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Morphometric Differentiation of Two Nigerian Fish Species (*Oreochromis niloticus* and *Lates niloticus*) Using Principal Components and Discriminant Analysis

Diferenciación Morfométrica de Dos Especies de Peces Nigerianos (*Oreochromis niloticus* y *Lates niloticus*) Utilizando Análisis de Componentes Principales y Análsis Discriminante

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SUMMARY: Morphological differences between two close fish populations were investigated in Doma Dam, north central Nigeria using principal component and discriminant analyses. The purpose was to identify the two species correctly and assign them into distinct genetic groups. To achieve this, seven morphometric measurements (body weight, standard length, total length, head length, body depth, dorsal fin length and caudal fin length) were made on each of the studied 252 fish specimens (153 *Oreochromis niloticus* and 99 *Lates niloticus*). The analysis of variance showed that significantly ($P<0.05$) higher values were recorded for *Lates niloticus* in all the body traits examined, except dorsal fin length. From the principal component analysis, the differences between the species resulted mainly from dorsal fin length, body weight, caudal fin length and head length. The stepwise discriminant analysis revealed that standard length, dorsal fin length and caudal fin length were the three most discriminating variables useful to distinguish clearly the two species at mature age. These three variables with strong discriminating power correctly classified 98.0% of individuals from the sample of known-fish populations. The classification accuracy was cross-validated using split-sample method, and indicated a 97.6% success rate (98.0% of *Oreochromis niloticus* and 97.0% of *Lates niloticus* were correctly assigned). The results can provide a sound scientific basis for the effective management and sustainable exploitation of the genetic resources of the two species under subtropical conditions.

KEY WORDS: Fish; Genotypes; Conservation; Morphological traits; Multivariate analysis; Nigeria.

INTRODUCTION

The quantification of specific characteristics of an individual, or group of individuals can demonstrate the degree of speciation induced by both biotic and abiotic conditions, and contribute to the definition of different stock of species (Bailey, 1997). Tilapia fish is an indigenous African fish that is widely cultivated especially in Asia and the Middle East (Machena & Moehl, 2001). It is much appreciated by consumers, being a good and affordable source of protein. *Oreochromis niloticus* (Nile Tilapia) and *Lates niloticus* (Nile Perch) are two important fish species found in aquatic environments in Nigeria. Due to their great similarity, the taxonomic relationship between the two fish species is controversial. Although they can be discriminated physiologically, it is very difficult to identify them with meristic index for they have many overlapping characters especially at the juveline stage. As a result of the tremendous pressure to capture *Lates niloticus*, an illegal mix with *Oreochromis niloticus* is often sold to unsuspecting consumers. This phenomenon not only damages the interests of the consumers, but is also very harmful to the protection and sustainable utilization of the two species. Thus it becomes imperative to discern their morphological differences and equally develop a practical method to identify them accurately. Scientifically, sound management of fish resources relies on information on the biology of the species, including information on population structure.

Morphometric variation between stocks can provide a basis for stock structure, and may be applicable for studying short-term, environmentally induced variation geared towards successful fisheries management (Murta, 2002; Pinheiro *et al.*, 2005). Morphometric measurements are

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widely used to identify differences between fish populations (Tzeng, 2004; Cheng *et al.*, 2005; Buj *et al*., 2008; Torres *et al*., 2010). On the other hand, the use of multivariate techniques such as principal components and discriminant analyses to quantify morphometric variables is receiving increased attention in stock identification (Kusznierz *et al.*, 2008; Bektas & Belduz, 2009; Specziar *et al*., 2009).

In Nigeria, an assessment of morphometric differentiation of indigenous fish species using multivariate mathematical approach has not been exploited. The present investigation therefore, aimed at examining the morphometric variability between *Oreochromis niloticus* and *Lates niloticus* in their natural fresh water habitat in north central, Nigeria. The results can provide a solid base for the identification, rational management, breeding and conservation of the two fish species.

