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Critically Small Contemporaneous Effective Population Sizes Estimated for Stocks of the African Bonytongue in Western Africa

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Abstract: Inland capture fisheries play a critical role in supporting food security and livelihoods in Africa. Therefore, it is important to evaluate the genetic health of exploited fish populations. The African bonytongue, *Heterotis niloticus*, supports important commercial and subsistence fisheries in western Africa. However, sharp declines in stocks have been reported. Herein, we estimate contemporary effective population sizes (*Ne*) of four *Heterotis* populations in Nigeria, three in Benin, and five in Cameroon using Linkage Disequilibrium methods. *Ne* estimates were used to assess genetic short-term (i.e., inbreeding depression) and long-term (i.e., loss of evolutionary potential) risks. *Ne* point estimates obtained with the best estimator (out of 16), as determined by computer simulations, were <50 (range = 5.1–36.2) for nine of the twelve populations examined, which is below the minimum recommended for avoiding the potential deleterious effects of inbreeding depression (original criterion $Ne \geq 50$, revised to $Ne \geq 100$); and well below the minimum recommended for populations to retain evolutionary potential (original criterion $Ne \geq 500$; revised to $Ne \geq 1000$). The lower bound of the confidence interval for two of the remaining populations was below the minimum recommended to retain evolutionary potential (with the point estimate of one of them also below this threshold), and for some methods, values were lower than the minimum recommended to avoid inbreeding depression. Accordingly, our results suggest that urgent conservation and management plans are needed to guarantee the persistence and sustainability of the *H. niloticus* populations examined.

Keywords: effective population size; inland capture fisheries; West Africa; *Heterotis*

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1. Introduction

Inland capture fisheries in Africa comprise 28% of the global total [1] and provide a critical source of protein, micronutrients and income for people in this continent [2–5]). The sustainability of these fisheries will be crucial for the food security of the rapidly growing human population of sub-Saharan Africa, which is projected to double by 2050 [6]. Multiple threats, however, are imperiling freshwater fish stocks in Africa, including overexploitation, habitat degradation, introduction of exotic species, and aquaculture [7–10]. Africa's most populous country, Nigeria (population ~225 million) in West Africa, ranked 9th globally for inland fisheries yield in 2020, accounting for 3% of the global total and ~11% of Africa's total (Table 1). Nigeria is one of the seven countries driving most of the growth in global inland fisheries [4]. Nigeria is also one of nine countries that collectively will contribute more than half the projected growth of the global human population between 2019 and 2050 [6], and could be one of the world's largest consumers of fish by that year [11]. Inland fisheries have been historically important in Nigeria's western neighbor Benin, a country with a much smaller population (13.7 million). During 1960–2013, the inland fishery sector of Benin produced an average 27,000 tons annually, employing 57,500 fishers and 40,000 women active in fish processing and marketing [12]. Benin has ranked 15th globally in inland capture fisheries production per capita, and 17th in inland capture fisheries production per unit area [13]. Nigeria's eastern neighbor Cameroon (population ~28.6 million), home to several large rivers, also has considerable inland capture production (Table 1). As of 2018, inland fisheries in this country were estimated to employ more than 177,000 fishers [14]. Despite the current and future significance of inland capture fisheries in these countries, and in Africa in general, very little is known about the status of fish stocks, including their effective population size (*Ne*), one of the most important parameters in evolutionary biology and conservation biology [15,16].

Table 1. Inland capture (live weight) of all fishes and *Heterotis niloticus* in Africa, Nigeria and Benin, and for all fishes in Cameroon.

	Production (Average per Year)				**Yearly Production**				
Region	**1980s**	**1990s**	**2000s**	**2010s**	**2017**	**2018**	**2019**	**2020**	**2021**
Africa	1.47M	1.89M	2.33M	2.87M	3.01M	3.02M	3.24M	3.21M	3.49M
Africa—*Heterotis*		5961	12,653	23,876	31,815	29,257	28,649	27,818	27,777
Nigeria	100,513	104,173	210,970	350,175	420,078	392,188	373,344	354,378	362,792
% of Africa	6.8%	5.3%	9.01%	12.2%	14.0%	12.9%	11.4%	10.9%	10.4%
Nigeria—*Heterotis*		4770	10,877	20,606	27,896	25,689	24,626	23,375	23,875
% of Africa		80.0%	86.0%	86.3%	87.7%	87.8%	86.0%	84.0%	86.0%
Benin	31,823	31,830	28,664	28,969	33,415	28,900	28,775	28,815	29,000
Benin—*Heterotis*	421	565	564	791	853	1398	925	1085	1095
Cameroon	201,000	316,000	666,580	423,913	30,292	30,636	30,985	30,630	31,550

M = million tons of live weight.

