg/µl DNA concentration. Generally, there were significant variations ($p<0.05$) among the mean values of the EE across all the treatments. The ether extract content, which reflects the lipid concentration in fish, showed noticeable ($p<0.05$) variations across the treatments. High lipid levels,

especially in fish containing 10 and 40 µg/µl DNA, may indicate altered lipid metabolism probably due to foreign DNA expression. The observed (9 - 13 %) EE may indicate that the foreign DNA could have influenced lipid metabolism in the Nile tilapia. Genetic modification, particularly through DNA/gene transfer, is known to impact lipid regulation pathways. A similar trend was observed in transgenic mice containing silencing genes (*SREBP-1c* and *PPARα*), which significantly changes lipid synthesis and fatty acid oxidation (Horton *et al.*, 2002). Devlin *et al.* (2001) demonstrated that transgenic salmon exhibited increased lipid accumulation due to enhanced growth signals, suggesting that similar mechanisms might explain the ether extract changes observed in this study. Therefore, while moderate increases in EE could enhance nutritional value, careful optimization may be required to balance consumer health concerns and product quality. Similarly, higher ether extract in the fish containing the foreign DNA may result in beneficial fatty acids such as Omega-3, which can enhance the fish's health value as suggested by Tocher (2003).

Crude fiber

Crude fibre was observed to be significantly ($p < 0.05$) higher ($1.64 \pm 0.03\%$) in fish injected with 10 µg/µl DNA compared to the entire treatment. Lowest ($0.92 \pm 0.01\%$) crude fibre content was observed in the group injected with 40 µg/µl DNA. The crude fibre observed in this study is higher than 0.98 % reported by Yuvarajan *et al.* (2019) in Genetically Improved Farmed Tilapia (GIFT) and 0.54 and 0.59 % reported by Olapode *et al.* (2016) in *O. niloticus* and hybrid red tilapia, respectively. The variation in the fibre content may be due to differences in the fish strain (GIFT, normal tilapia, and the hybrids). Although fish naturally contain low fiber, these changes may reflect alterations in gut microbiota or feed digestion due to genetic influence. Bolawa *et al.* (2011) reported very low crude fibre, while Otene *et al.* (2024) found fibre values consistently to be below 1 %, emphasizing that fish muscle is not a source of dietary fibre.

Moisture Content

Moisture content increases with an increase in DNA concentration. Moisture was significantly ($p < 0.05$) higher

(63.94%) in fish that received 40 µg/µl DNA. This trend suggests a positive relationship between DNA concentration and moisture retention in fish. The higher moisture contents observed in this study are lower than 81.39 and 80.09 % in *O. niloticus* and hybrids of red Tilapia, respectively, observed by Olopade *et al.* (2016). El-Hawarry (2012) reported 79.09, 79.12, and 80.45 % moisture contents of fillets of *O. niloticus*, *O. aureus*, and their hybrids, respectively. The high moisture content observed in fish injected with a higher dosage of DNA may indicate that the muscle tissue of the fish retains more water, which could affect texture, shelf life, and susceptibility to spoilage (perishability). Wang and Zheng (2025) emphasize that enzymatic autolysis, lipid oxidation, and microbial growth are the main drivers of fish spoilage, all of which are facilitated by high moisture levels in the fish. Elevated water retention may be associated with changes in muscle plasticity and gene regulation. Recent studies by Koganti *et al.* (2020) highlight the role of DNA methylation and microRNAs in modulating muscle growth and water-binding properties in fish. Significant variations ($p < 0.05$) were observed between the mean moisture contents of the fish treated with 10 µg/µl DNA compared to the entire treatments. However, no significant variations ($p > 0.05$) were observed among the moisture contents of fish treated with 0, 20, and 30 µg/µl DNA.

Metabolizable Energy

Metabolizable energy was significantly higher (3474.10 ± 91.36 Kcal) in fish treated with 40 µg/µl DNA, followed by (2970 ± 3.0 Kcal) in fish treated with 30 µg/µl DNA. The increase in the Metabolizable energy (ME) with an increase in concentration of the DNA may indicate improved nutrient density and energy utilization, which may reflect metabolic shifts which might have been influenced by the presence of foreign DNA sequences related to growth or feed efficiency. According to Thabet *et al.* (2025), foreign DNA can upregulate metabolic efficiency, thereby increasing metabolizable energy concentration. Konnert *et al.* (2022) opined that high ME indicates efficient nutrient conversion in the fish, which may translate to better protein and fat availability for human consumption.

Fingerprint Random Amplified Polymorphic DNA Analysis

The DNA purity used in this study was 1.76 ng/ μ L at A260/A280 and 1.09 at A260/A230. Fig. 3 is the RAPD gel representative of *O. niloticus* injected with *L. niloticus* DNA. Gel electrophoresis revealed distinct banding patterns. The control fish had 5 bands, while fish injected with 10,

20, 30, and 40 μ g/ μ L had 7, 6, 8, and 9 bands, respectively, suggesting probable differential integration or transient expression of *L. niloticus* DNA fragments among the treated fish. The donor fish DNA fragment was approximately 300 bp in size, based on its position relative to the 250 bp and 500 bp ladder bands.

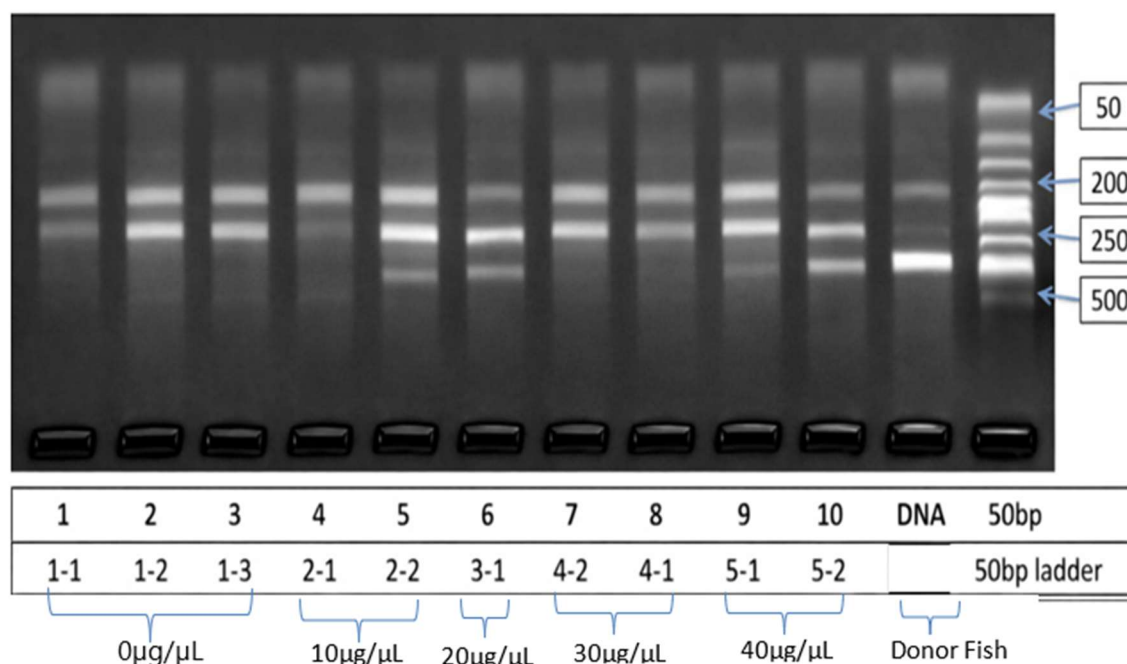


Fig. 3: Gel representative of the DNA fingerprint of *O. niloticus* containing *L. niloticus* DNA

