



Geometric Morphometric Analysis of Shape Variation of *Sardinella lemuru*

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Abstract

Zamboanga City is noted as the sardines capital of the Philippines, the largest producer of canned and bottled sardines in the country. Many species of *Sardinella* species are distinguished according to specific body features. In this study, geometric morphometric analysis was used to generate shape variation of *Sardinella lemuru*. A total of 120 samples were digitized and sexes were determined by direct gonadal examination. Images were processed using geometric thin-plate spline grids (TPS), partial warps (PW) and relative warps (RW) software. The generated relative warp scores were subjected to statistical analysis using the PAST software. Results revealed that the shape variation (90.86%) in the female was found in the curvature of the body and length area between posterior insertion of the first dorsal fin and posterior most body extremity at RW 1. In the males, shape variation (48.24%) was found in the mid-section of the body at RW 1. The body shape variation of *Sardinella lemuru* can be accounted to their genetics and evolutionary adaptation. Over fishing could have contributed to their differentiation. The phenotypic plasticity of *Sardinella lemuru* allows them to respond and adapt to environmental change by modifying their morphology and behaviour which eventually lead to changes in their morphology, reproduction, or survival that alleviate the effects of such environmental change.

Keywords: Geometric Morphometrics, variations, gonads, landmarks.

Background of the study

Philippine sardine biodiversity is among the highest in the world and includes the only known freshwater sardine species (Willette et al., 2011). It has been observed that in areas in the Philippines where there is high landing of sardines, there is also a high rate of primary productivity suggesting that there are numerous suitable sardine supporting habitats in the country (Cordero et al., 2003).

Sardinella is a genus of fishes in the family Clupeidae. These fish are generally coastal, form into large schools and abundant in warmer waters. The diversity

of *Sardinella* in the Philippines is the third highest in the world. Whitehead (1985) listed that there are 21 species of *Sardinella* worldwide. In the Philippines there are eleven commercially important species of *Sardinella* which thrive in marine waters and *S. tawilis* thrive in freshwaters. There are several species reported in Zamboanga City including *S. albella*, *S. gibbosa*, *S. fimbriata*, and *S. lemuru*, species having the greatest mean annual landing, according to the National Stock Assessment Program.

Zamboanga is noted as the sardine capital of the Philippines, the largest producer of canned and bottled sardines in the country. To increase the population of sardines and herring in Zamboanga City, the Bureau of Fisheries and Aquatic Resources (BFAR) implemented a four month closed season for sardines ban from December 1, 2012 to March 1, 2013 to allow the sardine species to breed and spawn during the period and eventually increase in population significantly.

Many species including *Sardinella* species are distinguished by their locations but may also be distinguished according to specific body features. It is common for species within the genus to be mistaken for one another. Delineation of species within the *Sardinella* genus is mostly based on morpho-anatomical features. Identification based on morphology is somewhat difficult because these species have very similar appearances and partly because the taxonomy of the genus *Sardinella* is not well-understood (Whitehead, 1985).

This study examines and quantifies the body shape variation of *Sardinella lemuru* species using geometric morphometric analysis (GM), where it reveals differences in body shape. This is a modern method for size and shape analysis that address the potentially serious problems of more traditional approaches by focusing on data and methods that completely and efficiently archive the geometric information recorded

from the specimens in a sample (Rohlf and Marcus, 1993).

This study aims to determine and compare morphological variation in terms of body shapes of *Sardinella lemuru* based on geometric morphometric analysis. The outcome of the study will generate an accurate identification of *Sardinella lemuru* based on shape variation in morphology, which is important in management of the fishery and has great implications to biodiversity. It is also an important character for the analysis of fish evolution especially for the analysis of spatial variation among fish populations and for theories about adaptation to local environmental conditions (Nieves and Monteiro, 2003).

Methodology

The 120 sexually mature fishes were collected at two different fish landings in Zamboanga City, Western Mindanao, Philippines. There were 60 males and 60 females of *Sardinella lemuru* were collected. Adult specimens ranging in size from 240 mm to 300 mm were used.

The left side of the body of fish were measured and photograph. The images were captured using a Canon Powershot A2200 Digital Camera (14.1 megapixels). The images were transferred to computer software for digitization of the body shape. Digitization was done in each fish sample. (Figure 1).



