

Mitochondrial DNA D-loop Genetic Relatedness and Characterization of Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) from Lakes Alau and Bako, Northeast, Nigeria

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ABSTRACT

This study investigated the genetic relatedness and characterization of Nile tilapia (*Oreochromis niloticus*) populations from Lake Bako, Mambilla Plateau and Lake Alau, Maiduguri using Mitochondrial DNA (mtDNA) D-loop sequences. Blood samples were collected from each individual of and preserved on an FTA card. DNA was extracted from the card using Aqualex extraction kits. Polymerase Chain Reaction was performed using a commercially available decamer primer: FISH COMUM D-LOOP F (5'-GGATTYTAACCCYTRCCC -3') and (R:) as the forward primer and *C. zilli_rev* (5' – AGTAAAGTCAGGACCAAGCC - 3') as reverse primer. The results showed that, in total 117 variable sites were observed and Hap_1_Hap_40 was detected. Lake Alau population has 65.50 % A+T and 34.5% G+C, while 65.5% and 34.9% were the nucleotide composition of Lake Bako population. Both populations have 1.00 Haplotypes diversity. The nucleotide diversity observed in Lake Bako population is 0.0342, while 0.0285 were recorded in Lake Bako population. The average number of nucleotide differences of 26.61053 and 31.98421 were observed in Lakes Bako and Alau populations, respectively with and overall value of 29.996. The Neighbour-Joining phylogenetic tree revealed two major clusters. The genetic variation among population is 95.31%and within population is 4.69%. Populations of *O. niloticus* in Lakes Bako and Alau are genetically different. Lake Bako population seemed to have intact genetic structure. Both populations have high genetic diversity, hence have high potential for genetic improvement and conservation.

Keywords: Genetic Diversity, Lake Alau, Lake Bako, Mitochondrial DNA, *Oreochromis niloticus*



Citation

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INTRODUCTION

Oreochromis niloticus is the next most commonly reared fish species worldwide after carp and the most important aquaculture species of the 21st century (Shelton 2002). The Nile Tilapia is widely produced due to its high reproductive rate, palatability, tolerance to harsh environments conditions, ability to withstand diseases and the prospect to be raised under numerous farming structures (Shahid *et al.*, 2019; Yosef, 2009). It was recently called the "aquatic Chicken" by Fitzsimmons (2005) because of its fast growth rate and worldwide acceptability comparable to the chicken. The yearly world production of farmed tilapia has increased over the years, with main world tilapia production coming from outside its origin, China in Asia. The production has exceeded 6 million tonnes since 2020 (Fletcher, 2020).

The native range of tilapias are Africa and other subtropical countries of the world. They enjoy different environments ranging from purely freshwaters to brackish waters. Among the most favoured aquaculture species is the Nile tilapia, *Oreochromis niloticus*. In its native range, the genetic characteristics of the species have not been given due attention. Studying the genetic structure of population at the molecular level benefits genetic breeding and conservation and it is of great importance in understanding the level of population genetic differentiation. Compared with nuclear DNA, Mitochondrial DNA can be an excellent tool for obtaining genetic information in a population, due to its unique characteristics of maternal transmission, fast rate of evolutionary change, high frequency of nucleotide transition or transversion and fast mutation rate during subculture (Teletchea, 2009; Oleinik *et al.*, 2007; Avise, 1994).

Most of the Nigerian inland waters are stressed due to climate change, over exploitation, anthropogenic activities that may release agro-waste into the water and destruction of habitats subsequently alters the fish population. All these may affect the diversity and genetic signature of the fish as a result of mutation in a quest to adjust to the aforementioned problem in the environment. Lower genetic diversity can affect the performance of the fish, more so that majority of the Tilapia cultured in Nigeria are sourced from the wild, hence their genetic purity is uncertain. For the fish to be effectively conserved and subsequently improved, their genetic status needs to be ascertained. Up till now, there is no information on the genetic status of *O. niloticus* from Northeast Nigeria. This study therefore aimed at investigating the genetic structure and diversity of *O. niloticus* from Lakes Bako and Alau with the aim of providing information for possible conservation and improvement of the in the region.

