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MORPHOMETRIC DIFFERENTIATION AMONG FOUR POPULATIONS OF RED TILAPIA (*Oreochromis spp.)*

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ABSTRACT

The study investigated the population structure of four red tilapias (*Oreochromis* spp.) populations from different Philippine fisheries institutions to provide baseline information in the morphometric differentiation of these populations. Forty-five (45) truss measurements were measured in each of the four populations. Multivariate analyses showed significant differences in morphometric characters for both male and female samples. Pairwise comparison revealed significant differences amongmale populations. NFFTC and BFS were the least significantly different populations in male and female samples suggesting these two to be the most similar pair of populations. Group membership prediction using discriminant function analysis for male and female individuals showed high number of correctly classified fish with both 99.0%. The discriminant analysis plot for male samples showed clear separation of FAC and RNT from the other populations and slight intermixing between the NFFTC and BFS with 94.4% total between-group variability. In female samples, the plot explaining 93.3% of the total between-group variability showed clear separation of RNT from the other three populations and a slight intermixing between the FAC and NFFTC and between the samples from NFFTC and BFS. The results agreed that morphometric differences between the populations may appeared due to either genetic differences and environmental factors.

Key words: Morphometric, *Oreochromis spp*., Multivariate analysis, Univariate analysis

INTRODUCTION

Red tilapia (*Oreochromis*spp.)have become objects of interest for culturists and researchers throughout the world [1]. In Asia, red tilapia has become popular due to its greater economic value relative to Nile tilapia [2]. Philippines is among the countries that expressed their strong interest in the development of this species [1]which is considered as cheaper alternative fish to high-priced marine red species [3]. Freshwater-reared red tilapia is a high-valued species distributed mostly in urban specialty markets in the Philippines [4]. Despite the high production cost in intensive culture, farming of this species is an economically feasible enterprise given the species' domestic market price that is roughly twice as much as the Nile tilapia [4].

As a potential cultivable species with important biological characteristics [5], management of red tilapia is needed. Management of fish resources relies on basic knowledge on the biology of the species, including information on population structure [6]. This can be obtained through morphological characterization such as morphometric, which has been commonly used in identification of fish stocks [7,8].Morphometric measurements are widely used in identifying differences between fish populations [9] and still one of the main approaches used in ichthyology and aquaculture because of its long tradition and simplicity [10]. Therefore, morphometric analysis of fish is an important key in the study of biology of fish [11]. Identifying stocks of species with unique morphological characters enables a better management of the species and ensures perpetuations of the resources[12]. In this context, morphometric data are of great importance for the improvement of aquaculture [13].

In the Philippines, there are several Asian red tilapia strains available for culture [4]. Virtually, nothing is known about the morphological population structure of red tilapia in the Philippines including the populations used in this study: Central Luzon State University-Freshwater Aquaculture Center (CLSU-FAC); Department of Agriculture-Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (DA-BFAR-NFFTC); red Nile tilapia ofCLSU-FAC; and Southeast Asian Fisheries Development Center-Aquaculture Department-Binangonan Freshwater Station (SEAFDEC-AQD-BFS).

The main objective of this study was to investigate the population structure of four red tilapia (*Oreochromis*spp.) populations from different fisheries institutions/stations namely CLSU-FAC,

DA-BFAR-NFFTC, and SEAFDEC-AQD-BFS using morphometric characters. Specifically, this study aimed to: (1) determine the morphometric characters of the samples from both sexes of the four populations; and (2) differentiate the morphometric characters among the four red tilapia populations.

MATERIALS AND METHODS

Positioning, pinning, measurement of weight and standard length, and documentation of each red tilapia population were conducted at the respective institution/station. Measurement and recording of morphometric variables were done at the Ichthyology Laboratory of CF-FAC, CLSU.

Collection and Conditioning of Fish Samples

Four red tilapia populations were used in this study. Among these were: (1) Central Luzon State University-Freshwater Aquaculture Center (FAC); (2) Department of Agriculture-Bureau of Fisheries and Aquatic Resources-National Freshwater Fisheries Technology Center (NFFTC); (3) red Nile tilapia from CLSU-FAC (RNT); and (4) Southeast Asian Fisheries Development Center-Aquaculture Department – Binangonan Freshwater Station (BFS).In each population, total samples of 50 fishes were measured (twenty-five (25) samples for each sex). Collection of measurement by sex was done since sexual dimorphism is evident in this group of fish. The collected samples were anesthetized using tricaine methane sulfonate (MS-222)

Positioning and Pinning

Cells and truss characters used in this study were modified but referenced according to the scheme described by Turan, 1999; such as, the distance between landmarks a and b was a truss character in the quadrilateral or cell with landmarks a, b, i, and j (Figure 1). On the other hand, the morphometric process and analyses followed were based from the description of Turan, 1999.Fish was placed on a 1-inch thick block of expanded polystyrene, and body posture and fins were teased into a natural position. Morphological features or landmarks that were distinctive and homologous from specimen to specimen were selected around the outline of the fish form. Each landmark was marked by piercing the expanded polystyrene with a dissecting needle. The locations of the ten (10) landmarks used in this study are illustrated in Figure 1.

