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Abstract: Despite the global contribution of aquaculture as an important pillar for food and nutritional security, its rapid growth has been hampered by inadequate and poor-quality seed due to inbreeding, hybridization of related stocks, and poor-quality broodstock. Phenotypic and genotypic characterization of Nile tilapia in natural water bodies of Uganda has been studied but research gaps remain on the morphometric characterization of farmed *O. niloticus* in Uganda, which hinders selective breeding initiatives. This study characterized *O. niloticus* from various farmers in Uganda's Southwestern Highland Agro-Ecological Zone using their morphometric traits. In this study, 8 morphometric traits were taken on each of the 258 fish samples collected with at least 30 samples sampled from each pond system in the zone. The morphometric data generated were analyzed using multivariate analysis. In order to delineate the populations from the different ponds, Discriminant Function analysis (DFA) was performed on the dataset and distribution plots generated to test on the purity of the strains. There were significant differences in the morphometric traits among the populations with the Victoria fish population having the highest standard length while the Kyoga population had the highest weight. Morphometric characterization grouped the fish into three clusters, indicative that fish farms in the zone possibly had different sources of fish seed. The condition factor, which is a measure of wellbeing of the fish, showed that all the populations displayed an isometric growth which is indicative of the fish growing in length as it is in weight. The results of this study provide evidence that local *O. niloticus* from different farms display differences in body shape as a result of using fish seed from different sources. This aspect can be applied in selective breeding programs after establishing the genetic structure of the farmed Nile tilapia stock.

Keywords: *O. niloticus*; strains; morphometry; variations; condition factor

Key Contribution: The manuscript contributes morphometric baseline information required for Nile tilapia selective breeding program. Furthermore, the knowledge generated is key while designing conservation measures of the Nile tilapia in aquatic ecosystems.

1. Introduction

Aquaculture is the world's fastest growing agricultural and food processing sector, and serves a critical role in developing economies through its value chain linkages in promoting food and nutrition security, rural development, and poverty alleviation [1]. Tilapia is the world's second most cultured fish species, after carp [2,3], and is currently cultured in more than 140 countries [4]. China, the Philippines, Taiwan, Indonesia, Thailand, and Egypt are the leading suppliers of this agro-food product, with an estimated global production of 179 million metric tons [5].

Nile tilapia is considered as the ideal fish species for aquaculture mainly due to its rapid growth, high fecundity, ability to resist poor water quality, and good performance under sub-optimal nutritional conditions [6–8]. Additionally, the fish possesses adaptive life history characteristics especially fast growth, high fecundity, and large egg sizes that have few predators, as well as high dietary overlap across size class, habitat, and season [9,10]. Its omnivorous nature further prompts it to utilize the available resources, making it easy and affordable to grow even for small-scale farmers. Apart from being a model aquaculture species, the expansion of tilapia culture globally is also fueled by high genetic diversity in the available natural tilapia germplasm [6] which makes it a good candidate for genetic manipulations for product improvement and thereafter the expansion of profit margins. Besides the economic returns from aquaculture, tilapiines have also been adopted for use as control agents for aquatic vegetation and elimination of unwanted aquatic fauna such as snails and mosquitoes [8].

The Nile tilapia (*Oreochromis niloticus* Linnaeus, 1758) is native to African freshwater systems and the Middle East, with its natural range encompassing the Nile River Basin, from the headwaters in East Africa to the Nile Delta in Egypt and lakes and streams connected to these drainages [11]. In East Africa, *O. niloticus* is native to Lakes; George, Edward, Albert, Kivu, Tanganyika, Turkana, and Baringo, and to River Nile [11], and the species has been introduced to almost every tropical country in the world for aquaculture purposes [12].

The fisheries sector in Uganda is the second largest exchange earner for the country after coffee and contributes 12% to the agricultural gross domestic product (GDP) and about 3.0% to the National GDP. The investment in the sector is estimated at $200 million with employment of over 700,000 people [13]. Uganda has more than 350 fish species; Nile perch and Tilapia remain the most important, making up to 46% and 38%, respectively, of the total fisheries production [13]. Aquaculture production stands at around 111,000 tons, of which Largemouth African catfish and Nile tilapia contribute over 90% in the country [14]. The sector has experienced a significant increase in production attributed to the commercialization of the fisheries sub-sector which has witnessed an increase in the number of people venturing into fish farming [5].

Despite the aquaculture subsector being identified as an important pillar for food and nutritional security in the country, the rapid growth of the subsector is curtailed by inadequate quality seed with most hatcheries being plagued by inbreeding, hybridization of related stocks, and poor-quality broodstock [15,16]. In the context of farmed Nile tilapia, many studies on the domestication of this fish species have shown phenotypic variations in the subsequent generations with respect to the pure Tilapia strains of the brood stock [8,14,16,17]. This may be attributed to speciation due to change in the environment or formation of hybrids as a result of species interbreeding.

Consequently, there are questions on the possible deviations in the genetic and morphometric features of the organisms after several generations of recycling. Furthermore, without a clear known origin of the brood stock, the fish are most likely sourced from stocks suffering from genetic founder effects, as is common among farmed tilapia sources globally [18], a practice that is difficult to avoid under farm conditions. Consequently, the fish suffer from high levels of inbreeding, which is frequently exacerbated by hybridization because tilapias are prolific breeders in culture conditions. Inbreeding leads to loss of genetic variability [19] and inbreeding depression associated with lower growth rates, reduced fitness, low survival, and low fecundity [19].

Morphometric and meristic methods remain the simplest and most direct way of species identification and can be used as a measure of delineating fish species into strains/types [12]. Characterization of *O. niloticus* based on morphometric and meristic traits have also been reported in several studies [6,8,12,17,20,21]. These studies report the existence of both genetic and morphometric differences in the cultured *O. niloticus* fish species from similar indigenous fish species in the wild.

While the population characteristics (biology, abundance, condition factors, and the reproductive biology) of *O. niloticus* in the three lakes have been fairly well studied by other researchers—L. Victoria [14,22,23], L. Albert [23,24], and L. Kyoga [22,23]—research work on the morphometric characterization of farmed *O. niloticus* sourced from the natural water bodies in Uganda is still understudied and this hinders development of selective breeding programs. Therefore, this study focused on comparing the possible morphometric variations between *O. niloticus* populations from isolated pond systems with strains from three lake systems that had been isolated for two years and compared them with strains of *O. niloticus* cultured by farmers in the region. The study hypothesized that the morphometric traits would show no significant differences among the populations investigated. This study is significant as it will generate and contribute baseline information for strain improvement of the *O. niloticus* breed in East Africa. The study will further provide a broader diversity of knowledge on the phenotypic characteristics of the different *O. niloticus* strains in aquatic systems that could be used when designing conservation measures.

