Aquaculture 535 (2021) 736432

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

Genetic structure of wild and farmed Nile tilapia (*Oreochromis niloticus*) populations in Benin based on genome wide SNP technology

Mohammed Nambyl A. Fagbémi [a,b,*], Lise-Marie Pigneur [c], Adrien André [c], Nathalie Smitz [d], Vincent Gennotte [a], Johan R. Michaux [c], Charles Mélard [a], Philippe A. Lalèyè [b], Carole Rougeot [a]

[a] Aquaculture Research and Education Center (CEFRA), Liège University, 10 Chemin de la Justice, B-4500 Tihange, Belgium
[b] Laboratory of Hydrobiology and Aquaculture (LHA), Faculty of Agricultural Sciences, University of Abomey-Calavi, 01 BP: 526 Cotonou, Benin
[c] Conservation Genetics Laboratory, Liège University, 4000 Liège, Belgium
[d] Royal Museum for Central Africa, Leuvensesteenweg 13-17, 3080 Tervuren, Belgium

ARTICLE INFO

Keywords:
Population genetic structure
Oreochromis niloticus
SNP
Benin

ABSTRACT

For the last three decades, Nile tilapia *Oreochromis niloticus* (Linnaeus, 1758) is considered as one of the most productive and internationally traded food fish. Although economically important, our knowledge on the genetic structure of natural and farmed populations is scarce, especially in Africa. Sustainable aquaculture however requires integrating genetic information to elaborate appropriate management practices. The aims of the present study were: (i) to characterize the genetic structure and diversity of several *O. niloticus* wild populations collected in four drainage basins: Mono, Niger, Ouémé and Volta in Benin; (ii) to compare the identified genetic profiles of these wild populations with domesticated strains bred in two Beninese fish farms and one Belgian aquaculture research center; (iii) and finally to use the data as a tool to improve management of wild genetic resources and domesticated farmed strains.

In order to characterize the genetic structure of the thirteen sampled populations of *O. niloticus* 2.950 SNPs were used to perform a clustering analysis and investigate the genetic diversities and population differentiations. Our results showed that, populations of *O. niloticus* collected in different basin and farms in Benin showed low to moderate genetic differentiation (F_{st} from 0.018 to 0.143) with the exception of the Nangbéto population of Mono basin, which is genetically more differentiated (F_{st} from 0.091 to 0.278). Compared to wild populations, there is a greater genetic proximity between the breeding populations of CRIAB and the Pendjari river population (F_{st} = 0.0 47), and between the Yohan-Esteve farm populations and the Gbassa population (F_{st} from 0.045 to 0.055). In view of the low level of inbreeding and the good growth and reproductive performance of the Togbadji population in the Mono basin, it would be a potential candidate for the development of a local strain of *O. niloticus* for aquaculture in Benin.

1. Introduction

Tilapias are fish of the Cichlidae family, including more than 3.000 species (Kocher 2004). Cichlids are widely distributed through Central and South America, Africa, Madagascar and southern India (Chakrabarty, 2004), living in coastal, brackish and freshwater habitats (Mcandrew and Beveridge, 2000). Some cichlid species like *O. niloticus* and *O. mossambicus* are farmed for food production (Babiker and Ibrahim, 1979; Arthington, 1986; Lowe-mcconnell, 2010). Over the last three decades, Nile tilapia *O. niloticus* farming has significantly grown worldwide. It is considered as one of the most productive and internationally traded food fish (Mires, 1982; Frei et al., 2007; El-Sayed and Kawanna, 2008; Ng and Wang, 2011; Hernández et al., 2013). Tilapia is the fourth most farmed aquatic species in the world, after the grass carp *Ctenopharyngodon idella*, the silver carp *Hypophthalmichthys molitrix* and the common carp *Cyprinus carpio*, with a production of 4.2 million tons in 2016, namely 8% of the total farmed aquaculture species (FAO, 2019).

Despite the importance of Nile tilapia as a protein source, our knowledge on the genetic structure of natural and farmed populations is scarce, especially in Africa (Agnése et al., 1999). Sustainable aquaculture requires integrating genetic information on the bred livestock to

* Corresponding author at: Aquaculture Research and Education Center (CEFRA), Liège University, 10 Chemin de la Justice, B-4500 Tihange, Belgium.
E-mail address: mohammedf21@hotmail.fr (M.N.A. Fagbémi).

https://doi.org/10.1016/j.aquaculture.2021.736432
Received 23 May 2020; Received in revised form 12 January 2021; Accepted 21 January 2021
Available online 26 January 2021

elaborate appropriate management practices. Knowledge of the population genetic structure of the Nile tilapia is economically important for several issues relevant for future development of aquaculture strains and management of fisheries (Hassanien et al., 2005). Identification of wild population genetic structure would provide biologically meaningful geographic boundaries for assessing a number of parameters, including genetic diversity. Investigating the genetic diversity of a species is for example important to detect specific genetic traits, which might promote distinct differences in life-history traits such as growth rate, fecundity, abundance and disease resistance (Stepien, 1995). It is therefore important to understand the broad genetic population groupings, especially when investigating domestication and production potential of wild strains for aquaculture.

In Benin, *O. niloticus* is the most farmed fish species (Rurangwa et al., 2014). Although in West Africa, its natural distribution area covers the Senegal, Nil, Gambia, Volta, Niger, Benue and Chad basins (Daget and Iltis, 1965; Lévêque et al., 1990; Ahouansou-Montcho et al., 2015), *O. niloticus* was introduced in southern Benin, in the Ouémé (Laleye et al., 2004) and in the Mono basins (Ahouansou-Montcho, 2003). The first transfer into the coastal region occurred in 1979 at the Aquaculture station of Godomey with fishes originated from the Tropical Forestry Technical Center (CTFT-Ivory-Cost). In 1992, *O. niloticus* was introduced at the Regional Agricultural Center for Rural Development from the Tropical Forestry Technical Center (CTFT-Burkina-Faso). Following the floods of lake Toho in 1995 (Ahouansou-Montcho, 2003), some fishes escaped from the ponds and entered into the lake, resulting in the introduction of the cultured strain in this tributary lake of the Mono basin (Ahouansou Montcho, 2003, 2011; Lederoun et al., 2018). The species colonized all freshwater areas of southern Benin. With the recent liberalization and development of the aquaculture sector in Benin, other strains were potentially introduced and their origins are not controlled (Ghanaian, Nigerian, etc.). Except in a few cases (e.g. Aquaculture Research and Incubation Center of Benin, CRIAB), the strains farmed in Benin often have an unknown origin. Some are suspected to come from small producers with restricted broodstock of unknown and potentially inbred lines.