Sardinella lemuru (Female)



Sardinella lemuru (Male)

Figure 1. Lateral images of the *Sardinella lemuru*.

Sexing was based by direct examination of the gonads after scanning. The resulting partial scores of the images were subjected to relative warp analysis, which quantifies body shape of the specimens. It also plots the landmarks in a three dimensional morpho-space warp grid where variations were shown as deformations of the grids. Images of different sex among selected existing species were separated for comparison of body shape between the sexes. The

PAST software (Paleontological Statistics Version 1.31) was used to test and visualize the shape differences of *S. lemuru*.

Landmarks were placed on significant points or features of the fish body for the acquisition of coordinate data that will be used to determine shape variations (Figure 2).



Figure 2. Landmarks' description of the *S. lemuru* with 10 landmarks as follows: (1) snouttip, (2) and (3), anterior and posterior insertion of the first dorsal fin,(4) and (6) dorsal and ventral region of the caudal peduncle, (5) posterior most body extremity, (7) and (8) insertion of the anal fin, (9) posterior extremity of premaxillar, and (10) centre of the eye.

Data Analysis

To determine morphological differences of *S. lemuru*, the data were subjected to Relative Warp Analysis. The data of the relative warp analysis were further confirmed using the scatter plot and Discriminant Function Analysis (DFA).

Results

The pattern of body shape variation within the population of the female *Sardinella lemuru* (Figure 3). The females show greater variation in the curvature of the body and in the distension in the mid-section of the body.

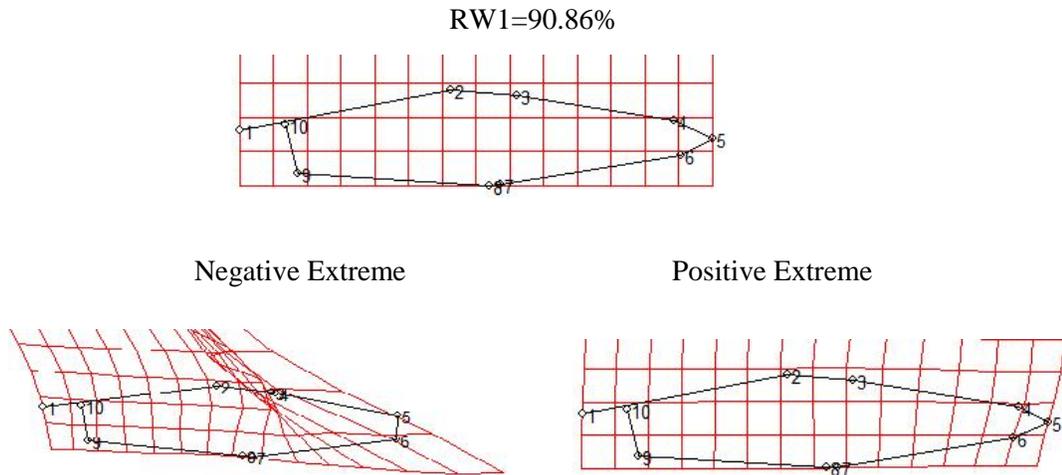


Figure 3. Mean shape of the female *Sardinella lemuru* with extreme positive and negative variation in shape.

The mean body shapes of the male populations of *S. lemuru* were projected and observed using the relative warp visualization plot. The transformation grids displaying shape changes from the mean to the extreme of RW 1 of the shape variation of the male

S. lemuru. In this population, the males show greater variation in the curvature of the body and variation in the distension of the mid-section of the body (Figure 4).

