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Morphological variation of *Oreochromis niloticus* (Lacépède, 1803) populations in stressed environment: Middle stream of Comoé River (Ivory Coast)

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Abstract

This work aims to assess the influence of environmental stress in the middle course of the Comoé River on the morphological characteristics of *Oreochromis niloticus* populations using the geometric morphometric approach. A total of 248 specimens of *O. niloticus* were collected from four sites (M'Basso, YèrèYèrè, Abradinou and Bettié). No specimen of *O. niloticus* has been collected from the Manzan River, which has a high level of gold panning activity. Nineteen (19) homologous landmarks were digitized on each individual. Data were studied using principal component analysis, canonical variable analysis, discriminant function analysis and allometric analysis. The PC1 and PC2 axes show an extension of the deformation grids at the level of the head and the caudal and dorsal fins. However, the PC3 axis almost exhibits a deformation grid that deforms at the caudal fin, pectoral fin, and dorsal fin. The results of the various analyzes showed significant differences between the morphology of the populations studied. These differences were observed mainly in the head, dorsal and pectoral fins. The disturbance of aquatic habitats has an impact on the morphology of specimens of *O. niloticus* from the middle course of the Comoé River. A slight deformation is recorded in the individuals of *O. niloticus* at M'Basso, considered the least disturbed site. On the other hand, a strong deformation is observed in specimens from the YèrèYèrè, Abradinou and Bettié sites which are more disturbed.

Keywords: Comoé; Disturbed habitat; Geometric morphometric; Ivory Coast; *Oreochromis niloticus*

1. Introduction

Fish species account for more than half of all vertebrates (Marchetti *et al.,* 2020). Fish has an important economic value and a valuable source of animal protein for humans, in addition to being an important component of biodiversity (Marchetti *et al.,* 2020). In West Africa and particularly in Ivory Coast, fish is the main source of animal protein for populations with an estimated consumption of 20 kg/inhabitant/year, i.e. an estimated annual national need of 300,000 t (Anonyme, 2015). In continental fishery, one of the most coveted species is *Oreochromis niloticus* (Lacépède, 1803), which belongs to the family Cichlidae. *O. niloticus* has an original strictly African distribution covering the Nile, Chad, Niger, Senegal, Gambia, Ivory Coast, Volta and Lake Tanganyika basins (Lévêque and Paugy, 2006). Due to its interest in fish farming, this species is one of the most important species in African fish farming (Ansah *et al.,* 2014). *O. niloticus* is known for its rapid growth and high reproductive potential (Doudet, 1992) and is likely to produce individuals with interesting zootechnical traits when crossed with *Sarotherodon melanotheron* (Bopo *et al.,* 2018). In addition, this fish abundant in Ivorian waters is sensitive to variations in climatic and environmental conditions due to anthropogenic activities (chemical pollution and gold panning) that plague Ivorian ecosystems, in this case the Comoé River. Several studies on the reproduction (Duponchelle and Panfili, 1998), diet (Madrid *et al.,* 2001) and ecology (Lacroix *et al.,* 2004)

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of *O. niloticus* have been conducted by the scientific community. Also, a morphometric study of *O. niloticus* population based on traditional morphometry was carried out by Gourène and Teugels (1993). Traditional morphometry which used linear distance such as length, width, height, etc. is strongly correlated with height. Therefore, measurements from two different sources can produce the same results and the same source can produce different results. Shape analysis therefore becomes very difficult. To overcome this, landmark-based geometric morphometry is used to study shape variation (Adams *et al.,* 2004). Shape variation using geometric morphometrics can help us understand phenotypic, ecological, and behavioral differences and can elucidate the evolutionary pathway (Klingenberg *et al.,* 2003). Moreover, the geometric morphometric method is a relatively modern method with many advantages including being cost effective, fast and useful (Bookstein, 1997) and has been used successfully in many studies (Banimasani *et al.,* 2017). To our knowledge, no geometric morphometric study based on landmarks has been conducted on *O. niloticus* from Ivory Coast. Analysis of the body shape of this species could be a major asset for understanding its biology. Indeed, body shape is one of the most obvious aspects of phenotype, potentially providing a link between genotype and environment (Ricklefs and Miles, 1994). Investigations based on body shape are able to provide valuable functional and taxonomic information (Adams *et al.,* 2004). The objective of this study is to evaluate the influence of the environmental stress that prevails in the middle course of the Comoé River, on the morphological characteristics of *O. niloticus* populations by the variation of body shape using the approach of geometric morphometrics based on landmarks.

2. Materials and Methods

2.1. Study environment and Choice of study stations

The town of Bettié is located in the south-east of Ivory Coast, in the heart of the sub-equatorial forest zone.

A total of five stations from upstream to downstream were selected in the middle reaches of the Comoé River (Figure 1). These are: M'basso, Manzan, YèrèYèrè, Abradinou and Bettié. The characteristics of each of the sampling stations are recorded in Table 1. For this study, the choice of stations was made considering their situation in relation to agglomerations, their accessibility at any period, the permanence of water and the importance of gold panning and other anthropogenic activities (fishing, presence of housing, dumping of domestic waste, presence of plantations and livestock activities).