To further investigate the genetic diversity of this economically important species, various genetic markers have been used in the past for the identification of Nile tilapia stocks, including: allozymes (eg: Pouyaud and Agnèse, 1995; Rognon et al., 1996), mitochondrial DNA restriction fragment length polymorphisms (mtDNA-RFLPs) (eg: Agnèse et al., 1997; Rognon and Guyomard, 1997), random amplification of polymorphic DNA (RAPD) (eg: Dinesh et al., 1996; Hassanien et al., 2004) and microsatellites which latter are versatile genetic markers frequently used to address species ecology, evolution and conservation related issues (Wirgin and Waldman, 1994; O'Reilly and Wright, 1995; Jarne and Lagoda, 1996; O'connell and Wright, 1997). For example, in 1996, Dinesh et al. used random amplified polymorphic DNA (RAPD) fingerprinting to estimate the genetic variation and species differentiation of three species of tilapia in Singapore (Nile tilapia, Mozambique tilapia and blue tilapia). This study reported a high genetic similarity within each tilapia species and a total of 13 RAPD markers differentiating the three species of tilapia were detected. Using the same method, Hassanien et al. (2004) assessed the genetic diversity of Nile tilapia collected from the Nile river (Cairo, Assuit and Qena) and two Delta lakes (Burullus and Manzalla) in Egypt. The molecular phylogenetic tree supported Manzalla and Burullus populations as being strongly linked, but genetically distant from the Assuit and Cairo populations, with Qena population as outgroup. Results on electrophoretic polymorphism at 30 protein loci in 27 wild and farmed populations of two tilapia species, *Tilapia zilii* and *O. niloticus* were also reported by Rognon et al. (1996). Single and joint segregations were analyzed at 12 polymorphic loci in full-sib families. A pronounced differentiation was observed between the Ivory Coast and Nilo-Sudanian *T. zilii* populations. Within the Nilo-Sudanian region, the level of variation observed in *O. niloticus* and *T. zilii* was rather low compared to other freshwater species. The same pattern of geographical differentiation was found between the West and East African populations in both species. In contrast to fish-farmed stocks analyzed in other countries, those from the Ivory Coast and Niger displayed neither evidence of loss of genetic diversity nor any trace of introgression with other cultured tilapia species, indicating that these stocks have been properly managed (Rognon, 1996). In 1997, Rognon and Guyomard studied the variation of the nad5–6 mtDNA fragment in six Nile tilapia populations using PCR and RFLP analysis. The observed variation allowed a strict discrimination between eastern and western African populations. Agnèse et al., (1997) analyzed the genetic differentiation among 17 wild populations of Nile tilapia distributed in West African, the Ethiopian and Kenyan rift valley and the Nile drainage using allozymes and mtDNA-RFLPs. Although these studies yielded interesting results, they were often based on limited sampling as well as on markers that were not polymorphic enough to give consistent results. It was therefore essential to extend these studies using more sensitive genetic markers.

Recently, new technologies (Next Generation Sequencing (NGS)) allow for the use of genome-wide genetic data, including the identification of Single Nucleotide Polymorphisms (SNPs) (Abdelrahman et al., 2017; Kumar and Kocour, 2017). These are helpful genetic markers that are currently proven to be successful for population genetic investigations. A SNP consists of a substitution of a single nucleotide that occurs at a specific position in the genome (for reviews, see Brumfield et al., 2003; Morin et al., 2004; Wayne and Morin, 2004). SNPs allow genome wide scans of selectively neutral or adaptive variation (Luikart et al., 2003; Wayne and Morin, 2004), with simple mutation models, powerful analytical methods (Ryman et al., 2006), and application to non-invasive and historical DNA (Morin and Mccarthy, 2007). The discovery of SNP markers in commercial species was facilitated by the availability of high quality reference genomes, as it is the case for *O. niloticus* (Lee et al., 2010; Guyon et al., 2012; Yáñez et al., 2019), Atlantic salmon (*Salmo salar*) (Lien et al., 2016) and rainbow trout (*Oncorhynchus mykiss*) (Berthelot et al., 2014). This information supported the development of dense SNP panels, which were already developed for the Atlantic salmon (Houston et al., 2014; Yáñez et al., 2016), the rainbow trout (Palti et al., 2015) and the channel catfish (*Ictalurus punctatus*) (Liu et al., 2014; Zeng et al., 2017). In *O. niloticus,* SNP genotyping was used in different approaches. For example, it was showed that the EPC1 gene may be a candidate gene related to osmoregulation in tilapia and could contribute to selection of a salt tolerant line by using marker-assisted selection technique (Gu et al., 2018). SNP genotyping was also used to highlight the conserved roles of TGF-β signaling pathway in fish sex determination (Li et al., 2015) and allowed, based on the number of independent chromosome segments, to show that at least 4.2 K SNPs might be required to implement genome-wide association studies (GWAS) and genomic prediction in the current Nile tilapia populations (Yoshida et al., 2019). The present study is one of the few studies (Lind et al., 2019; Yáñez et al., 2019) to use SNP genotypes for the identification of a genetic structure within several *O. niloticus* wild populations.

The aims of the present study were: (i) to characterize the genetic structure and diversity of several *O. niloticus* wild populations collected in four drainage basins: Mono, Niger, Ouémé and Volta in Benin; (ii) to compare the identified genetic profiles of these wild populations with domesticated strains bred in two Beninese fish farms and one Belgian aquaculture research center (Aquaculture Research and Education Center-University of Liège); (iii) and finally use the data as a tool to improve the management of wild genetic resource and domesticated farmed strains at least in Benin.

2. Material and methods

2.1. Sample collection

Fish were collected in eight sampling stations in Benin and one in

Togo, located close to the country border with Benin (Fig. 1): Gobé (GO) and Bétérou (BT) in the Ouémé basin; Nangbéto (NGT), Sohoumé (SH), Togbadji (TG), Toho (TH) and Djonougoui (DJ) in the Mono basin; Gbassa (GB) in the Niger basin; and Pendjari (PJ) in the Volta basin. In addition, domesticated fishes from two Beninese fish farms and one Belgian research center were sampled: CRIAB (CB) and Yohan-Esteve where fish farmers reported to rear two populations from distinct origins (YE1 and YE2), and CEFRA (CFT) (Table 1). The CEFRA strain originally came from the Stirling University and is of Egyptian origin and specifically from lake Manzala (Cefra archives). It was included in this study in order to compare it with the local wild populations of Benin.

A small piece of the pectoral fin was collected from anesthetized specimens. Seven to 30 individuals were sampled per station. The fin samples were stored in 1.5 ml Eppendorf tubes containing 90% ethanol and stored at 4 °C for further processing.

2.2. DNA isolation

Total genomic DNA (gDNA) was extracted using the DNeasy Blood and Tissue kit (Qiagen) according to manufacturer's protocol. The DNA was then quantified using the Quant-iT PicoGreen dsDNA Assay kit (Invitrogen). Afterwards, 120 ng of genomic gDNA was loaded on a 2% agarose gel.

Samples reaching a sufficient concentration (at least 10 ng/μl) and quality (intact high molecular weight DNA, no sign of fragmentation) were selected for the GBS (genotyping-by-sequencing) library preparation. In total, 171 samples were selected and used for the GBS library preparation (Table 1).

2.3. GBS library

GBS data was generated following Elshire et al. (2011) method with the following protocol changes: 100 ng of gDNA and 1.44 ng of total adapters were used for the library preparation; gDNA samples were restricted with EcoT22i enzyme and the library was amplified with 18 PCR cycles.

2.4. Data analysis

2.4.1. SNP identification

SNPs were identified from raw sequences using Tassel 5 GBS v2 Pipeline (https://bitbucket.org/tasseladmin/tassel-5-source/wiki/Tassel5GBSv2Pipeline). First, raw reads were demultiplexed using the axe-demux tool. Demutiplexed reads were then converted to tag counts using the GBSSeqToTagDB plugin. TagExportToFastq Plugin was used to pull the tags and export them in fastq format. Bowtie 2 (Langmead and Salzberg, 2012) was then used to align tags to the *O. niloticus* reference genome (https://www.ncbi.nlm.nih.gov/assembly/GCF_001858045.2). The resulting SAM file was run through SAMToGBSdbPlugin to store the position information for each aligned tag. DiscoverySNPCaller PluginV2 was then used to identify SNPs from aligned tags using the GBS DB. Finally, ProductionSNPCaller PluginV2 was used to convert data from fastq and keyfile to genotypes, and to add these to a genotype file in VCF format.

2.4.2. SNP filtering

The SNP table was filtered using Tassel 5 software (Glaubitz et al., 2014). First, individuals characterized by a heterozygosity below 1% were filtered out. Then, SNPs presenting a minor allele frequency under 16% and a heterozygous proportion under 20% were also filtered out (Yáñez et al., 2019).