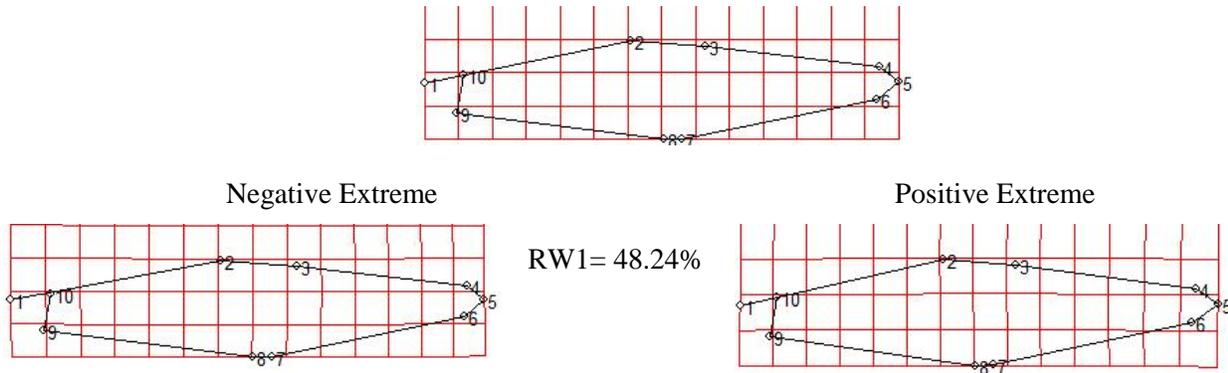


Figure 4. Shape variation of the male *Sardinella lemuru*.

Table 1 shows variation in the body shapes of *S. lemuru* of both male and female populations and the corresponding percentage variance. The shape variations (90.86%) were found in the curvature of the

body and posterior insertion of the first dorsal fin of the females. The shape variations (48.24%) were found in compression and decompression of the mid-section of the body.

Table 1. Variation in the body shapes of *S. lemuru* populations and the corresponding percentage variance.

RW	Female	RW	Male
1 90.86%	Shape variation in the curvature of the body and length area between posterior insertion of the first dorsal fin and posterior most body extremity. And change in the position of the dorsal region of the caudal peduncle in the negative extreme from the mean.	1 48.24%	Compression and decompression of the mid-section of the body. Negative extreme shows compression while positive extreme shows decompression which causes the body to be longer from the mean.
		2 18.89%	Variation in the length from the snout tip to posterior most body extremity and change in the caudal peduncle.
		3 9.63%	Body width changes, variation in the curvature of the body. And narrowing of caudal peduncle in the negative extreme.
		4 7.05%	Narrowing of the body width, change in the length from the origin to insertion of dorsal fin and from origin to insertion of anal fin.
		5 5.65%	Change in the length from the origin to insertion of dorsal fin and from origin to insertion of anal fin and change in the size of caudal peduncle.

The scatter plot of the relative warp scores of male and female *S. lemuru* body shapes. The relative position of the body shapes of *S. lemuru* at the x and y axis of the

scatter plot show variations exemplified by separation and overlapping among individuals of the male and female populations of *S. lemuru* (Figure 5).