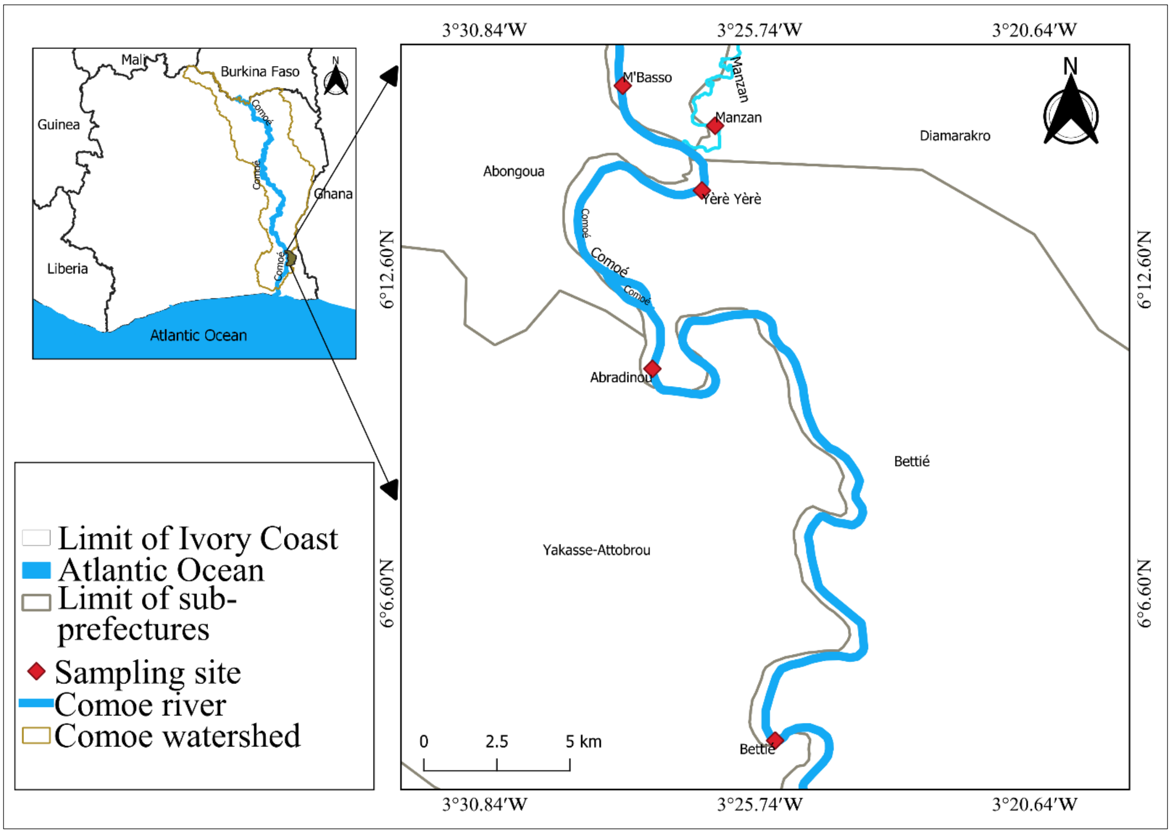

Figure 1 The sampling map of the study stations

2.2. Ichthyological sampling

The quarterly sampling method was conducted over one year, from March 2021 to March 2022. It is subdivided into 4 campaigns including 2 campaigns in the dry season and 2 other campaigns in the rainy season. A sampling campaign is carried out over 3 consecutive days. Sampling took place in the middle reaches of the Comoé River on the 5 experimental stations (M'Basso, Manzan, YèrèYèrè, Abradinou and Bettié). Catches are made using nets of different meshes (7, 10, 12, 13, 18, 20, 25, 28, 30, 35, 50, 60, and 70 mm) to capture the maximum number of specimens of *O. niloticus*. Inventory data from experimental fisheries were supplemented with inventory data from commercial fisheries to increase the likelihood of having a high diversity of fish. During this study, nets were laid only once per campaign at each site between 5 and 6 p.m. and surveyed the next day between 7 a.m. and 9 a.m. A total of 248 specimens of *O. niloticus* were collected at the sites of M'Basso, YèrèYèrè, Abradinou and Bettié. No specimens of *O. niloticus* have been captured on the Manzan River with high gold panning activity. The fish collected are sorted and divided into groups according to the identification keys of Paugy *et al.* (2003a and 2003b). These fish are then transported to the laboratory using coolers containing a mixture of ice and pooch-ice. Only individuals of *Oreochromis niloticus* identified in the captures were photographed and then subjected to geometric morphometric analysis.

Table 1 Characteristics of each of the sampling stations

Stations	**Geographical coordinates (Latitude / Longitude)**	**Land use**	**Substrate**
M'basso	06.24834° / -003.45189°	- Plantations (rubber, cocoa, bananas) - Village - Forest	Mud, clay, silt, gravel, sandstone and pebbles
YèrèYèrè	06.24566° / -003.44944°	- Plantations (rubber, cocoa, bananas) - Latrines near the river, - Livestock (bovine and ovine) - Village	Mud, Clay, Silt
Abradinou	06.17935° / -003.46151°	- Plantations (rubber, cocoa, bananas) - Village - sand extraction, -Pipe draining household water to the river	Mud, clay, silt, sand
Bettié	06.06382° / -003.42307°	- Plantations (rubber, cocoa, bananas), -Village, Water treatment plant - Bridge, - Pipeline	Mud, Clay, Silt

2.3. Image capture

The different photographs of each specimen were taken with a smartphone brand SONY model Xperia G3221, series RQ3006YEKA. The camera lens is positioned above each specimen and the vertical distance is kept constant (15 cm) at all image shots while maintaining the same magnification and brightness.

