Morphological Variation Assessment on Male and Female Tilapia, *Oreochromis niloticus* From Two Fish Farms in Negeri Sembilan, Malaysia

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Abstract. Tilapia is unique in that it continues to play a significant role in rural aquaculture, helping to improve farmers' livelihoods. The aim of this study was to calculate the distance between specified morphological characteristics of the Tilapia fish and to determine the inter-population morphometric variability of Tilapia from various fish farms using truss-morphometric characters. A total of 50 samples were collected using fish nets from two selected fish farms in Negeri Sembilan namely Ulu Bendul & Kuala Pilah, in which 25 samples were caught from each farm. The samples then were soaked with fish tranquilizer to prevent it from moving, as for morphometric measurement, it was measured by using digital calipers. The study discovered that the two red tilapia populations had considerable morphometric variability. For both male and female specimens, multivariate analysis revealed substantial variations in morphometric features between the two populations of red tilapia. For both genders, the discriminant analysis plot revealed a clear distinction between two separate groups. There is no overlapping among the two sexes of two different populations is shown through canonical variate analysis plot. The results revealed that both Function 1 and Function 2 was successful in classifying the individuals into four distinct groups (Ulu Bendul Male (UBM), Ulu Bendul Female (UBF), Kuala Pilah Male (KPM), and Kuala Pilah Female (KPF)). Species identification is an important part of biodiversity monitoring. It was able to examine morphometric character variation and fish growth using the ANOVA and t-test. The DFA demonstrated that it was beneficial in categorising the samples not only into species groups, but also into sexes.

Keywords: Tilapia, Morphometric variability, DFA, CFA

1 Introduction

Aquaculture is increasingly crucial in meeting the rising global demand for fishery products. It is one of the fastest-growing sectors within the livestock industry, particularly in Malaysia, where fish is a vital food source. Fish provides essential protein and minerals and is favoured over beef or chicken due to its lower lipid and

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higher water content. *Oreochromis niloticus*, commonly known as Tilapia, is one of Malaysia's most popular fish and a leading aquatic species worldwide. Tilapia includes various cichlid fish found in freshwater and some saltwater environments [1]. Its adaptability, marketability, and stable market value make it the world's second most extensively farmed fish, with productivity quadrupling in the past decade. Originally from Africa and the Middle East, Tilapia has been introduced to over 90 countries for aquaculture and commercial fishing.

Tilapias, once considered invasive, are now vital to aquaculture due to their adaptability and resilience [2]. They are the world's second most farmed fish after carps and are deemed the most crucial aquaculture species of the 21st century [3]. Nile tilapia, in particular, is popular in freshwater ponds due to their rapid growth, high survival rates, and suitability for both permanent and seasonal ponds. Tilapia can be distinguished from sunfish and crappie by the interruption of their lateral line, a characteristic of the Cichlidae family. They have a broad, flattened body with long, heavily spiny dorsal, pelvic, and anal fins. Broad vertical bars along the sides of fry, fingerlings, and sometimes adults further differentiate them [3].

Stock identification involves identifying self-sustaining components within natural populations, dividing them into groups with different growth rates and reproductive patterns. Morphometric variations are a useful approach for analysing and classifying populations [4]. It is critical for successful fisheries management and monitoring [5]. In aquatic ecosystems, fish populations are dispersed across large regions, separated by barriers like temperature, salinity, food, and predation. Stock identification helps manage each stock individually, estimate population abundance, and preserve sustainable productivity. It also determines stock reactions to exploitation and aids in fishery stock evaluation via modelling and understanding the stock structure of a species is essential.

2 Literature Review

Tilapia is a popular freshwater fish grown commercially in Malaysia and worldwide, including in China, the USA, the EU, and Japan. Nile tilapia, with its compact body and reddish fins, is a key species [6]. Tilapia is classified into three genera based on breeding habits: Oreochromis, Sarotherodon and Tilapia. Males grow faster and larger than females, with these differences influencing competition and mating [7]. Research shows environmental conditions significantly affect tilapia's physical traits that play a crucial role in nutrient cycling by consuming algae and detritus, improving water quality. Native to Africa, tilapia was introduced to Asia in Indonesia and later to Malaysia and adapts well to various aquaculture systems and is used for multiple purposes, from food to beauty products [8].

Morphometric characteristics like fin, body, and head length, along with meristic traits like the number of scales or vertebrae, are used to distinguish fish species. These traits are vital for taxonomy, species health, and reproduction, providing essential data for species identification and fisheries research [9]. Despite advances in genetic methods, morphologic diversity studies remain crucial for stock identification. A study

on red tilapia in Philippine fisheries showed morphometric differences among four groups, with some similarities between male and female samples likely due to genetic factors. Morphometric variations can arise from genetic or environmental factors [10].