Out of five loci scored, four were polymorphic, corresponding to 80% polymorphism. The 80% polymorphism observed in this study may be an indicator of genetic variability likely induced by the injected *L. niloticus* DNA. The observed polymorphism in RAPD profile may reflect genetic differences associated with introduction of *L. niloticus* DNA (Williams *et al.*, 1990). The 80 % polymorphysim observed in this study is higher than 50-70% for healthy fish populations as suggested by Ali *et al.* (2005). However, the high polymorphism observed in this study is less than 98.9% observed in Genetically Improved Farmed Tilapia (GIFT) reported by Oliveira *et al.* (2011) using RAPD. The variation could be due to

differences in the genetic improvement method; GIFT tilapia were improved through selection, which has high heritability, while DNA injected into the muscle is taken up by muscle cells and may be expressed locally, hence the effect may be somatic and transient (Heppell and Davis, 2000). The presence of polymorphic loci may suggest introgression of *L. niloticus* DNA. Genetic variability can enhance resilience to environmental stress and disease (Weising *et al.*, 2005), these findings align with findings of Sambrook and Russell (2001) who reports that RAPD analysis is effective in revealing high levels of polymorphism in fish.

The exhibition of different banding patterns may indicate genetic variation among the fish. The 250 bp band appears to relate with the increasing DNA concentration in *O. niloticus*, which may suggest a gene or DNA fragment silently or transiently expressed or integrated more at higher concentration (Kusa *et al.*, 2026). The 500 bp band, observed in fish injected with 30 and 40 µg/µL, might be a unique RAPD fragment only expressed or detectable at higher DNA concentrations, and is possibly a dose-sensitive gene or regulatory element activated at higher DNA loads.

The presence of distinct bands in the injected fish and their absence in controls may indicated transient DNA expression on DNA damage or stress caused by the foreign DNA. Intramuscular DNA delivery in fish is known to produce temporary expression of foreign genes. For instance, DNA vaccine studies in salmon and tilapia show that injected DNA can be transcribed in muscle fibers for a limited period before degradation (Heppell and Davis, 2000). Foreign DNA can activate DNA repair pathways in muscle cells and skeletal muscle exposed to exogenous DNA may undergo fragmentation due to nuclease activity and repair enzyme activation (Dey *et al.*, 2023). The distinct bands may be a stress response to the injected foreign DNA. According to Petitjean *et al.* (2019), stress in fish alters gene expression and DNA stability. The unique fragments may therefore reflect stress-induced molecular changes in tilapia muscle.

Assem and El-Zaeem (2005) reported integration of Shark DNA into the genome of *Tilapia zilli* (*Coptodon zillii*) after direct injection. In a similar vein, El-Zaeem *et al.* (2011) reported the random integration of sea bream and Artemia DNA into *O. niloticus* genomes. They claimed that the integrations could be a functional or a silent integration, as suggested by Yaping *et al.* (2001). Fujimura and Kocher (2011) demonstrated efficient transgenesis in *O. niloticus* using the Tol2 transposon system, achieving approximately 30% germline transmission and strong Green Fluorescent Protein (GFP) expression. Similarly,

Olabode *et al.* (2014) reported successful integration of growth hormone genes using Tol2-mediated microinjection, with PCR confirmation and enhanced growth in transgenic tilapia.

The *L. niloticus* DNA injected into *O. niloticus* may have induced genomic alterations or changes in RAPD amplification patterns, resulting in observed polymorphic RAPD profiles. Zhang *et al.* (1994) demonstrated that RAPD analysis detected genomic changes in tilapia following microinjection of foreign DNA, with altered banding patterns compared to non-injected controls.

RADP Banding and Fragment Size of *O. niloticus* Containing *L. niloticus* DNA

Table 5 shows the mean molecular weights of DNA bands in *O. niloticus* treated with *L. niloticus* DNA. Electrophoresis of *O. niloticus* containing *L. niloticus* DNA revealed DNA fragments between 430 and 540 bp, while the donor fish had an average of 565 bp. Lower concentrations (0 µg/µL) were associated with the smaller fragments (430 bp), while moderate doses (10–20 µg/µL) yielded larger fragments (502–540 bp). At higher concentrations (30–40 µg/µL), fragment sizes decreased slightly (452–475 bp), suggesting possible saturation or degradation effects. These results align with the principle that DNA migration is inversely related to fragment size (Lee *et al.*, 2012). The clustering of fragments near the donor reference (565 bp) suggests that injected DNA was maintained long enough to be detected, consistent with transient expression of foreign DNA (Li *et al.*, 2019). While Variability across concentrations may reflect epigenetic regulation, as mechanisms like DNA methylation and histone modification can silence or exert influence on the activities of the injected DNA (Bird, 2007; Zhang *et al.*, 2020). In tilapia, epigenetic responses to exogenous DNA have been shown to influence fragment stability and retention (Konstantinidis *et al.*, 2020). Thus, the observed patterns highlight the interplay between transient expression and epigenetic control in shaping the fate of donor DNA within host genomes.

Table 5: Mean Molecular Weights of DNA Bands in *O. niloticus* Injected with *L. niloticus* DNA

DNA Concentration ($\mu\text{g}/\mu\text{L}$)	Mean Fragment Size (bp)
0 (control)	430
10	502
20	540
30	475
40	452
Donor fish	565

Genetic Similarity Indices

The dendrogram generated from gel electrophoresis banding profiles using UPGMA (Fig. 4) cluster revealed distinct groupings among fish that received *L. niloticus* DNA. The control fish (0 $\mu\text{g}/\mu\text{L}$ of DNA) clustered separately from the donor fish, indicating genetic dissimilarity from their sibling that received DNA. In contrast, samples that received 10 to 20 $\mu\text{g}/\mu\text{L}$ DNA

showed progressive clustering toward *L. niloticus*, suggesting a dose-dependent assimilation of *L. niloticus* genetic material. *Oreochromis niloticus* injected with 30 and 40 $\mu\text{g}/\mu\text{L}$ DNA formed a close cluster, indicating close similarity. The donor fish (*L. niloticus*) clustered separately. The *O. niloticus* injected 10 $\mu\text{g}/\mu\text{L}$ exhibited moderate bootstrap, indicating that it is closer to *O. niloticus* than those injected with 30 and 40 $\mu\text{g}/\mu\text{L}$ DNA.

