DESCRIBING SHAPE VARIATIONS OF EUTHYNNUS AFFINIS (MACKEREL TUNA) USING LANDMARK-BASED GEOMETRIC MORPHOMETRIC ANALYSIS

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Name of the Authors:

Roldan T. Echem[1]
Ian J. Catubay[1]

[1]Department of Biology and Natural Science, College of Science and Mathematics, Western Mindanao State University, Normal Road, Baliwasan, Zamboanga City 7000, Philippines

Corresponding author: Roldan T. Echem[1]

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ABSTRACT

A new study shows that the impact of fishing for tuna and similar species during the last 50 years has lessened the abundance of all these populations by an average of 60%. Experts add that the majority of tuna fish have been exploited to the limits of sustainability. *Euthynnus affinis* is one of the commercially important tunas in the Philippines. A majority of tuna caught by the commercial fisheries of the country is exported. The tuna population has already shown signs of overexploitation and decline worldwide. Therefore, the tuna should be properly studied and managed to ensure its sustainability. In this study, landmark-based geometric morphometrics was used to primarily generate body shape variations among male, female and between male and female tuna species.A total of 300 samples were collected in Zamboanga City, Western Mindanao, Philippines. Samples were utilized for imaging and land markings. Sexes of fish samples were determined by direct examination of gonads. Images were processed using geometric thin-plate grids (TPS) and relative warps (RW) software. The data generated from relative warp were subjected to PAST software to established shape variations displayed in the transformation of grids of the relative warps. Results revealed that there were shape variations between sexes and among species of tuna. The relative warp analysis showed that the males shape variations were found on the snout tip and dorsal fin. For females, the body shape variations were localized on snout tip and caudal peduncle. Three shape variations occurred both male and female *E. affinis* namely; the snout tip, dorsal extremity of the caudal fin, and interior of the anal fin. Discriminant Analysis was used to confirm shape variations between sexes of mackerel tuna species and revealed highly significant differences. However, there were also shared characteristics between sexes. A variation on snout tips can be attributed to their habitat and eating habits of both males and females. The shape variation among females can be accounted to the production of offspring. The shape differences of *E. affinis* were mostly affected by their behaviors and as well adaptation in the environment.

Keywords: Geometric Morphometrics, gonads, *Euthynnus affinis*, landmarks, Variation

I. INTRODUCTION

Euthynnus affinis (Cantor, 1849), is a small epipelagic, migratory, neritic tuna is one of the major commercial tuna species being caught worldwide (Santos et al.,2010). A recent study concluded that populations of tuna and similar species have been cut by 60% on average throughout the world over the last century. Most of these populations have been exploited to the limits of sustainability, and there are many species that have been overexploited (Juan-Jorda et al., 2011). Mackerel have also experienced a significant reduction in abundance.

In the Philippines, tuna remains an important commercial fish species being landed in the country in terms of volume (BAS 2006). The majority of tuna caught by the commercial fisheries of the country is exported in processed/ canned form because there is no existing fresh or dried export market. On the other hand, catches from municipal fisheries goes directly to the domestic market for local consumption (Barut et al., 2010).

The majority of tuna fish have been exploited to the limits of sustainability. Mackerel tuna have also experienced a significant reduction in abundance. Serious efforts and effective action are needed to reduce global overfishing, to recover overexploited populations and regulate trade that endangers them(Jorda et al., 2011).

Morphological descriptions have been the basis of taxonomic classification and understanding of biological diversity and sexual dimorphism has been one of the most interesting sources of phenotypic variation in animals. phenotypic variation is the existence of differences in forms such as shapes and sizes(Benitez, 2013). In this study, the phenotypic variation is being used as one of the comparisons between sexes of *E. affinis.*

The main goal of geometric morphometrics is to study how shapes vary of the same species and between sexes. Morphometrics is mostly used in biology to describe organisms and it is very important because it allows quantitative descriptions (Gelsvartas, 2002). This quantitative approach will compare shapes of *E. affinis* much better than the traditional approach. In this study, the quantitative descriptions *E. affinis* was supported with statistical analysis methods that allowed accurate interpretation of the collected data.

