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Defining Management Units for Wild Nile Tilapia *Oreochromis niloticus* from Nine River Basins in Ghana

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Abstract: Despite the global importance of the Nile tilapia *Oreochromis niloticus*, especially to aquaculture, knowledge of genetic variability within native populations is still limited. While several studies have assessed genetic differentiation across the major drainage basins of Africa, relatively little effort has focused on characterizing genetic differentiation at finer scales. We assessed genetic variation in *O. niloticus* within and among nine drainage basins in Ghana using nuclear microsatellite DNA markers as the basis for identifying potential units of conservation among wild populations. We screened 312 wild individuals using eight nuclear microsatellite DNA markers. We found moderate genetic diversity within and differentiation among all wild populations studied, with strong signals of recent demographic bottlenecks in several populations. Genetic structure among 11 populations suggested the presence of up to ten management units (MUs). In particular, the Black Volta and the Tano–Asuhyea populations, which were the most genetically distinct and geographically isolated and may be most at risk of loss of genetic diversity over time, may well represent evolutionary significant units. Therefore, at the minimum, the Black Volta and Tano–Asuhyea populations should be prioritized for conservation actions to sustain them over the long-term.

Keywords: microsatellites; genetic bottlenecks; conservation units; management units; evolutionary significant units; population genetics

Citation: Anane-Taabeah Attu, G.; Frimpong, E.A.; Hallerman, E.M. Defining Management Units for Wild Nile Tilapia *Oreochromis niloticus* from Nine River Basins in Ghana. *Diversity* **2022**, *14*, 73. https://doi.org/10.3390/d14020073

Academic Editors: Michael Wink and Elmira Mohandesan

Received: 19 November 2021
Accepted: 14 January 2022
Published: 21 January 2022

1. Introduction

The Nile tilapia (*Oreochromis niloticus*) is the third most cultured species worldwide, with a 2018 production of 4.5 million metric tons [1]. Given the importance of *O. niloticus* to aquaculture and commercial fisheries, particularly in Africa, it is surprising that our knowledge of genetic diversity within and among populations is still rather limited. Most research has focused on characterizing aquaculture performance differences among geographically defined strains [2–4], with comparatively little attention to population genetic differentiation in the wild.

Several early studies have attempted to cover multiple major drainage basins in Africa where *O. niloticus* occurs. Allozyme markers showed genetic differentiation among western and eastern African populations [5,6], suggesting the effects of paleogeographic events. Allozyme and mitochondrial restriction fragment length polymorphisms showed genetic differentiation of western African, Ethiopian Rift Valley, and Nile drainage populations [7]. In addition to the effects of paleogeographic events, microsatellite DNA variation indicated more recent, isolation-driven differentiation between and within drainage basins [8]. Our understanding of differentiation among *O. niloticus* populations can be advanced by conducting drainage-scale, local and regional studies, which incorporate multiple, highly variable molecular markers [9–11]. This is especially useful for identifying populations that may be demographically isolated from other populations (as management units, or

MUs; [12]) or populations that are genetically distinct, have unique adaptations, and require conservation management (evolutionarily significant units, or ESUs; [13]).

Frimpong et al. [14] showed the importance of fine-scale population genetic studies within individual drainage basins as opposed to non-systematic sampling among multiple large rivers. The authors screened five microsatellite loci among collections of *O. niloticus* from three major tributaries of the Volta River system within Ghana (the Oti, White Volta, and Afram rivers). Their analysis revealed that the three wild populations showed moderate levels of genetic differentiation (Afram and White Volta, F_{ST} = 0.049; Afram and Oti, F_{ST} = 0.027; and White Volta and Oti, F_{ST} = 0.054), suggesting that widening the sampling effort to include multiple sites within different rivers would reveal patterns of significant genetic variation within and among populations. In Ghana, the largest populations of *O. niloticus* occur within the Volta system, which drains about 70% of the country. Not surprisingly, many genetics studies have focused on *O. niloticus* populations in the Volta system in West Africa [4,15,16]. However, *O. niloticus* also occurs in almost all Ghanaian rivers, including the Pra, Densu, Ankobra, and Bia systems [17,18], and should be studied holistically.

Given the considerable historical hydrological connectivity apparent within the Volta River basin [19] and the moderate genetic differentiation observed among *O. niloticus* populations from the Oti, White Volta, and Afram rivers within the Volta River system [14], we expect considerable genetic differentiation among populations among different river basins in Ghana. Genetic differentiation may be the result of both non-selective (isolation, random genetic drift) and adaptive (natural selection, coadaptation) population genetic processes. Many rivers in West Africa are stressed from increased anthropogenic activities, such as alluvial mining, overfishing, water abstraction, and deforestation within the catchment and riparian zones, which pose significant threats to aquatic life. Without timely intervention, uncharacterized populations of *O. niloticus* could become extirpated, posing the loss of valuable genetic resources. Understanding the genetic variability within and among populations is vital for promoting long-term persistence of *O. niloticus*, especially because of growing threats to their persistence in the wild. Rognon and Guyomard [20] reported widespread genetic introgression by *O. aureus* into several West African *O. niloticus* populations. Similar results were reported for wild tilapia samples collected in Ghana, with evidence showing that pure populations of *O. niloticus* still persist in Ghanaian rivers [21]. Analysis of mitochondrial DNA sequence data from wild and farmed *Oreochromis spp.* collected in Ghana revealed the presence of non-native tilapia on some aquaculture facilities, including individuals showing introgression from non-native *Oreochromis mossambicus*, from which some stocks have escaped into the wild and interbred with native populations [22]. Given these threats, it is imperative that we assess population genetic data for wild populations of *O. niloticus* in a timely manner to inform conservation planning and management. This study was conducted to assess population genetic variation in *O. niloticus* within and among drainage basins in Ghana using nuclear microsatellite DNA markers to identify potential units of conservation among wild populations.

