

Multivariate analysis using morphometric and ultrasound information for selection of tilapia (*Oreochromis niloticus*) breeders

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ABSTRACT - This study evaluated morphometric and ultrasound information of tilapia (*O. niloticus*) breeders through multivariate analysis. We applied correlation, clustering, and principal component analysis to a dataset composed of information from 222 male and female breeders of the improved GIFT strain. The body weight, objective of the breeding program, showed a high positive correlation with most of the morphometric parameters. The formation of clusters indicated characteristics responsible for muscle composition and carcass weight. Some characteristics showed a high correlation, such as body weight and fillet weight (0.98 and 0.94 for females and males, respectively), and a high contribution to the explanation of data variability; of the total characteristics evaluated for females, two explained 75% data variability and four explained 72% for males. We concluded that it is possible to reduce the number of characteristics measured, as well as use information of average daily weight gain and body weight to select female and male breeders, respectively, to drive genetic gains favoring more productive generations.

Keywords: aquaculture, breeding, fish farming, *Oreochromis sp.*, phenotypic selection

Introduction

The Brazilian aquaculture production in 2016 was 507.12 million tons, an increase of 4.4% over the previous year (IBGE, 2016). Tilapia was the most produced fish with 239,09 million tons, corresponding to 47.1% of all fish produced in Brazil (IBGE, 2016). Projections made for 2030 indicate that there will be a 38.32% increase in fish production, due to the demand for healthier foods and the improvement in the quality, standardization, and constancy of fish supply in the market (FAO, 2014). In this context, farming fish that present high growth performance under certain conditions should be prioritized, so that the selection of these genetically superior individuals can further maximize productivity and add value to the product (Resende et al., 2010).

The genetic improvement of the animals seeks, among other things, the productive increase of economically important characteristics, such as the body weight of fish. This genetic improvement involves the selection and mating of the animals that present the best performance for the desirable

trait, which can be directly measured or indirectly measured by means of others that are correlated with it. In the genetic breeding of fish in Brazil, such as Tilapia (*Oreochromis niloticus*), it is possible to use performance information such as weight, daily weight gain, morphometric measurements (Ribeiro and Legat, 2008), and ultrasound measurements (Bianchecci, 2011).

Some of these measurements have high genetic correlation (Kunita et al. 2013), and when there is a set of correlated variables, it is possible to transform the set of original data into another with uncorrelated variables, which will be derived linear of combinations of the original data, in descending order of importance. These combinations are the first possible variance in the first variables, called principal components (PC; Manly, 2008).

In principal component analysis (PCA), the variance contained in each principal component is expressed by the eigenvalues of the standardized matrix. The largest eigenvalue is associated with the first principal component, the second largest eigenvalue to the second principal component, until the smallest eigenvalue is associated with the last principal component, which places the first as the most important. Thus, the first principal components usually explain much of the variance of the original variables. This statistical tool can be used successfully to support decision-making (Yamaki et al., 2009; Silva and Sbrissia, 2010; Silva et al., 2015) in the choice of variables that effectively contribute to the explanation of the target trait of selection in breeding programs, such as tilapia in Brazil.

Given the above, the present study aimed to evaluate possible associations between morphometry and ultrasound characteristics obtained from tilapia specimens breeders by multivariate analysis.

Material and Methods

The data set was composed initially of 231 pieces of information of male and female breeders from a genetically improved fish generation (generation 2012/2013), GIFT strain from the Tilapia Breeding Program of the State University of Maringá (State of Paraná, Brazil), and were individualized by microchip, in which animals are selected by the genetic gain presented for growth rate (average daily gain – ADG). The average daily weight gain was obtained by dividing the final body weight (slaughter) by age (in days) of each animal studied.

The ultrasound device used for the measurements was the Digital Palmtop Ultrasound Scanner, version V1.10E-1, Model CUS-3000V (Shenzhen Carewell electronics Co, Shenzhen, China). Fish were killed by the destruction of the spinal cord. After slaughter, the animals were weighed on a precision weighing scale of 0.1 g. For filleting, fish processing was performed manually by the same operator. The filleting procedure followed the order: processing headless fish (dorsal-ventral cut), filleting, and skinning of fillet with knife.

