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Describing body shape within and between sexes and populations of the Mottled spinefoot fish, *Siganus fuscescens* (Houttuyn, 1782) collected from different bays in Mindanao Island, Philippines

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Abstract. The study was conducted to determine sexual dimorphism in body shapes of *S. fuscescens* in five selected bays in Mindanao Island, Philippines. Likewise, shape and size relationship was assessed within species and between populations of the fish. The method of landmark-based geometric morphometrics was used to describe the body shapes and size of the fish. Twenty-five landmark points were digitized from images of 194 individuals and relative warp analysis was done. Thin-plate spline and Manova/CVA were used in presenting shape variations, where in this study body shape characteristics of the fish from different populations were observed. Discriminant function analysis and Hotelling's tests however showed no sexual dimorphism in body shape for all the populations. Shape variations were observed to be size-dependent. Both sexes of *S. fuscescens* were different from all other populations for having small sizes.

Key Words: Mottled spinefoot, *Siganus fuscescens,* sexual dimorphism, body shapes, sexes.

Introduction. The Mottled spinefoot fish (*Siganus fuscescens*) is one of the common species of siganids in the Philippines. It is a reef-associated fish and is also overexploited (Pauly et al 2006). Studies regarding the influence of historical and contemporary factors on genetic structure, phylogeographic patterns based on mitochondrial control region sequences on this fish revealed three distinct lineages. One lineage was identified as the morphologically similar species *Siganus canaliculatus*, while two lineages are monophyletic with *S. fuscescens*. Fine-scale structure was of the South China Sea and south Philippine Sea, while Sulu Sea and inland seas were unstructured. Genetic structure across multiple spatial scales (archipelagic, regional, and fine-scale within basins) suggests the influence of vicariant barriers and contemporary limits to gene flow in *S. fuscescens* that may be influenced by oceanographic circulation, geographical distance between available habitats, and latitudinal temperature differences (Ravago-Gotanco et al 2010). These results may also reflect variations in the phenotype of the fish. Morphological variation dealt on the relationship between organism-environment and population divergence (Love & Chase 2009), and interactions among individuals within species and functional-performance lead to ecomorphology (Motta et al 1995; Utayopas 2001). This covers shape variation, due to the differences of topography, food availability (Chouinard & Bernatchez 1998; Lau et al 2008) and locomotion (Ristovska et al 2008). Several species undergo developmental modifications (Russo et al 2007) as organisms interact with the environment. Body structures develop according to their importance for the primary living functions, thus developmental modifications in several species may be closely linked to morphological changes in habitats (Russo et al 2007; Fox & Bellwood 2012). These interactions can be studied at multiple levels; among individuals within species, among species and higher taxa and among guilds of community (Motta et al 1995; Loy et al 2001) thus this study was conducted.

Since the body shape of an organism is a result of its genetic makeup (Will & Rubinoff 2004) and its response to the environment they inhabit (Costa & Cataudella 2007), there are possibilities that a genotype of an organism may change as its environmental conditions changes over time (Iwamoto et al 2012). For centuries, traditional morphometrics is practiced to determine biological aspect differences within and between populations. Advances in molecular methods show that genetic profiling among *S. fuscescens* can arise and is argued to be a good tool in describing variability in the species (Lacson & Nelson 1993; Ravago-Gotanco et al 2010; Magsino & Meñez 2007). Will & Rubinoff (2004) however argue that DNA barcodes cannot replace morphological studies for identification and classification since many taxonomists have focused their attention on the phenotypes especially based on the complex morphological characters rather than relying on a single gene marker. With advances in digital imaging, photography, mathematics and statistics, descriptions of characters have become more quantitative than qualitative thus moving the science of descriptions in biology to become more visual and highly quantitative. One of the new methods in morphometrics the landmark-based geometric morphometric approach was argued to be an effective tool in describing variations in morphological shapes within, between and among objects of interest (Kassam et al 2003; Adams et al 2004). This study is important in *S. fuscescens* stocks analysis and its biology as well as breeding selection since this species is also considered a good candidate for aquaculture (Wambiji et al 2008).

