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RESEARCH ARTICLE

Intraspecific shape analysis of Bali sardinella (*Sardinella lemuru*) using geometric morphometrics collected in the coast of Cabadbaran, Agusan Del Norte, Philippines

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ABSTRACT

Modern techniques are often applied to analyze the body shape differences among biological organisms. Also, taxonomy and systematics are two essential fields of Biology concerning shape discrimination. This study aims to identify the shape variations of *Sardinella lemuru* (Bali sardinella) using Symmetry Asymmetry Geometric Data (SAGE) Software Application. A total of 70 fish samples consisting of 35 males and 35 females were collected in Barangay Caasinan, Cabadbaran, Agusan Del Norte, Philippines. Standard laboratory procedures were done and fish samples were subjected to the analysis. Procrustes ANOVA revealed a highly significant difference ($P<0.0001$) among the components analyzed (individuals, sides, and individuals vs. sides). This implied that each of the fish samples exhibited different body shapes. Principal Component Analysis (PCA) obtained a high rate of Interaction/Fluctuating Asymmetry (76.79%) in males when compared to female samples (74.08%). The shape dissimilarities within the populations were associated with genetic components, ecological adaptations-swimming, predator escape, and resource competition. Thus, the present study identified shape disparity within the fish populations. The development of employing modern techniques enhances scientific methods to quantify shape dissimilarities among species individuals and assemblages.

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Introduction

Over the decades, using modern methods is a way of elaborating realistic shape analyses. It draws significant information to generate specific data useful to create knowledge. While determining shape discrimination with the use of modern techniques often utilized by many researchers. Form and shape transformation has been an ultimate requirement to evaluate biological phenotypes. Since then up to the present, detecting shapes and copying metric observations has been a challenge to recognize how various biological forms vary. A modern and systematic approach proved the connection between form and function. Thus, measuring the distinct factors could be used for detecting species characteristics (Richtsmeier et al., 2002). Body shape is an important aspect of identifying variations within the fish assemblages. It has been associated with numerous dynamic activities, such as food hunting, swimming, and escaping predators (Schoener, 1971; Webb, 1984; Martinez-Leiva et al., 2023). Additionally, fish body shape has also been influenced by mating (Martinez-Leiva et al., 2023). The shape of the fish was found to have phenotypic plasticity: this information showed the ability of single genotypes to create different phenotypes when open to ecological conditions (Pigliucci et al., 2006; Fusco & Minelli, 2010; Klingenberg, 2019).

Fish are commonly used as a biomarker of environmental status. They are the best sample for detecting conditions since they inhabit where most alteration occurs. Biological changes can affect its physiological activities and later may express its morphology. Ecological risks and anthropogenic activities may pose unfavorable conditions both in the environment and the organisms (Natividad et al., 2015). Over the years, aquatic habitat has become a place with a wide range of alterations (Dikshith et al., 1990). Aquatic modifications can be a factor in changing the genetic makeup of an organism and result in diversity and variation in the population (Trono et al., 2015). It causes intolerable effects, damaging the environmental state and leading to phenotypic differences (Duruibe et al., 2007). Adaptations are a key component that can alter the morphological traits of the aquatic organism (Jumawan et al., 2016). These are contributing factors that directly affect its state of well-being. The effect of adaptation may be described as morphological asymmetries through imperfect development (Cabuga et al., 2022). Individual and fish groups had a chance to adapt where necessary for survival. Ecological characteristics shown by juveniles and adults could be influenced by environmental factors through their embryonic development up to epigenetic modifications (Best et al., 2018; Jonnson & Jonnson, 2018). Moreover, morphological, sensorial, and behavioral changes occur during the fish's ontogenetic development dependent on exogenous influences such as temperature and food supply. Nonetheless, physiological traits (e.g., type of respiration and muscle reorganization) impact the metabolism activities of each sample (Burggren & Blank, 2009; Somarakis & Nikolioudakis, 2010; Biro & Stamps, 2010). The development of vital organs and sensory mechanisms is the result of metabolism changes that are associated with optimizing survival (Osse, 1997; Khemis et al., 2013).