The Truss Network System (TNS) is a landmark-based geometric morphometrics method that captures an individual's structural data without restricting variability regions or shape changes [11, 12]. It encompasses the entire fish within a consistent network, enhancing the ability to discern differences between samples particularly in threatened fish species [13]. TNS utilizes geometric morphometrics to measure the length and depth of the body along the longitudinal axis, providing crucial truss dimensions [13]. Truss network measurements consist of estimated distances between landmarks, forming linked quadrilaterals, making it an effective tool for stock management and conservation [4]. The dimensions obtained through TNS, such as the criss-cross pattern along the body, facilitate the identification of variations in slope, horizontal, and lateral directions, further contributing to its utility in studying form variations [14].

3 Methodology

3.1 Sampling Site

Study was carried out in Negeri Sembilan, with the locations for Tilapia sampling chosen from the Kuala Pilah area. The samples were collected with fish nets from two selected fish farms in Kuala Pilah. Antiseptics were used as a killing method for the tilapia.

Fig. 1. Map of sampling site of Kuala Pilah, Negeri Sembilan

Fig. 2. Map of sampling site of Ulu Bendul, Negeri Sembilan

3.2 Sampling

A total of 50 samples were collected using fish nets from two selected fish farms in Negeri Sembilan namely Ulu Bendul & Kuala Pilah, in which 25 samples were caught from each farm. The samples then were soaked with fish tranquilizer to prevent it from moving, due to its dorsal fins may cause injury and make it harder to place in a plastic bag. Then the plastic bags were placed inside a plastic container.

3.3 Sample keeping

The tilapias were washed properly to eliminate any debris with tap water. Then, the samples were weighed with electronic weighing scales as the weight of the tilapia is crucial to analyse the morphology of the fish. All samples were kept in a zip lock that were labelled in accordance with the location of the research site.

3.4 Collection of Morphometric Data

Total length (TL), standard length (SL), and 45 morphometric characteristics were measured in millimetres (mm) using digital callipers, with measurements taken to the nearest 0.01 mm. The right side of each fish was measured, and an electronic weighing balance with 0.01 g precision was used to determine total body weight. Morphometric values included the lengths between the following landmarks: (a) upper jaw anterior tip, (b) back of the neurocranium, (c) origin of the dorsal fin base, (d) base of the dorsal fin, (e) anterior attachment of dorsal membrane from caudal fin, (f) anterior attachment

of ventral membrane from caudal fin, (g) end of anal fin, (h) origin of anal fin, and (j) pectoral fin insertion. Measurements were taken under bright lighting to ensure clear visibility of all body parts.

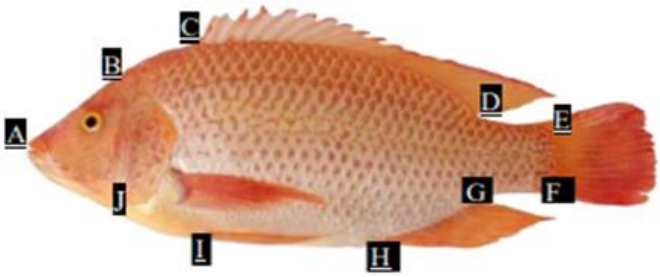

Fig. 3. Locations of the ten points for constructing the Truss Network on red tilapia

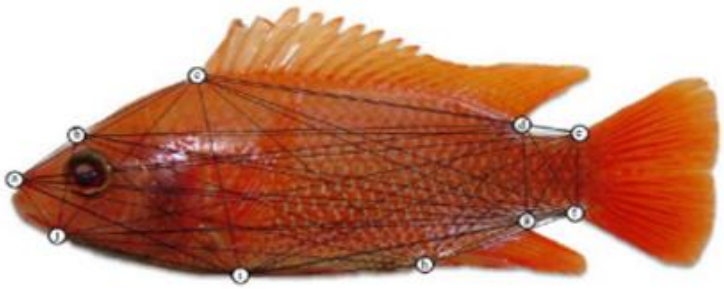

Fig. 4. Locations of the ten (10) landmarks for constructing the Truss Network (circles) and morphometric variables (lines) on red tilapia (Oreochromis spp.). Morphometric variables included the following : a-b, a-c, a-d, a-e, af, a-g, a-h, a-i, a-j, b-c, b-d, b-e, b-f, b-g, b-h, b-i, b-j, c-d, c-e, c-f, c-g, c-h, c-i, c-j, d-e, d-f, d-g, d-h, di, 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.

3.5 Analysis of Variance (ANOVA)

The analysis of variance (ANOVA) was used to analyse data on each morphometric trait in tilapia populations, which was performed using the Statistical Package for the Social Sciences (SPSS) system.