Material and Method

Fish sample collection. *S. fuscescens* populations were collected by commissioned fishermen from selected bays in Mindanao, Philippines (Figure 1). Collected fish were brought to the landing sites and immediately processed for image capture and analysis.

Sample preparation for imaging. Fish samples were pinned to a Styrofoam using insect pins. Formalin-seawater solution (10%) was applied in the fins of the samples collected to stiffen (Love & Chase 2009; Kassam et al 2003). Each fish sample was documented through scanning using the HP G2410 flatbed scanner with an optical scanning resolution of 1200 dpi.

Sex determination. Each sample was dissected to determine the sex. Females were identified by examining the eggs which were readily distinct in the ovaries. According to Calliet et al (1986) ovaries are tubular and normally pink, yellow or orange. In adult males, the testes were typically smooth, whitish and non granular in appearance.

Landmark selection and digitization. Homologous and non-homologous biological features among the left and the right images of the samples were set as the points for land marking using the software tpsDig version 2.12 (Adams et al 2004) to digitize the landmarks (Figure 2), which capture the general body shape of the specimens.

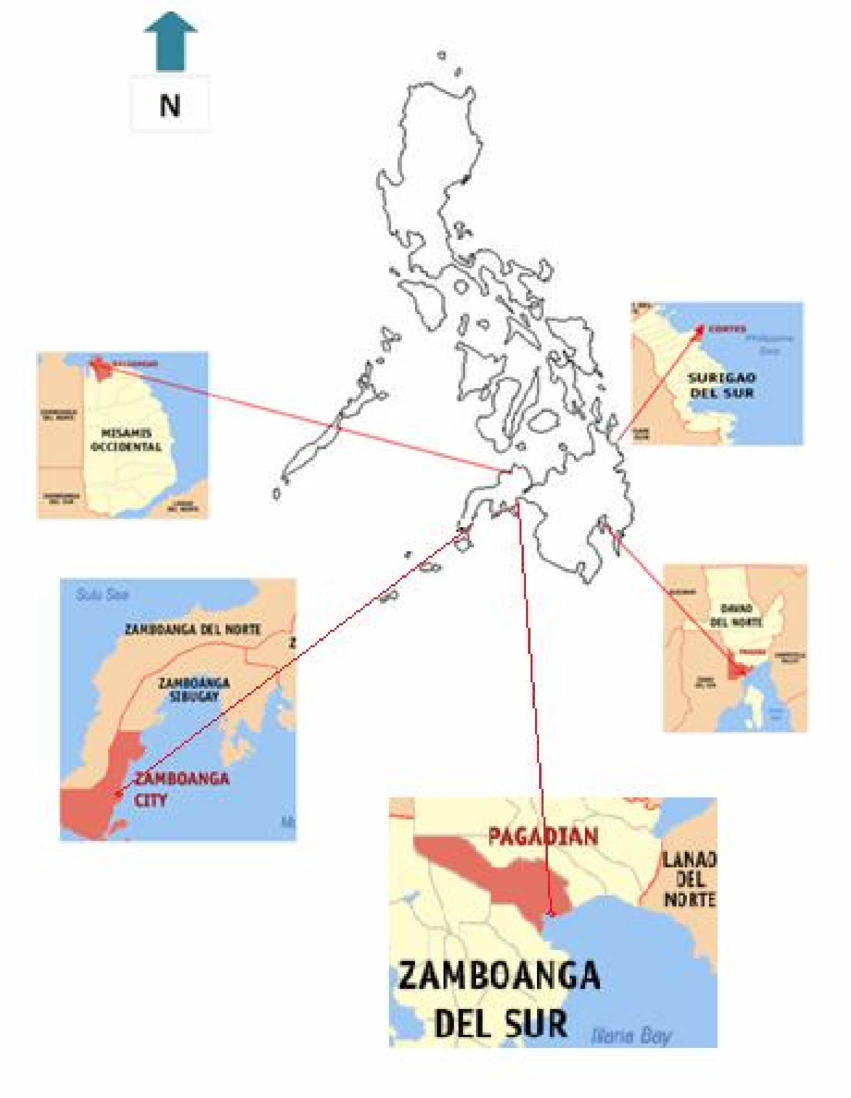

Figure 1. Geographic location of the study sites in Mindanao, Philippines.