To recognize the shape variation in fishes, Geometric Morphometric Analysis (GMA) was applied to demonstrate the unlike characteristic traits. Indeed, this was an effective tool to evaluate the developmental variability of an individual species as it represents the total population (Bergstrom & Reimchen, 2002). It serves as a significant mechanism to assess environmental pollutants that alter the species (Tomkins & Kotiaho, 2001). It is also identified as an efficient instrument for quantifying environmental conditions (Lecera et al., 2015). And a potential quantitative approach to assessing if the environment can provide ecological growth toward species (Angtuaco & Leyesa, 2004). In addition, GM was a simple and reliable means of identifying developmental instability (Ducos & Tabugo, 2015). It is widely known to describe indiscriminate nonconformities based on morphological traits (Swaddle, 2003). This application is widely recognized as it can deliberately identify the effects of several changes through species morphology (Jumawan et al., 2016). Furthermore, it is one of the most recognized scientific mechanisms because it can represent quantitative functions and analyze morphological shapes (Polly, 2012). This study utilized *Sardinella lemuru* a marine fish species called *Tamban* in the study area. A previous study was conducted by Luceño et al. (2014) employing the same species. The current study, however, would provide current information regarding the latter; as a result, this acts as the study's significance in examining the metric qualities of the fish samples. Therefore, this study used Geometric Morphometric to determine the differences in the body shape of both the male and female populations of *S. lemuru*.

Material and Method

Study Area

The study area was Barangay Caasinan, Cabadbaran City, Agusan Del Norte, Philippines (Figure 1). The fish collection was done in May 2023 with the aid of local fishermen utilizing motorized boats or bancas and gillnets as their catching gears.

Fish Collection, Processing and Sex Determination

Totally 70 adult fish samples consisting of 35 males and 35 females of the same size were randomly collected. The freshly caught samples were placed in an ice box and brought to the laboratory for further processing. Individually, the fish were sorted according to their size and positioned on the top of the Styrofoam. Each fish's fin was spread and pinned to make it wider and visible and applied with 10% formaldehyde using a small paintbrush. After this, the ruler was placed in the bottom portion of each sample to obtain the total length (Natividad et al., 2015). Lastly, the image of each sample was then captured using a digital camera. The sex of the samples was determined through internal examination by checking the genitalia. Females exhibited yellow to orange granular textures in the presence of ovaries. While, the testes of males, are smooth to white and have a non-granular texture (Requiron et al., 2012).

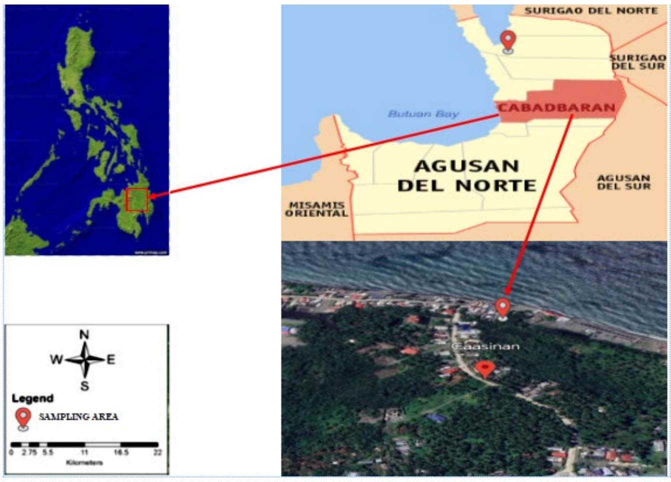

Figure 1. Map of the study area, Barangay Caasinan, Cabadbaran, Agusan Del Norte, Philippines

Landmark Selection and Digitation

The captured images were then categorized and sorted by sex. Then it was transferred and converted to a TPS file using the tpsUtil. The landmarking process of the samples was done through tpsDig2 (version 2, Rohlf, 2004). Sixteen (16) anatomical landmark points (Figure 2, Table 1) were utilized to digitize the samples of *S. lemuru.* To lessen the measurement error, the samples were tri-replicated. Its bilateral symmetry (left and right) was digitized using tpsDig2. The collected coordinates were then subjected to Symmetry and Asymmetry in Geometric Data (SAGE, version 1.04, Marquez, 2007) (Figure 3).