Ne represents a population's genetic finite sampling size between generations by comparison to a hypothetical idealized population (i.e., the Wright-Fisher model) where all genetic drift is represented by a sample of $2N$ gametes from one generation to establish the next generation. The Wright-Fisher model is used to predict the genetic consequences of genetic drift as well as to provide a common quantitative metric for drift that can be compared among otherwise heterogeneous populations. Estimates of *Ne* in an actual population are made using an observable genetic pattern, such as chance disequilibrium between pairs of unlinked loci that varies with the strength of drift. Estimates of *Ne* in natural populations predict the effects of genetic drift, including the magnitude of random changes in allele frequencies, the rate of random fixation and loss of alleles, and the loss of heterozygosity [15]. *Ne* predicts the rate of increase in homozygosity as alleles undergo fixation and loss, which increases the potential for inbreeding depression. *Ne* also predicts the ability of populations to respond to natural selection and undergo adaptation, also known as their evolutionary potential [17].

Franklin [18] and Soulé [19] proposed that $Ne \geq 50$ for a single isolated population is required for short-term persistence, because it minimizes the risk of deleterious effects from inbreeding depression. At $Ne < 50$, an isolated population can enter an "extinction vortex" [20]. Franklin [18] also proposed that $Ne \geq 500$ is needed for healthy evolutionary potential and long-term persistence. Known as the "50/500 rule", these thresholds have been used as a guiding principle in conservation for assessing minimum viable *Ne* and to determine the extinction risks of populations and species [21]. These minima, however, have been revised upward. Frankham et al. [22] indicate that $Ne = 50$ is inadequate for preventing inbreeding depression over five generations in the wild, with $Ne \geq 100$ being required to limit loss in total fitness to $\leq 10\%$. Caballero et al. [23] suggested that $Ne \approx 70$ is sufficient to avoid extinction due to inbreeding depression, when considering that deleterious recessive alleles in homozygous genotypes will be purged by natural selection, reducing inbreeding depression over time. Frankham et al. [22] argued that several independent lines of theoretical and empirical evidence indicate that at least $Ne = 1000$ is required to maintain initial evolutionary potential in perpetuity. Pérez-Pereira et al. [24] indicate $Ne \geq 500$ is necessary for long-term persistence for species with moderately high reproductive rates; and $Ne \geq 1000$ for species with low reproductive rates. Previously, Lande [25] argued that $Ne \geq 5000$ was necessary to retain evolutionary potential; and Lynch and Lande [26] suggested a range between 1000 and 5000 (see [21] for the meaning of these thresholds in conservation). *Ne* can be estimated from genetic data, which are slowly accruing for inland capture fisheries of Africa. Estimates of the *Ne* of fish stocks can be used to assess the genetic risk of extinction and to inform conservation and management strategies for sustainable use. The present study focuses on the estimation of *Ne* for an important inland fisheries species of western Africa.

The African bonytongue, *Heterotis niloticus* (Cuvier, 1829), supports significant commercial and subsistence fisheries in western Africa. Nigeria leads wild captures of this fish (Table 1; [27]), totaling 409,784 metric tons between 1990 and 2021 (85.3% of the total catch in Africa during that period), of which 23,875 metric tons were caught in 2021 (86% of the total catch in Africa that year). Benin ranked second for *Heterotis* yields, with 21,640 metric tons captured between 1987 and 2021 (5% of the total caught in Africa during that period), including 1095 tons in 2021 (Table 1). In Cameroon, this fish naturally occurs in the Sudano-Sahelian zone (Far North and North regions), from where it has been introduced to other regions in the country (i.e., Centre, South, and Littoral), where it supports subsistence fisheries [28,29]. Although FAO does not track capture statistics for *H. niloticus* in Cameroon, historically, it has been an important capture species in the Logone River in the Far North [30], and Benue River in the North [31] where it occurs naturally. This fish was introduced into the Nyong River (Centre region) in 1958 [32], where it supports an important fishery, with reports of 60 tons landed near Ayos in 1976 [32], ~616 tons/year prior to 1984 in Akonolinga, and ~240 tons/year from the middle Nyong River during 2004–2005 (reviewed in [28]). Overexploitation and declines in *Heterotis* stocks have been reported for Nigeria [33], Benin [34], and other West African countries [35].

Genetically differentiated populations of the African bonytongue, suggested to represent different conservation/management units, have been identified in Benin [36], Nigeria [37], and Cameroon [38]. These populations provide important food resources for local communities and the sustainability of some stocks appears threatened [36,37]. Herein, we estimate *Ne* for these populations to assess the risks of inbreeding depression and loss of evolutionary potential.

2. Methods

2.1. Datasets

We used published genotypic datasets of *Heterotis* populations (Figure 1; Table S1) from Benin [36], Nigeria [37], and Cameroon [38]. The study in Benin identified the following three genetically differentiated populations: Niger River at Malanville; Mono River; and Oueme-Sô river-floodplain system. The study in Nigeria identified the following four genetically differentiated populations: Kainji Lake; Epe Lagoon; Igbokoda River; and Ethiope River. The study in Cameroon examined the following five populations: Logone River (Far-North region); Benoue River (North region); Nyong River (Centre region); Dja and Lobo Rivers (South region); and Nkam River (Littoral region). Wikondi et al. [38] report no genetic differentiation among Centre, Littoral, and South populations. However, we assessed genetic differentiation among the five populations from Cameroon using the software GenAlEx v. 6.51b2n [39], and found significant differences in all pairwise comparisons (Table S2). Table 2 shows the year of collection for each locality, sample size, number of microsatellites, number of polymorphic microsatellites (only polymorphic microsatellites were used in our analyses), and genetic diversity measures.

