BODY SHAPE AND LENGTH-WEIGHT RELATIONSHIP OF VERMICULATED SPINEFOOT *SIGANUS VERMICULATUS* COLLECTED FROM THE MORO GULF, PHILIPPINE SEA AND PUJADA BAY OF MINDANAO, PHILIPPINES

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ABSTRACT: *The vermiculated spinefoot fish, Siganus vermiculatus is a species of Siganus that has been given importance in the field of fisheries and aquaculture for its premium meat and high market potential thus, an evaluations of body shape and length-weights of fish populations are important which could be of prime importance in aquaculture. This study explore the use of geometric morphometrics, a new tool in describing body shape variations and, length-weight relationship within and between sexes of the populations of the fish. Populations of the fish were collected from five different bays around Mindanao Island, Philippines.*

The use of geometric morphometrics in the analysis of body shape of S. vermiculatus made use of a total of 27 landmarks analyzed on Thin Plate Spline (tps) software. Results revealed that the inter-and intraspecific body shape variations did not show any significant difference and no sexual dimorphism between sexes (p value=1) Generally, the body shapes of the fish species slightly vary between sexes, the females of S. vermiculatus have bigger mouth, eyes and body depth are deeper compared to the male population. The heads of the females are dorsally located; however, the caudal peduncle of the male population is located dorsally than those of the female S. vermiculatus. S. vermiculatus are found to be size-shape dependent in both sexes. Smaller S. vermiculatus exhibit a deeper body depth and smaller head length however, as it grows larger in length, the body became elongated and having a slender and larger head length. This study on body shape of S, vermiculatus have important fitness consequences. The observed shape variations within and between the three populations collected from different bays may reflect significant differences in the ability of the fish to successfully occupy a particular habitat which the different bays represented. The results also imply that body shape can also be considered an important factor in fisheries and aquaculture.

Keywords: geometric morphometrics; sexual dimorphism; aquaculture; *S. vermiculatus*

INTRODUCTION

The species *S. vermiculatus* command a very high price and have long been considered as a good candidate for aquaculture aside from *S. guttatus* [1]. Observations on growth rates in nature and in captivity revealed that *S. vermiculatus* is a fast grower when compared to other siganid species [2].

S. vermiculatus has a vermiculated pattern in its head and body with a brownish color found on the darker areas and bluish above grading and silvery below on paler areas. The darker lines are much broader than paler ones on nape and head, about equal in breadth posteriorly with a golden flush on its head and blue lines on cheeks. Dark lines are found breaking into spots on caudal fin with four spots arranged in vertical rows. Dark spots also present on soft parts of dorsal and anal fins, arranged in rows, with a prominent proximal rows; other parts of median fins dusky; pectoral fins hyaline; pelvic fins dusky and golden yellow (Fig. 1). A distinct change in color pattern was noticed during pre-spawning and spawning behavior [2]. In both males and females the vermiculated pattern nearly disappeared and was replaced by a wide horizontal dark stripe which divided the body into two equal brighter sections. Moreover, the vermiculated pattern becomes more complex and intricate with size and age.

Siganids are highly specialized in terms of anatomy and feeding habits and it is therefore surprising that they occupy a remarkable wide diversity of shallow water habitats, including coral reefs, sandy and rocky bottom with or without vegetation, lagoons, river mouths and mangrove swamps [2]. The juveniles of *S. vermiculatus* live among mangroves then move out to lagoon and coastal reefs as they mature, it forms schools and feeds on algae growing on seagrasses, mangrove roots and rocks [3]. Adult fish >120mm long were observed in a variety of habitats, mainly in mangrove swamps and on coral reefs [2]. The members are almost all marine except *S. vermiculatus* which occurs in small schools in and about river mouths and brackish lagoons, as this species can tolerate very low salinities (2 ppt) and temperature up to 38^0C. These observations clearly indicate adaptation and genetic diversity.

There are several ways of studying diversity in organisms. Morphological, cytological, biochemical and molecular tools are commonly used. Describing morphological diversity was generally based on traditional means of qualitative descriptions. However, with advances in computer science,

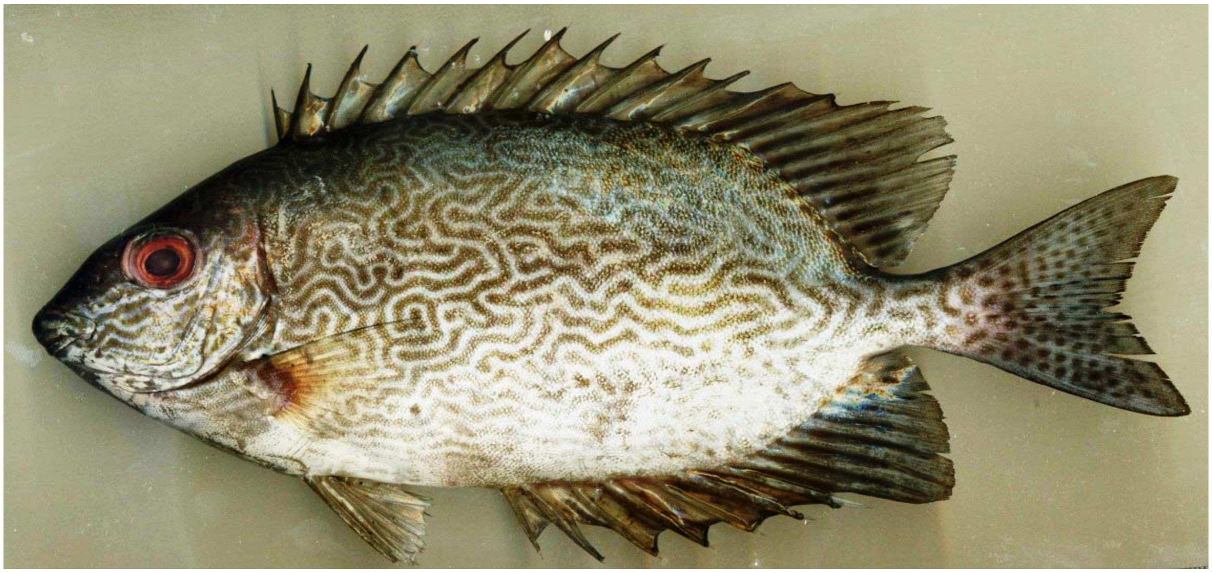

Figure 1. Adult *S. vermiculatus*.