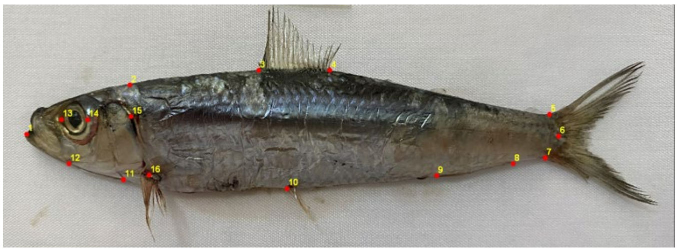

Figure 2. Digitized fish sample with sixteen anatomical landmarks

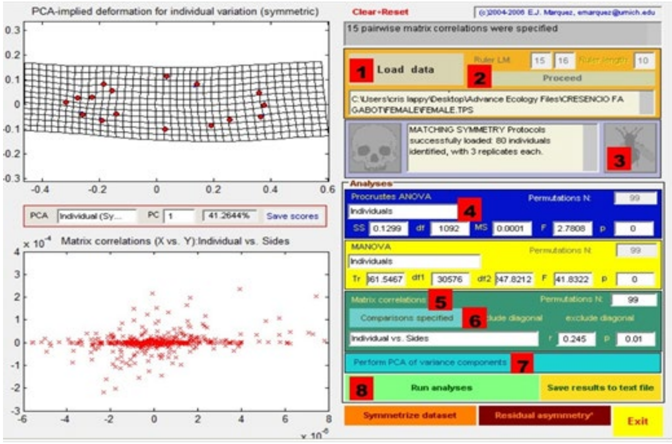

Figure 3. Symmetry and Asymmetry Geometric (SAGE) Data Software

Table 1. Description of the landmark points adapted from Paña et al. (2015)

Coordinates	Locations/Nomenclature
1	Snout tip
2	Posterior end of nuchal spine
3	Anterior insertion of dorsal fin
4	Posterior insertion of dorsal fin
5	Dorsal insertion of caudal fin
6	Midpoint or lateral line
7	Ventral insertion of caudal fin
8	Posterior insertion of anal fin
9	Anterior insertion of anal fin
10	Dorsal base of pelvic fin
11	Ventral end of lower jaw articulation
12	Posterior end of the premaxilla
13	Anterior margin through midline of orbit
14	Posterior margin through midline of orbit
15	Dorsal end of operculum
16	Dorsal base of pectoral fin

Shape Analysis and Data Generation

The Procrustes ANOVA test was applied to identify the significant difference in the symmetry of the three factors analyzed – individual, sides, and interaction of individuals and sides. The significant level was verified at $P<0.0001$. Along with this, the variances of its side and the estimation of directional asymmetry were also identified. The level of shape variations was specified through percentages (%) which were analyzed and compared between male and female samples (Natividad et al., 2015).

Results and Discussion

Table 2 shows the Procrustes ANOVA on the body shape of *Sardinella lemuru* for both the female and male sexes. Three parameters (individuals, sides, and individuals by sides) were evaluated to identify shape defects in the fish population. The analysis was applied to both female and male samples. Individual samples' left and right sides were also compared and examined. It was observed that highly significant differences (P<0.0001) occur in the individual fish of both sexes, resulting in body shape differences when one of the fish samples is compared to another. Additionally, its sides displayed a quite a substantial variance, indicating varying asymmetries on the left and right samples. The detected dissimilarities could be an indication that the species samples were under environmental stress in the area while others were associated with endo-parasites. Under typical circumstances, symmetrical appearances in fish species were anticipated. However, the poor water quality of the disturbed environment affected the morphological characteristics of the fish species during their development (Lytle & Poff, 2004). Thus, deformities developed by absorbing the environmental perturbations that ultimately changed an organism's developmental hemostasis and gave rise to diverse phenotypic traits (Parsons, 1990). Further, considering the scenario it includes a wide array of factors such as changes in temperature and length of the growing season. However, the differences were also related to other environmental issues like resource availability, and water velocity (Craig & Foote, 2001; Kishida et al., 2010).

