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Evaluation of the genetic diversity and population structure of dam-induced fragmented populations of *Oreochromis niloticus* (Linnaeus 1758) in Challawa and Kano Rivers

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Abstract

Anthropogenic interference with river systems can fragment populations into smaller sub-populations, leading to loss of their genetic diversity necessary to withstand environmental challenges and increasing their susceptibility to evolutionary pressures. This study assessed the genetic diversity and population structure of dam-induced populations of *Oreochromis niloticus* in the Challawa and Kano Rivers. Four polymorphic microsatellite primers-UNH 146, UNH 172, UNH 185, and UNH 995-were used to amplify extracted DNA of 87 fish samples from four populations located on the two rivers i.e., reservoir (above-dam) and below-dam. Band scores from gel electrophoresis were used for the genetic diversity analysis. The diversity indices, including the number of alleles (Na) per population, effective alleles (Ne), and Shannon's information index (I), revealed higher values for the reservoir population in the Challawa River compared to its below dam counterpart. Three of the populations displayed heterozygosity deficits (Ho<He), and all populations significantly deviated ($P < 0.05$) from Hardy-Weinberg Equilibrium (HWE). The genetic distance index (F_{ST}) between the reservoir and below dam sections of the Challawa and Kano Rivers was 0.129 and 0.182, respectively, indicating moderate and high levels of genetic differentiation between the respective sections. The Analysis of Molecular Variance (AMOVA) results confirmed a high level of genetic differentiation among the populations (23.0%), suggesting overall restricted gene flow (Nm = 1.078). Both the phylogenetic tree and structure analysis indicated that the reservoir and below dam populations are structured. The study affirms that damming did impede gene flow across the river sections, leading to reduced genetic variability of the fragmented populations. Continuous monitoring of genetic variability in these populations is recommended to ensure prompt management measures for the sustainability of the fisheries.

Key words: Anthropogenic, Connectivity, Differentiation, Founder, Heterozygosity, Inbreeding.

INTRODUCTION

Dams are constructed to store and conserve flows of rivers and surface runoffs from catchment areas to provide essential socio-economic services. Through this means, reliable means of water for irrigation farming, municipal use and fisheries are made available **(Tecle, 2017)**. Dams account for almost a fifth of the world's electricity power supply and useful in reducing risk of flood and mitigating effect of drought on agricultural productivity **(Akindele & Indabawa, 2015).**

However, river impoundment brought about by the constructed dams affects aquatic biota in diverse ways. Dam pose as barrier impeding upstream-downstream or inter-drainage movements and thus gene flow between fish populations are disconnected **(Winemiller *et al*., 2016)**. It also modifies riverine environments resulting into degradation of preferable habitats and creation of inhospitable environments for fish. The flow regimes of rivers are usually altered after impoundment thus changing the lotic condition of river into relatively stable water **(Lima *et al*., 2018).** This form of change in river hydrology often leads to the extirpation of some native species strictly adapted to riverine condition while allowing some non-endemic species to flourish **(Marques *et al*., 2018)**. The below-dam sections of impounded rivers usually experience changes in seasonal flow which in turn influences water temperature and chemistry that are important environmental factors controlling spawning and larvae development of fish **(Cheng *et al*., 2015)**.

Habitat fragmentation and population isolation brought about by damming of rivers have drastic effects on population genetic diversity and riverine biodiversity conservation **(Fluker *et al*., 2014).** Genetic diversity is an intrinsic means of evaluating ability of population to thrive and overcome evolution factors. Reduction in genetic diversity and increased genetic differentiation between fragmented populations are often resulted due to reduced interpopulation gene flow, increased random genetic drift and founder effect on the isolated populations **(Yamamoto *et al*., 2019)**.
Studying the genetic diversity of isolated populations provides information on their viability as it is often associated with key demographic characteristics such as growth potential, fecundity, survival and general ability to cope with future adverse environmental changes **(Yamamoto *et al*., 2019)**. Small isolated populations have high tendency of experiencing loss of genetic diversity and increased inbreeding level which are indicators of reduced fitness and ability to thrive and withstand environmental challenges **(Liu *et al*., 2020)**. It is therefore important to understand genetic diversity and evaluate level of gene-flow among fragmented populations. This would facilitate drawing of inferences on their general evolutionary potentials so as to proffer suggestions that would lead to effective management of the fisheries resources.

The dams constructed upstream of Hadejia River were meant to provide important socio-economic services such as irrigation farming, hydroelectric power generation, municipal water supply and fishing. The existence of these dams has produced inadequacies in the river flow, yield and composition of fishes inhabiting the Hadejia River **(Gana *et al*., 2018)**. Also in 1976, two years after the completion of Tiga dam, about 100 million cubic meters per year reduction of river flow was experienced in Gashua due to water diverted from the reservoir for irrigation purpose **(Gana *et al*., 2018).**

Oreochromis niloticus (Linnaeus 1758) also known as Nile Tilapia is a prominent member of the Cichlidae family native to Western Africa and the Nile basin **(Champneys *et al*., 2021)** The species accounts for approximately 80% of global tilapia aquaculture productions **(FAO, 2018).** It holds significant commercial values in Challawa and Kano Rivers, constituting approximately 21% and 8% of their respective fish biodiversity **(Abdul-Azeez *et al.*, 2024)**. Nile Tilapia is a popular aquaculture species due to its high environmental tolerance, fast growth rate and high fecundity **(Champneys *et al*., 2021; Mabrouk *et al*., 2021)**. This species is essentially a littoral dweller **(Bandara & Amarasinghe, 2017)** and considered a species with limited dispersal as its migration for spawning is mostly restricted to the littoral areas **(Muhtadi *et al*., 2022).** Utilizing a species with limited dispersal, such as *O. niloticus*, in studying the effects of habitat fragmentation on genetic diversity facilitate the detection of the potentially severe impact of fragmentation on the inhabitant populations' genetic diversity **(Fluker *et al*., 2014)**. Species with limited dispersal often face challenge in connecting with adjacent populations, thereby compromising their genetic exchange with neighbouring populations.

To date, there is dearth of information from published literature on the effect of these upstream dams on the fish genetic diversity of the below-dam sections of any of the tributaries. Challawa and Kano Rivers being the major upstream tributaries of the Hadejia River are hereby selected for this study. It is expected that rapid population expansion in new water bodies created by dams often leads to increased genetic diversity of species that easily get adapted to the new environment **(Bbole *et al*., 2020)**. We therefore hypothesized that *O. niloticus* populations residing in the reservoirs created by river damming will exhibit greater genetic variability compared to those in the below-dam sections of the rivers. The present study investigated effect of the damming of these two rivers on the genetic diversity and differentiation of the dam-induced fragmented populations of *O. niloticus*. Outcomes of this study would provide baseline information for the management programs of the fisheries and point of reference for future investigations.

MATERIALS AND METHODS

Study Area

Kano and Challawa Rivers are the major upstream tributaries of Hadejia River. Tiga Dam (storage capacity: 1492 x 10^6 m^3) and Challawa gorge- Dam (storage capacity: 972 x $10^6 m^3$) were respectively constructed in 1974 and 1992 primarily for irrigation, municipal water supply and hydro-electricity power generation. The two are located within Kano State in the Northwestern Nigeria. Kano State is a prominent commercial center in the northern Nigeria and located between latitude 10º33‘and 12º27‘North of the equator and longitude 7º34‘and 9º29‘East of the Greenwich meridian **(Abdulazeez, 2018; Mohammed *et al*., 2020)**.

