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Article

Sexual dimorphism, asymmetry, and allometry in the shell shape of *Modiolus metcalfei* (Hanley, 1843) collected from Dumangas, Iloilo, Philippines: A geometric morphometric approach

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Abstract
The shell is the most conspicuous and variable part of a bivalve and is largely affected by environmental conditions. Thus, this study was conducted to describe the changes in the shell shape of the horse mussel *Modiolus metcalfei* from Dumangas, Iloilo. Allometric changes in shape, sex-induced shape differences, and asymmetry were investigated using geometric morphometric methods. A total of 12 landmarks where used to obtain biological shape information from 60 horse mussel individuals. Multivariate analysis of variance revealed significant differences of shell shapes between sexes (Wilk's λ=0.01, p=1.26E-219). Generally, males have an expanded shell but compressed mid-shell region while females have compressed shell but expanded mid- and postero-ventral region. Moreover, the multivariate regression of shape on centroid size was statistically significant (p<0.001, Goodall's F-test). Smaller individuals were slender and elongate while larger individuals were slightly rounded and curved.Furthermore, directional and fluctuating asymmetry were highly significant (p<0.0001, Procrustes ANOVA), indicating developmental instability probably caused by the ecological health of the coastal waters of Dumangas, Iloilo. The differences in shape between sexes were attributed to the differences in reproductive roles while allometric shell morphology changes reflected its adaptation to the environment.

Keywords horse mussels; morphology; ecological stress; environmental adaptation.

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1 Introduction

Shape is the geometric information which remains after the effects of location, scale, and rotation are removed from an object (Kendall, 1977). Within a species, adult shape is conserved and phenotypically integrated (Strauss and Bookstein, 1982; Rohlf, 1990; Rohlf and Marcus, 1993; Adams et al., 2004), often persisting throughout the geographical range of the species (Kelly et al., 2013; Lima-Filho et al., 2017) and over extended geological time (Walker and Bell, 2000). In mollusks, the shell is most conspicuous and variable. Genetic differentiation and phenotypic plasticity contribute to variations in shell morphology (Marquez et al., 2010). Therefore, shell shape is an important morphological trait in assessing the phylogenetic history, function, and life habit of a mollusk (Crampton and Maxwell, 2000).

Fluctuating asymmetry is the random deviation from perfect symmetry (Graham et al., 2010). It reflects both genetic and environmental perturbations experienced by an organism making it a reliable indicator of developmental instability and ecological stress (Klingenberg and McIntyre, 1998; Graham et al., 2010; Savriama and Klingenberg, 2011; Lajus et al., 2015). In a homologous environment, the same external factors affect both sides of an organism as they are replicas of same developmental event and share the same genotype (Dongen, 2006). When genetic and environmental perturbations occur, an organism should adequately buffer itself in order to achieve developmental stability. Failure to buffer such perturbations will result to developmental instability (Parsons, 1990).

At present, little is known on the dimorphic trends in the shell shapes of mollusks. Currently, there is no study on the sexual shape dimorphism in mytilid bivalves. In addition, allometric studies on the shell shape changes is limited (Marquez et al., 2010, 2018). The advent of geometric morphometric methods have revolutionized the investigation of shape differences within and among populations as subtle differences are detected. It provides statistically robust and visual methods for shape analysis. Moreover, the clear mathematical separation of size and shape enables the study of its variation and relationships (Zelditch et al., 2012).

Dumangas is a coastal town in the province of Iloilo in the island of Panay, Philippines. It has a total coastline of 21.6 km with seven major rivers traversing the municipality. Its coastal waters are rich in horse mussel resources where its exploitation has been practiced since the 1970s. In addition, it has a wide area of marine ponds utilized for the production of milkfish. Moreover, it has one of the major ports in the province where Roll-on Roll-off vessels operate to carry passengers and cargoes.

We hypothesized that the physical disturbance in the habitat brought about by the continuous exploitation of horse mussels and effluents from marine ponds, passenger and cargo vessels, and domestic wastes have contributed to the deterioration of the quality of the environment subjecting *M. metcalfei* to ecological stress thereby, promoting developmental instability in the organism. Furthermore, we expected that morphological variation is affected by sex due to the dioecious nature of the horse mussel and allometry should reflect the environmental constraints and adaptation of the species.