The morphometrics used in this study permits the quantitative analysis of variation in size and shape of *E. affinis* that give contributions in the field evolution and developmental mechanisms. This morphometric combine a geometric concept of shape with the procedures of multivariate statistics and constitute a powerful and flexible set of tools for analyzing morphological variation. Therefore, information on phenotypic traits is an important component of comparative studies of development (Klingenberg, 2001).

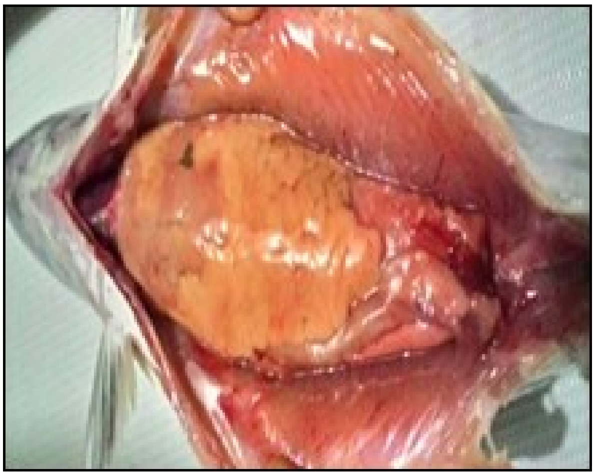
II. MATERIALS AND METHODS

A total of 300 matured *Euthynnus affinis* samples were used in this study. There were 150 males and 150 females collected from Baliwasan Seaside, Zamboanga City(Figure 1).This fishes were caught in Maluso, Basilan Strait. The size ranges from 12 cm-13 cm.

Figure 1.Map of the Philippines showing fish landing sites in Baliwasan Seaside, Zamboanga Cityand Maluso,Basilan Province.

The samples were laid in a styrofoamand were photographed using a high resolution single-lens reflex (DSLR Canon ef 50mm f/1.8). The lens was mounted on Canon EOS 1100D camera at a uniform focusing distance of 0.8 m and the same contrast of the light. Image processing was done before the sexing of gonads in order not to destroy the outer rim of the mackerel tuna. The images of the samples were numbered respectively to identify the samples after the sexing of gonads.

Determination of sexes was done by direct examination of gonads. The *E. affinis* samples were cut from the middle part of the ventral region to the anus (Amtyaz et al.,2013). The different stages of the gonads were also determined (Figure 2).



a) Female Gonad

b) Male Gonad

Figure 2. Gonads of female and male *E. affinis*.

The images of the samples were digitized using thin-plate splines-Utility (tpsUtil) and saved as thin-plate splines (TPS) files. Landmark points were digitized using thin-plate splines-Digitized (tpsDig) ver. 2.15 software (Rohlf, 2008). Fifty-two (52) landmark points were used to illustrate the shape variations of the samples (Figure 3).

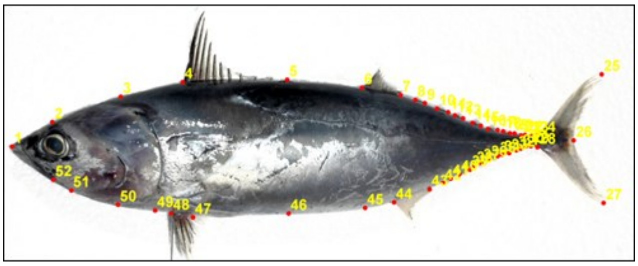


Figure3. Digitized image of E. affinis showing the landmark points with corresponding numbers.

Relative warp analysis using thin- plate splines-Relative Warps (tpsRelw) was used to give information in the variation in the local shape. This involves in fitting and interpolation function to homologous landmarks for each specimen. Results of the relative warps of the shape were generated using Paleontological Statistics (PAST) software.

To analyze the data, statistical analyses were employed. The data among sexes of *E. affinis* were subjected to normality test. The most informative warp scores were then subjected to Principal Components Analysis (PCA) between sexes. PCA determines which landmark points has the highest Eigen values and is used to confirm the relative warps. PCA is performed based on the variance-covariance of the coefficients (Torreset al.,2008).