2. Materials and Methods

2.1. Fish Sampling and DNA Extraction

Fish morphologically identified as Nile tilapia *Oreochromis niloticus* were sampled from eight major rivers and one coastal lagoon in Ghana: Afram River, Oti River, White Volta River (two sites), Black Volta River (three sites), Pra River, Tano River (two sites), Ankobra River, Lower Volta River, and Juen Lagoon (Figure 1). A total of 312 individual fish collected from December 2014 through July 2017 (Table 1) were analyzed. Due to small sample sizes in the two Black Volta sites (Talewona and Lawra), all Black Volta individuals were combined and analyzed as one population represented by only one site location, Kantu, as seen in Figure 1. Total genomic DNA from fin-clips previously stored in paper envelopes was extracted at Virginia Tech, USA, following the methods described by Anane-Taabeah et al. [22].

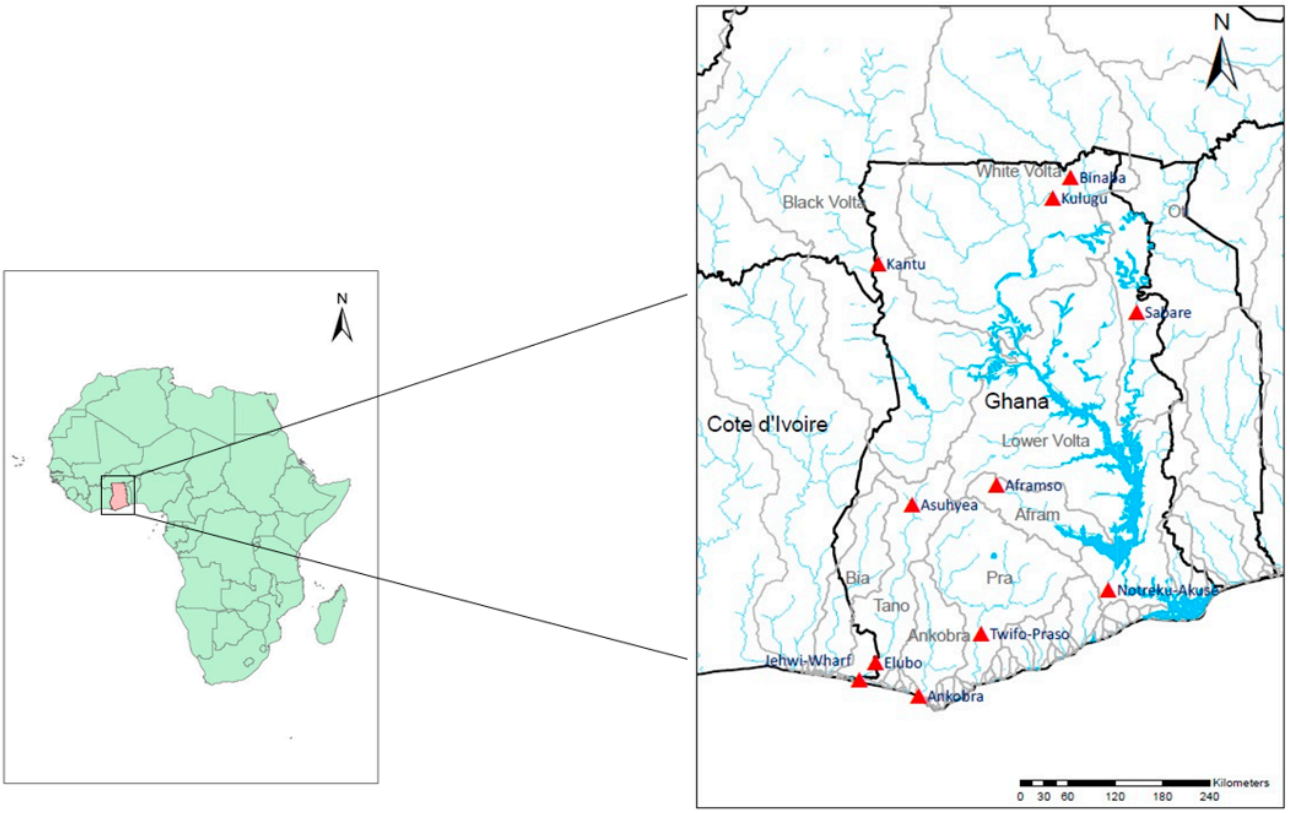

Figure 1. Sampling locations for wild *Oreochromis niloticus* collected in Ghana at 11 sites from eight rivers and one coastal lagoon from December 2014 to July 2017. Sampling sites are indicated with red triangles.

Table 1. Summary of genetic variation among eight microsatellite DNA loci examined in wild tilapia populations from 11 sites (nine river basins) collected in Ghana from December 2014 to July 2017. N = number of individuals genotyped per locus, H_o = observed heterozygosity, H_e = expected heterozygosity, A = mean number of observed alleles per locus, Ar = allelic richness, PrA = number of private alleles, Range = base-pair differences between the shortest and longest microsatellite alleles observed per locus, M-ratio = approximate ratio of A and Range, and F_{IS} = inbreeding coefficient.

Basin	Sampling Location	Year Sampled	Population ID	N	H_o	H_e	A	Ar	PrA	Range	M-Ratio	F_{IS}
Afram River	Aframso	2014; 2015	AF	30	0.75	0.73	9.00	6.75	2	15.55	0.49	−0.03
White Volta River	Binaba	2014; 2015	WB	30	0.65	0.72	7.38	5.87	0	16.75	0.41	0.10
White Volta River	Kulugu	2014; 2015	WK	30	0.63	0.70	8.38	6.27	3	17.63	0.44	0.09
Oti River	Sabare	2014; 2015	OT	30	0.67	0.68	8.63	6.07	4	19.88	0.41	0.02
Pra River	Twifo-Praso	2017	PR	30	0.67	0.65	8.38	6.10	2	18.00	0.46	−0.03
Ankobra River	Ankobra	2017	AN	30	0.60	0.58	6.63	4.90	1	16.75	0.39	−0.02
Tano River	Asuhyea	2017	TA	30	0.65	0.64	5.88	4.78	0	15.63	0.37	−0.02
Tano River	Elubo	2017	TE	18	0.82	0.79	8.38	7.48	5	18.50	0.45	−0.04
Juen Lagoon	Jehwi-Wharf	2017	JU	12	0.69	0.74	7.63	7.63	6	18.13	0.39	0.07
Black Volta River	Kantu **	2017	BV	39	0.60	0.71	9.25	6.37	8	20.75	0.45	0.16
Lower Volta River	Notreku-Akuse	2017	LV	33	0.69	0.72	9.75	6.77	4	18.13	0.39	0.05

** The majority of Black Volta River samples were obtained from Kantu (N = 30). Samples were also collected from Lawra (N = 3) and Talewona (N = 6).