MATERIALS AND METHODS

Materials

Wild *Oreochromis niloticus* were collected from Lake Alau and Lake Bako, Mambila Plateau in Taraba State. Lake Alau is situated between latitude 11°39' 4"N and 11° 40', 02"N and longitude 13°39' 92"E at 120m above sea level. While Lake Alua Maiduguri has typical tropical climate with temperatures between 13 to 41°C with April being the hottest month (40°C) and January -December the coolest with 13°C (Weather and Climate.com, 2021). It has two seasons, wet and dry seasons. The wet season starts in May and ends in September, with its peak in August. Lake Bako in Mambila is a large concrete dam located between latitude 11° 21'8"E, 11° 21' 20"E and longitude 6°42' 52"N, 6° 43' 10" N, above sea level. The climatic characteristics of Mambila Plateau are of a typical temperate region (Bako *et al.*, 2016). The rain starts in February to November with an annual mean value of 1850mm (Bako *et al.*, 2016).



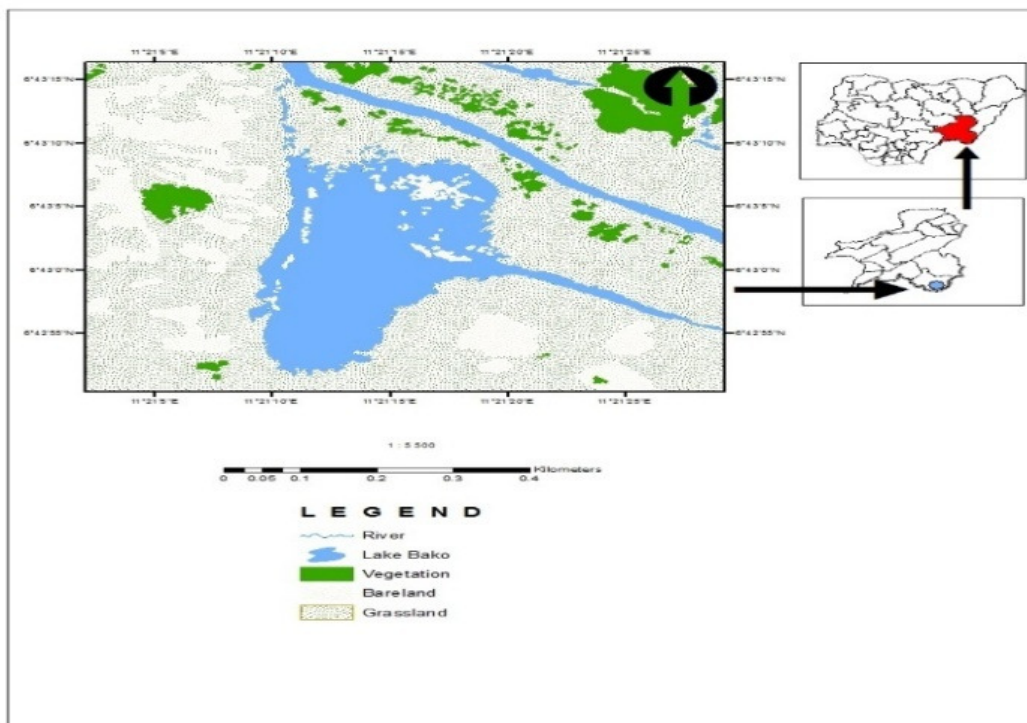


Figure 3.1 Lake Bako, Taraba State, Nigeria, inset Taraba showing Lake Bako, Nigeria showing Taraba State. Source : Edited from Google Earth image (2019).