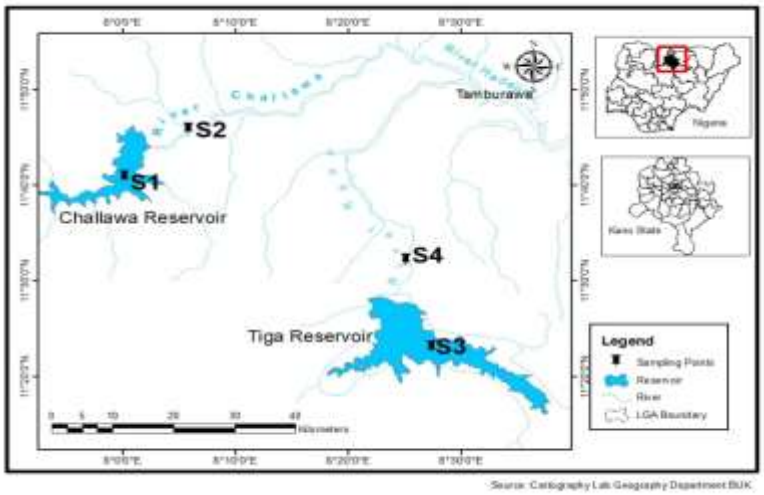

Figure 1.0 Map Showing Hadejia-Jama'are River system in Nigeria.

Sampling location

Two sampling locations were identified on each of the Challawa and Kano Rivers. On each of the rivers, one location was selected above the Dam (i.e., on the reservoir) and below the dam as indicated on the map (Figure 1). Their coordinates were taken using global positioning system (GPS Waypoints, Version 3.11) and recorded (Table 1).

Table 1: Description of the sampling locations

River	Name of Location	Location on River	Location Symbol	Latitude	Longitude	Elevation
Challawa	Daura	Reservoir	S1	11.708888	7.986813	518.7m
Challawa	Kwarin Isau	Below dam	S2	11.762055	8.095992	471.0m
Kano	Rurum	Reservoir	S3	11.410552	8.448852	510.4m
Kano	Gargai	Below dam	S4	11.536472	8.416765	480.4m

Sample collection and DNA isolation.

This study was based on 87 samples of *O. niloticus* collected from the catches of local fishers from four sampling locations. The samples were transported under chilled conditions to the Molecular Nutrition Laboratory of the Biochemistry Department, Bayero University Kano, Nigeria, for molecular analyses. About 45mm^2 of flesh tissue was collected from each sampled fish into a 1.5 ml Eppendorf tube and stored at 4°C**.** DNA extraction was performed using a modified version of the Livak protocol **(Livak, 1984)**.

The grind buffer was prepared as follows: 1.6 ml 5M NaCl, 5.48 g sucrose, 1.57 g Tris-HCl (pH 9), 10.16 ml 0.5M EDTA, 2.5 ml 20% SDS, bringing the volume to 100 ml, and filter sterilizing

it. 5 ml aliquots were stored at -20°C. The buffer was heated in a water bath at 65°C for 5 min. and mixed before each use to re-dissolve precipitates.

Each tissue sample was homogenized in 100 µl of preheated grind buffer in a 1.5 ml Eppendorf tube. It was then incubated at 65°C for 30 min. The samples were centrifuged for 30s to collect condensation. Then, 14 µl of 8M K-acetate (to a final concentration of 1M) was added, mixed, and incubated on ice for 30 min. The samples were centrifuged for 20 min. at 4°C. The supernatant was transferred into a new 1.5 ml Eppendorf tube. 200 µl of 100% ethanol was added and the mixture was vortexed for 15 min. at 4°C. The supernatant was removed and discarded. The remaining pellet was rinsed with approximately 100 µl of ice-cold 70% ethanol. The tube was left open on the bench top for one hour to dry the pellet. The pellet was then suspended in 100 µl of dH_2O and incubated at 65°C for 10 minutes. DNA quality was determined by visualization in an agarose gel electrophoresis (2.0%).

PCR amplification and gel separation

Amplification of the eluted DNA from *Oreochromis niloticus* species was carried out using four microsatellite primers originally developed for Tilapias **(Lee *et al*., 2005).** The PCR amplification was performed using Thermal cycler (Borad T100^{TM}) involving 15 µl reactions with a final concentration of 1X Taq buffer, 1.5 mM $MgCl_2$, 0.12 µl 200 nM (each) dNTPs, 0.5µl of each primer, 0.12 µl Kapa Taq DNA polymerase (KAPA Biosystems, Wilmington, MA, USA), 1.5 µl DNA, and 9.99 µl sterile H_2O. The PCR conditions used were: initial denaturation and enzyme activation at 95°C for 15 min, followed by 35 cycles of 94°C for 30 s, 55°C for 1 min, and 72°C for 1 min, with a final extension at 72°C for 20 min. Amplicon separation was done in 2.0% agarose gel electrophoresis containing 0.5X TAE buffer and ethidium bromide (10 µg/ml). Features of the markers are presented in **Table 2**. Bands were scanned with Gel documentation system (Ingenius 3, Model IG3/1459. UK).

Table 2: Markers' nucleotide sequences, band size and annealing temperature

Primer	Nucleotides sequence (5'-3')	Size range(bp)	Annealing temperature (ºC)
UNH146	F:CCACTCTGCCTGCCCTCTAT R:AGCTGCGTCAAACTCTCAAAAG	100-230	55
UNH172	F:AATGCCTTTAAATGCCTTCA R:CTTTTATAGTCGCCCTTTGTTA	100-335	55
UNH185	F:CAGACACACTAGACACATTCTA R:GTGTTTCCATGTGTCTGTAC	100-200	55
UNH995	F:CCAGCCCTCTGCATAAAGAC R:GCAGCACAACCACAGTGCTA	100-300	55

Data collection

Band scores from the gel-scanned images were input into GenAlex v 6.5, **(Peakall & Smouse, 2012)** and preliminary data validation exercises were performed before further analysis was undertaken.

Data Analyses

Genetic diversity

Allelic properties of the four loci across the four populations, such as the number of alleles (Na), effective alleles (Ne), Shannon diversity index (I), observed, expected, and unbiased expected heterozygosity (Ho, He, and uHe respectively), fixation index (F), number of migrants alleles (Nm), and percentage polymorphism were calculated using GenAlex. Deviation from the Hardy-Weinberg Equilibrium (HWE) and genetic linkage disequilibrium (LD) tests were performed using the Genepop R package v.1.2.2 **(Raymond & Rousset, 1995)** based on Chi-squared statistics. Genepop uses the Markov chain algorithm with 10,000 dememorization steps, 100 batches, and 5,000 iterations to run the LD test. The Hardy-Weinberg probability test was performed with 10,000 dememorization steps, 20 batches, and 5,000 iterations.