3.6 Canonical Variate (CVA) and Discriminant Function Analysis (DFA)

Principal components analysis (PCA) and discriminant function analysis (DFA) were used to examine the modified data. The variation among samples for size-adjusted truss measures was also compared using univariate analysis of variance. The number of substantially different morphometric features among pairs of samples was also determined using a post hoc multiple comparison test. The significance of differences among the samples in the data set was tested using multivariate analysis of variance.

3.7 Cluster Analysis

Truss-morphometric variables were standardised independently for each area to remove the influence of fish size upon those variables. The meristic traits will not be standardised because there was no substantial relationship between them and tilapia body size [15]. The below allometric equation will be used to normalise the variables.

$$Vtrans = \log V – â(\log SL – \log SLmean) \quad (1)$$

Where Vtrans is the morphometric variable that has been modified, V stands for the untransformed variable, SL stands for each fish's standard length, SL mean is the overall mean standard length of all the fish from each group (region), and β is the slope of the relation between log V and log SL.

3.8 Identification of Gender

Fish are notoriously difficult to sex, and separating male from female tilapia is no exception. The genital papilla, which was positioned right behind the anus, can be used to detect the sex of a tilapia. Female tilapia has a more spherical form with a triangular indent in the centre, while males have a tapering shape below the anus. The genital of the tilapia was examined by using bare hands. The weight and the sizes of the tilapia can be used to examine the gender of tilapia, as the female ones are larger than the males.

4 Findings

The univariate analysis of variance (ANOVA) was used to study two populations of tilapia fish, focusing into the connection between measurement and sexes. The major focus of analysis is on differences in group means, indicating that ANOVA is focused with variance differences. As for this experiment, one-way ANOVA was applied. When the data is separated into groups based on only one factor which is population, a one-way analysis of variance is employed. a-b, a-c, a-d, a-e, af, 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 is the ten landmarks for constructing the truss network and morphometric variables on the tilapia.

Table 1. Univariate Analysis of Variance (ANOVA) testing the significant difference between male and female morphometric measurements

Variable	P	Variable	P	Variable
a_b	0.533	b_i	0.736	e_f
a_c	0.723	b_j	0.029*	e_g
a_d	0.006	c_d	0.010*	e_h
a_e	0.540	c_e	0.009*	e_i
a_f	0.049*	c_f	0.003*	e_j
a_g	0.117	c_g	0.003*	f_g
a_h	0.885	c_h	0.040*	f_h
a_i	0.814	c_i	0.348	f_i
a_j	0.208	c_j	0.123	f_j
b_c	0.688	d_e	0.028*	g_h
b_d	0.021*	d_f	0.858	g_i
b_e	0.198	d_g	0.683	g_j
b_f	0.014*	d_h	0.132	h_i
b_g	0.049*	d_i	0.760	h_j
b_h	0.097	d_j	0.010*	i_j

Notes: Significance level (P) is presented with an asterisk for variable that is significantly different among populations. * for $P < 0.05$

Upon testing for 22 interactions between variables and gender in 50 samples, 16 out of 45 characters, specifically characters a_f, b_d, b_f, b_g, b_j, c_d, c_e, c_f, c_g, c_h, d_e, d_j, e_f, e_h, e_j, and f_h were significantly distinct between gender (P=0.000) as shown in Table 1. Similar to the interaction of factors and sex from 200 samples of tilapia fish revealed that 30 out of 45 characters were significantly varied across sexes, according to a prior study reference [16].

Table 2. T-test analysis for male tilapia using two populations Ulu Bendul and Kuala Pilah

Variable	P	Variable	P	Variable	P
a_b	0.028*	b_i	0.380	e_f	0.041*
a_c	0.317	b_j	0.282	e_g	0.960
a_d	0.225	c_d	0.458	e_h	0.698
a_e	0.249	c_e	0.951	e_i	0.304
a_f	0.428	c_f	0.998	e_j	0.658
a_g	0.697	c_g	0.462	f_g	0.738
a_h	0.642	c_h	0.652	f_h	0.220
a_i	0.843	c_i	0.788	f_i	0.130
a_j	0.376	c_j	0.523	f_j	0.202
b_c	0.003*	d_e	0.432	g_h	0.168
b_d	0.158	d_f	0.960	g_i	0.192
b_e	0.946	d_g	0.019*	g_j	0.342
b_f	0.280	d_h	0.772	h_i	0.659
b_g	0.855	d_i	0.298	h_j	0.176
b_h	0.606	d_j	0.252	i_j	0.834

Notes: For variables that differ significantly within populations, the significance level (P) is shown with an asterisk: * for the significance level < 0.05