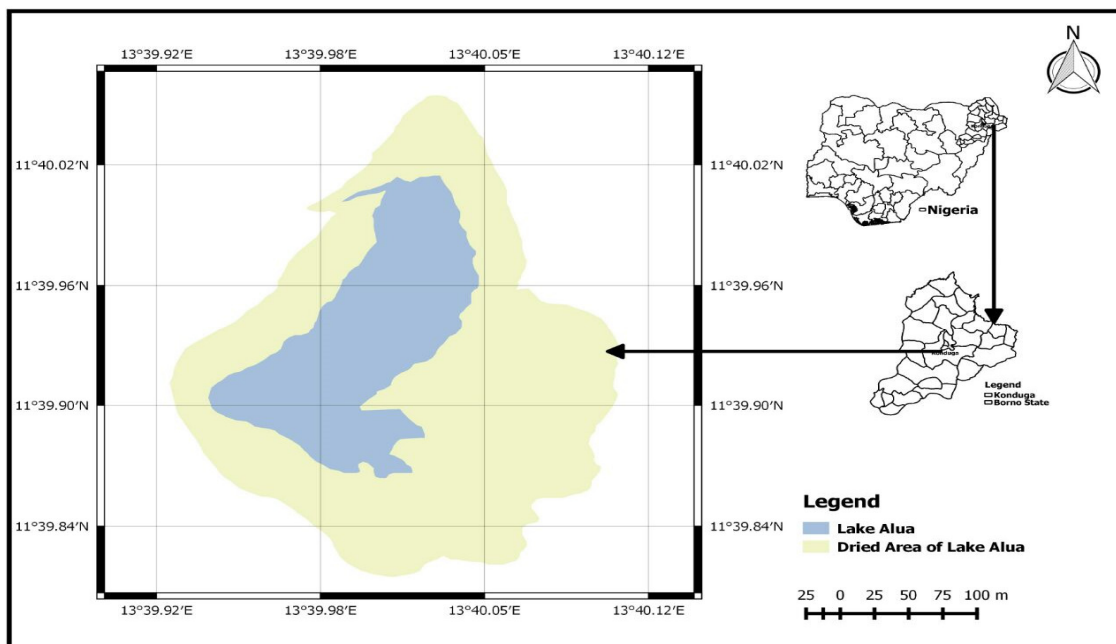


Fig. 1. Map of Lake Alau showing the sampling site

Sample collection

The samples were collected during rainy season (August) and identified following the method described by Teugels *et al.* (1992). Blood samples (0.5ml) were collected from each individual fish according to Aqualex kit manufacturer's instructions. The blood samples were dropped immediately on to an FTA Card (Model: FTA micro Card, Whatman Bioscience USA) in concentric manner air-dried and packed into nylon envelope containing silicon beads until required. The blood samples on the FTA card were transported to the Animal Biotechnology and Genetic Centre, Department of Animal Science, Federal University of Agriculture Abeokuta, Nigeria for mitochondrial DNA (mtDNA) extraction.

Extraction of DNA

The genomic DNA extraction from blood-stained FTA® Classical card was carried out using the manufacturer's recommended protocol (Whatman® Bioscience, UK) with modification previously described by Ajibike (2016). Five (5) 1mm disk was punched out from each blood-stained FTA® cards using Harris micro-punch and placed in Eppendorf tubes. 100 mM of Tris-BASE and 0.1% Sodium Dodecyl Sulphate (SDS) was added into each Eppendorf tubes containing the samples discs. The tubes were dropped into bath containing water, agitated gently for 30 minutes at 30 °C on a mixer. After which, solution was carefully emptied leaving the discs in the tubes. 0.5 ml of 5M Guanidine thiocyanate was added into each tube and mixed for 10 minutes for second wash. After removing as much as possible the guanidine thiocyanate solution, leaving the discs in the tubes. 0.5 ml of distilled water was added into each tube and gently agitated for 10 minutes. Another 0.5 ml of distilled water was added again after the removal of the first added distilled water and left to stand for 10 minutes. The tubes, containing the discs and 0.5 ml of distilled water, were dropped in water bath at 90 °C for 10 minutes after which they were taken to the laboratory for analysis. The last added distilled water was left in the Eppendorf tubes and used for PCR amplification. The DNA concentration and purity, A260/A280 ratio between 1.8 and 2.0 were assessed using a NanoDrop 1000 Spectrophotometer. Potential DNA degradation was visualized on 2% agarose gel.