2. Materials and Methods

2.1. The Study Area

The fish samples in this study were collected from different farms located in South Western Highland Agro-Ecological Zone (SWHAEZ) in the districts of Kanungu and Kabale (Figure 1). SWHAEZ, formerly known as the Kigezi sub-region, consists of five districts; Kabale, Rubanda, Kisoro, Kanungu, Rukungiri, and Rukiga, located in southwestern Uganda. The zone lies between altitudes of 1262 and 3883 m above sea level. The zone experiences a bimodal rainfall pattern that ranges from 1000 to 1500 mm per annum. Kanungu and Rukungiri districts have analogous annual temperature in the range of 20.56 °C to 30.56 °C, while other districts in the region have an annual temperature range of 13.00 °C to 23.21 °C [25]. Therefore, these districts (Kabale and Kanungu) were selected owing to varying climatic conditions and as representation of the other districts in the zone. Both private and government fish farms were incorporated in the study. The private farms included; Butare, Rwabirundo and Nyamabare in Kabale district; Masya and Kambuga in Kanungu district (Figure 1). These farms were selected on account of their vast experience in fish farming and business for more than 10 years. Moreover, fish stocking data and farm history availed by the district fisheries officers and farmers in the respective districts indicated that stocking dates of the preferred farms were in a very close range; therefore, it was assumed that the fish sampled were in the same age bracket. At the time of sampling, all the fish in distinct localities had spent seven months under captive management.

Furthermore, Kyanamira aquaculture fish facility, located in the Kabale district, was also included in the study area. The facility is superintended by Kachwekano Zonal Agricultural Research and Development Institute of National Agricultural Research Organization. Most pertinently, the fish populations at Kyanamira facility were stocked and raised in independent pond systems with the native brood stock originating from three freshwater lakes in Uganda, namely, L. Victoria (Central Uganda), L. Kyoga (Central Uganda), and L. Kayumbu (South Western Uganda). The pond systems at the facility have a surface area of 800 m^2 with a stocking rate of 4.0 fish/m^{-3}. The water temperature in the ponds is maintained in a range of 21.13 °C to 23.03 °C while the dissolved oxygen (DO) level ranges between 4.24 and 5.76 mg/L. All the chosen fish farms were closely monitored where monthly sampling was performed to facilitate fish grading and sorting for invariable growth patterns.

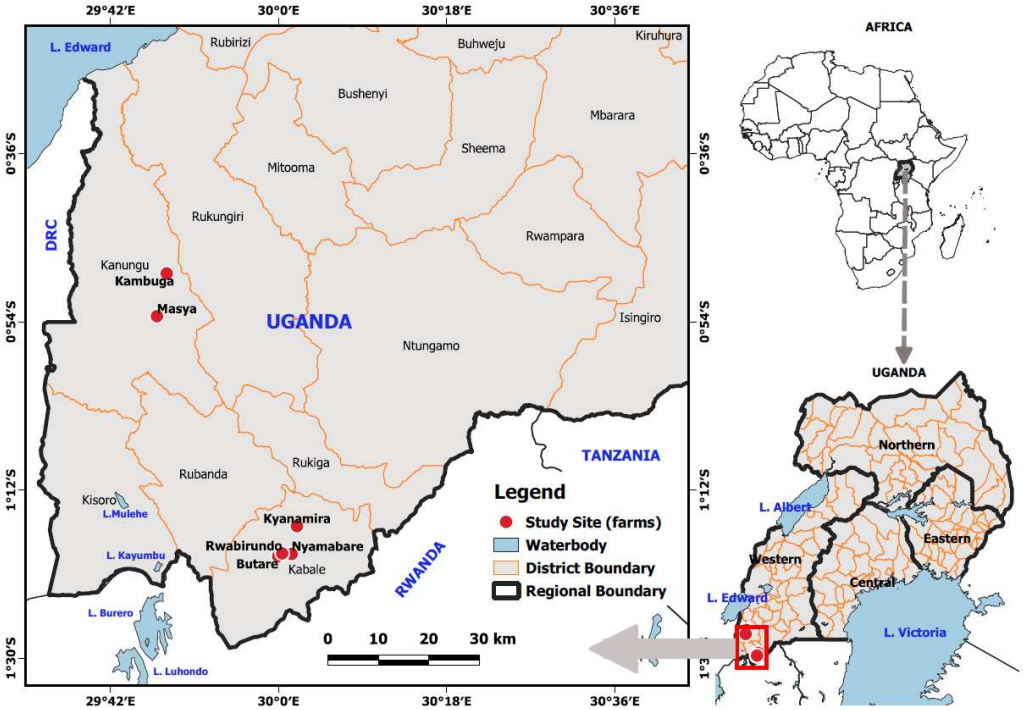

Figure 1. Map showing the sampling fish farms.

2.2. Research Design

This study was conducted by taking nine different body measurements of *O. niloticus* fish samples collected from three ponds systems between February and April, 2022. The fish samples were obtained from Kyanamira fish facility ponds holding the fish populations of three lakes, Kyoga, L. Victoria, and L. Kayumbu. Additionally, samples were obtained from five randomly selected farmers from Kabale and Kanungu districts in the region. The selected fish were those at maturity, swollen stomachs for the females and males with tapered shapes with well-developed external reproductive organs. A total of 258 specimens were obtained and morphometric measurements were taken onsite. The fish populations were analyzed as a whole and populations were compared without discriminating the sex. Any variations that would arise from sexual dimorphism in tilapia were catered for by applying the same data collection criteria for the fish across populations from the different lakes and farms. Morphometric traits were measured using a centimeter scale to the nearest 0.1 cm except for body weight (BW) which was measured to the nearest 0.1 g using a digital balance. The morphometric traits were: Total Length (TL), Standard Length (SL), Body Width (WID), Caudal fin Length (CL), Head Length (HL), Dorsal Length (DL), Pectoral fin Length (PEC), and Pelvic fin Length (PEL) (Figure 2). Additionally, the body weight of the fish samples was also measured separately. These morphometric traits were chosen because they are easily measured longitudinal traits and normally present moderate to high heritability (10, 26). The body characters viz. TL, HL, DL, BD, CL, PEC, and PEL were expressed as percent to Standard Length of the fish to correct size dependent variation in morphometric characters [26].