Measurement of the Fish Samples

Photographic images of the marked and pinned expanded polystyrene with the positioned and teased fish were taken using a digital camera. Morphometric variables were measured from each specimen using ImageJ software (file

version 1.4.3.67; product version 2006.02.01).

Measurements were taken on the left side of fish throughout the sampling and variables were measured to the nearest 0.00001 mm.The morphometric variables included the distances between the following landmarks (Figure 1): (a) anterior tip of snout at upper jaw; (b) most posterior aspect of neurocranium (beginning of scaled nape); (c) origin of dorsal fin; (d) insertion of dorsal fin; (e) anterior attachment of dorsal membrane from caudal fin; (f) anterior attachment of ventral membrane from caudal fin; (g) insertion of anal fin; (h) origin of anal fin; (i) insertion of pelvic fin; and (j) posteriormost point of maxillary. For landmarks b, c, d, g, h, i, and j, points were made at their respective positions at the closest point to the body on a line perpendicular to the horizontal axis of the fish. Distances between landmarks were presented as x-y (where x and y were both landmarks). Forty-five (45) variables/measurements were determined.

Data Analysis

The samples for all populations were took randomly and size effects were minimized, thus, the formula for logarithm of ratio transformation below was used.

$$M_{adj}=logM/logL_s$$

Where:
M_{adj}= the size adjusted truss measurement
logM= the logarithmic transformation of original truss measurement
$logL_s$= the logarithmic transformation of standard length

Since morphological differentiation may vary between the sexes in some fish species [12], the interaction between variables and sexes was also tested. In the case of the present study, females and males were treated separately in multivariate analyses to remove the effect of sex from the result.

Statistical Analyses

The transformed data were analyzed through principal components analysis (PCA) and multiple-discriminant function analysis (DFA). Univariate analysis of variance was also used to compare the variation among samples for size-adjusted truss measurements. Moreover, post hoc multiple comparison test was performed to find the number of significantly different morphometric characters between pairs of samples.Multivariate analysis of variance was performed to test the significance of differences among the samples in the data set.

RESULTS AND DISCUSSION

Interaction of Variables and Sex

Prior to analyses, the interaction between morphometric variables and sex was tested to know whether the analyses would be done separately for male and female or data for the two sexes would be

combined for single analyses. This is because morphological differentiation may vary between the sexes in some fish species [14].

Distinction in morphometric attributes between sexes of *Hypseleotrisagilis*and *Salmo truttafario*respectively[15,16].In this study, testing the interaction of variables and sex from the 200 samples revealed that 30 out of 45 characters, namely a-b, a-c, a-d,b-i, b-j, c-d, e-f, e-g, e-h, c-f, c-g, c-h, c-i, c-j, e-j, f-h, a-i, a-j, f-j, g-h, d-f, d-g, b-e, b-f, g-j, d-h, d-i, d-j, h-j, and i-j, were significantly different between sexes (Table 1) and 21 of which were highly significantly different (P=0.000). Thus, the effect of sex on morphological variation was considered and analyses for morphometric variables of male and female were done separately.

Morphometric studies can be used in determining the shape dimorphism between sexes [17] did in their study on *Oreochromismossambicus*from Lake Lanao, Philippines. Therefore, this result can be attributed to the sexual dimorphism evident to some species belonging to genus *Oreochromis*[18]. Thai red tilapia (*Oreochromis* sp.), for instance, has sexual growth dimorphism [19].

Univariate Statistics Analysis

Univariate statistics analysis showed that 36 out of 45 truss measurements, namely a-b, a-c,a-h, a-i, a-j, b-h, b-i, b-j, c-d, c-e, c-f, c-g, c-h, c-i, c-j, d-e, d-f, d-g, d-h, d-i, d-j, e-f, e-g, e-h, e-i, a-f, f-g, f-h, f-i, f-j, g-h, g-i, g-j, h-i, h-j, and i-j, were significantly different among male populations (Table 2) while 41 truss measurements, namely a-b, a-c, a-f, a-g, a-h, a-i, a-j, b-c, b-d, b-e, b-f, b-g, b-h, b-i, b-j, c-d, c-e, c-f, c-g, c-h, c-i, c-j, d-e, d-f, d-g, d-h, d-i, e-f, e-g, e-h, e-i, f-g, f-h, f-i, f-j, g-h, g-i, g-j, h-i, h-j, and i-j, were significantly different among female samples (Table 3). Likewise, weaker separation of male strains of Nile tilapia was observed by Velasco *et al.,* 1996.