2.4. Geometric morphometry

Three software programs (tpsUTIL, tpsDIG 2 and MorphoJ) were used for the processing of morphological data. The tpsUtil software (version 1.80) is used to create an input file for the data acquisition program. The tpsDig software (version 2.31) is used to retrieve images and scan landmarks on images that will then be used to record the x and y coordinates of reference points (Rohlf, 2015). MorphoJ software (version 1.07a) (Klingenberg, 2011) is used for data analysis. A total of 19 homologous reference points are chosen to describe the actual shape and size of each *O. niloticus* specimen in this study (Figure 2). The benchmarks used in this study (Table 2) are chosen on the basis of the amounts of information they contain according to the first work in geometric morphometry, carried out on cichlids by Rüber and Adams (2001), as well as on the basis of the typology of reference points defined by Bookstein (1997). Before the coordinates were applied, the scale factor for the measurement was defined in the tpsDig software using the graph paper included in the background of each image. An identification code is placed near each specimen before the image is captured.

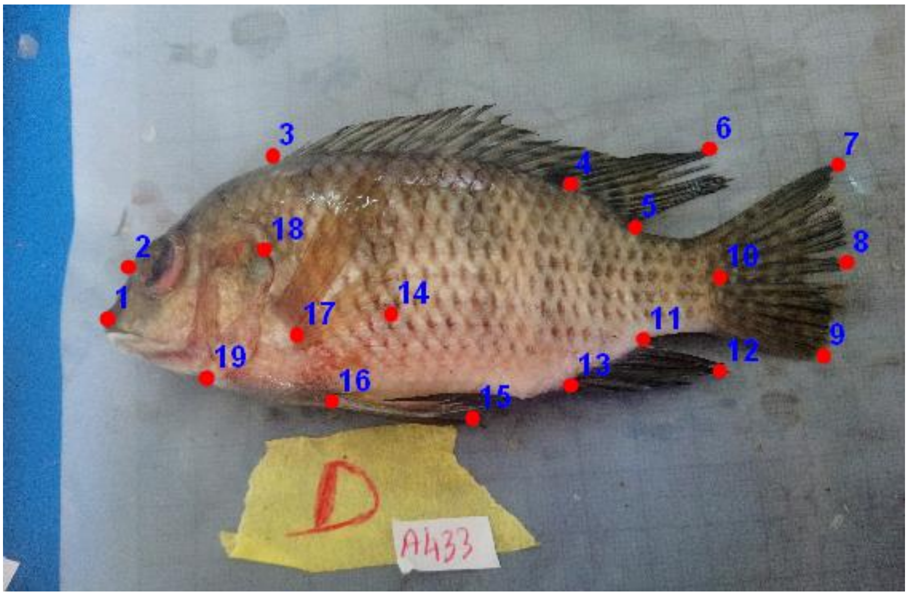

Figure 2 Digitization of 19 reference points on the right side (D) of the fish *Oreochromis niloticus* using tpsDig2 software

Table 2 Description of the selected landmarks

Landmarks	Descriptions
L1	Top of the upper lip
L2	Nose opening
L3	Anterior insertion of dorsal fin
L4	Posterior base of last hard dorsal fin ray
L5	Posterior insertion of dorsal fin
L6	Top of dorsal fin soft rays
L7	Anterior end of caudal fin
L8	Posterior margin of caudal fin
L9	Posterior end of caudal fin
L10	Posterior margin of tail
L11	Posterior insertion of anal fin
L12	Upper end of anal fin
L13	Anterior insertion of anal fin
L14	Upper end of pectoral fin
L16	Anterior insertion of pelvic fin
L17	Lower end of pectoral fin
L18	Upper point of curvature of operculum
L19	Lower point of curvature of operculum

2.5. Statistical analysis of data

After the Procrustes superposition which eliminates position, scale, and orientation effects, principal component analysis (PCA) is applied to the new coordinates called the Procrustes coordinates of the reference points using the covariance matrix of the Procrustes coordinates. This process identifies the main orthogonal axes (principal components) that explain most of the variation in fish shape. A canonical analysis of variance (CVA) is then performed on the Procrustes coordinates of everyone. The site in which the fish evolved is considered an independent factor. This analysis makes it possible to visualize the differences in fish shape between the different groups considered (Klingenberg and Monteiro, 2005). The biological hypotheses explaining the separation of the groups are then constructed by analyzing the relative position of each group (Albrecht, 1980). Mahalanobi distances are measured between sites two by two to see if populations are close. The Procuste distance, which can be defined by an angle separating each group in multidimensional space, can be directly converted to similarity or difference in shape between groups. The significance of these distances is evaluated by a permutation test (10.000 permutations). Finally, the analyses by discriminant functions (DFA) are then carried out to test the divergences between the groups taken two-to-two. Allometric effects are tested through regression between shape PC scores and centroid size followed by a permutation test (10.000 permutations). All these analyses were conducted using MorphoJ software (Klingenberg, 2011).