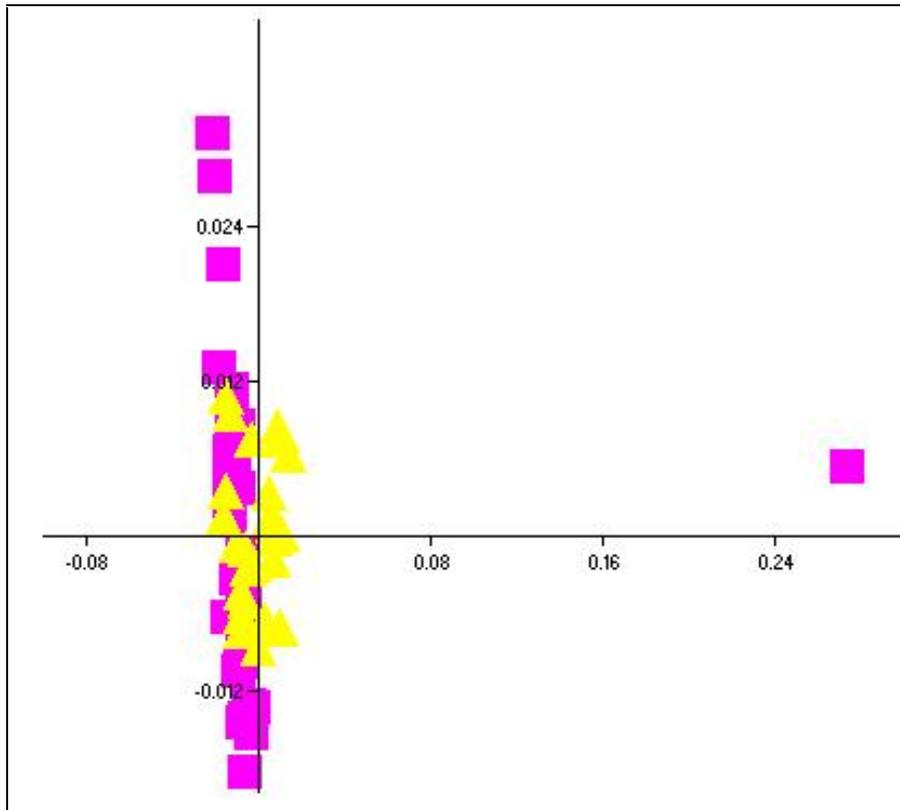


Figure 5. Scatterplot of the relative warp scores of male and female *S. lemuru* body shapes.

Figure 6 shows the histogram of the discrimination of body shape of *S. lemuru*. At 51.67% shape variations

are correctly classified and such variations were very minimal and not insignificant ($p = 0.051$).

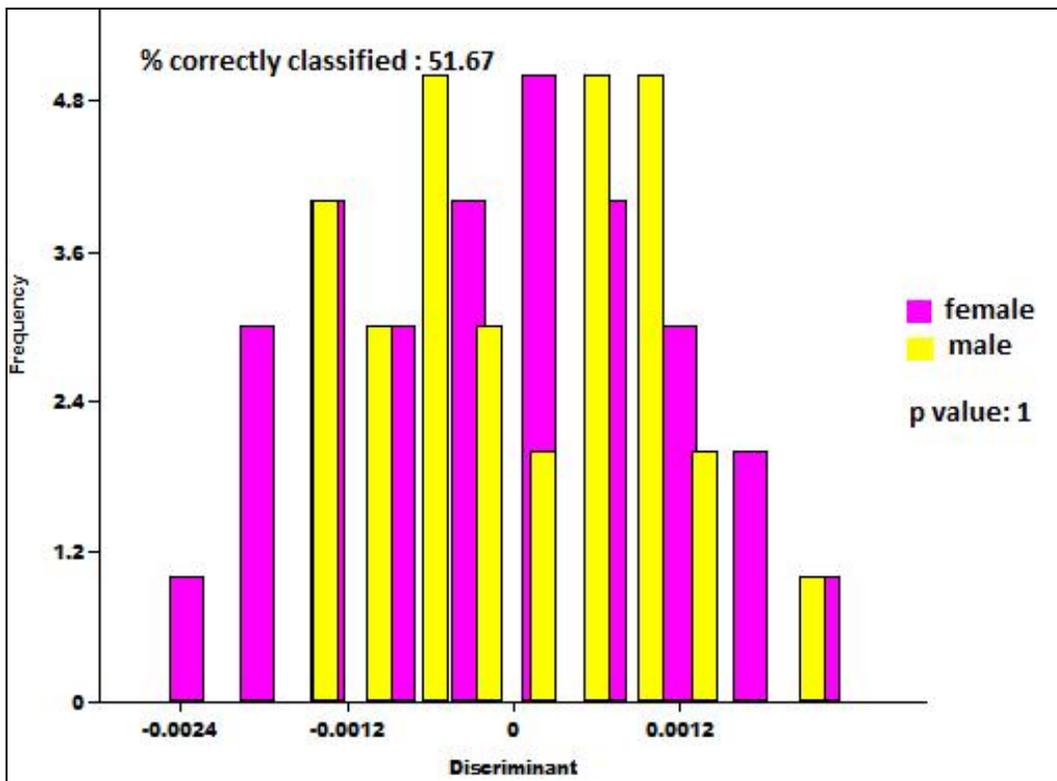


Figure 6. Histogram of the body shape of *S. lemuru*.

Discussion

The differences in body shape variation among male and female population *Sardinella lemuru* can be observed on the ordination plots of the relative warp. The females showed greater variation at the curvature and width of the body. The relative warp analysis showed shape variation in the curvature of the body and length area between posterior insertion of the first dorsal fin and posterior most body extremity. The males *S. lemuru* shows transformation grids displaying shape changes in the compression and decompression of the mid-section of the body.