2 Materials and Methods

2.1 Sample collection and image acquisition

Sixty male and female individuals of *M. metcalfei* were collected from the coastal waters of Dumangas, Iloilo (Fig. 1). Male and female sexes were identified based on the coloration of the gonads: males have creamy white testis while females have bright orange ovary.Prior to photographing, each specimen was cleaned off from their soft tissues and sun-dried. Photographs of the left and right valves of the shell were then taken in the same position and orientation with a ruler as a measurement reference using a digital camera.

2.2 Landmark selection and digitization

Images were compiled, scaled, and digitized using the tpsDig version 2.12 (Rohlf, 2008a) and tpsUtil version 1.44 (Rohlf, 2009b) software. Seven type 1 (anatomical) and five type 2 (mathematical) homologous landmarks (Table 1) in the inner valves were landmarked as illustrated in Fig. 2. Landmark positions were selected according to Valladares et al. (2010) with some modifications. Landmarking per specimen was done in triplicates at different days to minimize measurement error and biases.The x and y coordinates of these landmarks on the images were used to analyze asymmetry, allometry, and sexual shape dimorphism.

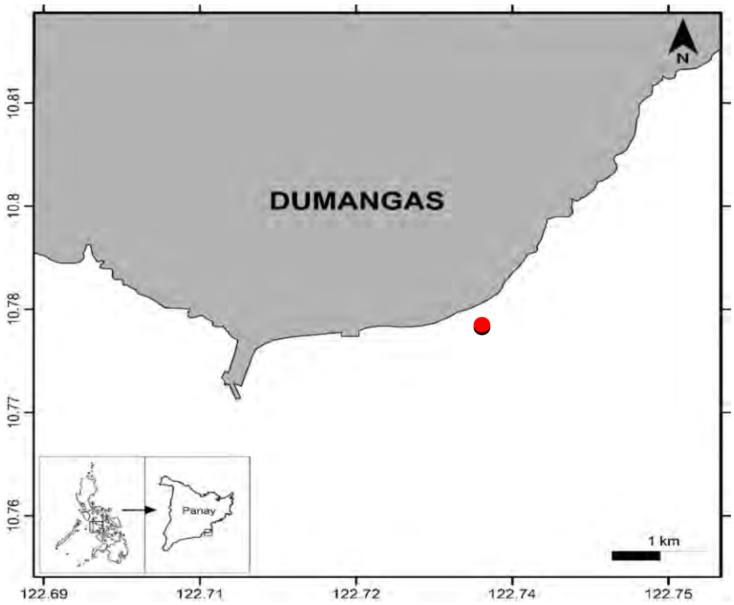

Fig. 1 Map showing the location of the study site. Red dot indicates the location of the sampling site.

Table 1 Landmarks used for the geometric morphometric analyses of *Modiolus metcalfei*.

Landmark	Description	Type
1	Umbo	I
2	Start of ligament	I
3	End of ligament	I
4	Dorsal margin maxima	II
5	Posterior Adductor 1	I
6	Posterior Adductor 2	I
7	Posterior Adductor 3	I
8	Posterior Adductor 4	I
9	Posterior margin maxima	II
10	Projection	II
11	Projection	II
12	Anterior margin maxima	II

2.3 Shape analysis

The x and y coordinates of the 12 landmarks were analyzed using Symmetry and Asymmetry in Geometric Data software version 1.21 (Marquez, 2014). Given that the right and left valves are pairs of separated structures (Savriama and Klingenberg, 2011) the analysis of fluctuating asymmetry was based on matching symmetry. Therefore, to study the left-right asymmetry, the reflection was removed by transforming all configurations from one body side to their mirror images (Klingenberg and McIntyre, 1998). After superimposition, pure shape information was preserved in the specimens' aligned landmarks, and variation around the mean shape in the sample (consensus) was decomposed into a symmetric and an asymmetric component (Klingenberg and McIntyre, 1998; Klingenberg et al., 2002).