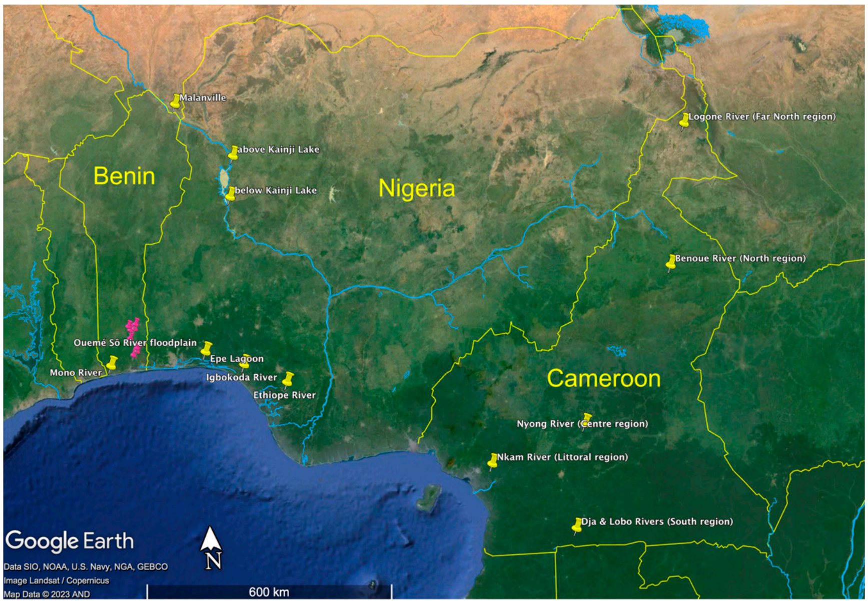

Figure 1. Map of the study area depicting sampled localities in Benin, Nigeria, and Cameroon. Collectively, the pink pins represent the Ouemé-Sô river-floodplain system.

Table 2. Information of Benin, Nigeria, and Cameroon datasets.

Country	Population	Year	SSRs	N	Nt	*Na*	*Nea*	*Ho*	*He*	*uHe*	*F*
Benin											
	Ouemé-Sô river-floodplain system	2008 2010	8	184	74	9.25	3.66	0.60	0.69	0.69	0.12
	Mono River	2009	8	15	47	5.88	3.67	0.59	0.65	0.67	0.11
	Malanville, Niger River	2009	6 (2)	12	28	3.50	2.05	0.34	0.41	0.43	0.21
Nigeria											
	Kainji Lake	2018	9	23	75	8.33	4.56	0.73	0.70	0.72	−0.03
	Epe Lagoon	2018	8 (1)	20	46	5.11	2.65	0.44	0.47	0.48	0.06
	Igbokoda	2018	9	15	51	5.67	2.55	0.47	0.54	0.56	0.18
	Ethiope River	2018	8 (1)	19	44	4.89	2.58	0.50	0.49	0.51	−0.01
Cameroon											
	Logone River (Far-North)	2020	7	18	41	5.86	3.20	0.71	0.64	0.66	−0.14
	Benoue River (North)	2020	7	24	42	6.00	3.67	0.66	0.69	0.71	0.04
	Nyong River (Centre)	2020	7	20	25	3.57	2.67	0.63	0.56	0.58	−0.07
	Nkam River (Littoral)	2020	6 (1)	22	26	3.71	2.70	0.51	0.53	0.55	0.03
	Dja and Lobo rivers (South)	2020	7	12	24	3.43	2.31	0.42	0.47	0.49	0.06

Year = collection year; SSRs = number of polymorphic microsatellites used (number of monomorphic loci in original study in parenthesis); N = sample size; Nt = total number of alleles; *Na* = average number of alleles; *Nea* = effective number of alleles; *Ho* = observed heterozygosity; *He* = expected heterozygosity; *uHe* = unbiased expected heterozygosity; *F* = inbreeding coefficient.

2.2. Evaluation of Ne Estimators for Genotype Data

Because there are numerous estimators of *Ne* that employ two-locus disequilibrium (r^2), we carried out simulations using SpEED-Ne [40] to evaluate which estimators exhibited the greatest accuracy and precision with sample sizes of individuals, loci, and numbers of alleles per locus that approximated those of sampled populations. Time-forward simulations were carried out following Hamilton et al. [40], generating microsatellite genotype data sets with true *Ne* equal to 10, 25, 50, 100 and 250, each with 250 independent replicates. Simulated data sets all had eight loci with six initially equally frequent alleles per locus, a 5% rate of null alleles, and a sample size of individuals equal to true *Ne*.