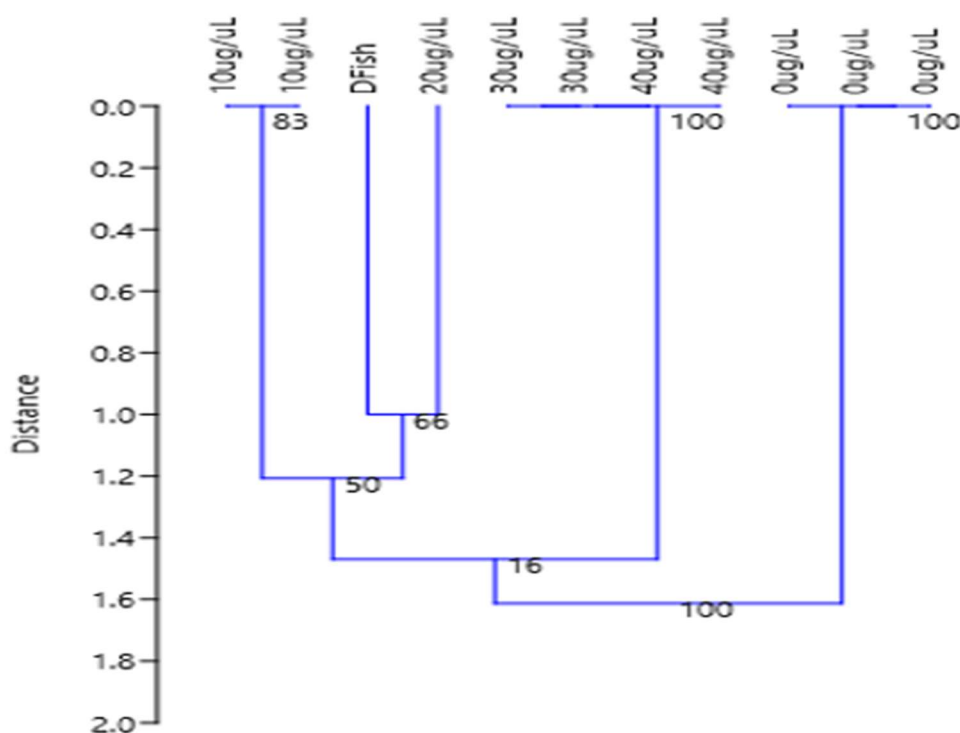


Fig. 4: Phylogenetic tree based on Nei's (1972) standard genetic distances using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method generated from RAPD data.

Key: DFish = Donor fish (*Lates niloticus*).

Overall, the dendrogram supports the hypothesis that the increasing DNA concentration enhances slight genetic similarity to the source organism, with 30 – 40 µg/µL representing a potential threshold for effective transient transformation.

Genetic Variability

This pattern of closer cluster formation of fish that received higher DNA concentration implies that higher DNA concentrations could have enhanced genomic integration or expression, which is consistent with previous findings in transgenic fish by Dai *et al.* (2010). In Zebra fish. The moderate bootstrap exhibited by fish injected with DNA closer to the donor fish, (*L. niloticus*), suggests partial uptake or variability in transformation efficiency. These findings align with earlier reports by Sneath and Sokal (1973), Fu *et al.* (2021), and Georgieva (2022) that hierarchical clustering can effectively resolve genetic relationships in electrophoretic data.

CONCLUSION

This study revealed that fragmented DNA materials from *Lates niloticus* may potentially improve growth in *Oreochromis niloticus*. Fragment of *L. niloticus* DNA transiently integrated into the *O. niloticus* genome. The DNA of *L. niloticus* has altered the proximate composition of Nile tilapia, especially by enhancing protein content and metabolizable energy. These changes could offer nutritional, economic, and production benefits in Tilapia aquaculture, though further investigation into safety, long-term performance, and consumer acceptance is essential.

Limitation of the Study

Although the present study demonstrates that injection of Nile perch DNA can enhance growth and proximate composition in Nile tilapia, several limitations may restrict its direct application to aquaculture. Only sham (fish injected buffer solution) were used as control. No plain control to rule out injection stress was used. The injection method is labor-intensive and impractical for large-scale farming, while handling stress and mortality may reduce survival under commercial conditions. Furthermore, the stability of DNA integration and long-term inheritance is

yet to be confirmed, raising questions about the sustainability of the observed improvements. Economic feasibility, food safety, and consumer acceptance of fish containing exogenous DNA also remain unresolved. Finally, comparative evaluation against established improvement methods such as selective breeding or hormonal sex reversal was not undertaken. The integration of DNA be confirmed the DNA integration through qPCR or Insitu-hybridization as a viable strategy for aquaculture are yet to be confirmed. These limitations highlight the need for further study before *Lates niloticus* DNA transfer can be considered.

Ethics Consideration

The experiment was conducted following the approval of the Health Research Ethics Committee (HREC) No. UNIMAID/HREC/VOL.1/2022/04 guiding the use of animals in scientific research at the University of Maiduguri, Nigeria.

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Author(s) Contributions,

DIYAWARE, M.Y.: Conceptualized, designed the methodology, supervised, and wrote the manuscript text. WAZIRI, A.M.: Ran the experiment. MOHAMMED, Z.B. and HASSAN, M.Z.: Collected the data and monitored the water quality parameters in the culture tank.



ABUBAKAR, M.B., BUKAR, M.: Conducted the laboratory work.

AJIBIKE, A.B.: Did the Gel scoring and data analysis.

6UMAR, D.M., ONYIA, L.U., and SULUMBE, I.M.: Proofread the manuscript.

All authors read, edited, and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