The fish studied were under the same culture conditions, with an average temperature of 22 °C. The measurements of the samples were all obtained in sequence at the time of slaughter.

To standardize the variables and remove the effect of scales, a correlation matrix was used, as suggested by Souza (2000), which presented non-zero correlation coefficients. Performance, morphometric, and ultrasound characteristics were analyzed (Table 1).

Data were adjusted, removing nine pieces of information from typing errors, which resulted in 222 pieces of information. Prior to the application of the multivariate analyses, the data were subjected to the KMO (Kaiser-Meyer-Olkin) statistic, with KMO being greater than 0.80 (in males and females), and Bartlett's test was significant. These statistics indicate that data can be analyzed by multivariate analysis.

For the application of multivariate analysis (cluster analysis and PCA), the data set was standardized; each descriptor, thus, had null mean and unit variance. This analysis allowed to reduce the space of original variables into a smaller set, preserving the maximum of the original variability of the data. The results of Pearson's linear correlation were analyzed by the F test, at the 5% probability level. All statistical analyses were performed in software R 3.2.2 (R Development Core Team, 2008), with the use of vegan packages (Oksanen et al., 2010), using method WARD for the Euclidean Distance (Cruz et al., 2004).

Table 1 - Description of the characteristics used in the study

Variable	Abbreviation	Description
Body weight (g)	BW	Measurement on an analytical balance
Standard length (cm)	SL	From the anterior end of the head to the beginning of the caudal fin insertion
Total length (cm)	TL	From the anterior end of the head to the end of the caudal fin
Height 1 (cm)	H1	Front height of the first ray of the dorsal fin
Width 1 (cm)	W1	Measured from the front of the first ray of the dorsal fin
Head size (cm)	HS	Measured between the anterior end of the head and the caudal border of the operculum
Height 2 (cm)	H2	Height between the final insertion of the anal and dorsal fins
Width 2 (cm)	W2	Width between the final insertion of the anal and dorsal fins
Ultrasound height 1 (mm)	UH1	Left side area of the base of the insertion of the pelvic fin to the anterior base of the dorsal fin
Ultrasound width 1 (mm)	UW1	Width from the base of the insertion of the pelvic fin to the anterior base of the dorsal fin
Ultrasound area 1 (mm ²)	UA1	Height from the base of the insertion of the pelvic fin to the anterior base of the dorsal fin
Ultrasound height 2 (mm)	UH2	Left side area between the final insertion of the anal and dorsal fins
Ultrasound width 2 (mm)	UW2	Width between the final insertion of the anal and dorsal fins
Ultrasound area 2 (mm ²)	UA2	Height between the final insertion of the anal and dorsal fins
Fillet weight (g)	FW	Final fillet weight, after filleting procedure; measured on an analytical balance
Fillet yield (%)	FY	Ratio between fillet weight and fish weight
Average daily gain (g)	ADG	Rate of weight gain per day (final body weight/age)

Results

The descriptive statistics of the characteristics (Table 2) show that males presented higher values for all the characteristics studied, such as average weight (1551.0 g) in relation to females (761.0 g), with only one exception, the characteristic ultrasound height 1 (UH1), to which, in this case, the opposite occurs.

Table 2 - Mean, maximum, and minimum values for morphometric and ultrasound measurements for males and females