In post hoc multiple comparison tests, FAC samples showed the highest number of significantly different characters among the four populations of male red tilapia (24, 31, and 23 against NFFTC, RNT, and BFS respectively) followed by the RNT samples with 31, 22, and 24significantly different characters when compared to FAC, NFFTC, and BFSrespectively (Table 4). On the other hand, RNT showed the highest number of significantly different characters among the female samples (31, 31, and 32 against FAC, NFFTC, and BFSrespectively); FAC samples obtained the second highest number (21, 31, and 26 against NFFTC, RNT, and BFS respectively).

In this analysis, the populations with the highest number of significantly

different truss characters were FAC red tilapia (highest for male and second highest for female) and RNT (highest for female and second highest for male). Thus, these two populations were the most distinct in terms of number of significantly different variables.

Principal Component and Multiple-Discriminant Function Analyses

Principal component analysis (PCA) combined and summarized the variation associated with each of a number of measured variables into a smaller number of principal components (PC) which were a linear combination of the variables that described the shape variations in the pooled sample [14]. In this analysis, only the components with eigenvalues exceeding 1.00 were included which was also stated in the study of [21]. In the present study, only the first PC was interpreted.

The first component extracted in a principal component analysis accounts for a maximal amount of total variance among the observed variables. The remaining components that are extracted account for a maximal amount of variance in the observed variables that was not accounted for by the preceding components [22]. Principal component analysis proceeds in this manner with each new component accounting for progressively smaller amounts of variance. This is why only the first few components are retained and interpreted [22].

Discriminant function analysis (DFA), which was used to discriminate the samples according to the variables, calculated functions discriminating between samples of known identity and then reclassified the individuals into the designated groups on the bases of these functions [14]. The percentage of correctly classified individuals gave a measure of the morphological distinctness of the samples [14]. However, contributions of variables for the first function were also considered to support the results of PCA[14,21].

Examination of the contribution of each variable to PC1 (accounting for 29.2% of the variation) for male red tilapia showed a high contribution from 14 measurements namely c-e (0.698), c-f (0.746), c-i (0.775), d-e (0.745), d-f (0.769), d-g (0.731), d-h (0.863), e-f (0.722), e-g (0.837), e-h (0.898), e-i (0.771), f-g (0.725), f-h (0.797), and g-h (0.734) which were mostly from the posterior part of the body of fish (Table 5).

On the other hand, the truss measurements with significant loadings on the first discriminant function (DF1) were a-b, a-c, a-j, b-j, c-e, c-d, c-f, c-i, c-j, d-e, d-f, d-g, d-h, e-g, e-h, e-i, f-g, f-h, and g-h (Table 6) which were also mostly from the measurements taken from the posterior

part. However, it also included measurements taken from the anterior and dorsal parts indicating these regions and the posterior body part to be important in the description of male population characteristics.

For female red tilapia, contribution of variables to PC1 (Table 7) were mostly from the measurements taken from the anterior and posterior parts of the fish body (a-b (0.792), a-c (0.758), a-i (0.771), b-i (0.881), b-j (0.855), c-i (0.850), c-j (0.864), d-e (0.782), d-f (0.866), d-g (0.875), d-h (0.887), e-f (0.903), e-g (0.883), and e-h (0.883)) and from the dorsal part of the fish (c-d (0.629) and c-e (0.724)).

The first discriminant function (DF1) also showed a high loading of mostly the same variables (a-b, a-c, a-h, b-i, b-j, c-e, c-d, c-f, c-i, c-j, d-e, d-f, d-g, d-h, e-f, e-g, e-h) which indicated that these regions are important in the description of characteristics of female samples (Table 8).

Most of the truss measurements that greatly contributed to the principal component loadings and discriminant functions, for both male and female samples, were from the anterior part (head), posterior part (tail) and dorsal part of the body of fish. Therefore, these regions can be considered as important in differentiating the four populations of red tilapia used in this study.Previous morphometric studies also showed the importance of these regions in population discreteness. Morphometric differentiation betweenthe dorsal fin length (c-d) is one of the main indicators of difference between *Oreochromis niloticus* and *Latesniloticus*[6]. Morphometrics of different populations of African catfish (*Clariasgariepinus*) and Atlantic herring (*Clupeaharengus*) respectively, observed differences were both mainly from measurements taken from the head of fish, indicating this region to be important in the description of population characteristics[14,24]. Several principal components showed great contribution of truss measurements from the tail region, together with the head portion, especially in differentiation of sexes between the eight strains of Nile tilapia (*Oreochromis niloticus*) [20].

