CrossMark

PRIMARY RESEARCH PAPER

Fluctuating asymmetry in the shell shape of the Atlantic Patagonian mussel, *Mytilus platensis,* generated by habitat-specific constraints

María Magdalena Trivellini · Silvina Van der Molen · Federico Márquez

Abstract Fluctuating asymmetry (FA) consists of random deviations from perfect symmetry, and it is a useful trait to monitor developmental stability and ecological stress. The principal goal in this work was to study shell shape FA in *Mytilus platensis* as a measure of stress of living in intertidal wild areas compared to subtidal cultured ones. Shell shape asymmetry was studied by geometric morphometrics using landmarks and semilandmarks from internal and external anatomical structures. We analyzed FA comparing shell shape from 452 individuals living at different habitats (intertidal rocky shores vs. subtidal longlines) in two Patagonian sites. We predict that mussels living in the intertidal present higher levels of morphological alterations (i.e., FA) than those from the subtidal, where the environmental stress is lower. We based the asymmetry analysis on the FA scores from Procrustes ANOVA; all effects of the model were statistically significant. The Procrustes FA scores differed significantly between habitats: intertidal populations presented higher FA scores than subtidal ones. As predicted, shell shape in *Mytilus platensis* is influenced by the habitat and these differences add evidence to the fact that FA is a suitable measure of developmental instability, indicating the inability of species to buffer stress in its developmental pathways.

Keywords Asymmetry · Physical stress · Modularity · Geometric morphometrics · Intertidal · Subtidal · Sexual dimorphism

M. M. Trivellini · S. Van der Molen · F. Márquez (✉)
IBIOMAR, Instituto de Biología de Organismos Marinos - CONICET, Blvd. Brown 2915,
U9120ACD Puerto Madryn, Argentina
e-mail: fede@cenpat-conicet.gob.ar

M. M. Trivellini
UNC, Universidad Nacional de Córdoba, Av. Vélez Sarsfield 299, Córdoba, Argentina

F. Márquez
UNPSJB, Universidad Nacional de la Patagonia San Juan Bosco, Blvd. Brown 3100, Puerto Madryn, Argentina

Introduction

Biological shape is one of the most conspicuous aspects of an organism's phenotype and provides a link between the genotype and the environment (Ricklefs & Miles, 1994; Monteiro et al., 2002). Through the ages, symmetry was one of the ideas by which man has tried to comprehend and create order, beauty, and perfection (Weyl, 1989). The most common and simplest type of symmetry in biological structures is the bilateral symmetry, when left and

Springer

right sides are mirror images of each other (Savriama & Klingenberg, 2011). Symmetry is classified into two categories: matching symmetry, when there are pairs of separate structures on the left and right sides, and object symmetry when there is a single structure internally symmetric (Mardia et al., 2000; Savriama & Klingenberg, 2011). There are three known variation patterns of the differences between the right and left sides (Palmer, 1994): if the variation is normally distributed around a mean of zero, fluctuating asymmetry (FA) is present; when the variation is normally distributed around a mean that is significantly different from zero, it is called directional asymmetry (DA); and when the frequency distribution departs from normality in the direction of platykurtosis or bimodality, it is known as antisymmetry (AA). The asymmetry types have different statistical properties as well as distinct biological origins and implications (Klingenberg, 2015). Moreover, these three types of asymmetries can occur together in the same trait (Van Valen, 1962).

Fluctuating asymmetry reflects a population's adaptation and coadaptation status (Graham et al., 2010), and it is considered as a measure of developmental noise and developmental instability (DI) (Klingenberg & McIntyre, 1998; Graham et al., 2010; Savriama & Klingenberg, 2011; Lajus et al., 2015). The FA is used as a proxy of DI since both sides of an organism can be viewed as independent replicas of the same developmental event (Dongen, 2006). Both sides share the same genotype and, in a homogeneous environment, they are under the influence of the same external factors. According to Markow (1995), the developmental stability is the situation achieved when an organism has adequately buffered itself against epigenetic perturbations, displaying its developmentally programmed phenotype. When an organism has failed to buffer such disturbances, it may display signs of DI. Organisms experiencing genetic or environmental stress have lower developmental stability and exhibit greater levels of FA. Thus, FA is often monitored to detect populations under stress (Lajus et al., 2015) and has been correlated with physiological stress caused by extreme environmental conditions (e.g., Beardmore, 1960; Hosken et al., 2000; Briones & Guiñez, 2005). Many tools have been developed in the field of geometric morphometrics for the study of shape

asymmetry (Klingenberg & McIntyre, 1998; Mardia et al., 2000; Klingenberg, 2015). Ducos & Tabugo (2015), comparing two sites with different contamination levels, demonstrated that FA is a stress bioindicator and a measure of DI in the bivalve *Gafrarium tumidum*.

Different ecological factors are known to influence shell shape in mussels (Valladares et al., 2010; Márquez et al., 2018). The conditions of rocky intertidal habitats, that generally include periodic exposure to desiccation, extreme temperatures, and wave exposure (Alunno-Bruscia et al., 2001; Steffani & Branch, 2003; Kirk et al., 2007; Márquez et al., 2015), generate more fluctuating habitats when compared to subtidal ones, where the physical conditions are more homogenous and stable. Probably, these differences between habitats is showed in trade-offs; for example, between protection from the destructive force of waves and lower food supply in subtidal environments. These trade-offs can affect the availability of surplus energy and the relative use of this energy for the different body parts (Steffani & Branch, 2003). Bivalve molluscs, and particularly mussels, are characterized by high phenotypic plasticity in response to all these variations in local environmental conditions, and to mitigate the effects of intra-specific competition at the individual level (Beadman et al., 2003; Steffani & Branch, 2003; Kirk et al., 2007; Funk & Reckendorfer, 2008; Cubillo et al., 2012). Therefore, wide variation in shell morphology is to be expected, even in animals from the same locality (Seed, 1968).