With multilocus genotype data, two-locus disequilibrium is used to estimate *Ne* according to $1/[3r^2 - (r^2\ correction)]$ where the r^2 *correction* is used to subtract disequilibrium caused by mechanisms other than genetic drift, such as finite sample size or within-locus disequilibrium [40]. We compared four categories of *Ne* estimators based on (1) disequilibrium adjusted for within-locus excess homozygosity (r^2_Δ) and r^2 *correction* equal to the expected disequilibrium among unlinked loci in a finite sample of individuals, $E\left[r^2\right]$; (2) disequilibrium estimated with a composite haplotype table (r^2_c) and $E\left[r^2\right]$ as the r^2 *correction*; (3) r^2_Δ and the sample size of individuals as independent variables in second-order regression equation fits proposed by Waples [41] to adjust for bias and $E\left[r^2\right]$ as the r^2 *correction*; and (4) r^2_c and an r^2 *correction* estimated by median r^2_c observed for 5000 random permutations of genotypes (pm). Within each of the four categories, estimates of disequilibrium were either allele frequency thresholded (AFT), using AFT = 0.05, or weighted (AFW), to adjust for the disproportionate impact of low-frequency alleles, and either lacked or included (UB) a small sample bias adjustment $S/(S-1)$ where S is the average of the number of sampled genotypes for each locus pair. Detailed descriptions of these estimators are given in the study by Hamilton et al. [40].

We report *Ne* point estimates and corresponding confidence intervals (CI) obtained with the estimator(s) that performed best in simulations parameterized with sample sizes of individuals and numbers of loci similar to those for most populations in our study. Reported confidence intervals were obtained using the following three jackknife confidence interval estimation methods implemented in SpEED-Ne: over all locus pairs; over individuals; and over loci. We updated SpEED-Ne to version 2.6 [42] to provide jackknife confidence intervals where the upper and lower bound r^2 estimates have an $S/(S-1)$ adjustment for small sample size bias.

For comparison, *Ne* was also estimated for all populations using the widely applied second-order regression equation fits using sample size and r^2 as independent variables [41,43], as implemented in the program NeEstimator v.2.1 [44]. *Ne* values were estimated assuming random mating and using a minimum allele frequency (MAF) threshold value of 0.05. Confidence intervals for *Ne* values were estimated using NeEstimator v.2.1 based on a delete-one-locus jackknife to estimate the variance in r^2 to give a parametric confidence interval using the chi-square distribution ([43], see [40]). We used SpEED-Ne to estimate the effective number of independent locus pair comparisons (n') used for this confidence interval, and compared it with the actual number of unordered pairwise comparisons of loci ($n_{pw} = [n(n-1)]/2$; where n is the number of loci used) to evaluate how well the assumptions of the chi-square CI were met (i.e., $n \leq n' \leq n_{pw}$; [40]). In our datasets, n varied between 6 and 9, resulting in n_{pw} of 15–36 unordered locus pairs.

3. Results

Simulated genotype data (Figures S1–S3) showed that for true *Ne* values of 10, 25, and 50, the r^2_Δ UB-AFT estimator, followed by the r^2_Δ UB-AFW estimator, provide the most accurate estimates among the 16 compared (i.e., median slightly biased, smaller quartile ranges, and markedly fewer outliers). At higher true *Ne* values (i.e., 100 and 250; Figures S4 and S5), an increasing number of the 16 estimators showed improvement in accuracy and precision. As nine of the *Ne* point estimates obtained with the empirical data were <50, we report results with the r^2_Δ UB estimators (Table 3).

Table 3. Genetic effective population size (*Ne*) estimates and 95% confidence intervals (CIs) in parenthesis. Estimates employed a minimum allele frequency threshold of 5% (AFT) or were allele-frequency-weighted (AFW) to adjust for the influence of low-frequency alleles. For the first two columns, *Ne* estimates were adjusted for small sample bias by $S/(S-1)$, where S is the average of the number of sampled genotypes for each locus pair, and percentile jackknife CIs are shown in this order: over all locus pairs; over individuals; and over loci. For the third column, *Ne* estimates were based on second-order regression equation fits using sample size and r^2 as independent variables [41], and CIs were based on a jackknife over loci and a parametric chi-square distribution based on an effective number of independent locus comparisons (n').