Multivariate Statistics

For multivariate analysis, Wilks' λ tests were done using the discriminant scores from functions. The Wilks' λ tests of discriminant analysis indicated highly significant differencesin morphometric characters of all the populations for both male and female red tilapia (Table 9)[21].

Pairwise comparison between samples using the first function revealed significant differences among the four male populations (Table 10). For female

samples, all populations except NFFTC and BFS red tilapia were distinct from each other. Using the second function, insignificant differences were found between FAC and RNT populations for male samples and between NFFTC and RNT for female samples (Table 10).The analysis showed that there were different results between male and female samples, thus, differentiation of this species must be done separately. The pattern of variable loadings across the principal components indicated significant differences between males and females of the eight strains of Nile tilapia [20].

Using the first function, there were no comparable populations in male group while NFFTC and BFS populations showed insignificant difference in female group. However, it can be observed that NFFTC and BFS were the least significantly different populations in male samples suggesting these populations to be the most similar pair of populations especially among females. Since these two populations were reared in different locations with different culture environments (earthen ponds for NFFTC; concrete tanks for BFS), their poor distinctness can be attributed to their genetic similarity considering they both belong to the genus *Oreochromis*. Similar observation was found wherein female populations of brown trout (*Salmo trutta{fario*) were clustered in two distinct groups with the second group consisting of geographically far apart populations [16].

Though fish are known to exhibit a high component of environmentally induced morphological variation, morphometric differentiation may still be of genetic basis [14]. Samaradivakara *et al.,* 2012 recommended in their study to examine the genetic component of phenotypic discreteness after finding some intermingling between four tilapia populations in selected reservoirs in Sri Lanka. Moreover, the second function showed insignificant differences betweenRNTand FAC for male and between RNT and NFFTC for female. Though RNT population has an obvious genetic distinctness, it still showed that environment is a strong contributor of morphological variation in fish [14] since RNT, FAC, and NFFTC populations were reared in the same vicinity.

Group Membership Prediction and Discriminant Analysis Plot

As mentioned earlier, DFA was used to measure the morphological distinctness of the samples. This was done by using the calculated functions in discriminating between samples of known identity and reclassifying the individuals into the designated groups on the bases of these functions [14]. The overall random assignments of male and female

individuals into their original populationwere high (both 99.0%) (Table 11). The percentage of correctly classified BFS male red tilapia samples to their original group was lowest (96.0%) showing the least clear separation from the other three populations which all obtained 100% correct classification of individuals into their original group. However, this value (96%) can be still considered high since Turan, 1997 described 84% as high overall random assignment of individuals in his study.

On the other hand, NFFTC population showed the lowest percentage of correctly classified female samples to their original group, which is 96% (Table 11). All other female samples were correctly classified into their original group.

Plotting of DF1 and DF2 showed a clear between-sample differentiation among male samples (Figure 2). The first DF accounted for 80.5% and the second accounted for 13.9% of the between-group variability, explaining 94.4% of the total between-group variability (Figure 2). The plot showed clear separation of FAC from the other three populations; the same observation was also showed by the RNT samples. However, slight intermingling was observed between the male samples from NFFTC and BFS, which can also be observed in Table 13 showing the misclassified BFS male red tilapia under the NFFTC column.

In female samples, DF1 accounted for 78.0% while DF2 accounted for 15.3% of the between-group variability that explained 93.3% of the total between-group variability (Figure 3). The plot showed clear separation of RNT from the other three populations. However, there was also a slight intermingling observed between the female samples from FAC and NFFTC and between the samples from NFFTC and BFS. The plot also showed the misclassified NFFTC fish plotted within the points representing the BFS samples.

Generally, all the four populations of red tilapia showed distinctness both in the group membership predictions and in the discriminant analysis plots. However, NFFTC and BFS populations somehow exhibited intermingling both in male and female samples. Since these two populations were reared in different environments, slight morphometric invariability can be due to its genetic similarity. Morphometric differences between the populations may have appeared due to either genetic differences [13], which was observed with RNT population and can be incomplete between NFFTC and BFS populations, or environmental factors, which can be the

reason of poor discreteness between FAC and NFFTC female populations.

Morphometric difference between FAC and BFS male populations can be also attributed to environmental factors. The morphometric analysis of selected species of serranid fishes wherein they considered the differences in habitat and feeding ecology of each species as factors for the shape differences [23]. The detected differences among the morphometric characters of African catfish (*Clarias gariepinus*) populations in Turkey may be related to differential environmental conditions such as temperature, turbidity, food availability, and water depth [24].