2.2. Polymerase Chain Reaction and Genotyping

All fish samples were genotyped using polymerase chain reaction (PCR) at ten polymorphic microsatellite DNA loci developed for *O. niloticus*, which have shown high allelic variation in many studies [10,11,14,22,23]. Technical information for the respective microsatellite markers is presented in Table S1.

The final volume of 11 µL PCR amplification reactions consisted of 1 µL of 50–100 ng of genomic DNA, 0.1 µL of 5 U/µL of *Taq* DNA polymerase (Promega, Madison, WI, USA), 2 µL of 5× PCR buffer, 1 µL of 25 mM $MgCl_2$, 0.4 µL of 2.5 mM dNTP mix, 0.4 µL of 1× bovine serum albumin, and 0.4 µL each of 5 µM of forward and reverse primers and 5.3 µL of dH_2O. The following thermal cycling conditions were used: 94 °C for 3 min; 35 cycles of 94 °C for 40 s, 54–58 °C depending on marker for 30 s, and 72 °C for 1 min; a final extension of 72 °C for 5 min; and a 4 °C hold. PCR products were visualized using agarose gel electrophoresis to confirm amplification and amplicon sizes prior to genotyping with an ABI3730 automated DNA sequencer at the Virginia Tech Biocomplexity Institute (Blacksburg, VA, USA) or Cornell University (Ithaca, NY, USA). Amplification products were visualized with GeneMarker version 2.6.4 (SoftGenetics, State College, PA, USA) and scored by eye.

2.3. Data Analysis

Prior to analysis, we screened the data following the methods described in Anane-Taabeah et al. [22]. We checked for genotyping errors in the data using the program MICROCHECKER [24]. We then screened populations for deviation from linkage equilibrium [25] and Hardy–Weinberg equilibrium (HWE) at each locus using the program ARLEQUIN, version 3.1 [26]. The significance of departures from HWE was assessed using Fisher's exact test with a Markov chain of 1,000,000 steps and 100,000 dememorization steps [27]. The significance of pairwise linkage tests was determined using the likelihood ratio test with 10,000 permutations [28]. Finally, we used the sequential Bonferroni correction to account for possible Type 1 errors associated with multiple pairwise evaluations [29].

We quantified genetic variation at the ten microsatellite loci using number of alleles per locus, observed and expected heterozygosities, allele frequencies at each locus, and number of private alleles. Allelic richness was calculated using FSTAT [30]. We used the Garza–Williamson index (*M*-ratio, the ratio of allelic richness to allelic range, the latter being the difference between the largest allele and the smallest allele per locus, [31]) to assess random genetic drift and F_{IS} inbreeding coefficients to assess inbreeding within populations. We also estimated effective population size (N_e) using the program NeEstimator version 2.1 [32] and tested for recent bottleneck using the program BOTTLENECK [33].

We quantified population differentiation and structure using multiple methods. We used the program ARLEQUIN to calculate the F_{ST} [34] metric of differentiation and perform analysis of molecular variance (AMOVA, [35]). The null hypothesis was that all populations belong to a single group (no population differentiation) evident by high within-population variation, and the alternate hypothesis was significant population differentiation evident by high among-population variation. We calculated locus-by-locus genic differentiation using the Fisher's exact *G* test executed in Genepop on the web version 4.2 (available at: http://genepop.curtin.edu.au/genepop_op3.html; accessed on 12 March 2018, [36]). We also calculated locus-by-locus F_{ST} estimates in ARLEQUIN. We further estimated $G_{ST'}$, a standardized measure of differentiation developed by Hendrick [37] and Jost's *D* [38], an actual measure of differentiation, i.e., metrics which are not limited by the level of heterozygosity as is F_{ST}. Both $G_{ST'}$ and *D* were calculated using the program POPTREE2 [39]. We then compared the locus-by-locus F_{ST} estimates to the Fisher's exact *G* test, $G_{ST'}$, and *D* estimates.

We assessed population structure among populations using the program STRUCTURE version 2.3.4 [40]. We used both the admixture and no-admixture models both using location as a prior and not doing so in order to assess underlying patterns in the structure of the populations. We executed the models with 100,000 burn-in and 500,000 MCMC replicates with the ancestry-correlated option using default settings. We set the number of clusters *K* sequentially from 1 to 11 populations to aid in individual assignment analyses. The most likely *K* value was selected using both the Evanno et al. [41] and Pritchard and Wen [42] methods implemented online in STRUCTURE HARVESTER [43]. The Clumpak program was used to visualize the Structure plots [44]. We also tested for the likelihood

of isolation by distance (IBD), that is, whether genetic dissimilarity among populations increased with increasing geographic distances among sites. We performed a Mantel test to assess IBD using Genepop on the web [36] and R© statistical software version 3.5.1 [45]. The general equation for a Mantel test statistic is:

$$Zm = \sum_{i=1}^{n} \sum_{j=1}^{n} g_{ij} \times d_{ij}$$

where g_{ij} and d_{ij} are the genetic and geographic distances, respectively, between populations i and j, given n populations [46]. For genetic distances, we used pairwise F_{ST} values transformed as $F_{ST}(1 - F_{ST})$; and for the geographic distances, we used both the raw distances between sites (km) and the log (ln)-transformed distances.