MATERIAL AND METHOD

Study area. The study was carried out in Doma Dam, Nasarawa State, north central Nigeria (Sub-Saharan Africa). The State falls within the guinea savanna agro-ecological zone, and is found between latitudes 07º52'N and 08º56'N and longitudes 07º25'E and 09º37'E, respectively. It has two distinct seasons. The wet season lasts from about the beginning of May and ends in October. The dry season is experienced between November and April. Annual rainfall figures range from 1100 to 2000mm. The mean monthly temperatures in the State range between 20º and 34ºC, with the hottest months being March/April and the coolest months being December/January (Lyam, 2000).

Doma Dam was established in 1985 by the Federal Government of Nigeria. The Dam lies between latitude 08º 33'N and longitude 08º 32'E. The altitude is 181.53 above mean sea level. It is broadly divided into two sections: The side Dam is 320m long while the main Dam is 520m long, with an average height of 27m. The storage capacity of the reservoir is 3,000,000cm^3, with two spill wheels that are used to regulate the amount of water in the Dam during the rainy season. The Dam, supervised by the Lower Benue River Development Authority (LBRDA), is aimed primarily at making a pull of water available for the irrigation of agricultural crops. However, different fish species naturally found their way into the Dam (NADP, 2006).

Data collection. Two hundred and fifty two mature fish samples comprising 153 *Oreochromis niloticus* and 99 *Lates niloticus* of both sexes were harvested in Doma Dam from May to June, 2009. The minimum and maximum atmospheric temperatures during the study period were 23.7 and 31.0ºC, respectively. The water (Dam) average temperature and pH were 28ºC and 7.5, respectively. Fishing was done by means of cast nets of 25 and 30mm mesh sizes. The fish specimens were transported in ice chest to the laboratory where measurements started immediately to avoid shrinkage. Each fish specimen was subsequently given a serial identification number after it was drained off using filter papers.

Seven morphological measurements were made on each specimen. The body parts were measured following standard anatomical reference points (Omoniyi & Agbon, 2004; Alp & Kara, 2007). The body traits measured were body weight (BW); measured as total weight including gut and gonads; standard length (SL), measured from the maxilla to the end of the caudal peduncle; total length (TL), taken from the maxilla to the end of the caudal lobe; head length (HL), horizontal distance from the maxilla to the most posterior point of the opercula on the ventral side; body depth (BD), taken from the level of the origin of the anal fin; dorsal fin length (DL), measured from the origin of the dorsal fin to its end and caudal fin length (CL), taken from the beginning of the caudal fin to its end. Body weight was taken using a 2-kg electronic balance, while the rest of the morphometric traits were taken using digital callipers and determined to the nearest millimetre on a measuring board.

Statistical analysis. Data on the morphometric measurements (body weight, standard length, total length, head length, body depth, dorsal length and caudal length) of the two fish species were analyzed using univariate (analysis of variance (ANOVA), with means separated using the two-tailed, two-sample t-test) and multivariate statistical methods. The multivariate techniques involved the use of principal components and canonical discriminant analyses. Principal components (PC) are a weighted linear combination of correlated variables, explaining a maximal amount of variance of the variables thereby aiding in data reduction. In the PC analysis, determinant of the correlation matrix was used to test for multicollinearity and singularity. Anti-image correlations, Kaiser-Meyer- Olkin measures of sampling adequacy and Bartlett's Test of Sphericity (tests the null hypothesis that the original correlation matrix is an identity matrix) were computed to test the validity of the principal component factor analysis of the data set. Cumulative proportion of variance criterion was employed in determining the number of factors to extract. Reproduced and residual correlations were used to test the appropriateness of the number of factors extracted. The varimax criterion of the orthogonal rotation method was employed in the rotation of the factor matrix to enhance the interpretability of the factor analysis.