Table 2. Procrustes ANOVA test for samples of *S. lemuru* in terms of sexes

Factors	SS	DF	MS	F	P-value
Female					
Individuals	0.1485	952	0.0001	2.5945	0.0001**
Sides	0.0302	28	0.0011	18.7075	0.0001**
Individual x Sides	0.0549	952	0.0001	7.0073	0.0001**
Measurement Error	0.0161	1960	0	--	--
Male					
Individuals	0.1553	952	0.0002	2.5775	0.0001**
Sides	0.026	28	0.0009	14.6622	0.0001**
Individual x Sides	0.0603	952	0.0001	5.5827	0.0001**
Measurement Error	0.0222	1960	0	--	--

Note: *Side = directional asymmetry; individual x sides interaction = fluctuating asymmetry; * P<0.0001 significant, ns – statistically insignificant (P>0.05); significance was tested with 99 permutations.*

Table 3. Principal Component Analysis of *S. lemuru* in terms of sexes

PCA	Individual	Sides	Interaction (FA)	Affected Landmarks
		Female		
PC1	51.60%	100%	49.51%	1, 12, 14, 15, 16
PC2	11.54%		11.54%	1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 13, 16
PC3	8.36%		7.58%	1, 2, 3, 4, 9, 10, 11, 12, 13, 14, 15, 16
PC4	6.50%		5.45%	8, 9, 10, 11, 13, 14
Total	78.00%		74.08%	
		Male		
PC1	53.06%	100%	45.72%	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 16
PC2	13.79%		14.38%	1, 3, 4, 5, 6, 8, 9, 10, 16
PC3	7.84%		10.85%	1, 4, 7, 8, 9, 10, 12, 15
PC4	5.06%		5.84%	1, 4, 9, 11
Total	79.75%		76.79%	

Note: Individual = individual samples, Sides= Left & Right, Interaction= Fluctuating Asymmetry.

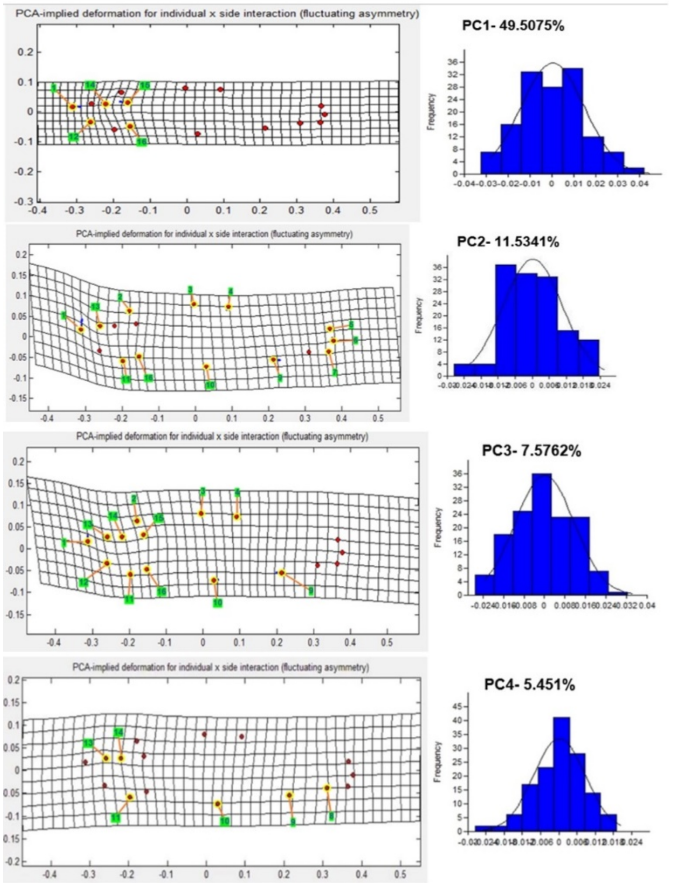

Figure 4. Principal Components (PC) implied deformation grid and a histogram of individual (symmetric) in *S. lemuru* female