statistics, geometry and imaging tools, descriptions have become more quantitatively done. One of the most popularly investigated is the body shape. The body shape in fishes has been a subject of research investigations for over a century [4] as it is an important character for the analysis of fish evolution as it influences locomotion efficiency in different environments, particularly foraging and predator evasion [5]. The use of geometric morphometry on the study of shape has caused a revolution on the biometric analysis thus was used in this study to describe adult *S. vermiculatus* collected from different coastal bays. Shape analysis is a fundamental part of much biological research and is 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 [5].

Efforts have already been made to culture *S. vermiculatus* in the field and in captivity that are already been proven successful [7]-[9]. The characteristics of *S. vermiculatus* as fast grower [2], easily bred and have good market value [7]-[9]; and can tolerate extreme physico-chemical parameters made it a desirable candidate for aquaculture. In addition, *S. vermiculatus* has been one of the major fish culture commodities in the tropics. The environment plays an important role affecting the habit and other important ecological traits such as feeding efficiency, locomotor performance, vulnerability to predators and reproductive success. It is also reported to directly influence the organisms' phenotype [10]. Thus, the study of morphological variations of *S. vermiculatus* can help determine species adaptation and fitness involving a geographical location.

MATERIALS AND METHODS

Sample Collection

Fish samples were collected from the three (3) different coastal bays in Mindanao (Fig. 2). Samples were brought to the laboratory for sex determination and examination of shape variation.

Individual *S. vermiculatus* was weighed, measured and pinned to a dissecting pan. Fins were then treated with 5% formalin for stiffening ready for image scanning using (Hp G2410 flatbed scanner, with an optical resolution of 1200 dpi). The digitization of lateral left and right side images of *S. vermiculatus* were taken for geometric morphometric analysis. The images of the scanned samples were analyzed on a computer using TPSdig (version 2.04) [11].

Following the captured image, sexes of *S. vermiculatus* were determined through direct examination of the gonads through dissection using a reference [12]. The gonads were visually determined by picking them with forceps in the fish internal body.

Landmark Selection

The landmarks were assigned on prominent features of the fish body, which are precise locations on biological forms that hold some developmental, functional, structural, or evolutionary significance. The landmark locations are recorded as two- or three-dimensional coordinates resulting in spatial map of the relative location of the chosen points [13]. These landmarks were then assigned to homologous structures found on the lateral orientation of the fish. This is to ensure consistency in the number of landmarks since they are variables in the statistical analyses.

The digitization of landmark points was in tri replicates on both lateral sides for each fish sample using the TpsDig ver.2.10 [11]. A total of 27 morphological landmarks were used to capture the shape of each individual using the TPSdig (Fig. 3). The images of *S. vermiculatus* populations from Moro Gulf, Philippine Sea and Pujada Bay were separated. This was done to produce a consensus configuration per sex relative to their body shape that would serve as basis for comparison between the sexes of each population. In able to determine the shape variability of each population, the images between the two sexes were pooled into one analysis.

