

# Genetic Variation Studies of Four Selected Populations of *Oreochromis Niloticus* In Nigeria

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**Abstract-** The genomes of a natural and a cultured population of *Oreochromis niloticus* from each of Northern and Southern Nigeria were comparatively assessed in this study to determine the levels of genetic variation and establish their suitability as sources of broodstocks for aquaculture. DNA was obtained from clipped caudal fins and analysed in a Multigene Gradient thermocycler using 8 microsatellite markers. The amplicons generated were subjected to fluorescent genotyping and the corresponding allele scored. The data obtained was used to determine genetic parameters. While the heterozygosities of the two populations from the wild deviated from Hardy-Weinberg equilibrium (HWE), the heterozygosities of the cultured populations were within HWE. Three populations were inbred while one population was outbred. The fish obtained from these fish farms are generally not managed based on modern population genetic principles although the wild population from New Bussa is a suitable source of Nile Tilapia broodstocks for aquaculture.

**Index Terms-** *Oreochromis niloticus*, Genetic variation, Diversity, Aquaculture, Heterozygosity, Microsatellites

## I. INTRODUCTION

The Nile Tilapia is a commercially important fish with the potential of becoming one of the most productive and internationally traded food fishes in the world (Gupta and Acosta, 2004). In Nigeria, this fish is largely under-cultured as a result of challenges in its production with most of its supply gotten from the wild. It is however noteworthy according to Bickerton (2017) that the Nile tilapia being a fast-growing fish eating a largely plant diet can be grown for much lesser production outlay yielding a turn over faster for a profit relatively compared to other fish species due largely to its inexpensive price. Equally, their large mass, fast growing behaviour and well-liked taste have made them the third most important fish cultured globally. They are also a good source of protein since with respect to other fishes, they occupy a lower trophic level and owing to their fast growth, tilapias do not generally accumulate contaminants in their tissues under natural conditions with the Nile Tilapia being also used in recycling wastes into high quality flesh (Alkobaby *et al.*, 2008). According to Hamed *et al.* (2010), the contribution of Nigeria's cichlid aquaculture to the world's total output is nil in spite of the

potentials and available resources. This they attributed to include stunting, low market value and the lack of developed commercially viable species which are adaptable to their cultured environment.

As consumer demand for fish protein increases, it becomes vital that culture operations successfully meet market demand. Therefore, optimization of hatchery efficiency is of paramount importance if production is to be maximised and maintained. Broodstock productivity is reported to represent the most significant constraint on commercial tilapia production. This has been confirmed locally (Anyanwu, *et al.*, 1991) and globally (Rana, 1997). Locally, there is a heavy dependence on fry and fingerlings from the wild, and fish farmers are usually confronted with the problem of identification of good strains of tilapia "seeds" for cultivation. Improvement in the methodologies of screening of cultivar stocks is clearly needed with better understanding of the factors modifying broodstocks output being of great significance to the added growth of tilapia culture. Broodstock selection and management is thus an important area of aquaculture where modern knowledge and techniques have been applied. The established good practice in the selection of broodstock is to ensure that the genetic diversity of the fish selected is representative of that of the wild, to ensure high levels of diversity in subsequent generations. This is necessary to form a good genetic base to create an enhanced strain of the fish being cultured (Hill, 2000). A high diversity is not only vital for the enhancement of stocks, but will also increase the ability of the population to resist diseases and allow adaptation to possible environmental changes (Gamfeldt and Kallstrom, 2007).

Concurrently, Monsen-Collar and Dolcemascolo (2010) noted that historical events leave distinct signatures in the molecules of organisms that can be accurately interpreted. Therefore information about past environmental activities in natural habitats including anthropogenic ones can be deduced from DNA molecular analyses especially microsatellites which are simple repetitive sequences which have rapid mutation rates,

This study is therefore aimed at comparing the levels of genetic variation such as heterozygosities of the major natural populations of Nile Tilapia of Nigeria in order to assess their potential for sustainable existence in these habitats and their suitability as broodstocks for aquaculture. Information obtained will also be

utilized for the futuristic and sustainable management of the fish species and these natural water bodies.