Observable differences in body shapes among individuals of *S. lemuru* are further supported using scatterplot. It revealed variations as exemplified by separation and overlapping among individuals of the male and female populations of *S. lemuru*. This indicates that both males and females have more similarity in shapes. According to Requieron *et al.* (2010), the variations between sexes of *S. lemuru* were also observed based on differences in their abdominal region. The female *Sardinella* have stout body outline compared to that of males which have slender body shape. Females with larger abdomen might be due to sexual maturation.

Discriminant Function Analysis (DFA) was used to further emphasize the comparison between the differences of the body shapes of *S. lemuru*. It can be seen that the females and males overlap showing minimal overlap allowing separation of the two sexes. Thus, it suggests that there are differences between the two sexes which can be attributed to sexual dimorphism. The overlapping and separation of population showing body shape variation can be accounted to their genetics, evolutionary adaptation and response to its surrounding habitat. According to Luceño *et al.* (2013), one example of selection pressure that could have contributed to their differentiation is overfishing/overexploitation since *Sardinella* is one of those fishes in the Philippines reported to be under heavy fishing pressure. Consequently, the phenotypic plasticity of fish thereby allows them to respond and adapt to environmental change by modifying their morphology and behaviour which eventually lead to changes in their morphology, reproduction, or survival that alleviate the effects of such environmental change.

Body shape in fishes is attributed to a variety of environmental influences, including temperature (Martin, 1949; Beacham, 1990; Loy *et al.*, 1996), water velocity (Imre *et al.*, 2002), and quantity of food

(Currens *et al.*, 1989) and type of food or feeding mode (e.g. Meyer, 1987; Wimberger, 1992; Day *et al.*, 1994; Robinson & Wilson, 1995).

Demonstrations of variation have been in physiological traits such as growth rate (e.g. Conover & Present, 1990; Parsons, 1997; Arendt & Wilson, 1999; Trussell, 2002; Salvanes *et al.*, 2004), developmental rate (Berven *et al.*, 1979; Laugen *et al.*, 2003), and reproductive rate (Kokita, 2004). Differences in body shapes have evolved to compensate for environmental effects, inducing phenotypic variation within geographic range. Most of these cases involve range gradients in temperature and length of the growing season, though variation has also been demonstrated for other environmental factors such as resource availability (Craig & Foote, 2001) and water velocity (Trussell, 2002).

Recent studies on shape variation on fish have reported that variation in morphological characters such as body shape have resulted to genetic effects and plastic responses which reinforces each other (e.g. Day *et al.*, 1994; Robinson & Wilson, 1996; Parsons, 1997). In difference in body shape (phenotypic character) are developed in favoured in the different environments, and both genetic divergence and adaptive phenotypic responses have evolved in response to contrasting selection pressures. Such pressure is associated to overfishing, coastal run-off and discharges, oceanographic effect brought by climate change (Reid *et al.*, 2010).

However, variation in morphological traits is still thought to be predominantly not well understood (e.g. Parsons 1997; Trussell 2002). This may be because environmental effects on traits like body shape are less well known than the effects of factors like temperature on rates of physiological processes, so that phenotypic similarity in body shape between areas or populations continues to attract little attention. However, given the demonstrated influences of the environment on body shape in fishes, phenotypic similarity between populations occurring in different habitats is more likely to reflect genetic differentiation than genetic homogeneity. Many marine fishes occur across a broad range of distribution (and thus environments) but fail to show the striking phenotypic diversity that is evident in many freshwater fishes. Marcil *et al.*, (2006) suggested that much of the genetic diversity in these widespread marine fishes may be overlooked, with stabilizing selection for the same optimal phenotype resulting in genetic differentiation to counteract the environmental differences between populations.

Conclusion

The findings of this study suggest that although there are notable pattern of morphological variations between sexes *Sardinella lemuru* as suggested by the landmark-based geometric morphometrics and multivariate analysis tools, however, such variation is not statistically significant. Most of the variation is mainly due to differences in body width, compression/decompression in the mid-section of the body, on the curvature of the body, change in the dorsal fin length, change in caudal peduncle, and change in the snout region. And that the variation of body shapes of male and female *Sardinella lemuru* can be attributed to the population's response to their present environment and could be accounted to their genetics and evolutionary adaptation.

Recommendation

Aside from landmarks, other characters should also be considered such as scale morphology, body coloration pattern, and internal landmarks. Furthermore, assurance that prospective researchers should undergo thorough training on geometric morphometric.

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