Shape analysis. Geometric configurations were obtained from the digitized landmarks generated by the image analysis and processing software. The direct analysis of the landmark coordinates were not done as they contained components of both shape and non-shape variation such as position, orientation and size (Adams et al 2004). All non-shape related information was removed from the dataset by applying the Generalized Procrustes Analysis (GPA) in tpsRelw (version 1.49) software (Rohlf 2005). The generalized Procrustes analysis produces general mean shape of all populations, which then was used as a reference to all subsequent analyses. The TpsRelw was used to perform a principal component analysis on the partial warp scores, yielding relative warp scores as descriptors for the variation in shape (Kassam et al 2003; Adams et al 2004). The generated relative warp scores was used in Canonical variance analysis (CVA) and the centroid size scores which was also generated in TpsRelw was used to test for the relationship between shape and size using TpsPLS. To test for the shape variation Thin-plate spline was used (Bookstein 1991).

To determine sexual dimorphism in the body shape among samples, the relative warp scores generated was subjected to Discriminant Function Analysis (DFA) and Hotelling's test (Hammer et al 2001).

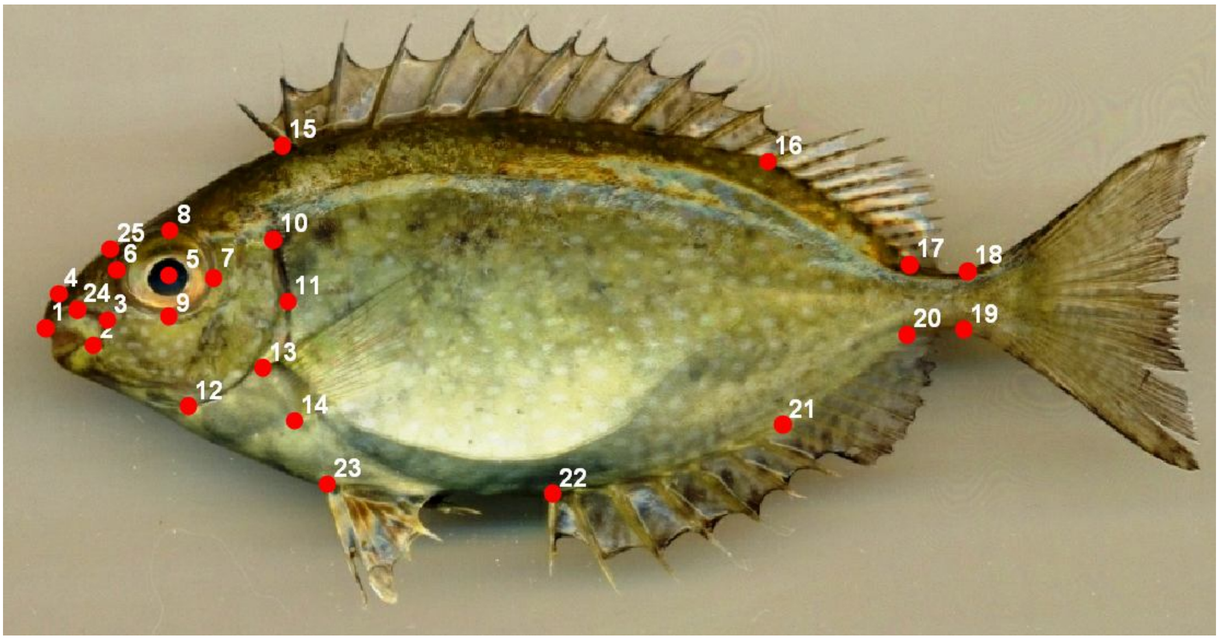

Figure 2. Twenty-five (25) homologous landmarks used for the shape analysis. (1) Lower anterior of the premaxilla; (2) Lower posterior of the premaxilla; (3) Upper posterior of the premaxilla; (4) Upper anterior of the premaxilla; (5) Center of eye; (6) Anterior margin through the midline of the eye; (7) Posterior margin through the midline of the eye; (8) Superior margin of the eye; (9) Inferior margin through the midline of the eye; (10) Dorso-lateral angle