A T-test analysis revealed that 4 out of 45 truss measurements, namely a_b, b_c, d_g, and e_f, were significantly distinct between male and female populations (Table 2). From the data itself there are only a few variables that 24 differ significantly within the populations. These 4 parts show significant differences as these parts are crucial for differentiating the morphology of the tilapia. This confirmed that both of the populations are from the same species. The result shows that there are 4 variables with significant levels less than $P < 0.05$. According to reference [17], when the p-value of a T-test is less than $P < 0.05$, the result is considered statistically significant. The result is insignificant if the p-value is bigger than 0.05. The same T-test was also tested on female populations, and revealed that 3 out of 45 truss measurements, namely a_j, e_g, and g_h were significantly distinct between the female populations (Table 3). So, there is no significant difference between the two populations, due to it only producing 3 measurements.

Table 3. T-test analysis for female tilapia using two populations Ulu Bendul and Kuala Pilah

Variable	P	Variable	P	Variable	P
a_b	0.221	b_i	0.095	e_f	0.652
a_c	0.801	b_j	0.759	e_g	0.011*
a_d	0.312	c_d	0.253	e_h	0.126
a_e	0.235	c_e	0.351	e_i	0.258
a_f	0.835	c_f	0.117	e_j	0.277
a_g	0.193	c_g	0.187	f_g	0.454
a_h	0.220	c_h	0.572	f_h	0.268
a_i	0.269	c_i	0.927	f_i	0.433
a_j	0.002*	c_j	0.123	f_j	0.248
b_c	0.362	d_e	0.121	g_h	0.042*
b_d	0.989	d_f	0.076	g_i	0.989
b_e	0.872	d_g	0.112	g_j	0.963
b_f	0.836	d_h	0.848	h_i	0.723
b_g	0.223	d_i	0.097	h_j	0.714
b_h	0.317	d_j	0.806	i_j	0.710

Notes: Significance level (P) is presented with asterisk for variable that is significantly different among populations: * for $P<0.05$

PCA is used to extract the most significant data from a data table and to represent this data as a series of orthogonal variables that were known as principal components. According to reference [18], PCA's main goal is to minimise the dimensionality of a data set with a huge number of connected variables while preserving as much variance as possible. It's a technique for detecting patterns in data and displaying them in a way that emphasises their similarities and dissimilarities.

Table 4. Loadings of the first of twelve principal components (PC1) formophometric characters of male tilapia (accounting of 47.2% of the variance)

Variable	P	Variable	P	Variable	P
a_b	0.747*	b_i	0.864	e_f	0.597*
a_c	0.523	b_j	0.561	e_g	0.662*
a_d	0.705	c_d	0.820	e_h	0.727
a_e	0.848	c_e	0.605	e_i	0.791
a_f	0.756	c_f	0.830	e_j	0.849
a_g	0.835	c_g	0.879	f_g	-0.280
a_h	0.729	c_h	0.824	f_h	0.651
a_i	0.706	c_i	0.663	f_i	0.758
a_j	0.555	c_j	0.364	f_j	0.475
b_c	0.461	d_e	0.511*	g_h	0.750
b_d	0.781	d_f	0.687*	g_i	0.532
b_e	0.439	d_g	0.457	g_j	0.904
b_f	0.926	d_h	0.926	h_i	-0.692*
b_g	0.927	d_i	0.727	h_j	0.779
b_h	0.873	d_j	0.863	i_j	0.497*

Notes: An asterisk (*) is used to indicate a coefficient that has a large contribution to the component

For male red tilapia, the contribution of each variable to PC1 (which accounts for 47.2 %) revealed a strong contribution from 7 measurements, including a_b (0.747), d_e (0.511), d_f (0.687), e_f (0.597), e_g (0.662), h_i (0.692) and i_j (04.97) which were mostly from the posterior part of the body of fish (Table 4).

Discriminant function analysis (DFA) is a statistical approach for classifying unknown individuals and the probability of being classified into a specific group. The sample is properly distributed for the characteristic, according to DFA. DFA has been used to determine the morphological diversity of putatively different taxonomic groups [19].

Table 5. Contribution of each variable of the discriminant function (DF1) for male samples (accounting for 100% of the variation)

Variable	P	Variable	P	Variable	P
a_b	0.430	b_i	0.139*	e_f	-0.053*
a_c	0.335	b_j	-0.138	e_g	-0.053
a_d	0.317	c_d	0.126	e_h	0.052*
a_e	0.313	c_e	0.118*	e_i	-0.043
a_f	0.290	c_f	0.116*	e_j	0.039
a_g	0.288	c_g	0.115	f_g	0.029
a_h	0.263	c_h	0.113*	f_h	0.028
a_i	0.245	c_i	0.102*	f_i	-0.028
a_j	0.231	c_j	0.099	f_j	0.024
b_c	0.206	d_e	0.095	g_h	-0.024
b_d	0.190	d_f	-0.088*	g_i	-0.014*
b_e	-0.174	d_g	0.088	g_j	-0.011*
b_f	0.161	d_h	0.080	h_i	-0.007
b_g	0.155	d_i	-0.077*	h_j	-0.007
b_h	0.151*	d_j	0.068*	i_j	0.005*

Notes: An asterisk (*) is used to indicate a coefficient that has a large contribution to the function.

