Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
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www.academicjournals.com

OPEN ACCESS **Journal of Fisheries and Aquatic Science**

ISSN 1816-4927
DOI: 10.3923/jfas.2020.59.68

Research Article

Multivariate Morphometric Analysis of Riverine and Lacustrine *Barbus altianalis* populations in Uganda

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Abstract

Background and Objective: Human-induced physical and environmental barriers especially hydropower dams and dense anoxic vegetation along riverine-lacustrine connectivity are known to isolate and fragment fish populations, leading to variation. This study explored possible morphological variations among populations of *Barbus altianalis* from Uganda with the intent to provide a more cost-effective and reliable platform for elucidating the stocks under combined environmental and anthropogenic influence as a tool for improved fisheries management and conservation. **Materials and Methods:** Multivariate analysis of morphometric variables was performed to determine the morphological variation and discreteness among three populations of *B. altianalis* (N = 251) from the lacustrine (Lake Edward) and riverine (River Nile and Kazinga channel) systems in Uganda. Morphological variation was determined using a multivariate analysis of advanced truss and non-truss network systems on all individuals collected from commercial catches. **Results:** There was a significantly ($p \leq 0.001$) higher level of heterogeneity among populations. Group means on the DFA1 showed that River Nile (-0.89) separated furthest from Lake Edward (0.79) and the Kazinga channel (0.68). River Nile had the highest number (80.5%) of individuals correctly classified while Lake Edward (67.2%) and Kazinga channel (60.5%) had more misclassifications between them, an indication that they could be much harder to separate. **Conclusion:** The observed patterns of variation were consistent with the geographical locations of the different water bodies, that the isolating mechanisms are subjecting the different populations to different selection pressure hence different phenotypic responses by the populations.

Key words: Discriminant function analysis, Lake Victoria, Edward basins, phenotypic variation, stock identification

Citation: Ondhoro, C.C., G.E. Maes, N.W. Nkalubo, J.K. Walakira, M.T. Mwanja, E. Ganda, G.K. Kawooya, J. Efitre and C. Masembe, 2020. Multivariate morphometric analysis of riverine and lacustrine *Barbus altianalis* populations in Uganda. J. Fish. Aquat. Sci., 15: 59-68.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The barbel fish *Barbus altianalis*, locally known as "Kisinja" in Uganda, is a commercially important freshwater fish that grows to a large size (maximum reported size about 90 cm total length) and is considered a culinary delicacy among riparian communities in East Africa[1,2]. This cyprinid is an omnivore, widely distributed in Lakes Kyoga, Edward, Albert and George, as well as in the River Nile and others that drain into Lake Victoria[3]. Although the taxonomic status of *B. altianalis* is debatable, we used the traditional nomenclature which recognizes the species as *B. altianalis* [4-6].

The populations of *B. altianalis* are under threat from over fishing, erecting hydropower dam barriers along migration routes and degraded aquatic ecosystems[7]. However, various conservation aspects such as population structure, variability and resilience to harvest pressure have not been satisfactorily evaluated[8]. Prior to the construction of the Owen Falls dam on the River Nile in 1954, large congregations of *B. altianalis* were common at the foot of Ripon falls[1-8]. However, due to over fishing and environmental changes, coupled with vulnerable reproductive and feeding behavior, the species is under threat of extinction[5-7]. Being a partial batch spawner, the fish migrates up and downstream throughout the year to spawn. However, this behavior exposes it to overexploitation along the migration routes[9,10]. While off-shore trawl surveys through Lake Victoria in 1975 and 1985 showed fluctuations in Catch per Unit Effort (CPUE) of between 0.2 to 0.5 kg/h[5,8], the current CPUE in the Victoria and Edward basins is estimated to average 0.06 and 1.50 kg/h respectively[11-13].

In addition, physical barriers including hydropower dams along the Nile River and anoxic papyrus swamps along the riverine-lacustrine borders between Lakes Victoria and Albert have isolated populations of *B. altianalis*[4,2].