2.3. Data Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 23.0. IBM Corp (Armonk, NY, USA) developed by IBM for data management and Microsoft Excel. One-way Analysis of Variance (ANOVA) followed by Tukey post hoc test was used to test the significance of differences in the morphometric traits between populations ($p < 0.05$). Prior to the analyses, normal distributions were investigated using Kolmogorov–Smirnov test and homogeneity of variances was checked with Levene's

test. Morphological trait values were taken as dependent variables and populations as explanatory variables.

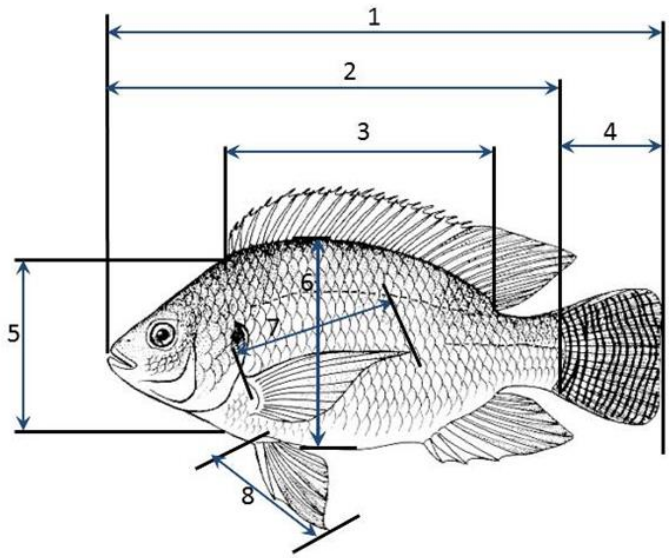

Figure 2. Morphometric measurements of *O. niloticus*: 1. Total length, 2. Standard Length, 3. Dorsal fin length, 4. Caudal fin length, 5. Head length, 6. Body width, 7. Pectoral fin length, 8. Pelvic fin length (Source: Author photo).

Morphometric variation among the eight populations of *O. niloticus* was used to test for the presence of stock structuring using discriminant function analysis (DFA) [27,28]. Stepwise DFA on morphometric data was used to select all of the traits. A univariate analysis of seven measurements expressed as percentages of the standard length of fish was run to estimate the importance of the morphometric measurements for species identification.

The length–weight relationship of the fish was calculated using the equation by [29] in the form of: W = aL^b to estimate the length–weight relationship of the fish in each population. The length–weight relationship of the collected fish samples was calculated based on the logarithmic transformation of the data. When analyzing the length–weight relationships between the fish samples, the data were logarithmically transformed to stabilize the variance that could result from size-dependent weight measurements [30]. Fish body weight and standard length were used in this analysis.

W = body weight of fish in grams,

L = Standard length in centimeters,

"a" is the intercept,

"b" is the slope of the regression line,

The fish Condition Factor (K); Relative condition [30] factor which is an expression of the degree of the wellbeing of fishes in their habitat was calculated as

K = W/W′ where:

W = Actual weight of Fish

W′ = Calculated weight of the fish.

3. Results

3.1. Morphometric Traits of the O. niloticus Populations

One-way analysis of variance to test the significant differences in the morphometric traits among the *O. niloticus* populations revealed significant differences in all traits among the populations. The morphometric traits: Standard length, Body weight, Total length, Dorsal length, Caudal fin length, Head length, Body width, Pectoral fin length, and Pelvic fin length were significantly different among the populations ($p < 0.05$) (Table 1).

Table 1. Mean ± SD for the different morphometric characters of *O. niloticus* population (significantly different ($p < 0.05$) (SL = Standard Length, BW = Body weight, TL = Total Length, DL = Dorsal fin Length, CL = Caudal fin Length, HL = Head Length, WID = Body Width, PEC = Pectoral fin Length, PEL = Pelvic fin Length).

Populations	SL	BW	TL	DL	CL	HL	WID	PEC	PEL
Kyoga	18.68 ± 3.19	283.20 ± 91.41	80.74 ± 13.62	84.92 ± 12.64	27.53 ± 2.05	32.49 ± 2.53	42.02 ± 3.38	39.59 ± 2.61	28.50 ± 3.10
Kayumbu	15.88 ± 2.81	167.05 ± 87.86	87.98 ± 52.91	67.28 ± 8.43	27.89 ± 5.39	33.09 ± 3.04	40.10 ± 4.12	36.48 ± 3.81	27.64 ± 2.57
Victoria	18.98 ± 2.16	281.69 ± 89.73	78.24 ± 6.45	67.49 ± 7.01	27.14 ± 3.06	33.86 ± 3.14	41.35 ± 2.54	36.29 ± 3.03	28.28 ± 3.58
Kambuga	17.27 ± 2.21	233.39 ± 78.01	82.06 ± 1.42	56.33 ± 3.55	24.31 ± 2.25	32.66 ± 2.19	36.57 ± 6.95	39.18 ± 2.91	27.948 ± 2.25
Masya	18.47 ± 1.70	274.12 ± 58.45	80.49 ± 1.36	59.01 ± 2.40	26.09 ± 1.53	30.42 ± 1.36	39.48 ± 2.71	33.38 ± 2.54	33.72 ± 3.24
Rwabirundo	15.69 ± 0.76	143.18 ± 21.10	79.07 ± 4.23	75.88 ± 4.02	29.24 ± 1.28	33.97 ± 1.31	40.96 ± 1.70	42.38 ± 1.73	30.35 ± 1.72
Nyamabare	17.68 ± 1.41	195.33 ± 43.21	78.83 ± 1.86	76.12 ± 4.10	27.92 ± 1.63	32.46 ± 1.70	40.35 ± 1.74	39.99 ± 2.82	29.16 ± 2.26
Butare	17.64 ± 1.64	215.80 ± 53.44	83.74 ± 14.34	66.81 ± 15.51	26.53 ± 4.51	31.22 ± 2.90	40.44 ± 4.34	34.57 ± 7.23	28.88 ± 4.78
Average	17.56 ± 2.29	227.95 ± 86.43	87.53 ± 25.09	68.92 ± 10.56	27.02 ± 3.34	32.49 ± 2.63	40.11 ± 4.14	37.65 ± 674	29.30 ± 3.58
$P_{7.250}$	0.000	0.000	0.000	0.040	0.000	0.000	0.000	0.000	0.000
F	11.30	17.84	20.74	2.13	7.54	8.34	6.13	21.19	13.31

The *O. niloticus* population from Victoria had the highest mean standard-length value (18.98 ± 2.161) followed by Kyoga (18.68 ± 3.19) while Rwabirundo (15.69 ± 0.76) had the least mean standard-length value. Post hoc using Tukey HSD showed that the mean values for standard length between; Kyoga and Kayumbu, Kyoga and Rwabirundo, Kayumbu and Victoria, Kayumbu and Masya, Kayumbu and Nyamabare, Kayumbu and Butare, Victoria and Kambuga, Victoria and Rwabirundo, Kambuga and Rawbirundo, Masya and Rwabirundo, Rwabirundo and Nyamabare were significantly different ($p = 0.00$) while other populations' standard length were not significantly different ($p = 0.05$) (Table 1).