3. Results

3.1. Comparison of the mean population form of *Oreochromis niloticus* by Principal Component Analysis (PCA)

The main component analysis is carried out based on the data of the nineteen benchmarks obtained at the level of the body conformation of the individuals of *O. niloticus* collected from the sampling sites of Abradinou, Bettié, M'basso and YèrèYèrè) excluding the Manzan site, because no specimens of *O. niloticus* were collected at this site. Figure 3 shows the morphological space defined by the axes CP1 (19.74%), CP2 (11.15%) and CP3 (9.94%) which account for 40.83% (Table 3) of the total variability of the conformation. We notice that the distinction between groups is not very clear, all groups indeed overlap. Deformation grids associated with the main components (PC) make it possible to determine the morphology of specimens (Figure 4). The PC1 and PC2 axes show an extension of the deformation grids at the level of the head, caudal, dorsal and fins. However, the PC3 axis has almost a deformation grid that deforms at the caudal fin, the pectoral fin and the dorsal fin.

Table 3 Percentage variance of the first three axes of the principal component analysis

CP	Eigenvalues %	Variance %	% Cumulative variation
1.	0.00138894	19.744	19.744
2.	0.00078421	11.147	30.891
3.	0.00069926	9.940	40.831

CP: Principal Component

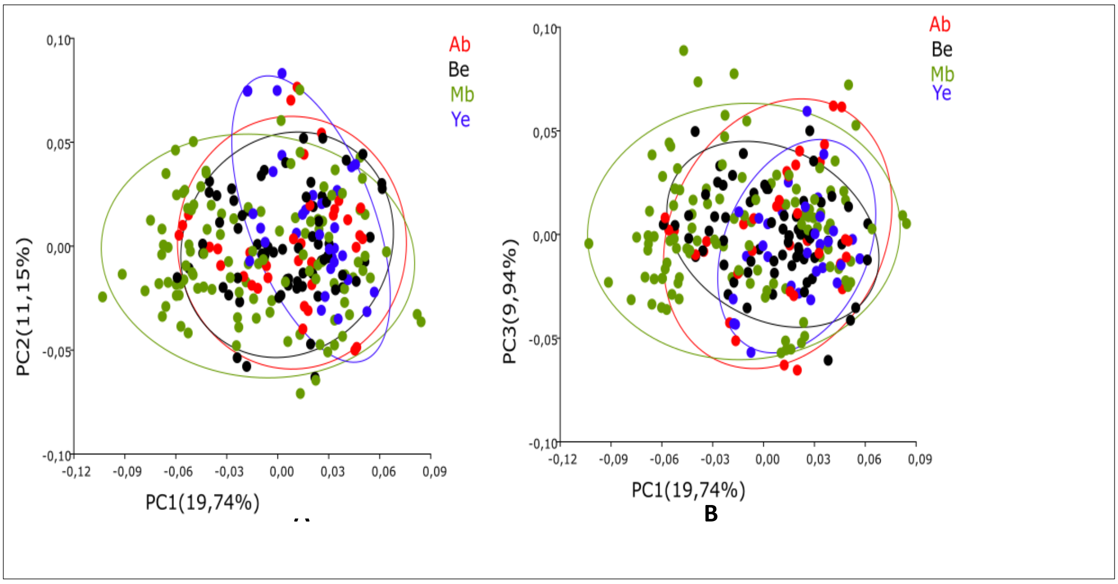

Figure 3 Principal Component Analysis Based on Procrusteal Residuals of the Benchmark Point Configurations of the entire *Oreochromis niloticus* sample explained by the selected axes: (A) CP1 and CP2 then (B) CP1 and CP3 for 248 observations of the four Ab sites: Abradinou (red dots), Be: Bettié (black dots), Mb (green dots) and Ye: YèrèYèrè (blue dots). 90% confidence ellipses (p = 0.900).