Examination of systemic morphometric variation, therefore, gives prospects for population structure of *B. altianalis* stocks from different water bodies. This is premised on the affirmation that variations among fish populations provide a basis for fish population structure and a foundation for documenting environmentally induced variance[14]. Furthermore, morphological divergence is important in fish stock management as a marker of changes in environmental quality associated with evolution. Knowledge of fish morphometric variations therefore, facilitates the development of optimal strategies for efficient management of stocks, reacting independently to fishing pressure and other ecosystem dynamics[14,15]. Changes in body forms of fish populations exposed to different environmental conditions could be used to improve fisheries management and conservation[16]. However, phenotypic features, more so the morphology of fishes are interlinked and therefore, use of single parameter as had been before, could be misleading[16]. For example, the shortcoming with single morphological parameters is related to the existence of temporary morphotypes such as deformities originating from either early ontogenetic development such as juvenile pigmentation in some tilapia species, commonly known as "Tilapia mark", or those inflicted by type II cannibalism, common with African catfish[17]. Therefore, unless a given morphometric feature is known to be genetically linked, multivariate morphometric approach remains the most reliable technique for inferring population morphometric variations[18-23]. The method has been widely applied in a number of recent studies to study taxonomic contradictions on fish stocks[20,15,16-24]. Application of multivariate morphometric analysis on the current stocks of *B. altianalis* is a cost-effective alternative to generate valuable management information for monitoring and designing appropriate conservation strategies for different water bodies[23].

The aim of this study was therefore, to examine the morphometric variation among populations of *B. altianalis* from River Nile, Lake Edward and Kazinga channel Uganda using multiple morphometric variables to inform conservation and management of the species.

MATERIALS AND METHODS

Study area: The study was carried out in selected fish landing sites along River Nile, Lake Edward and the Kazinga channel in 2013.

Fish specimens used in this study were obtained from four landing sites in the River Nile (upstream - Ripon and downstream -Kiira, Kalange and Bukeka) of the Owen Falls dam. On Lake, Edward samples were obtained from Katwe and Kayanja landing sites. Fish were also sampled from two landing sites of Katunguru and Kazinga in the Kazinga channel, upstream Lake Edward, Uganda. Given its location, the fishers at the Ripon landing site conduct their fishing activities both in the lacustrine and the riverine systems. The fisherfolk, fishing vessels and gears in Kiira, Kalange and Bukeka landing sites are restricted to fishing within the riverine system between the dams along River Nile. Katwe, Kayanja, Katunguru and Kazinga are situated in water bodies located within Queen Elizabeth National Park, where fishing activities have some level of restriction given their location in a protected area (Fig. 1).