3. Results

3.1. Genetic Diversity within Wild Populations

We found that all ten microsatellite loci screened were polymorphic. Table S2 provides the allele frequencies for each of the eight microsatellite loci across sites. After analysis with MICROCHECKER, we found evidence of segregation of null alleles at locus *UNH925* for 7 of the 11 populations from the wild, and departure from HWE consistently across all sites. We also found evidence of segregation of null alleles consistent with departure from HWE at locus *UNH130* in some populations. We therefore excluded data from loci *UNH925* and *UNH130* from downstream analysis.

After Bonferroni correction ($\alpha = 0.05/28$ pairs of comparison; adjusted $\alpha = 0.0018$), significant linkage disequilibrium was observed at one pair of loci for the Tano (Elubo) and four pairs of loci for the Black Volta populations. Significant departures from HWE ($\alpha = 0.05$) were observed in the Afram population at *UNH180*; White Volta–Binaba at *UNH123*, *UNH858*, *UNH898*, and *UNH991*; White Volta (Kulugu) at *UNH123*, *UNH178*, and *UNH898*; Oti at *UNH123* and *UNH180*; Ankobra at *UNH178*; Tano (Asuhyea) at *UNH178*, Juen at *UNH991*; Black Volta at all loci except *UNH991*; and Lower Volta at *UNH123*, *UNH180*, and *UNH934*. Because such departures were distributed among the respective populations, data for all loci were retained in the analysis.

The summary statistics quantifying genetic variation across the eight loci for each site are provided in Table 1. Mean observed and expected heterozygosities were moderate to high across sites and ranged between 0.60 and 0.82 and 0.58 and 0.79, respectively (Table 1). The Black Volta, Ankobra, and Tano–Asuhyea populations had the lowest values, while the Afram and Tano-Elubo populations showed the highest heterozygosities. Similarly, the mean number of alleles per locus was moderate to high across sites and ranged from 5.88 to 9.75; the Tano–Asuhyea population showed the lowest number of alleles per locus, while Black Volta, Afram, and Lower Volta populations showed the highest mean number of alleles across the eight loci ($\geq$9.0). Private alleles were observed at all loci for all sites except White Volta–Binaba and Tano–Asuhyea; numbers of private alleles ranged from one (Ankobra) to eight (Black Volta). The highest numbers of private alleles were observed at loci *UNH123* (9) and *UNH180* (7; Table S2). Locus-by-locus values of genetic diversity metrics are presented in Table S3.

Effective population sizes estimated using the linkage disequilibrium method ranged from 21 to infinity (Table 2). Seven populations—Aframso, White Volta–Binaba, Oti, Tano-Elubo, Juen, Black Volta, and Lower Volta rivers—had N_e estimates ranging between 21.4 and 178.2. Lower confidence interval estimates were sufficiently small that inbreeding and random drift could be strong population genetic forces within these populations. Upper confidence interval estimates for five populations were in the tens or low hundreds, suggesting a limited scope for adaptation over the long term. Upper confidence interval estimates for six populations, indicated as infinity, might better be regarded as undefined. These estimates could be bounded by the addition of more marker loci or more individuals to the analysis.

Table 2. Effective population size (N_e) estimates and 95% confidence intervals for wild *O. niloticus* population samples [1] collected in Ghana. ∞ = infinite. AF = Afram, WB = White Volta–Binaba, WK = White Volta–Kulugu, OT = Oti, AN = Ankobra, TA = Tano–Asuhyea, TE = Tano–Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta.

Population ID	Estimated N_e
AF	43.3 (25.1, 102.6)
WB	21.4 (14.0, 36.2)
WK	∞ (104.4, ∞)
OT	46.4 (23.7, 182.2)
PR	1184.4 (52.8, ∞)
AN	∞ (58.1, ∞)
TA	∞ (120.3, ∞)
TE	28.1 (15.8, 74.1)
JU	50.1 (12.4, ∞)
BV	22.8 (14.9, 37.6)
LV	178.2 (43.2, ∞)

[1] AF = Afram, WB = White Volta–Binaba, WK = White Volta–Kulugu, OT = Oti, AN = Ankobra, TA = Tano–Asuhyea, TE = Tano–Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta.

The results of the bottleneck tests show that the stepwise mutation model (SMM) was the more conservative in characterizing recent demographic declines within the populations than the infinite allele model (IAM). Three out of the four tests, including the sign test, standard differences test, and the Wilcoxon two-tailed test, under the SMM showed several bottlenecked populations. The sign test suggests that the White Volta–Kulugu, Oti, Pra, Ankobra, Black Volta and Lower Volta populations were bottlenecked (Table 3). The standard differences test suggested that all populations were bottlenecked, while the Wilcoxon two-tailed test suggested that all but the White Volta–Binaba and Tano–Asuhyea populations were bottlenecked. Mean *M*-ratios ranged between 0.37 and 0.49 for all populations across the eight loci; these low ratios (<0.7) supported the inference that genetic bottlenecks had occurred recently. Overall F_{IS} ranged from −0.02 to 0.16 (Table 1); positive values of F_{IS} indicate an excess of homozygotes, suggesting the occurrence of inbreeding. Locus-by-locus F_{IS} values are reported in Table S3.

Table 3. Summary statistics and *p*-values for bottleneck tests conducted for wild *O. niloticus* populations [1]. Tests with significant *p*-values (α = 0.05) are indicated in bold. Sign = sign test, stdv = standard differences test, W_1t = Wilcoxon one-tailed test, W_2t = Wilcoxon two-tailed test. IAM = infinite allele model, SMM = stepwise mutation model.