REFERENCES

- Abaho, I., Kwikiriza, G., Atukwatse, F., Izaara, A. A., Ekwangu, J., Baguma, S. D., Kubiriba, J., and Kasozi, N. (2025). Selective Breeding for Genetic Improvement of Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) in Uganda: Current Status, Challenges, and Future Perspectives. *Animals*, 15(2), 142. <https://doi.org/10.3390/ani15020142>
- Abatcha, I., Mustapha, A., and Barkindo, A. (2024). Comprehensive analysis of rainfall variability in urban Maiduguri, Nigeria: Implications for climate resilience and sustainable development. *International Journal of Environment and Climate Change*, 14(3), 149-159.
- Abd El Hamied, A. M. L. (2009). Effect of foreign DNA injection on growth performance and feed utilization in Nile tilapia (*Oreochromis niloticus*). *Egyptian Journal of Aquatic Biology and Fisheries*, 13(2), 123-134.
- Abdel-Tawwab, M., Ahmad, M. H., Khattab, Y. A. E., and Shalaby, A. M. E. (2010). Effect of Dietary Protein Levels on Growth Performance and Protein Utilization in Nile tilapia (*Oreochromis niloticus*). *Aquaculture Research*, 41(8), 1265-1271.
- Ahmed, I., Jan, K., Fatma, S., and Dawood, M. A. O. (2022). Muscle proximate composition of various food fish species and their nutritional significance: A review. *Journal of Animal Physiology and Animal Nutrition*, 106(5), 13711. <https://doi.org/10.1111/jpn.13711>.
- Ahmed, R., and Siriwardena, S. (2023). *Tilapia Nigeria*. Tilapia Aquaculture Developers Association Nigeria (TADAN). Available at: <https://www.tadan.org>
- Ali, B.A., Ahmed, M.M.M., and El-Zaeem, S.Y. (2004). Application of RAPD markers in fish: Part I—Some genera (Tilapia, Sarotherodon, and Oreochromis) and Species (*Oreochromis aureus* and *Oreochromis niloticus*) of Tilapia. *International Journal of Biotechnology*, 6(1), 86-93. <https://doi.org/10.1504/IJBT.2004.004615>
- Ali, B. A., Huang, T., Qin, D., and Wang, X. (2005). A Review of Random Amplified Polymorphic DNA (RAPD) Markers in Fish Research. *Reviews in Fish Biology and Fisheries*, 14, 443-453.
- Anderson, E.D., Mourich, D.V., Fahrenkrug, S.C., LaPatra, S., Shepherd, J., and Leong, J.A. (1996). Gene expression in rainbow trout (*Oncorhynchus mykiss*) following Intramuscular Injection of DNA. *Molecular Marine Biology and Biotechnology* 5, 105-113.
- Andri, I., Zairin, M., and Harton, A. (2010). *The Effectiveness of Bull Testes Meal Extract on Sex Reversal of Nile Tilapia, Oreochromis niloticus by Immersion Technique*. MT-Fisheries IPB University Scientific Repository. 3076]. <http://repository.ipb.ac.id/handle/123456789/56745>
- Assem, S. S. and El-Zaeem, S. Y. (2005). Application of Biotechnology in Fish Breeding. II: Production of Highly Immune Genetically Modified Redbelly Tilapia, *Tilapia zilli*. *African Journal of Biotechnology* 4(5), 449-459.
- Association of Official Analytical Chemists (AOAC) International (2023). *Official methods of analysis of AOAC International* (22nd ed.). AOAC International. <https://www.aoac.org>
- Bardacki, F., and Skibinski, D.O.F. (1994). Application of the RAPD Technique in Tilapia Fish: Species and



- Subspecies Identification. *Heredity*, 73, 117-123. <https://doi.org/10.1038/hdy.1994.110>
- Bird, A. (2007). Perceptions of epigenetics. *Nature*, 447(7143), 396–398.
- Bolawa, O. E., Gbenle, G. O., and Adewusi, A. (2011). Proximate composition and nutritional evaluation of tilapia species from Lagos Lagoon, Nigeria. *African Journal of Food Science*, 5(2), 123–127
- Boyd, C. E., and Tucker, C. S. (1998). *Pond aquaculture water quality management*. New York, NY: Springer, p.700.
- Busacker, G. P., Adelman, I. R., and Goolish, E. M. (2012). Growth. In C. B. Schreck, L. Bernier, & P. B. Moyle (Eds.), *Methods for fish biology* (pp. 363–387).
- Cebeci, A., Ihan Aydın, I., and Goddard, A. (2020). Bigger, Stronger, Better: Fish Transgenesis Applications and Methods. *Biotechnology Studies* 29(2), 85-97. <http://doi.org/10.38042/biost.2020.29.02.05>.
- Chatakondi, N., Lovell, R.T., Duncan, P.L., Hayat, M., Chen, T.T., Powers, D.A., Weete, J.D., Cummins, K., and Dunham, R.A. (1995). Body Composition of Transgenic Common Carp (*Cyprinus carpio*) Containing Rainbow Trout Growth Hormone Gene. *Aquaculture* 138, 99-109.
- Chukwu, J., Diyaware, M. Y. and Ude, E. F. (2012). Evaluation of Pawpaw (*Carica papaya*) seed for controlling reproduction in the Nile Tilapia (*Oreochromis niloticus*). *Journal of the Science of Agriculture, Food Technology, and Environment (JSAFE)*, 12(1), 1–8.
- Dai, J., Cui, X., Zhu, Z., and Hu, W. (2010). Non-Homologous End Joining Plays a Key Role in Transgene Concatemer Formation in Transgenic Zebrafish Embryos. *Int J Biol Sci.*, 6(7),756-768. <https://doi.org/10.7150/ijbs.6.756>.
- Devlin, R. H., Yesaki, T. Y., Donaldson, E. M., Du, S. J., and Hew, C. L. (2001). Growth Performance of Coho Salmon Transgenic for an Exogenous Growth Hormone Gene After Oral Administration of A DNA Vaccine. *Aquaculture*, 194(1-2), 107–121. [https://doi.org/10.1016/S0044-8486\(00\)00509-0](https://doi.org/10.1016/S0044-8486(00)00509-0).
- Dey, S., Sahoo, L., and Sahoo, P. K. (2023). DNA repair genes play a variety of roles in the development of fish embryos. *Frontiers in Cell and Developmental Biology*, 11, 1123456. <https://doi.org/10.3389/fcell.2023.1123456>
- 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. *Aquaculture Research*, 10(5), 849–854.
- Diyaware, M. Y., Nwafili, S. A., Haruna, A. and Omorodion, N.G. (2016). The effect of graded levels of ram testes on sex, growth, and Survival of *Oreochromis niloticus* (Linnaeus, 1856). *Nigerian Journal of Fisheries*, 13(1 and 2), 1085–1092.
- Diyaware, M.Y., Suleiman, S. B., Akinwande, A. A., and Aliyu, M. (2017). Anesthetic Effects of Clove (*Eugenia aromaticum*) Seed Extract on *Clarias gariepinus* (Burchell, 1822) Fingerlings Under Semi-Arid Conditions. *Journal of Agricultural Sciences, Belgrade* 62(4), 411-421. <https://doi.org/10.2298/jas1704411d>.
- Du, J., Zhu, T., Tian, T., Song, H., Lei, C., J. Tian, J., Han, L., and Li, S. (2025). Genetic diversity analysis and DNA fingerprinting of different populations of largemouth bass (*Micropterus salmoides*) in China with fluorescence-labeled microsatellite markers. *BMC Genomics* 26, 531
- Dunham, R. A. (2011). *Aquaculture and Fisheries Biotechnology: Genetic Approaches* (2nd ed.). Wallingford, Oxfordshire, UK; Cambridge, MA: CABI. *Biotechnology* 4, 604-611.
- Dunham, R.A., Chatakondi, N., Nichols, A.J., Kucuktas, H., Chen, T.T., Powers, D.A., Weete, J.D., Cummins, K., and Lovell, R.T. (2002). Effect of Rainbow Trout Growth Hormone Complementary DNA on Body Shape, Carcass Yield, and Carcass Composition of F1 and F2 Transgenic Common Carp (*Cyprinus carpio*). *Marine*
- El-Hawarry, W.N. (2012). Growth Performance, Proximate Muscle Composition and Dress-Out Percentage of Nile Tilapia (*Oreochromis niloticus*), Blue Tilapia (*O. aureus*) and their Interspecific Hybrid (σ *O. aureus* X φ *O. niloticus*) Cultured in Semi-Intensive Culture System. *World's Veterinary Journal*, 2(2), 17-22.
- El-Sayed, A.F.M. (2020). Aquaculture in Africa: Status, Challenges, and Outlook. *FAO Fisheries and Aquaculture Circular*, 11-15.