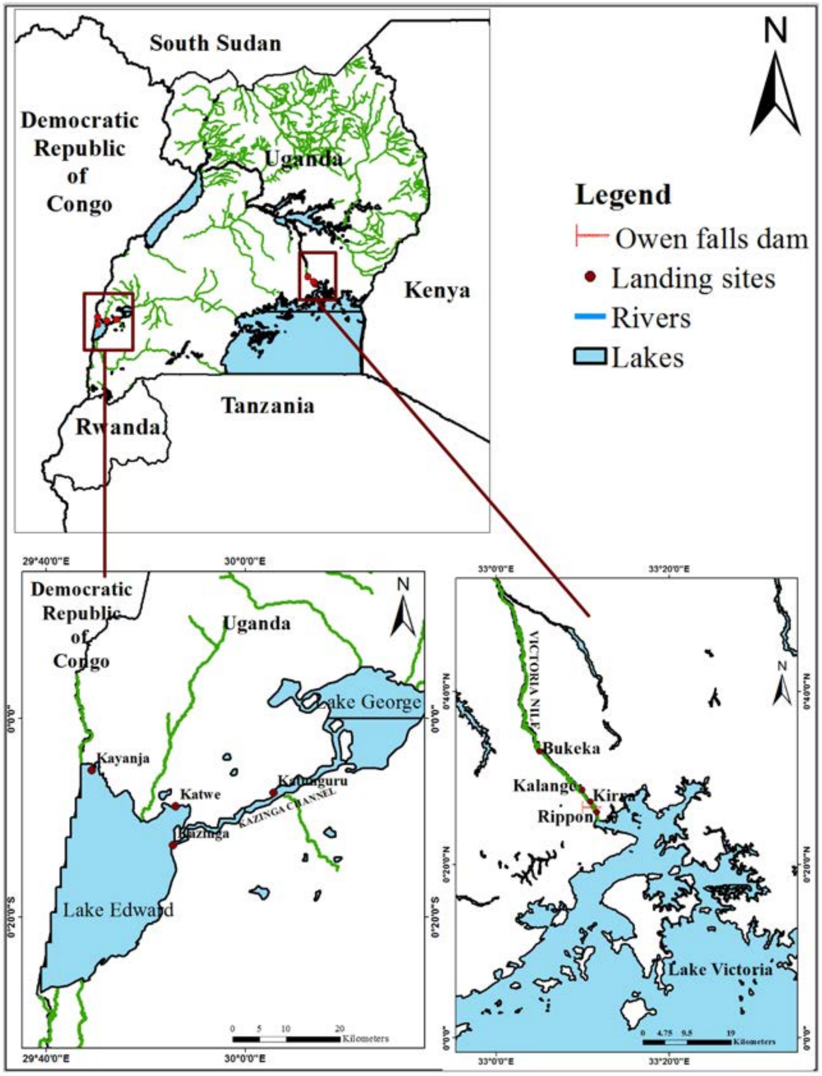

Fig. 1: Map indicating the location of the sampled landing sites in River Nile, Lake Edward and Kazinga channel. Source: personal data

Sample and morphometric data collection: Fish specimens were obtained from commercial catches in December 2013. Samples from all the landing sites from the same water body were pooled into one unit to generate three discrete populations of the River Nile- Population one, Lake Edward- population two and Kazinga channel- population three. Pooling of samples was done mainly to limit the study to population level that was designated by water bodies under investigation. Fish samples were collected within the same period (December 2013) to minimize variation contributed by seasonal variation in food, water quality, reproduction and other allochthonous influence[25].

A truss dimension of each fish was constructed from the left side of the fish, using Vernier callipers, following landmarks adopted from the previous studies[26,27,15]. A total of 13 trusses and 6 non-truss morphometric variables were measured on each specimen to the nearest 0.1 cm. Truss and non-truss network systems were adopted because they are able to discriminate phenotypic stocks by the configuration of the constructed landmarks that cover the entire fish body with no loss of information and being more sensitive to changes[26-29] (Table 1).

Construction of truss and non-truss dimensions: A truss dimension is series of measured distances calculated between landmarks that form a regular pattern of connected quadrilaterals or cells across the body form as described by Strauss and Bookstein[27]; Turan[15]. The truss procedure used for this study was based on 10 landmarks, whereby, the truss dimensions were constructed by interconnecting landmarks