The specific objectives of this study are to:

- i. compare the genetic variations of the populations;
- ii. compare the expected genetic diversity existing within each populations;
- iii. determine the proportion of the microsatellite loci that are heterozygous in the populations;
- iv. determine the proportion of the microsatellite loci expected to be heterozygous under Hardy Weinberg equilibrium across each of the populations;
- v. determine the genetic and evolutionary significance of the expected level of heterozygosity in the populations.

## II. METHODOLOGY

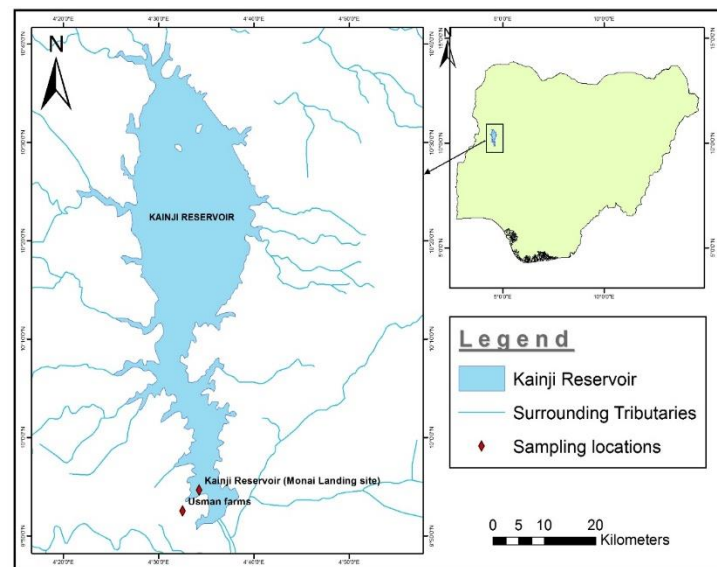
The populations assessed in this study are:

### 1. The Kainji Reservoir

The Kainji dam was built in 1968. It has a maximum depth of 12.1m. The dam is 85.5m (215ft) in height and about 8km (5 miles) in length. The reservoir stretches some 136km upstream and has a breadth of 24km at its widest point. The maximum head elevation is 141.7m (465 ft) and maximum tail elevation is 104m (330 ft) with a total capacity of 15 billion cubic metres covering an area of 1270 km<sup>2</sup> (Sarafinchin, 2012) . The point of capture of the Nile Tilapia population is at the Monai landing station just on the outskirts of New Bussa town. It has the coordinates 009<sup>0</sup> 054' 038.51'' N, 004<sup>0</sup> 034' 015.87'' E.

### 2. Usman farms, Monai Village, New Bussa, Nigeria

The Usman farm is a private farm in the Monai village of New Bussa which engages in the semi-intensive monoculture of Catfish and Tilapia fishes. The farm has the coordinates 009<sup>0</sup> 052' 031.14'', 004<sup>0</sup> 032' 031.29'' E. The farm has four large earthen ponds, three of which are exclusively for catfishes while the fourth is solely for Tilapia fishes, from which the samples used were collected.



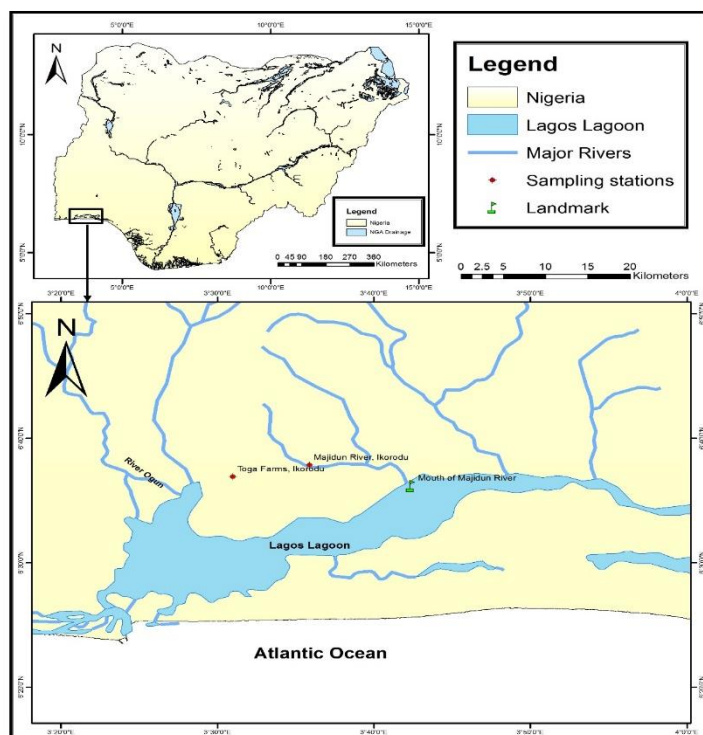
**Fig. 1: Map of Nigeria showing inset the New Bussa Area and the sampling locations (Google Maps, 2021a)**