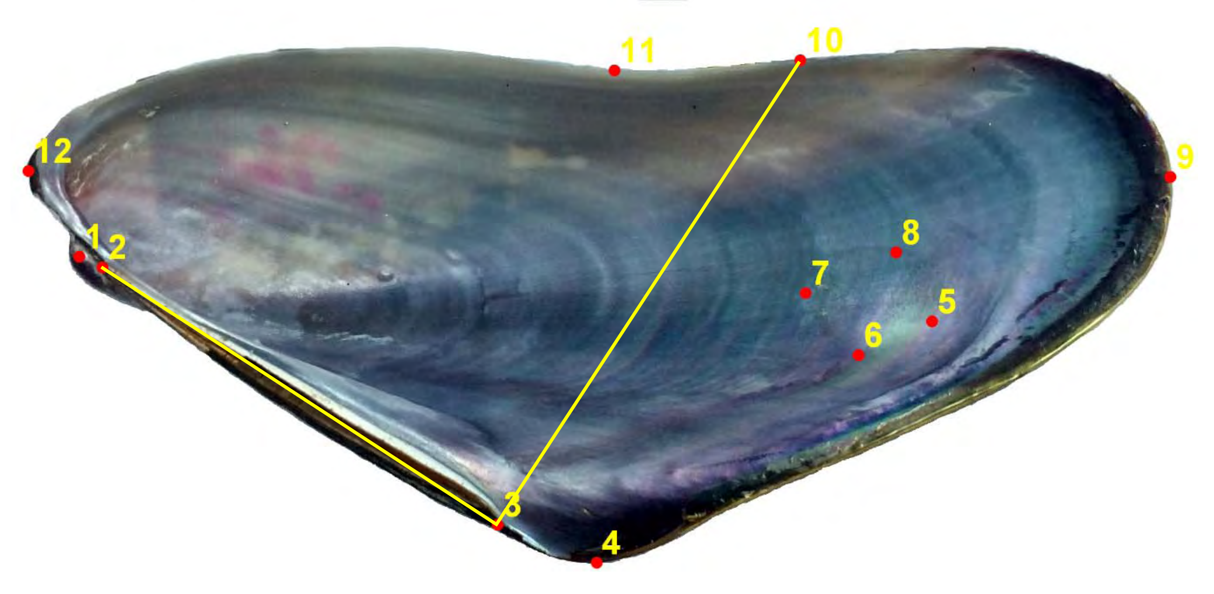

Fig. 2 Photograph of the inner left valve of *Modiolus metcalfei* showing the position of 12 landmarks.

The superimposed landmark configurations were analyzed through Procrustes analysis of variance (ANOVA) with 99 permutations to analyze fluctuating asymmetry (measured by side x individual interaction), directional asymmetry (expressed by the main effect for sides), and measurement error (expressed by the residual term). The individuals and the valve side were entered as random and fixed effects, respectively. The degrees of freedom are those for ordinary ANOVA multiplied by the shape dimensions, which is twice the number of the landmarks minus the four degrees of freedom that are lost during superimposition (two degrees lost during translation, one degree for each of the two dimensions, and one each for scaling and rotation).

Moreover, to detect the components of variances and deviations, a principal component analysis of the covariance matrix associated with the component of fluctuating asymmetry variation was also performed to carry out an interpolation based on a thin-plate spline and then visualize shape changes as landmark displacement in the deformation grid.

2.4 Analysis of shape difference between sexes

The degree of shape difference between sexes were visualized using thin-plate spline deformation plots implied from the principal components analysis. Multivariate analysis of variance was used to analyzed significant shape difference between sexes using Paleontological Statistics software version 3.18 (Hammer et al., 2001).

2.5 Analysis of allometry

To explore how shape varies with size, shape variables which include all partial warps and uniform components were subjected to a multivariate regression on centroid size using tpsRegr version 1.36 (Rohlf, 2009a). The fit of the regression models was evaluated using Goodall's F-test. Centroid size was calculated from the raw coordinates of the landmarks by using tpsRelw version 1.46 (Rohlf, 2008b).

3 Results and Discussion

3.1 Sexual shape dimorphism

Sexual shape dimorphism was detected in *M. metcalfei*. Variation in the shell shape of males and females was significantly different (Wilk's λ=0.01, *p*=1.26E-219, MANOVA). The results of the canonical variates analysis clearly separated the shape variables of the sexes as shown in Fig. 3.