2.4.3. Clustering analysis

Bayesian clustering of SNP genotypes were performed using Structure v2.3.4 (Pritchard et al., 2000). Due to large computation time, burn-in period was set at 50.000 and the number of MCMC chains at 500.000. The number of clusters (K) tested for sample grouping ranged from 1 to 10, with five iterations for each K. Individuals were pooled together independently to their spatial origin. The results and visual output of the five iterations for each K value were summarized using CLUMPAK (Kopelman et al., 2015). The optimal number of clusters was assessed based on the method defined by Evanno et al. (2005). The highest probability for each sample to belong to each cluster was used to determine its affiliation for the subsequent analyses.

2.4.4. Genetic diversity and population differentiation

Summary statistics were estimated for both populations (based on the geographic origin of the specimens) and clusters (based on the Structure assignment results). Expected (H_E) and observed (H_O) heterozygosities, as well as inbreeding coefficients (F_{IS}) were estimated using Genetix v4.05 software (Belkhir et al., 2004) with 1.000 permutations for significance. An exact test of population differentiation of pairwise weighted mean F_{ST} was performed using Arlequin v3.5 (Excoffier and Lischer, 2010) (10.000 permutations for significance, with an allowed missing data level of 0.05). F_{ST} heatmaps were reconstructed with RStudio v3.3.1 (Team, R. Core, 2014) using the heatmap.plus package v2.18.0 (https://github.com/alexploner/Heatplus).

3. Results

3.1. SNP identification

The SNP identification step resulted in a matrix of 171 individuals and 29.302 SNP genotypes. After the filtering steps, 2.950 SNPs were retained and used as input for further analyses.

3.2. Clustering analyses

Using Structure v2.3.4, the best K value was 2 after Evanno's correction (Evanno et al., 2005), with indications of a substructure for K = 4 (Supplemental data).

For K = 2 (Fig. 2a), the first cluster (K1) included all (but one) specimens from the CEFRA research station (Belgium), while most other samples were predominantly assigned to the second cluster (K2). However, several individuals showed an admixed pattern ($q < 0.9$, $N = 91/171$), especially in the fish farm YE1 and YE2, in the Niger and Mono basins.

For K = 4 (Fig. 2b), the most differentiated cluster included the specimens from CFT, followed by a second cluster only including the NGT samples. The specimens from PJ and CB have a shared genetic signature, and were grouped together in one cluster. The last cluster mostly included specimens collected at the GB site, as well as at the fish farm Yohan Esteve (YE1 and YE2). The genetic signature of this last cluster was also observed in other areas (i.e. sites SH, TG and DJ of the Mono Basin; sites GO and BT from Ouémé basin), mixed with other cluster assignments.

3.3. Population differentiation and genetic diversity

Considering a genetic structure based on two clusters (K = 2), the genetic differentiation between K1 (mainly representing fishes from the CEFRA in Belgium) and K2 was relatively low ($F_{st} = 0.096$) (Fig. 2a.). Similarly, based on a hypothesized structure with K = 4 (Table 2 and Fig. 3A.), genetic differentiation between clusters was low, except between the cluster 1 (K1 in orange), and the other ones (F_{st} values between 0.126 and 0.251). The assessment of the genetic differentiation between the different collection sites revealed low to moderate F_{st} values (from 0.018 to 0.143) among sites, at the exception of the Belgian research facility (CFT, F_{st} from to 0.135 to 0.278) and the NGT (F_{st} from 0.091 to 0.278) (Table 3 and Fig. 3B.) locality situated in the Mono basin. Both areas displayed a higher level of differentiation compared to

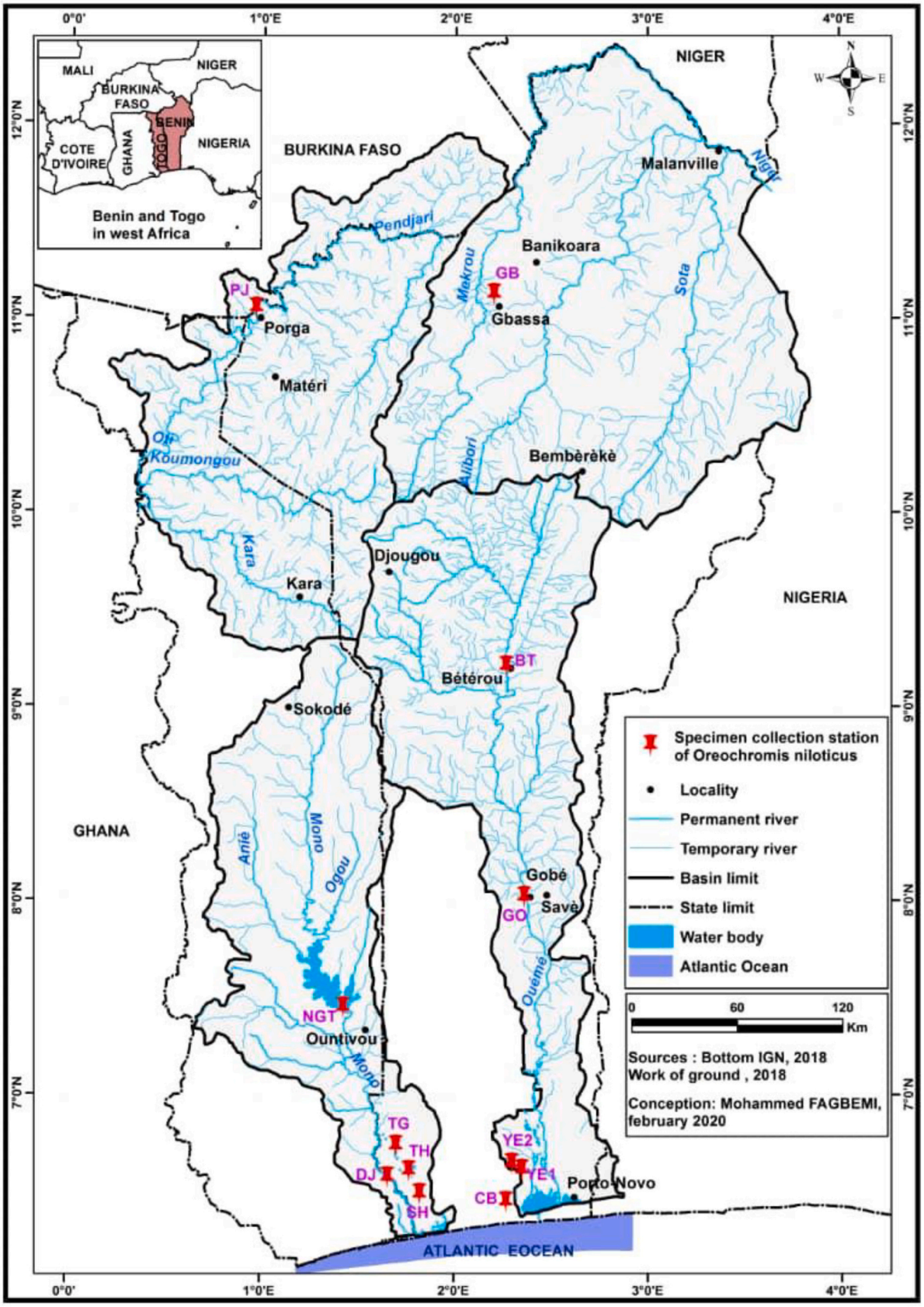

Fig. 1. Map of sampling sites in Benin and Togo. BT: Bétérou, CB: CRIAB, DJ: Djonougoui, GB: Gbassa, GO: Gobé, NGT: Nangbéto, PJ: Pendjari, SH: Sohoumè, TG: Togbadji, TH: Toho, YE1: Yohan-Esteve 1, YE2: Yohan-Esteve 2.