Body weight among the populations was significantly different at $p < 0.05$ level [df (791,365.55), f = 17.84, $p = 0.00$] (Table 1). The population from Kyoga had the highest mean body weight (283.19 ± 91.41) followed by Victoria (281.69 ± 89.73), while Rwabirundo (143.18 ± 21.10) had the least mean body weight value. Body weight among the populations indicated that Kyoga and Kayumbu, Kyoga and Rwabirundo, Kyoga and Kyamabare, Kyoga and Butare, Kayumbu and Victoria, Kayumbu and Kambuga, Kayumbu and Masya, Victoria and Rwabirundo, Victoria and Nyamabare, Kambuga and Rwabirundo, Kambuga and Nyamabare, Masya and Rwabirundo, and Masya and Nyamabare were significantly different ($p = 0.00$), while other populations did not show any significant differences ($p = 0.05$).

3.2. Length–Weight Relationship and Condition Factor

The *O. niloticus* populations from Kyoga had a higher mean standard length and body weight (18.68 ± 3.19 cm and 283.20 ± 130.41 g, respectively) followed by Victoria with 18.98 ± 2.16 cm and 281.69 ± 89.73 g, respectively, while Rwabirundo had the least mean values (15.69 ± 0.76 cm and 143.18 ± 21.10 g, respectively) (Table 2). The parameter 'b' of the fish samples ranged from a minimum of 1.14 to a maximum of 2.91, with a mean value of 2.24 (Table 2). Populations from Kayumbu, Kyoga, and Butare showed isometric growth with values of regression slope b; 2.91, 2.65, and 2.54, respectively, (b = 3) while other populations (Victoria, Kambuga, Masya, Rwabirundo, and Nyamabare) showed negative allometric growth pattern recording values of b < 3 (Table 2, Figure 3).

Table 2. Length–weight relationships of the different populations.

Population	N	Standard Length (cm)	Body Weight (g)	a	b
Kyoga	32	18.68 ± 3.19	283.20 ± 130.41	−0.96	2.65
Kayumbu	32	15.88 ± 2.81	167.05 ± 87.86	−1.31	2.91
Victoria	30	18.98 ± 2.16	281.69 ± 89.73	−1.08	2.75
Kambuga	35	17.27 ± 2.21	233.39 ± 78.01	−0.43	2.25
Masya	33	18.47 ± 1.70	274.12 ± 58.45	−0.35	2.20
Rwabirundo	30	15.69 ± 0.76	143.18 ± 21.10	−0.74	2.42
Nyamabare	30	17.68 ± 1.41	195.33 ± 43.21	−0.39	2.14
Butare	34	17.64 ± 1.64	215.80 ± 53.44	−0.85	2.54

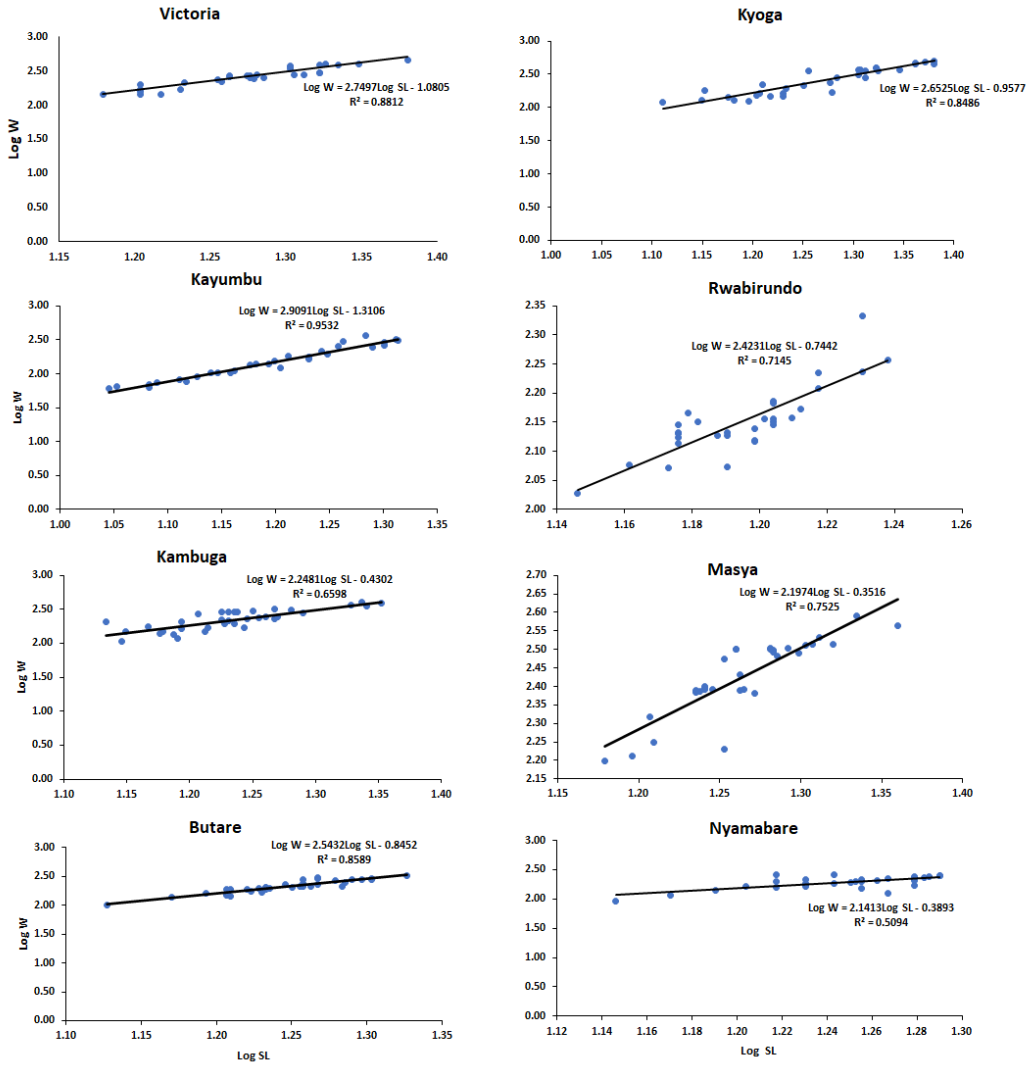

Figure 3. Length–weight relationships of the population of *O. niloticus* in the sampling sites.