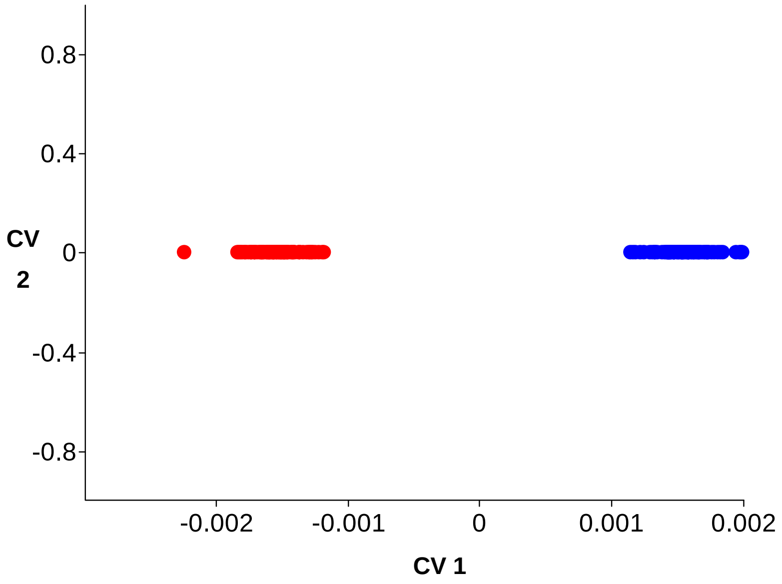

Fig. 3 Canonical variate analysis of the shell shape showing the differences between sexes of *Modiolus metcalfei* (blue: male; red: female).

The variations in the shell shape of male and female *M. metcalfei* are shown in Fig. 4. In males, certain individuals had shown pronounced expansion in the anterior region and pronounced compression in the posterior region as shown in the first relative warp which accounted for 39.96% of the variation (Fig. 4A). Other individuals had shown expansion throughout the shell but in the mid-ventral region as shown in the second relative warp which accounted for 24.91% of the variation (Fig. 4B). Both relative warps accounted for 64.86% of the variation in shell shape of male *M. metcalfei*.

In females, the trend in shell variation was different. Certain individuals had shown pronounced compression throughout the shell but the mid-ventral region where expansion was prevalent as shown in the first relative warp which accounted for 39.25% of the variation (Fig. 4C). Other individuals had shown pronounced compression in the anterior region and weak compression in the posterodorsal region but pronounced expansion in the mid-ventral region as shown in the second relative warp which accounted for 26.55% of the variation (Fig. 4D). Both relative warps accounted for 65.79% of the variation in the shell shape of female *M. metcalfei*.

Dimorphic trends among bivalves, in contrast to vertebrates, are poorly understood. Different reproductive roles may influence the occurrence of dimorphic trends in bivalve shell shape. In

Anodontaanatina, females were significantly wider than males, probably as a result of altered shell growth to accommodate marsupial gills. Some populations have females which are significantly thinner than those of males, which could be a result of resource depletion by offspring production (Zieritz and Aldridge, 2011). Zieritz and Aldridge (2011) further argued that the sexual dimorphism in this species is a reflection of the overarching effect of habitat on morphology. Accordingly, populations in the most favorable habitats exhibit faster growth rates, attain larger maximum sizes and produce more offspring, which result in more swollen gills and consequently more inflated shells of gravid females compared to less fecund populations. In the same species, shell shape was also affected by parasitism wherein infected specimens grow wider and more elongated.