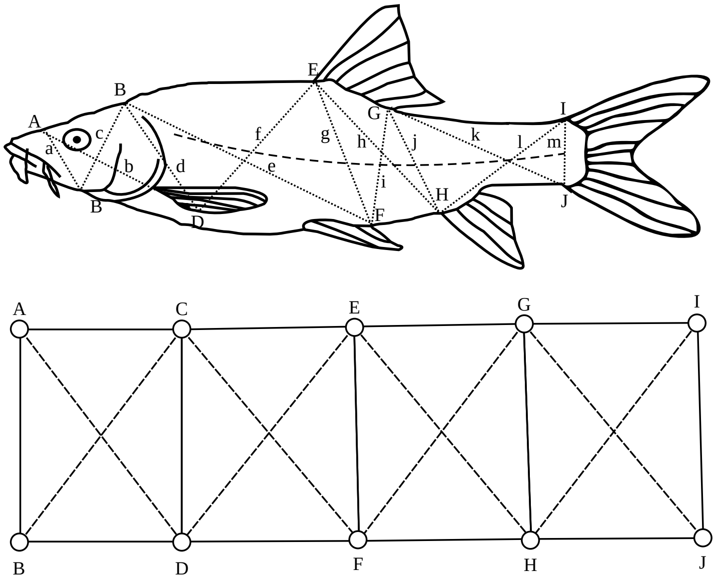

Fig. 2: Data recording procedure, Top 19 distance parameters recorded on vernier calipers, Top marked in lower case letters are distances measured between landmarks and marked in upper case letters are the morphometric land marks in *B. altianalis*
a: Snout, b: Ventral junction of operculum junction, c: Supra orbital, d: Lower pectoral fin insertion, e: Anterior dorsal fin insertion, f: Posterior end of Pelvic Fin insertion, g: Posterior end of dorsal fin insertion, h: Anterior end of anal fin insertion, i: Dorsal caudal fin Insertion, j: Ventral caudal fin insertion, k: Posterior dorsal fin insertion to ventral caudal fin insertion, l: Dorsal caudal fin insertion to ventral caudal fin insertion, m: Anterior anal fin insertion to dorsal caudal fin insertion, Bottom: Geometric representation of the data recording procedure, Each single cell is determined by four sides and two diagonals, Modified from Corti *et al.*[30]

Table 1: Truss and non-truss dimensions measured for the morphometric analysis of *B. altianalis* collected from River Nile, Lake Edward and Kazinga channel as in Fig. 2

Truss	Non-truss
Snout to ventral operculum junction distance (a)	Orbital distance (obd)
Snout to lower pectoral fin insertion (b)	Pectoral fin length (pfl)
Snout to posterior end of supra orbital (c)	Dorsal fin length (dfl)
Posterior end of supra orbital to lower pectoral fin insertion (d)	Pelvic fin length (pvfl)
Posterior end of supra orbital- posterior end of pelvic fin origin (e)	Anal fin length (afl)
Anterior dorsal fin to lower pectoral fin insertion (f)	Caudal fin length (cfl)
Anterior dorsal fin to posterior end of pelvic fin origin (g)	
Anterior dorsal fin to anterior anal fin insertion (h)	
Posterior dorsal fin insertions to posterior pelvic fin insertion (i)	
Posterior dorsal fin insertion to anterior anal fin insertion (j)	
Posterior dorsal fin insertion to ventral caudal fin insertion (k)	
Dorsal caudal fin insertion to ventral caudal fin insertion (l)	
Anterior anal fin insertion to dorsal caudal fin insertion (m)	

to form a total of 13 measurements. The measurements were conducted by inserting a pin on an insertion of the first landmark (A-snout) followed by a similar insertion on the second landmark (B-Ventral junction of operculum). The readings were then taken by vernier callipers along a straight edge. The same procedure were repeated for all other measurements along different landmarks. The measurements of non-truss (measured distances along the length of a single landmark i.e. fin length), were done by inserting a pin on a point of attachment of the fin to the trunk and a vernier calliper then run through a straight edge to the tip and a record of the readings was taken (Fig. 2)[26,27].

Data analysis: Size-dependent morphometric variables were log-transformed to normalize the dataset. The log-transformed data were each correlated to fish total length to establish the relationship between total length and each morphometric variables. After establishing the relationship between total length and morphometric variables, the log-transformed variables were then corrected to eliminate the correlation of the variables with size. This was done by adopting an allometric method suggested by Elliot *et al.*[31], Turan[15] and Mir *et al.*[16], using the formula:

$$\text{Madj} = \text{M}\,(\text{L}_s/\text{L}_o)^{\text{b}}$$

where, M is the original truss and non-truss measurement of the morphometric variable, Madj is the size adjusted truss and non-truss measurement of the morphometric variable, L_o is the total length of the fish, L_s is the overall mean total length and b was estimated for each variable from the observed data as the slope of the regression of log M on log L_o using the fish from every population.

The results derived from the allometric method were confirmed by testing the correlation between transformed variables and total length (L_o).

The differentiation among the populations was based on the percentage of correctly and incorrectly classified fish. The degree of intermingling among the populations as indicated by the number of misclassified individuals. The first and the second F-ratios that test both canonical correlations were explored for significance to describe the differences among the three populations. The discriminant function for dimensions one and two was explored.

The separation among the populations was explored using a discriminant function analysis score plot.

In order to identify the contribution of each morphometric variable to the observed variation, loading plot that provides the graphical way of looking at the standardized discriminant function coefficient was used. Log transformed morphometric variables were used for the production of the loading plot and the correlation table. The transformed variables are therefore, denoted by capital letters to differentiate it from 'adj' (adjusted).