Relative Condition Factor (K) for the *O. niloticus* did not differ significantly among the populations ($p > 0.05$) [df (8,279), F = 0.27, $p = 0.98$] (Figure 4). All the populations had an average condition factor (K = 1) (Figure 4).

3.3. Similitude in the Morphometric Traits among the Oreochromis niloticus Populations

3.3.1. Discriminant Function Analysis (DFA) of different *Oreochromis niloticus* populations

In this study, all the traits were selected by stepwise DFA on morphometric data. The analysis of the morphometric variables produced seven significant discriminants functions (Table 3). The first function accounted for 40.5% of the variance and the second function 24.2% for the traits (Table 3). According to the canonical discriminant function coefficients obtained for morphometric data, the most influential variables for function 1 were standard length, total length, caudal fin length, and head length, while body weight and pectoral fin length were influential in function 2. Total length was more influential at function 3 while pelvic fin length was more influential at function 5. The canonical discriminant function 1 and canonical discriminant function 2 were plotted to allow visual examination of the distribution of each sample in the populations. Populations of Kambuga, Masya, and Rwabirundo were very distinct and highly isolated while populations of Victoria, Nyamabare, Butare, Kyoga, and Kayumbu were overlapping and distributed in the study (Figure 5).

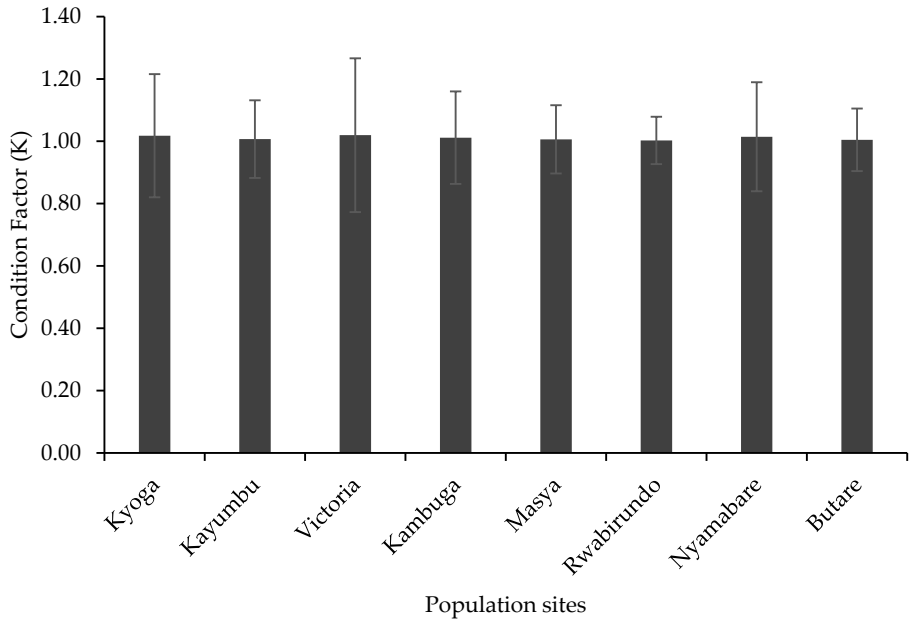

Figure 4. Relative condition factor of the different populations.

The overall random assignment of individuals into their original group was 65.5% (Table 4) with Victoria and Rwabirundo having a higher correct classification 90.6 and 90.0%, respectively, while Kayumbu had the least classification (43.8%) (Table 4). The proportion of individuals correctly classified into their original group, i.e., Kyoga, Kayumbu, Victoria, Kambuga, Masya, Rwabirundo, Nyamabare, and Butare was 43.8%, 40.6%, 90.6%, 77.7%, 84.8%, 90.0%, 50.0%, and 47.1%, respectively.

Three distinct groups were observed among the Kabale sub populations with a few samples overlapping (Figure 6). Few samples from Nyamabare were overlapping in the populations of Rwabirundo and Butare. Of the samples, 83.0% were randomly assigned into their groups (Figure 6). The proportion of individuals correctly classified into their original group, in Butare, Rwabirundo, and Nyamabare, were 88.2%, 96.7%, and 63.3%, respectively (Table 5).

Table 3. Standardized Canonical Discriminant Function Coefficients for Morphological characters of *O. niloticus* populations.

	Function						
	1	**2**	**3**	**4**	**5**	**6**	**7**
SL	1.35	−1.11	−0.15	0.62	−0.44	−1.29	0.25
BW	−1.72	1.17	0.49	0.08	0.04	1.09	0.09
TL	0.18	−0.03	0.79	0.08	0.44	−0.03	−0.15
DL	0.06	0.03	0.01	0.29	−0.30	0.20	0.13
CL	0.50	−0.08	−0.07	0.13	−0.02	0.38	0.69
HL	0.46	0.35	0.19	−0.02	0.16	0.11	0.26
WID	0.34	−0.62	0.10	0.00	−0.27	0.43	−0.68
PEC	0.29	0.78	−0.19	0.47	0.16	−0.19	−0.23
PEL	−0.33	−0.29	−0.44	0.46	0.67	0.06	−0.07
Eigenvalue	1.51 [a]	0.89 [a]	0.80 [a]	0.25 [a]	0.18 [a]	0.08 [a]	0.00 [a]
% of Variance	40.50	24.20	21.60	6.80	4.70	2.10	0.10
Cumulative %	40.50	64.70	86.30	93.10	97.90	99.90	100.00
Canonical Correlation	0.775	0.69	0.69	0.45	0.39	0.27	0.06

[a.] Eigen value. The eigen values are related with the canonical correlations and describe how much discriminating ability a function possesses. The higher the value, the more discriminating it is thus function 1 (1.51) is more discriminating than other functions.