Table 1
Sampling locations and number of analyzed specimens.

Basin/sampling station		Geographic coordinates (WGS84)	Countries	Habitat type	Number of collected specimens	Number of genotyped specimens
Mono	Djonougoui (DJ)	X: 351859 Y: 726900	Togo	Wild	34	13
	Nangbéto (NGT)	X: 326763 Y: 823660	Togo	Wild	34	10
	Sohoumè (SH)	X: 370050 Y: 717554	Benin	Wild	31	8
	Togbadji (TG)	X: 356686 Y: 744835	Benin	Wild	30	9
	Toho (TH)	X: 364129 Y: 730540	Benin	Wild	30	4
Niger	Gbassa (GB)	X: 412808 Y: 1229684	Benin	Wild	32	18
Ouémé	Bétérou (BT)	X: 590828 Y:-2252089	Benin	Wild	7	3
	Gobé (GO)	X: 430018 Y: 886561	Benin	Wild	30	16
Volta	Pendjari (PJ)	X: 277559 Y: 1222019	Benin	Wild	30	14
Yohan-Esteve	I (YEI)	X: 428524 Y: 731114	Benin	Farm	30	12
	II (YEII)	X: 428524 Y: 731114	Benin	Farm	30	15
CRIAB	(CB)	NA	Benin	Farm	34	29
CEFRA/Belgium	(CFT)	X: 860639 Y: 7050962	Belgium	Research center	20	20

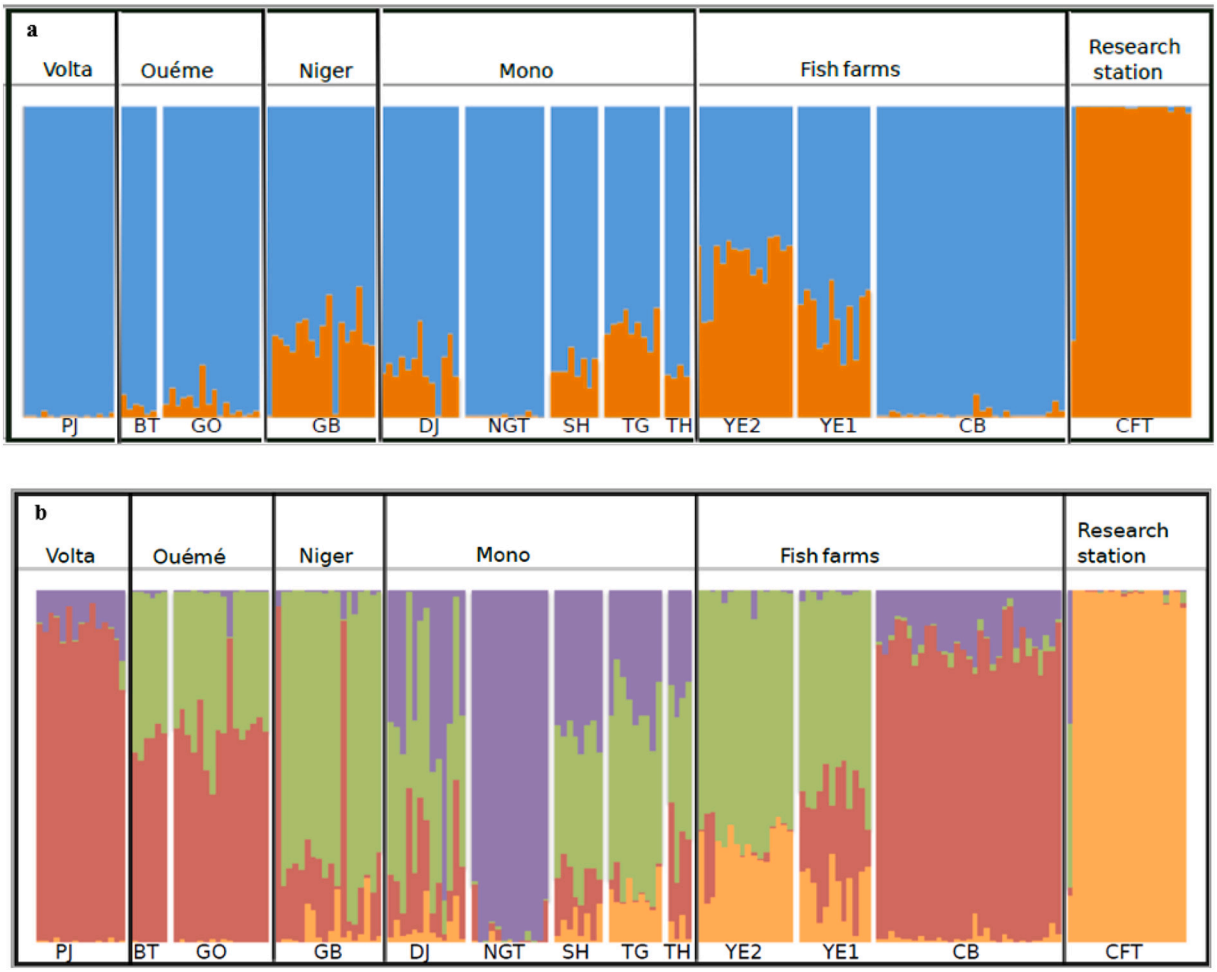

Fig. 2. Structure assignement results (a) following K = 2 (K1 in orange and K2 in blue), (b) following K = 4 (K1 in orange, K2 in red, K3 in green, K4 in violet). The cluster membership of each specimen is shown by the color composition of the vertical lines, with the length of each color being proportional to the estimated membership coefficient. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 2
F_{st} estimates for the clusters according to Structure under the hypothesis of K = 4. (***) *p*-value <0.001.

Cluster	K1	K2	K3	K4
K1	0	0.180	0.126	***
K2	***	0	0.058	***
K3	***	***	0	***
K4	0.251	0.087	0.090	0

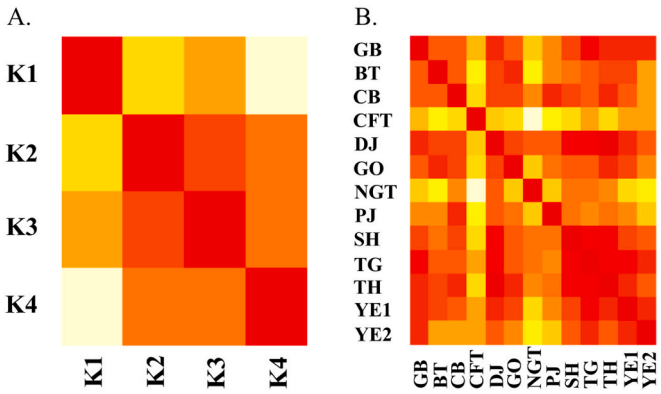

Fig. 3. Heatmap illustrating pairwise F_{st} values among (A.) the four identified clusters, and (B.) sampling locations. High F_{st} values are shown in yellow and low F_{st} values are displayed in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

all the other sampling sites.

The inbreeding coefficients (F_{is}) and levels of expected (H_E) and observed (H_O) heterozygosities, are given in Table 4 for the 13 geographic sampling locations, and in Tables 5 and 6 for the identified clusters following Structure (K = 2 and K = 4). Considering the sampling locations, some sites showed high F_{is} indices (from 0.131 to 0.288) suggesting a higher risk of inbreeding depression (e.g. SH, NGT, CFT, DJ, TH). A particularly high level of inbreeding was noticed within the NGT and SH populations (F_{is} = 0.206 and 0.288). In contrast, other sites were characterized by negative or low F_{is} values (e.g. BG, BT, CB, PJ, TG).