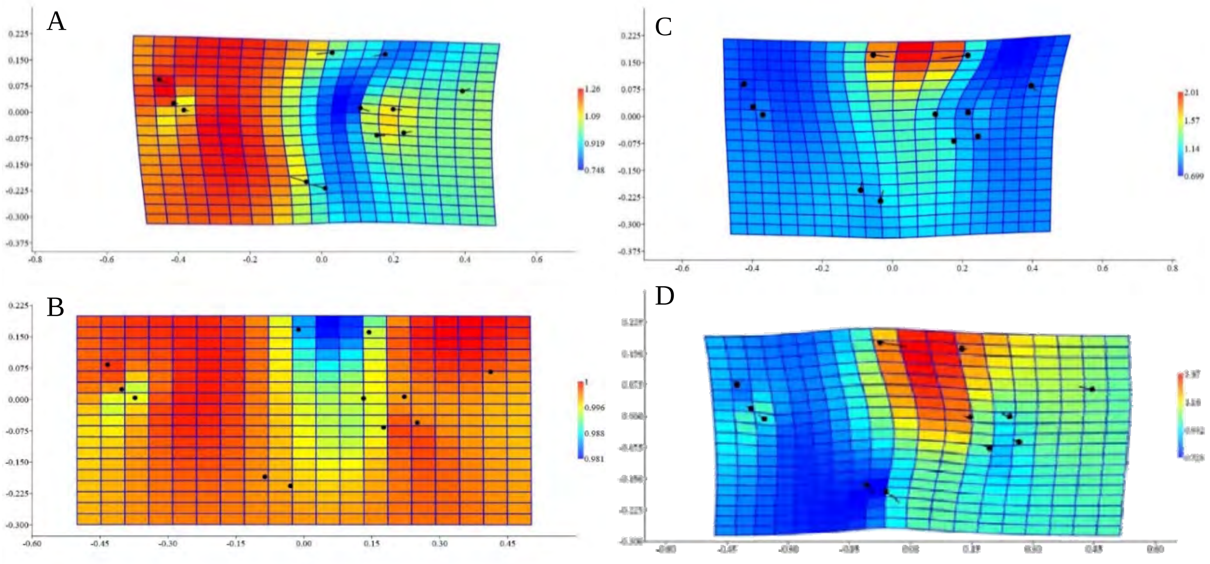

Fig. 4 Thin-plate spline of the first two relative warps of male (A-B) and female (C-D) *Modiolus metcalfei*. Colors indicate area expansion or compression (i.e. the degree of local growth). Red represents expansion, blue represents compression.

3.2 Asymmetry

An examination of symmetry in *M. metcalfei* showed size and shape variations among individuals indicating genotypic differences within the population. Moreover, a significant difference in the variations on the left and right valves indicating directional symmetry were also observed. However, the individual x side interaction was also significant indicating that the differences in the shapes between the left and right valves can be attributed to the failure of the effect of individual valves to be the same from side to side (Table 2). This indicates fluctuating asymmetry in *M. metcalfei*.

The individual x sides interaction is a measure of fluctuating asymmetry and antisymmetry, hence, a mixed effect. The failure of the effect of individuals that is the same from side to side is the interaction of individual by sides. It is the only measure which indicates fluctuating asymmetry. Therefore, the *F*-value shows highly significant fluctuating asymmetry for the population of *M. metcalfei* in Dumangas, Iloilo (Table 3). The effect called “sides” which refer to the variation between the two sides, a measure of directional asymmetry, was found to be significant in *M. metcalfei*. The directional asymmetry is mainly related to a pronounced dislocation in the posterior region and anterior region produced by landmarks 1, 2, 4, 5, 6, 7, 8, 9, 10, and 12 as shown in Fig. 5.

Table 2 Analysis of shape asymmetry. The result of Procrustes two-way, mixed model analysis of variance (ANOVA) results of symmetry of the population of *Modiolus metcalfei* Dumangas, Iloilo.

Effect	Sum of Squares	Degrees of Freedom	Mean of Squares	*F*-value	*p*-value
Individuals	0.8599	1180	0.0007	9.233	<0.0001*
Sides	0.0166	20	0.0008	10.521	<0.0001*
Individuals x Sides	0.0931	1180	7.89E-05	5.199	<0.0001*
Measurement Error	0.0729	4800	1.52E-05		