Variable	Females			Males		
	Mean	Min - max	SD	Mean	Min - max	SD
Body weight (g)	761.0	668 - 1,245	196.45	1551	894 - 1,875	281.61
Standard length (cm)	27.49	24.7 - 36.5	1.84	33.47	28 - 38	1.91
Total length (cm)	34.35	31 - 42.9	2.09	42.21	36 - 47	2.19
Height 1 (cm)	10.34	7.2 - 15.7	1.00	13.41	8.10 - 16.30	1.31
Width 1 (cm)	4.53	3.4 - 5.4	0.22	5.07	5 - 7	0.22
Head size (cm)	8.99	7.1 - 11.2	0.61	10.69	7.2 - 12	0.83
Height 2 (cm)	3.70	3.7 - 6.8	0.38	5.03	4.7 - 7	0.41
Width 2 (cm)	1.73	1.1 - 2.7	0.19	2.22	1.3 - 3.6	0.19
Ultrasound area 1 (mm ²)	20.47	18 - 26	1.59	25.73	22 - 32	1.59
Ultrasound width 1 (mm)	30.75	28 - 39	2.88	40.45	32 - 52	2.88
Ultrasound height 1 (mm)	66.48	62 - 81	3.47	63.53	60 - 81	3.47
Ultrasound area 2 (mm ²)	14.55	11 - 18	1.30	18.89	18 - 26	1.30
Ultrasound width 2 (mm)	38.70	31 - 49	4.03	50.25	32 - 60	4.03
Ultrasound height 2 (mm)	37.38	31 - 46	2.36	37.36	33 - 62	2.36
Fillet weight (g)	257.10	189 - 481	72.15	543.40	290 - 870	113.35
Fillet yield (%)	33.15	0.28 - 0.38	1.26	34.93	0.32 - 0.47	2.70
Average daily gain (g)	1.57	1.04 - 3.19	0.38	3.53	2.09 - 4.76	0.56

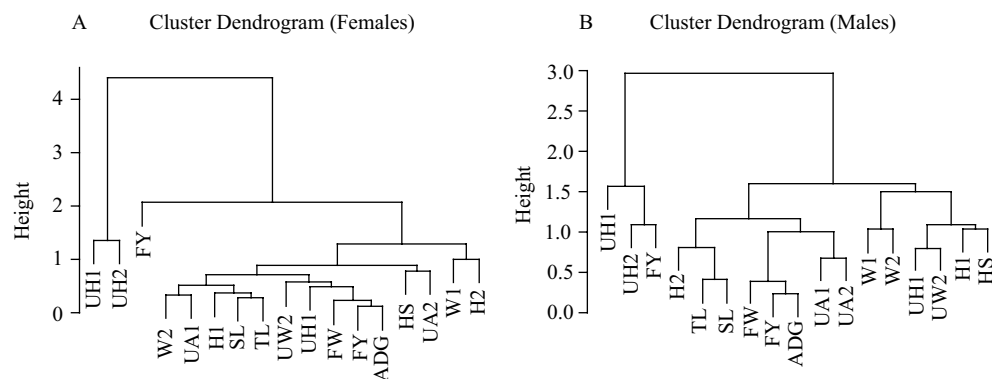
SD - standard deviation.

In cluster analysis for females (Figure 1), three main groups were formed: the first group, UH1 and ultrasound height 2 (UH2); the second group, fillet yield (FY); and the third group formed by the other characteristics in the study. For males, two groups were found: one consisting of UH2, FY, and UH1 and the other, of the other parameters.

The results for the principal components (Table 3) show that, for females, 75% of the variation is explained considering two PC; in turn, for males, 72% variation can be explained considering four components. Some characteristics presented similar values of the total variation, as observed in the first principal component (PC1) for females, in which the characteristics of body weight (BW), fillet weight (FW), and ADG have the same magnitude (0.29), as well as height 1 (H1), width 2 (W2), ultrasound area 1 (UA1), ultrasound width 1 (UW1), ultrasound width 2 (UW2), and total length (TL) (0.27). A similar trend was found for males with close values in PC1 for BW (0.31) and FW (0.30) and the same for standard length (SL), H1, UW1, UW2, and TL (0.28). For females, important characteristics as UH1, UH2, and FY showed magnitude of -0.06 , -0.08 , and 0.13 , respectively, and these characteristics for males showed a magnitude of 0.11 , 0.06 , and 0.01 , respectively (Table 3). The spatial projection of the vectors of the studied variables, considering two main components for females and for males is shown in Figure 2.