of the operculum; (11) Posterior margin of the operculum; (12) Isthmus; (13) Origin of pectoral fin; (14) Insertion of pectoral fin; (15) Origin of the dorsal fin; (16) Origin of dorsal unbranched rays; (17) Insertion of dorsal fin; (18) Superior insertion of caudal peduncle; (19) Upper tip of the caudal fin; (20) Lower tip of the caudal fin; (21) Origin of anal unbranched rays; (22) Origin of anal fin; (23)Origin of pelvic fin; (24) Mid-superior margin of the upper lip; (25) Hump (Love & Chase 2009).

Results and Discussion. Analysis of shape is a fundamental part of biological research (Adams et al 2004). The use of landamrk-based geometric morphometrics in this study have provided a visual and statistical means to describe and identify shape variations between sexes, within, between and among populations of *S. fuscescens*. Differences in shape of the body of the fish were described in terms of distinction in the deformation of grids depicting the characters (Adams et al 2004) for both the left and right sides of the image of the body of *S. fuscescens* fish samples (Figure 4 and 5). The analysis of the departures from left-right body shapes or fluctuating asymmetry are considered to result from stress during development (Nosil & Reimchen 2001; Russo et al 2007).

It was hypothesized that body shapes could differ between sexes of individuals. Sexual dimorphism often occurs in most of the major freshwater and marine fish species (Echeverria 1986) thus shapes between sexes of the fish were compared within and between populations. Using the twenty-five landmarks, Discriminant analysis showed no significant differences in shapes between sexes (Figure 3).

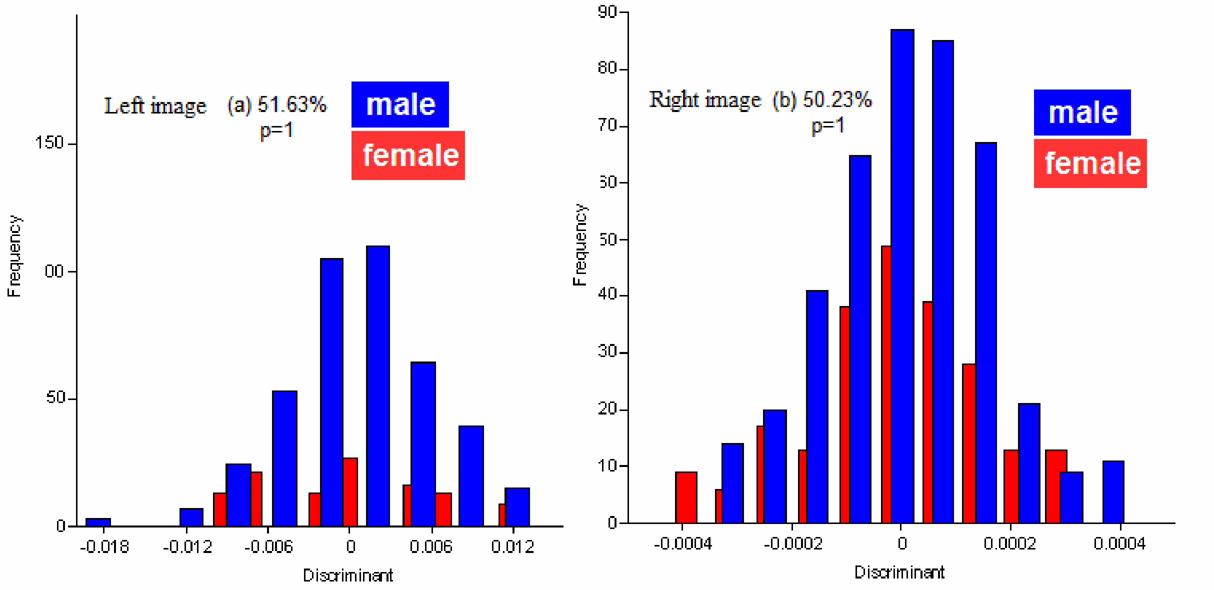

Figure 3. Discriminant Function Analysis (DFA) / Hotelling's test of male and female populations of *S. fuscescens.*