Moreover, the observed differences in body shapes were associated with the influence of genetics and ecological factors i.e., feeding habits, prey and predator relationship, mobility, and the aging process (Cabuga et al., 2018). The study showed that female *S. lemuru* revealed a stout body outline compared to that of males which had a slender body shape. Evidently, female populations indicate larger abdomens which is linked to sexual maturation (Echem, 2016). Additionally, evidence in the body shape variations correlated with physiological traits such as growth, development, and reproductive stage (Parsons, 1987; Arendt & Wilson, 1999; Laugen et al., 2003; Salvanes et al., 2004; Conover et al., 2006; Kakioca, 2013). Previously, Thompson's work, which referred to the ideas of Galileo and Goethe on morphology and of Russell on functionalism, was the first to hypothesize that physical forces and transformations result in morphological space (Abzhanov, 2017). While the theory of morphology expresses that shape is a reflection of an organism to the ecology, evolution, and phylogenetic processes (Karr & James, 1975; Winemiller, 1991; Wainwright et al., 2002; Neige, 2003; Kerschbaumer & Sturmbauer, 2011; Price et al., 2011). Thus, measuring the distance and applying geometric morphological analysis (GMA) is the common technique for computing the degree of variation in shape, and the latter is the most forceful for depicting different visual patterns (Bookstein, 1991; James Rohlf & Marcus, 1993). Generally, numerous studies inferred that a fish with a more streamlined body shape exhibited maximum metabolic rates than a deep-bodied one within intra and interspecific levels (Petterson & Brönmark, 1999; Killen et al., 2016; Sánchez-González & Nicieza, 2022). These conditions were associated with lengthy swimming ability but the existing gap during these stages are shape and metabolism which may contribute to morphological differences (Latorre et al., 2020).

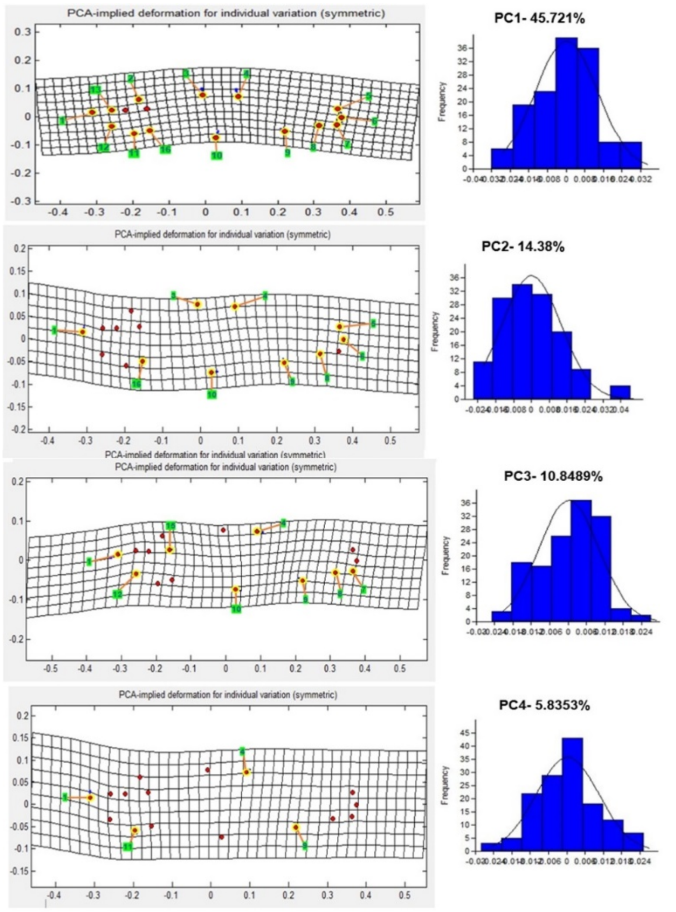

Figure 5. Principal components (PC) implied deformation grid and a histogram of individual (symmetric) in *S. lemuru* male