### 3. Majidun River

Majidun River is located in Ikorodu, Lagos State in Southern Nigeria coastline. It is a relatively small, narrow and shallow water body with an average depth of 3 m. It is one of the numerous aquatic habitats constituting the Lagos Lagoon complex with others adjoining Majidun and forming the complex including Ologe, Lagos, Lekki and Epe Lagoons, Yewa and Ogun rivers and the Badagry and Ogudu creeks (Lawson et al, 2013). The Majidun River drains directly into the Lagos Lagoon and empties into the Atlantic Ocean. Its importance is seen in the areas of artisanal fisheries, transportation, sand mining and logging activities carried out in and around the water body. The main source of water into the river is the Ogun River with seasonal flooding being experienced which carries with it a lot of detritus, domestic and industrial waste and pollutants from land. The point of capture for this study spans the coordinates 006<sup>0</sup> 037' 009'' N, 003<sup>0</sup> 032' 020'' E and 006<sup>0</sup> 037' 011'' N, 003<sup>0</sup> 028' 009'' E.

### 4. Toga farm, Ikorodu, Lagos State, Nigeria

Toga farm is a private fish farm running intensive monoculture of Tilapia and Catfishes in the Ikorodu area of Lagos State. The point of capture on the farm spans the coordinates 006<sup>0</sup> 036' 056'' N, 003<sup>0</sup> 030' 059'' E and 006<sup>0</sup> 036' 057'' N, 003<sup>0</sup> 030' 059'' E. The Toga farm has 8 medium-sized concrete ponds, 2 of which are devoted exclusively to grow-out adult Tilapia fishes from which the sample used in this study were collected.



**Fig. 2: Map of Nigeria showing inset the Ikorodu Area and the sampling locations (Google Maps (2021b))**

Twenty *Oreochromis niloticus* specimens were collected from both wild and cultured sources of two locations (New Bussa (Niger State) and Ikorodu (Lagos State)) for the study. The fish

samples from the wild were bought from landings of artisanal fishermen while the cultured samples were obtained from the fish farms described above. Caudal fin clips of the samples were harvested and kept in 95% ethanol. They were then transferred to a refrigerator in the fish culture laboratory of the Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria set to a temperature of 4°C until DNA extraction.



**Figure 3: Picture showing *Oreochromis niloticus* collected from Kainji Reservoir**

DNA extraction was performed using the DNeasy Blood and Tissue kit (Qiagen, 2006). The extraction was carried out by first macerating the fins separately using different sterile razor blades. The fragmented fin clips were differently placed in a 2 ml micro-centrifuge tube and 180 µl Buffer ATL (lysis buffer) and 20 µl proteinase K added to it. The mixture was vortexed, and placed in a water-bath (equipped with a mechanical shaker) which was used to incubate the mixture at 56°C for 3 hours. The resultant lysate solution was mixed thoroughly by vortexing for 15 seconds. The solution was then mixed with 200 µl Buffer AL and vortexed. Cold 96% ethanol was added to the homogenous solution and mixed thoroughly by vortexing for 30s. The resultant mixture was pipetted into a mini-spin column placed in a 2ml collection tube and centrifuged at 8000 rpm for 1 min. The flow through collection tube was then discarded after which the spin column was placed in a new 2 ml tube, 500 Buffer AW1 added and centrifuged for 1 min at 8000 rpm. The filtrate was discarded again. The spin column was placed in another new 2 ml tube, 500 µl of Buffer AW2 added and centrifuged for 3 minutes at 14000 rpm. The resultant filtrate was discarded. The spin column was placed in another new clean 2 ml micro-centrifuge tube and 200 µl of Buffer AE pipetted directly onto the DNeasy membrane, incubated at room temperature for 1 minute and then centrifuged for 1 min at 8000 rpm to elute.