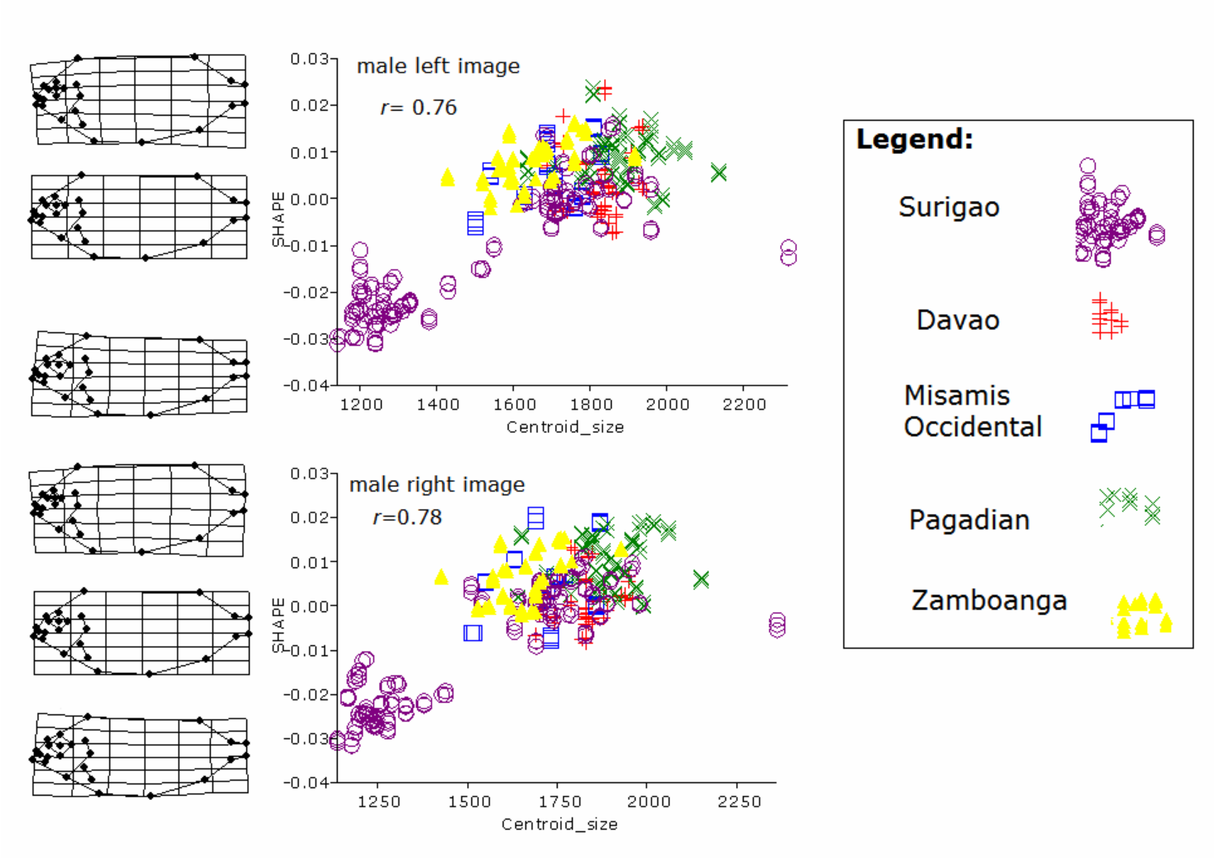

Figure 4. Relationship between shape and size of male *S. fuscescens* population.