The study of Pearson's correlation coefficient showed a correlation between the morphometric measurements (Table 4). Some characteristics presented a moderate correlation, such as 0.55 between FY and FW (females) and others showed a low correlation, such as 0.03 between BW and UH1 (males), indicating that these characteristics act independently, that is, the selection of one does not influence the selection of the other, unlike strongly correlated characteristics such as ADG and BW of 0.99 in females. Low correlation may also indicate that some characteristics are orthogonal, that is, they are not in the same component and may be in another of smaller magnitude. Other characteristics presented a negative correlation, indicating that the impact of the selection performed may act differently in each one, while one suffers increase, another may suffer reduction, such as -0.25 between BW and UH1 in females.

For females (Table 4), some parameters such as BW, FW, and ADG showed a high correlation (above 0.95). In the same way, males presented the same parameters with correlation above 0.89 . Fillet yield is a trait with significant economic impact and presented the highest correlation with fillet weight (FW) in both females (0.55) and males (0.60), and a weaker correlation with width 1 (W1) and UH1 (females -0.03 and 0.05) and also head size (HS) and UH2 (males -0.03 and 0.08); studies to select fish with higher FY may contribute to the improvement of performance of this trait.



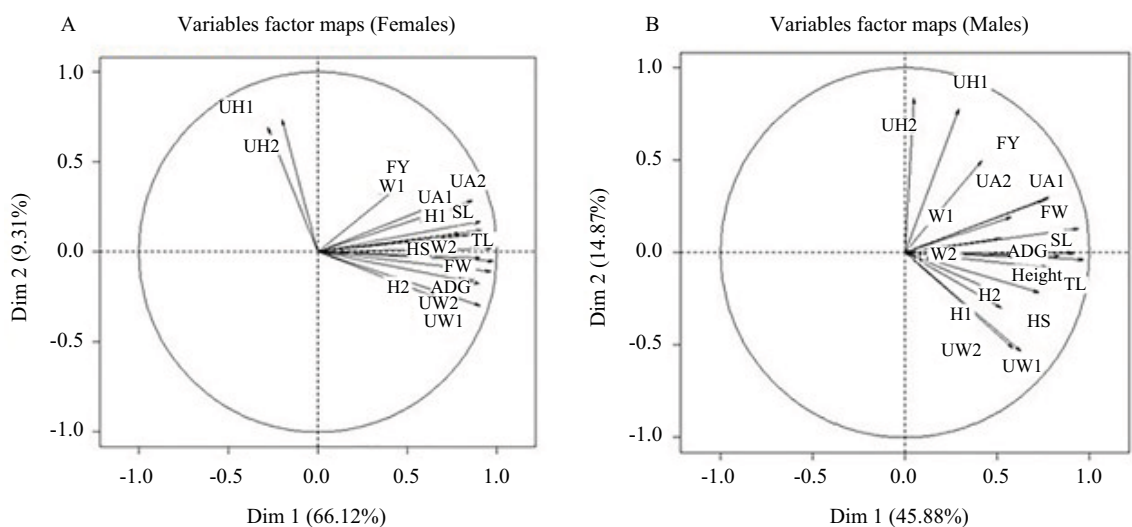
BW - body weight; SL - standard length; H1 - height 1; W1 - width 1; HS - head size; H2 - height 2; W2 - width 2; UA1 - ultrasound area 1; UW1 - ultrasound width 1; UH1 - ultrasound height 1; UA2 - ultrasound area 2; UW2 - ultrasound width 2; UH2 - ultrasound height 2; TL - total length; FW - fillet weight; FY - fillet yield; ADG - average daily gain.

Figure 1 - Dendrogram between the characteristics of females (A) and males (B).