Measurements of trusses with statistically significant loadings on the first discriminant function (DF1) suggest that 14 variables are involved with these findings including a_b, a_d, a_c, a_e, a_f, a_g, a_h, a_i, a_j, b_d, b_c, b_e, b_f, and b_h were found to be significant which were derived mostly from measurements collected from the posterior region of the body (Table 4.5) is a data-reduction approach for determining the membership of naturally occurring groups. For male 30 samples, DFA might be used to look into the contributions of each DF1 variable. Measurements of trusses with statistically significant loadings on the first discriminant function (DF1) suggest that 14 variables are involved with these findings including a_b, a_d, a_c, a_e, a_f, a_g, a_h, a_i, a_j, b_d, b_c, b_e, b_f, and b_h were found to be significant which were derived mostly from measurements collected from the posterior region of the body (Table 5) is a data-reduction approach for determining the membership of naturally occurring groups.

Table 6. Loadings of the principal components (PC1) for morphometric characters of female tilapia (accounting for 43.9% of the variance)

Variable	P	Variable	P	Variable	P
a_b	0.478*	b_i	0.519	e_f	0.599
a_c	0.696	b_j	0.719	e_g	0.522
a_d	0.953	c_d	0.811	e_h	0.755
a_e	0.528	c_e	0.896	e_i	0.355
a_f	0.923	c_f	0.942	e_j	0.864
a_g	0.926	c_g	0.945	f_g	0.503
a_h	0.530	c_h	0.889	f_h	0.463
a_i	0.442	c_i	0.408	f_i	0.693
a_j	-0.525*	c_j	0.369	f_j	0.795
b_c	-0.399*	d_e	-0.478*	g_h	0.564
b_d	0.871	d_f	0.206	g_i	0.766
b_e	0.848	d_g	0.229	g_j	0.861
b_f	0.936	d_h	0.427	h_i	0.526*
b_g	0.745	d_i	0.475	h_j	0.615*
b_h	0.501	d_j	0.906	i_j	0.578

Notes: An asterisk (*) is used to indicate a coefficient that has a large contribution to the component

Variables contributing to PC1 (Table 6) for female red tilapia came mostly from measurements obtained at the front and posterior sections of their bodies that were namely, a_b (0.478), a_j (0.525), b_c (0.399), d_e (0.478), h_i (0.526) and h_j (0.615). An asterisk (*) indicates a coefficient that makes a significant contribution to the component. The findings demonstrated that a maximum of two principal components (PC) explained 43.9 % in the data sets for female tilapia morphometric characters. PCA helps to improve interpretability while retaining as much information as possible. This is accomplished by generating new variables that are unrelated to one another. Since technological variables are built to adapt to varied data kinds and structures, it may be considered an adaptive data analysis technology [20].

Table 7. Contribution of each variable of the discriminant function (DF1) for female samples (accounting 100% of the variation)

Variable	P	Variable	P	Variable	P
a_b	0.195	b_i	0.064*	e_f	0.028*
a_c	0.189	b_j	0.063	e_g	0.019*
a_d	-0.188	c_d	0.061*	e_h	0.018*
a_e	0.124	c_e	0.060*	e_i	0.017*
a_f	0.123	c_f	0.059*	e_j	-0.016*
a_g	0.106*	c_g	0.058*	f_g	-0.016*
a_h	0.091	c_h	0.058*	f_h	0.015*
a_i	-0.087	c_i	0.055*	f_i	-0.013
a_j	0.082	c_j	0.055*	f_j	0.009*
b_c	0.081*	d_e	0.053*	g_h	-0.007*
b_d	0.076*	d_f	0.050	g_i	-0.006*
b_e	0.076*	d_g	0.049*	g_j	-0.005
b_f	0.071*	d_h	0.044*	h_i	0.004*
b_g	0.065*	d_i	0.034*	h_j	-0.004*
b_h	0.065	d_j	-0.033*	i_j	0.004*

Notes: An asterisk (*) is used to indicate a coefficient that has a large contribution to the function.