Results suggest no sexual dimorphism in body shapes among *S. fuscescens* collected from different geographical areas in Mindanao. This result may conform to the conclusion of Thresher (1984) that there is no obvious sexual dimorphic pattern in body shapes in this species. Scatterplots shown in Figure 4 and 5 revealed no clear pattern that suggest sexual dimorphism in body shapes for both male and female populations. The study, however, shows that variation in the body shapes of this species is size-dependent (Figure 4 and 5) and is indicating that the species follow one single allometric pattern (Lleonart et al 2000). Size dependency has impacts on reproductive success among populations (Uusi-Heikkila et al 2010).

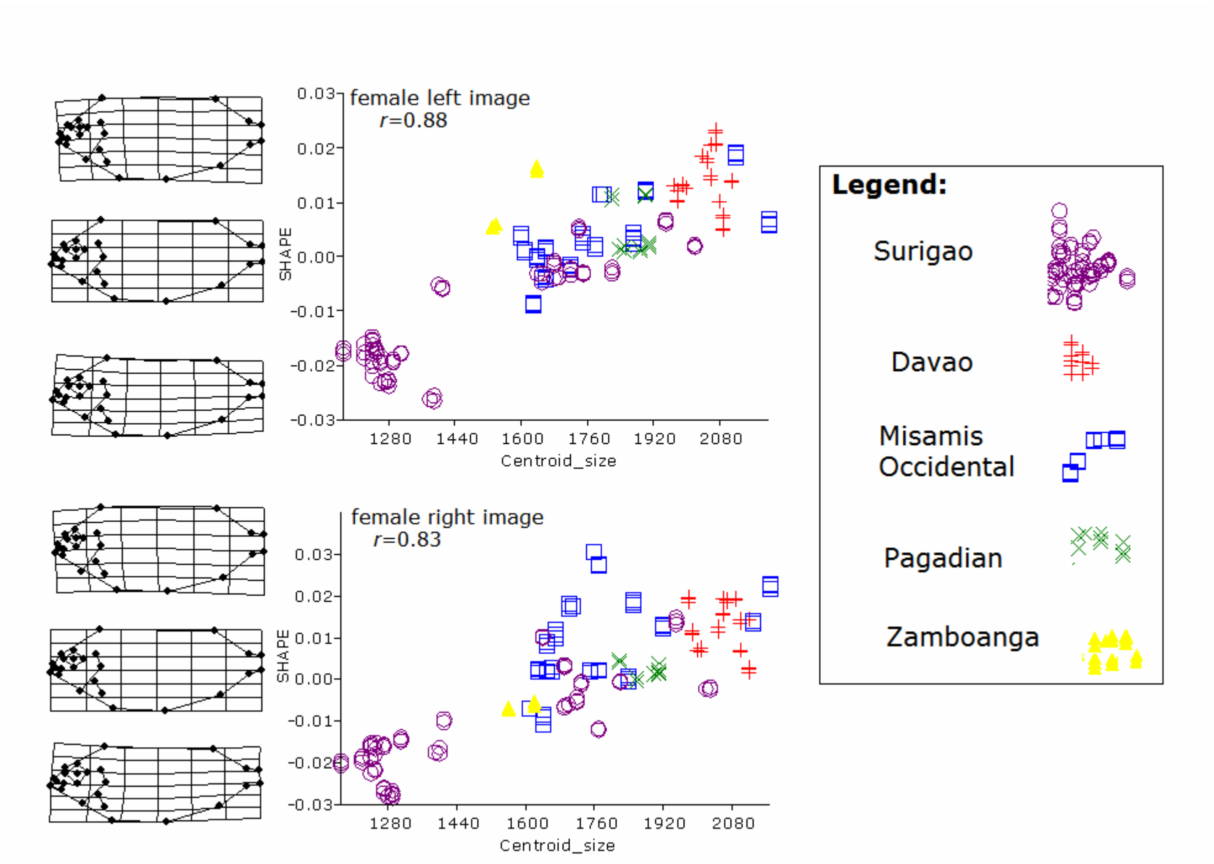

Figure 5. Relationship between shape and size of female *S. fuscescens* population.

Results of the CVA show significant similar variation for both left and right images of the population (Figure 6). While there are overlaps in the scatterplots, it is clear that there are observable variations in body shapes which are caused by external environmental forces such as currents, or by forces produced by their own fins during maneuvers (Standen & Lauder 2005).