CONCLUSION

The present study revealed evidence of highly significant morphometric heterogeneity among the four red tilapia populations. Multivariate analyses showed that there were significant differences in morphometric characters of the four populations of red tilapia for both male and female samples.

Truss measurements taken from the anterior, dorsal, and posterior regions were found important in differentiating the four populations. Comparison between samples using the first and second discriminant scores mostly revealed significant differences.

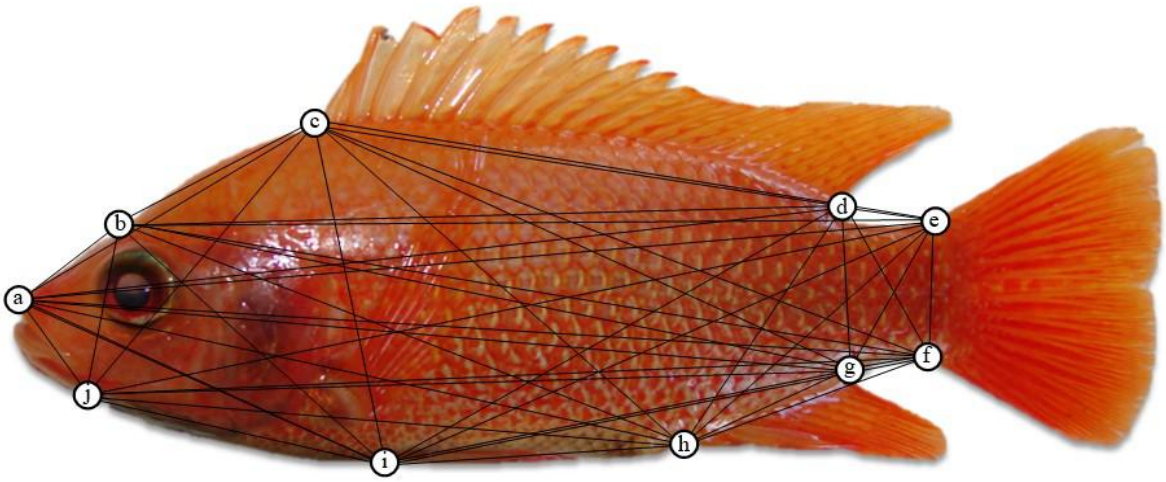

Figure 1: Locations of the ten (10) landmarks for constructing the truss network (circles) and morphometric variables (lines) on red tilapia (*Oreochromis*spp.). Morphometric variables included the following: a-b, a-c, a-d, a-e, a-f, a-g, a-h, a-i, a-j, b-c, b-d, b-e, b-f, b-g, b-h, b-i, b-j, c-d, c-e, c-f, c-g, c-h, c-i, c-j, d-e, d-f, d-g, d-h, d-i, d-j, e-f, e-g, e-h, e-i, e-j, f-g, f-h, f-i, f-j, g-h, g-i, g-j, h-i, h-j, and i-j.

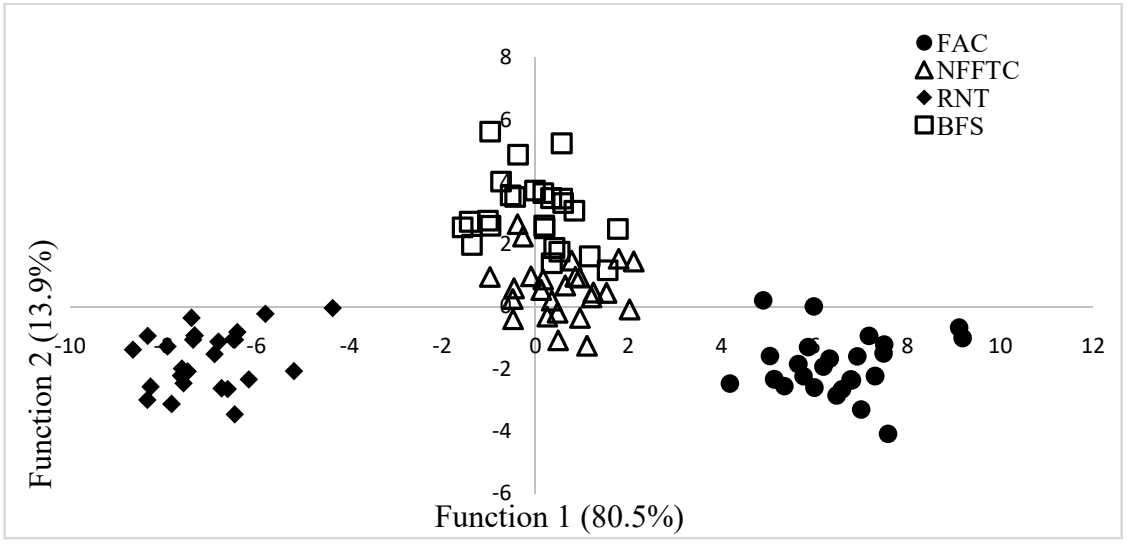

Figure 2: Discriminant analysis plot for male samples of the four populations of red tilapia