DFA revealed a single component that accounted for 100% of the variation. The first discriminant function (DF1) similarly showed a significant loading of variables that were generally the same, which is 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_j, and i_e which revealed that these locations are significant in the description of the features of female samples (Table 7). Discriminant function analysis produced one discriminant function which is DF1 for both morphometric and landmark measurements. For morphometric and landmark measurements, the first DF for both male and female accounted for 100%. The connection between discriminant variables and DF indicated that morphometric measurements such as Total Length (TL) and Standard Length (SL) contributed to the DF1 as shown in Table 7. As a result, these areas may be regarded as crucial in distinguishing the two red tilapia populations studied in this research.

Table 8. Result of multivariate tests for male and female samples

Effect	Male			Female		
	Value	F	P	Value	F	P
Wilk's Lambda	0.00	29.933	0.32	0.00	39.449	0.25

Notes: Wilks' Lambda values that were used in this research (*significant at $P < 0.001$)

As shown in Table 8, the significant or P values are larger than 0.05, so it means that the data is normally distributed and it was accepted. Wilks' tests were performed on the discriminant results acquired from the functions in order 36 to conduct multivariate analysis. The tests of both male and female shows the value of Wilk's Lambda values that were used is 0.00 and for male it has a probability of $P = 0.32$ meanwhile the female has a probability of $P=0.25$. As for both of these variables have a significance level of $P < 0.001$. From the results, it shows that the data is well accepted, due to P values for male is 0.32 and for female is 0.25, both of its P values are larger than 0.05.

Table 9. Count and percentage of gender populations sample in each population for morphometric measurement

Gender Population	**Predicted Group Membership**				
	UBM	**UBF**	**KPM**	**KPF**	**Total**
UBM	5	0	0	0	5
UBF	0	20	0	0	20
KPM	0	0	11	0	11
KPF	0	0	0	14	14
UBM	100.0	.0	.0	.0	100.0
UBF	.0	100.0	.0	.0	100.0
KPM	.0	.0	100.0	.0	100.0
KPF	.0	.0	.0	100.0	100.0

Notes: UBM = Ulu Bendul Male, UBF= Ulu Bendul Female, KPM = Kuala Pilah Male, and KPF = Kuala Pilah Female.

Discriminant function analysis (DFA) is a data-reduction approach for determining the membership of naturally occurring groups. It can provide answers to theoretical concerns, but it has shown to be particularly beneficial in practical research. Individuals were assigned to their original lineup using the DFA [21]. DFA also helps to identify the factors that predict group membership from a collection of predictors by selecting a linear combination of variables that maximize the distinctions between natural group

means. It helps to identify which factors are the greatest discriminators between groups is important for predicting group membership [22].

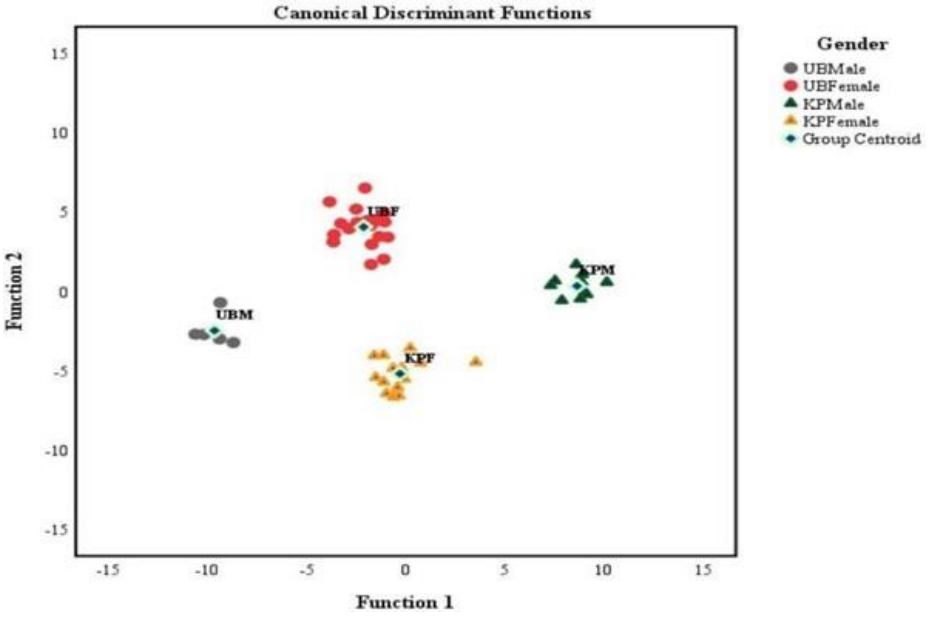

Fig. 5. Discriminant analysis plot for female and male samples of the two populations of red tilapia

Figure 5 depicts that there is no overlapping among the two sexes of two different populations as shown through canonical variate analysis plot, the variables that were evaluated were 45 values of Function 1 and Function 2 generated from discriminant function analysis (DFA). The results revealed that both Function 1 and Function 2 was successful in classifying the individuals into four distinct groups (Ulu Bendul Male (UBM), Ulu Bendul Female (UBF), Kuala Pilah Male (KPM), and Kuala Pilah Female (KPF).