- El-Sayed, A-FM. (2020). The Role of Tilapia Culture in Rural Development. In: El-Sayed A-FM, ed. *Tilapia Culture*. 2nd ed. Elsevier/Academic Press, 75-295.
- Elwan, R.I.B. (2009). Manipulation of Biotechnology for the Production of Genetically Modified Nile Tilapia (*Oreochromis niloticus*). *M.Sc. Thesis*, (Saba-Bacha), Faculty of Agriculture (Saba-Bacha), Alex. University, Alexandria, Egypt.
- El-Zaeem, S. Y. (2012). Extraordinary mullet growth through direct injection of foreign DNA. *African Journal of Biotechnology*, 11(33), 8295-8301. DOI: 10.5897/AJB11.4085
- El-Zaeem, S. Y., El-Tawil, N. E., and Amer, T. N. (2009). Effect of direct injection of shark DNA into skeletal muscles on growth performance, body composition, and feed utilization of Red Tilapia. *Journal of the Arabian Aquaculture Society*, 4(2), 103-118.
- El-Zaeem, S. Y., El-Tawil, N.E. and Amer, T. N. (2023). Effect of Direct Injection of Shark DNA into Skeletal Muscles on the Productive Performance Characteristics of Red Tilapia (*Oreochromis* sp.) Fed at Different Dietary Regimes. *African Journal of Fisheries Science*, 11(2), 001-007. www.internationalscholarsjournals.org.
- El-Zaeem, S.Y. (2001). *Breeding Studies in Tilapia*. Ph.D. Thesis, Faculty of Agriculture (Saba-Bacha), Alex. Univ. Alexandria, Egypt.
- El-Zaeem, S.Y. (2004). Alteration of the Productive Performance Characteristics of *Oreochromis niloticus* and *Tilapia zillii* Under the Effect of Foreign DNA Injection. *Journal of Aquatic Biology and Fisheries*, 8(1), 261- 278.
- El-Zaeem, S.Y. and Assem, S.S. (2004). Application of Biotechnology in Fish Breeding: I- Production of Highly Immune Genetically Modified Nile tilapia, *Oreochromis niloticus*, with Accelerated Growth by Direct Injection of Shark DNA into Skeletal Muscles. *Egypt. Journal of Aquatic Biology and Fisheries* 8(3), 67-92.
- El-Zaeem, S.Y., Amer, T.N., and El-Tawil, N.E. (2012). Evaluation of the Productive Performance Characteristics of Red Tilapia (*Oreochromis* sp.) Injected Shark DNA into Skeletal Muscles and Maintained Diets Containing Different Levels of Probiotics and Amino Yeast. *African Journal of Biotechnology*, 9(12), 476-487.
- El-Zaeem, S.Y., Mohammed, M. A., Mohammed, E.S., and Hamad, A.E. (2011). Production of salinity-tolerant Nile Tilapia, *Oreochromis niloticus* Through Traditional and Modern Breeding Methods: II. Application of Genetically Modified Breeding by Introducing Foreign DNA into Fish Gonads. *African Journal of Biotechnology*, 10(4), 684-695.
- Food and Agriculture Organization (FAO) (2025). *Global Production – March 2025 Update*. Food and Agriculture Organization of the United Nations.
- Fu, C., Cui, Y., Hung, S.S.O. and Zu, Z. (1998). Growth and Feed Utilization by F₄ Human Growth Hormone Transgenic Carp Fed Diet with Different Protein Levels. *Journal of Fish Biology*, 53, 115-129.
- Fu, L., Wang, Y., Li, T. and Hu, Y-Q (2021). A Novel Approach Integrating Hierarchical Clustering and Weighted Combination for Association Study of Multiple Phenotypes and a Genetic Variant. *Front. Genet.* 12:654804. <https://doi.org/10.3389/fgene.2021.654804>.
- Fujimura, K., and Kocher, T. D. (2011). *Tol2 transposon-mediated transgenesis in tilapia (Oreochromis niloticus)*. *Aquaculture*, 319(3-4), 342-346. <https://doi.org/10.1016/j.aquaculture.2011.07.021>.
- Georgieva, O. (2022). Clustering for Gene Expression Analysis. *CEUR Workshop Proceedings (CEUR-WS.org) Education and Research in the Information Society, October 13-14, 2022, Plovdiv, Bulgaria*. 17-24.
- Gomez-Chiarri, M., Livingston, S., Muro-Cacho, M., Sanders, S., and Levine, R.P. (1996). Introduction of foreign genes into the tissue of living fish by direct injection and particle bombardment. *Diseases of Aquatic Organisms*, 27(1), 5-12. <https://doi.org/10.3354/dao027005>.
- Gultom, V. D. N. (2023). *Past, present, and prospects on microinjection gene transfer in aquaculture*. IOP Conference Series: *Earth and Environmental Science*, 1137(1), 012040.