Population	p_sign_IAM	p_sign_SMM	p_stdv_IAM	p_stdv_SMM	p_W_1t_IAM	p_W_1t_SMM	p_W_2t_IAM	p_W_2t_SMM
AF	0.309	0.058	0.183	**0.000**	0.156	0.990	0.313	**0.027**
WB	0.097	0.186	**0.035**	**0.027**	**0.020**	0.770	**0.039**	0.547
WK	0.580	**0.001**	0.292	**0.000**	0.156	1.000	0.313	**0.004**
OT	0.429	**0.001**	0.489	**0.000**	0.578	1.000	0.945	**0.004**
PR	0.407	**0.001**	**0.047**	**0.000**	0.727	1.000	0.641	**0.004**
AN	0.455	**0.010**	0.183	**0.000**	0.680	0.996	0.742	**0.012**
TA	0.252	0.426	0.065	**0.007**	**0.027**	0.809	0.055	0.461
TE	0.106	0.054	0.059	**0.031**	**0.010**	0.986	**0.020**	**0.039**
JU	0.595	0.052	0.463	**0.001**	0.527	0.986	1.000	**0.039**
BV	0.574	**0.001**	0.469	**0.000**	0.320	1.000	0.641	**0.004**
LV	0.173	**0.010**	0.303	**0.000**	0.680	0.994	0.742	**0.020**

[1] AF = Afram, WB = White Volta–Binaba, WK = White Volta–Kulugu, OT = Oti, AN = Ankobra, TA = Tano–Asuhyea, TE = Tano–Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta.

3.2. Genetic Differentiation among Wild Populations

Pairwise F_{ST} estimates revealed low to moderate genetic differentiation among sites (Table S4), which was statistically significant (p = 0.000). In general, Tano–Asuhyea was differentiated from all but the Tano–Elubo and Juen populations. The lowest differentiation

was observed between the Oti and Afram populations (F_{ST} = 0.02), while the highest differentiation was observed between those of Tano–Asuhyea and Oti (F_{ST} = 0.18). The AMOVA results also support moderate to high differentiation among populations, with over 8% of the variance explained by differences among populations (Table S5). Genic differentiation (Fisher's exact *G* test) and the locus-by-locus F_{ST} estimates all showed highly significant statistical differences ($p < 0.000$) across all loci for all sites combined (Table S4). Similarly, both Hendrick's $G_{ST'}$ and Jost's *D* supported high genetic differentiation among populations (Table S6).

STRUCTURE analysis showed significant differentiation among the populations at the 11 sites. After applying multiple analytic approaches as described in Methods above, the best-supported model was no admixture using location as a prior. The admixture model showed the highest support for grouping populations into four clusters (*K* = 4, results not shown), with several apparent migrants and admixed individuals at several sites. Results from use of the no-admixture model using location information, while generally comparable to those of the admixture model, were better supported in terms of higher Ln*P*(*D*) values. The best-supported model for genetic structure using the Evanno et al. [41] method was *K* = 5, suggesting five clusters within and among individuals from the 11 sites (Figure 2). Genetic differentiation is often hierarchically organized, and it is often unrealistic to expect that there is one 'true' *K* that is best for modeling a particular data set (J. Pritchard, quoted in [47]). Therefore, viewing and reporting plots for multiple *K*-values is appropriate [48] because different values of *K* can give insights into different levels of structure. The Ln*P*(*D*) values suggested that the most probable number of clusters was *K* = 10 (Figure 2, Table S7). STRUCTURE plots for both *K* = 5 and *K* = 10 are presented for comparison (Figure 2).

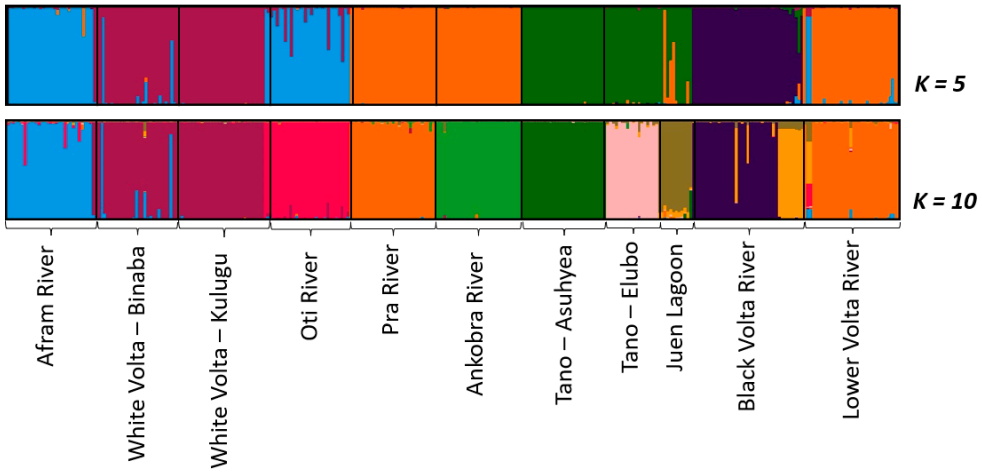

Figure 2. Analyses of wild samples of *O. niloticus* sampled from Ghana with STRUCTURE results showing the proportion of individuals from each wild *O. niloticus* population's ancestry inferred from each of *K* = 5 (**top plot**) and *K* = 10 (**bottom plot**) clusters (MCMC = 500,000).

At *K* = 5 (Figure 2, top plot), the first cluster (shown in magenta) contained individuals from the two White Volta sites (*Q*, the level of individual membership in the inferred cluster, ranged between 0.92 and 0.99) and the Oti River (*Q* = 0.11). The second cluster (shown in blue) contained individuals mainly from Afram River (*Q* = 0.98) and Oti River (*Q* = 0.88). The third cluster (shown in purple) was almost exclusive to the Black Volta River (*Q* = 0.98). The fourth cluster (shown in orange) contained individuals from Pra River (*Q* = 0.97), Ankobra River (*Q* = 0. 98), Juen (*Q* = 0.20), and Lower Volta (*Q* = 0.91). Nearly 10% of individuals from the Lower Volta shared genotypes with individuals in the White Volta–Oti and Afram–Oti cluster, while almost no individuals shared genotypes with individuals from the Black Volta (*Q* = 0.0001). The fifth cluster (shown in green) contained individuals from Tano–Asuhyea River (*Q* ranged between 0.98 and 1.00), Tano–Elubo (*Q* = 0.97), and

Juen (Q = 0.76). There was no signal of admixture or introgression of genes from the Black Volta population into individuals in the fifth cluster (Q = 0.0000), even though very weak signals were detected in the reverse direction (Q ranged between 0.0004 and 0.0079).