Considering the genetic clusters (Tables 6 and 7 for K = 2 and K = 4 respectively), the F_{is} values were high for K1 (0.177) and K4 (0.274) in which inbreeding level and heterozygosity imbalance were more marked with values close to 1.

4. Discussion

The present study aims to characterize the genetic structure and diversity of several *O. niloticus* wild and farmed populations collected in Benin, and in one Belgian aquaculture research center (Aquaculture Research and Education Center-University of Liège) and to use the data as a tool to improve management of wild genetic resource and domesticated farmed strains.

The genetic structure of *O. niloticus* populations collected in Benin shows that between the Gbassa populations of the Niger Basin and the Pendjari populations of the Volta Basin, there is a moderate differentiation (F_{st} = 0.122) which could be explained by the fact that the Black Volta and the Pendjari Rivers were tributaries of the Niger River in the past (Lévêque, 1997). The low level of inbreeding noted in the Pendjari population of the Volta basin (F_{is} = 0.055) and Gbassa population of Niger basin (F_{is} = −0.037) was previously highlighted by Van Bers et al. (2012) and recently confirmed by Lind et al. (2019). This shows the importance of this populations and the significant size of the Volta and Niger basins in Benin (about 13,600 and 44,313 km^2, respectively). There is a moderate genetic differentiation between the populations of the Volta, Mono, Ouémé and Niger basins, probably due to the short geographical distance separating these basins from each other. The location of these different basins in a very open environment where no barriers exist would favour gene flow between the different basins. This observation is confirmed with the Nangbéto population in the Mono basin, which remains the only population to display significant differentiation compared to the other basins (F_{ST} varied from 0.091 to 0.278). This would be due to the physical isolation of this population following the installation of the Nangbéto hydroelectric dam (1987). The

Table 4
F_{is}, H_E and H_O indice, calculated for the 13 geographic sampling locations.

Sampling location	N	F_{IS}	H_E (standard deviation)	H_O (standard deviation)
GB	18	−0.037	0.390 ± 0.126	0.417 ± 0.211
BT	6	0.061	0.327 ± 0.181	0.342 ± 0.289
CB	31	−0.014	0.394 ± 0.120	0.406 ± 0.191
CFT	20	0.171	0.274 ± 0.183	0.237 ± 0.215
DJ	13	0.153	0.380 ± 0.129	0.341 ± 0.206
GO	16	0.114	0.370 ± 0.138	0.342 ± 0.201
NGT	13	0.206	0.274 ± 0.190	0.233 ± 0.223
PJ	15	0.055	0.351 ± 0.147	0.346 ± 0.217
SH	8	0.288	0.352 ± 0.153	0.279 ± 0.218
TG	9	−0.049	0.388 ± 0.132	0.431 ± 0.231
TH	4	0.134	0.348 ± 0.167	0.362 ± 0.288
YE1	12	0.131	0.375 ± 0.134	0.345 ± 0.206
YE2	16	0.070	0.364 ± 0.144	0.352 ± 0.211

Table 5
F_{is}, H_E and H_O indices calculated for the two clusters as proposed by the Structure analysis (K = 2).

Cluster	N	F_{IS}	H_E (standard deviation)	H_O (standarda deviation)
K1a	30	0.177	0.333 ± 0.152	0.280 ± 0.186
K2a	151	0.146	0.422 ± 0.084	0.362 ± 0.140

Table 3
F_{st} values sorted according to the 13 geographic collecting sites. (*) p-value <0.05, (**) p-value <0.01, (***) p-value <0.001.

	GB	BT	CB	CFT	DJ	GO	NGT	PJ	SH	TG	TH	YE1	YE2
GB	0	***	***	***	***	***	***	***	***	***	**	***	***
BT	0.087	0	***	***	***	***	***	***	***	***	**	***	***
CB	0.089	0.079	0	***	***	***	***	***	***	***	***	***	***
CFT	0.159	0.231	0.199	0	***	***	***	***	***	***	***	***	***
DJ	0.053	0.072	0.056	0.170	0	***	***	***	***	***	*	***	***
GO	0.075	0.038	0.068	0.190	0.072	0	***	***	***	***	***	***	***
NGT	0.184	0.227	0.122	0.278	0.091	0.185	0	***	***	***	***	***	***
PJ	0.122	0.128	0.047	0.237	0.092	0.100	0.174	0	***	***	***	***	***
SH	0.070	0.103	0.070	0.191	0.032	0.085	0.110	0.108	0	***	*	***	***
TG	0.032	0.086	0.091	0.142	0.031	0.075	0.109	0.127	0.029	0	**	***	***
TH	0.046	0.064	0.050	0.203	0.018	0.047	0.129	0.094	0.019	0.021	0	***	***
YE1	0.045	0.062	0.084	0.148	0.052	0.065	0.198	0.124	0.068	0.036	0.051	0	***
YE2	0.055	0.135	0.143	0.135	0.085	0.124	0.215	0.172	0.079	0.053	0.086	0.053	0

Table 6
F_{is}, H_E and H_O indice calculated for the four clusters proposed by the Structure analysis (K = 4).

Cluster	N	F_{IS}	H_E (standard deviation)	H_O (standard deviation)
K1	19	0.141	0.258 ± 0.199	0.231 ± 0.219
K2	69	0.076	0.406 ± 0.105	0.378 ± 0.167
K3	73	0.118	0.415 ± 0.097	0.370 ± 0.150
K4	20	0.274	0.331 ± 0.152	0.251 ± 0.193

variability of the inbreeding level within the different basins could be linked to the respective size of these basins, with 13,600 km^2, 50,000 km^2, 2416 km^2 and 44,313 km^2 respectively for the Volta, Ouémé, Mono and Niger in Benin (Ahouansou-Montcho, 2011; Chikou, 2006; Lederoun et al., 2018; Lokonon, 2019). With a few exceptions (Ouémé and Togbadji in the Mono basin), there is a low level of inbreeding according to the significance of the size of the basin and vice versa. In a larger area, individuals in a population would have a greater chance of mating randomly and thus limit the risk of inbreeding in the population.

Within the Mono Basin populations, Nangbéto samples, collected on a dam of the Mono river located upstream of all other stations (Djonougoui, Sohoumè, Togbadji, Toho) are particularly distinct (Table 4). So we would note to varying degrees, gene flows between the different river basins except with the Nangbéto population, despite the distance between all sampling points. Indeed, the Nangbéto hydroelectric dam forms a water reservoir on the upstream part of Mono River, in Togo, where anthropic activities are highly regulated. The Djonougoui, Sohoumè, Togbadji and Toho stations located downstream of the Nangbéto station are supplied with water from the Mono River during the rainy season. Thus, this could favour a flow of genes from the Nangbéto station upstream to the other stations downstream, but not in the opposite direction and would explain the strong presence of the Nangbéto genetic signal in all the other populations of this basin (Fig. 2b.). Since the building of the dam (1987), the Mono river was divided into three distinct zones: upstream of the dam, the reservoir (sampling station) and downstream of the dam. However, the lack of devices to allow mobility between different parts of the Mono river (Lederoun et al., 2018) has led to ecological break on the course of the Mono river between the upstream and the area qualified as low valley of Mono, where one finds a string of small lakes (among which Sohoumè, Togbadji and Toho and Djonougoui which is a quarry lake) tributaries of the Mono river during floods. Therefore, the genetic structure of Nile tilapia populations in Benin suggests two major groups in the Mono basin, the Nangbéto group and the group of the lower valley of Mono: Djonougoui, Sohoumè, Togbadji and Toho. This genetic separation could be explained by the ecological rupture induced by the construction of the dam 30 years ago. The control of fishing activities (control of fishing periods and equipment) on the Nangbéto reservoir probably contributed to an isolation of this population compared to other ones, which seem to be more subject to hybridization. Nevertheless, escaped *O. niloticus* specimens from farm were reported in Toho lake (Ahouansou-Montcho, 2003, 2011; Lederoun et al., 2018), which is the largest of the tributary lakes of the Mono River (Pliya, 1980). All these lakes communicate with each other during floods, and are isolated from the Nangbéto reservoir.