The asterisks represent statistical significance

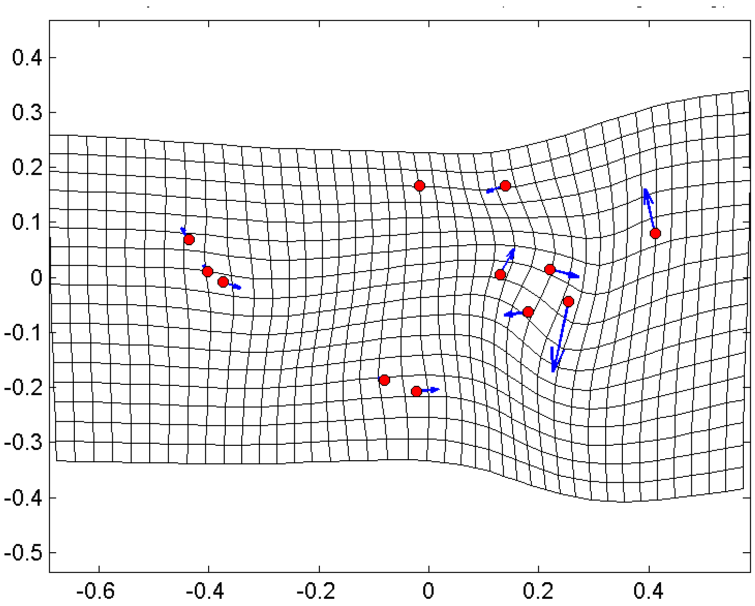

Fig. 5 Principal components analysis-implied deformation for side variation (directional asymmetry) in *Modiolus metcalfei*.

To determine the affected landmarks, a principal components analysis was employed. The result showed a total of 64.54% of fluctuating asymmetry interaction from the upper 5% effective principal components (PC) from PC1 to PC4 (Table 3). PC1 and PC2 had shown that all areas covered by these landmarks were found to have greater asymmetry while PC3 and PC4 had shown that the majority of the landmarks had greater asymmetry. Principal components analysis-implied deformations of the symmetry and fluctuating asymmetry in the shell of *M. metcalfei* are shown in Fig. 6.

The results of the present study revealed highly significant fluctuating asymmetry in *M. metcalfei* in Dumangas, Iloilo. Procrustes ANOVA with individual and side as the main effect was used to explore asymmetries in the samples. It also allowed the detection of measurement error on fluctuating asymmetry estimates (Klingenberg, 2015). Results showed that measurement error was negligible in relation to fluctuating asymmetry, indicating the importance of the exhaustive digitization protocol carried out. In addition, individual and side (directional asymmetry) effects were also found to be significant. A significant fluctuating and directional asymmetry indicated that generations of phenotypes are interacting in a perturbed environment, hence, convey the interplay of both genotype and environment in a more stressful condition.

Table 3 Principal component scores reflecting the symmetry and asymmetry score values as well as the summary of the affected landmarks.

PC	Individual (Symmetry)	Sides (Directional Asymmetry)	Interaction (Fluctuating Asymmetry)	Affected Landmarks
1	41.91%	100%	26.15%	All
2	27.13%	-	14.62%	All
3	10.42%	-	12.71%	1,2,5,6,7,8,9,10,11,12
4	5.11%	-	11.06%	3,4,5,6,7,8,9,10,11
Total	84.57%	100%	64.54%	