Population	r^2_Δ **AFT** N_e	r^2_Δ **AFW** N_e	r^2_Δ **AFT** N_e **Waples**
Nigeria Populations:			
Kainji Lake	22.7 (20.8–25.6) (16.9–23.8) (20.4–28.1)	24.9 (23.3–29.0) (19.3–24.9) (21.1–32.9)	23.7 (9.3–278.0) n' = 460.8
Epe Lagoon	19.1 (17.5–22.2) (13.9–19.1) (17.5–21.7)	19.6 (17.7–23.8) (13.9–21.3) (15.3–25.3)	23.1 (8.4–∞) n' = 351.5
Igbokoda	36.1 (30.2–74.6) (15.0–49.3) (27.1–89.6)	47.4 (38.6–624.8) (16.6–38.0) (32.0–∞)	288.0 (11.6–∞) n' = 249.0
Ethiope River	24.4 (23.0–29.2) (16.3–31.2) (20.9–34.7)	25.5 (23.1–45.4) (15.6–29.1) (20.3–∞)	42.2 (8.2–∞) n' = 464.9
Benin Populations:			
Oueme-Sô river-floodplain	1867.8 (1292.1–31,044.5) (1083.2–2750.7) (984.5–∞)	∞ (∞–∞) (5583.6–∞) (1518.8–∞)	∞ (448.0–∞) n' = 545.8
Mono River	15.0 (13.8–17.2) (10.3–13.7) (11.8–18.3)	17.8 (16.3–20.1) (12.3–17.3) (15.9–23.2)	22.9 (9.1–2310.1) n' = 409.0
Malanville	9.6 (7.8–35.1) (5.8–9.1) (5.9–∞)	8.9 (7.3–13.8) (6.0–10.6) (7.0–44.1)	27.0 (2.1–∞) n' = 30.4
Cameroon Populations:			
Logone Riv. (Far-North)	27.8 (24.7–37.3) (17.6–33.9) (21.7–40.0)	40.6 (33.6–73.2) (23.1–41.5) (28.9–178.8)	61.3 (11.1–∞) n' = 376.0
Benoue River (North)	36.2 (32.9–42.0) (23.8–36.1) (29.9–45.1)	29.3 (27.5–36.1) (19.0–39.5) (23.2–40.9)	48.0 (14.6–∞) n' = 753.1
Nkam River (Littoral)	∞ (181.7–∞) (57.1–∞) (71.4–∞)	546.6 (125.9–∞) (41.5–∞) (70.9–∞)	∞ (11.3–∞) n' = 186.2
Dja and Lobo Riv. (South)	5.1 (4.8–5.9) (3.5–6.1) (4.8–5.6)	4.9 (4.5–5.6) (3.4–6.9) (4.5–5.8)	2.6 (0.7–∞) n' = 175.9
Nyong River (Centre)	171.2 (112.1–2392.1) (40.8–∞) (64.2–∞)	221.4 (103.9–∞) (30.5–∞) (109.1–∞)	∞ (12.6–∞) n' = 334.2

Ne point estimates < 50 were obtained for the following nine localities ordered from smallest to largest (estimations using r^2_Δ UB with AFT; CIs for jackknifing over all locus pairs): Dja and Lobo rivers 5.1 (4.8–5.9); Malanville 9.6 (7.8–35.1); Mono River 15.0 (13.8–17.2); Epe Lagoon 19.1 (17.5–22.2); Kainji Lake 22.7 (20.8–25.6); Ethiope River 24.4 (23.0–29.2); Logone River 27.8 (24.7–37.3); Igbokoda River 36.1 (30.2–74.6); and Benoue River 36.2 (32.9–42.0). *Ne* point estimates for the three remaining populations were 171.2 (112.1–2392.1) for Nyong River; ∞ (181.7–∞) for Nkam River; and 1867.8 (1292.1–31,044.5) for the Oueme-Sô river-floodplain system.

For comparison, we also reported *Ne* estimated with Waples [41] regression equation fits implemented in NeEstimator v2.1 (Table 3). The chi-square CI often had an infinite upper bound and effective sample of locus pairs (n') much greater than the actual number of locus pairs (n_{pw} = 15–36) used to estimate r^2_{Δ}.

4. Discussion

Ne point estimates for nine of the twelve *H. niloticus* populations examined are below the minimum recommended to avoid the potential deleterious effects of inbreeding depression (original criterion $Ne \geq 50$, revised to $Ne \geq 100$; [22]); and, well below the minimum recommended for populations to retain evolutionary potential (original criterion $Ne \geq 500$; revised to $Ne \geq 1000$ [22]; and $Ne \geq 5000$ [25]). These populations are Malanville and Mono River, in Benin; Kainji Lake, Epe Lagoon, Ethiope River and Igbokoda River, in Nigeria; and Logone River, Benoue River, and Dja and Lobo rivers, in Cameroon. The CI lower bounds for Nyong River and Nkam River, in Cameroon, were below the recommended values to retain evolutionary potential, and for some methods, below the recommended values to avoid inbreeding depression. In addition, the *Ne* point estimate for Nyong River (171.2) was well below the '500' threshold to retain evolutionary potential, whereas the *Ne* point estimate for Nkam using AFW (*Ne* = 547) was below the '1000' threshold. Nyong River and Nkam River populations are introduced (see below). The CI lower bounds for the Oueme-Sô river-floodplain system population were below or closely above the '1000' threshold to retain evolutionary potential. Therefore, our results suggest that urgent conservation and management plans are needed to guarantee the survival and sustainability of most of the *H. niloticus* populations examined.