Similarly, the Pendjari population of the Volta basin (F_{st} varying from 0.092 to 0.237) is also genetically isolated, probably due to their specific situation. This station is located in a protected area in the North of Benin with highly regulated access where the fishing season and fishing areas are well defined, and fishing activities are scrupulously supervised. We also note a high level of inbreeding in all the populations of the Mono basin except in Togbadji (F_{is} = −0.049) contrary to the populations collected in the northern region of Benin (Gbassa, Pendjari and Bétérou) (F_{is} varying from −0.037 to 0.061). It should be noted that the tributary lakes of the Mono River, Toho, Sohoumè and Djonougoui are small lakes (Pliya, 1980) where it would be less likely to observe a large genetic diversity given the surface area and size of these populations.

In our study, sampling was also carried out in the breeding farms of Yohan-Esteve, CRIAB and in the research centre of the University of Liège in Belgium. It appears that the specimens from CEFRA (Belgium) were the most differentiated as compared to all other sampling sites (Benin and Togo). They probably originate from Lake Manzala in Egypt and were sampled in the stock of the CEFRA research center in Belgium. The founding stock was constituted in 1986 from individuals brought from Stirling University (CEFRA archives). This major genetic differentiation could result from a strong genetic drift, which appears after breeding selections from a small number of founder individuals. It should also be noted that among the populations collected on the farms, the one of CRIAB remains genetically close to Pendjari collected in the Pendjari River. This farm reared a strain coming from the Volta Basin in Ghana. It is important to underline the good management of the stock of this farm which shows a low level of inbreeding (F_{is} = −0.014). The low inbreeding levels for the populations of CRIAB, Gbassa and Togbadji, could also be due to the mixture between populations which presented different genetic signatures. The populations sampled in the Yohan-Esteve farm (YE1 and YE2), show a higher genetic proximity with the Gbassa population of the Niger basin. YE1 also remains genetically close to the Djonougoui Togbadji and Toho populations of the Mono basin while YE2 shows a low genetic differentiation compared to the Togbadji population of the Mono. Unfortunately we have no precise information on the origin of these two populations. Globally, our study shows that even if the Togbadji population of Mono presents a low genetic differentiation with the Djonougoui, Toho, Sohoumè populations and moderate with Nangbéto, it displays a strong heterozygosity. Moreover, this population presents both good growth (unpublished data) and reproduction (Fagbémi et al., 2019) performances and would deserve particular attention in the Mono basin within the framework of a programme for the implementation of a local strain for the development of aquaculture in Benin. In the Niger and Ouémé basins, given the level of heterozygosity of the Gbassa and Bétérou populations, they could also be included in a programme for the development of local strains.

All these observations raise the thorny issue of the management of genetic resources, particularly fisheries resources. It should be noted that all populations where anthropogenic activities were not subject to regulations and resource management are subject to a large genetic flow between indigenous populations and other populations with unknown origins. For example, in the Nangbéto station where the species was introduced (Paugy and Bénech, 1989), the population slightly differs from the Djonougoui, Sohoumè, Togbadji and Toho populations (F_{st} NGT-TH = 0.129). Escapes of farmed fish into Toho lake after a flood could be the cause of an introgression of this population from the Mono basin. In Ouémé basin, the Betérou and Gobé populations geographically distant from 165 km are poorly differentiated (F_{st} GO-BT = 0.038). This low differentiation could be explained by the proximity of stations located on the same watercourse with Bétérou upstream and by the low level of fishing activities in these regions. In the South of Benin, where the other stations are located, there is a development of fishing and fish farming activities with fish of unknow origin. There is a risk that these genetically unknown individuals escape in the wild and contribute to genetic pollution. However, they remain poorly differentiated and genetically distant form the Nangbéto, Pendjari and Sohoumè populations. In the Mono basin, the zootechnical data on both growth and reproduction (unpublished data; (Fagbémi et al., 2019)) confirmed that the Nangbéto population considerably differs from the other populations.

5. Conclusion

In conclusion, our study showed that populations of *O. niloticus* collected in different basins in Benin showed moderate genetic differentiations at the exception of the Nangbéto population in the Mono basin, which is more genetically differentiated. For further aquaculture

development, the results of this study combined with the good results of growth (unpublished data) and reproduction performance studies (Fagbémi et al., 2019) in recirculating system show that the Togbadji population of the Mono basin could be a potential candidate for the development of a local strain of *O. niloticus* in Benin.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Mohammed N. A. FAGBEMI is a PhD grant holder from ARES-CCD in PRD project (OpTil-Benin). Our acknowledgements to "Académie de Recherche et d'Enseignement Supérieur" (ARES-Belgium) which funded this research. We would like to thank Dr. Djiman LEDEROUN for providing us with his address book in the Mono and for his advice; our field driver Emmanuel AGOSSOU; technicians specializing in fishery production from the localities of Porga, Banikoara in 2016-2017 and fishermen from the various collection localities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2021.736432.

References

FAO, 2019. FAO Yearbook. Fishery and Aquaculture Statistics 2017/FAO Annuaire. Statistiques Des Pêches et de l'aquaculture 2017/ FAO Anuario. Estadísticas de Pesca y Acuicultura 2017. Rome. also available at. www.fao.org/documents/card/en/c/ca5495t.

Abdelrahman, H., ElHady, M., Alcivar-Warren, A., Allen, S., Al-Tobasei, R., Bao, L.Zhou, 2017. Aquaculture genomics, genetics and breeding in the United States: Current status, challenges, and priorities for future research. BMC Genomics 18 (1), 1–23. https://doi.org/10.1186/s12864-017-3557-1.

Agnèse, J.F., Gourène, B.A., Abban, E.K., Fermon, Y., 1997. Genetic differentiation among natural populations of the Nile Tilapia *Oreochromis Niloticus* (Teleostei, Cichlidae). Heredity 79 (1), 88–96.

Agnése, J.F., Gourène, B.A., Owino, J., Pouyaud, L., Aman, R., 1999. Genetic characterization of a pure relict population of *Oreochromis Esculentus*, an endangered Tilapia. J. Fish Biol. 54 (5), 1119–1123.

Ahouansou-Montcho, S., 2003. Etude de l'écologie et de La Production Halieutique Du Lac Toho Au Bénin. Mémoire de DESS, Faculté Des Sciences Agronomiques, Université d'Abomey-Calavi, Bénin 88.

Ahouansou-Montcho, S., 2011. Diversité et Exploitation Des Poissons de La Rivière Pendjari (Bénin, Afrique de l'Ouest). Thèse de Doctorat. Université d'Abomey-Calavi, Bénin.

Ahouansou-Montcho, S., Agadjihouèdé, H., Montchowui, E., Lalèyè, P.A., Moreau, J., 2015. Population parameters of *Oreochromis Niloticus* (Cichlidae) recently introduced in Lake Toho (Benin, West Africa). International Journal of Fisheries and Aquatic Studies 2 (3), 141–145.

Arthington, A.H., 1986. Introduced Cichlid Fish in Australian Inland Waters. In: Limnology in Australia. Springer, pp. 239–248.

Babiker, M.M., Ibrahim, H., 1979. Studies on the biology of reproduction in the cichlid Tilapia Nilotica (L.): gonadal maturation and fecundity. J. Fish Biol. 14 (5), 437–448.