Stepwise discriminant analysis was used to identify the combination of variables that best separate the two genetic groups. The relative importance of the morphometric variables in discriminating the two fish populations was assessed using the F-to-remove statistic. Collinearity among the variables used in the discriminant model was evaluated using the tolerance statistic. For species identification, the unstandardized discriminant function procedure was employed. The ability of this function to identify *Oreochromis niloticus* and *Lates niloticus* was indicated as the percentage of individuals correctly classified from the sample that generated the function. The honesty (reliability testing) of the function was validated using split-sample validation (cross-validation) (Poulet *et al*., 2005). In cross-validation, one individual is removed from the original matrix. The discriminant analysis is then performed from the remaining observations and used to classify the omitted individual. The proportion of individuals correctly re-allocated is taken as a measurement of the integrity of that group. The number of misclassified individuals indicates the degree of intermingling between the populations. All analyses were done using SPSS (2001) statistical package.

RESULTS AND DISCUSSION

The unequal sample sizes of the two species reflect the population structure and relative abundance of the two genetic types. The means, standard deviations and coefficients of variation of the morphological measurements of the species are presented in Table I. Higher mean values ($P<0.05$) were recorded for *Lates niloticus* in body weight, standard length, total length, head length, body depth and caudal fin length. However, their *Oreochromis niloticus* counterparts had significantly ($P<0.05$) higher dorsal fin value. The superiority exhibited by *Lates niloticus* may be as a result of its genetic attributes. Genetic variation in weights and body yields of Nile Tilapia strains has been reported (Diodatti *et al*., 2008). In a similar study, Turan (2004), reported that phenotypic and genetic differentiation may occur among fish populations, which may be recognizable as a basis for separation and management of distinct populations

The apparent smaller body stature (configuration) exhibited by the two species compared to their genetic potential might be attributed to low quality and quantity of available feeding materials since the fishes only made do with whatever they came across in the Dam. This situation could have been worsened by the act of predation, whereby some fishes preyed on others (there are different fish species in Doma Dam). Predation pressure and habitat conditions could influence growth and survival. Svänback & Eklöv (2006) reported that most lakes are not single-species lakes and the density fluctuations of all species with time will influence available resources and the feeding regime, which could engender predation risk. Overfishing is another factor that could be responsible for the seemingly small body structure. The burning desire of the members of the communities within and around the Dam to make ends meet led to indiscriminate fishing activities. As a result of this, there was the tendency of harvesting most of the fishes of bigger sizes.

In the principal component analysis, the determinant of the correlation matrix (0.001) was greater than 0.00001 (which is the standard), while anti – image correlations computed showed that partial correlations were low, indicating that true principal component factors existed in the data. This was buttressed by Kaiser-Meyer- Olkin measure of sampling adequacy studied from the diagonal of partial correlation, revealing the proportion of the variance in the body measurements caused by the underlying factor. The present value of 0.87 was found to be sufficiently high for all the body traits. The overall significance of the correlation matrix tested with Bartlett's Test of Sphericity for the morphometric traits of the two fish populations (chi-

Table I. Basic statistics for the morphometric characters of *Oreochromis niloticus* and *Lates niloticus*.

Traits	*Oreochromis niloticus*			*Lates niloticus*		
	Mean±SE	**SD**	**CV (%)**	**Mean±SE**	**SD**	**CV (%)**
BW (g)	488.89±12.32^{b}	152.42	31.18	638.41±11.46	114.04^{a}	17.86
SL (cm)	19.42±0.27^{b}	3.40	17.51	27.22±0.29	2.92^{a}	10.73
TL (cm)	23.77±0.29^{b}	3.55	14.93	31.66±0.29	2.92^{a}	9.22
HL (cm)	7.06±0.10^{b}	1.27	17.99	8.78±0.16	1.62^{a}	18.45
BD (cm)	9.30±0.12^{b}	1.47	15.81	11.10±0.19	1.87^{a}	16.85
DL (cm)	12.89±0.22^{a}	2.73	21.18	12.24±0.27	2.72^{b}	22.22
CL (cm)	5.95±0.13^{b}	1.59	26.72	8.23±0.18	1.82^{a}	22.11

SD: Standard deviation; CV: Coefficient of variation. Means within the same row bearing different superscript differ significantly ($P<0.05$).

square = 2061.99; P < 0.01), provided support for the validity of the factor analysis of the data set. The communalities, which represent the proportion of the variance in the original variables that is accounted for by the factor solution ranged from 0.753-0.987. This further lent credence to the appropriateness of the factor analysis. Estimates of the residual correlation matrix were low enough.