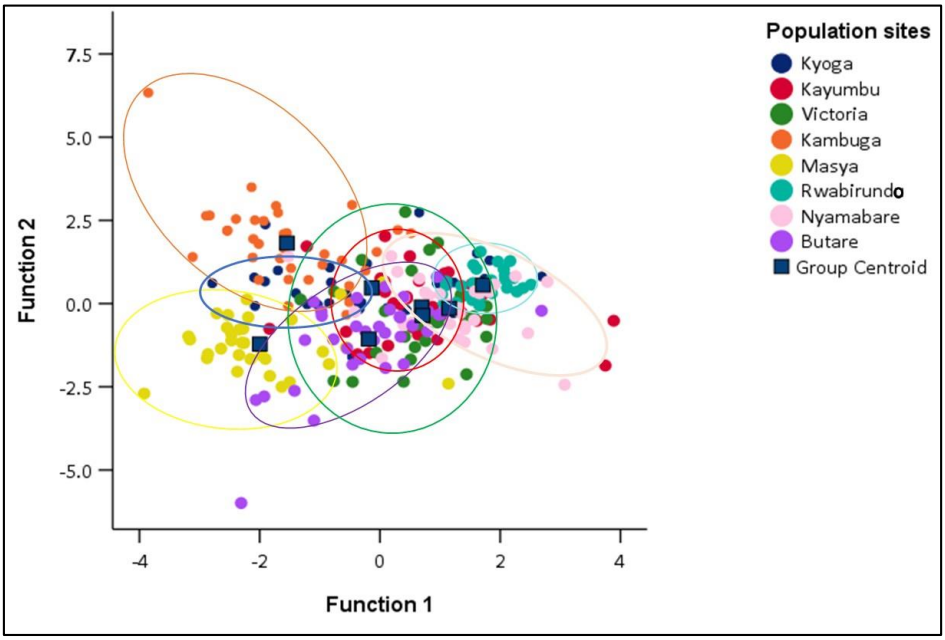

Figure 5. Canonical Discriminant Functions for different populations.

Table 4. Classification Results for all the populations.

		Predicted Group Membership								
		Kyoga	**Kayumbu**	**Victoria**	**Kambuga**	**Masya**	**Rwabirundo**	**Nyamabare**	**Butare**	**Total**
Count	Kyoga	14	2	1	2	1	6	4	2	32
	Kayumbu	4	13	1	1	0	3	7	3	32
	Victoria	0	1	29	1	0	0	0	1	32
	Kambuga	5	0	0	27	1	0	1	1	35
	Masya	0	1	0	0	28	0	1	3	33
	Rwabirundo	0	0	0	0	0	27	3	0	30
	Nyamabare	1	2	0	1	0	8	15	3	30
	Butare	3	4	0	0	6	1	4	16	34
%	Kyoga	43.8	6.3	3.1	6.3	3.1	18.8	12.5	6.3	100.0
	Kayumbu	12.5	40.6	3.1	3.1	0.0	9.4	21.9	9.4	100.0
	Victoria	0.0	3.1	90.6	3.1	0.0	0.0	0.0	3.1	100.0
	Kambuga	14.3	0.0	0.0	77.1	2.9	0.0	2.9	2.9	100.0
	Masya	0.0	3.0	0.0	0.0	84.8	0.0	3.0	9.1	100.0
	Rwabirundo	0.0	0.0	0.0	0.0	0.0	90.0	10.0	0.0	100.0
	Nyamabare	3.3	6.7	0.0	3.3	0.0	26.7	50.0	10.0	100.0
	Butare	8.8	11.8	0.0	0.0	17.6	2.9	11.8	47.1	100.0

65.5% of original grouped cases were correctly classified.

Table 5. Classification Results for Kabale sub populations.

		Predicted Membership			
	Population Sites	**Butare**	**Rwabirundo**	**Nyamabare**	**Total**
Count	Butare	30	0	4	34
	Rwabirundo	0	29	1	30
	Nyamabare	5	6	19	30
%	Butare	88.2	0.0	11.8	100.0
	Rwabirundo	0.0	96.7	3.3	100.0
	Nyamabare	16.7	20.0	63.3	100.0

Of original grouped cases, 83.0% were correctly classified.

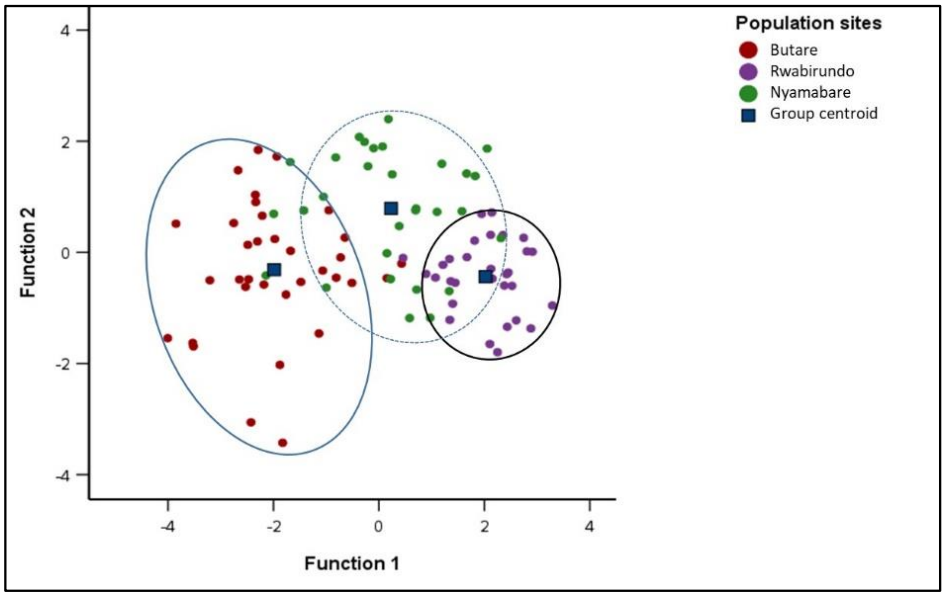

Figure 6. Canonical Discriminant Functions for the sub populations of Kabale.

The population of Kayumbu in Kyanamira farm was very distinct and highly isolated from populations of Victoria and Kyoga. Additionally, more than 60% of the populations of Kyoga and Victoria were also distinct. Some samples were shared among the populations (Figure 7). The overall random assignment of individuals into their original group was high (81.1%) (Table 6). The proportion of individuals correctly classified into their original group, i.e., Victoria, Kayumbu, and Kyoga was 80.0%, 81.3%, and 81.8%, respectively (Table 6).