Polymerase Chain Reaction and Electrophoresis

The DNA amplification from the D-loop region of the *O. niloticus* mitochondrial genome were performed using Fish_comum_Dloop_F (5' – GGATTYTAACCCYTRCCCC – 3') as the forward primer and *C. zilli_Rev* (5' – AGTAAAGTCAGGACCAAGCC – 3') as reverse primer (Bingjie *et al.*, 2019). All PCR were performed in a 30 µL reaction volume containing 5 µL of 150-200 ng genomic DNA, and 25 µL of PCR mix (0.2 mM of each dNTPs, 0.4 µM of each primer, 1.5 mM of MgCl₂, 1 × PCR buffer and 2 U Surf Hot *Taq* DNA) (Stab Vida, Spain). The PCR was carried out on Agilent Surecycler 8800 thermocycler (Stab Vida, Spain). Polymerase Chain Reaction conditions were: Initial denaturation at 96 °C for 15 minutes, followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing was at 60 °C for 30 seconds and extension at 70 °C for 1-minute 30 seconds, and final extension was at 70 °C for 5 minutes.

Data Analysis

Genetic distance (D) within and among populations were estimated using the Kimura-2- model. The generated mtDNA sequence of the D-loop region constituting the entire length of the region was used for the analyses. Viewing and editing of the sequences were performed using ChromasPro® (Technelysium Pty., Tewantin, Australia). Polymorphic sites were identified using DnaSP v. 5.10.01 (Rozas *et al.*, 2003), and visualised using Jalveiw software version 2 (Waterhouse *et al.* 2009). Haplotype (H) and nucleotide diversity (π), Haplotypes and polymorphic site were determined using DnaSp 5.10.01 software. Nucleotide diversity, variance and standard deviation were estimated according to Tajima (1983) and Nei (1987) using Arlequin 3.5.1.3 (Excoffiere and Lischer, 2005). Theta (Θ) was estimated using the method described by Chakraborty and Weiss (1991) with the aid of Arlequin 3.5.1.3 (Excoffiere and Lischer, 2005). The observed haplotypes and bootstrapping were estimated using Neighbour Joining (NJ) tree Tamura and Nei (1993) in MEGA 6.06. The neutrality test of the pairwise differences of the two populations was performed to infer historical demographic and deviation of sequence variation from evolutionary neutrality. To test for past population expansion, statistical tests Tajima's D (Tajima, 1989) and



Fu's FS (Fu, 1997) were used via DNASP. In addition, the mismatch frequency, graphs were plotted in DnaSP ver 5.10.01 to determine whether the populations exhibited evidence of spatial range expansion or a stationary population history (Tajima, 1989).

RESULTS AND DISCUSSION

Fig. 2 shows aligned variable sites of D-loop sequences of *Oreochromis niloticus* population from Lake Alau (LA), Maiduguri Borno State and Lake Bako, Mambila Plateau (MP), Taraba State. The length of the amplified D-loop segment was observed to be 934 bp, which is longer than 927 bp and 932 bp reported by He *et al.* (2010) for *O. niloticus* and *O. aureus*, respectively.

The nucleotide compositions of Lakes Alau and Bako *O. niloticus* population are rich in A+T, constituting 65.5% and 65.1% for Lake Alau and Bako, respectively. The G+C content were 34.5% and 34.9%, respectively for Lakes Bako and Alau. The nucleotide compositions of both populations observed in this study are closer to 64.5% A+T and 35.7% G+C documented by Jiang *et al.* (2019) in Tilapia. These D-loop sequence observed in this study are in congruent with the distribution characteristics of the

base content in the D-loop region of most fish species (Broughton *et al.*, 2001). The nucleotide compositions were consistent in both populations in the following order of T>A>C>G. Obviously is 65.2% average value of A+T which is higher than 34.8% G + C. A total of 40 haplotypes were identified in the 40 individuals and were defined by 129 polymorphic (segregating) sites and 100 Parsimony informative sites (Fig. 2).