Genetic differentiation and population structure

The F_{ST} and Nei's genetic distance matrix of the four populations, the Analysis of Molecular Variation (AMOVA), and Mantel's test to investigate whether genetic differentiation followed the isolation by distance model were all done in GenAlex. The Nei genetic distance matrix was imported into MEGA v..X. **(Kumar *et al*., 2018)** and a phylogenetic tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA). The program STRUCTURE v.2.3.4.**(Pritchard *et al*., 2000)** which uses a Bayesian clustering approach, was employed to probabilistically assign individuals to clusters using allele frequency data. An admixture model and correlated allele frequency were used to determine group membership probability (Q) of each individual for K=1 to K=10. Ten replicates of each level of K with 300,000 Markov-Chain Monte Carlo (MCMC) iterations were set after a 150,000 iteration burn-in period. The program Structure Selector **(Li & Liu, 2018)** was used to determine the most likely number of clusters the populations are structured into. The Puechmaille method **(Puechmaille, 2016)**, which uses parameters (MedMeaK, MaxMeaK, MedMedK, MaxMedK) in considering influence of uneven genotypic dataset in the determination of appropriate number of clusters (K) was employed.

Recent bottleneck

Tests for evidence of a recent bottleneck, an indication of a recent drastic decline in effective population size (Ne), were performed using the BOTTLENECK v.2.3.4 **(Piry *et al*., 1999)** program. The test operates on the principle of a population that has recently experienced reduction in Ne would have significantly higher observed genetic heterozygosity (He) than expected under the mutation-drift equilibrium (Heq). The determination of heterozygosity excess was performed using the stepwise mutation model (SMM) and the two-phase model of mutation (TPM). The default settings for TPM (variance of 30, with 70% of mutations foll owing SMM) were adopted as they were considered most extreme mutation models appropriate for microsatellites **(Bbole *et al*., 2020)**. A two-tailed Wilcoxon's signed-rank test was used to determine the significance of heterozygosity excess at 1,000 iterations.

RESULTS

Allelic diversity of the four markers

The allelic diversity analysis of the four polymorphic microsatellite markers revealed that the average number of alleles (Na) ranged from 2.250 to 3.500. The Shannon index values varied between 0.666 and 1.006. Observed heterozygosity (Ho) ranged from 0.169 to 0.654, while

expected heterozygosity under Hardy-Weinberg equilibrium (He) was between 0.418 and 0.582. The unbiased expected heterozygosity ranged from 0.429 to 0.598. The analysis identified an excess of heterozygosity (Ho > He) at loci UNH146 and UNH185, and a deficit (Ho < He) at loci UNH172 and UNH995. All loci showed significant deviations ($P < 0.05$) from Hardy-Weinberg equilibrium (Table 3).

Table 3: Allelic diversity indices at the four loci

	Na	Ne	I	Ho	He	uHe	HWE	F_{IS}	F_{IT}	F_{ST}	Nm
UNH146	3.50	2.43	1.01	0.61	0.58	0.60	0.00*	-0.05	0.08	0.13	1.74
UNH172	3.00	1.74	0.70	0.27	0.42	0.43	0.00*	0.36	0.46	0.15	1.40
UNH185	2.50	1.92	0.72	0.65	0.46	0.47	0.00*	-0.43	-0.27	0.11	2.00
UNH995	2.25	1.97	0.67	0.17	0.42	0.43	0.00*	0.60	0.79	0.47	0.28
Mean	2.81	2.02	0.77	0.43	0.47	0.48	0.00*	0.12	0.26	0.22	1.36

Na= Average number of alleles, Ne= Effective alleles, I= Shannon's information index, Ho=observed heterozygosity, He=Expected heterozygosity, UHe=Unbiased expected heterozygosity, HWE=Hardy-Weinberg equilibrium, F- statistics (F_{IS}, F_{IT} and F_{ST}), Nm=Number of migrant alleles.

At population level, the genetic diversity indices are summarized in Table 4. The number of alleles (A) across the four populations ranged from 9 to 13, effective alleles (Ne) ranged from 1.856 to 2.277, and the Shannon information index (I) ranged from 0.609 to 0.925. The reservoir population of *O. niloticus* in the Challawa River (S1) had higher values for A, Ne, and I compared to its below dam counterpart (S2). Conversely, the population below dam on the Kano River (S4) had higher values for these indices compared to its counterpart (S3) in the reservoir. Higher private alleles (PA) were observed in the below dam population on the Challawa River, while lower value of the same parameter was found in the below dam population of the Kano River. The markers showed high polymorphism across all populations (Figure 2). Three of the four populations exhibited heterozygosity deficits, with observed heterozygosity (Ho) less than expected heterozygosity (He). Below dam population on the Kano River alone experienced excess heterozygosity. Hardy-Weinberg equilibrium (HWE) exact tests revealed significant deviations ($P < 0.05$) from equilibrium in all populations. A positive Wright fixation index (F) in three populations indicates some level of homozygosity. Genotypic linkage disequilibrium (LD) tests showed LD in only three (12.5%) of the 24 pairwise locus comparisons (Table 5). Specifically, locus UNH146 was significantly in LD with UNH185 in population S1 and with UNH172 and UNH995 in populations S3 and S4, respectively.

Table 4: Genetic diversity parameters of the *O. niloticus* populations

River	Site	N	A	Ne	I	PA	%P	Ho	He	UHe	F	HWE
	Reservoir	20.00	13.00	2.28	0.93	5.00	100.0	0.50	0.55	0.56	0.15	0.00*

Challawa	Below dam	23.00	10.00	1.88	0.69	26.00	100.0	0.29	0.43	0.44	0.22	0.00*
	Reservoir	23.00	9.00	1.86	0.61	46.00	75.0	0.39	0.40	0.40	0.03	0.00*
Kano	Below dam	21.00	13.00	2.05	0.86	7.00	100.0	0.53	0.50	0.52	-0.07	0.00*
	Mean	21.75	11.25	2.02	0.77	21.00	93.75	0.43	0.47	0.48	0.08	0.00

N= Number of sample, A= Number of alleles, Ne= Effective alleles, I= Shannon's information index, PA=Private alleles, %P=percentage polymorphism of the markers, Ho=observed heterozygosity, He=Expected heterozygosity, UHe=Unbiased expected heterozygosity, F=Fixation index, HWE=Hardy-Weinberg equilibrium.