The computer simulations we conducted indicated that *Ne* estimates obtained using ${r^2}_{\Delta}$ with AFT and the small sample size bias correction (UB) are expected to be fairly precise for the genotypic data used, especially when $Ne \leq 50$, which encompasses the *Ne* estimates we obtained for nine populations. This is consistent with expectations on the performance of the disequilibrium N_e estimation approach, with which precise estimates for relatively small (*Ne* < 200) isolated populations can be obtained, and small populations are not likely to be mistaken for large ones [45]. Waples (Guest Box 10 in [17]) indicates that if *Ne* is relatively small (<100), reasonably precise estimates can be obtained using samples of 25–50 individuals with 5–10 moderately variable loci; however, considerably more data are needed to achieve comparable precision if the population is relatively large (*Ne* ∼ 500–1000 or higher). Gilbert and Whitlock [46] used simulations to compare the performance of seven *Ne* estimation methods under different scenarios of migration with three different *Ne* values (50, 500 and 5000), and found that the disequilibrium method (implemented in NeEstimator v2.0 in their study) outperformed the other methods in conditions of isolation for *Ne* = 50. This method also performed well in scenarios of low migration and small *Ne*. Accordingly, because the *Heterotis* populations studied herein exhibit genetic differentiation consistent with isolation [36,37], the small *Ne* estimates we obtained are likely reflective of the true *Ne*. Nonetheless, in taxa with overlapping generations, *Ne* estimates based on mixed-age (i.e., multi-cohort) adult samples, which is likely the case for the *Heterotis* samples examined herein, tend to be biased downwardly [47]. The simulations of Waples et al. [47], based on random samples of adults from 22 taxa representing different taxonomic groups with different life histories (invertebrates, plants, and major vertebrate groups, including fishes), revealed that for most, estimated *Ne* was 9–35% below true *Ne*, with only two outliers (mosquito and primrose) below this range (52% and 45% below true *Ne*, respectively). The life history of *Heterotis* likely resembles more closely that of the non-outlier taxa of Waples et al. [47]. However, even if we were to consider a downward bias as extreme as 60%, our *Ne* < 50 estimates would be upwardly corrected to a range between 13 (for Dja and Lobo rivers, whose *Ne* point estimate was 5.1) and 90 (for Igbokoda and Benoue rivers, whose *Ne* point estimates were ~36), and thus fall below the 100 threshold for inbreeding depression.

Our computer simulations show that the *Ne* estimator used in NeEstimator (defined in [43], Table 1) performed much worse for true *Ne* values of 10, 25, and 50 than the r^2_Δ UB estimators with AFT or AFW, and all other estimates in general (Figures S1–S3). Hamilton et al. [40] made similar observations with simulated data for small *Ne* but different sample sizes of simulated microsatellite loci with a range of null allele frequencies. Nonetheless, critically small *Ne* point estimates (range 2.6–61.3) were obtained with NeEstimator for the populations for which *Ne* was <50 with r^2_Δ UB estimators, with the exception of Igbokoda, for which NeEstimator's *Ne* = 288 (Table 3). *Ne* point estimates obtained with NeEstimator were infinity for Nyong River, Nkam River, and the Ouemé-Sô river-floodplain system, the populations for which the largest (and in some cases infinite) r^2_Δ UB estimates were obtained. Several observations suggest that Igbokoda is a small (*Ne* < 50) population (i.e., concordant with SpEED-Ne's r^2_Δ UB point estimates). Firstly, the Igbokoda sample was small (n = 15), and its inbreeding estimate was high *(F* = 0.18; Table 2), features that could have biased estimates by NeEstimator. Nonetheless, NeEstimator yielded a very small CI lower bound (i.e., 11.6). Secondly, Igbokoda is located halfway between the other two southern Nigeria populations examined (separated by ~100 Km of each), Epe Lagoon and Ethiope River, for which *Ne* point estimates < 50 were obtained. For these populations, we had larger sample sizes (n = 20 and 19, respectively), and their inbreeding coefficient *F* was lower (0.06 and −0.01, respectively; Table 2). There is no reason to expect a larger *Ne* for the Igbokoda population, as fish from this locality face severe threats for their conservation (see discussion below). Finally, the high level of inbreeding that Igbokoda appears to be experiencing is inconsistent with a large *Ne*.

Compared to the jackknife percentile CIs from SpEED-Ne, NeEstimator yielded much larger upper bound estimates for two populations, and infinite upper CI bounds for the remaining ten populations. A tendency to report infinite upper CI values using the method implemented in NeEstimator has been observed in other fish studies (e.g., [48]). This has also been observed in very small endangered populations (i.e., several tens of individuals), for which the *Ne* point estimates and CI lower bounds were finite and consistent with historical census size estimations, and comparable to those obtained with other *Ne* estimation methods [36]. Disequilibrium methods can produce negative estimates, which are reported as infinite. This occurs because to estimate *Ne*, the expected contribution of disequilibrium from sources other than drift is subtracted from the total estimated disequilibrium or r^2. Accordingly, if the sample of individuals and loci is relatively small given the true *Ne*, mean disequilibrium (r^2) can be smaller than the correction for disequilibrium due to sources such as a finite sample size, leading to a negative *Ne* estimate [45]. Nonetheless, even in cases where the point estimate is negative, the CI lower bound generally will be finite and can provide useful information about plausible limits of *Ne* [45]. The CI method implemented by NeEstimator is considered a 'pseudo-jackknife', in that the variance of r^2 is estimated by resampling but then used to construct a parametric confidence interval [40,49]. In the present study, the effective number of unordered locus pairs (n'; Table 3) was much greater than the actual number (n_{pw} = 15–36), showing that the assumptions of the chi-square CIs were not met. The standard delete-one jackknife confidence intervals in SpEED-Ne provided alternatives to estimate confidence intervals with fewer distributional assumptions. Hamilton et al. (2018) showed that percentile CIs jackknifing over loci and jackknifing over individuals for the r^2_Δ estimators provided >97% confidence interval coverage for *Ne* from 10 to 250. For this study, we expanded SpEED-Ne to compute jackknife CIs for disequilibrium estimates with a simple adjustment for small sample sizes. The SpEED-Ne jackknifing methods should therefore be suitable for datasets with the characteristics (i.e., sample size, number of loci, number of alleles) similar to those of this study.