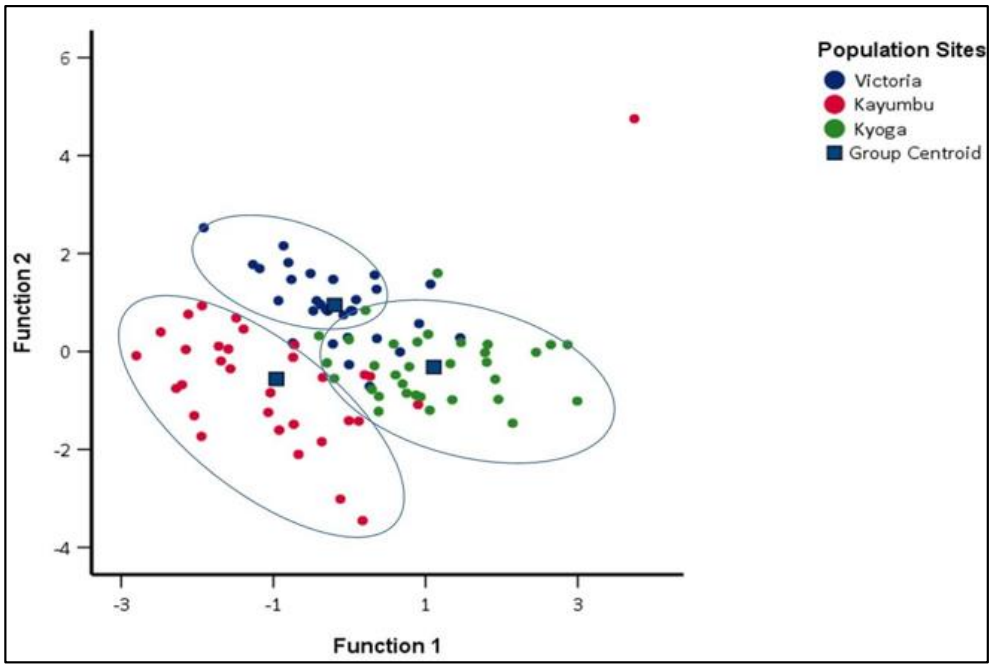

Figure 7. Canonical Discriminant Functions for the sub populations of Kyanamira.

Table 6. Classification Results of Kyanamira sub populations.

		Predicted Group Membership			
	Site	**Victoria**	**Kayumbu**	**Kyoga**	**Total**
Count	Victoria	24	2	4	30
	Kayumbu	3	26	3	32
	Kyoga	4	2	27	33
%	Victoria	80.0	6.7	13.3	100.0
	Kayumbu	9.4	81.3	9.4	100.0
	Kyoga	12.1	6.1	81.8	100.0

81.1% of original grouped cases were correctly classified.

3.3.2. Hierarchical Cluster Analysis

Hierarchical cluster analysis performed on the sampled sites displayed two distinct clusters with Kyoga, Victoria, Kambuga and Masya populations of *O. niloticus* grouping together in one cluster. The second cluster consisted of two other sub-clusters that comprised one cluster with Kayumbu and Rwabirundo populations, while the other consisted of Nyamabare and Butare populations (Figure 8).

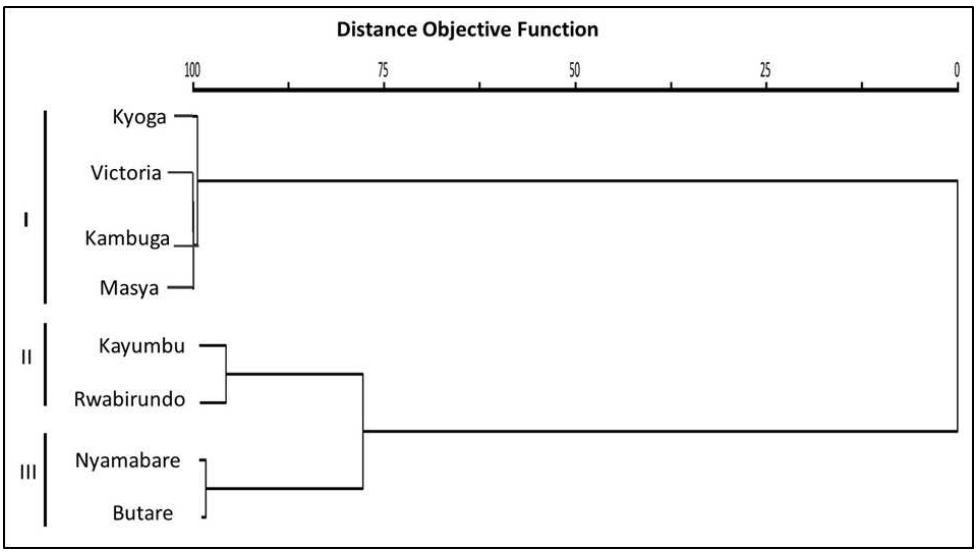

Figure 8. Cluster dendogram of the *O. niloticus* populations. I. II and III are the clusters generated from the *O. niloticus* populations in the different sites.

4. Discussion

This work was carried out to evaluate the morphometric variations of *O. niloticus* strains cultured by various farmers in Uganda's South Western Highland Agro-Ecological Zone (SWHAEZ). The results showed clear and distinct morphological differences between populations across the tested environments, thus negating the hypothesis set earlier that the morphometric traits would show no significant differences among the populations.

4.1. Morphometric Traits of the O. niloticus Populations

Recent studies [6,8,12,31] have documented the existence of morphological differences in the farmed *O. niloticus* populations and strains across a range of different environments. These studies align with the current findings which show clear and distinct morphological differences observed among populations obtained from different farms. The morphometric variabilities among populations of *O. niloticus* under culture have been reported to be influenced by genetic differentiations, food composition, depth, stocking density, farming method (pond or cage), habitats, and water quality [8,32]. Fish samples from the same

gene pool, but cultured in different aquatic environments, have reported to show explicit differences in body shape [8,33].

The difference in the morphological traits in our study could be a factor of environmental variation as the farms were located within two districts (Kanungu and Kabale) of varying climatic conditions. In the current study, Rwabirundo, Butare, and Nyamabare populations, despite having been stocked with fish from the same source, exhibited morphological variations which can be attributed to environmental changes as a result of the location of the farm, as well as their culture and management conditions. Notably, the results of the Discriminant Function Analysis (DFA) showed a high level of differentiation and morphometric variability between the populations of Masya and Kambuga sampled sites (located in Kanungu) compared to other stations. The findings align with those of studies by [34] which report that the morphological differences observed in fish are strongly influenced by water quality and habitat conditions. These differences are strongly suspected to be as a result of environmental conditions such as temperature, which is higher in Kanungu district compared to Kabale district. Such temperature differences influence behavior feeding, metabolic rate, growth, and reproduction, thus affecting overall growth of fish directly.