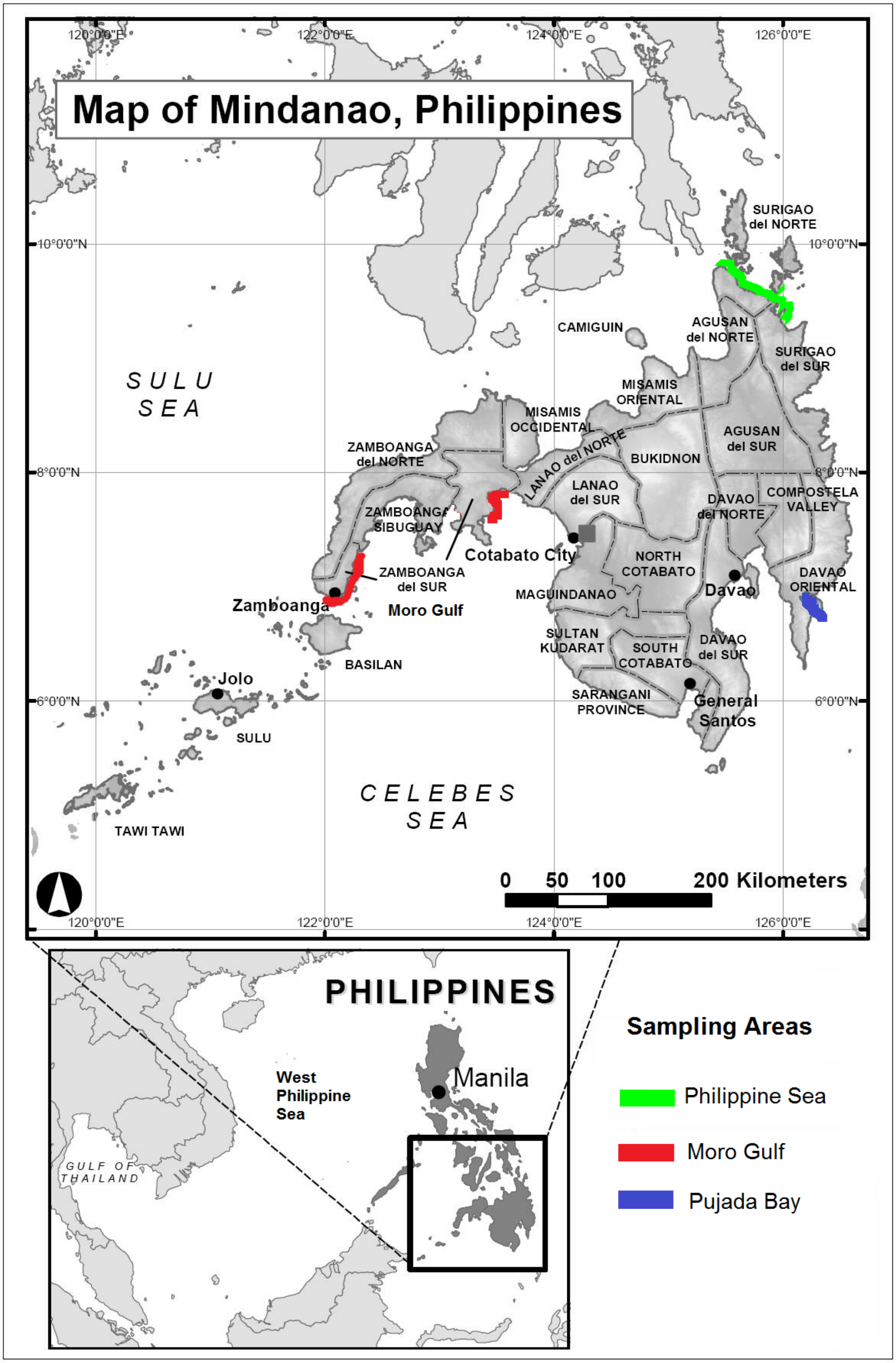

Figure 2. Map of Mindanao Island, Philippines showing the sampling areas.

Sample Preparation for Imaging and Sex Determination

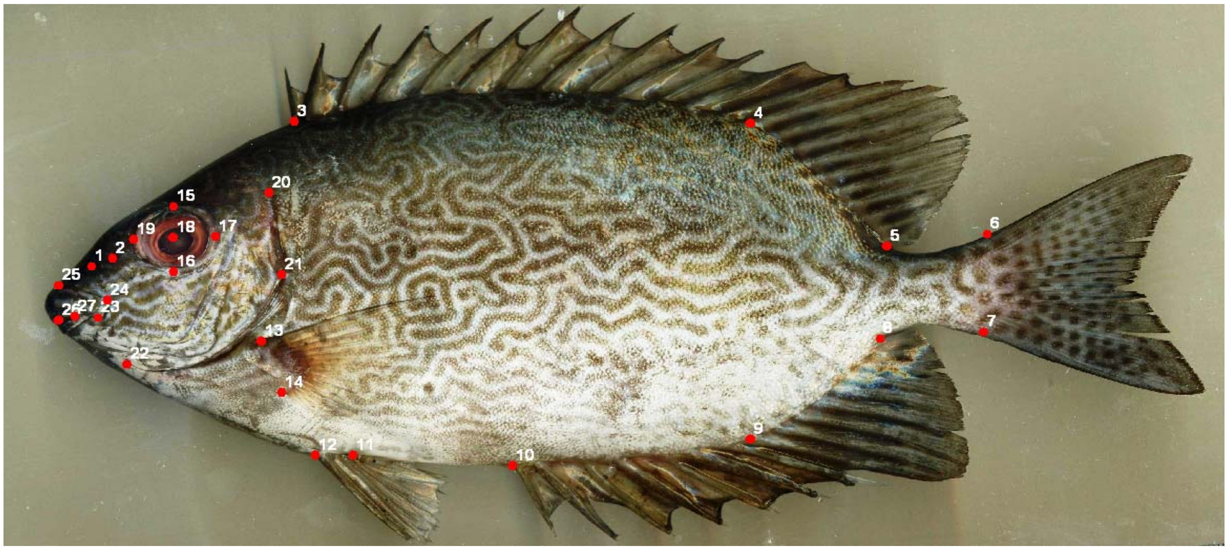

Figure 3.The assignment of landmarks to the lateral orientation of *Siganus vermiculatus*. (1 &2) anterior and posterior nostril, (3) Origin of the dorsal fin, (4) First base of the dorsal ray, (5) Insertion of the dorsal fin, (6& 7) 1st Dorsal and ventral ray of the caudal fin, (8) Insertion of the anal fin, (9) Base of the 1st anal fin ray, (10) Origin of the anal fin, (11 & 12) Insertion and origin of the pelvic fin, (13 &14) Origin and Insertion of the pectoral fin, (15 &16) Superior and Inferior margin of the orbit through the midline of the eye, (17) Posterior margin of the orbit through the midline of the eye, (18) Middle of the eye, (19) Anterior margin of the orbit through the midline of the eye, (20, 21 &22) Dorso-lateral angle, posterior margin and ventral conjunction of the operculum, (23) Junction between the posterior end of the maxilla, (24, 25, 26 and 27) Posterior end, superior margin, inferior margin and mid-inferior margin of the maxilla.

Landmark Method for Shape Analysis

Geometric morphometrics is considered to be one of the most powerful tools in biological shape analysis. It is a fusion of geometry and biology, morphometrics deals with the study of form in two- or three- dimensional space [13].

The x and y coordinates of the landmark points that were digitized from the left and right images of the fishes was generated using image analysis and processing software (tpsDig ver.2.10) [11]. The non- shape components were removed prior to analysis via Generalized Procrustes Analysis (GPA) using TpsRelw version1.36 [14]. The GPA were aligned in all the specimens in morpho- space, eliminating size and rotational/translational differences [15]. The fish landmarks were reflected on one side of the body to their mirror image to align corresponding landmarks on both sides. Configurations on the lateral left and right sides were then scaled to achieve the same unit centroid size. Procrustes Superimposition was then process to generate coordinate data, which involves the arrangement of landmark data from two forms into the same coordinate space. This is done by shifting the left and right centroids to a common set of x and y coordinates [16].