It is possible that *Ne* of the Nyong River and Nkam River populations are critically small, as suggested by the CI lower bounds, but limited data prevented accurate point estimates. These populations, along with the population of Dja and Lobo rivers (South region), are introduced, probably from wild populations in northern Cameroon. According to Depierre and Vivien [32], *Heterotis* was introduced from northern Cameroon populations to a fish farming station in Melen, a suburb of Yaounde, in 1955, and from there, ~20 fry were released into the Nyong River in 1958. Subsequently, in 1961, an accidental spill from a fishpond released several hundred fry into the Nyong River. These authors also indicate that *Heterotis* appeared in the Lower Sanaga River (Littoral region) around 1968, probably from the lower course of the Nyong, as the two rivers are connected at the mouth of the Sanaga through mangrove channels during periods of high water. It is possible that from the Sanaga, *Heterotis* colonized other rivers in the Littoral region, such as the Nkam, and that the Dja and Lobo rivers were also colonized by individuals related to the Nyong population. Indeed, STRUCTURE analyses of the five Cameroon populations found two main clusters, one corresponding to the native populations (Benoue and Logone rivers), and the other to the introduced populations, suggesting a common origin for the introduced populations [38]. Small *Ne* point estimates were obtained for Benoue River (36.2) and Logone River (27.8); thus, it is reasonable to expect the *Ne* of the introduced populations to be smaller, due to expected founder events typically associated with introductions. The three Cameroon introduced populations have notably lower allelic diversity and heterozygosity (*Na* = 3.4–3.7; *He* = 0.47–0.56; Table 2) than their northern Cameroon native counterparts (*Na* = 5.9–6.0; *He* = 0.64–0.69; Table 2); consistent with founder events. In addition, for Nkam River, one of the seven loci used by Wikondi et al. [38] was monomorphic, and therefore was discarded for *Ne* estimations. The negative (infinity) point estimate obtained for Nkam River may stem from its limited genetic information, and more individuals and/or microsatellites may be needed to obtain a reliable estimate. Nonetheless, some of the CI lower bounds of Nkam River and Nyong River indicate that it is plausible these populations also may be at risk of inbreeding depression. Congruent with a history of introductions and genetic diversity reduction, a very small *Ne* estimate, the smallest in this study, was obtained for Dja and Lobo rivers (5.1).

The Ouemé-Sô river-floodplain appears to have the largest *Ne* (1868) of all populations examined. The area sampled is vast (~1680 Km^2; with sampling localities separated by up to 75 Km) with many interconnected water bodies, including the two major channels, five permanent lakes, and numerous secondary channels and seasonal floodplain pools. Nonetheless, CI lower bounds were close to the threshold for maintaining evolutionary potential. We note that the Benin samples used to estimate *Ne* were collected between 2008 and 2009, and the ones from Nigeria were collected in 2018. Thus, it is possible that the present-day *Ne* of these populations is even smaller than our estimates, especially for the Benin stocks, which used samples collected over a decade ago, and considering that fishing pressure and habitat impacts have continued or increased since then.

Ne estimates of *Heterotis* populations in West Africa are, in general, much lower than those reported for the region's wild populations of Nile tilapia (*Oreochromis niloticus*), an important species supporting capture fisheries and aquaculture production [50]. For Nile tilapia examined across West Africa, including eight countries representing the major catchments of the Volta, Niger, Senegal, and Gambia river basins, *Ne* was below the threshold for long-term genetic risks (range: 56–352 individuals), whereas 10 stocks (43.5%) were below the revised threshold of 100 for short-term genetic risk; and 14 (60.9%) had CI lower bounds below 100 (the lowest was 30.3). The *Ne* point estimate for Nile tilapia at Malanville was much larger (*Ne* = 236) than that for *Heterotis* from the same location (*Ne* = 9.6). Nile tilapia at Malanville might be part of a more widely distributed metapopulation, whereas *Heterotis* at this locality might correspond to a more isolated population. Nile tilapia from Malanville and Mopti (Mali), another locality in the Niger River located ~1400 Km upstream, show high genetic similarity, suggesting high levels of gene flow. Nile tilapia *Ne* estimated at Mopti is very similar (*Ne* = 289) to that in Malanville; thus, it is possible that such *Ne* estimates reflect the *Ne* of a broader metapopulation. According to Waples and England [51], "LD estimates of *Ne* accurately reflect local (subpopulation) *Ne* unless $m \geq$ 5–10%. With higher m, *Ne* converges on the global (metapopulation) *Ne*". For *Heterotis*, high genetic differentiation appears to occur at comparatively shorter distances within the Niger River, i.e., between Malanville and Kainji Lake (~230 km), and between Kainji Lake and the lower Niger portion (~700 km).

We note that heterozygosity and allelic diversity are poor predictors of short- and long-term genetic risks, underscoring the importance of *Ne* estimates. Heterozygosity in Kainji Lake was the highest among all populations (*uHe* = 0.72), with the second highest allelic diversity (*Na* = 8.33), yet *Ne* was only 23. Similarly, for Nile tilapia, the locality with the highest estimated *Ne* had comparatively low heterozygosity and allelic diversity among the Nile tilapia populations examined. Severe declines in *Ne* can occur without a significant loss of genetic diversity [52].