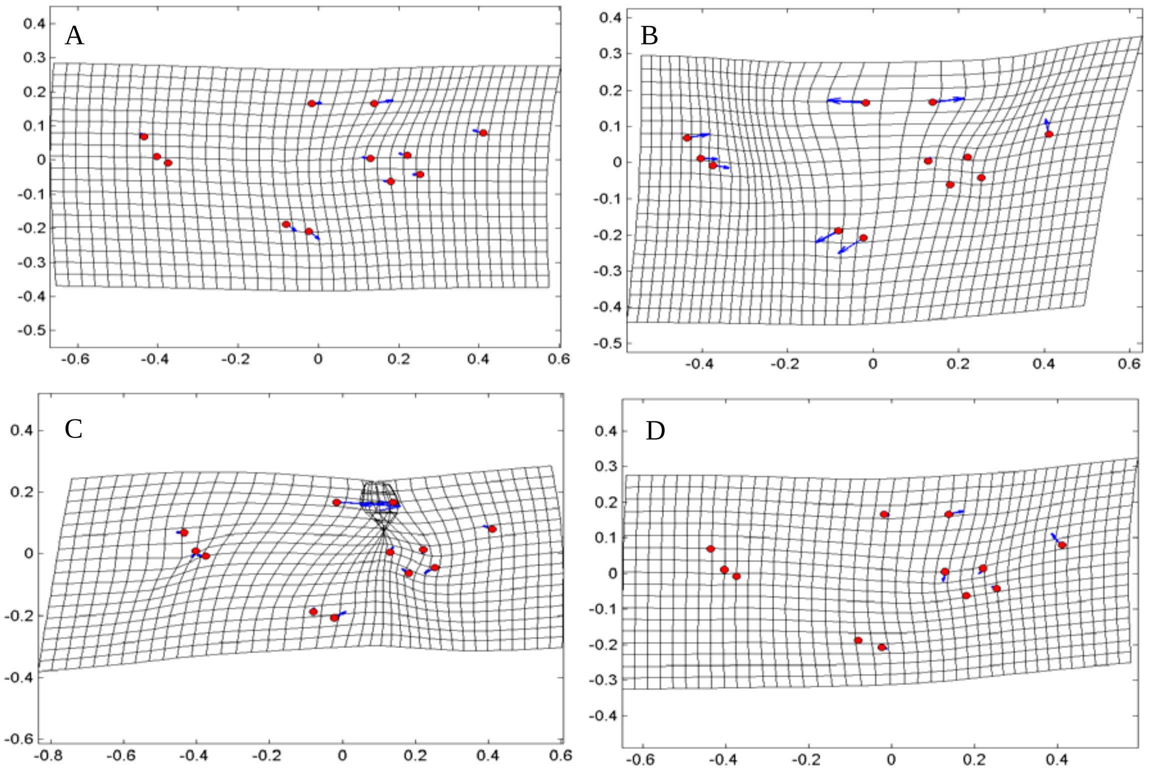

Fig. 6 Principal components analysis-implied deformation of the individual (A-B) and individual x side variation (C-D) in *Modiolus metcalfei*.

Fluctuating asymmetry in *M. metcalfei* in Dumangas, Iloilo can be explained by both genetic and environmental perturbations. The deviation from bilateral symmetry may be due to exposure to developmental perturbations in their early life. Sources of developmental perturbations include exogenous and indigenous stress such as low habitat quality to low genetic heterozygosity among others (Utayopas, 2001).

In addition, a wide range of environmental factors results in fluctuating asymmetry. In Atlantic Patagonian mussel *Mytilus platensis*, habitat-specific constraints resulted in fluctuating asymmetry. Accordingly, the intertidal environment is more disturbed and variable than the shallow subtidal environment reflecting higher fluctuating asymmetry values in mussels thriving in the intertidal zone (Trivellini et al., 2018). Pollution resulted to shell fluctuating asymmetry in *M. galloprovincialis* (Scalici et al., 2017), *Perna viridis* (Borlaza and Tabugo, 2018), *Gafrarium tumidum* (Ducos and Tabugo, 2014, 2015), *Venerupsis philippinarum* (Cabiluna and Tabugo, 2017), *Dreissena polymorpha* (Yavnyuk et al., 2009). Salinity is also an environmental factor resulting in fluctuating asymmetry. Lajus et al. (2015) reported lower fluctuating asymmetry values in *Mytilus* mussels from estuarine areas (lower salinity) compared to those from the Barents Sea (with oceanic salinity).

On-site observation points out to two stressors affecting *M. metcalfei* in Dumangas, Iloilo. First, the physical habitat disturbance brought about by the exploitation of this resource. Gatherers collect *M. metcalfei* daily for their livelihood thus mussels are physically disturbed by trampling and scouring of the mussel bed. Previous studies indicated that hydrodynamic forces affect mussel morphology (Akester and Martel, 2000; Steffani and Branch, 2003; Selin, 2013; Moschino et al., 2015: Hodgson et al., 2018). Even wave activity caused by anthropogenic boating activities can cause variation in *M. galloprovincialis* shell shape (Moschino et al., 2015). Thus, this repeated physical disturbance directly to the mussels may have a profound effect on their physiology resulting in developmental instability, hence, fluctuating asymmetry. Second, pollution brought about by the effluents from marine ponds, passenger and cargo vessels, and domestic wastes in the area. Aquaculture wastes (i.e. leached chemicals from feeds, chemotherapeutants, fish wastes, etc.) are released as effluents to the sea daily. Mussel beds are exposed to these effluents as they are located near shore (≈ 500 m). In addition, domestic wastes and wastes from passenger and cargo vessels may have contributed to the pollution in the area. The close association between the environmental conditions and fluctuating asymmetry levels has been considered as evidence of a direct effect of environmental stress. Evidence in the literature suggests that chemical pollution results in fluctuating asymmetry in mussels. Although the present study did not measure these pollutants, the results suggest that their quantities have already affected the mussels in the area