At K = 10 (Figure 2, bottom plot), each population clustered distinctly, with the exception of the two White Volta sites, the Pra, the Lower Volta, and the Black Volta. The two White Volta sites clustered together, with Q values similar to those observed when K = 5. The Pra and the Lower Volta clustered together. Interestingly, individuals in the Black Volta population grouped into two clusters. The first Black Volta cluster comprised 30 individuals originally sampled from Kantu. The second cluster comprised nine individuals sampled from Lawra and Talewona, which also shared genotypes with some individuals from Juen Lagoon (Q ranged between 0.14 and 0.20).

The Mantel test showed a significant positive relationship between genetic distances and geographic distances and provided modest support for isolation by distance (IBD) (adjusted r^2 = 0.08, and p = 0.023, Figure 3). However, the genetic differentiation across space showed a weak relationship between genetic distances and geographic distances, even after log-transforming the geographic distances (adjusted r^2 = 0.10, and p = 0.012) (Figure S1).

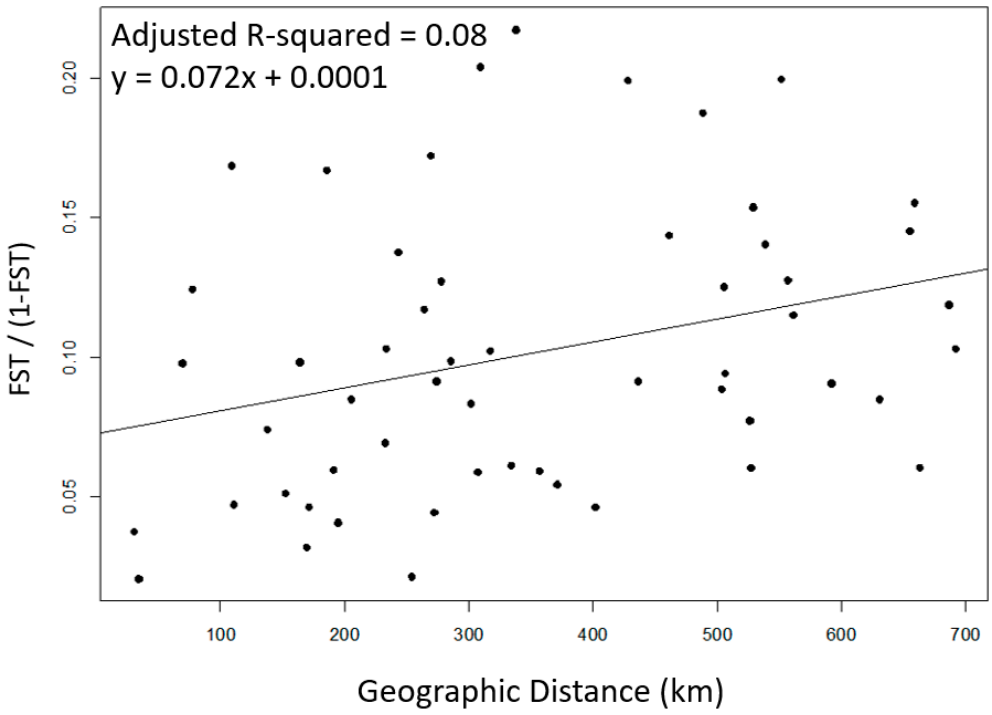

Figure 3. Relationship between genetic distance and geographic distance among wild *Oreochromis niloticus* collected from 11 sites in Ghana from December 2014 and July 2017.

4. Discussion

4.1. Genetic Diversity within Wild O. niloticus Populations: Evidence of Recent Bottlenecks

The genetic diversity observed among wild populations of Nile tilapia in this study was higher than that found in a similar study conducted in Ghana narrowly within the Volta River basin [15]. However, the finding that significant departures from Hardy–Weinberg equilibrium (HWE) occurred at several loci for all populations except Pra River and Ankobra, and significant linkage disequilibrium (LD) observed, especially in the Black Volta River, suggests the occurrence of recent genetic bottlenecks within the populations. Further, the results of bottleneck tests conducted suggest that even the Ankobra and Pra populations were bottlenecked, and the mean M-ratio estimates in this study were below the empirical 0.7 threshold value used to assess population bottlenecks within populations [31]. It is plausible that there were natural historical bottlenecks and that the resulting demographic effect on populations was exacerbated with recent anthropogenic

impacts. Effective population size estimates also suggested declining population numbers at several sites, which is alarming for a highly fecund species such as *O. niloticus*. Inbreeding among relatives is also likely due to the low to moderate and positive values of the inbreeding index (F_{IS} ranged between 0.04 and 0.16). Deviations from HWE may be due to multiple causes, including genetic drift, inbreeding, or mixing of differentiated populations [21]. The inference of segregation of null alleles at three loci (*UNH178*, *UNH203*, and *UNH898*) in the Black Volta population also could have contributed to the departures from HWE and linkage equilibrium observed. Nonetheless, the Black Volta population requires further assessment and monitoring in order to understand the genetic dynamics within the population. This is especially pertinent since many private alleles were observed in the Black Volta population, as well as private mitochondrial DNA (mtDNA) haplotypes; and they are likely the main ancestral population of *O. niloticus* in Ghana [22].