Discriminant Function Analysis (DFA) was used in this study. DFA was employed not only to determine equality of the means of the two groups but also to reclassify specimens to previously defined groups. This analysis is a standard method for visually confirming or rejecting the hypothesis that two groups are morphologically distinct (Hammer et al., 2001). Relative warp scores were also subjected to Multivariate Analysis of Variance (MANOVA) to determine the significant variation in the body shape between sexes of *E. affinis.*

RESULTS AND DISCUSSION

In this study, the difference in the body shape among males, females, and between the male and female of *E. affinis* was determined using Geometric morphometrics (GM). Procrustes fit was used to remove the unnecessary points unrelated to the shape, remove biases and also used for scaling. The variations in the body shape of both male and female *Euthynnus affinis* will be determine based on the relative warp and its corresponding percentage of variance.

For the relative warps of males, shape variations were attributed to the constriction of snout tip, narrower body size and constriction of dorsal and ventral tip of caudal fin. The boxplots and histograms in the body shape variations among male *E. affinis* species showed bimodal and multimodal variations on the body shapes. The results revealed highly significant variations occurred among males of *E. affinis* species(Figure 4).

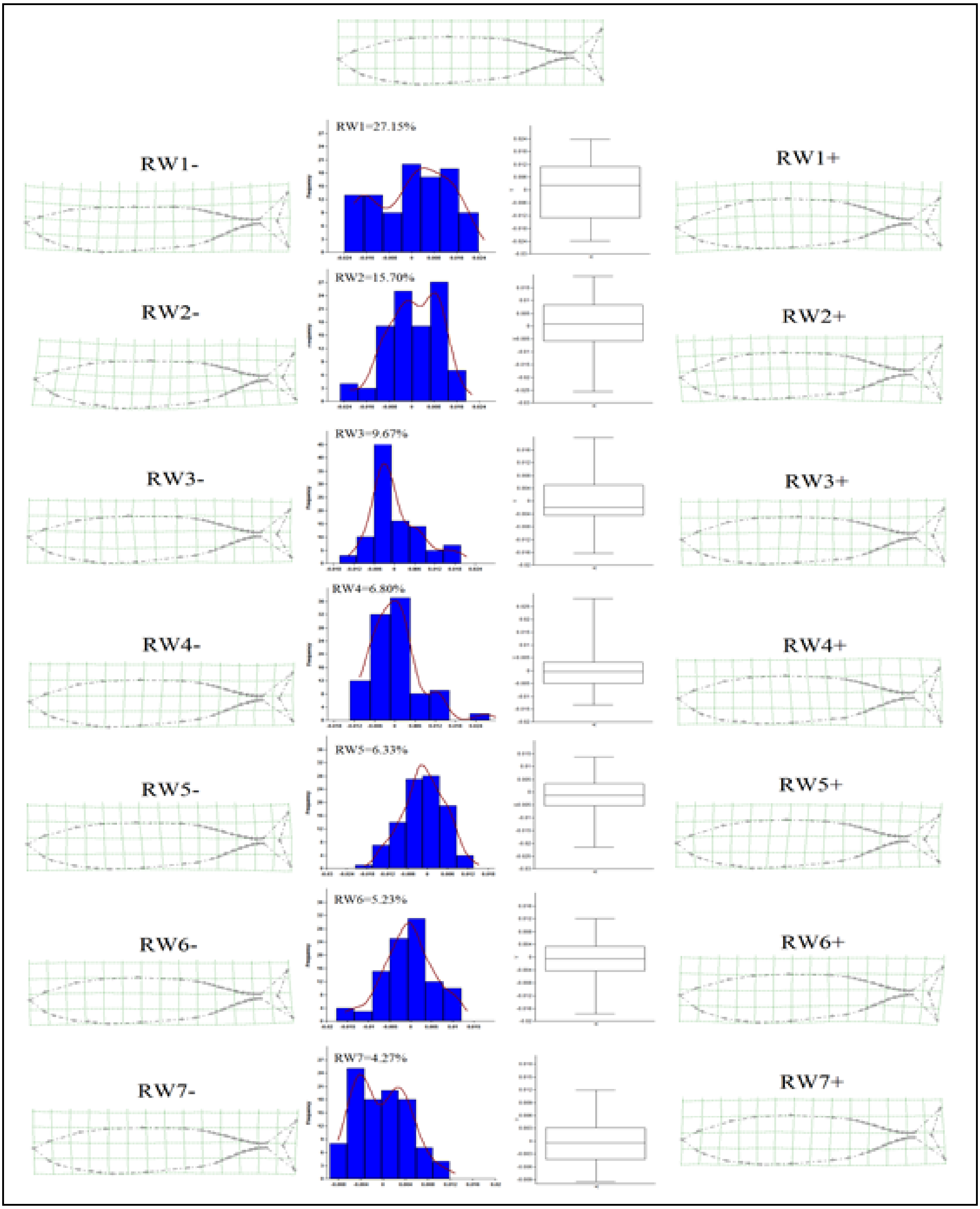


Figure 4. Summary of geometric morphometric analysis showing the consensus morphology and frequency histogram and boxplot of male *E. affinis.*