Belkhir, K., Borsa, P., Chikhi, L., Raufaste, N., Bonhomme, F., 2004. Genetix 4.05, Population Genetics Software for Windows TM. Université de Montpellier II. Montpellier.

Berthelot, C., Brunet, F., Chalopin, D., Juanchich, A., Bernard, M., Noël, B., Bento, P., Da Silva, C., Labadie, K., Alberti, A., Aury, J.M., Louis, A., Dehais, P., Bardou, P., Montfort, J., Klopp, C., Cabau, C., Gaspin, C., Thorgaard, G.H., Boussaha, M., Quillet, E., Guyomard, R., Galiana, D., Bobe, J., Volff, J.N., Genêt, C., Wincker, P., Jaillon, O., Crollius, H.R., Guiguen, Y., 2014. The rainbow trout genome provides novel insights into evolution after whole-genome duplication in vertebrates. Nat. Commun. 5.

Brumfield, R.T., Beerli, P., Nickerson, D.A., Edwards, S.V., 2003. The utility of single nucleotide polymorphisms in inferences of population history. Trends in Ecology and Evolution 18 (5), 249–256.

Chakrabarty, Prosanta, 2004. Cichlid biogeography: comment and review. Fish Fish. 5 (2), 97–119.

Chikou, A., 2006. Etude de La Démographie et de l'exploitation Halieutique de Six Espèces de Poissons-Chats (Teleostei, Siluriformes). In: Dans Le Delta de l'Ouémé Au Bénin, p. 459.

Daget, J., Iltis, A., 1965. Poissons de Côte d'Ivoire (Eaux Douces et Saumâtres).

Dinesh, K.R., Lim, T.M., Chan, W.K., Phang, V.P.E., 1996. Genetic variation inferred from RAPD fingerprinting in three species of Tilapia. Aquac. Int. 4 (1), 19–30.

Elshire, R.J., Glaubitz, J.C., Sun, Q., Poland, J.A., Kawamoto, K., Buckler, E.S., Mitchell, S.E., 2011. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. PloS One 6 (5).

El-Sayed, A.-F.M., Kawanna, M., 2008. Optimum water temperature boosts the growth performance of Nile Tilapia (*Oreochromis Niloticus*) fry reared in a recycling system. Aquac. Res. 39 (6), 670–672.

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.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite Ver 3.5: a new series of programs to perform population genetics analyses under Linux and windows. Mol. Ecol. Resour. 10 (3), 564–567.

Fagbémi, M.N.A., Oloukoule, R., Lederoun, D., Mélard, C., Lalèyè, A.P., Rougeot, C., 2019. Comparative study of the breeding performances of five F1 strain of *Oreochromis niloticus* in Benin. In: Aquac. Eur. Int. Congr. Berlin, Ger. from Oct. 7th to 10th 2019.

Frei, M., Razzak, M.A., Hossain, M.M., Oehme, M., Dewan, S., Becker, K., 2007. Performance of common carp, Cyprinus Carpio L. and Nile Tilapia, *Oreochromis Niloticus* (L.) in integrated Rice-fish culture in Bangladesh. Aquaculture 262 (2–4), 250–259.

Glaubitz, J.C., Casstevens, T.M., Lu, F., Harriman, J., Elshire, R.J., Sun, Q., Buckler, E.S., 2014. TASSEL-GBS: a high capacity genotyping by sequencing analysis pipeline. PLoS One 9 (2), e90346.

Gu, X.H., Lin, H.R., Xia, J.H., 2018. Significant association of SNP polymorphism in the Tilapia enhancer of Polycomb homolog 1 gene with salt tolerance. Aquac. Res. 49 (4), 1690–1698.

Guyon, R., Rakotomanga, M., Azzouzi, N., Coutanceau, J.P., Bonillo, C., D'Cotta, H., Pepey, E., Soler, L., Rodier-Goud, M., D'Hont, A., Conte, M.A., Van Bers, N.E.M., Penman, D.J., Hitte, C., Crooijmans, R.P.M.A., Kocher, T.D., Ozouf-Costaz, C., Baroiller, J.F., Galibert, F., 2012. A high-resolution map of the Nile Tilapia genome: a resource for studying cichlids and other Percomorphs. BMC Genomics 13 (1), 1–17.

Hassanien, H., Abdallah, M., Elnady, A., Obeida, H., Itriby, 2004. Genetic diversity of Nile Tilapia populations revealed by randomly amplified polymorphic DNA (RAPD). Aquac. Res. 35 (6), 587–593.

Hassanien, H., Abdallah, Gilbey, J., 2005. Genetic diversity and differentiation of Nile Tilapia (*Oreochromis Niloticus*) revealed by DNA microsatellites. Aquac. Res. 36 (14), 1450–1457.

Hernández, M., G-leyva, E., Milstein, A., 2013. Polyculture of mixed-sex and male populations of Nile Tilapia (*Oreochromis Niloticus*) with the Mayan cichlid (*Cichlasoma Urophthalmus*). Aquaculture 418–419, 26–31.

Houston, R.D., Taggart, J.B., Cézard, T., Bekaert, M., Lowe, N.R., Downing, A., Talbot, R., Bishop, S.C., Archibald, A.L., Bron, J.E., Penman, D.J., Davassi, A., Brew, F., Tinch, A.E., Gharbi, K., Hamilton, A., 2014. Development and validation of a high density SNP genotyping Array for Atlantic Salmon (*Salmo Salar*). BMC Genomics 15 (1), 1–13.

Jarne, P., Lagoda, P.J.L., 1996. Microsatellites, from molecules to populations and Back. Trends Ecol. Evol. 11 (10), 424–429.

Kopelman, N.M., Mayzel, J., Jakobsson, M., Rosenberg, N.A., Mayrose, I., 2015. Clumpak: a program for identifying clustering modes and packaging population structure inferences across K. Mol. Ecol. Resour. 15 (5), 1179–1191.

Kumar, G., Kocour, M., 2017. Applications of next-generation sequencing in fisheries research: a review. Fish. Res. 186, 11–22.

Laleye, P.A., Chikou, A., Philippart, J.-C., Teugels, G., Vandewalle, P., 2004. Etude de La Diversité Ichtyologique Du Bassin Du Fleuve Ouémé Au Bénin (Afrique de l'Ouest). Cybium 28 (4), 329–339.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with bowtie 2. Nat meth. Nature Research 9, 357–359.

Lederoun, D., Snoeks, J., Lalèyè, P.A., Vandewalle, P., Vreven, E., 2018. An updated checklist of the Ichthyofauna of the Mono River basin (Benin and Togo: West Africa). Ichthyological Exploration of Freshwaters 28 (2), 135–156.

Lee, B.Y., Howe, A.E., Conte, M.A., D'Cotta, H., Pepey, E., Baroiller, J.F., di Palma, F., Carleton, K.L., Kocher, T.D., 2010. An EST resource for Tilapia based on 17 normalized libraries and assembly of 116,899 sequence tags. BMC Genomics 11 (1).

Lévêque, C., 1997. Biodiversity Dynamics and Conservation: The Freshwater Fish of Tropical Africa. Cambridge University Press.

Lévêque, C., Paugy, D., Teugels, G.G., 1990. Faune Des Poissons d'eaux Douces et Saumâtres de l'Afrique de l'Ouest, The Fresh and Brackish Water Fishes of West Africa. Tome 1.

Li, M., Sun, Y., Zhao, J., Shi, H., Zeng, S., Ye, K., Jiang, D., Zhou, L., Sun, L., Tao, W., Nagahama, Y., Kocher, T.D., Wang, D., 2015. A tandem duplicate of anti-Müllerian hormone with a missense SNP on the Y chromosome is essential for male sex determination in Nile Tilapia, *Oreochromis Niloticus*. PLoS Genet. 11 (11), 1–23.