The recent genetic bottlenecks inferred within the study populations may be the consequence of anthropogenic impacts upon the river systems in question. Many tilapia populations have been subject to heavy exploitation. Further, within the last decade, drastic habitat alteration has occurred in almost all freshwater habitats in Ghana, especially due to illegal alluvial gold mining. Turbidity levels of many rivers in Ghana are significantly above recommended limits for aquatic life, and cichlids in particular do not thrive under such environmental conditions. Dankwa et al. [49] found that in heavily mined areas (with turbidity levels between 100 and 1110 NTU), cichlids were completely absent, as were their planktonic prey. Physical and environmental impacts can affect reproduction and long-term persistence of *O. niloticus* in Ghana. High turbidity could affect the selection of mates, which also could result in inbreeding. Management effort should therefore focus on addressing these ecological stressors to ensure the long-term persistence of *O. niloticus*.

4.2. Genetic Differentiation among Wild Populations: Barriers to Dispersal and a Unique Dispersal Mechanism

The metrics used to assess genetic differentiation and structure among populations showed moderate genetic differentiation as a function of geographic location. The pairwise F_{ST} estimates showed that the Afram and Oti populations were the least differentiated. This finding was supported by the clustering of Afram River and Oti River populations into the same group by the STRUCTURE analysis. Even though moderately differentiated, some level of gene flow was apparent between *O. niloticus* populations in the Afram and Oti rivers across a distance of about 254 km (158 mi) between the two populations.

The clustering of the Oti and the White Volta populations was not as surprising because the distance between the Oti site and the farthest of the two White Volta sites (Binaba) is relatively short, 172 km (106 mi). The distance between the Afram site and the Lower Volta site is about 195 km (121 mi). The finding that some level of genetic exchange among Nile tilapia occurred between the Lower Volta and all the rivers within the Volta basin (except the Black Volta) suggests that isolation-by-distance is not as important in mediating gene flow within the Volta basin as the presence of physical barriers such as the 57-year-old Akosombo Dam. The presence of the Akosombo Dam and other irrigation reservoirs also could explain why the Black Volta River populations are highly differentiated from all other populations within the Volta basin. The results of the Mantel test further support the interpretation that isolation by distance alone could not explain the genetic structuring observed across space for the populations. For instance, significant genetic structuring was observed between the Black Volta sites even though two of the sites (Kantu and Talewona) are only 13 km (about 8 mi) apart.

On the other hand, both isolation-by-distance and physical barriers could be operating together to limit gene flow between the Black Volta and all sites outside the Volta basin. However, we observed that the Black Volta was more differentiated from the Tano–Asuhyea (F_{ST} = 0.143) than from the Tano–Elubo (F_{ST} = 0.083) population, which suggests some genetic exchange between the latter two population pairs. This result corroborates findings that the Black Volta population shared mitochondrial *COI* haplotypes with Tano–Elubo

population and samples from Cote d'Ivoire [21,22] and suggests *O. niloticus* movement, likely through human intervention, between Ghana and Cote d'Ivoire at the shared border along the Black Volta River.

The pairwise F_{ST} estimates also revealed that the Tano–Asuhyea population was very distinct from all populations outside the Tano basin and moderately differentiated from the Tano–Elubo and Juen Lagoon populations. The apparent genetic differentiation from other populations, relatively low allelic richness, and the lack of private alleles within the Tano–Asuhyea population suggest the loss of diversity within the population due to genetic drift. This interpretation is supported by the low mean *M*-ratio estimated for the Tano–Asuhyea population (0.37) and significant bottleneck test results (Table 3). Occurring in the headwaters of the Tano River, the Tano–Asuhyea population is relatively isolated and may be exchanging relatively few migrants with the Tano–Elubo population.

STRUCTURE analysis also showed clustering of individuals from the Tano basin (Tano–Asuhyea, Tano–Elubo, and Juen Lagoon) and individuals from the Pra, Ankobra, and the Lower Volta rivers. Given that the Pra, Ankobra, Tano, and the Lower Volta rivers all run parallel to one another and drain into the Atlantic Ocean, oceanic dispersal appears to be a plausible mechanism for the genetic exchange apparent among *O. niloticus* populations. It appears that these populations are migrating from one freshwater environment through brackish water into another freshwater environment. Oceanic dispersal is possible because tilapias, in general, are euryhaline [50,51], and the salinity tolerance in *O. niloticus* has been documented [52–54]. Genetic data have been useful in detecting oceanic dispersal for species presumed to be restricted to freshwater environments, such as river sharks [55]. Oceanic dispersal also may explain the colonization of *O. niloticus* in the Ankobra and Pra rivers (from the Tano River [26]) and further explain why *Tilapia pra* is restricted to the Ankobra, Tano, and Pra rivers [18].

4.3. Defining Conservation Units for Wild Oreochromis niloticus from Nine River Basins in Ghana

Taken together, the genetic diversity and differentiation observed within and among the wild populations of Nile tilapia provide support for developing effective management and conservation plans for preserving these populations. The STRUCTURE results suggest that of the eleven populations studied, there may be as many as ten populations requiring separate management, which should be designated as management units (MUs). Additionally, the genetic distinctiveness of the Tano–Asuhyea and Black Volta populations and their isolation from other populations suggests the possibility that these populations may represent evolutionary significant units (ESUs) within *O. niloticus* populations in Ghana. Further studies focusing upon elucidation of any adaptive differences among candidate ESUs should help establish this possibility.