5 Discussion

5.1 Tilapia's morphological variation

The influence of sex on morphological variation was taken into consideration and analyses for morphometric variables in male and female subjects were conducted individually. This finding is supported by the morphometric analyses on tilapia from Lake Lanao, Philippines, on morphological landmarks to analyse the shape dimorphism between sexes [23]. Individual differences in growth related to sex have significant production implications, such as substantial variations in sizes or unsaleable little fish. In tilapia (*Oreochromis spp*.), males are typically greater than females when it comes

to sizes [24]. These characteristics are widely linked to the sexual dimorphism seen in species related to the genus Oreochromis. However, a contradicting finding was found by previous study, revealing a significant difference (P=0.001) between four river populations of *Anodontostoma chacunda* in all morphometric features. Males and females had no statistically significant differences in morphometric characteristics (ANOVA, P=0.05), hence the sexes were mixed for further research.

However, a contradicting finding was found by former study, revealing a significant difference (P=0.001) between four river populations of *Anodontostoma chacunda* in all morphometric features. Males and females had no statistically significant differences in morphometric characteristics (ANOVA, P=0.05), hence the sexes were mixed for further research. Even so, most studies benefited from this method and conduct, in which from one of the previous study, its data revealed that out of 25 standardized truss characteristics, there were significant differences (P=0.05) between the means of the four groups, 13 for male and 23 for female.

5.2 T-test for growth of male and female tilapia

On average length, two sample T-tests were performed, indicating that the Gache Gache and Fothergill regions have significant variances in mean lengths. In the Fothergill unfished region, *L. miodon, P. philander, S. zambezensis, H. vittatus,* and *Brycinus lateralis* all had longer mean lengths. The catch per unit effort (CPUE) T-test between Fothergill and Gache Gache also reveals some significant changes in CPUE between unfished and fished regions. The P value is stated as under the hypothesis that there is either no effect or no difference (null hypothesis). To measure the P values, which are likely to observe any differences between groups due to the chances. The P value can have any value between 0 and 1 since it is a probability. Close to zero values imply that the observed difference is considered to be due to chance, meanwhile a P value close to 1 indicates that there is no difference in outcomes other than chance. According to reference [17], if all of the assumptions used to produce the data (including the test hypothesis) were accurate, a lower P value simply identifies the data as unusual due to a major random error or a violation of a hypothesis other than the test hypothesis. As the P value obtained from this study was less than 0.05, it was assumed that it was not chosen for presentation. So, from the results, it shows that 3 out of 45 variables are significantly different among populations and have a $P < 0.05$. Higher p-values, also known as p-scores, indicate a significant difference between the two sample sets. The lesser the p-value, the closer the two sample sets to each other. The presence of a significant t-score shows that the groups are distinct. The groupings are comparable if the t-score is modest.

5.3 Classification of tilapia into gender

PCA is used to extract the most significant data from a data table and to represent this data as a series of orthogonal variables that were known as principal components. Fish that have a high score on the fourth PC with 6.87% of total variance display a little forward rotation of the mouth, a reduction in dorsal fin basal length, and an increase in

anal fin basal length. In this study, eigenvalues greater than one were accepted, whereas eigenvalues less than one were excluded. The first principal component (F1) accounted for 58.59 % variation and was significantly linked to features relating to head shape, which produced 5 variables, and body shape, which produced 15 variables. The second principal component (F2), which accounted for 7.46 % variance and was significantly related with the caudal peduncle region producing just one variable, contributed for 7.46 percent of the total variance. The more significant the corresponding variable is in defining the common component, the bigger the absolute value of the correlation [25]. The variables' high loading indicated that these regions are crucial in describing male sample characteristics.

Discriminant function analysis (DFA) is a statistical approach for classifying unknown individuals and the probability of being classified into a specific group. Based on the results that were done by previous study on gizzard shad, *A. chacunda*. It has two results for DFA, which is DF1 and DF2. The first and second discriminant functions of the study revealed that (DF1 and DF2) were significant for 98.58% of total variance (94.01% and 4.57%, 31 respectively), suggesting that the first two canonical variables were responsible for a number of the total variance. However, the total random allocation of individuals into their original data set was low, with males accounting for 54.1 %. In the male population, the proportion of properly categorised individuals in their initial samples demonstrated considerable inter-mixing.