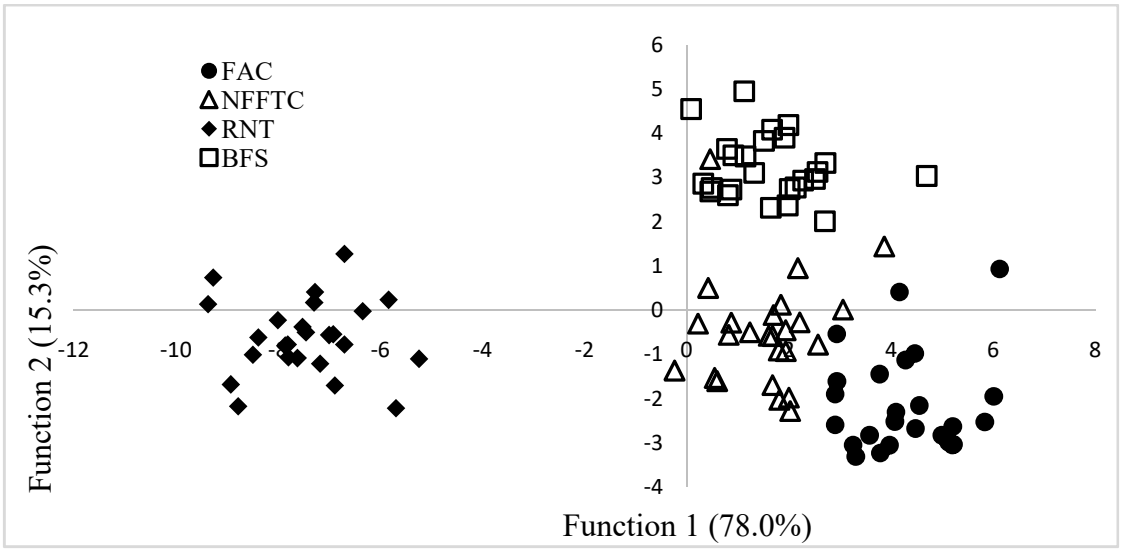

Figure 3: Discriminant analysis plot for female samples of the four populations of red tilapia

Table 1: Univariate analysis of variance (ANOVA) testing the interaction between measurements and sexes

Variable	P	Variable	P	Variable	P	Variable	P	Variable	P
a-b	0.000***	b-c	0.757	c-e	0.253	d-h	0.000***	f-h	0.033*
a-c	0.000***	b-d	0.431	c-f	0.002**	d-i	0.005**	f-i	0.883
a-d	0.000***	b-e	0.000***	c-g	0.000***	d-j	0.000***	f-j	0.000***
a-e	0.133	b-f	0.006**	c-h	0.000***	e-f	0.000***	g-h	0.000***
a-f	0.790	b-g	0.883	c-i	0.000***	e-g	0.012*	g-i	0.060
a-g	0.069	b-h	0.696	c-j	0.046*	e-h	0.000***	g-j	0.000**
a-h	0.227	b-i	0.007**	d-e	0.193	e-i	0.774	h-i	0.922
a-i	0.000***	b-j	0.000***	d-f	0.000***	e-j	0.000***	h-j	0.000***
a-j	0.000***	c-d	0.000***	d-g	0.000***	f-g	0.409	i-j	0.015*

Note: Significance level (P) is presented with superscript for variable that is significantly different among populations:* for P<0.05; ** for P<0.01; and * for P<0.001.**

Table 2: Analysis of variance comparing variables among male red tilapia samples

Variable	P	Variable	P	Variable	P	Variable	P	Variable	P
a-b	0.000***	b-c	0.659	c-e	0.000***	d-h	0.000***	f-h	0.000***
a-c	0.005**	b-d	0.589	c-f	0.000***	d-i	0.000***	f-i	0.000***
a-d	0.052	b-e	0.462	c-g	0.000***	d-j	0.002**	f-j	0.023*
a-e	0.210	b-f	0.077	c-h	0.000***	e-f	0.000***	g-h	0.000***
a-f	0.005**	b-g	0.195	c-i	0.000***	e-g	0.000***	g-i	0.000***
a-g	0.137	b-h	0.009**	c-j	0.000***	e-h	0.000***	g-j	0.006**
a-h	0.041*	b-i	0.024*	d-e	0.000***	e-i	0.000***	h-i	0.000***
a-i	0.000***	b-j	0.000***	d-f	0.000***	e-j	0.092	h-j	0.002**
a-j	0.000***	c-d	0.000***	d-g	0.000***	f-g	0.000***	i-j	0.008**