- <https://doi.org/10.1088/1755-1315/1137/1/012040>
- Guo, W., Lele, Fu., Wu, Y., Liu, H., Yang, Y., Hu., W., and Xie, S. (2021). Effects of dietary protein levels on growth and feed utilization in non-transgenic and growth-hormone-gene transgenic common carp (*Cyprinus carpio* L.). *Aquaculture Reports*, 21, 00854 p.10.
- Hansen, E., Fernandes, K., Goldspink, G., Butterworth, P., Umeda, P.K., and Chang, K.C. (1991). Strong Expression of Foreign Genes Following Direct Injection into Fish Muscle. *FEBS Letters*, 290, 73–76.
- Haruna, M.A., Salisu, I.B., and Zannah, U.B. (2025). Genetic Improvement of African catfish (*Clarias gariepinus*) using Nile perch (*Lates niloticus*) Deoxyribonucleic Acid (DNA). *Agriculture, Food and Natural Resources Journal*, 4 (1), 108–115.
- Hemeida, A.A., Riad, S.A., and El-Zaeem, S.Y. (2004). Genetic Alteration Following the Production of Genetically Modified Nile Tilapia (*Oreochromis niloticus*). *Egypt. J. Genet. Cytol.*, 33: 369–387.
- Heppell, J., Davis, H.L. (2000). Intramuscular Injection of DNA Vaccines in Fish. In: Lowrie, D.B., Whalen, R.G. (eds) *DNA Vaccines. Methods in Molecular Medicine™*, vol 29. Humana Press. <https://doi.org/10.1385/1-59259-688-6:99>
- Horton, J. D., Goldstein, J. L., and Brown, M. S. (2002). SREBPs: Activators of the complete program of cholesterol and fatty acid synthesis in the liver. *Journal of Clinical Investigation*, 109(9), 1125–1131. <https://doi.org/10.1172/JCI15593>.
- Jannatun, F., Rahman, M., Karim, S., and Ali, H. (2023). Proximate analysis of fish: Nutritional profiling and quality indicators for human consumption. *Journal of Aquatic Food Product Technology*, 32(4), 215–225.
- Job, B.E., Antai, E.E., Inyang-Etoh, A.P., Ootogo, G.A., and Ezekiel, H.S. (2015). Proximate Composition and Mineral Contents of Cultured and Wild Tilapia (*Oreochromis niloticus*) (Pisces: Cichlidae) (Linnaeus, 1758). *Pakistan Journal of Nutrition*, 14, 195–200. <https://doi.org/10.3923/pjn.2015.195.200>.
- Karaket, T., Reungkhajorn, A., and Ponza, P. (2023). The Optimum Dose and Period of 17 α -methyltestosterone Immersion on Masculinization of Red Tilapia (*Oreochromis spp.*). *Aquaculture and Fisheries* 8:174–179. <https://doi.org/10.1016/j.aaf.2021.09.001>.
- Kaufman, L. (1992). *Catastrophic change in species-rich freshwater ecosystems: The lessons of Lake Victoria*. *BioScience*, 42(11), 846–858. <https://doi.org/10.2307/1312084>
- Khatei, A., Tripathy, P.S., and Parhi, J. (2021). Molecular Markers in Aquaculture. In: Pandey, P.K., Parhi, J. (eds) *Advances in Fisheries Biotechnology*. Springer, Singapore, pp.165–174. https://doi.org/10.1007/978-981-16-3215-0_11.
- Koganti, P. P., Wang, J., & Wang, H. (2020). *Epigenetic regulation of muscle growth and water-holding capacity in fish: Role of DNA methylation and microRNAs*. *Aquaculture Reports*, 18, 100445.
- Konnert, G. D. P., Gerrits, W.J.J., Gussekloo, S.W. S., and Schrama, J. W. (2022). Balancing and Energy in Nile Tilapia Feeds: A Meta-analysis. *Rev. Aquac.*, 14,1757–1778. <https://doi.org/10.1111/raq.12671>.
- Konstantinidis, I., Sætrum, P., Mjelle, R., Nedoluzhko, A. V., Robledo, D., a Fernandes, J. M. O. (2020). Major gene expression changes and epigenetic remodelling in Nile tilapia muscle after one generation of domestication. *Epigenetics*, 15(10), 1052–1067.
- Krivins, A. H. (2024). Comparative Study on the Efficiency Of Feed Conversion In Nile Tilapia (*Oreochromis Niloticus*) Fed with Different Feed Types. *International Journal of Agriculture and Plant Science*, 6(2), 22–24. www.agriculturejournal.in.
- Kusa, T. Diyaware, M.Y., Suleiman, S.B., Aliyu, M., Mohammed, Z.B., Biola, A.A., Popoola, M.O., and Haruna, M.Y. (2025). Growth response and some spawning indices of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758) containing piscine DNA. *The Journal of Basic and Applied Zoology*, 86, 105 <https://doi.org/10.1186/s41936-025-00525-7>
- Kusa, T., Diyaware, M. Y., Suleiman, S. B., Aliyu, M., Mohammed, Z. B., Ajibike, A. B., Popoola, O. M.,



- and Haruna, M. Y. (2026). Growth response and some spawning indices of Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758) containing piscine DNA. *The Journal of Basic and Applied Zoology*, 87(1). <https://doi.org/10.1186/s41936-026-00547-9>
- Kyari, A. U., Alkali, M. I., Ibrahim, H. H., & Abacha, I. U. (2026). Spatio-Temporal Variation of Rainfall, Temperature, and Vegetation in Maiduguri and Environs. *International Journal of Research and Innovation in Social Science*, 9(12), 3887–3893.
- Lee, L., Hui, C., Chuang, C., and Chen, J. (2013). Electrotransfer of the epinecidin-1 gene into skeletal muscle enhances the antibacterial and immunomodulatory functions of a marine fish, grouper (*Epinephelus coioides*). *Fish & Shellfish Immunology*, 35(5), 1359–1368. <http://dx.doi.org/10.1016/j.fsi.2013.07.050>
- Lee, P.Y., Costumbrado, J., Hsu, C.Y., and Kim, Y.H. (2012). *Agarose Gel Electrophoresis for the Separation of DNA Fragments*. *J Vis Exp*, 62, 3923. <https://doi.org/10.3791/3923>
- Li, J., Liu, Y., Zhang, H., Wang, Q., & Chen, X. (2019). Transient expression of foreign DNA in fish cells: Mechanisms and applications. *Aquaculture International*, 27(3), 567–579. <https://doi.org/10.1007/s10499-018-0329-7>
- Liu, H. (2008). The fresh and brackish water fishes of Lower Guinea, West-Central Africa. Latidae. p. 404–406. In: M.L.J. Stiassny, G.G. Teugels and C.D. Hopkins (eds.) Volume II. Collection Faune et Flore tropicales 42. Institut de Recherche pour le Développement, Paris, France, Muséum National d'Histoire Naturelle, Paris, France, and Musée Royal de l'Afrique Centrale, Tervuren, Belgium. pp. 603.
- Martinez, R., Juncal, J., Zaldivar, C., Arenal, A., Guillen, I., Morera, V., Carrillo, O., Estrada, M., Morales, A., and Estrada, M. P. (2000). Growth Efficiency in Transgenic Tilapia (*Oreochromis* sp.) Carrying a Single Copy of a Homologous cDNA Growth Hormone. *Biochemical and Biophysical Research Communications*, 267(1), 466–472.
- Mlalila, N., Mahika, C., Kalombo, L., Swai, H., and Hilonga, A. (2015). Human food safety and environmental hazards associated with the use of methyltestosterone and other steroids in production of all-male tilapia. *Environmental Science and Pollution Research*, 22(7), 4922–4931. <https://doi.org/10.1007/s11356-015-4133-3>.
- Mohammed, Z. B., Aliyu, M., Raji, A.O., and Diyaware, M.Y. (2024). Evaluation of Camel Testes for Masculinisation of the Nile Tilapia (*Oreochromis niloticus*, Linnaeus, 1758). *Nigerian Journal of Fisheries and Aquaculture* 12(2), 70 – 85.
- Mukti, A. T., Carman, O., Alimuddin, A., Zairin, M. J.R., and Suprayudi, M. A. (2020). Growth Performance, Survival Rate, Flesh, and Proximate Composition of Sex-grouped Triploid and Diploid Nile Tilapia (*Oreochromis niloticus*). *Turk. J. Vet Anim. Sci.*, 44:290–298. <https://doi.org/10.3906/vet-1905-79>.
- Mwaura, J. G., Wekesa, C., Ogotu, P. A., & Okoth, P. (2023). Whole Transcriptome Analysis of Differentially Expressed Genes in Cultured Nile Tilapia (*O. niloticus*) Subjected to Chronic Stress Reveals Signaling Pathways Associated with Depressed Growth. *Genes*, 14(4), 795. <https://doi.org/10.3390/genes14040795>.
- Nei, M. (1972). Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences*, 70(12), 3321–3323. <https://doi.org/10.1073/pnas.70.12.3321>
- Ogbuebunu, K. E. and Awodiran, M. O. (2017). Molecular Characterization of *Lates niloticus* (Perciformes, Latidae) Populations from Three Nigerian Waterbodies Using Random Amplified Polymorphic DNA and Microsatellite Markers. *Vestnik zoologii*, 51(1), 31–36. <https://doi.org/10.1515/vzoo-2017-0005>
- Olabode, O. S., Goland, M., and Levavi-Sivan, B. (2014). Preliminary Report on the Production of transgenic *Oreochromis niloticus* using Tol2 kit. *Nigerian Journal of Biotechnology*, 27, 34–39.
- Olapode, O. S., Oladunjoye, I. O., and Akinyemi, A. F. (2016). Proximate composition and nutrient evaluation of Nile tilapia (*Oreochromis niloticus*)