Agarose gel electrophoresis was used to confirm the presence of DNA in the extract. To prepare 1% agarose gel, two (2) 0.5g tablets were dissolved in 100ml of 0.5X TBE buffer (comprising

of Trizma base, Boric acid and EDTA) inside a conical flask. The mixture was heated in an EMS-9000 Precision Pulsed Laboratory microwave oven for about 4 minutes and cooled under tap water. After cooling, a drop of the ethidium bromide dye was added to the gel and poured into the casting tray containing sample combs. The gel was allowed to solidify at room temperature for 10-15 minutes after which the sample combs were carefully removed. The casting tray containing the solidified gel was horizontally placed in an electrophoresis tank and covered with 0.5X TBE buffer. A measure of 3 µl of the DNA sample mixed with 3 µl of 6X loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol and 30% glycerol) was carefully pipetted into the wells of the gel electrophoresis apparatus. Then, a lid was placed on the electrophoresis chamber and the power leads connected to electric current running at a voltage of 90 V. After 20 minutes, the gel was removed and viewed under Trans-illuminator monitor (Euduro GDS, aplegen UV light, Labnet International technologies).

The quantity (concentration) and quality (purity) of DNA was measured using NanoDrops (ND-1000) which is a full-spectrum (220-750nm) spectrophotometer. The ratio of value of absorption at 260nm to value of absorption at 280nm was used to determine the purity of each DNA sample. A value of 1.8 or just above (1.8-2.0) means pure DNA while a value less than 1.8 (Other contaminants including protein) and above 2.0 (RNA contamination) means impure DNA. After measuring the DNA in nanogram/ml (ng/ml), the volume required to make the required concentration of DNA (10ng/ml) to be added to make up the fixed volume of the reaction mixture (100 µl) was calculated by the mole ratio method.

The microsatellites primers used in this study were selected based on the level of polymorphism and excellent amplification obtained from previous studies (Kunkle et al, 2010). The microsatellite loci used were GM 166, GM201, GM284, GM385, UNH 919, UNH 934, UNH 937, UNH 974 (Table 1).

GeneAmp 9700 thermal cycler (Applied Biosystems, USA) using the eight (8) microsatellite markers previously selected. The PCR mix was a total of 10 µl which include 3.0 µl of 25ng genomic DNA, 1.0 µl of 10X promega buffer (10 mM Tris-HCl pH 9.0, 50mM KCL, 0.01 % gelatin), 0.8 µl of 2.5mM MgCl<sub>2</sub>, 0.5 µl of 2.5mM dideoxynucleotide triphosphates (dNTPs), 0.8 µl of 5% T<sub>20</sub>, 2.5 µl of H<sub>2</sub>O (distilled water), 0.5 µl of each Primer (Primer 1; forward and Primer 2; reverse) for each locus, and 0.4 µl of *Thermus aquaticus*

**Table 1: Micratellite markers used**

	Microsatellite locus	Genbank Accession #	Forward Primer Sequence(5' – 3')	Reverse Primer Sequence (5' – 3')	Annealing temperature (°C)
1	GM 166	BV005579	TGTGAGGCGTGTA ATGCTAAA	AAGAGCGGTGGGT GGAC	68 – 72
2	GM 201	BV005353	TATTCAGGCTCTT CTTTTGCT	CAGAATGAACTCC CTCCAG	68 – 72
3	GM 284	BV005395	AGCCGGTTTCCAG TGAGAA	CGCAGCTTTGATT CATACCCT	68 – 72
4	GM 385	G68259	GGTGGGCAGTGT GTGTTTTT	TTTTCATCCAGGC CTCAGTT	68 – 72
5	UNH 919	G68230	TGACAGCCTGGC ATAATGAG	CACTGAGACTGGA AGGCACA	68 – 72
6	UNH 934	G68240	ACTGCAATGAAA TGCTGCTT	CCATTCCTCAGAG CACAACA	68 – 72
7	UNH 937	G68241	CGCTTGCTAAGGC TATCGTC	TGTCAGCAAATCA AACACATACA	68 – 72
8	UNH 974	G68261	GCACGTCTGAGA GTGTGGAA	CAGCTTTCACACC AGCCTAA	68 – 72