The number of haplotypes (20) in Mambila Plateau (MP) population is defined by 93 variable (polymorphic) sites and 66 Parsimony informative while in Lake Alau (LA) population is 20 defined by 108 polymorphic sites and 82 parsimony informative sites. The high number of haplotypes observed in both populations during this study are higher than 11 reported by Abdel-Hamid *et al.* (2014) for *O. niloticus* from Egypt and 14 haplotypes reported by Agbebi *et al.* (2016) for *Oreochromis niloticus* from FUNAAB reservoir, Nigeria. The two population share the same number of haplotypes and is in line with the report of Agnese *et al.* (1999). The high number of haplotypes observed in the present study could be due to the high mutation rate of the mtDNAD-loop (Freeland *et al.*, 2011).



Genetic diversity

The estimates of genetic diversity in Lakes Alau and Bako strains of *O. niloticus* are shown in Fig. 2. Higher (1.00) haplotype diversity (H_d) was observed in both populations, indicating high gene flow among the populations; that is depicting high genetic diversity. The Haplotype diversity observed in this study are higher than 0.829 observed for *O. niloticus* from Lake Victoria by Angienda *et al.* (2011). Fish population with high genetic diversity indicates that the population have high potentials to adapt and persist longer in that environment (Greenbaum *et al.* 2014; Caballero and Garcia-Dorado 2013).

Nucleotide diversity (π) was higher (0.0342) in Lake Bako population compared to that of Lake Alau (0.02849) populations. The low nucleotide diversity recorded in Lake

Alau population suggests that there are likelihood bottleneck affecting the population. The sign of bottle neck observed in Lake Alau population could have been initiated by high fishing pressure and the yearly flooding of the lake as result of release of water from Lagdo Dam, Cameroun coupled with climate change and ecological pressures suggested by Bassita *et al.* (2018). However, the nucleotide diversity observed among the two populations during this study are higher than the nucleotide diversity range of 0.005 to 0.012 reported by Angienda *et al.* (2011) for *O. niloticus* population from Lake Victoria. High nucleotide diversity according to Stepien (1999) suggests large, stable, and effective population sizes over time. Abdel Hamid *et al.* (2014) documented π of 0.276 in *O. niloticus* from the Nile River between Giza and Maserregion, El –Kadima, Egypt.

Table 1: The Genetic diversity of *O. Niloticus* from Lakes Alau and Bako

Population	N	Nh	Np.	H	π	k	Ts	Tr'	Kp
Lake Alau	20	20	93	1.00	0.02849	26.61053	33	51	
Lake Bako	20	20	107	1.00	0.03424	31.98421	36	65	
Combined	40	40	129	1.00	0.03212	29.996	48	73	30.660

N=sample size, Nh=number of haplotypes, Np=number of polymorphic sites; H=haplotype diversity; π = nucleotide diversity, k=average number of nucleotide differences, Ts=number of transitions, Tr'=Number of transversion, Dxy=Average number of nucleotide substitution. per site between populations, kp=Average number of nucleotide differences between populations

Haplotypes diversity recorded in this study varied from 80% reported by Abdel-Hamid *et al.* (2014). The 100% haplotypes diversity observed in this study suggested that there is stable and effective population size as suggested by Zhou *et al.* (2010). Joshi *et al.* (2013) opined that high number of haplotypes is due to the high substitution rate, because D-loop region has tendency of higher substitution rates than the rest of mtDNA genome (Cann *et al.*, 1984). However, the high haplotypes diversity observed in this study, indicated that the populations contain an abundance of genetic resources for further possible exploitation in breeding of the species (Chaobian *et al.*, 2019). Bingjie *et al.* (2019) also observed 0.834 (83.4%) H_d in *Coptodon zillii* population in China. The combination of high haplotype diversity and low

nucleotide diversity is reportedly common in some species, especially pelagic marine fishes (Liu and Fu., 2015), due to rapid demographic expansion from a small effective population size. This is under the assumptions of Grant and Bowen (1998), Avise (2000) and Lowe *et al.* (2004), that there is sufficient time for the number of haplotypes to increase through mutation but insufficient for accumulation of large sequence differences. The high haplotype and nucleotide diversity observed in this study, indicates the abundant genetic resources of two populations that could be exploited for selective breeding (Jiang *et al.* 2019).