Table 3 - Principal components (PC), eigenvalues (λ), percentage of variance explained by components (VCP), and cumulative variance (VCPA) in morphometric and ultrasound variables

PC	Females				Males			
	PC1	PC2	PC3	PC4	PC1	PC2	PC3	PC4
BW	0.29	0.04	0.03	0.01	0.31	0.05	-0.07	0.10
SL	0.28	-0.08	0.06	-0.06	0.28	0.07	-0.29	0.10
H1	0.27	-0.08	-0.08	0.05	0.28	0.11	-0.12	0.12
W1	0.20	-0.21	-0.18	-0.57	0.19	0.18	-0.24	-0.39
HS	0.24	0.08	0.16	-0.09	0.27	-0.08	-0.13	-0.21
H2	0.20	-0.21	-0.28	-0.19	0.23	0.27	0.06	0.02
W2	0.27	-0.10	0.01	0.12	0.25	0.03	-0.20	0.08
UA1	0.27	0.13	-0.13	-0.13	0.25	-0.28	-0.25	-0.06
UW1	0.27	0.24	-0.17	-0.02	0.28	-0.17	0.26	-0.09
UH1	-0.06	-0.59	0.49	0.20	0.11	0.51	0.06	0.21
UA2	0.26	0.23	-0.12	-0.23	0.27	-0.25	-0.08	-0.03
UW2	0.27	0.14	-0.20	0.01	0.28	-0.19	0.10	-0.15
UH2	-0.08	-0.55	0.49	-0.36	0.06	0.52	0.26	-0.26
TL	0.27	0.03	-0.17	0.07	0.28	-0.03	-0.26	0.10
FW	0.29	-0.02	-0.12	-0.09	0.30	-0.16	0.15	0.11
FY	0.13	-0.29	-0.48	-0.60	0.01	-0.32	0.68	0.01
ADG	0.29	0.09	0.07	0.03	0.18	0.01	-0.00	0.77
λ	3.35	1.26	1.06	0.99	2.79	1.59	1.02	0.96
VCP	0.66	0.09	0.06	0.05	0.46	0.15	0.06	0.05
VCPA	0.66	0.75	0.82	0.87	0.46	0.61	0.67	0.72

BW - body weight; SL - standard length; H1 - height 1; W1 - width 1; HS - head size; H2 - height 2; W2 - width 2; UA1 - ultrasound area 1; UW1 - ultrasound width 1; UH1 - ultrasound height 1; UA2 - ultrasound area 2; UW2 - ultrasound width 2; UH2 - ultrasound height 2; TL - total length; FW - fillet weight; FY - fillet yield; ADG - average daily gain.



BW - body weight; SL - standard length; H1 - height 1; W1 - width 1; HS - head size; H2 - height 2; W2 - width 2; UA1 - ultrasound area 1; UW1 - ultrasound width 1; UH1 - ultrasound height 1; UA2 - ultrasound area 2; UW2 - ultrasound width 2; UH2 - ultrasound height 2; TL - total length; FW - fillet weight; FY - fillet yield; ADG - average daily gain.

Figure 2 - Spatial projection of the vectors of the studied variables, considering two principal components (DIM1 = PC1 and DIM2 = PC2), for females (A) and males (B).

Table 4 - Correlation between morphometric and ultrasound measurements for females (above the main diagonal) and for males (below diagonal)