The variability observed among male species of *E. affinis* in terms of body size, snout tip and length of the caudal fins can be explained as difference of habit or of preferred habitat. The slimmer body of the males being adapted to a more active habit or a habitat with fast water flows (Nacua et al., 2010). And also, males have adaptations that increase the probability of acquiring mates and of success at male-male competition (Torres et al., 2010).

There were significant variations in the 7Relative Warps of the male *E. affinis* with a total variations of 75.15%. Relative Warp one (1) has the highest percent variation (27.15%) attributed in the snout tip of the male *E. affinis* (Table 2).

Table 2. Variability in the morphological body shape of male *E. Affinis*

Relative warp	% Variation	Description of Variations
1	**27.15%**	Variation in body size; narrower body and longer snout tip in the (-) extreme than that on the (+) extreme
2	**15.70%**	Wider in body size in (-) extreme than that on the (+) extreme -variation also occur in the snout tip
3	**9.67%**	Variation in caudal fin; longer dorsal tip of the (+) extreme than that on the (-) extreme
4	**6.80%**	Variation in caudal fin; longer dorsal and ventral tip of the (-) extreme than that of the (+) extreme
5	**6.33%**	Variation in caudal fin; longer dorsal tip of the (-) extreme than that of the (+) extreme
6	**5.23%**	Variation in caudal fin; longer ventral tip of the (+) extreme than that of the (-) extreme
7	**4.27%**	Variation in caudal fin; longer dorsal tip of the (-) extreme than that of the (+) extreme

Normality tests of the Principal Component scores show that there were extremely significant differences in the distribution of the variations in the body shapes of the male *E. affinis*. Principal Component 3(Shapiro- Wilk W = 0.93; *P*= 0.00004) and Principal Component 4(Shapiro- WIlk W = 0.93; *P* = 0.00009) has the highest variations.Test for normality of the Principal Component scores shows the distribution in variations in the body shape of male *E. affinis* along Principal Component 3 is highly significant which contributed in the snout tip and Principal Component 4, shape variation can be attributed to caudal fin(Table 3).

Table 3. Normality test for significant distribution of variation in the body shape of male *E. affinis.*

PC	Shapiro-wilk W	P(normal)	Remarks
1	0.95	0.0008	Significant
2	0.97	0.033	Significant
3	0.93	0.00004	Extremely Significant
4	0.93	0.00009	Extremely Significant
5	0.99	0.67	Not Significant
6	0.99	0.46	Not Significant
7	0.97	0.03	Significant

Variations observed among female *E. affinis* species can be inferred along the snout tip, ventral region of caudal peduncle and corpulent body size. The boxplots and histograms in the body shape variations among female *E. affinis* species showed bimodal and multimodal variations on the body shapes. The results revealed highly significant variations occurred among females of *E. affinis* species (Figure 5).