- and hybrid red tilapia. *International Journal of Fisheries and Aquatic Studies*, 4(2), 123–128.
- Oliveira, S. N., Ribeiro, R. P., Lopera, N. M., Candioto, F. B., Resende, E. K., and Legat, A. P. (2011). Análise genética de três gerações de tilápia do Nilo (linhagem GIFT) utilizando o marcador RAPD. *Acta Scientiarum. (English Abstract) Animal Sciences*, 33(2), 207–212. <https://doi.org/10.4025/actascianimsci.v33i2.9202>
- Otene, B.B., Iorchor, S. I., and Amadi, G. A. (2024). Spatio-Temporal Assessment of Proximate Composition of Tilapia (*Oreochromis niloticus*) and Silver Catfish (*Chrysichthys nigrodigitatus*), Tombia Segment, New Calabar River, Port Harcourt, Nigeria. *International Journal of Innovative Food, Nutrition and Sustainable Agriculture*, 12(3), 28–34.
- Otero, O. (2004). Anatomy, systematics, and phylogeny of both recent and fossil latid fishes (Teleostei, Perciformes, Latidae). *Zool. J. Linn. Soc.* 141(1), 81–133.
- Paugy, D. (2003). The fresh and brackish water fishes of West Africa. Centropomidae. p. 451–453. In D. Paugy, C. Lévêque and G.G Teugels (eds.) Volume 2. Coll. faune et flore tropicales 40. Institut de recherche de développement, Paris, France, Muséum national d'histoire naturelle, Paris, France and Musée royal de l'Afrique Central, Tervuren, Belgium, 815p.
- Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: Genetic Analysis in Excel. *Bioinformatics*, 28(19), 2537–2539. <https://doi.org/10.1093/bioinformatics/bts460>
- Petitjean, Q., Jean, S., Veyssière, C., and Laffaille, P. (2019). Stress responses in fish: From molecular to evolutionary processes. *Science of the Total Environment*, 684, 427–438.
- Premarathna, A.D., Kumara, A.M.C.P., Jayasooriya, A.P., Satharasinghe, D. A. and Pathirana, E. (2020). Nutritional Analyses of Black Tiger Prawn (*Monodon penaeus*) and Nile tilapia (*Oreochromis niloticus*) from Sri Lanka. *Research Square*, 1–10.
- Raatz, S. K., Silverstein, J. T., Jahns, L., and Picklo, M. J. (2013). Issues of Fish Consumption for Cardiovascular Disease Risk Reduction. *Nutrients*, 5(4), 1081–1097. <https://doi.org/10.3390/nu5041081>
- Rahman, M. A. R., Ronyai, A., Engidaw, B. Z., Jauncey, K., Hwang, G-L, Smith, A., Roderick, E., Penmad, D., Varadi, L., and Maclean, N. (2001). Growth and Nutritional Trials on Transgenic Nile Tilapia Containing an Exogenous Fish Growth Hormone Gene. *Journal of Fish Biology*, 59, 62–78.
- Rahman, M. A., Arshad, A., Marimuthu, K., Ara, R., and Amin, S. M. N. (2013). Inter-specific Hybridization and its Potential For Aquaculture of Fin Fishes. *Asian Journal of Animal and Veterinary Advances*, 8, 139–153. <https://doi.org/10.3923/ajava.2013.139.153>
- Rahman, M.A., and Maclean, N. (1992). Production of transgenic tilapia (*Oreochromis niloticus*) by one-cell-stage micro-injection. *Aquaculture* 105, 219–232.
- Rahman, M.A., Mak, R., Ayad, H., Smith, A., and Maclean, N. (1998). Expression of a Novel Piscine Growth Hormone Gene Results in Growth Enhancement in Transgenic Tilapia (*Oreochromis niloticus*). *Transgenic Research*, 7(5), 357–369.
- Roderick, E. (2025, February 4). *The global tilapia*. Aqua Culture Asia Pacific. Retrieved from <https://aquaasiapac.com/2025/02/04/the-global-tilapia/>
- Sambrook, J., and Russell, D. W. (2001). *Molecular cloning: A laboratory manual* (3rd ed.), Vol. 1. Cold Spring Harbor Laboratory Press, New York, USA
- Sánchez-Peña, M.J., Márquez-Sandoval, F., Ramírez-Anguiano, A.C., Velasco-Ramírez, S.F., Macedo-Ojeda, G., and González-Ortiz, L.J. (2017). Calculating the metabolizable energy of macronutrients: a critical review of Atwater's results. *Nutr Rev.* 75(1), 37–48. <https://doi.org/10.1093/nutrit/nuw044>.
- Shakweer, W. M. E., Krivoruchko, A. Y., Dessouki, Sh.M., and Khattab, A. A. (2023). A Review of Transgenic Animal Techniques and Their Applications. *Journal of Genetic Engineering and Biotechnology* 21(1), 55. <https://doi.org/10.1186/s43141-023-00502-z>