Note: Significance level (P) is presented with superscript for variable that is significantly different among populations:
*** for P<0.05; ** for P<0.01; and *** for P<0.001.**

Table 3: Analysis of variance comparing variables among female red tilapia samples

Variable	P	Variable	P	Variable	P	Variable	P	Variable	P
a-b	0.000***	b-c	0.000***	c-e	0.000***	d-h	0.000***	f-h	0.000***
a-c	0.000***	b-d	0.014*	c-f	0.000***	d-i	0.000***	f-i	0.000***
a-d	0.001**	b-e	0.006**	c-g	0.001**	d-j	0.308	f-j	0.018*
a-e	0.028*	b-f	0.001**	c-h	0.000***	e-f	0.000***	g-h	0.000***
a-f	0.000***	b-g	0.002**	c-i	0.000***	e-g	0.000***	g-i	0.000***
a-g	0.665	b-h	0.000***	c-j	0.000***	e-h	0.000***	g-j	0.007**
a-h	0.178	b-i	0.000***	d-e	0.000***	e-i	0.000***	h-i	0.000***
a-i	0.000***	b-j	0.000***	d-f	0.000***	e-j	0.478	h-j	0.000***
a-j	0.000***	c-d	0.000***	d-g	0.000***	f-g	0.000***	i-j	0.000***

Note: Significance level (P) is presented with superscript for variable that is significantly different among populations: * for P<0.05; ** for P<0.01; and * for P<0.001.**

Table 4: Post hoc multiple comparison tests of morphometric variables between pairs of populations

Sex	Population	FAC	NFFTC	RNT
Male	NFFTC	24	--	
	RNT	31	22	--
	BFS	23	4	24
Female	NFFTC	21	--	
	RNT	31	31	--
	BFS	26	15	32

Note: Values presented are the number of significant variables observed out of 45 morphometric variables for corresponding populations.

Table 5: Loadings of the first of nine principal components (PC1) for morphometric characters of male red tilapia (accounting for 29.2% of the variation)

Variable	PC1	Variable	PC1	Variable	PC1	Variable	PC1	Variable	PC1
a-b	0.575	b-c	0.092	c-e	0.698*	d-h	0.863*	f-h	0.797*
a-c	0.368	b-d	0.044	c-f	0.746*	d-i	0.655	f-i	0.689
a-d	0.018	b-e	0.246	c-g	0.656	d-j	-0.088	f-j	0.084
a-e	0.308	b-f	0.230	c-h	0.617	e-f	0.722*	g-h	0.734*
a-f	0.279	b-g	0.048	c-i	0.775*	e-g	0.837*	g-i	0.569
a-g	0.033	b-h	0.193	c-j	0.536	e-h	0.898*	g-j	-0.081
a-h	0.173	b-i	0.280	d-e	0.745*	e-i	0.771*	h-i	0.455
a-i	0.410	b-j	0.643	d-f	0.769*	e-j	0.146	h-j	-0.025
a-j	0.540	c-d	0.635	d-g	0.731*	f-g	0.725*	i-j	0.045

Note: Coefficient with large contribution to the component is presented with superscript (*).

Table 6: Contribution of each variable to the first of three discriminant functions (DF1) for male samples (accounting for 80.5% of the variation)

Variable	DF1	Variable	DF1	Variable	DF1	Variable	DF1	Variable	DF1
e-h	0.333*	b-j	0.185*	e-i	0.180	a-d	-0.035	f-g	0.223
f-h	0.306*	c-e	0.164*	h-i	0.067	d-j	-0.072	e-f	0.201
d-h	0.262*	c-f	0.155*	g-i	0.107	e-j	-0.036	b-f	0.000
e-g	0.252*	d-g	0.153*	a-i	0.140	g-j	-0.066	f-j	-0.044
g-h	0.247*	c-d	0.151*	f-i	0.142	a-g	-0.036	b-i	0.052
d-e	0.214*	a-b	0.139*	i-j	-0.002	b-g	-0.033	c-g	0.126
a-j	0.207*	c-j	0.118*	b-h	-0.013	b-c	0.015	a-e	0.014
c-i	0.194*	a-c	0.074*	a-h	-0.012	a-f	0.001	b-e	0.019
d-f	0.190*	d-i	0.138	h-j	-0.062	c-h	0.101	b-d	-0.015

Note: Coefficient with large contribution to the function is presented with superscript (*).