Using the symmetry and asymmetry scores, principal component analysis was applied to determine the Interaction (Fluctuating Asymmetry) and affected landmarks among the fish samples. Four principal components (PC) were considered in male and female samples (PC1-PC4). The four principal components (PC) accounted for 77.9544% of the total variation in female samples. PC1 accounted for 51.5961%, which has the highest variation. Unexpectedly, there were no commonly affected landmarks in female samples for the four principal component scores (Table 3). Subsequently, in male samples, the four PCs also constituted 79.7482% of the cumulative variations. PC1 contributed the highest accounted variation with 53.055%. The commonly affected landmarks in male samples for the four PC scores were landmarks 1, 4, and 9 (Table 3). These were a portion of the snout tip, posterior insertion of the dorsal fin, and anterior insertion of the anal fin.

Male *S. lemuru* were observed to reveal higher affected landmarks with 79.7482% from the upper 5% of composite principal components from PC1 to PC4. PC1 reveals that all affected landmarks were found to have greater asymmetry. This means that male phenotypic variability tends to be high under conditions of environmental stress (Parsons, 1987; Holloway et al., 1990; Hoffmann & Parsons, 1991). On the other hand, female *Sardinella lemuru* reveals none affected landmark than to males from PC1 to PC4 with a total of 77.9544%. It was interesting to note that the affected landmarks were only observed in males than in female samples of *S. lemuru*. These affected landmarks were further shown in the deformation grid, and the histogram of the values revealed skewness, suggesting asymmetry in body form (Figures 4 & 5). This shows the anatomical landmark points affecting the male *S. lemuru.*

Moreover, it was also observed that male samples have the highest Interaction or Fluctuating Asymmetry (FA) at 76.79% compared to female samples at 74.08%. This suggests shape variances among the sexes. The higher the FA the more altered the body shape which was seen in the histogram provided in Figure 6. In general, those affected landmarks among the male and female fish samples could be attributed to their mobility and interaction within the environment. Further, energy utilization as a source for swimming could affect the physical traits of the species. While, reduced locomotion can be a reason for gaining higher speed (Dabrowski, 1986; Khemis et al., 2013; Nemova, 2016). Study shows that affected anatomical regions were significant for body movement during swimming and requires high protein content and oxygen supply. Nonetheless, this constituted the development of the axial musculature and was connected to the increased swimming activity due to avoiding predation. Nevertheless, given the limits of geometric morphometric analysis, all indication implies that distinct growth in body shape elongation may be more noteworthy than the ontogenetic period (Martinez-Leiva et al., 2023). During this transition, the body shape of fish modifies to develop deeper and laterally compressed, which is more suitable for speedy swimming (Koumoundouros et al., 2009; Kourkouta et al., 2021; Downie et al., 2021). Thus, the importance of identifying shape dissimilarities within the fish species could be visualized through patterns. Finally, with the aid of Geometric Morphometrics, it is now possible to get precise information on how two unique species within populations differ from one another in terms of physical features.