- Sharma, P., and Sharma, S. B. (2021). Data analysis of protein, moisture, ash, and fat profiles of the fishes of the upper Ganga. *International Journal of Fisheries and Aquatic Research*, 6(2), 24–26.
- Shears, M.A., Fletcher, G.L., Hew, C.L., Gauthier, S., and Davies, P.L. (1991). Transfer, Expression and Stable Inheritance of Antifreeze Protein Genes in Atlantic Salmon (*Salmo salar*). *Mol. Mar. Biol. Biotechnol.* 1, 58–63.
- Sneath, P. H. A., and Sokal, R. R. (1973). *Numerical taxonomy: The principles and practice of numerical classification*. San Francisco, CA: W. H. Freeman.
- Steffens, B. (2024). Tilapia's Role in Global Aquaculture and Food Security. *Fish Aqua J.*, 15:381. <https://doi.org/10.35248/2150-3508.24.15.381>
- Sudha, P.M., Low, S., Kwang, J., Gong, Z. (2001). Multiple Tissue Transformation in Adult Zebra Fish by Gene Gun Bombardment and Muscular Injection of Naked DNA. *Mar. Biotechnol.*, 3, 119-125.
- Suwannatrai, K., Namwongsa, K., Phanomkhet, N., Tawil, S., and Roschat, W. (2023). Analysis of the Nile Tilapia Fish's (*Oreochromis niloticus* L.) Proximate Composition in Sakon Nakhon Province, Thailand. *Creative Science*, 15(2), 1-9.
- Tan, J.H., and Chan, W.K. (1997). Efficient Gene Transfer into Zebrafish Skeletal Muscle by intramuscular injection of plasmid DNA. *Molecular Marine Biology and Biotechnology* 6, 98–109.
- Thabet, D. M., Abou Zied, R.M., Hassan, G.M., and El-Zaeem, Y.S. (2025). Genetic improvement of cold tolerance and growth of Nile tilapia (*Oreochromis niloticus*). *Mediterranean Aquaculture Journal* 12 (1): 1-12.
- Tocher, D. R. (2003). Metabolism and Functions of Lipids and Fatty Acids in Teleost Fish. *Reviews in Fisheries Science*, 11(2), 107–184. <https://doi.org/10.1080/713610925>
- Wang, Y. and Zheng, Y. (2025). *Spoilage mechanisms of fish: Enzymatic autolysis, lipid oxidation, and microbial growth*. Food Science and Technology International, 31(2), 145–158.
- Wasso, D.S., Ayagirwe, R.B.B., Oluwamuyiwa, O.E., and Kassam, D. (2024). Nile Tilapia Farming and Diversity in Sub-Saharan Africa: A Review. *Indian Journal of Animal Research*, 1-10. <https://doi.org/10.18805/IJAR.BF-1769>
- Weising, K., Nybom, H., Wolff, K., and Kahl, G. (2005). *DNA fingerprinting in plants: Principles, methods, and applications* (2nd ed.). Boca Raton, FL: CRC Press. <https://doi.org/10.1201/9781420040043>
- Welsh, J., and McClelland, M. (1990). *Fingerprinting genomes using PCR with arbitrary primers*. *Nucleic Acids Research*, 18(24), 7213–7218. <https://doi.org/10.1093/nar/18.24.7213>.
- Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1990). DNA Polymorphisms Amplified by Arbitrary Primers are Useful as Genetic Markers. *Nucleic Acids Research*, 18(22), 6531–6535.
- Wolff, J.A., Malone, R.W., Williams, P., Chong, W., Acsadi, G., Jani, A., and Felgner, P.L. (1990). Direct Gene Transfer into Mouse Muscle in Vivo. *Science*, 247, 1465-1468.
- Wood (1983). *The Guinness Book of Animal Facts and Feats*. Sterling Pub Co Inc.
- Xu, Y., Tian, H.L., Chan, C.H., Liao, J., Yan, T., Lam, T.J., and Gong, Z. (1999). Fast skeletal Muscle-specific Expression of a Zebra Fish Myosin Light Chain 2 Gene and Characterization of its Promoter by Direct Injection into Skeletal Muscle DNA. *Cell Biol.*, 18(1), 85-95.
- Yaping, W., Wei, H., Gang, W., Yonghua, S., Shangping, C., Fuying, Z., Zuoyan, Z., Jianxin, F., and Zirui, Z. (2001). Genetic Analysis of all Fish Growth Hormone Gene Transferred carp (*Cyprinus carpio* L.) and its F₁ Generation. *Chinese Sci. Bull.* 46 (14), 1174-1177.
- Yuvarajan, P., Felix, S., Stephen, J., Kumar, S.S., Ahilan, B., Mahadevi, and Kannan, B. (2019). Tilapia Low Cost! High Nutrient! Alleviate Malnutrition. *Int. J. Curr. Microbiol. App. Sci.*, 8(7), 2154-2157.
- Zhang, P., Hayat, M., and Chen, S. (1994). RAPD analysis of genomic changes in transgenic fish. *Molecular Marine Biology and Biotechnology*, 3(3), 148–153.
- Zhang, Y., Li, J., Chen, S., and Wang, Y. (2020). Exogenous DNA integration and epigenetic regulation in fish genomes. *Aquaculture Research*, 51(12), 4875–4886. <https://doi.org/10.1111/are.14876>.



Abbreviations

List of Abbreviations

bp	Base pair	NIR	Near-Infrared	
cDNA	Complementary Deoxyribonucleic Acid	RAPD	Random Amplified Polymorphic	
EDTA	Ethylenediaminetetraacetic acid		Deoxyribonucleic Acid	
DNA	Deoxyribonucleic Acid	SDS	Sodium Dodecyl Sulphate	
GIFT	Genetically Improved Farmed Tilapia	SSC	Sodium Citrate	
gDNA	Genomic Deoxyribonucleic Acid	UPGMA	Unweighted Pair Group Method with	
			Arithmetic Mean	



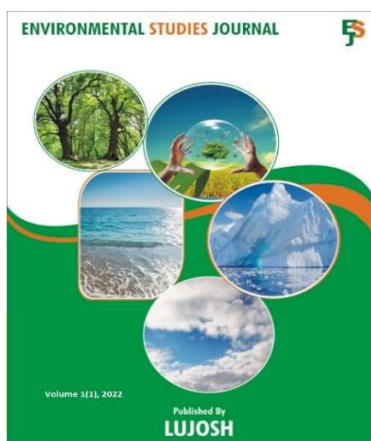


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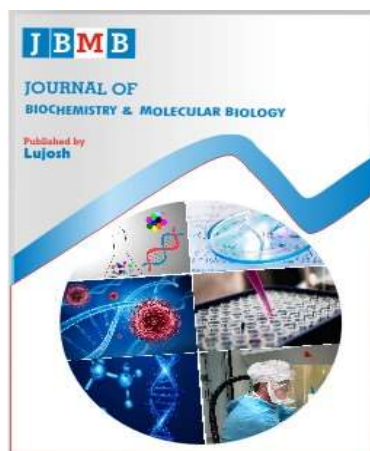
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