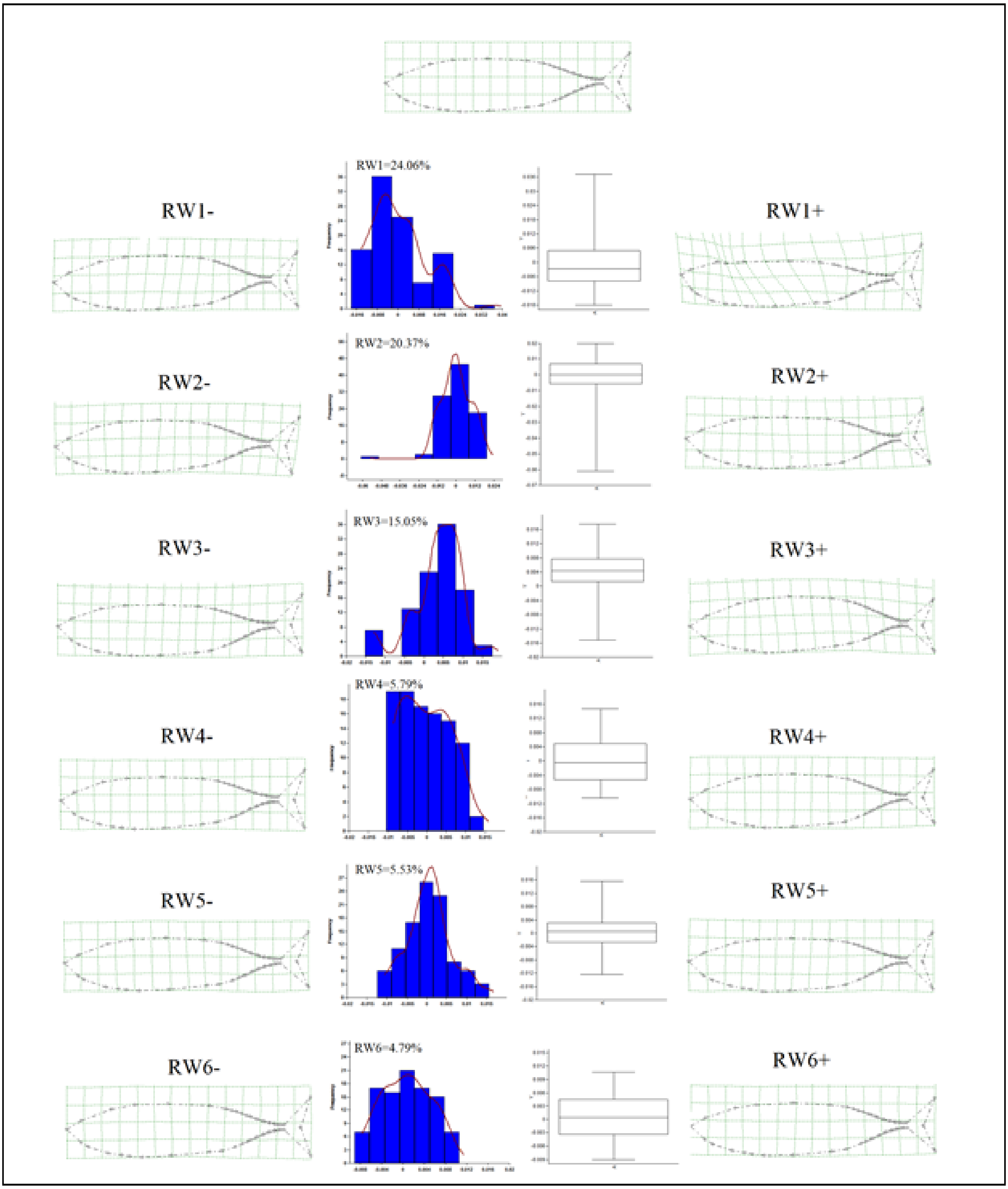


Figure 5. Summary of geometric morphometric analysis showing the consensus morphology and frequency histogram and boxplot of female *E. affinis.*

Most differences were confined to the head and the mid body region implying that the females *E. affinis* were affected on their behaviours and as well as adaptation in the environment (Requiron et al., 2010). The trend in shape variation among the female reflects selection towards bodies capable of acquiring, processing and storing energy to facilitate the production of offspring. Another feature is their difference in caudal peduncle where female have broader, which suggest the efficient fanning and guarding of eggs laid by the females to protect from predators (Ceniza et al., 2012).

There were significant variations in the six (6) Relative Warps of female *E. affinis* with a total variation of 75.59%.Relative Warp one (1) has the highest percent variation (24. 06%) in the snout tip of the female *E. affinis* (Table 4).

Table 4. Variability in the morphological body shape of female *E. Affinis.*

Relative warp	% Variation	Description of Variations
1	**24.06%**	There is a pronounced deformation along the snout tip in the (+) extreme than that on the (-) extreme
2	**20.37%**	A prominent deformation along the ventral region of caudal peduncle and abdominal region in the (-) extreme than that on the (+) extreme
3	**15.05%**	Variation on the body size; narrower body of the (-) extreme than that of the (+) extreme
4	**5.79%**	Minimal deformation in the (-) extreme and (+) extreme
5	**5.53%**	Slight variations was inferred in the (-) extreme and (+) extreme
6	**4.79%**	Least deformation was assumed in the (-) extreme and (+) extreme

Table 5 revealed that the normality tests of the principal component scores were highly significant difference in the distribution of the variations in the body shapes of the female *E. affinis*. Principal Component2(Shapiro- Wilk W = 0.89; *P*= 0.0000003)and Principal Component 3(Shapiro- Wilk W = 0.92; *P*= 0.000008).