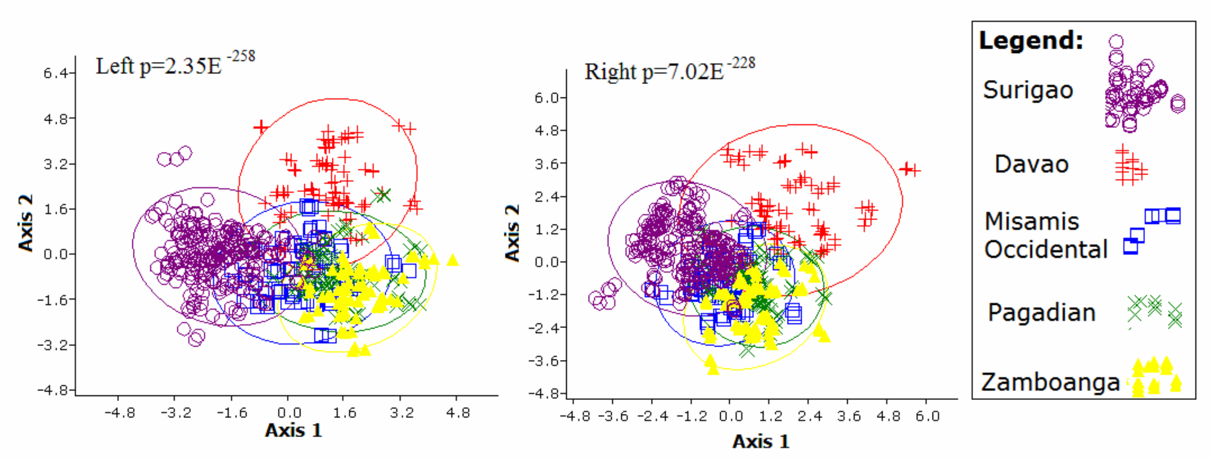

Figure 6. CVA scatter plot of left and right images of samples collected from the five sites. (1) Davao Gulf – RED; (2) Mis. Occ – Blue; (3) Illana Bay – Violet; (4) Zamboanga – Green; (5) Surigao – Yellow.

Removing the role of gender in shape variations in the different populations of the fish, describing the shapes of the fish within and between populations will provide an idea of the differences in the ecological characteristics of the bays. Results of the thin plate splines (Figure 7) show that *S. fuscescens* from Surigao have compressed dorsal fin, elongated body and narrow body depth compared to other populations as shown by differences in vector direction for landmarks 15-22. This body shape featuring the trunk and tail of the fish may be linked to the accelerated swimming ability of the fish since acceleration are generated using the body and median fins to avoid predators which may be high in the area (Pulcini et al 2008). Davao Gulf population shape variation was more on the feeding morphology, due to the direction of vectors in landmarks 1-4 and 24 which focused on the mouth region (Figure 7). Result show that fish collected from the area have bigger mouths, eyes, wide operculum and broader bodies compared to samples from other bays. They also have their insertion of the pectoral and the origin of pelvic fin posteriorly located, and shortened but blunted caudal region. The populations from Davo Gulf and Surigao have similarities for having bigger heads, eyes and mouth parts. Since these characters may have developed to distinguish food in turbid waters and to nibble more encrusted food in the area, it is hypothesized that both populations may have similar water physical characteristics. According to Harder (1975), visual acuity among fishes is precise in clear water. In turbid areas, where visibility is limited, the quantity of rods augment along with the area of the retina on which light impinges and so the eyes are bigger. Despite of enlargement of the eyes, the precision of targeting prey is still reduced, thus this is compensated with the widening of the mouth (Russo et al 2007; Pulcini et al 2008).

Shape variation of samples from Iligan Bay was more in the head region (Figure 7). The constricted feature of the eye region may define that the bay area has clear water bodies, however, expansion of the mouth may suggest also to their feeding activity (Pulcini et al 2008). The expansion of the caudal peduncle and anteriorward direction of their pectoral fin and origin of the pelvic fin may suggest that this area has fewer predators (Standen & Lauder 2005; Pulcini et al 2008).