(Taq) polymerase. PCR amplification was carried out in the thermal cycler using a touch-down program that had been programmed to perform:

- an initial denaturation step at 94°C for 5 minutes,
- 25 cycles of denaturation at 94°C for 30 seconds, annealing at a suitable temperature and elongation at 72°C for 45seconds,
- final elongation or extension step at 72°C for 7minutes at 30-35 cycles.

Each primer has a different annealing temperature but the same denaturation and elongation temperature.

Electrophoresis and genotyping were carried out using ABI Genetic Analyzer which is an automated capillary electrophoresis system that can separate, detect, and analyze up to 16 capillaries of fluorescently labeled DNA fragments in one run. The PCR amplicons generated were mixed with GENE Scan 500 Liz size standard (Applied Biosystems) and were transferred to the ABI PRISM 3100 genetic analyzer for capillary electrophoresis and subjected to fluorescent genotyping with the corresponding allele scored using GeneMapper Software version 7. When the alleles (PCR product fragment sizes) were scored with the GeneMapper software, strict criteria were followed to classify the peaks as true

alleles. In the case of two peaks, it was only considered a heterozygote if the two peaks did not differ with more than 2/3 in height. When the first peak had a considerably smaller height than the second, the first peak was considered to be a stutter band. Data were successfully processed into Microsoft Excel 2007 and converted to the input format for subsequent analysis.

The data obtained was used to determine the following genetic variation parameters: Observed Heterozygosity ( $H_o$ ), Expected heterozygosity ( $H_e$ ), Deviations from Hardy-Weinberg equilibrium, Allelic richness ( $A_e$ ), Effective number of alleles ( $N_e$ ) and Inbreeding coefficient ( $F_{IS}$ ). The HWE were determined using Genepop Version 4.3 (Rousset, 2008) while the genetic distance and the corresponding dendrogram were computed using the Fstat Software Version 2.9.3 (Goudet, 2002). Other parameters were determined using Popgen version 1.31 (Yeh *et al.*, 1999)

### III. RESULTS

#### Measure of intra population genetic variation

The population genetic variations were estimated by means of the observed and expected heterozygosities, inbreeding coefficient, number of alleles (allelic richness), and effective number of alleles. From Table 2, the observed heterozygosities across all loci in *O. niloticus* captured population from Kainji

reservoir (CAPK) ranged from 0.2500 to 1.0000 while that of the cultured populations of the same fish species from New Busa - Usman farms (CUPN) ranged from 0.2857 to 1.0000. On the other hand, the observed heterozygosities of the captured population of *O. niloticus* from Ikorodu - Majidun River (CAPI) ranged from 0.0000 to 1.0000 while the population of the cultured populations of the same fish species from Toga farms (CUPT) ranged from 0.0000 to 1.0000. In addition, from Table 3, the average value of the observed heterozygosity of CAPK is 0.6004; CUPN 0.6042; CAPI 0.4125 while CUPT is 0.5179.

The expected heterozygosities across all loci in CAPK ranged from 0.5417 through 0.8929 while that of CUPN ranged from 0.4394 to 0.9231. The expected heterozygosity of CUPT ranged from 0.0000 to 0.7778 while that of CUPT ranged from 0.1429 to 0.8352. The expected heterozygosity values of all populations across all loci were generally higher than the observed heterozygosity except the CUPN where the observed heterozygosity (0.5179) is more than the expected heterozygosity (0.4533). From Table 3, the average expected heterozygosity for CAPK, CUPN, CAPI and CUPT are 0.7488, 0.7043, 0.5954 and 0.4533 respectively. In addition, the two populations from the wild (CAPI and CAPK) showed deviations from Hardy-Weinberg equilibrium (HWE) while the cultured populations were within the HWE equilibrium. The CAPK deviated from HWE at loci GM201, GM284, and UNH974 while the CAPI deviate from HWE loci UNH919, GM166, and UNH934.