Critically small values of *Ne* have also been reported for populations of *Arapaima gigas*, the African bonytongue's closest living relative, and the only other member of Arapaiminae, which is distributed throughout the Amazon River basin in South America. Farias et al. [53] estimated *Ne* for 19 populations of this fish along the Amazon River basin and three locations in the Araguaia-Tocantins basin. For 19 populations, *Ne* ranged between 0.8 and 48.8 (average = 15.6); for an additional population, *Ne* was 95; and for the remaining two, *Ne* was undetermined (infinity), probably due to small sample sizes and limited genetic diversity. Similar to *Heterotis*, high levels of genetic population differentiation were detected among these *Arapaima* populations; 226 of 231 pairwise F_{ST} comparisons were significant (range = 0.02–0.57; average = 0.22). An effect of floodplain connectivity and geographic scale on the patterns of genetic differentiation is observed for both species [36,37,53,54]. Genetic homogeneity occurs for both species at a fine scale (e.g., within the same floodplain, such as in the Oueme-Sô river-floodplain system for *Heterotis*); at a meso-scale (e.g., in separate floodplain systems, such as in the southern Nigerian locations for *Heterotis*), they exhibit low but significant values of genetic differentiation; and the highest levels of genetic differentiation occur at the largest geographic scale (e.g., >1300 km in *Arapaima* and >510 km in *Heterotis*). These patterns suggest limited migration, with individuals of both species probably confined mainly to a single floodplain [34,55]. High isolation, overexploitation and critically small *Ne* of populations is concerning for both species, the only members of Arapaiminae.

Implications for Conservation and Management

Overfishing and habitat destruction likely pose the greatest threats for *Heterotis* stocks. This species is highly sought because of its high protein content and firm flesh [56]. Overfishing has been documented in Kainji Lake [57,58], Malanville [59], Oueme-Sô river-floodplain system [60], Mono River [61], and Epe Lagoon [62]. Illegal fishing activities exacerbate this problem. In Kainji Lake, illegal practices include the use of prohibited gear (e.g., small mesh size nets and destructive fishing gear), fish poisoning, and explosives [63]. In the Oueme-Sô river-floodplain system, fish traps are placed near active *Heterotis* nests to capture brooding adults [34]. Pollution also impacts *Heterotis* stocks in some regions [64–67].

Our results indicate that *Ne* values for most populations of *Heterotis niloticus* in Nigeria, Benin, and Cameroon are below the recommended thresholds to avoid short- and long-term genetic risks. In addition, high inbreeding (F_{IS}) was reported for the following four local stocks [36,37]: Igbokoda (F_{IS} = 0.18; the highest inbreeding among Nigerian populations); Malanville (F_{IS} = 0.20); Mono River (F_{IS} = 0.13); and Oueme-Sô river-floodplain (F_{IS} = 0.13). *Ne* estimates of *Heterotis* are comparable to those of endangered species [68,69]. For example, *Ne*, heterozygosity, and F_{IS} values estimated for populations of an endangered snake [70] were similar to estimates for African *Heterotis*. Small *Ne* estimates of genetically differentiated *Heterotis* populations indicate that conservation and management actions are urgently needed, with special consideration of risks associated with inbreeding depression (but see [71]).

Heterotis niloticus is currently listed as a species of *Least Concern* by the International Union for Conservation of Nature (IUCN) [72], a designation that applies to the species as a whole. It is important that future assessments of this species consider (1) that this species is comprised of multiple genetically differentiated populations with small ranges relative to the species' global distribution, many of which face multiple threats (i.e., overexploitation, habitat destruction, pollution); (2) most populations examined to date reveal *Ne* point estimates below the critical long-term threshold of 500, revised to 1000, for retaining evolutionary potential, and most are also below the critical short-term threshold of 50 and 100 for preventing inbreeding depression; and (3) when considering the *Ne* CI lower bounds of most populations, their vulnerability to inbreeding depression appears more serious.

Growth of isolated populations may allow their *Ne* to increase [73], and fishing restrictions may be required to allow stocks to recover [74]. Gene flow between populations may also boost *Ne* [75]; however, given that stocks are genetically differentiated, gene flow could result in outbreeding depression [17] and erosion of locally adapted variation. Furthermore, translocation of stocks erodes distinct population genetic structures, which runs counter to a goal of the Convention on Biological Diversity's post-2020 global biodiversity framework [76]. Results of the present study provide baseline information for continued monitoring of *Ne*. Future research should also examine genetic diversity of other *Heterotis* populations, as well as monitor *Ne* of genetically distinct populations. *Heterotis* is just one of several fishes important for subsistence and commercial fisheries in Africa, and the genetic diversity of other fish species also needs to be investigated.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes9060196/s1, Figures S1–S5: *Ne* estimates from simulated microsatellite genotype data with true *Ne* = 10, 25, 50, 100, and 250, respectively; Table S1: Individual genotypes and allele frequencies per population per country; Table S2: Pairwise genetic differentiation among populations from Cameroon.

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