	BW	SL	H1	W1	HS	H2	W2	UA1	UW1	UH1	UA2	UH2	UW2	TL	FW	FY	ADG
BW	1	0.94	0.84	0.63	0.73	0.65	0.89	0.87	0.91	-0.25	0.83	0.87	-0.28	0.90	0.98	0.41	0.99
SL	0.84	1	0.83	0.64	0.74	0.55	0.86	0.82	0.77	-0.11	0.81	0.79	-0.16	0.94	0.91	0.34	0.92
H1	0.49	0.39	1	0.59	0.88	0.54	0.80	0.79	0.75	-0.10	0.79	0.80	-0.22	0.84	0.82	0.41	0.83
W1	0.53	0.38	0.21	1	0.56	0.48	0.60	0.63	0.53	0.06	0.72	0.59	0.04	0.54	0.59	0.03	0.60
HS	0.49	0.47	0.28	0.24	1	0.32	0.73	0.67	0.65	-0.12	0.62	0.65	-0.21	0.81	0.72	0.35	0.70
H2	0.67	0.56	0.40	0.38	0.37	1	0.53	0.58	0.72	-0.36	0.60	0.65	-0.24	0.50	0.64	0.31	0.69
W2	0.48	0.38	0.24	0.32	0.26	0.36	1	0.87	0.80	-0.09	0.76	0.77	-0.21	0.87	0.89	0.49	0.86
UA1	0.72	0.53	0.38	0.52	0.27	0.45	0.37	1	0.81	0.10	0.79	0.84	-0.28	0.78	0.87	0.47	0.83
UW1	0.58	0.43	0.40	0.22	0.38	0.51	0.28	0.48	1	-0.50	0.73	0.84	-0.36	0.76	0.89	0.38	0.91
UH1	0.03	0.01	-0.08	0.24	-0.15	-0.11	0.05	0.41	-0.59	1	-0.05	-0.15	0.18	-0.16	-0.22	0.05	-0.31
UA2	0.66	0.54	0.30	0.46	0.27	0.38	0.36	0.63	0.38	0.15	1	0.79	0.09	0.68	0.82	0.42	0.82
UH2	0.55	0.38	0.37	0.30	0.36	0.45	0.21	0.35	0.56	-0.29	0.49	1	-0.53	0.77	0.83	0.26	0.87
UW2	0.22	0.26	0.00	0.22	0.01	0.03	0.21	0.37	-0.09	0.45	0.56	-0.40	1	-0.34	-0.22	0.14	-0.29
TL	0.75	0.81	0.37	0.35	0.40	0.65	0.30	0.47	0.39	0.01	0.43	0.36	0.16	1	0.86	0.30	0.88
FW	0.94	0.78	0.44	0.49	0.41	0.62	0.46	0.74	0.51	0.12	0.70	0.48	0.31	0.63	1	0.55	0.97
FY	0.32	0.25	0.06	0.15	-0.03	0.22	0.19	0.41	0.09	0.27	0.47	0.08	0.42	0.17	0.60	1	0.39
ADG	0.93	0.79	0.46	0.47	0.43	0.60	0.39	0.53	0.53	0.05	0.50	0.50	0.23	0.71	0.89	0.35	1

BW - body weight; SL - standard length; H1 - height 1; W1 - width 1; HS - head size; H2 - height 2; W2 - width 2; UA1 - ultrasound area 1; UW1 - ultrasound width 1; UH1 - ultrasound height 1; UA2 - ultrasound area 2; UW2 - ultrasound width 2; UH2 - ultrasound height 2; TL - total length; FW - fillet weight; FY - fillet yield; ADG - average daily gain.

Discussion

The formation of the cluster composed of UH1 (height near the head), UH2 (caudal peduncle height), and FY indicates that these characteristics are related to the muscular composition of fish, while the other characteristics of the study are related to carcass weight. The FY trait is a separate component, corroborating its differentiation from the other characteristics, since it depends on some biological factors such as the anatomical shape of the animal and the presence or absence of intramuscular bones, head size, and number of viscera (Adames et al., 2014). A 1993 study (Eyo, 1993) found that fillet yield is influenced by anatomical shape and that large-headed fish exhibit lower fillet yields when compared with small-headed fish, and that other human or mechanical factors also interfere with fish yield (Pineiro et al., 2006). It also depends on the filleting method (Bombardelli and Sanches, 2008) and type of cut for removal of the head, skin, and fins (Macedo-Viégas and Souza, 2004).

Ultrasound characteristics UH1 and UH2 were also studied in work with common carp (*Cyprinus carpio*), in which the influence of these regions on FY was verified (Cibert et al., 1999). In another study by Bianchecci (2011), the authors found a similar response with tilapia (*O. niloticus*), a fact that suggests a strong relation between the muscle portion of these regions and FY in different species, indicating that morphological differentiation influences the definition of the best regions to obtain ultrasound images that are related to FY. This can explain the differentiation of these characteristics (UH1, UH2, and FY), being more clustered (as for the result found for males), or in groups separated from the other morphometric measures (as for the result observed for females), observed in the dendrogram.