4.2. Length–Weight Relationship and Condition Factor

The length–weight relationship provides useful information on fish growth and varies depending on the aquatic environment [32]. Such variations result from the differences in habitat/culture systems, gonad maturity, sex, nutritional content of the feeds, stomach fullness, health status of the fish, and differences in environmental conditions [6,32]. The value of the exponent 'b' in the parabolic equation (W = aLˆb) describing the length–weight relationship in *O. niloticus* lies between 2.5 and 4 (30). Based on the deviations of b value, fish can be categorized into: (i) isometric (b = 3), where the body form of fish remains constant at different lengths; (ii) b < 3, when fish become slenderer as the length increases; and (iii) allometric (b > 3), when fish grow more stout with an increase in length [32].

In the current study, most of the populations did not obey cube law which assumes that the regression slope b = 3. Most of the fish showed negative allometric growth (b < 3) except for Kayumbu that showed isometric growth (b = 3), therefore obeying cube law [35]. This (b < 3) could mean that large fish had more an elongated body shape or that smaller fish were in better nutritional condition at the time of sampling [35]. The negative allometry in the length–weight relationship of fish was an indication that the population of the species in these sites had heterogeneous groups with body weights varying differently with the cube of total length. On the other hand, isometric growth shown by Kayumbu was an indication that the pond has homogenous fishes in their populations.

The condition factor (K) of fish is a measure of the species' wellness and robustness, particularly the fish's health status, sexual maturity, and feeding status, and it predicts the species' adaptability by determining the individual's status as weight at a given length. An overall fitness for fish species is assumed when relative condition factor values are equal or close to 1 [36]. In all the eight farms, the condition factor was not significantly different among the populations with the fish having K values above 1, which is an indication of the fish being in good condition. The condition factor of *O. niloticus* has been recorded to be around 1 in normally growing fish and increases when sexual maturation approaches [8,37]. The higher condition factor in the present study is an indication of proper management practices especially proper feeding, good water quality that enhance plankton production, use of quality and quantity feed, and proper stocking densities that limits competition among the fish. These factors have been reported to contribute significantly to the robustness in fish conditions as it lowers stress levels that would otherwise impact negatively on the K value [36].

4.3. Similitude in the Morphometric Traits among the Oreochromis niloticus Populations

Significant heterogeneity ($p < 0.05$) in morphology among the eight *O. niloticus* populations was revealed by univariate and multivariate (DFA) statistics. The results indicate the presence of morphologically distinct (Kambuga, Masya, and Rwabirundo) and overlapping (Victoria, Nyamabare, Butare, Kyoga, and Kayumbu) populations. The overlaps could indicate that there is a high possibility of these fish originating from the same source and, thus, have a similar genetic makeup. However, these farms are located in different locations and under different management conditions leading to the observed differences. This further points to the possibility of farmers keeping more than one strain, and, consequently, the possibility of interstrain hybridization. This has been reported in similar works such as those of [8,12,38] showing close genetic distances and morphometric similarities among populations sharing a common ancestry or lineage.

The sub-population clustering of Kyoga, Victoria, and Kayumbu populations indicates the closeness in the morphological characteristics between these populations. Similarly, the populations of Butare, Rwabirundo, and Nyamabare in Kabale district showed low-level scale overlaps. Their occurrence in one region could explain the similarities in morphometric characters and explains the overlaps as environmental influence has been reported to be of importance during the development of fish [39].

Results from cluster dendrogram revealed the existence of three distinct clusters with Kyoga, Victoria, Kambuga, and Masya forming one cluster, Kayumbu and Rwabirundo forming the second cluster, and, lastly, Butare and Nyamabare forming another cluster. The most remarkable closer morphotypes between Butare and Nyamabare might be linked with the source of the fry and genetic makeup. The farms under study obtained their foundation stock through government programs aimed at improving agricultural production. In this production system, fish would be supplied by a single supplier across an entire district, indicating that there is a high possibility of these fish originating from the same source and, thus, have a similar genetic makeup. A similar explanation is given for the close linkages in the populations of Kambuga and Masya. These farms are located in a different district and, thus, a possibility of a different supplier sourcing fish from one seed source and supplying these farms.

The broodstock populations of Kyoga, Kayumbu, and Victoria were sourced from the lakes, Kyoga, Kayumbu, and Victoria, respectively, and managed in independent ponds at Kyanamira fish facility. Notably, from the close linkage between Kambuga, Masya, and Victoria, it could be assumed that these fish can trace their foundation stock to Lake Victoria. Since the source of Victoria population is clearly known, it means Kambuga and Masya share some similar source with Victoria populations. The close linkage between Kyoga and Victoria, with no history of nativity of *O. niloticus* strains having been introduced into these lakes [40], can be explained by the fact that cases of fish translocations have taken place between these lakes thus increasing cases of admixtures. Similarly, the population in Rwabinduru was morphometrically closer to the population in Kayumbu. This points to the possibility of these populations being genetically linked and sourced from Lake Kayumbu.

5. Conclusions

In this study, we show the existence of morphological variations in the populations of *O. niloticus* in Uganda. This study delineated *O. niloticus* populations from eight pond systems stocked with fish from three different lakes in Uganda and five fish farms in the western region of Uganda based on morphometric measurements. Biotic and abiotic factors, geography and associated climatic conditions, anthropogenic activities, genetics, and their interactions are believed to be the main cause of the observed variations. Application of molecular genetic markers such as microsatellites, cytochrome c oxidase subunit 1 (CO 1) gene, and the displacement loop (D-loop) region would be effective methods of examining the discreteness of *O. niloticus* strains and facilitate the development of species-specific management strategies. Therefore, future work should include experiments such

as progeny tests to clarify the influence of genetics, environment, and their interaction in the body shape of this species.

Author Contributions: G.K. conceived the study, designed the methods and study design, performed field work, performed data analysis, and drafted and edited the original manuscript. M.J.Y. performed data analysis, and drafted and edited the original manuscript. A.B. (Apulnal Beingana). and N.B. performed fieldwork, performed sample and data analysis, and commented and edited the manuscript. F.A. collected samples, entered raw data, performed the part of the analysis, and reviewed and edited the manuscript. A.B. (Alex Barekye) supervised the work, and commented on and edited the manuscript. H.M. and J.K.N. conducted thorough review, editing, and manuscript write up. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable. Ethical review and approval were waived for this study because field excursions were conducted together with respective authorities per district and therefore no special permission was required. Throughout the sampling regimes, fish were always bought from the farmers; thus, no special treatment for the animals was administered in the process.

Data Availability Statement: The data can be accessed via KG by emailing: gerald.kwikiriza@students.boku.ac.at.

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