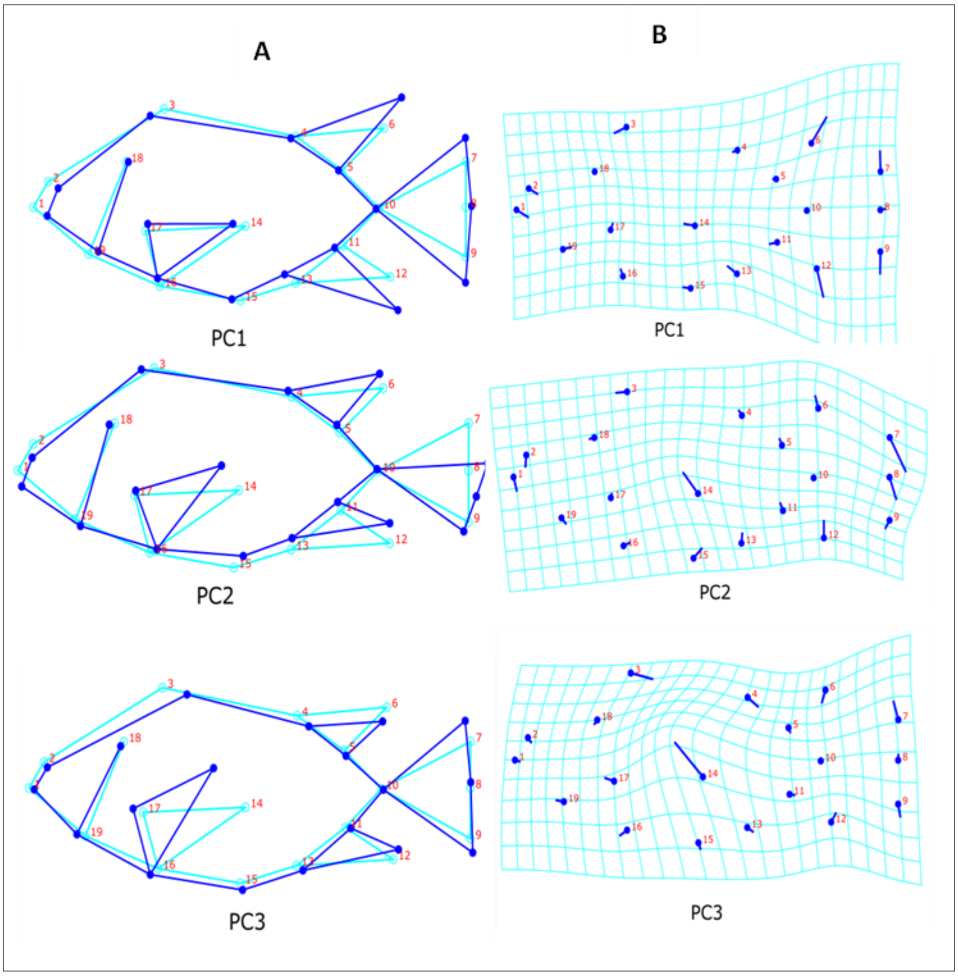

Figure 4 Visualization of the conformation change of *Oreochromis niloticus* along the PC1, PC2 and PC3 axes. A: "wireframe graph" and B: "transformation grid"

3.2. Comparison of the mean population form of *Oreochromis niloticus* by canonical variable analysis (CVA)

An analysis of canonical variables is performed on the mean form of the 248 individuals of *O. niloticus*. The graphical result is shown in Figure 5. The plan considered is structured around the two axes CV1 and CV2 which explain 89.76% (Table 4) of the total variability observed. The first canonical variable (CV1) explains 54.84% of the variation between groups. This axis splits the individuals of YèrèYèrè and M'Basso located on the positive side with the individuals of *O. niloticus* from the Bettié and Abradinou sites located on the negative side of axis 1. The second canonical variable (CV2) explains 34.92% of the total variation. It locates the individuals of *O. niloticus* from Abradinou and YèrèYèrè in the positive plane of axis 2 and those of Bettié and M'Basso on the negative side of the same Axis. Considering the cumulation of axes, the specimens of *O. niloticus* of Bettié are in the plane (-CV1; -CV2) and those of M'Basso in the plane (+CV1; -CV2). As for the individuals of YèrèYèrè, they are in the landmark (+CV1; +CV2) and finally the populations of Abradinou are spotted (-CV1; +CV2).

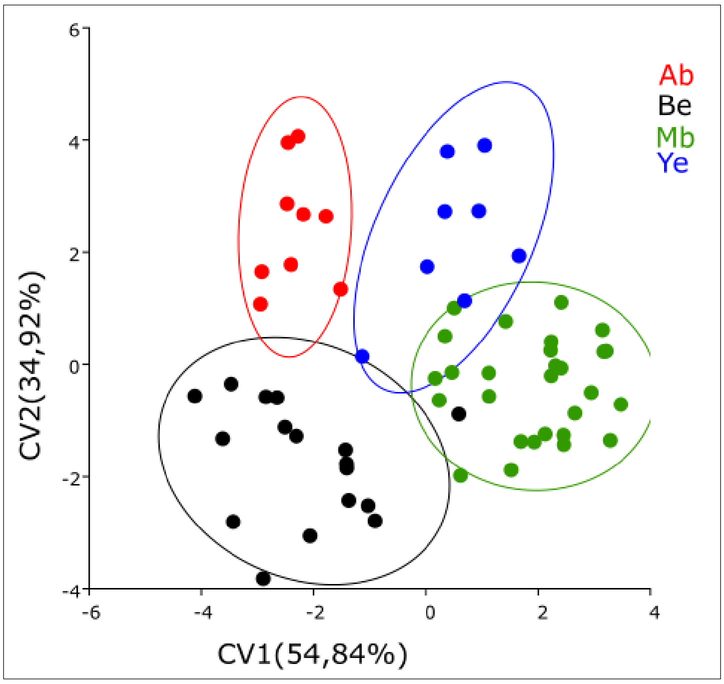

Figure 5 Results of the canonical variable analysis on the first two axes for the average of 248 individuals of *Oreochromis niloticus* caught at the different sites (Ab: Abradinou, Be: Bettié, Mb: M'Basso and Ye: YèrèYèrè), 90% confidence ellipses (p = 0.900).