After subjecting the landmark coordinate to procrustes fitting, the shape residuals was subjected to geometric morphometric analyses such as Thin-plate Splines (tps) [17]. Using the thin- plate spline equation and the standard formula for uniform shape components, a weight matrix (containing uniform and non- uniform shape components) from the aligned specimens were generated [15]. The differences in body shapes were then examined via relative warp (RW) analysis of the set of uniform and non-uniform components of shape using tpsRelw 1.36 [14]. The mean shape differences amongst area were evaluated using, TpsSplin and Cluster Analysis.

Relative warps generated for both male and female sexes were subjected to Discriminant Analysis (DA)/Hotelling's Test using the Microsoft Excel and PAST (Paleontological Statistics) version 2 softwares [18] were used to test for sexual dimorphism. TPS Partial Least Squares (tpspls) analysis was also applied to determine the size-shape sexual dimorphism on male and female *S. vermiculatus*.

RESULTS AND DISCUSSION

General observations in *S. vermiculatus* revealed no variations in color patterns between sexes, however, it was observed that smaller sized samples (≤120mm) displayed more intricate vermiculated patterns with darker lines and golden yellowish color on the dorsal portion of the head and body and whitish grey background shade on the ventral part of the body. The bigger size samples (>200 mm long) had golden yellowish background and brown with silvery bluish black. These observations were similarly noted describing that the vermiculated pattern of *S. vermiculatus* becomes more complex and intricate with size and age and a distinct change in the color pattern was then noticed during the pre-spawning and spawning behavior in both sexes [2].

Variability in Weight, Length and Carcass of *S. Vermiculatus* from Different Bays

Based on weight, length and carcass of the fish collected from the three bays, the samples collected from the Moro Gulf were generally smaller in terms of weight, length and carcass area (Fig. 4). *S. vermiculatus* that were bigger in length, weight and in carcass area were consistently observed on Philippine Sea (female population) and Pujada.

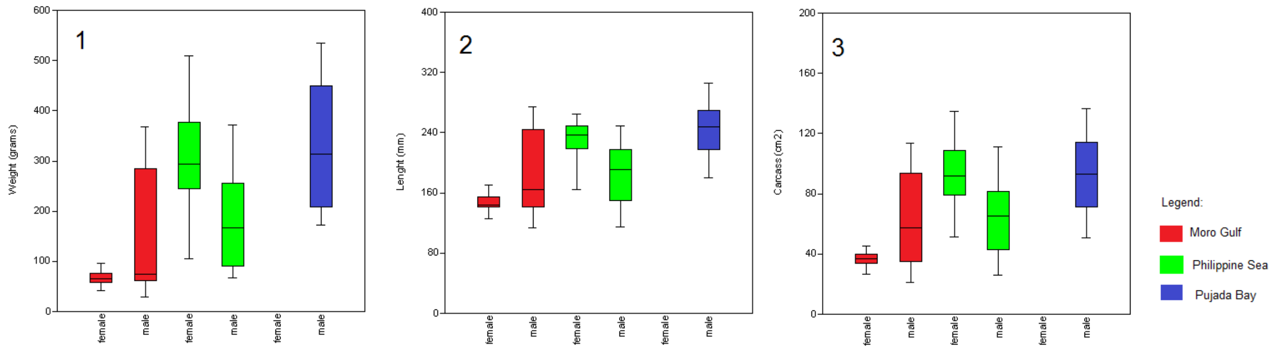

Figure 4. Box plot of (1) weight (grms.), (2) length (mm) and (3) carcass (cm^2) of female and male *S. vermiculatus* population.

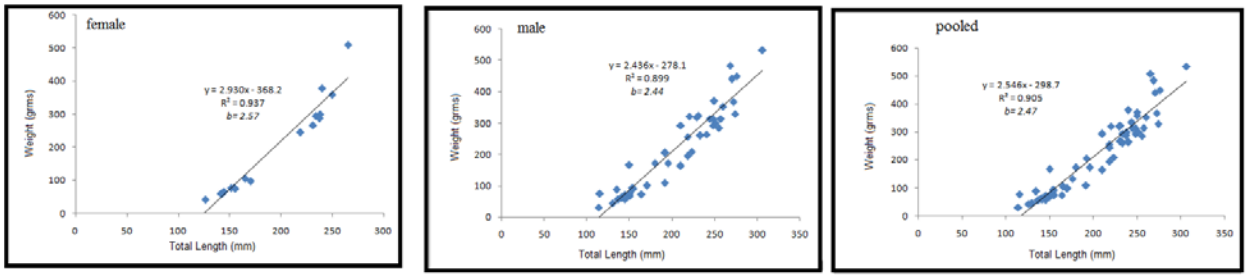

Figure 5. Length-Weight relationship of female (A), male (B) and pooled (C) *S. vermiculatus* population.

Bay (male population). On the other hand, the male samples from Pujada Bay cannot be compared to female samples in the area since there were no female samples collected to represent in the study. Based on length- weight relationship, *b* values which indicates growth patterns of *S. vermiculatus* gives a negative allometric growth for both sexes (Fig. 5). The regression (r^2) values from the length- weight relationship of *S. vermiculatus* among the male and female population ranged from 0.89 and 0.94, which indicated high and very high relationship, respectively.

From a series of all samples collected, results from the box plot analysis on weight, length and and carcass area of *S. vermiculatus* (Figure 6) had shown consistency that male and female samples from Moro Gulf were smaller compared to the samples collected from Philippine Sea and Pujada Bay, it suggests that male populations were larger than female population. However, this was not the case when samples were examined per area, between sexes. The sample specimen collected in Philippine Sea reveals that bigger in length and weight belongs to the female population. While samples from Moro Gulf however, had smaller in length had deeper body depth in both sexes. Sample specimen of *S. vermiculatus* from Pujada bay showed biggest and heaviest compared to the other two areas, only that female samples were not quantified because of the absence of female samples during the time of collection. It was observed that the *"b"* values obtained in these species exhibit negative allometric growth in both sexes. This indicates that weight grows slowly relative to the length of *S. vermiculatus,* and may also indicate on the relative health and condition of the fish [19]. However, this present study slightly differs from the results obtained which gave a positive allometric growth [2]. Deviation in growth pattern may be due to variations in ecology of the geographical locations, food availability and different environmental conditions [20].