After varimax rotation of the factor axes, three principal components were extracted which accounted for 90.92% of the total variance of the original seven morphological variables (Table II). The first principal component (PC1), which explained 75.23% of the total variation, represents allometric size factor, whereas the second and third principal components (PC2 and PC3) most often lack correlation to size and they are the most informative (Delling *et al*., 2000). Therefore, the highest character loadings in PC2 and PC3 should be taken into consideration to indicate the differences between the two species. Factor pattern coefficients of the rotated factors show the relative contribution of each trait to a particular component. Dorsal fin length and body weight had the highest loadings on PC2, while PC3 was characterized by caudal fin length and head length. This is an indication that the differences between the two fish populations resulted mainly from dorsal fin length, body weight, caudal fin length and head length. In a related study, Specziar *et al*. submitted that morphometric characters enabled a clear separation among genotypes; with the results of the principal component analysis suggesting that together with some other morphometric traits, fin morphology has a special importance in assigning genotypes into distinct groups. However, Turan *et al*. (2005) reported that the differences between fish species arose mainly from head measurements. In another study, principal component analysis was used to discern morphological differences in river blenny (Alp & Kara).

Results of the stepwise discriminant analysis showing Wilk's Lambda values, probability and tolerance statistics are presented in Table III. When the three most important morphometric traits (standard length, dorsal fin length and caudal fin length) for separating the two species were selected, Wilk's Lambda dropped to 0.205 with a significant difference between the two species (F = 43.304; P<0.001). The tolerance values obtained; 0.294-0.432 were greater than 0.1 indicating that there was no collinearity problem among the three most discriminating morphometric variables. Like the results obtained from principal components, the discriminant analysis also proved the high discriminating power of fin related characters. While standard length and caudal fin length are longer in *Lates niloticus*, dorsal fin length is higher in *Oreochromis niloticus*. Discriminant analysis is a common method used to identify fish populations (Maric *et al*., 2004; Bektas & Belduz).

Table II. Explained variation associated with rotated factors along with factor loadings and communality for the morphometric measurements of *Oreochromis niloticus* and *Lates niloticus*.

Variables	**PC1**	**PC2**	**PC3**	**Communality**
Body weight	0.669	0.574	0.360	0.884
Standard length	0.895	0.186	0.366	0.952
Total length	0.917	0.127	0.311	0.924
Head length	0.590	0.343	0.595	0.798
Body depth	0.600	0.480	0.474	0.815
Dorsal fin length	0.131	0.944	0.227	0.940
Caudalfin length	0.420	0.303	0.819	0.758
Eigenvalue	5.266	0.805	0.293	
% variance	75.23	11.50	4.19	

Table III. Morphometric characters selected by stepwise discriminant analysis to separate *Oreochromis niloticus* and *Lates niloticus*.

Variable	**Wilk's Lambda**	**F-remove**	**P-level**	**Tolerance**
Standard length	0.433	368.564	0.0001	0.415
Dorsal fin length	0.414	341.228	0.0001	0.294
Caudal fin length	0.205	43.304	0.0001	0.432