3.3 Allometry

To visualize allometric patterns in *M. metcalfei*, a multivariate regression of the shell shape versus the centroid size was conducted. The null hypothesis of isometry was rejected since the multivariate regression was statistically significant ($p<0.001$, Goodall’s F-test). The deformations in coordinate configurations related to the centroid size in *M. metcalfei* are shown in Fig. 7. The allometric deformation in shell shape in smaller individuals was dominated by pronounced anterior-posterior compression with an expansion in the ventral and dorsal parts of the shell resulting to slightly rounded and curved form while in larger individuals there was ventral and dorsal compression resulting to slender and elongate form.

Morphometric changes throughout ontogeny are clearly genotype driven (Gosling, 2015) but can also be modulated by environmental conditions (Claxton et al., 1998; Trussel, 2002; Hornbach et al., 2010; Rufino et al., 2012; Caill-Milly et al., 2014; Morais et al., 2014; Amini-Yekta et al., 2019; Whelan et al., 2019).

In some bivalve species, burrowing ability decreases during ontogeny. In these taxa, juveniles are relatively streamlined, an important burrowing advantage. However, adult shell morphology changes during growth as they inhabit increasingly deeper burrows, reducing their burrowing ability (Pohlo, 1964). This is clearly supported by the findings of the present study.

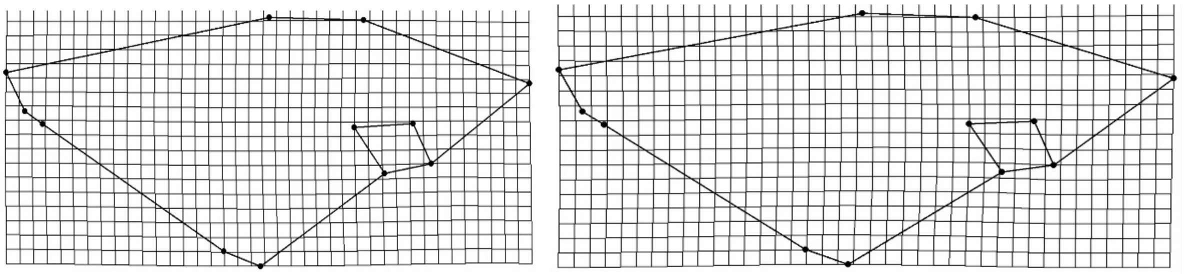

Fig. 7 Deformation grids of shell shapes related to centroid sizes of *Modiolus metcalfei*. From left to right respectively: smallest and largest.

Existing literature on allometric studies in tropical modiolids was in line with the results of the present study. Negative allometry was observed in the length-width and length-height relationships of *M. auriculatus* in Red sea (Abu-Zaid et al., 2014) and Byndoor, Karnataka, India (Singh et al., 2013). Whereas in temperate modiolid species, the results are in contrast to the present study. The length-weight, length-width, and length-height relationships of *M. adriaticus* in Algarve coast, southern Portugal were found to be isometric (Gaspar et al., 2001, 2002). Isometry in this modiolid species is explained by the species autecology, living attached by byssus threads to rocks, debris, and other bivalve shells.

Geometric morphometric methods have been used to investigate the morphology of aquatic organisms in the Philippines. Much have focused on the analysis of inter- and intra-population shape differences (Cabuga et al., 2016, 2017b, 2018; Tabugo-Rico et al., 2017; Presilda et al., 2018), fractal analysis (Tabugo et al., 2019), and asymmetry (Presilda et al., 2016; Cabuga et al., 2017a) in fishes, aquatic plants, and gastropods. The present study investigated the morphology of a commercially important bivalve with the combined use of existing software for morphological analysis in order to describe the morphological variations in the organism which has profound implications on the understanding of its ecology and biology.

4 Conclusion

The use of geometric morphometric methods revealed sexual shape dimorphism in *M. metcalfei*. This is the first report on sexual shape dimorphism on Mytilid bivalves. Moreover, the findings of allometry revealed the species autecology and adaptation to environmental conditions. In addition, the high fluctuating asymmetry values resulting from the variations in the left and right sides of the shell indicated ecological stress and developmental instability.

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