Table 4 Percentage variance of the first two axes of the canonical variable analysis

CV	Eigenvalues %	Variance %	% Cumulative variation
1.	1.32822542	54.84	54.84
2.	0.51552358	34.92	89.76

CV: canonical variable

Figure 6 shows the wireframe graphs and deformation grids of *O. niloticus* individuals. Among the 19 landmarks or Landmarks (L) used to assess the morphology of *O. niloticus*, 9 of them are associated with canonical variable 1 (CV1): "L1, top of the upper lip", "L4, posterior insertion of the dorsal fin", "L8, posterior limit of the caudal fin", "L9, posterior end of the caudal fin", "L11, posterior insertion of the fin", "L12, upper end of fin", "L14, upper end of pectoral fin", "L15, lower end of pelvic fin", and "L18, upper point of operculum curvature". The Landmarks associated with the canonical variable 2 (CV2) are 10 in number: "L2, opening of the nose", "L3, anterior insertion of the dorsal fin", "L5, Posterior insertion of the dorsal fin", "L6, dorsal fin soft ray top", "L7, caudal fin anterior end", "L10, posterior tail boundary", "L13, anterior fin insertion", "L16, anterior pelvic fin insertion", "L17, lower pectoral fin end" and "L19, lower operculum insertion". The ACV shows that the reference points influencing the morphology of YèrèYèrè individuals are L1, L3, L9, L10, L14, L17. Points L2, L4, L5, L6, L12, L15 and L18 are the variables that separate Bettié populations from other sites. As for the specimens of M'Basso three characters participate in their deformation, it is the anterior end of the caudal fin

(L7), the posterior insertion of the fin (L11) and the anterior insertion of the pelvic fin. The reference points that position the individuals of Abradinou *O. niloticus* in the plane (-CV1; +CV2), are L8 L13 and L19. Moreover, these differences were significant after the permutation test using Mahalanobis distances during LCA. The Procrustean ANOVA conducted to assess measurement error showed that the mean squared value of individuals was greater than the measurement error at all study sites.

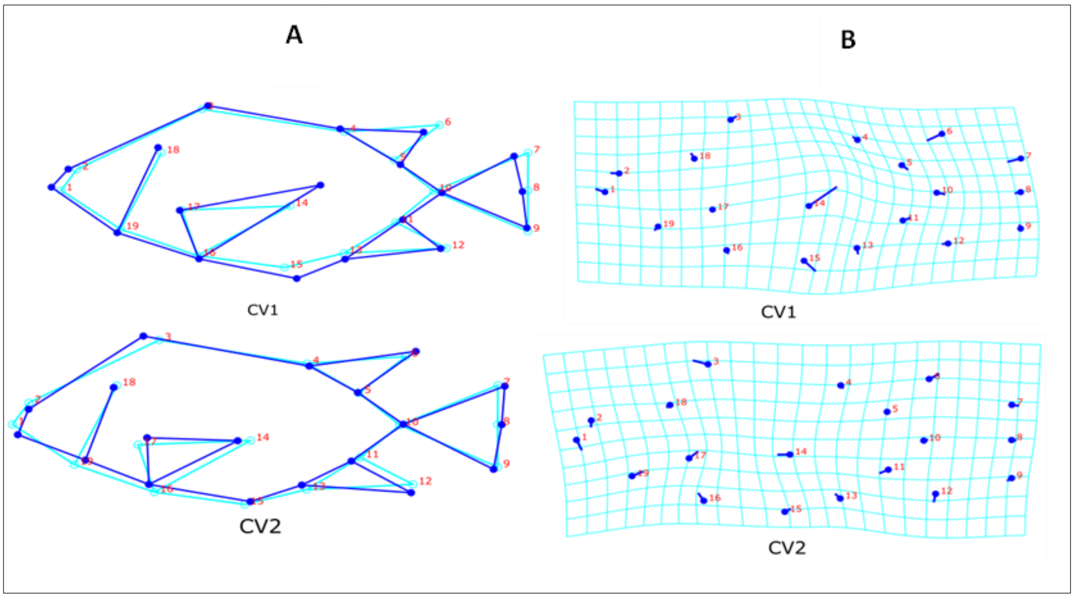

Figure 6 Wireframe graph (A) and Warp grids (B) representing the shape changes of *Oreochromis niloticus* associated with CV1 (above) and CV2 (below).

3.3. Distance from Mahalanobis and distance from Procruste

Table 5 shows the matrix of Mahalanobis distances from *O. niloticus* sampling sites. The population of Abradinou is significantly distant from that of M'Basso. Indeed, these two populations have the greatest distance from Mahalanobis (5.18). Specimens of *O. niloticus* from YèrèYèrè and those from M'Basso are significantly closest to each other (3.70). The permutation test indicates a significant difference between these two population groups (P<0.0001, per 10.000 towers). The average Procrustean distance between each site is calculated for the four sites considered.

Table 5 Distance of Mahalanobis between different populations of *Oreochromis niloticus.*

	Ab	Be	Mb
Be	4.44*		
Mb	5.18*	4.36*	
Ye	4.1047	5.03*	3.70*

The starred results (*) are significant (Ab: Abradinou, Be: Bettié, Mb: M'Basso and Ye: YèrèYèrè)

Table 6 Distance of Procrutes between the different populations of *Oreochromis niloticus*

	Ab	Be	Mb
Be	0.0225		
Mb	0.0411	0.0318	
Ye	0.0369	0.0305	0.0406

Ab: Abradinou, Be: Bettié, Mb: M'Basso and Ye: YèrèYèrè

The results are presented in Table 6. We notice that individuals from Abradinou and Bettié have the smallest distance from Procruste (0.0225), so they are morphologically similar. On the other hand, the population of Abradinou and that of M'Basso have the greatest distance from Procruste (0.0411). However, there is no significant difference in Procruste distances between these different populations of *O. niloticus* (P>0.0001).