The characteristics that showed close or equal impact on the total variation in females and males were corroborated by the spatial projection of the vector ordination (Figure 2), in which several characteristics are visualized in the same quadrant and are practically overlapping. This result

indicates that some variables have the same impact in explaining the total variation of the data and, in some cases, behave in a practically identical way. This similar behavior can be explained due to the high correlation existing among the characteristics such as BW, FW, and ADG in both sexes. The selection of animals can be directed to the genetic improvement of fillet yield, but it is difficult because it is a trait measured only after slaughter, which makes it important to know its association with other trait(s) that may be obtained without the death of individual candidates for selection. Information associated with this trait that can avoid animal sacrifice is extremely relevant for breeding programs (Turra et al., 2010).

Other researchers (Porto et al., 2015), who evaluated tilapia, also found high association between characteristics, both with genetic information, in which body weight and ADG had a 0.99 correlation and BW association above 0.77, as well as with morphometric information. Oliveira et al. (2016) observed that the high association between characteristics are maintained throughout the generations in genetically improved fish.

Knowing the profile of the variables possible to be obtained makes the collection and data set efficient and low-cost, since it allows to disregard some information known to have low or no influence on the total variation of the data, which can be grouped so closely, saving time, equipment, and labor, without losing accuracy in data analysis.

From the 14 variables present in the dataset for both sexes, it is possible to reduce this value to two and four in females and males, respectively, choosing the characteristics according to the methodology proposed by Jolliffe (2002), in which we can discard as many characteristics as the number of components with a variation of less than 70%. However, the discard of variables and the interpretation of the results must have criteria and caution, and it is not absolutely guaranteed that a chosen subset after the discard of variables can really be the best, since a selected subset in a sample of the present may be insufficient for analyzes of other samples under future conditions.

In the case of the present study, through the methodology used and the results obtained, we observed that it is possible to reduce the size of the data set without causing a decrease in the precision of the analysis, which is due to the high degree of association found between the variables, as observed with production animals (Yamaki et al., 2009), eliminating redundant information detected through correlation coefficients between characteristics. This analysis has been used to explain structural relationships between animal body measurements and discard of variables, in which most variance components can be discarded.

Considering the results of the principal components (Table 3) to select females, the most relevant (magnitude) characteristics in order of priority are: ADG (PC1), UH1 (PC2), UH2 (PC3), and FY (PC4), which is strengthened by the dendrogram analysis (Figure 1A) and by the relationship between the characteristics (Table 4). It is noticed that in PC4, the W1 characteristic, which is morphometric, indicates that the next component will have more relevant morphometric information. Knowing that 75% of the data variation can be explained by two PC, the ADG and UH1 characteristics can be used to evaluate and select females. To select males, the most relevant characteristics are: BW (PC1), UH2 (PC2), FY (PC3), and ADG (PC4), corresponding to the four PC explaining 72% of the data variation (Table 3), and both cluster analysis (Figure 1) and correlation analysis (Table 4) corroborate these results.

The main component analysis is a statistical approach, which can be used to analyze the interrelationships between variables by condensing the information contained in them into one smaller set of statistical variables, with minimal loss of information, minimizing redundancies. The analysis of clusters is a classification technique typology of data that considers the similarity between the observed characteristics, calculated based on a distance, in which the elements with closest Euclidean distances are being grouped sequentially to a single group to be formed. The results can easily be graphically displayed in diagrams called dendrograms; in this way, working with both methodologies can strengthen the results found, as well as facilitate understanding.

For the genetic improvement of fish, the analysis of main components can act as a tool, helping breeders to choose the most relevant variables to be used in breeding programs (Cruz et al., 2004), since this methodology allows judging the importance of available variables in a more productive, fast, objective, and efficient way, separating the important information from the redundant and random ones.

Conclusions

The most relevant characteristics for the selection of breeders with higher productive performance are the average daily gain for females and body weight for males, and ultrasound information may aid in selection for tilapia breeders. It is possible to reduce the data set of the 14 characteristics used to evaluate both sexes, in only two representing 75% of the total variation of the data and in four explaining 72% for females and males, respectively.

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