Body Shape Variation

Thin-plate splines generated the mean shapes of the body of the fish (Fig. 6, 7). Variability between the head and the body was observed between sexes. The females of *S. vermiculatus* have bigger mouth, eyes and the body depth is deeper compared to the males. The caudal peduncle of the male is located more dorsally than those of the female *S. vermiculatus*. The variation among the head especially on the functional traits (eyes and mouth) of *S. vermiculatus* suggests differences in the physical characteristics of the environment [21].

The cluster analysis in body shapes of *S. vermiculatus* samples per area are shown in Figure 8 (lateral left side) and Figure 9 (lateral right side) generated using the TPS spline. The figures of the left side body shape relationship, shows that Moro Gulf and Philippine Sea *S. vermiculatus* specimens are more similar in terms of body shape than to those *S. vermiculatus* from Pujada Bay The same pattern was observed on the lateral right side image of *S. vermiculatus* shown in Figure 9. The displacements of 20 landmarks observed in Moro Gulf were detected on the nostrils (2 & 3), dorsal fin and insertion of dorsal rays (3,4&5) caudal fin (6 &7), anal fin (10), pelvic fin (11 & 12), pectoral fin (13 &14), operculum (20,21 &22) and mouth (23, 24, 25, 26 &27). Generally, the body shape of *S. vermiculatus* in this area was observed to have slightly

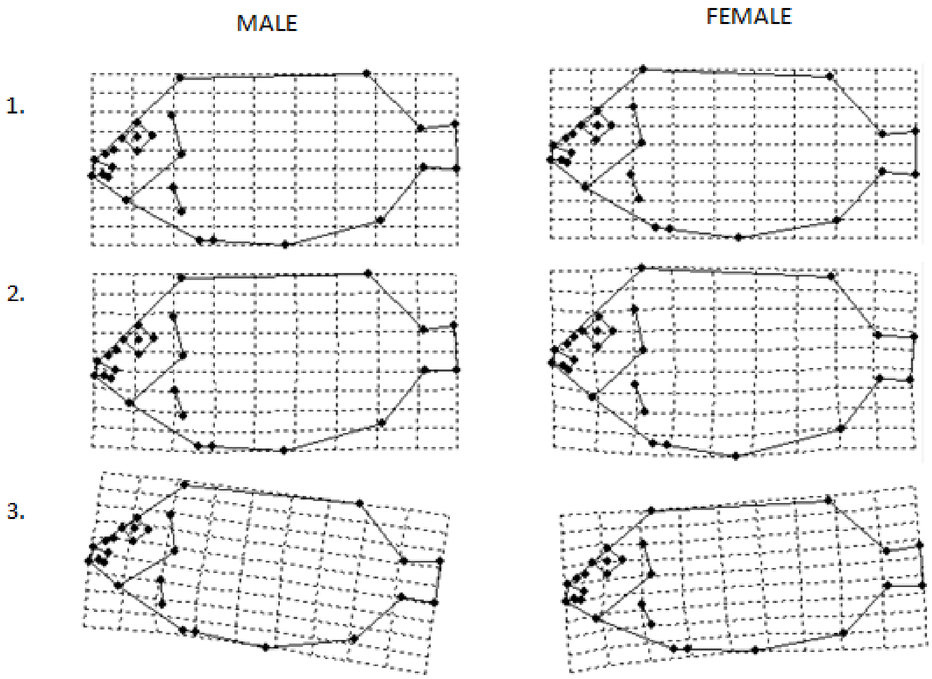

Figure 6. Mean Body Shape of Male and Female *S. vermiculatus* population from Philippine Sea (1), Moro Gulf (2), and Pujada Bay(3).

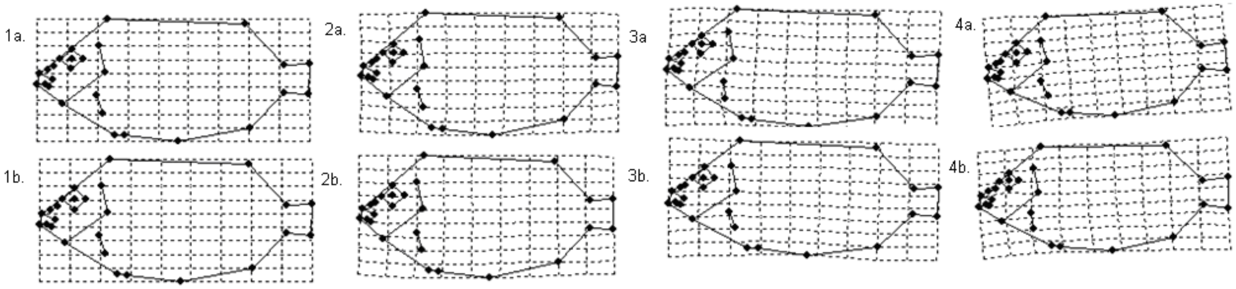

Figure 7. Mean body shapes showing the lateral left (a) and right (b) sides of *S. vermiculatus* population. (1) mean body shape, (2) Moro Gulf, (3) Philippine Sea and (4) Pujada Bay.

bigger head, mouth and a slight constriction of the caudal region. Also, the body depth is deeper and has shorter body length compared to the overall mean body shape, Philippine Sea and Pujada Bay *S. vermiculatus* samples as shown on Figure 8. Moreover, *S. vermiculatus* in Philippine Sea has 25 landmark points moved except for the dorso-lateral angle of the operculum (20) and posterior margin of the operculum (21) which remains stable. Specimen collected in the area shows a larger body length compared to Moro Gulf specimens; an enlargement of the eyes, mouth and nostrils was observed. The body and caudal region is slender compared to Moro Gulf specimens and a lot more similar to the pooled left mean body shape. The lateral left pooled mean shape image of Pujada Bay has 26 landmark points that obviously changed in position except to the posterior margin of the operculum (21). This makes the body shapes of *S. vermiculatus* in Pujada Bay more elongated. The head is smaller which follows a smaller eyes and mouth compared to Philippine Sea Moro Gulf and the overall mean body shape.