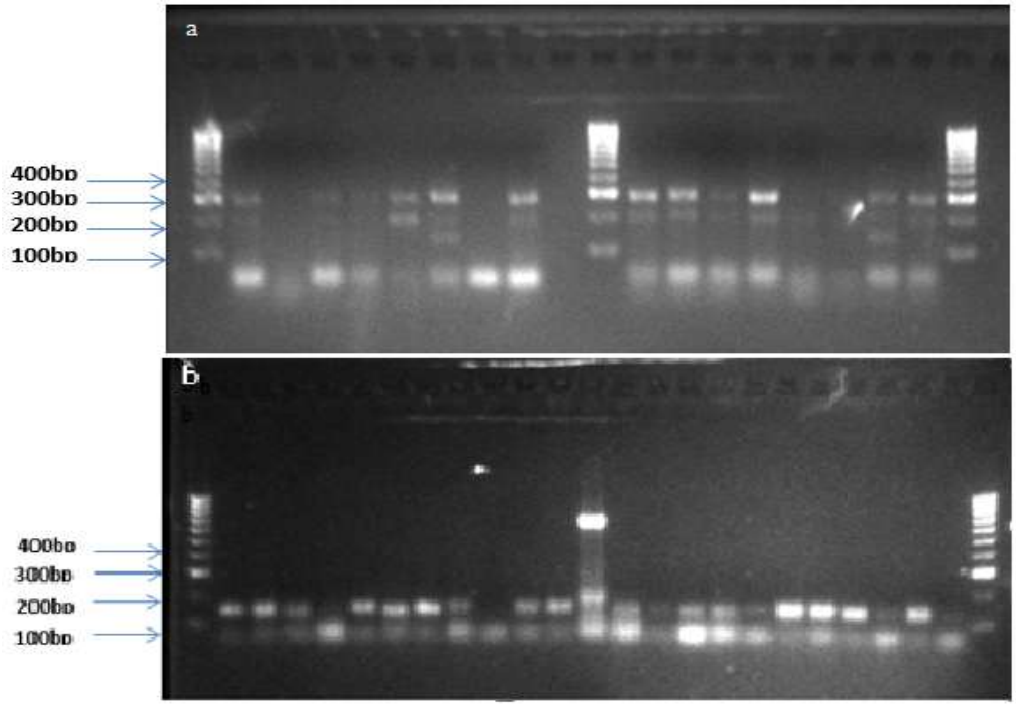

Figure 2: (a) Amplified DNA from S2 population of *O. niloticus* using UNH 995 marker (b). Amplified DNA from S3 using UNH 185 marker

Table 5: Linkage disequilibrium (LD) between loci pairs

Population	Locus pairs		P-Value	S.E.	Switches
S1	UNH146	UNH172	0.635814	0.003590	70875
S1	UNH146	UNH185	0.002714	0.000451	69444
S1	UNH172	UNH185	0.821322	0.002771	71114
S1	UNH146	UNH995	0.296076	0.003299	116402
S1	UNH172	UNH995	0.081536	0.001428	123885
S1	UNH185	UNH995	0.215834	0.002883	131405

S2	UNH146	UNH172	1.000000	0.000000	295947
S2	UNH146	UNH185	0.565416	0.000813	282293
S2	UNH172	UNH185	0.244938	0.001073	310774
S2	UNH146	UNH995	0.266902	0.000846	315808
S2	UNH172	UNH995	1.000000	0.000000	216975
S2	UNH185	UNH995	1.000000	0.000000	116048
S3	UNH146	UNH172	0.040138	0.000566	223163
S3	UNH146	UNH185	1.000000	0.000000	135358
S3	UNH172	UNH185	1.000000	0.000000	319754
S3	UNH146	UNH995			
S3	UNH172	UNH995	No contingency table		
S3	UNH185	UNH995			
S4	UNH146	UNH172	0.328564	0.003782	190316
S4	UNH146	UNH185	1.000000	0.000000	190316
S4	UNH172	UNH185	1.000000	0.000000	182836
S4	UNH146	UNH995	0.045974	0.001077	158603
S4	UNH172	UNH995	0.313698	0.001997	168210
S4	UNH185	UNH995	0.570504	0.001154	233552

Genetic differentiation and population structure

Genetic differentiation, as indicated by F_{ST} and Nei's genetic distance, was 0.129 and 0.318, respectively, between the fragmented *O. niloticus* populations on the Challawa River (S1 & S2). In contrast, higher genetic differentiation was observed between the fragmented populations on the Kano River (S3 and S4), with F_{ST} of 0.182 and Nei's genetic distance of 0.496 (Table 6). Analysis of Molecular Variance (AMOVA) showed high genetic variation both among populations (23%) and within populations (22%), with the highest variation occurring between individuals (55%). This pattern was supported by Wright's statistics, which indicated the highest genetic variation between individuals (F_{IT} = 0.451) and significant ($P < 0.05$) variation within populations (F_{IS} = 0.229) and among populations (F_{ST}=0.227) (Table 7). The study also found a substantial gene flow, with more than one migrant per generation (Nm = 1.078) among the populations.

Cluster analysis, represented by the dendrogram, showed populations S1 and S3 of *O. niloticus*, located within the reservoirs of the Challawa and Kano Rivers, are genetically distinct from their respective below dam populations (S2 and S4). However, S3 appears to be more distinct from the others, while S4 is more genetically similar to S1 and S2 than to its reservoir counterpart (S3) on the same river (Figure 3). This pattern is also reflected in the Structure barplots, which identified three clusters (Figure 4). However, the use of Puechmaille Method in determining optimal cluster number (K) between uneven populations distinguished four distinct clusters (Figure 5). Mantel's test confirmed a significant ($P < 0.05$) but weak positive correlation (r = 0.326, R^2 = 0.1062) between genetic and geographical distances among all populations (Figure 6).

Table 6: F_{ST} genetic distance (below diagonal) and Nei' genetic distance (above diagonal) among *O. niloticus* populations

	Challawa River		Kano River	
Populations	**Reservoir (S1)**	**Below dam (S2)**	**Reservoir (S3)**	**Below dam (S4)**
Reservoir (S1)		0.345	0.610	0.292
Below dam(S2)	0.129		0.499	0.733
Reservoir (S3)	0.200	0.247		0.596
Below dam(S4)	0.122	0.122	0.182	

Table 7: Analysis of molecular variance (AMOVA) of *O. niloticus* populations based on genotypes

Source of variation	**Df**	**SS**	**MS**	**Est. var.**	**%**	**F-Statistics**	**Value**	**p-value**
Among Pops	3	46.834	15.611	0.326	23.00	F_{st}	0.227	0.001
Among Indiv.	83	118.649	1.430	0.321	22.00	F_{is}	0.290	0.001
Within Indiv.	87	68.500	0.787	0.787	55.00	F_{it}	0.451	0.001
Total	**173**	**233.983**		**1.435**				

Est. var.=estimated variance

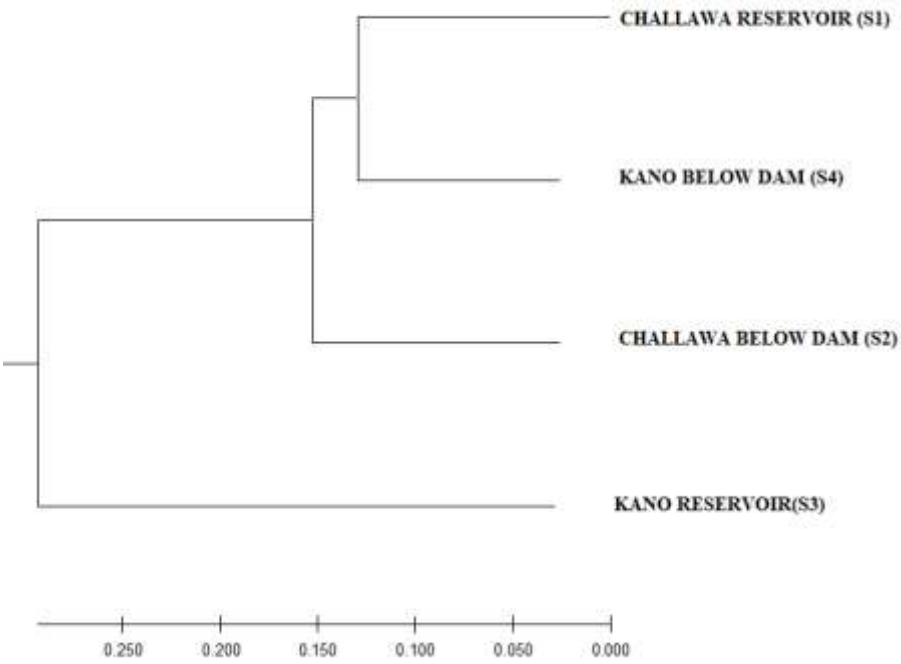

Figure 3: UPGMA genetic dendrogram of the *O. niloticus* populations using Nei genetic distance matrix