Statistical analysis: Morphometric truss and non-truss parameters were subjected to Discriminant Function Analysis (DFA) to explore possible separation among the three populations using the software STATA, version 12. The percentage of correctly classified individuals was used as a measure of the morphological distinctness of the samples pooled within groups. Correlations between variables and discriminant scores were used to interpret canonical variants among populations[16]. Significant ($p<0.001$) morphometric differences and the separation of the three populations away from each other were confirmed by Wilk's lambda (λ) test[31].

RESULTS

Fish population characteristics: A total of 251 freshly caught fish samples were obtained; 113 specimens from River Nile, (only four from the lake-river interface upstream from the Owen Falls hydropower dam and 109 downstream the dam); 61 were from Lake Edward while 77 from Kazinga channel (Table 2).

All the morphometric characters were linearly correlated to total length, indicating significant relationship between respective variables with size before allometric transformation (Table 3).

The size correlated morphometric variables showed no relationship with size after allometric transformation. None of the 19 morphometric variables showed a significant correlation with total length, indicating the size effect was successfully removed from the dataset as showed by standardized discriminant function coefficients below. None of the variables showed a significant correlation of $\geq$0.9 in both function one (DFA 1) and two (DFA 2) Table 4.

Morphometric variation: The re-substitution classification table (confusion matrix) indicated population one had the highest number (80.53%) of individuals classified correctly

Table 2: Sampling sites, fishing effort and size (±SD) of *B. altianalis* from River Nile, Lake Edward and Kazinga

Water body	Landing Site	Shoreline coordinates	Sample size	Fishing duration (days)	Fishmass (kg)	Fish total length (cm)
Nile river			113	13	Average	Average
	Kalange	N00.47690 E033.16570	34	3	2.52±0.7	57.62±4.8
	Bukeka	N00.55221 E033.08302	36	1	0.83±0.5	36.95±10.5
	Kiira	N00.45398 E033.18178	39	2	2.55±0.9	57.69±7.0
	Ripon	N00.43393 E033.19382	04	7	1.65±1.5	47.78±10.7
Kazinga channel			77	4	Average	Average
	Katunguru	S00.12522 E030.04763	37	2	0.19±0.2	24.58±5.1
	Kazinga	S00.20813 E29.74357	40	2	0.32±0.1	29.45±7.9
Lake Edward			61	6	Average	Average
	Katwe	S00.14745 E029.88352	31	3	2.20±2.0	49.46±21.4
	Kayanja	S00.08747 E029.74357	30	3	2.60±1.9	53.20±19.8

while populations two (67.2%) and three (60.5%) had more misclassifications between them indicating that the latter groups could be harder to separate (Table 5).

Among the 19 variables, orbital distance, pelvic fin and caudal fin lengths (OBDadj, PVFLadj and CFLadj) respectively, were not significantly (p>0.001) different in population one. Wilk's lambda (λ) test of DFA showed significant (p<0.001) differences in morphometric variables and confirmed significant separation of the three populations from each other (Table 6).

The results of the factor analysis between the three populations indicated that the two factors (DFA1 and DFA2) collectively explained 100% of the total morphometric variation. With exception of OBDadj, PVFLadj and CFLadj, all other variables had higher loadings. Those with higher loadings accounted for 84% of the variance than the second-factor loading accounted for 16% of the variance (Table 7).

The group means on the first function showed that population one (River Nile (-0.8869) separated farthest from populations two (Lake Edward (0.796) and three (Kazinga channel (0.6796). The score plot illustrates the populations being reasonably well separated from each other with minimum intermingling. There is clear separation in the second dimension between the lacustrine (Lake Edward) from riverine (River Nile and Kazinga channel) but with insubstantial overlap (Fig. 3).

Observations of the loading plot indicated variables OBDadj, PVFLadj and CFLadj near the origin, implying that they provide almost no discriminating ability in comparison to the other discriminating variables (Fig. 4).