The number of alleles of all populations studied across each locus ranged from 1 to 8. The highest number of alleles was found in CAPK followed by the CUPN. The least diverse populations were however CAPI and CUPT. The inbreeding coefficient ( $F_{IS}$ ) is a measure of the level of inbreeding in a population. It is the probability that an individual possesses two alleles at a locus that are the same and as present in the parents. In CAPK, Inbreeding coefficient ( $F_{IS}$ ) values ranged from -0.493 to 0.750 across the loci, in CUPN, it ranges from -0.154 to 0.600 while in CAPI it ranges from -0.412 to 1.000 with CUPT having values from -0.500 to 0.280. On the average, the population with the highest level of inbreeding is captured Ikorodu (CAPI) with 0.3330 followed by CAPK with 0.2180 and then CUPN with 0.1620 while the least inbred and the only outbred population is CUPT with an inbreeding coefficient ( $F_{IS}$ ) value of -0.1560.

The heterozygosities of the wild populations (CAPK and CAPI) were generally not in HWE while the heterozygosities of the cultured population (CUPN and CUPT) were within HWE. The heterozygosity values that are not within HWE were either heterozygote deficiencies (observed heterozygotes less than expected heterozygotes) or heterozygote excess (observed heterozygotes more than expected heterozygotes). The heterozygote deficiencies were at loci GM201 and UNH974 in CAPK and loci GM166 and GM385 in CAPI. The heterozygote excesses were at locus GM284 in CAPK and UNH919 in CAPI.

**Table 2: Observed and expected heterozygosities and test of deviation from Hardy-Weinberg equilibrium of the populations at each locus**

Locus		Captured New Busa (CAPK)	Cultured New Busa (CUPN)	Captured Ikorodu (CAPI)	Cultured Ikorodu (CUPT)
GM201	N	16	14	6	14
	Ho	0.5000	1.0000	0.3333	0.4286
	He	<b>0.8083</b>	0.8901	0.6000	0.5824
	P	0.031944	0.839299	0.148954	0.497636
	N <sub>a</sub>	6.0000	7.0000	3.0000	3.0000
	N <sub>e</sub>	4.1290	5.7647	2.0000	2.1778
	F <sub>IS</sub>	0.398	-0.135	0.500	0.280
GM284	N	16	14	12	14
	Ho	<b>1.0000</b>	0.8571	0.8333	1.0000
	He	0.6917	0.9231	0.7576	0.8352
	P	0.036713	0.274511	0.597149	0.578174
	N <sub>a</sub>	4.0000	8.0000	6.0000	6.0000
	N <sub>e</sub>	2.8444	7.0000	3.2727	4.4545
	F <sub>IS</sub>	-0.493	0.077	-0.111	-0.217
UNH919	N	16	14	10	14
	Ho	0.7500	0.8571	0.8000	0.7143
	He	0.8167	0.8022	<b>0.7778</b>	0.5385
	P	0.756494	0.767889	0.043036	0.350748
	N <sub>a</sub>	5.0000	5.0000	4.0000	2.0000
	N <sub>e</sub>	4.2667	3.9200	3.3333	2.0000
	F <sub>IS</sub>	0.087	-0.075	-0.032	-0.364
UNH937	N	16	14	12	14
	Ho	0.8750	0.4286	0.3333	0.4286
	He	0.7000	0.6484	0.6212	0.3846
	P	0.881433	0.190085	0.316465	0.954820
	N <sub>a</sub>	5.0000	3.0000	3.0000	3.0000
	N <sub>e</sub>	2.9091	2.5128	2.3226	1.5556