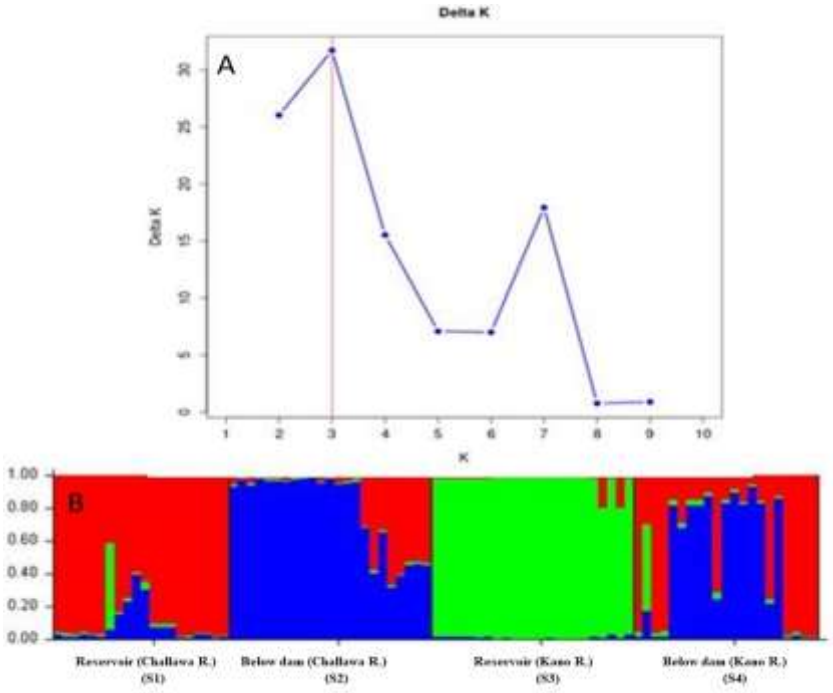

Figure 4: ΔK, K graph of Evanno's method to determine the best K values for the 87 individuals of four *O. niloticus* populations using four microsatellite markers

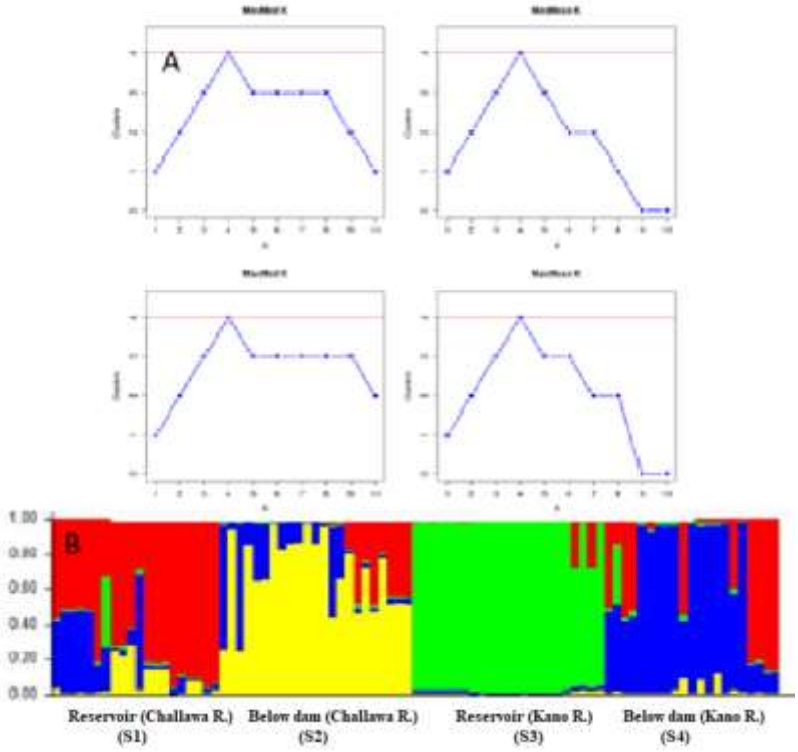

Figure 5: Bayesian Structure Analysis of *O. niloticus* Populations a. Optimal K value determination using the Puechmaille Method (MedMeaK, MaxMeaK, MedMedK, MaxMedK) via the Structure Selector. **b**. Barplots showing the genetic structure of populations with K = 4, where each bar represents an individual and colors indicate the proportion of individuals assigned to each of the four clusters.

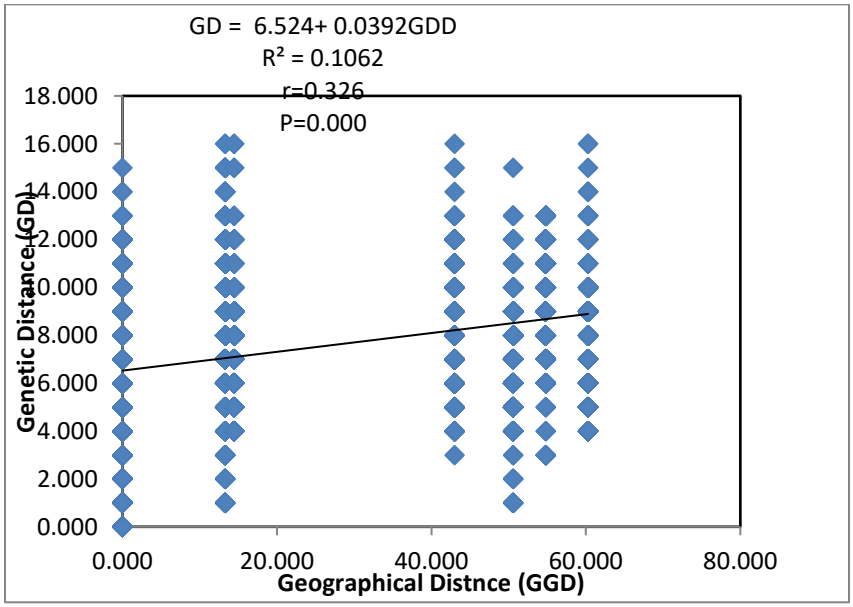

Figure 6: Relationship between the Geographical Distance (GGD) and Genetic Distance (GD) of the *O. niloticus* populations

Recent bottleneck analysis

The test of determining recent decline of effective population size (Ne) using the WILCOXON TEST under the stepwise mutation model (SMM) and the two-phase model of mutation (TPM) provided no evidence of such (Table 8). There was no significant difference between the observed heterozygosity (He) and the expected under the mutation-drift equilibrium (Heq)

Table 8: BOTTLENECK results of the fragmented populations of *O. niloticus*.