The unstandardized stepwise discriminant function was used to classify individual fishes. The three discriminating variables were the variables included in the discriminant (D) equation below:

$$D = -4.692 + 0.410SL + 0.384CL – 0.567DL$$

The classification function could be directly used to identify the two species, since positive D scores indicate *Lates niloticus* and negative D scores indicate *Oreochromis niloticus*. This function was able to classify correctly 247 of the 252 fishes (i.e. 98.0%) (Table IV). Cross-validation with the split-sample method indicated a 97.6% overall success rate (98% of *Oreochromis niloticus* and 97% of *Lates niloticus* were correctly assigned). The three discriminating variables therefore, were sufficiently robust to be used in the field to identify and determine the population structure of the fish species. This is an indication that morphological measurements could be taken into consideration to increase the consistency of individual fish identification especially by farmers, livestock extension officers, researchers and most especially consumers who are not familiar with the two fish species. In a related investigation, Turan *et al*. (2005) was able to correctly classify overall 78% of six populations of *Clarias gariepinus*. Similarly, Pollar *et al*. (2007) reported that the discriminant analysis correctly classified 95.6% of *Tor tambroides*, while the cross-validation testing procedure correctly assigned 93.1% of the fishes into determined populations. The discriminating variables obtained in this study might not be exhaustive as the inclusion of other conventional and non-conventional variables might produce more discriminants. More evidences drawn from biochemical and molecular genetics will also consolidate information arising from morphological variability. However, these will be a subject for future investigation.

Table IV. Classification results for the discriminant analysis. Species 1.00= *Oreochromis niloticus*; 2.00= *Lates niloticus*. 98.0% of the original grouped cases correctly classified. 97.6% of cross-validated grouped cases correctly classified.

		Predicted Group Membership		
	Species	**1.00**	**2.00**	**Total**
Original count	1.00	150	3	153
	2.00	2	97	99
%	1.00	98.0	2.0	100.0
	2.00	2.0	98.0	100.0
Cross validated count	1.00	150	3	153
	2.00	3	96	99
%	1.00	98.0	2.0	100.0
	2.00	3.0	97.0	100.0

CONCLUSION

This study provided the fish farmers, researchers, extension workers and consumers a useful tool for the identification and assessment of stock status of *Oreochromis niloticus* and *Lates niloticus*. This involved using a small set of morphometric characters (variables with high discriminating power) that are easy-to-operate with and that are easily accessible in field work. These variables were obtained via principal component and discriminant analyses. Since the identification of populations and their connectivity between each other is a major point for management, breeding and conservation of species, the use of standard length, dorsal fin length and caudal fin length for this purpose under the present subtropical conditions appears promising.

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RESUMEN: Las diferencias morfológicas entre dos poblaciones de peces similares se han investigado en Doma Dam, centro-norte de Nigeria utilizando análisis de componentes principales y análisis discriminante. El propósito fue identificar correctamente las dos especies y asignarlas a los distintos grupos genéticos. Para lograr esto, siete medidas morfométricas (peso corporal, longitud estándar, longitud total, longitud de la cabeza, profundidad del cuerpo, longitud de la aleta dorsal y la longitud de la aleta caudal) se realizaron en cada una de las muestras de los 252 peces estudiados (153 *Oreochromis niloticus* y 99 *Lates niloticus*) . El análisis de varianza mostró que de forma significativa ($p<0,05$) los valores más altos se registraron en *Lates niloticus*, en todos los rasgos del cuerpo examinado con excepción de la longitud de la aleta dorsal. A partir del análisis de componentes principales, las diferencias entre las especies se observó principalmente en la longitud de la aleta dorsal, el peso corporal, la longitud de la aleta caudal y longitud de la cabeza. El análisis discriminante reveló que la longitud estándar, longitud aleta dorsal y caudal son las tres variables más discriminatorias de utilidad para distinguir claramente las dos especies en la edad madura. Estas tres variables con fuerte poder de discriminación clasificaron correctamente el 98,0% de los individuos de la muestra de las poblaciones conocidas de peces. La exactitud de la clasificación fue sometida a una validación cruzada utilizando el método de la muestra dividida, la que indicó una tasa de éxito del 97,6% (98,0% de *Oreochromis niloticus* y 97,0% de *Lates niloticus* asignados correctamente). Los resultados pueden proporcionar una base científica sólida para el manejo eficaz y la explotación sostenible de los recursos genéticos de las dos especies en condiciones subtropicales.

PALABRAS CLAVE: Pescado; Genotipos; Conservación; Características morfológicas; Análisis multivariado; Nigeria.

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