PCA helps to improve interpretability while retaining as much information as possible. This is accomplished by generating new variables that are unrelated to one another. Since technological variables are built to adapt to varied data kinds and structures, it may be considered an adaptive data analysis technology [20]. The PCA is able to isolate the species variation, which accounts for around 43.9% of the variance in the original data, meanwhile for the study conducted by reference [16] was 35%. In this study, DFA revealed a single component that accounted for 100% of the variation. Discriminant function analysis produced one discriminant function which is DF1 for both morphometric and landmark measurements. For morphometric and landmark measurements, the first DF for both male and female accounted for 100%. The connection between discriminant variables and DF indicated that morphometric measurements such as Total Length (TL) and Standard Length (SL) contributed to the DF1 as shown in Table 4.7. As a result, these areas may be regarded as crucial in distinguishing the two red tilapia populations studied in this research.

According to a former study, 100% of the initial grouped cases were successfully categorised freshwater Murrel, *Channa punctatus* into their appropriate subpopulations using discriminant function analysis (DFA). Two discriminant functions were created as a result (DF 1 and DF 2). The two functions represented 100% of the difference between the groups, with DF1 and DF2 accounting for 99.6% and 0.4% of the between-group variability in morphometric data, respectively. As three separate group centroids were produced in DFA, the combined group plot of DF 1 on DF 2 discovered the presence of three distinct phenotypic stocks of *C. punctatus*. Based on the previous study conducted on *Sattar snowtrout, Schizothorax curvifrons*, DFA discovered two 35 morphological variables that described 78.6% and 21.4 % of the morphological variance among the populations. There were 28 measurements of truss distances with

relevant loading on the first factor (DF1), that represented 78.6% of the total variance. These 28 measures define the parameters that embrace the fish both crosswise and horizontally. The second factor (DF2) represented 21.4 % variance and generated three variables due to the relevant truss distance loadings on this factor. These loadings were primarily limited to the fish's front and midsection area.

5.4 Validity of Truss Network

Multivariate analysis is a field of statistics concerned with the summaries, representation, and interpretations of the data drawn from populations in which each experimental unit is evaluated for multiple characteristics. As for the multivariate analysis, Shapiro-Wilk Test or Wilk's Test were used for testing the normality data. The Shapiro– Wilk test, among others, is a common method for testing the normality of continuous data. The Shapiro–Wilk test is better suited for smaller sample sizes (less than 50 samples), although it may also be used with larger samples. The null hypothesis proposes that the data was collected from a normal distributed population. The hypothesis was accepted if $P > 0.05$, and the data are said to be normally distributed [26].

5.5 Group Membership Tilapia's

The discriminant functions were made based on the sexes, which is female and male for two different populations. The huge plot difference between the genders of the same population shows that it is related to sexual dimorphism. Sexual dimorphism means that the exterior appearance of a species' two sexes differs. Many additional characteristics, including internal anatomy, behaviour, fragrance, sound, physiological, life history, and ecology, may differ between the sexes. Sexual selection was responsible for the majority of sexual dimorphism, in which selection pressure acts on the sexes independently, favouring features that offer an individual a benefit over those of the same sex in terms of mating chances [27]. The morphology of the fish, in contrast to its size or weight, might well be important, for example, fish with a deeper and broader body 39 appear to have more meat than fish with a shorter and thinner body [28]. Based on the study that was done by reference [21], DFA of the data yielded three DFs as a result of the study's findings. The first DF (DF1) contributed for 76.1 % of the total variance, but the second (DF2) and third DFs (DF3) represented for 17.9% and 6% of group diversity within populations, respectively. Allocation performance was high across the field, with the DFA accurately classifying 98.7% of people into their original category.

6 Conclusion & Recommendations

The study revealed a significant morphometric variability between the two red tilapia populations, with substantial differences observed in both male and female specimens. Accurate species identification is crucial for biodiversity monitoring, informing

conservation efforts, aquaculture practices, and long-term fisheries management. Misidentification can pose risks to both species and the environment, leading to inaccurate monitoring, inefficient resource allocation, and declining fish stocks. Additionally, Truss morphometric analysis effectively confirmed the identity of tilapias from Kuala Pilah and Ulu Bendul as *O. niloticus*, accurately grouping them by sex with no errors using canonical discriminant function. Additionally, ANOVA and T-tests assessed morphological variation and growth, with the truss morphometric proving useful in Tilapia fish stock assessment. The analysis successfully classified samples not only by species but also by gender, as demonstrated by DFA. The study highlights the reliability of truss morphometric analysis in determining interpopulation morphometric variability in tilapia fish. While most statistical analyses showed no significant differences in fish growth and size, further studies should employ additional morphometric measurements to explore potential disparities between populations.

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