The landmark displacements of the lateral right side image of *S. vermiculatus* were observed on Figure 9. An obvious landmark displacement found in Moro Gulf samples was on the pelvic fins (11 & 12) and pectoral fin (13 &14). The fish specimens collected in the area have smaller body length however, deeper body depth, caudal peduncle is shorter and mouth is similar to the total mean (right) shape. The Philippine Sea specimens have 14) landmark displacements,that are mostly on the body region. The displacements of landmarks (5, 6, 7 &8) positioned the caudal region ventrally. Landmarks that are located on the dorsal region (3 & 4) and those on the ventral region (landmarks 9, 10, 11 & 12) moved towards the center of the body, pectoral fins landmarks (13 &14) were also positioned dorsally. The following displacements of the landmarks

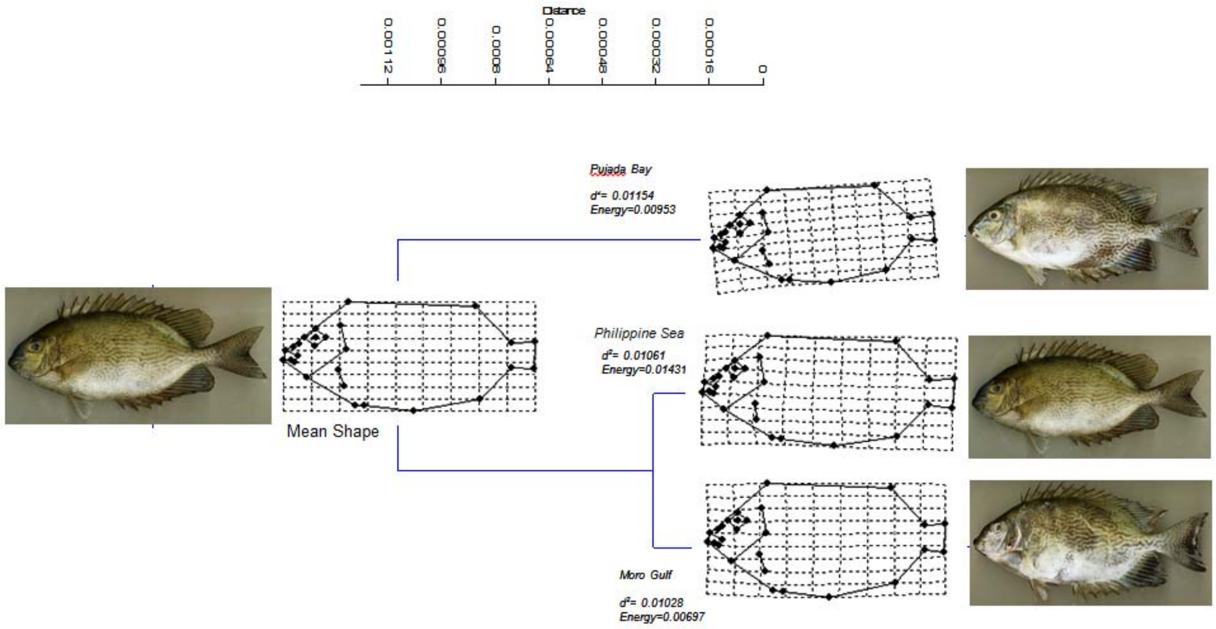

Figure 8. Cluster analysis of mean shapes of lateral left side among *S. vermiculatus* population from Mindanao Is., Philippines.