3.4. Comparison of the mean form of *Oreochromis niloticus* populations by discriminant factor analysis (DFA)

Figure 7 presents the results of the discriminant function of the population of *O. niloticus*. According to the analysis of the discriminant function, individuals of *O. niloticus* who are morphologically close come from Abradinou and YèrèYèrè (2.933). On the other hand, the populations of Abradinou and M'Basso are very remote (72.04). The Procruste and Mahalanobis distances observed in the different groups are not significantly different (10.000 turns permutation test, P > 0.0001) between localities (Table 7).

Table 7 *Oreochromis niloticus* groups formed from discriminant factor analysis

Groups	D. Procruste	D. Mahalanobis	P
Ab-Be	0.0225	3.93	0.981
Ab-Ye	0.0369	2.933	0.9748
Be-Ye	0.0305	4.60	0.9570
Ab-Mb	0.0411	72.04	0.017
Be-Mb	0.0318	6.88	0.0215
Mb-Ye	0.0406	25.77	0.3997

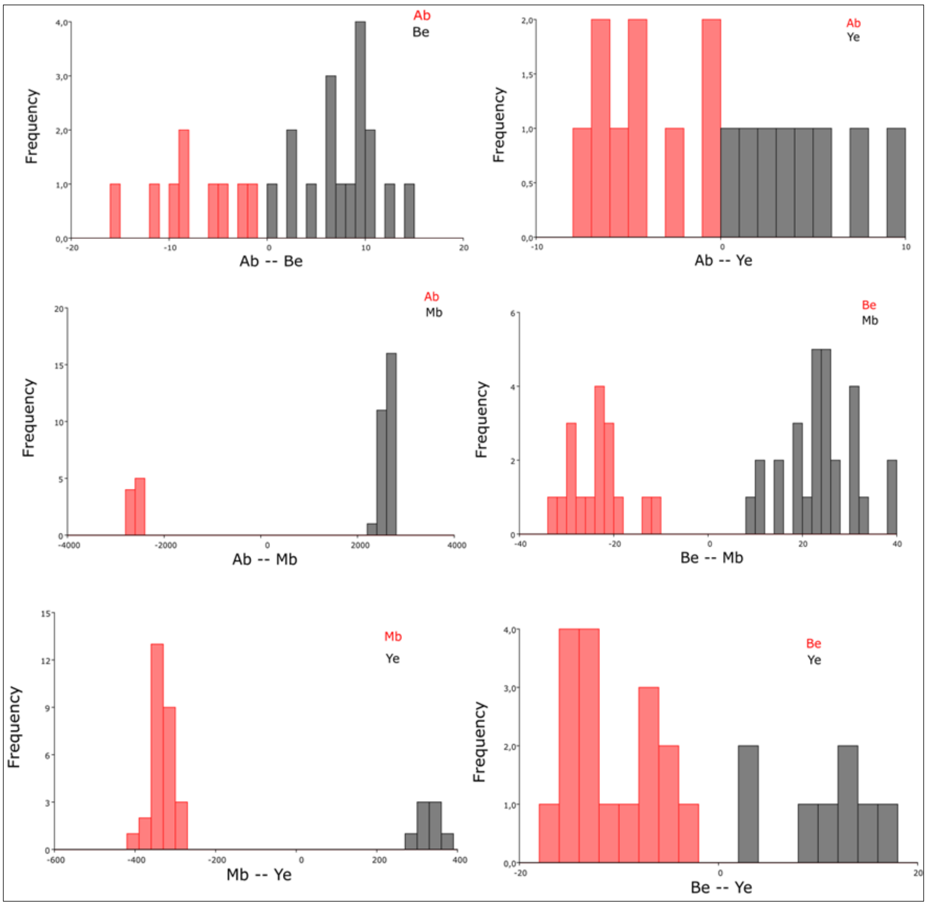

Figure 7 Discriminant Function Analysis (DFA) graph for *Oreochromis niloticus* populations for the sampling site category (Be: Bettié, Ab: Abradinou, Mb: M'Basso and Ye: YèrèYèrè).

3.5. Effect of allometry of *Oreochromis niloticus*

Allometry is the effect of size on conformation. It was evaluated on specimens of *O. niloticus* from the sampling sites of Bettié, Abradinou M'Basso and YèrèYèrè. The regression of the Procrustes coordinates on the centroid size of the fish *O. niloticus* is shown in Figure 8. It appears from this analysis that the allometry is significant (permutation test 10.000 revolutions, P<0.0001) for all specimens of *O. niloticus* from the study sites. However, the shape of individuals showed changes depending on height overall, with only 1.59% obtained as a prediction value in the multiple regression analysis. In addition, the relationship between the shape and size of the *O. niloticus* fish in each group showed that the variation in the shape of the fish was not significantly related to its size in each study site (permutation test with 10.000 turns, P >0.0001). But the percentage change in fish shape explained by size changes was 5.23% respectively; 3.28%, 5.26% and 2.05% in individuals from Abradinou, Bettié, YèrèYèrè and M'Basso (Table 8).