Table 5. Normality test for significant distribution of variation in the body shapeof female *E. affinis*

PC	Shapiro-wilk W	P(normal)	Remarks
1	0.95	0.001	Significant
2	0.89	0.0000003	Extremely Significant
3	0.92	0.0000008	Extremely Significant
4	0.96	0.007	Significant
5	1.0	1.0	Not Significant
6	0.98	0.16	Not Significant

The body shape variations between male and female *E. affinis* with the used of transformation grids supported by the frequency histograms and boxplots have sustained consistency to variation in body shapesin the snout tip. Histograms showed bimodal and multimodal variations on the body shapes of pooled *E. affinis* species (Figure 6).

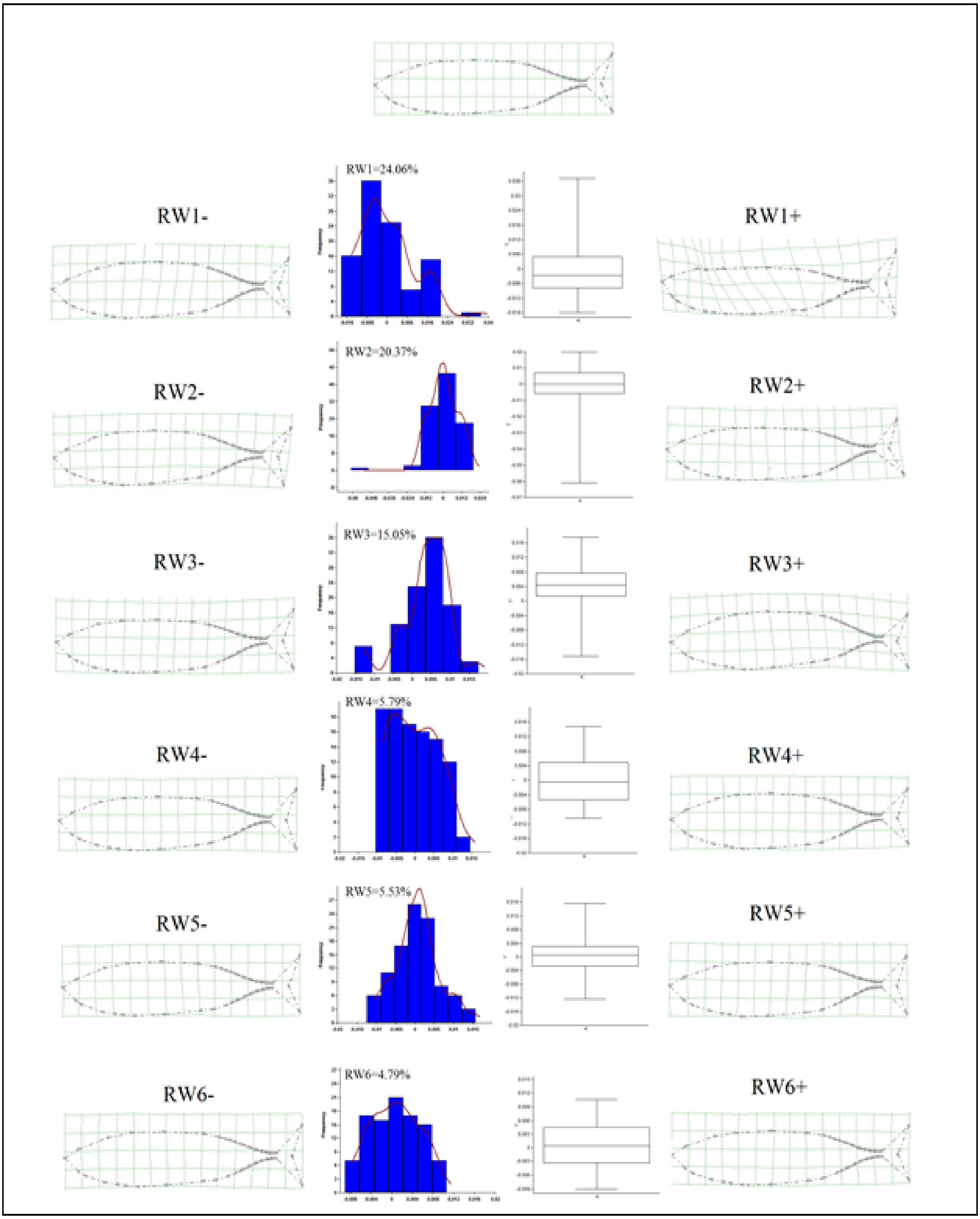


Figure 6. Summary of geometric morphometric analysis showing the consensus morphology and frequency histogram and boxplot of pooled *E. affinis.*

There were significant variations of the female *E. affinis* with a total variation of 75.37%. Relative Warp one (1) has the highest percent variation (21.35 %) in the snout tip of pooled *E. affinis* (Table 6).

Table 6. Variability in the morphological body shape of the pooled *E. affinis*

Relative warp	Variation %	Description of Variations
1	21.35%	Variation in body size; narrower body in the (+) extreme than that on the (-) extreme Wider snout tip in the (-) extreme than that of the (+) extreme
2	15.68%	Variation in body size; wider body in (+) extreme than that of (-) extreme Variation in caudal fin; longer and wider in (+) extreme than that of (-) extreme
3	13.22%	Deformation of the grid in the anterior of 1st dorsal fin along the (+) extreme than that of (-) extreme Variation in caudal fin; longer in the (-) extreme than that of the (+) extreme
4	7.07%	Variation in body size; broader body in (-) extreme than that of (+) extreme Variation in caudal fin; longer dorsal and ventral tip in (-) extreme than that of (+) extreme