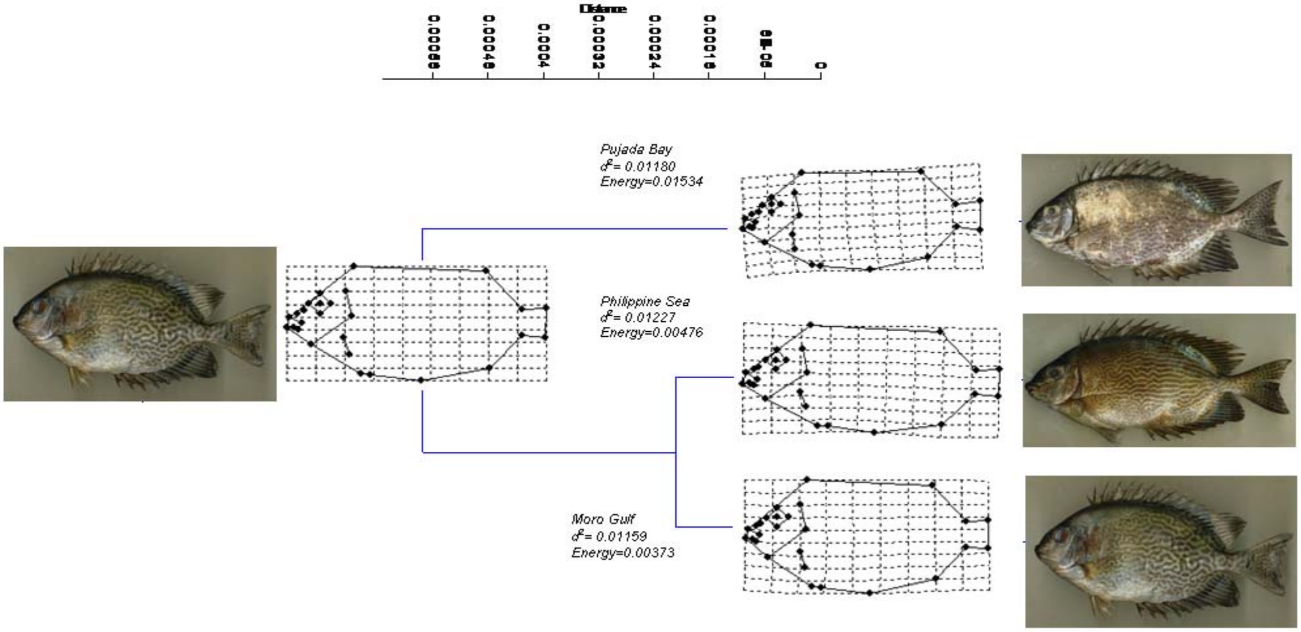

Figure 9. Cluster analysis of mean shapes of lateral right side among *S. vermiculatus* population from Mindanao Is., Philippines.

makes the bodies of *S. vermiculatus* appear elongated than those from Moro Gulf but not as elongated with the *S. vermiculatus* from Pujada Bay. The *S. vermiculatus* specimens from Pujada Bay appears to have smaller head, all landmark points found in the head moved ventrally and making the eyes, mouth and nostril constrict. The caudal region is wider compared to Philippine Sea and Moro Gulf and the bodies are more elongated and longer than those *S. vermiculatus* found in Moro ⌒ lf.

The size- shape relationship of *S. vermiculatus* of female and male population were shown on Figures 10 and 11 using the TPS partial least squares (TPSpls). In this analysis, the centroid size of individual fish specimen was used, which is a useful measure of overall size of a landmark configuration [22]. Both sexes are observed to be size-shape dependent with high to very high regression values for female (r= 0.88, left and r=0.89, right) and male (r=0.74, left and r= 0.79, right). Generally, and age [2].

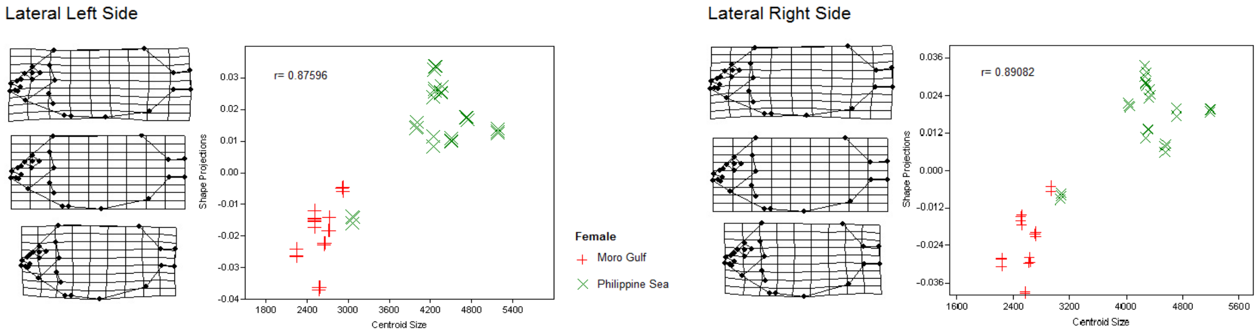

Figure 10. Relationship of shape projection and centroid size of female *S. vermiculatus* collected from the sampling areas.

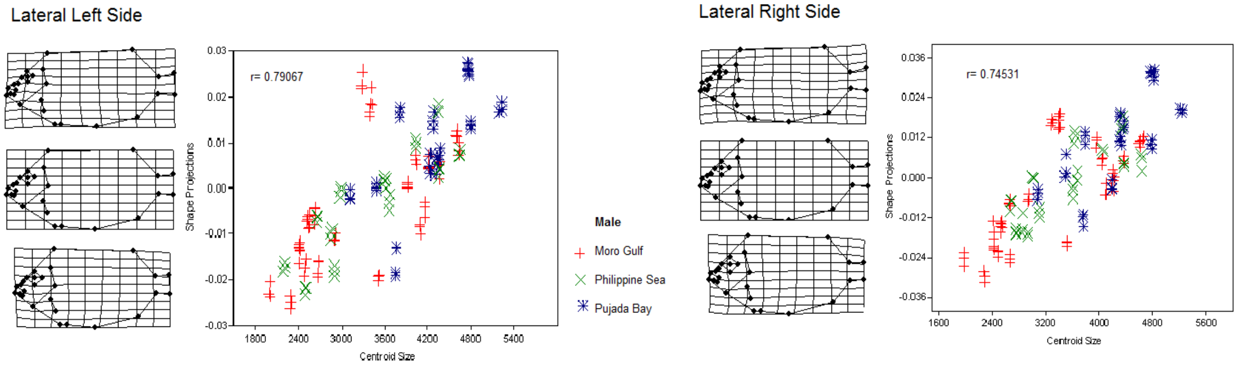

Figure 11. Relationship of shape projection and centroid size of male *S. vermiculatus* collected from the sampling areas.

the figure explains that the body shape in relation to size do not vary between sexes. Smaller *S. vermiculatus* exhibit a deeper body depth and smaller head length however, as it grows larger in length, the body became elongated and having a slender and larger head length. Also, the distance between the upper and the lower of the caudal fin (landmark 6 and 7) widens as it grows in size. The vermiculation patterns and coloration of the fish species between sexes also does not follow a pattern into which distinguishes a female from a male. Only that the vermiculated pattern becomes more complex and intricate with size