Table 3: Regression models of TL against morphometric variables of *B. altianalis*

Relationship	Equation	r^2
TL and a	a = 0.2+0.14TL	0.9
TL and b	b = -0.08+0.16TL	0.9
TL and c	c = -0.84+0.21TL	0.93
TL and d	d = 0.48+0.23TL	0.93
TL and e	e = 0.47+0.28TL	0.94
TL and f	f = -0.12+0.27TL	0.91
TL and g	g = -0.01+0.39TL	0.94
TL and h	h = 0.004+0.24TL	0.89
TL and i	i = 0.002+0.23TL	0.92
TL and j	j = 0.33+0.32TL	0.91
TL and k	k = -0.01+0.25TL	0.94
TL and l	l = -0.29+0.13TL	0.83
TL and m	m = 0.71+0.31TL	0.87
TL and obd	obd = -0.58+0.10TL	0.58
TL and pfl	pfl = 0.36+0.15TL	0.93
TL and dfl	dfl = 1.50+0.13TL	0.93
TL and pvfl	pvfl = 0.85+0.13TL	0.5
TL and afl	afl = 0.52+0.14TL	0.95
TL and cfl	cfl = 1.64+0.17TL	0.41

Regression model of TL, truss and non-truss variables (p≤0.0 5), TL is total length of each fish sample obtained from respective landing sites, The different letters marked in lower case represent respective truss and non-truss variables measured from each fish sample as elaborated in Table 1 above

Table 4: Coefficients of correlation of adjusted morphometric variables with discriminant functions of *B. altianali*

Size adjusted morphometric variable	DFA 1	DFA 2
Aadj	-0.0582117	0.4408181
Badj	0.0782379	0.4325121
Cadj	0.0012114	0.2965586
Dadj	-0.0501821	0.2630142
Eadj	0.0903308	0.2694192
Fadj	0.0535007	0.1789762
Gadj	0.0138467	0.2338929
Hadj	0.1330427	0.2639298
Iadj	0.1095107	0.3081632
Jadj	0.0019803	0.2394415
Kadj	-0.0323128	0.3549375
Ladj	-0.0644008	0.1503315
Madj	0.0069744	0.4603842
OBDadj	-0.0129624	0.1976076
PFLadj	-0.1129453	0.2749095
DFLadj	-0.2550584	0.304675
PVFLadj	-0.2550584	0.1493597
AFLadj	-0.1048381	0.314537

The different letters marked in upper case represent respective size adjusted/corrected truss and non-truss variables measured from each fish sample as elaborated in Table 1 above

Table 5: Re-substitution classification summary of three populations of *B. altianalis*

	Classified			
True population	1	2	3	Total
1	91.0	10.0	12.0	113
	80.5	8.9	10.6	100
2	7.0	41.0	13.0	61
	11.5	67.21	21.3	100
3	13.0	17.0	46.0	76
	17.1	22.4	60.5	100
Total	111	68	71	250
	44.4	27.2	28.4	100
Priors	0.33	0.33	0.33	

Table 6: Wilk's lambda tests (function 1 and 2) for the verifying and classifying differences among three populations of *B altianalis*

Test of function	Wilk's lambda (λ)	Chi-square	Df	p-value
1 through 2	0.08	209.76	458	0.0000
2	0.53	529.0	230	0.005

Table 7: Canonical linear discriminant analysis of morphometric variables of *B. altianalis* from River Nile, Lake Edward and Kazinga channel

DFA	Canonical correlation	Eigen value	Variance	Likelihood ratio	F	Df1	Df2	p-value
1	0.6301	0.658569	0.8396	0.5356	4.4168	38	458	0.000
2	0.3343	0.125794	0.1604	0.8883	1.6074	18	230	0.059

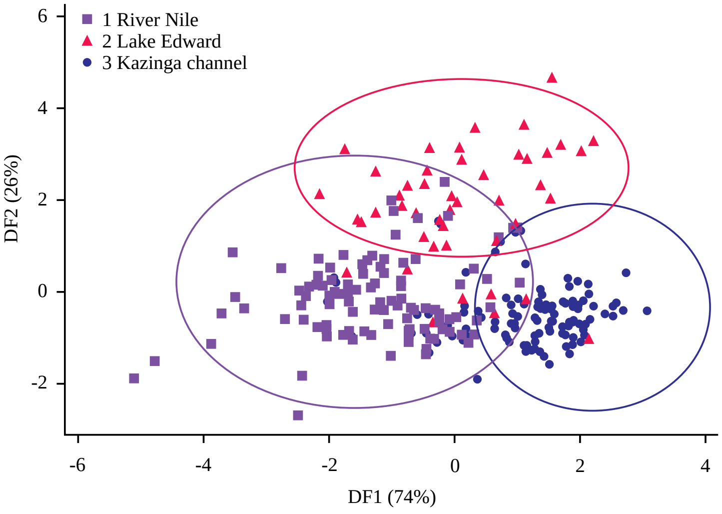

Fig. 3: DFA graph of 19 morphometric variables used for *B. altianalis* from River Nile, Lake Edward and Kazinga channel. Labels 1, 2 and 3 represent River Nile, Lake Edward and Kazinga Channel populations respectively