5	5.73%	Wider snout tip in the (-) extreme than that of the (+) extreme Variations in caudal fin; longer dorsal tip in the (-) extreme than that of the (+) extreme
6	4.64%	Variations in the caudal fin; longer in the (+) extreme than that of the (-) extreme
7	4.32%	Variations in body size; broader body in the (+) extreme than that of (-) extreme
8	3.36%	Variations in body size; wider body in the (-) extreme Variations in caudal fin; longer in the ventral tip of (-) extreme than that of the (+) extreme

Principal Components Analysis (PCA) revealed that there were highly shape variations between male and female *E. affinis.* Principal Component 1 that can be attributed to the snout showed the highest percent variance (31.81 %)and Principal Component 2 has a percent variance of 23.29% that can be attributed to the caudal fin. The results were significant because the equivalent Eigen values were higher than the Jolliffe cut- off value of 0.00004(Table 7).

Table 7. Principal Component Analysis (PCA) and the corresponding Eigen values and percent variance.

PC	Eigen Value	% Variance
1	0.0001	31.81
2	0.0001	23.29
3	0.00005	11.06
4	0.00005	10.41
5	0.00003	7.17
6	0.00003	6.52
7	0.00002	5.23
8	0.00002	4.52

Jolliffe Cut-off: 0.00004

Multivariate Analysis of Variance (MANOVA) revealed highly significant variation (Wilk's lambda (Λ) = 0.75; P = 0.00000000209) in the body shape of the male and female*E. affinis* (Table 8).

Table 8. MANOVA test for significant variation in the body shape between male and female *E. affinis.*

Source of Variation	Wilk's lambda (Λ)	df1	df2	F	P (same)
Body shape	0.75	8	191	8.104	0.00000000209

It can be observed in the histogram of the shape characters of male and female *E. affinis* that the five coloured bins overlap at some point implying no complete separation of data sets. This entails that there is a variation between male and female