River	Location on river	TPM	SMM	Shifted mode
Challawa	Reservoir	0.187500	0.875000	NO
Challawa	Below dam	0.187500	0.875000	NO
Kano	Reservoir	0.125000	0.125000	NO
Kano	Below dam	0.312500	1.000000	NO

Probability of significant($P<0.05$) Heterozygosity excess according to WILCOXON TEST conducted under Two-phased model of mutation (TPM) and Step-wise mutation model (SMM).

DISCUSSION

Effect of the dams on the genetic diversity

Understanding the impact of human-induced habitat fragmentation on species sustainability is crucial for implementing effective management measures **(Fluker *et al*., 2014)**. Genetic diversity approaches are used to detect populations that have lost genetic diversity due to their isolation from larger ecosystems caused by various human activities. Maintaining genetic diversity is therefore essential for species conservation, as a loss or significant reduction in genetic diversity indicates a diminished evolutionary potential **(Changadeya *et al*., 2013)**.

The evidence of the populations' departure from Hardy-Weinberg Equilibrium (HWE) obtained from this study and the lack of linkage disequilibrium (LD) in most pairwise locus tests, could be due to founder effect on the reduced and disconnected populations **(Bbole *et al*., 2020)**. In addition, the positive Wright's fixation index (F) values recorded, suggests that the fragmented populations have experienced increased inbreeding due to resulting mating between closely related individuals **(Nousias *et al*., 2021)**. Other contributory factors could be presence of null alleles s results of non-amplification of some of the microsatellite markers (Rico *et al.*, 2017) and genetic drift resulting from the reduced population size **(Johnson *et al.,* 2018)**. However, the evidence of LD in 12.5% of the pairwise locus comparisons could indicate possibility of certain level of gene flow, in one time or the other, among the fragmented populations **(Cure *et al*., 2017).**

The observed heterozygosity deficiency (Ho < He) in three of the four populations supports the notion of increased inbreeding within the fragmented populations **(Montoya-Lopez *et al*., 2019)**. The lower inbreeding coefficients values (F=0.150) recorded for S1 population against S2 (F=0.215) at the below dam section of Challawa River align with our hypothesis that the impoundments could facilitate rapid population expansion thereby leading to improvements in the genetic diversity of the species in the lakes **(Bbole *et al*., 2020)**. Comparison of the genetic diversity parameters recorded in this study shows that the observed heterozygosity (Ho) recorded (0.294 to 0.525) is lower than that of wild and cultured O. *niloticus* from New Bussa, Niger State (0.6004 &0.6042 respectively). The values are also partly lower than the those from Ikorodu (0.4125&0.5179), Lagos State **(Oyawoye, 2023)**. Additionally, the average effective allele (Ne) in this study (1.856 to 2.277) is lower (2.0878 to 3.4872) than the findings in the referred study **(Oyawoye, 2023)**. The reduced genetic diversity observed in this study may be attributed to the fragmentation caused by the damming, which likely restricted their connectivity and gene flow **(Pavlova *et al*., 2017)**. Additionally, fishing pressure could also be a contributing factor to the low genetic diversity in these populations **(Domingues *et al*., 2018)**.

Effect of the dams on genetic differentiations and population structure

Mantel's test indicated that the populations are not significantly differentiated based on the isolation-by-distance (ISD) model. The AMOVA analysis confirmed high genetic differentiation among the populations (23.0%) but registered greatest genetic variation between individuals (55.0%). This suggests some degree of random mating, likely due to the relatively large size of the populations within the impounded parts of the rivers **(Pertiwi *et al*., 2017)**.

Genetic differentiation (F_{ST}) between the fragmented populations was moderately high (F_{ST} = 0.129) for the Challawa River populations (S1 and S2) and highly differentiated (F_{ST} = 0.182) in the Kano River populations. Differentiations index greater than 15% are considered to be high, implying low gene flow between these populations **(Changadeya *et al*., 2013)**. The overall substantial gene flow of more than one migrant per generation among the *O. niloticus* populations may be attributed to their connection through a shared river basin among some of the populations **(Lind *et al*., 2019).**

Interestingly, below dam population (S4) on Kano River indicated lower genetic differentiation with populations outside its river basin (S1 and S2). The observed level of similarities between population S4 and the other two could be the possible reason the Evanno method **(Evanno *et al*., 2005)** suggested 3 clusters (Figure 4) for the populations. However, the Puechmaille method, takes the uneven nature of the data into consideration in determining the appropriate K value **(Puechmaille, 2016)**. The close genetic similarity between S4 and S2, despite their geographical distance, could be due to shared ancestral population with common alleles. This finding aligns with other studies where genetic similarity was observed between populations of *O. niloticus* separated by over 1400 km but connected by a common river **(Lind *et al*., 2019)**. Considering that the two rivers converge at Tamburawa (Lat.11.8695º Long.8.5318º), which is approximately 57.7km from S2 and 52.8km from S4, historical connectivity between these populations is likely to have existed.

It is important to note that the use of only four polymorphic microsatellite markers for this genetic study might be a limitation reflecting in its outcomes. While previous studies have successfully characterized genetic diversity with a similar number of loci **(Adelieje *et al*., 2020;**

Thai *et al*., 2007; Vajed Ebrahimi *et al.*, 2017; Yamamoto *et al.*, 2004), some researchers, such as **Mittell *et al* (2015)**, recommended a higher number of loci (e.g., ten) for more robust genetic assessments. The limited number of markers used in this study was due to resource constraints, which might have affected the resolution of genetic diversity and population structure, potentially underestimating finer-scale genetic variations. However, by focusing on highly polymorphic loci, we aimed to capture meaningful genetic patterns within the *O. niloticus* populations despite this limitation.

Recent bottleneck of *O. niloticus*

Analyzing genetic bottlenecks is crucial for effective conservation planning. A population that has recently experienced a bottleneck has undergone a severe reduction in its effective population size. This reduction can lead to various genetic issues that may increase the risk of extinction **(Bbole *et al*., 2020)**. Following a recent bottleneck, the number of alleles in a population typically decreases more rapidly than heterozygosity at polymorphic loci (He). As a result, the observed heterozygosity (He) will often be higher than the expected heterozygosity under mutation-drift equilibrium (Heq) (Piry *et al*., 1999). However, increased heterozygosity and deviations from Hardy-Weinberg equilibrium (HWE) could be attributed to factors such as null alleles, the Wahlund effect, selection against heterozygotes, population admixture, or a combination of these factors **(Coleman *et al*., 2018)**.

The bottleneck analysis in this study found no evidence of recent bottlenecks in the four populations. Although, heterozygosity deficits (Ho < He) and significant linkage disequilibrium (LD) in most of the pairwise locus comparisons (87.5%) support lack of evidence of a recent reduction in effective population size. However, the results of the bottleneck analysis require further review. **Piry *et al*. (1999)** indicated that the Bottleneck program requires a minimum of four polymorphic loci and 20-30 individuals per sample to detect recent reductions in effective population size. However, the effectiveness of bottleneck simulations using microsatellite markers can be limited with less than 9 markers and sample sizes less than 31 individuals **(Changadeya *et al*., 2013)** .