	$F_{IS}$	-0.273	0.357	0.487	-0.125
GM166	N	16	12	10	14
	$H_o$	0.5000	0.5000	0.0000	0.7143
	$H_e$	0.5417	0.4394	<b>0.7111</b>	0.6484
	P	0.419198	0.936778	0.001134	0.577363
	$N_a$	6.0000	3.0000	3.0000	3.0000
	$N_e$	2.0317	1.6744	2.7778	2.5128
GM385	$F_{IS}$	0.082	-0.154	1.000	-0.111
	N	16	14	12	14
	$H_o$	0.5000	0.5714	0.0000	0.1429
	$H_e$	0.7917	0.7582	<b>0.5455</b>	0.1429
	P	0.081092	0.058550	0.007290	1.000000
	$N_a$	7.0000	7.0000	2.0000	2.0000
UNH934	$F_{IS}$	0.385	0.262	1.000	0.000
	N	14	14	8	14
	$H_o$	0.4286	0.2857	1.0000	0.0000
	$H_e$	0.7473	0.4396	0.7500	0.0000
	P	0.463852	0.296718	0.808847	-*
	$N_a$	6.0000	2.0000	4.0000	1.0000
UNH974	$F_{IS}$	0.446	0.368	-0.412	NA
	N	8	6	4	14
	$H_o$	0.2500	0.3333	0.0000	0.7143
	$H_e$	<b>0.8929</b>	0.7333	0.0000	0.4945
	P	0.028351	0.148954	-*	0.196706
	$N_a$	5.0000	3.0000	1.0000	2.0000
	$N_e$	4.5714	2.5714	1.0000	1.8491
	$F_{IS}$	0.750	0.600	NA	-0.500

\* - Monomorphic locus

**Table 3: Mean observed and expected heterozygosities, observed and effective number of alleles and inbreeding coefficient of the populations across all the loci.**

	Captured New Bussa	Cultured New Bussa	Captured Ikorodu	Cultured Ikorodu
N	15	13	9	14
$H_o$	0.6004	0.6042	0.4125	0.5179
$H_e$	0.7488	0.7043	0.5954	0.4533
$N_a$	5.5000	4.7500	3.2500	2.7500
$N_e$	3.4872	3.5640	2.4519	2.0878
$F_{IS}$	0.2180	0.1620	0.3330	-0.1560

N= Sample size,  $H_o$ = Observed heterozygosities,  $H_e$  = Expected heterozygosities,  $N_a$ =Allelic richness,  $N_e$ = Effective number of alleles and  $F_{IS}$ = Inbreeding coefficient

#### IV. DISCUSSION

##### Conformity/ deviation from Hardy-Weinberg equilibrium

The Hardy-Weinberg law states that under certain conditions, allele and genotypic frequencies do not change from generation to generation. Although the alleles may not be expressed (as determined by Mendel's laws of inheritance), they are still present

in the population in the same frequencies (Bader, 1998). These conditions include the population being infinitely large, mating being random, no genetic drift, gene flow from other populations and no other evolutionary forces affecting the population. Thus, the observed genotypic frequencies of the heterozygotes are tested against the expected genotypic frequencies of the heterozygotes; to determine if there is a deviation from the Hardy-Weinberg equilibrium. If the genotypic frequencies of the observed and expected heterozygotes are significantly different, then we can assume that the HWE conditions highlighted above have been violated. In this study, in both captured populations, New Bussa and Ikorodu (CAPK and CAPI), the expected and observed heterozygote frequencies of some loci differ significantly while those of some are within the HWE. On the other hand, the observed and expected heterozygote frequencies of the cultured populations from New Bussa and Ikorodu (CUPN and CUPT) are within the HWE. The deviations from HWE of the captured populations implies that the conditions or assumptions of the Hardy-Weinberg law have been violated. These forces again are non-random mating, gene flow and selection. Other outlier factors that can cause significant difference between observed and expected heterozygosity is either null alleles or Wahlund effect. Null allele is a term most commonly used to describe microsatellite alleles that do not amplify during PCR. The most common cause of this is a mutation in one or both of the primer binding sequences. If only one allele of a heterozygote is amplified then it will be erroneously genotyped as homozygote. On the other hand, Wahlund effect is as a result of taking samples from one or more different population which has different allele frequencies. This normally happens because boundaries of populations can sometimes be very difficult to identify. The proportion of homozygote in the aggregate sample ends up being higher than it would have been if the populations were analyzed separately.