Table 7: Loadings of the first of ten principal components (PC1) for morphometric characters of female red tilapia (accounting for 35.0% of the variation)

Variable	PC1	Variable	PC1	Variable	PC1	Variable	PC1	Variable	PC1
a-b	0.792*	b-c	0.566	c-e	0.724*	d-h	0.887*	f-h	0.667
a-c	0.758*	b-d	-0.006	c-f	0.680	d-i	0.460	f-i	0.457
a-d	0.000	b-e	0.096	c-g	0.473	d-j	0.012	f-j	-0.176
a-e	0.064	b-f	-0.118	c-h	0.647	e-f	0.903*	g-h	0.624
a-f	-0.247	b-g	-0.239	c-i	0.850*	e-g	0.883*	g-i	0.254
a-g	0.029	b-h	0.321	c-j	0.864*	e-h	0.883*	g-j	-0.264
a-h	0.141	b-i	0.881*	d-e	0.782*	e-i	0.401	h-i	0.350
a-i	0.771*	b-j	0.855*	d-f	0.866*	e-j	0.016	h-j	0.167
a-j	0.707	c-d	0.629*	d-g	0.875*	f-g	0.660	i-j	0.635

Note: Coefficient with large contribution to the component is presented with superscript (*).

Table 8: Contribution of each variable to the first of three discriminant functions (DF1) for female samples (accounting for 78.0% of the variation)

Variable	DF1	Variable	DF1	Variable	DF1	Variable	DF1	Variable	DF1
b-j	0.362*	b-i	0.294*	b-h	0.086	a-d	-0.009	e-i	0.091
e-g	0.352*	d-e	0.272*	a-j	0.261	g-h	0.171	b-c	0.167
c-j	0.350*	d-g	0.269*	a-i	0.259	g-i	0.061	f-i	0.110
e-f	0.348*	c-i	0.268*	h-i	0.104	b-g	-0.067	a-e	0.000
e-h	0.334*	c-e	0.165*	c-h	0.182	d-j	-0.015	c-g	0.080
a-c	0.307*	c-f	0.140*	d-i	0.113	g-j	-0.072	a-f	-0.077
a-b	0.303*	c-d	0.133*	f-g	0.201	a-g	0.020	b-d	-0.011
d-f	0.301*	a-h	0.046*	i-j	0.162	b-f	-0.046	e-j	-0.015
d-h	0.301*	f-h	0.228	h-j	0.037	b-e	0.008	f-j	-0.058

Note: Coefficient with large contribution to the function is presented with superscript (*).

Table 9: Result of multivariate tests for male and female samples

Effect	Male			Female		
	Value	F	P	Value	F	P
Wilks' Lambda	0.003	254.374	0.000*	0.003	239.684	0.000*

Note: This study considered Wilks' Lambda's values (*significant at P<0.001).

Table 10: Pairwise comparison (MANOVA) among four populations using the discriminant scores from function 1 and function 2

Sex	Population	Function 1			Function 2		
		FAC	NFFTC	RNT	FAC	NFFTC	RNT
Male	NFFTC	5.937**	--		2.434**	--	
	RNT	13.541**	7.604**	--	0.189ns	2.245**	--
	BFS	6.500**	0.562*	7.042**	4.906**	2.471**	4.717**
Female	NFFTC	2.736**	--		1.626**	--	
	RNT	11.742**	9.006**	--	1.499**	0.127ns	--
	BFS	2.664**	0.072ns	9.078**	5.341**	3.715**	3.842**

Note: Mean difference is presented with superscript for populations that are significantly different among populations: *for P<0.05; and ** for P<0.001. For not significantly different populations, mean difference is presented with superscript ns.

Table 11: Count and percentage of male and female samples classified in each population for morphometric measurements (99.0% of original grouped cases correctly classified)

Sex	Population	No. of cases	Predicted Group Membership			
			FAC	NFFTC	RNT	BFS
Male	FAC	25	25 100.0%	0 0.0%	0 0.0%	0 0.0%
	NFFTC	25	0 0.0%	25 100.0%	0 0.0%	0 0.0%
	US-UK	25	0 0.0%	0 0.0%	25 100.0%	0 0.0%
	BFS	25	0 0.0%	1 4.0%	0 0.0%	24 96.0%
Female	FAC	25	25 100.0%	0 0.0%	0 0.0%	0 0.0%
	NFFTC	25	0 0.0%	24 96.0%	0 0.0%	1 4.0%
	RNT	25	0 0.0%	0 0.0%	25 100.0%	0 0.0%
	BFS	25	0 0.0%	0 0.0%	0 0.0%	25 100.0%

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