CONCLUSION

This study highlighted the significant impact of the constructed dams on the Challawa and Kano Rivers by restricting gene flow among the fragmented *O. niloticus* populations and thus affecting their genetic diversity. It was observed that most of the populations exhibited heterozygosity deficits suggesting accumulation of inbreeding within the populations. The study also found evidence of substantial gene flow between some of the fragmented populations that have possible connectivity due to shared river basin. However, the moderate to high level of genetic differentiation among the populations could be the main factor behind the observed reduced genetic variability. Although the use of only four polymorphic microsatellite markers was a limitation due to resource constraints, the findings provide valuable baseline data for fisheries management. There is need to set up ongoing genetic monitoring program to promptly identify and address any future reductions in genetic diversity.

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Conflict of interests
The authors declared no conflict of interest.

References

Abdul-Azeez, H., Jere, W.W., Abdussamad, A.M. & Kassam, D. (2024). Fish Biodiversity in Modified Ecosystems: A Case Study of Nigeria's Challawa and Tiga Dams in the Challawa and Kano Rivers. *African Journal of Food, Agriculture, Nutrition and Development.*, *24*(5), 26333–26350.

Abdulazeez, A. (2018). A Spatial Assessment and Mapping of Groundwater Potentials in Parts of North-Western Kano State, Nigeria. *Techno Science Africana Journal.*, *14*(1), 102–117.

Adelieje, C., Uyoh, E. A., Ntui, V. O. & Ama-Abasi, D. (2020). Morphological and Genetic Diversity Studies of Chrysichthys nigrodigitatus from the Cross River, Nigeria, Using Microsatellite Markers. *Annual Research & Review in Biology*, *35*(5), 42–52. https://doi.org/10.9734/arrb/2020/v35i530224

Akindele, E. O. & Indabawa, I. I. (2015). A review of the effects of dams on the hydrology , water quality and invertebrate fauna of some Nigerian freshwaters. *The Zoologist*, *13*, 29–36.

Bandara, K. V. S. N., & Amarasinghe, U. S. (2017). Factors Related to Nesting Sites of Oreochromis niloticus (Linnaeus 1758; cichlidae) in Irrigation Reservoirs, Sri Lanka. *Asian Fisheries Science*, *30*(4), 319–335. https://doi.org/10.33997/j.afs.2017.30.4.008

Bbole, I., Zhao, J. L., Tang, S.-J. & Katongo, C. (2020). Genetic Diversity of Two Southern African Cichlids (Oreochromis andersonii and Oreochromis macrochir) in the Zambezi and Congo River Basins. *Journal of Applied Ichthyology*, *36*(1), 62–74. https://doi.org/10.1111/jai.13993

Champneys, T., Genner, M. J. & Ioannou, C. C. (2021). Invasive Nile Tilapia Dominates a Threatened Indigenous Tilapia in Competition over Shelter. *Hydrobiologia*, *848*(16), 3747–3762. https://doi.org/10.1007/s10750-020-04341-8

Changadeya, W., Stima, H., Kassam, D., Kaunda, E. & Jere, W. (2013). Genetic Diversity and Structure of Potamodromous Opsaridium microlepis (Gnther) Populations in the in-let Rivers of Lake Malawi. *African Journal of Biotechnology*, *12*(48), 6709–6717. https://doi.org/10.5897/ajb2013.12459

Cheng, F., Li, W., Castello, L., Murphy, B. R. & Xie, S. (2015). Potential Effects of Dam Cascade on Fish : lessons from the Yangtze River. *Reviews in Fish Biology and Fisheries*, *25*, 569–585. https://doi.org/10.1007/s11160-015-9395-9

Coleman, R. A., Gauffre, B., Pavlova, A., Beheregaray, L.B., Kearns, J., Lyon, J., Sasaki, M., Leblois, R., Sgro, C. & Sunnucks, P. (2018). Artificial Barriers Prevent Genetic Recovery

of Small Isolated Populations of a Low-mobility Freshwater Fish. *Heredity*, *120*, 515–532. https://doi.org/10.1038/s41437-017-0008-3

Cure, K., Thomas, L., Hobbs, J. P. A., Fairclough, D. V. & Kennington, W. J. (2017). Genomic Signatures of Local Adaptation Reveal Source-sink Dynamics in a High Gene Flow Fish Species. *Scientific Reports*, *7*(1), 1–10. https://doi.org/10.1038/s41598-017-09224-y

Domingues, R. R., Hilsdorf, A. W. S. & Gadig, O. B. F. (2018). The Importance of Considering Genetic Diversity in Shark and Ray Conservation Policies. *Conservation Genetics*, *19*(3), 501–525. https://doi.org/10.1007/s10592-017-1038-3

Evanno, G, Regnaut, S. & Goudet, J. (2005). Detecting the Number of Clusters of Individuals using the Software Structure: A simulation study. *Mol Ecol*, *14*(8), 2611-2620.

FAO. (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals.*

Fluker, B. L., Kuhajda, B. R. & Harris, P. M. (2014). The Effects of Riverine Impoundment on Genetic Structure and Gene Flow in Two Stream Fishes in the Mobile River Basin. *Freshwater Biology*, *59*(3), 526–543. https://doi.org/10.1111/fwb.12283

Gana, B. A., Harir, A. I., Bogoro, A. G. & Oladosu, R. O. (2018). Stream Ordering As a Tool For Effective River Basin Development : Examples From Komadugu – Yobe River Basin ,. *Journal of Resources Development and Management*, *44*, 67–78.

Johnson, M. T. J., Prashad, C. M., Lavoignat, M. & Saini, H. S. (2018). Contrasting the effects of natural selection, genetic drift and gene flow on urban evolution in white clover (Trifolium repens). *Proceedings of the Royal Society B: Biological Sciences*, *285*(1883), 1–10. https://doi.org/10.1098/rspb.2018.1019

Kassam, D., Changadeya, W., Stima, H., Jere, W. & Kaunda, E. (2017). Morphological and Genetic Variability among Mpasa (Opsaridium microlepis Gnther, 1864) Populations from the Inflow Rivers of Lake Malawi. *International Journal of Fisheries and Aquaculture*, *9*(5), 52–58. https://doi.org/10.5897/ijfa2017.0626

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution*, *35*, 1547–1549.

Lee, B-Y., Lee, W-J., Streelman, J, T., Carleton, K, L., Howe, A, E., Hulata, G., Slettan, A., Stern, J, E., Terai, Y. & Kocher, T, D. (2005). A Second-generation Genetic Linkage Map of Tilapia (Oreochromis spp.). *Genetics*, *170*(1), 237-244.

Li, Y.L. & Liu, J. X. (2018). StructureSelector: A Web Based Software to Select and Visualize the Optimal Number of Clusters using Multiple Methods. *Molecular Ecology Resources, 8*, 176–177.

Lima, A. C., Sayanda, D., Agostinho, C. S., Machado, A. L., Soares, A. M. V. M. & Monaghan, K. A. (2018). Using a Trait-based Approach to Measure the Impact of Dam Closure in Fish Communities of a Neotropical River. *Ecology of Freshwater Fish*, *27*(1), 408–420.

https://doi.org/10.1111/eff.12356

Lind, C. E., Agyakwah, S. K., Attipoe, F. Y., Nugent, C., Crooijmans, R. P.M.A. & Toguyeni, A. (2019). Genetic diversity of Nile tilapia (*Oreochromis niloticus*) throughout West Africa. *Scientific Reports*, *9*(1), 1–12. https://doi.org/10.1038/s41598-019-53295-y

Liu, D., Li, X. & Song, Z. (2020). No decline of genetic diversity in elongate loach (Leptobotia elongata) with a tendency to form population structure in the upper Yangtze River. *Global Ecology and Conservation*, *23*, e01072. https://doi.org/10.1016/j.gecco.2020.e01072

Livak, K. J. (1984). Organization and Mapping of a Sequence on the Drosophila melanogaster X and Y Chromosomes that is Transcribed during Spermatogenesis. *Genetics*, *107*(August), 611–634.