Lien, S., Koop, B.F., Sandve, S.R., Miller, J.R., Kent, M.P., Nome, T., Hvidsten, T.R., Leong, J.S., Minkley, D.R., Zimin, A., Grammes, F., Grove, H., Gjuvsland, A., Walenz, B., Hermansen, R.A., Von Schalburg, K., Rondeau, E.B., Di Genova, A., Samy, J.K.A., Vik, J.O., Vigeland, M.D., Caler, L., Grimholt, U., Jentoft, S., Våge, D.I., De Jong, P., Moen, T., Baranski, M., Palti, Y., Smith, D.R., Yorke, J.A., Nederbragt, A. J., Tooming-Klunderud, A., Jakobsen, K.S., Jiang, X., Fan, D., Hu, Y., Liberles, D.A., Vidal, R., Iturra, P., Jones, S.J.M., Jonassen, I., Maass, A., Omholt, S.W., Davidson, W.S., 2016. The Atlantic Salmon genome provides insights into Rediploidization. Nature 533 (7602), 200–205.

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. Sci. Rep. 1–12.

Liu, S., Sun, L., Li, Y., Sun, F., Jiang, Y., Zhang, Y., Zhang, J., Feng, J., Kaltenboeck, L., Kucuktas, H., Liu, Z., 2014. Development of the catfish 250K SNP Array for genome-wide association studies. BMC Research Notes 7 (1), 1–12.
Lokonon, B.O.K., 2019. Farmers' vulnerability to climate shocks: insights from the Niger Basin of Benin. Clim. Dev. 11 (7), 585–596.
Lowe-mcconnell, R., 2010. Recent research in the African Great Lakes: fisheries, biodiversity and cichlid evolution. In: Freshwater Forum, vol. 20.
Luikart, G., England, P.R., Tallmon, D., Jordan, S., Taberlet, P., 2003. The power and promise of population genomics: from genotyping to genome typing. Nat. Rev. Genet. 4 (12), 981–994.
Mcandrew, B.J., Beveridge, M.C.M., 2000. Tilapias: Biology and Exploitation. Kluwer Academic Publishers.
Mires, D., 1982. A study of the problems of the mass production of hybrid tilapia fry. In: International Conference on the Biology and Culture of Tilapias, Bellagio (Italy), 2–5 Sep 1980.
Morin, P.A., Mccarthy, M., 2007. Highly accurate SNP genotyping from historical and low-quality samples. Mol. Ecol. Notes 7 (6), 937–946.
Morin, P.A., Luikart, G., Wayne, R.K., 2004. Snps in ecology, evolution and conservation. Trends in Ecology and Evolution 19 (4), 208–216.
Ng, W.K., Wang, Y., 2011. Inclusion of crude Palm oil in the Broodstock diets of female Nile Tilapia, *Oreochromis Niloticus*, resulted in enhanced reproductive performance compared to Broodfish fed diets with added fish oil or linseed oil. Aquaculture 314 (1–4), 122–131.
O'connell, M., Wright, J.M., 1997. Microsatellite DNA in fishes. Rev. Fish Biol. Fish. 7 (3), 331–363.
O'Reilly, P., Wright, J.M., 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. 47, 29–55.
Palti, Y., Gao, G., Liu, S., Kent, M.P., Lien, S., Miller, M.R., Rexroad, C.E., Moen, T., 2015. The development and characterization of a 57K single nucleotide polymorphism Array for rainbow trout. Mol. Ecol. Resour. 15 (3), 662–672.
Paugy, D., Bénech, V., 1989. Les Poissons Des Bassins d'eau Douce Des Bassins Côtiers Du Togo (Afrique de l'Ouest). Revue d'Hydrobiologie Tropicale 22 (4), 295–316.
Pliya, J., 1980. La Peche Dans Le Sud-Ouest Du Benin; Etude de Geographie Appliquee Sur La Peche Continentale et Maritime.
Pouyaud, L., Agnèse, J.-F., 1995. Phylogenetic relationships between 21 species of three Tilapiine genera Tilapia, Sarotherodon and Oreochromis using Allozyme data. J. Fish Biol. 47 (1), 26–38.
Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155 (2), 945–959.
Rognon, X., Guyomard, R., 1997. Mitochondrial DNA differentiation among east and west African Nile Tilapia populations. J. Fish Biol. 51 (1), 204–207.
Rognon, X., Andriamanga, M., Mcandrew, B., Guyomard, R., 1996. Allozyme variation in natural and cultured populations in two Tilapia species: *Oreochromis Niloticus* and Tilapia Zillii. Heredity 76 (6), 640.
Rurangwa, E., Van Den Berg, J., Laleye, P.A., Van Duijn, A.P., Rothuis, A., 2014. Un Quick Scan Du Secteur Pour Des Possibilités d'interventions.
Ryman, N., Palm, S., André, C., Carvalho, G.R., Dahlgren, T.G., Jorde, P.E., Laikre, L., Larsson, L.C., Palmé, A., Ruzzante, D.E., 2006. Power for detecting genetic divergence: differences between statistical methods and marker loci. Mol. Ecol. 15 (8), 2031–2045.
Stepien, C.A., 1995. Population Genetic Divergence and Geographic Patterns from DNA Sequences: Examples from Marine and Freshwater Fishes. In: American Fisheries Society Symposium[AM. FISH. SOC. SYMP.]. 1995.
Team, R. Core, 2014. "R Core Team (2014)." R: A Language and Environment for Statistical Computing. R Found Stat Comput, Vienna, Austria.
Van Bers, N.E.M., Crooijmans, R.P.M.A., Groenen, M.A.M., Dibbits, B.W., Komen, J., 2012. SNP marker detection and genotyping in Tilapia. Mol. Ecol. Resour. 12 (5), 932–941.
Wayne, R.K., Morin, P.A., 2004. Conservation genetics in the new molecular age. Front. Ecol. Environ. 2 (2), 89–97.
Wirgin, I.I., Waldman, J.R., 1994. What DNA can do for you. Fisheries 19 (7), 16–27.
Yáñez, J.M., Naswa, S., López, M.E., Bassini, L., Correa, K., Gilbey, J., Bernatchez, L., Norris, A., Neira, R., Lhorente, J.P., Schnable, P.S., Newman, S., Mileham, A., Deeb, N., Di Genova, A., Maass, A., 2016. Genomewide single nucleotide polymorphism discovery in Atlantic Salmon (*Salmo Salar)*: validation in wild and farmed American and European populations. Mol. Ecol. Resour. 16 (4), 1002–1011.
Yáñez, J.M., Yoshida, G., Barria, A., Palma-Véjares, R., Travisany, D., Díaz, D., Cáceres, G., Cádiz, M.I., López, M.E., Lhorente, J.P., Jedlicki, A., Soto, J., Salas, D., Maass, A., 2019. High-throughput single nucleotide polymorphism (SNP) discovery and validation through whole-genome Resequencing of hundreds of individuals in Nile Tilapia (*Oreochromis Niloticus*). Biorxiv 594671.
Yoshida, G.M., Barria, A., Correa, K., Cáceres, G., Jedlicki, A., Cadiz, M.I., Lhorente, J.P., Yáñez, J.M., 2019. Genome-wide patterns of population structure and linkage disequilibrium in farmed Nile Tilapia (*Oreochromis niloticus*). Frontiers in Genetics 10.
Zeng, Q., Fu, Q., Li, Y., Waldbieser, G., Bosworth, B., Liu, S., Yang, Y., Bao, L., Yuan, Z., Li, N., Liu, Z., 2017. Development of a 690 K SNP Array in catfish and its application for genetic mapping and validation of the reference genome sequence. Sci. Rep. 7 (December 2016), 1–14.