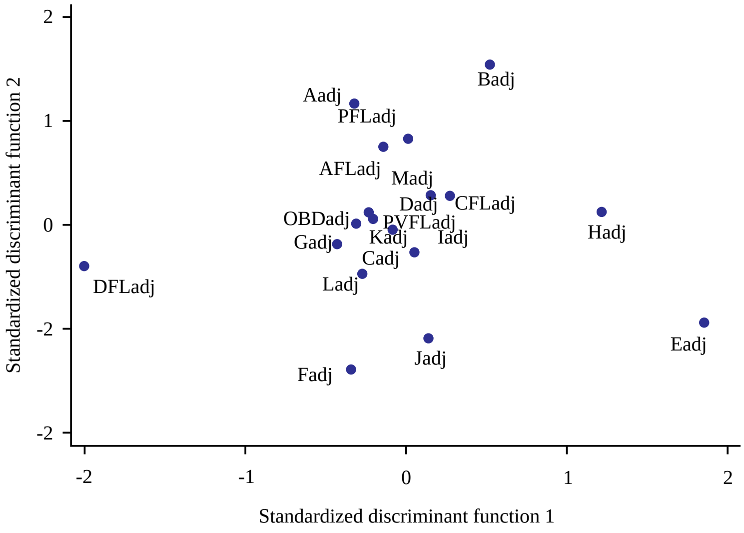

Fig. 4: Standardized discriminant factor loading plot (source; personal data)

a: Snout, b: Ventral junction of operculum junction, c: Supra orbital, d: Lower pectoral fin insertion, e: Anterior dorsal fin insertion, f: Posterior end of pelvic fin insertion, g: Posterior end of dorsal fin insertion, h: Anterior end of anal fin insertion, i: Dorsal caudal fin Insertion, j: Ventral caudal fin insertion, k: Posterior dorsal fin insertion to ventral caudal fin insertion, l: Dorsal caudal fin insertion to ventral caudal fin insertion: m: Anterior anal fin insertion to dorsal caudal fin insertion, Here variables are denoted by uppercase letters to differentiate them from 'adj' (adjusted)

DISCUSSION

There was no significant overlap among the River Nile, Lake Edward and the Kazinga channel populations. Intersect among the populations as revealed by the score plot, collectively showed no intermingling, implying significant heterogeneity among populations (Fig. 3). The observed variations were largely explained by both truss and non-truss morphometric variables. Exceptions were with the distance between the eyes, referred to as orbital distance (OBDadj), pelvic fin (PVFLadj) and caudal fin (CFLadj) lengths.

Group means of the first function further indicated that the River Nile population (-0.89) was further separated from Lake Edward (0.79) and Kazinga channel (0.68). Similarly, the matrix also puts River Nile population much further morphologically from the Lake Edward and Kazinga channel counterparts. Over 80.53% of individuals were correctly classified in River Nile, contrary to 67.2 and 60.5% in Lake

Edward and Kazinga channel populations respectively. The smaller margin of variance between Lake Edward and Kazinga channel populations implies the two populations could be much harder to separate. The variations were however, more concentrated on the anterior side of the specimens. As indicated in Table 4, the snout length and other parts of the head region contributed the largest variance in dimensions one (-0.06) and two (0.44).

Similar phenotypic variations among populations had earlier been observed with the variation in snout configuration in samples obtained from Lakes Edward and Victoria basins[6,2]. However, these earlier studies relied mainly on single parameter (snout), contrary to the current study which used multiple variables.

Morphological variation among fish populations due to deviation in head and middle regions of the fish are useful indicators of differentiation among respective populations, mainly as a result of geographical and topographical deviations that compel the different populations to adapt differently to physiological and morphological requirements of the habitat. Moreover, variation in the snout form reveals differences in the methods of obtaining food, following dietary shifts among populations. These forms therefore, induce evolution of body shape, influencing selection of food and prey catching mechanisms[16,32].