Mabrouk, M. M., Mansour, A. T., Abdelhamid, A. F., Abualnaja, K. M., Mamoon, A., Gado, W. S., Matter, A. F. & Ayoub, H. F. (2021). Impact of Aqueous Exposure to Silver Nanoparticles on Growth Performance, Redox Status, Non-specific Immunity, and Histopathological Changes of Nile Tilapia, *Oreochromis niloticus*, Challenged with Aeromonas hydrophila. *Aquaculture Reports*, *21*, 1–11. https://doi.org/10.1016/j.aqrep.2021.100816

Marques, H., Henrique, J. P. D., Perbiche-neves, G., Antoniassi, E. K., L. & Paiva, I., R. (2018). Importance of Dam-free Tributaries for Conserving Fish Biodiversity in Neotropical Reservoirs. *Biological Conservation*, *224*(March), 347–354. https://doi.org/10.1016/j.biocon.2018.05.027

Mittell, E.A., Nakagawa, S. & Hadfield, J. D. (2015). Are Molecular Markers Useful Predictors of Adaptive Potential? *Ecol Lett*, *18*, 772–778.

Mohammed, A., Muhammad, M. . M., Adeogun, B. . K., Abdullahi, S. . A.& Idris, U. . D. (2020). Application of the Weap Model for Future Water Allocation from Tiga Dam. *Nigerian Journal of Engineering*, *27*(2), 2–11. https://www.researchgate.net/publication/352523698

Montoya-Lopez, A.F., Tarazona-Morales, A.M., Olivera-angel, M. & Betancur-lópez, J. J. (2019). Genetic Diversity of Four Broodstocks of Tilapia (Oreochromis sp .) from Antioquia , Colombia. *Rev. Colomb Cienc Pecu.*, *32*(3), 201–213. https://doi.org/https://doi.org/10.17533/udea.rccp.v32n3a05

Muhtadi, A., Nur, M., Latuconsina, H. & Hidayat, T. (2022). Population Dynamics and Feeding Habit of Oreochromis niloticus and O. mossambicus in Siombak Tropical Coastal Lake, North Sumatra, Indonesia. *Biodiversitas*, *23*(1), 151–160. https://doi.org/10.13057/biodiv/d230119

Nousias, O., Tzokas, K., Papaharisis, L., Ekonomaki, K., Chatziplis, D., Batargias, C. & Tsigenopoulos, C. S. (2021). Genetic Variability, Population Structure, and Relatedness Analysis of Meagre Stocks as an Informative Basis for New Breeding Schemes. *Fishes*, *6*(4). https://doi.org/10.3390/fishes6040078

Oyawoye, T. O. (2023). Genetic Variation Studies of Four Selected Populations of *Oreochromis niloticus* in Nigeria. *International Journal of Scientific and Research Publication*, *13*(5), 75–82. https://doi.org/10.29322/IJSRP.13.04.2023.p13710

Pavlova, A., Beheregaray, L. B., Coleman, R., Gilligan, D., Harrisson, K. A., Ingram, B. A., Kearns, J., Lamb, A. M., Lintermans, M., Lyon, J., Nguyen, T. T. T., Sasaki, M., Tonkin, Z., Yen, J. D. L. & Sunnucks, P. (2017). Severe Consequences of Habitat Fragmentation on Genetic Diversity of an Endangered Australian Freshwater Fish: A call for assisted gene flow. *Evolutionary Applications*, *10*(6), 531–550. https://doi.org/10.1111/eva.12484

Peakall, R. & Smouse, P. E. (2012). GenAlEx 6.5: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research – an update. *Bioinformatics*, *28*, 2537-2539.

Pertiwi, N. P. D., Nugraha, B., Sulistyaningsih, R. K., Jatmiko, I., Sembiring, A., Mahardini, A., Cahyani, N. K. D., Anggoro, A. W., Madduppa, H. H., Ambariyanto, A., Barber, P. H. & Mahardika, G. N. (2017). Short Communication: Lack of differentiation within the bigeye tuna population of Indonesia. *Biodiversitas*, *18*(4), 1406–1413. https://doi.org/10.13057/biodiv/d180416

Piry, S., Luikart, G.& Cornuet, J. M. (1999). BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. *Journal of Heredity*, *90*(4), 502–503. https://doi.org/10.1093/jhered/90.4.502

Pritchard, J.K., Stephens, M. & Donnelly, P. . (2000). Inference of Population Structure using Multilocus Genotype Data. *Genetics*, *155*, 945–959.

Puechmaille, S. (2016). The Program Structure Does not Reliably Recover the Correct Population Structure When Sampling is Uneven: subsampling and new estimators alleviate the problem. *Molecular Ecology Resources*, *16*, 608–627.

Raymond, M. & Rousset, F. (1995). GENEPOP: a population genetics software for exact test sand ecumenicism. *J.Hered.*, *86*, 248–249.

Rico, C., Cuesta, J. A., Drake, P., Macpherson, E., Bernatchez, L. & Marie, A. D. (2017). Null alleles are ubiquitous at microsatellite loci in the Wedge Clam (Donax trunculus). *PeerJ*, *2017*(4), 1–21. https://doi.org/10.7717/peerj.3188

Tecle, A. (2017). Downstream Effects of Damming the Colorado River. *International Journal of Lakes and Rivers.*, *10*(1), 7–33.

Thai, B. T., Burridge, C. P. & Austin, C. M. (2007). Genetic Diversity of Common Carp (Cyprinus carpio L .) in Vietnam using Four microsatellite loci. *Aquaculture*, *269*, 174–186. https://doi.org/10.1016/j.aquaculture.2007.05.017

Vajed Ebrahimi, M. T., Mohammadreza, M. & Ali, E. (2017). Using Microsatellite Markers to Analyze Genetic Diversity in 14 Sheep Types in Iran. *Arch. Anim. Breed*, *60*, 183–189.

Winemiller, K. O., McIntyre, P. B., Castello, L., Fluet-Chouinard, E., Giarrizzo, T., Nam, S., ... & Sáenz, L. (2016). 128-129. (2016). Balancing Hydropower and Biodiversity in the Amazon, Congo, and Mekong. *Science*, *351*, 128–129.

Yamamoto, S., Morita, K. & Sahashi, G. (2019). Spatial and Temporal Changes in Genetic Structure and Diversity of Isolated White-spotted Charr (Salvelinus leucomaenis) Populations. *Hydrobiologia*, *840*, 35–48. https://doi.org/10.1007/s10750-019-3924-9

Yamamoto, S., Morita, K., Koizumi, I. & Maekawa, K. (2004). Genetic Differentiation of White-spotted Charr (Salvelinus leucomaenis) Populations after Habitat Fragmentation : Spatial – temporal changes in gene frequencies. *Conservation Genetics*, *5*, 529–538.