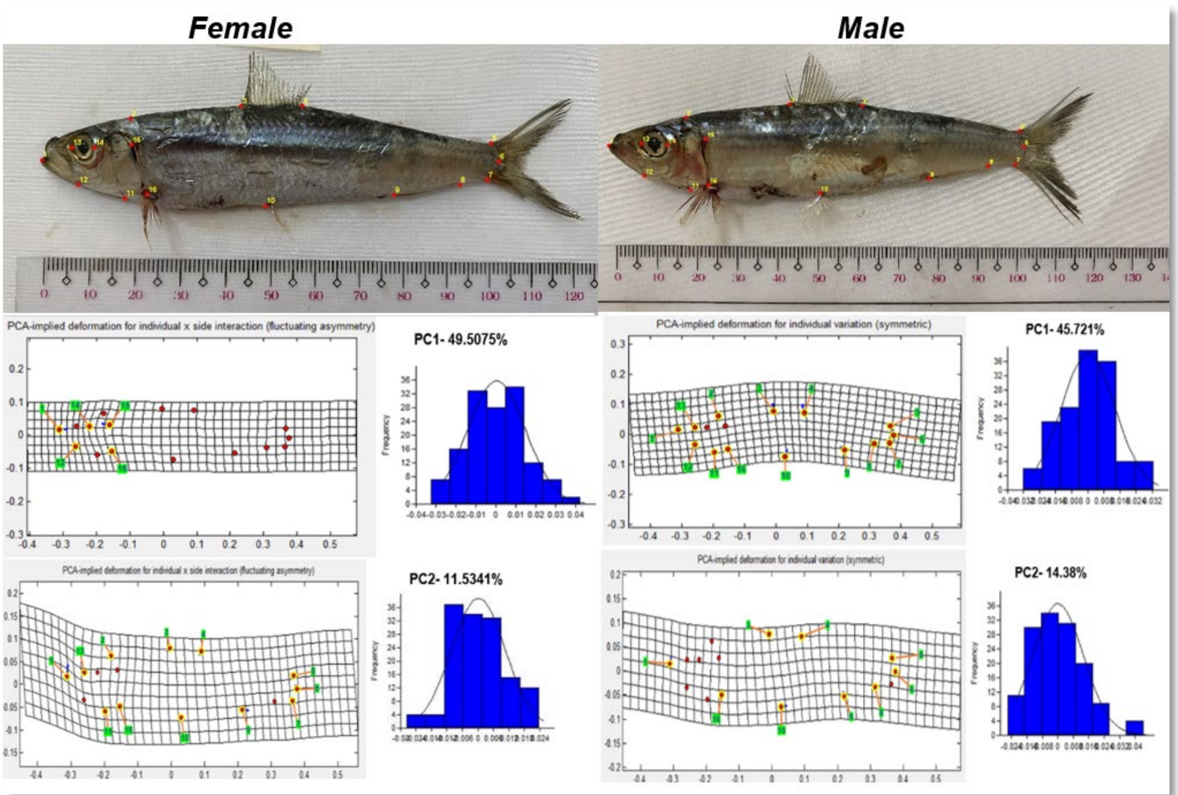

Figure 6. Actualized picture of digitized male and female fish with the affected landmarks shown in the PCA-deformation grid for PC1 and PC2

Conclusion

The present study has identified the intraspecific shape differentiation between the female and male *S. lemuru*. Procrustes ANOVA revealed a highly significant difference (P<0.0001), indicating morphological differences between sexes. While Principal Component Analysis shows that males exhibited a 76.69% rate of interaction (Fluctuating Asymmetry), which is higher compared to female with 74.07%. Further, several different anatomical landmarks points were affected among the fish samples. This suggests a disparity in the body shape that occurs among species of the same population. The implication suggests that phenotypic plasticity could play a significant role in the longevity of fishery resources. Evidently, using Symmetry and Asymmetry Geometric (SAGE) Data software applications enables one to draw vital information to understand shape variances within the same fish type and even more to numerous fish assemblages.

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Compliance With Ethical Standards

Authors' Contributions

CC: Designed the study and wrote the first draft of the manuscript.

JMP, RP, AJC, and PE: Performed the sampling and laboratory procedures.

MKA: Performed the Data Analysis

OLO: Wrote the Data Interpretation

All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

Data Availability Statement

All generated data and analysis are included in this published article. The generated data during the analysis are stipulated in the current study and available from the corresponding author on reasonable request.

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