Average number of nucleotide differences of 26.61053 and 31.98421 were observed in Lakes Bako and Alau



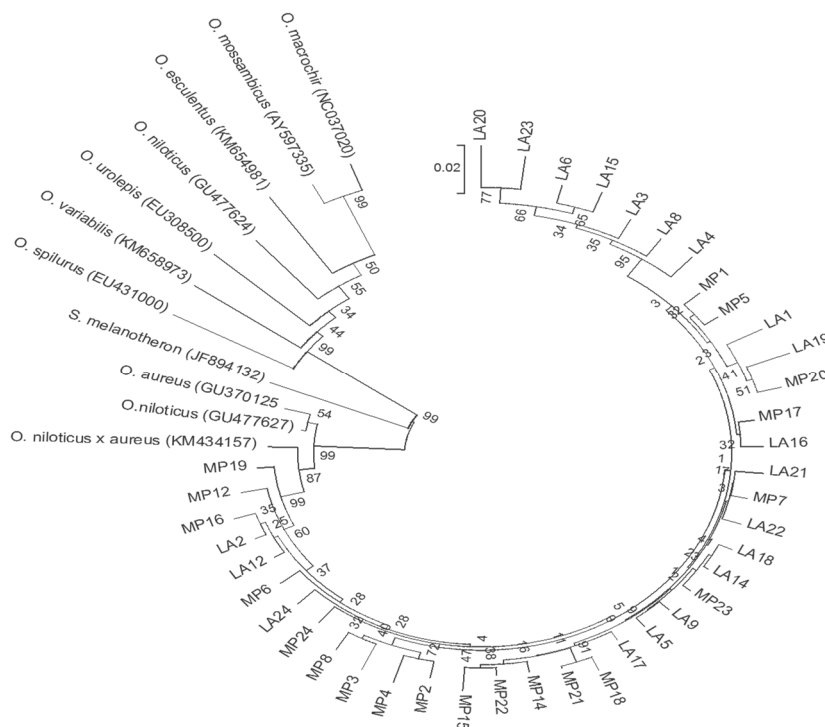
populations, respectively, while the overall nucleotide difference observed was 29.996. The Average number of nucleotide differences for both populations are lower than 24.286 reported by Zou *et al.* (2014) for Nile Tilapia from Freshwater Fisheries Research Centre of China.

Genetic distance of *O. niloticus* from Lakes Alau and Bako

The phylogenetic relationship between *O. Niloticus* from Lakes Alau and Bako is shown in Fig. 3. The neighbour-joining tree showed that the two populations were divided into two main clusters, indicating that both populations are from one primary maternal ancestor.

Seven individuals from Lake Alau formed a cluster, while the rest of Lake Alau together with Lake Bako population formed the second cluster. Mireku *et al.* (2018) also observed two cluster in *O. niloticus* in Volta Lake of Ghana using microsatellite markers. The sequences tend to cluster with a hybrid between *O. niloticus* and *O. aureus*

than the pure *O. niloticus*. This could suggest the two populations may be genetically different, although they may share a common ancestor. The cluster group mean genetic distance observed in this study were estimated at 0.048 and coefficient of evolutionary differentiation is 0.159 ± 0.046 . Both populations have 99% bootstrap, which differs from 77% bootstrap observed in same fish species in Volta Lake, Ghana documented by Rutten *et al.* (2004). Abdel-Hamid *et al.* (2014) also observed two clusters in *O. niloticus* population caught from Lake Qarun, Egypt. The finding here confirmed the observation of Ekerette *et al.* (2017) for *Oreochromis* species in Nigeria. Agn se *et al.* (1999), Ndiwa *et al.* (2014) and Shahid *et al.* (2019) reported 3 clusters for *O. niloticus* population from Riyadh, Saudi Arabia and Lake Bogoria, Kenya and Rift Valley Ethiopia, respectively. Agbebi *et al.* (2016) observed six clusters with no clear sub-structuring among *O. niloticus* population in Southwest Nigeria. These variations in the number of clusters could be due to regional differences.



Key: LA = Lake Alau, MP = Lake Bako

Fig. 3: Phylogenetic relationship between *Oreochromis niloticus* from Lakes Alau and Bako population in Nigeria.