E. affinis. However, there are shared characteristics between the two because of the overlapping bins (Figure 11).

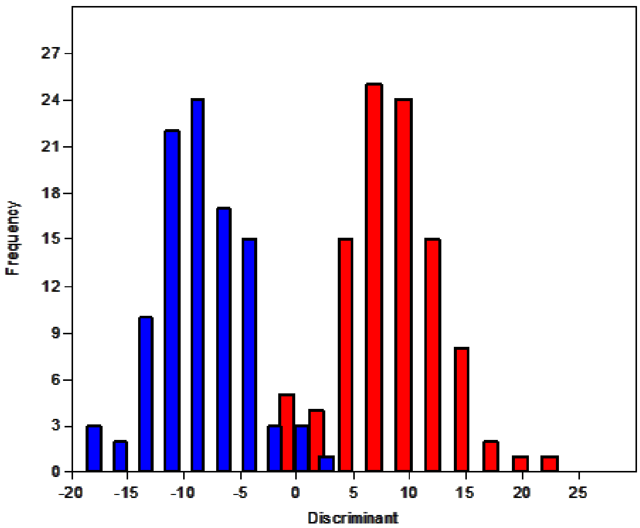


Figure 11. Histogram of shape characters of male (blue) and female (red) *E. affinis*

To further clarify the differences in body shapes between species of *E. affinis,* data were subjected to Relative Warp Analysis producing a scatter plot of points depicting the spread of the individual specimens. The spread of the points depicts the distance between species and subsequently the amount of similarity between species. Scatter plot shows that only few of male and female shared similar characteristics which can be interpreted as a presence of pronounced variation in the body shape and there were also extreme shapes that can observe for *E. affinis* species (Figure 12).

According to Turan et al. (2003) that the differences in morphology between sexes can be explained to the environmental adaptations need not be reflected into genetic changes of a population but may involve modifications in their morphology that would result into changes in their physiology and behaviour. Such changes may effectively separate them from interacting with the original population resulting into the formation of sub-populations. In addition, the frequency histograms of the relative scores also reveal that females exhibit greater degree of curvature of the body than the males. This could be attributed to the bulkier bellies of females as a consequence of their reproductive role (Dorado et al., 2012).

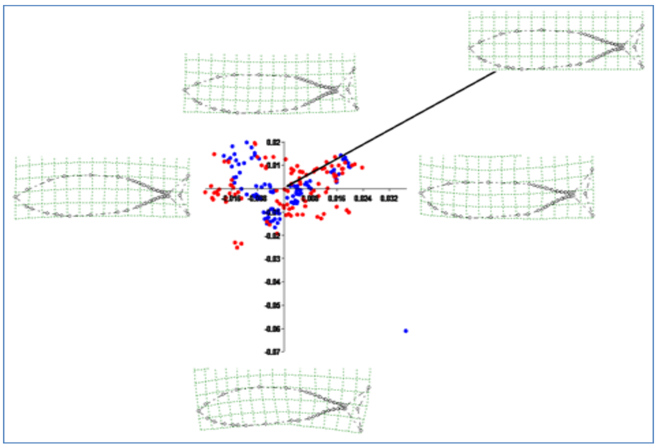


Figure 12. Scatter plot of the first two relative warps of male and female *E. affinis*.

CONCLUSION AND RECOMMENDATIONS

Based from the results, the study revealed highly significant shape variations in the morphological body shape among males, females and between sexes of *E. affinis* using the landmark-based geometric morphometric and multivariate statistical analyses. The shape variation among females can be accounted to the production of offspring. Three main variations occurred in the male and female *E. affinis* namely; the snout tip, dorsal extremity of caudal fin, and anterior of anal fin. A variation on snout tips can be attributed with their eating habits and preferred habitats both males and females. Normality test supported by boxplot and histograms were accurate in describing shape variation among sexes of *E. affinis*. The normality test showed the highest significant differences for the male and female. For the pooled samples, Principal Components Analysis (PCA) showed the highest percent variance. Discriminant Function Analysis (DFA) revealed that there were also variations between sexes. However, there were also shared characteristics between sexes of *E. affinis.*

It is recommended that further studies regarding geometric morphometric analysis will be employed and sexual dimorphism of *E. affinis* species particularly on the whole morphological body shape including the fins on the species of *E. affinis*. It is also recommended that another geometric morphometric study will be conducted on the fins of *E. affinis* using landmark-based geometric morphometric analysis to detect the sexual dimorphism.

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[16] Christian Peter Klingenberg*Morphometrics and the role of the phenotype in studies of the evolution ofdevelopmental mechanisms

Christian Peter Klingenberg*
Laboratory of Development and Evolution, University Museum of Zoology, Department of Zoology, Downing Street, Cambridge CB2 3EJ, United Kingdom

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