It was believed that the differences in the reproductive roles between sexes (sexual dimorphism) may influence patterns of selection and could lead to sex differences in morphological attributes such as the shape of its body [23]. It was argued that lack of significant sexual differences in siganids makes the interpretation of body condition factors difficult [24] [25]. Interestingly, using the 27 landmarks, the discriminant analysis (DA)/hotelling's test showed evidence of sexual dimorphism in body shape for *S. vermiculatus*. Figure 12 showed the Discriminant Function Analysis (DFA) of the relative warp scores between sexes, showing the lateral right, left and both sides, respectively for male and female *S. vermiculatus* collected in Moro Gulf and Philippine Sea. Samples collected from Pujada bay were not included in the analysis due to deficient female samples. Generally, the females of *S. vermiculatus* have bigger mouth, eyes and body depth are deeper compared to the male population. The heads of the females are dorsally located; however, the caudal peduncle of the male population is located dorsally than those of the female *S. vermiculatus*. Comparing the two areas, generally Moro Gulf populations have deeper body depth, bigger heads while Philippine Sea has elongated body and more tapered heads. The body length on the other hand, is shorter in Moro Gulf while longer body length in Philippine Sea

Nocturnal fishes like *S. vermiculatus* relatively exhibit larger eyes compared to the diurnal fish species and the structural variation that was observed on the species suggest on the constraining requirements on eye shape. Moreover, fish specimens that exhibit larger mouths are most likely associated in feeding habits as siganids are known to be voracious eater. The broadening of the body possibly suggests gravid female and food availability however, the elongation or narrowing of the body may not always suggest scarcity of food but rather an adaptation to escape prey interaction, making its body more fit to escape from prey.

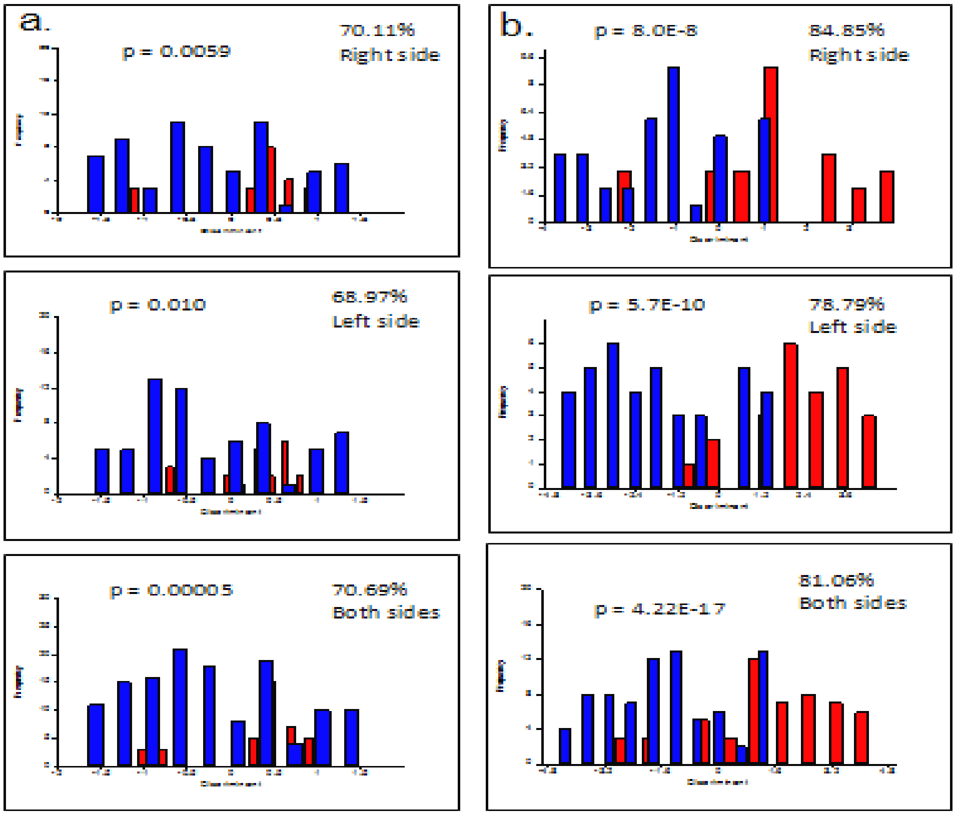

Figure 12. Discriminant Function Analysis (DFA)/ Hotelling's test of male (blue) and female (red) *S. vermiculatus* from (a) Moro Gulf and (b) Philippine Sea.

The morphological variations observed suggest on how well adaptive and fit the species lived in a particular area. *S. vermiculatus* that manifest larger traits/characters could be a result of structural adaptation to enhance their survival in a particular environment or their body adapting to manage stress. In the wild population of most bisexual species, phenotypic variation is determined by two factors: first, is the genotypic difference between the individuals, and second, is caused by diversity of environmental conditions during the formation of the phenotypic trait and manifest itself as phenotypic plasticity [26]. Moreover, the difference in geographical locations, food availability and environmental conditions affects body shape of an organism. The interspecific variation has two basic components: "geographic" variation among populations due to genetic divergence of phenotypic response to environmental factors, and within population variation [27] [28]. The second component can be partitioned into body size variation, sexual dimorphism, and functional effects correlated with age, seasonality and nutrition.

The morphological divergence in shape among conspecific populations may be caused by a phenotypic response to local environmental differences [29] or be an adaptation resulting from differences in resource use and fitness [30]. Therefore, the mechanistic underpinning of eco-morphology is that some aspects of inter-individual morphological variation will lead to functional and performance differences that result in differences in how these individuals use the available resources. In turn, ecological factors may influence further morphological changes (1) over evolutionary time as selection on these characters leads to changes in gene frequencies in a population or through extinction/speciation of taxa or, (2) over the lifespan of the organism through use-induced changes in morphological structures [30].

In conclusion, the results of this study show that an evaluation of body shape and length-weight relationships of fish populations is important to be able to determine the extent of variability in the fish which could be of prime importance in aquaculture.

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