Population genetic structure and demographic history

The Analysis of Molecular Variance (AMOVA) of *O. niloticus* populations from Lakes Alau and Bako based on D-loop sequences is shown in Table 2. The AMOVA showed high (95.31%) percentage genetic variation among population and lower between population. The high percentage genetic variation among the Lakes Alau and Bako is an indication of the presence high level of genetic diversity which could be due to geographical distances (separation). High genetic diversity among

individuals is an indication that the population will be able to adapt to certain environmental factors and as well as ability to resist emerging disease outbreak Boundless (2022), hence makes a good cross breeding stock. This outcome of this study agrees with expectations of freshwater species where differences tend to occur between populations. The high genetic variation among the population could be an indication of strong population differentiation between the two strains.

Table 2: Analysis of Molecular Variance (AMOVA) *O. niloticus* populations from Lakes Alau and Bako based on D-loop sequences.

Source of variation	DF	SS	Variance Components	% Variation	F _{ST}
Within populations	1	25.875	0.64197	4.69	0.047
Among populations	38	495.350	13.03553	95.31	
Total	39	521.225	13.67750	100	

Key: DF = degree of freedom, SS = Sum of squares, F_{ST} = fixation index

Ekerette *et al.* (2017) observed variation within population and high variation among population in the *O. niloticus* from University of Calabar Reservoir, Nigeria. The molecular variation among population may be due to natural selection or genetic drift, which are hinged on evolution via random mutation. Shahid *et al.* (2019) documented 99.99% genetic variation within population of *O. niloticus*, which differed with the outcome of this study. This might have been caused by population diffusion. Brinez (2011) reported 92.8% genetic variation of within population and low variation of 12% between population of *O. niloticus*. F_{ST} values are often used to infer gene flow, in which a lower F_{ST} value indicates low genetic divergence and higher gene flow. F_{ST} values in this study was 0.05 between Lake Bako (MP) and Lake Alau (LA) populations, indicating little genetic divergence, probably due to active exchange of genetic material between the populations through breeding.

CONCLUSION

The two populations are genetically different although they might share common ancestral lineage. The two population are stable and have effective population sizes over time. Lake Bako population seemed to have intact

genetic structure and could be used for selection breeding or hybridization and as well as conserved for future improvement.

AUTHOR 'S CONTRIBUTION

NYAKU, E.R.: Performed the experiment, data curation and manuscript draft.

DIYAWARE, M.Y.: Conceptualization, Dissertation Supervision, data analysis, and critical review of the manuscript draft.

SULEIMAN, S.B.: contributed to manuscript writing and proofreading.

NWAFILI, S.A.: manuscript editing and literature Contribution.

All authors have read and give the final approval for publication.

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

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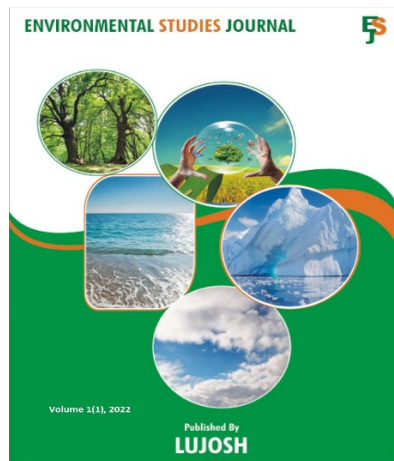


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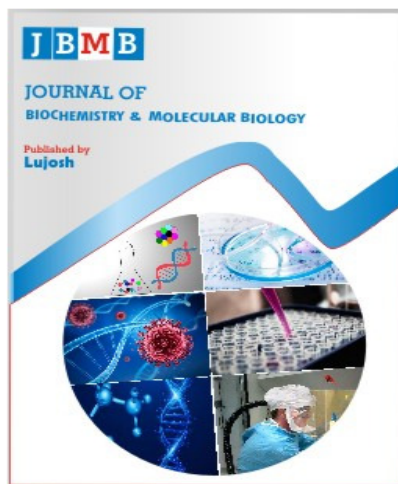
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