Illana Bay population was observed to have widening of the caudal peduncle but shortened base of the anal fin, and broadening of the trunk region (Figure 7). According to Standen & Lauder (2005), fish use their fins to control the body posture, and the position of the fins to the center of the mass is important to determine forces to be exerted during maneuvers. In addition, fishes with shorter caudal peduncle may rely their swimming propulsion generated by their median and paired fins, thus they are slow swimmers (Pulcini et al 2008). This body shape of the fish may indicate that in Illana Bay, predators may be less.

Zamboanga population has wider pectoral fin base as their caudal peduncle lengthens and broadens; however, they possess small eyes. Their anal fin and isthmus are anteriorly pointed, resulting to have their head length (HL) shorter but have increased width of their body depth (BD) (Figure 7). These variations observed among Zamboanga samples may suggest that this area have also clear water bodies but the lengthening of their caudal peduncle may signify that they were actively swimming and evading many predators. According to Lingham-Soliar (2005), swimming oscillations are generated backward, increasing the propulsion and maneuverability at high speeds when fishes have longer caudal peduncle. Results of the study that show general variations in the left and right body shape patterns may explain that if stimulus is presented on the left side, fish will execute a yawing turn to the right. The left pectoral fin would generate a laterally directed force, anterior to the center of the mass that yaw the body to the right (Lauder & Drucker 2004).

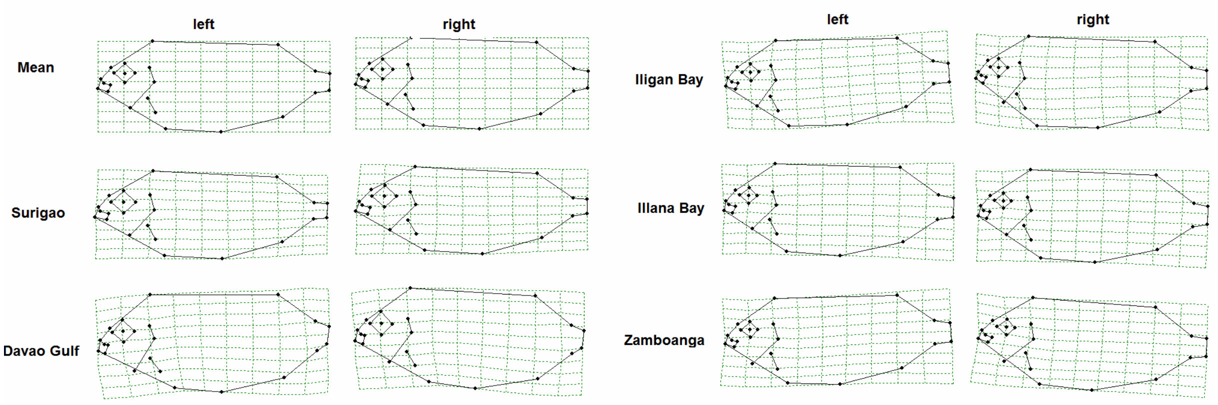

Figure 7. Thin Plate Spline plot of *S. fuscescens* collected from the five areas; Surigao, Davao Gulf, Iligan Bay, Illana Bay and Zamboanga, showing the shape relatedness among population.

Conclusions. The results generated in this study revealed that morphological variation may have interaction or relationship with ecological diversity among populations of this fish species of *Siganus* as shown by observed differences in retinal morphology, body shape, position and shape of the fins (Motta et al 1995). Shape variations observed in *S. fuscescens,* could possibly be associated to their adaptation to the primary habitat of the fish which in this case, differences in the ecological profile of the different bays where the fish populations were collected. A detailed physical, biological, and chemical oceanographic profiling of the different bays therefore is needed to be able to correlate the variations observed in the body shapes of the fish.

Results of the study show the importance of using landmark-based geometric morphometrics in describing variations in morphological shapes of the fish. The application of the method is important to have a better quantitative assessment of variations in body shapes of the fish which may have importance in the proper management of stocks in this species.

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