Table 8 Linear regressions of conformation variables as a function of centroid size of *Oreochromis niloticus* of sampling sites

Groups	Effectives	Frequencies (%)	P
Abradinou	36	5.23	0.055
Bettié	68	3.28	0.021
YèrèYèrè	32	5.26	0.089
M'Basso	112	2.05	0.018

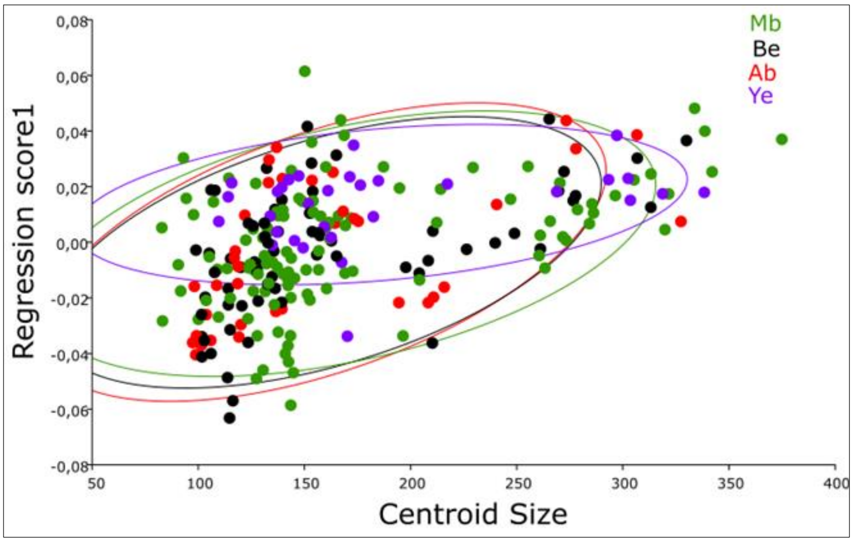

Figure 8 Multivariate regression of Procrustes coordinates of the shape of the fish *Oreochromis niloticus* on its centroid size (Mb: M'Basso, Be: Bettié, Ab: Abradinou and Ye: YèrèYèrè). 90% confidence ellipses (p = 0.900).

4. Discussion

In this study, multivariate analyses based on data from nineteen benchmarks obtained at the level of body conformation of *Oreochromis niloticus* individuals collected at the sampling sites (Abradinou, Bettié, M'basso and YèrèYèrè) were performed. We excluded the Manzan site because no individuals of *O. niloticus* have been collected from this site. The results of the discriminant analyses (LCA and AFD) show a clear separation and tight grouping between populations of *O. niloticus* from the sampling sites. The results of LCA and DFA confirmed the significant distinction between the population form of *O. niloticus* from M'Basso with moderate pollution used as a reference site and specimens of *O. niloticus* from the sites of YèrèYèrè, Abradinou and Bettié with intense pollution. Allometry is significant (10.000 turns permutation test, P<0.0001) for all *O. niloticus* specimens from the study sites. The different morphological characteristics of populations of a single species may be due to genetic differentiation or phenotypic plasticity in response to the environmental parameters of their habitat (Schluter 2009). Since habitat factors have a significant effect on the morphology of fish species (Baumgartner *et al.* 1988) therefore, morphological differences in *O. niloticus* observed in the middle Comoé River may be related to habitat diversity or sampling sites. Sampling sites have different coloration and water depth. The chemical stress caused by agricultural inputs, wastewater, and the phenomenon of gold panning in the middle reaches of the Comoé River must have influenced the development of organisms. It is possible that the mechanisms of correction of morphological traits engaged by the processes of homeostasis of the development of the body of the fins have been ineffective. This could result in the production of different phenotypes at the morphological level. According to Hoffmann *et al.* (2002), disturbance during development can result in the production of different phenotypes, especially since pollutants in the aquatic environment are considered major disruptors that can act on the development process of organisms. However, DeWitt *et al.* (1998) state that when environmental stress is large and persists over a long period of time, phenotypic plasticity remains limited. This can result in high mortality of plastic individuals and overall decline in reproduction and growth. It is possible that this situation is that of natural fish populations in the study area where massive mortalities of aquatic organisms have been observed since the advent of gold panning and rubber cultivation. In addition, in their study on homologous point morphometry of the invasive fish *Lepomis gibbosus*, Yerli *et al.* (2016) noted that this species tended to adopt a morphotype specific to each different habitat in order to adapt to it. These results would lead to the conclusion that the morphometric dissimilarity of the different fish populations in our study could be caused by an adaptation phenomenon related to environmental pressures of the habitat of the fish population studied.

5. Conclusion

This study is the first attempt at a geometric morphometric approach conducted on *Oreochromis niloticus* individuals in the waters of the middle Comoé River. Canonical analysis of variance and analysis of discriminant function successfully revealed significant variations in body shape between the four populations of *O. niloticus* examined. Inter-population variation in *O. niloticus* may indicate that individuals are phenotypically sensitive to environmental factors. Further molecular genetic studies should combine morphometric approaches to achieve accurate discrimination among *O. niloticus* populations.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors declare that there are no conflicts of interest

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