Fishing pressure is a major factor that has been reported as constituting strong evolutionary force which can change genetic diversity or structure of a population. Generally, most Nigerian water bodies are reportedly overfished though the intensity may vary from one region to the other. Fishing is almost always non-random. Typically, gear is designed to remove some kinds of individuals in preference to others, usually individuals that are larger. Fishing mortality is therefore selective with respect both to species and to phenotypic variation within species (Stokes *et al.*, 1993; Jennings *et al.*, 1998). Hence, if the characters or phenotypes of fishes being selected are due to genetic differences between individuals, then fishing will act as selective force leading to evolutionary change. Most phenotypic changes in this context are related to yield, such as growth rate, length- and age-at-sexual maturation, and fecundity. Therefore, simply through the action of fishing, fishers generate selection, causing evolution that changes the sustainable yield. The loci that are not within HWE in the wild populations of this study are thus suggested to have been affected by selective force occasioned by fishing pressure. In addition, since the region of the DNA analyzed in this study is primarily not under natural selection these loci therefore must be associated with regions of the genome under the influence of natural selection.

### Heterozygosity and inbreeding

Microsatellite heterozygosity (genetic diversity) is one of the most important attributes of any population. Environments are

constantly changing, and genetic diversity is necessary if populations are to evolve continuously and adapt to new situations. Furthermore, low genetic diversity typically leads to increased levels of inbreeding, which can reduce the fitness of individuals and populations. An assessment of genetic diversity is therefore central to population genetics and has extremely important applications. The populations studied showed a pattern of distribution of level of microsatellite heterozygosity where the northernmost samples of New Bussa had higher heterozygosity while the southernmost samples of Ikorodu showed reduced values. This same pattern is depicted in the values obtained for allelic richness and effective number of alleles.

Furthermore, the cultured populations from Ikorodu have the lowest value of expected heterozygosity despite having the lowest value of inbreeding; that is; though mating was random, the level of heterozygosity obtained was low. This suggest a low or reduced population size; that is; the size of the base population of the cultured populations from Ikorodu is small (founder effect). When population size is reduced allelic diversity usually decreases. This is often associated with a drop in expected heterozygosity because fewer alleles typically lead to reduced expectations of heterozygosity under HWE. At the same time, observed heterozygosity values may not deplete and in fact there may be a temporary increase in observed heterozygosity compared with expectations under HWE. In some cases, observed heterozygosity excess can be a useful indicator of past bottlenecks, although this test requires a large number of polymorphic loci and can detect only relatively recent bottlenecks (Luikart and Cornuet, 1998). On the other hand, the high level of inbreeding in the wild populations may be because of the reproductive behaviour of Nile Tilapia. Female Nile Tilapias spawn asynchronously and the operational sex ratio, therefore, is male biased (operational sex ratio is the sex ratio of animals actively participating in reproduction). A male-biased sex ratio together with the aggressive nature of males is expected to result in strong reproductive competition leading to high variance in male reproductive success. These differences in male reproductive success were reported to lead to increase in the level of inbreeding in the populations of the Nile tilapia.

### Implications of the findings of this study for aquaculture

The findings of this study have profound implications for aquaculture in the respective sites of this study and also to other aquaculture water bodies in Nigeria as a whole. First, the study provides information on level of genetic diversity of selected wild populations of *O. niloticus*. Since the base populations or individuals to be used as broodstock are expected to have higher levels of heterozygosity, the wild population of *O. niloticus* from Ikorodu is not a good source of broodstock. Secondly, the values of heterozygosity of the cultured populations were generally not different from those of the wild population. This study did not have any information on how the broodstock or base populations were selected in the respective farms but the fact that the values of heterozygosity of both wild and cultured species are relatively the same means the cultured populations have been well managed unknowingly or knowingly in terms of broodstock selection.

No single one of the four populations is genetically diverse enough to be left without considerable action taken on it. However, Kainji Reservoir is considered good enough to be a future source of cultured broodstocks. Measures which would

restore the genetic variability including reduction of anthropogenic activities such as pollution and fishing pressure should therefore be embarked upon towards re-establishing the potentials of these populations. Adequate number of individuals should also be used as founder stock in establishing a culture population. Likewise, the level of genetic diversity of base populations should always be considered before utilizing them.

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