Whereas the posterior part of the fish did not significantly contribute to the variation, the stability of the caudal (CFLadj) and the pelvic (PVFLadj) fin lengths across different water bodies shows the traits have been conserved across populations. Notably, in bony fishes, the caudal fins are mainly used for propulsion to move the fish forward, while the pelvic fin provides the ability during sharp turns, quick and vertical movement along the water column. As reported by Ogutu-Owhayo *et al.*[33], the smothering effects of siltation of River Katonga that used to provide a direct connection between Lakes Victoria and Edward basins blocked upstream migration of aerophilic *Barbus altianalis*, rendering them more or less obligate lacustrine or riverine species.

Similarly, as observed, the low abundance of *B. altianalis* upstream Owen-falls dam is characteristic of the species that is extinct from the lacustrine side of River Nile. Owen-falls hydropower dam and the dense anoxic conditions along the riverine habitats have barred the fish from migrating; subjecting individuals blocked upstream the reservoir to high mortality rates due to predation and high fishing pressure. Recent study on *B. altianalis* fishery reveals deteriorated environmental quality and high fishing effort against the species across River Nile, Lake Edward and Kazinga channel[34]. Similarly, Ochumba and Manyala[11] observed that, habitat alteration through anthropogenic processes caused the growth of dense papyrus at the mouth of River Sundo-Miru in Kenya, hampering up and downstream migration among potamodromous fish species. Likewise, such vegetation also limited the entry of newly hatched fry into the lake from the river, predisposing the fry to higher predation pressure and lack of suitable diet[29].

These finding may be useful to fisheries management and aquaculture production by providing baseline information on inter-population morphological variation in *B. altianalis*. Coupled with the findings of Ondhoro *et al.*[34] that presents data on fishing effort and environmental parameters, this information could assist in generating guidelines for location-specific management based on the prevailing environmental conditions and stock structure. A similar morphological study was applied to identify hatchery against wild stocks of Coho salmon (*Oncorhynchus kisutch*) [17,18,20]. Spawning experiments with *B. altianalis* concur with the current findings following survival variation in several spawning trials of wild specimens collected from the same study sites[10]. Individuals from the Lake Victoria basin had better spawning characteristics than their Lake Edward basin counterparts that experienced high broodstock mortality and very low larval survival rates[10].

Despite these apparently good findings, the shortcoming with morphological characterization still rests in its inability to resolve variation due to genetic influence. The use of nuclear markers such as SNP analysis is recommended for future population studies.

CONCLUSION

This study shows *B. altianalis* stocks in riverine and lacustrine systems are morphologically distinct and is very rare in the Lake Victoria- River Nile interface mainly due to its stocks being locked upstream by the dam, facing high fishing pressure and low rate of recruitment. This information is therefore, relevant for the development of appropriate capture fisheries and ecological monitoring policy based on periodic integrated multivariate approaches.

SIGNIFICANCE STATEMENT

This study discovers and provides the first construction of truss and non-truss dimensions for *B. altianalis* multivariate morphological discrimination. The study generated baseline data for monitoring the effects of isolating mechanisms such as hydropower dams on migrating fish species. With the emergency of more hydropower dams along the River Nile, the information generated in this study will provide a baseline for future monitoring of *Barbus* populations in Uganda and beyond.

ACKNOWLEDGMENT

Financial support for this study was provided through the National Fisheries Resources Research Institute-Uganda, by the Agricultural Technology Agri-business Advisory Services (World Bank ATAAS) Project (P.109224), administered by the National Agricultural Research Organization (NARO). Director of Research Buginyanya ZARDI (Dr. Lawrence Owere) provided additional financial support in processing the manuscript. We acknowledge the efforts of EA. Nyboer of McGill University Canada, for proof-reading the manuscript. Special thanks to the field staff; R. Wasswa and P. Odongo for guidance and coordination with fish folk in Kasese and Jinja districts respectively. M. Turyashemererwa of Aquaculture Research and Development Centre (ARDC) Kajjansi offered information and communication technology (ICT) support.

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