Conserving the Black Volta population in particular is important because of earlier findings from microsatellite data, which showed a lack of evidence of admixed individuals in the population, and from mtDNA analysis, which revealed that escaped, non-native, farmed tilapias are restricted to the Lower Volta and Afram rivers within the Volta basin [24]. The Lower Volta River is downstream of aquaculture operations on the Volta Lake, while there are aquaculture facilities in close proximity to the Afram River. On the other hand, the Black Volta River is relatively isolated from aquaculture farms even though cage farming in irrigation reservoirs is increasing in the region. Given the evidence of a possible human-mediated movement of *O. niloticus* between Ghana and Cote d'Ivoire at the shared border near the Black Volta River, if no conservation measures are instituted, it is only a matter of time before pure strains of *O. niloticus* in the Black Volta River are lost. Additionally, the possibility of ocean dispersal as a mechanism for migration of *O. niloticus* to otherwise isolated basins, restricting the dispersal of non-native tilapia populations in Ghana and along the West African coastline would be very challenging if non-native fish continue to escape from farms. The results from this study also provide crucial baseline information necessary for continuous monitoring of rivers close to aquaculture operations to conserve native *O. niloticus* populations.

4.4. Conclusions and Implications

The objective of this study was to assess genetic variation in *O. niloticus* within and among drainage basins in Ghana using nuclear microsatellite DNA markers to aid in detecting potential units of conservation within wild populations. We found moderate to high genetic diversity within and differentiation among wild populations, and we also detected strong signals of recent demographic and genetic bottlenecks in several populations. We also found significant genetic structure among the populations, suggesting the presence of up to 10 management units (MUs) within the 11 wild populations studied. In particular, the Black Volta and the Tano–Asuhyea populations, which were the most genetically distinct and geographically isolated and may be most at risk of loss of genetic diversity over time, may potentially represent evolutionary significant units. Therefore, at the minimum, both the Black Volta and Tano–Asuhyea populations should be designated as management units (MUs) and prioritized for conservation to sustain them in the long term.

Previous studies using allozyme [5] or mitochondrial DNA [20,22] markers showed introgression *of O. aureus* and *O. mossambicus* into some but not all western African *O. niloticus* populations. The microsatellite markers that we used are not in themselves powerful for detecting hybridization among *Oreochromis* species; mitochondrial and single-nucleotide polymorphism markers would be needed for assessment of interspecific hybridization.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/d14020073/s1, Table S1: Technical details for amplification of ten microsatellite loci for Ghanaian tilapias (*Oreochromis* spp.) collected from eight rivers and one coastal lagoon from December 2014 to July 2017 in Ghana, Table S2: Allele frequencies (%) across 8 nuclear microsatellite DNA loci for wild *O. niloticus* populations sampled from 11 sites (9 river basins) in Ghana from 2014 to 2017. AF = Afram, WB = White Volta–Binaba, WK = White Volta–Kulugu, OT = Oti, AN = Ankobra, TA = Tano–Asuhyea, TE = Tano–Elubo, JU = Juen, BV = Black Volta, and LV = Lower Volta. Private alleles are indicated in Bold, Table S3: Locus-by-locus genetic diversity metrics for all populations. Values in bold are significantly different from each other ($p < 0.05$), Table S4: Pairwise F_{ST} values from nuclear microsatellite DNA sequences for wild *O. niloticus* populations sampled from 11 sites (nine river basins) in Ghana from December 2014 to July 2017. All F_{ST} values were significant, Table S5: AMOVA for 8 nuclear DNA microsatellites loci in wild tilapia populations collected from 11 sites in Ghana from December 2014 to July 2017, Table S6: Genetic divergence statistics for each locus of a given sample size (n). Genic differentiation (G) from Fisher's exact test, Hendrick's $G_{ST'}$, and Jost's D are compared with F_{ST} values, Table S7: STRUCTURE results with estimates for probable K clusters for *O. niloticus* from Ghana. $K = 10$ as the most likely number of clusters is indicated in bold, Figure S1: Relationship between genetic distances and logarithm of geographic distances among *Oreochromis niloticus* collected from 11 sites in Ghana from December 2014 to July 2017.

Author Contributions: Conceptualization, G.A.-T.A., E.A.F. and E.M.H., methodology, G.A.-T.A., E.A.F. and E.M.H.; application of software, G.A.-T.A.; formal analysis, G.A.-T.A.; validation, G.A.-T.A., E.A.F. and E.M.H.; investigation, G.A.-T.A.; resources, G.A.-T.A., E.A.F. and E.M.H.; data curation, G.A.-T.A.; writing—original draft preparation, G.A.-T.A.; writing—review and editing, G.A.-T.A., E.A.F. and E.M.H.; visualization, G.A.-T.A., E.A.F., and E.M.H.; supervision, E.A.F. and E.M.H.; project administration, G.A.-T.A., E.A.F., and E.M.H.; funding acquisition, G.A.-T.A. and E.A.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded in part by the AquaFish Innovation Lab, supported by the U.S. Agency for International Development (USAID) under award number CA/LWA EPP-A-00-06-0012-00, and the Rufford Foundation (UK), under grant number 22083-1. G.A.-T.A. was supported in part by the Global Change Center (Virginia Tech Graduate School); Aquaculture Association of Ghana; Raanan Fish Feed, Ghana; Consulting in Aquaculture Research and Training for Development (CART4D); and the Burd Sheldon McGinnes Graduate Fellowship through the Department of Fish and Wildlife Conservation, Virginia Tech.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Ethics Committee of Kwame Nkrumah University of Science and Technology in a written letter in September 2014 (Protocol code number is not applicable).

Data Availability Statement: The data from this study have not been made publicly available yet. Some of the data can be accessed with written request to the authors.

Acknowledgments: We thank all the fishers, local guides, and personnel from the Fisheries Commission, Ghana, who collaborated on this project. We are grateful to the following individuals for their immense support in the field work in Ghana: Akwasi Ampofo-Yeboah, Nathaniel Gyasi Adjei, Francis Adjei, Anthony Aliebe, Abigail Ebachie Tarchie, and Emmanuel Nyamekye. We thank Miluska Olivera-Hyde and Sheila Harris (formerly of Virginia Tech) and Clay Ferguson of Virginia Tech assisted with laboratory work. Jess Jones and Donald Orth reviewed earlier versions of the manuscript and provided useful feedback.

Conflicts of Interest: The authors declare no conflict of interest, except those which may arise through the funding relationships described above.

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