The *Mytilus* genus represents an important component of the intertidal and subtidal communities and their species are distributed in a wide spectrum of environments in both the Northern and Southern hemispheres. The members of the *Mytilus edulis* species complex are *Mytilus edulis* Linnaeus, 1758, *M. galloprovincialis* Lamarck, 1819 and *M. trossulus* Gould, 1850, but nowadays the taxonomy and whether it is a native or invasive species are under discussion (McDonald et al., 1991; Westfall & Gardner, 2010). Several studies have led to contradictory results depending on the type of molecular marker used. For example, Astorga et al. (2015), on the basis of COI mitochondrial genes and 16RNA sequences study, point out that the samples from the southern cone of South America should be identified as *M. planulatus*

Lamarck, 1819 or *M. platensis* d' Orbigny, 1842. However, recently, Zbawicka et al. (2018) in single nucleotide polymorphism analysis concluded that the mussels from South American Atlantic coasts are *Mytilus platensis* (or *Mytilus edulis platensis*) and they confirmed that this species is a native of this area. Up to now, a lot of studies have been carried out to determine which *Mytilus* species is the one present in Argentinean coasts. For this reason, in the current work we have decided to use the name *Mytilus platensis*, as it is proposed by Zbawicka et al. (2018), although this classification is still in debate.

The principal goal was to test if the *Mytilus platensis* mussels exhibit FA as a result of environmental stress: we analyzed FA comparing shell shapes from individuals living at different environments (intertidal rocky shores vs. subtidal longlines). The coexistence of cultured and wild mussel populations at the same location provides a unique scenario to address comparative morphological studies of sessile marine species during the adult stages. Our prediction is that wild mussels living in the intertidal rocky shores will show higher levels of morphological alterations (i.e., FA) than cultured ones in subtidal longlines, where the environmental stress is lower.

Materials and methods

Sampling

Mussel samples were obtained from intertidal rocky shores and subtidal longlines at two sites of Chubut province, Argentinian Patagonia: Bahía Camarones (S44°54.098′, W65°35.472′), and 240 km further south, at Comodoro Rivadavia (S45°57.752′, W67°33.575′) (Fig. 1). A total of 452 adult mussels were collected from both sites. Wild ones from the intertidal (Bahía Camarones wild-CaBN; $n = 114$ and Comodoro Rivadavia wild-CoBN $n = 116$) were manually collected using quadrats of 25×25 cm, and subtidal cultured samples (Bahía Camarones cultured-CaCS; $n = 107$ and Comodoro Rivadavia cultured-CoCM; $n = 115$) were collected by lifting the longlines and taking mussels from the first 25 cm of the rope. The distance between the intertidal wild and the subtidal cultured samples, at each site, was less than 3 km.

The valves were separated and the sex was estimated by visual observations of gonad's color and texture (Zaixso, 2015). Regarding density, comparisons were done within each habitat for the two sites (Bahía Camarones/Comodoro Rivadavia), and statistical significance of the differences between the samples was evaluated by Kruskal–Wallis test (Zar, 1999). The shells were dissected, carefully cleaned and dried. Both valves from each individual were labeled with a single code including site/habitat/sex/side/n° ind. They were scanned with the inner surface parallel to the plane of scanning with an Epson Perfection v350 scanner with a 600 dpi resolution.

Geometric morphometric analyses

Data acquisition

Shell shape analyses were performed by means of geometric morphometric (GM) techniques using the Cartesian coordinates of a two-dimensional configuration of anatomical landmarks and semilandmarks. Images were compiled, scaled and digitized using the TpsDig2 and TpsUtil software (Rohlf, 2017a, b). The shape variables were obtained by randomly digitizing individuals from the two sites (Bahía Camarones/Comodoro Rivadavia), habitat (intertidal rocky shores/subtidal longlines), sex (M/F), and side (left/right). In each valve (right and left), 8 landmarks (1, umbo; 2, ligament; 3, pedal retractor muscle scar; 4, posterior adductor muscle scar, anterior part; 5, posterior adductor muscle scar, posterior part; 6, posterior border; 7, projection at 270° of the vector formed between the landmark 1 and 2 on the outline of the shell; 8, anterior adductor muscle scar) and 22 semilandmarks (9–13 the boundary between the landmarks 1 and 2; 14–20 between landmarks 2 and 6; 21 between landmarks 6 and 7, and 22–27 between landmarks 7 and 1, 28–30 between landmarks 4 and 5) were digitized by one observer (MMT) using TpsDig2 (Fig. 2a). Landmark positions were selected according to Valladares et al. (2010) with some modifications.

To quantify and minimize measurement error (ME), a subsample of 15 individuals from each site (total = 60 individuals) was digitized twice. The semilandmarks were aligned using TpsRelw software (Rohlf, 2017c) to minimize the bending energy of the configurations (Gunz & Mitteroecker, 2013). Subsequently, the landmark configurations were

Fig. 1 Sample collection sites at Chubut province. Triangles: cities, stars: sampling sites
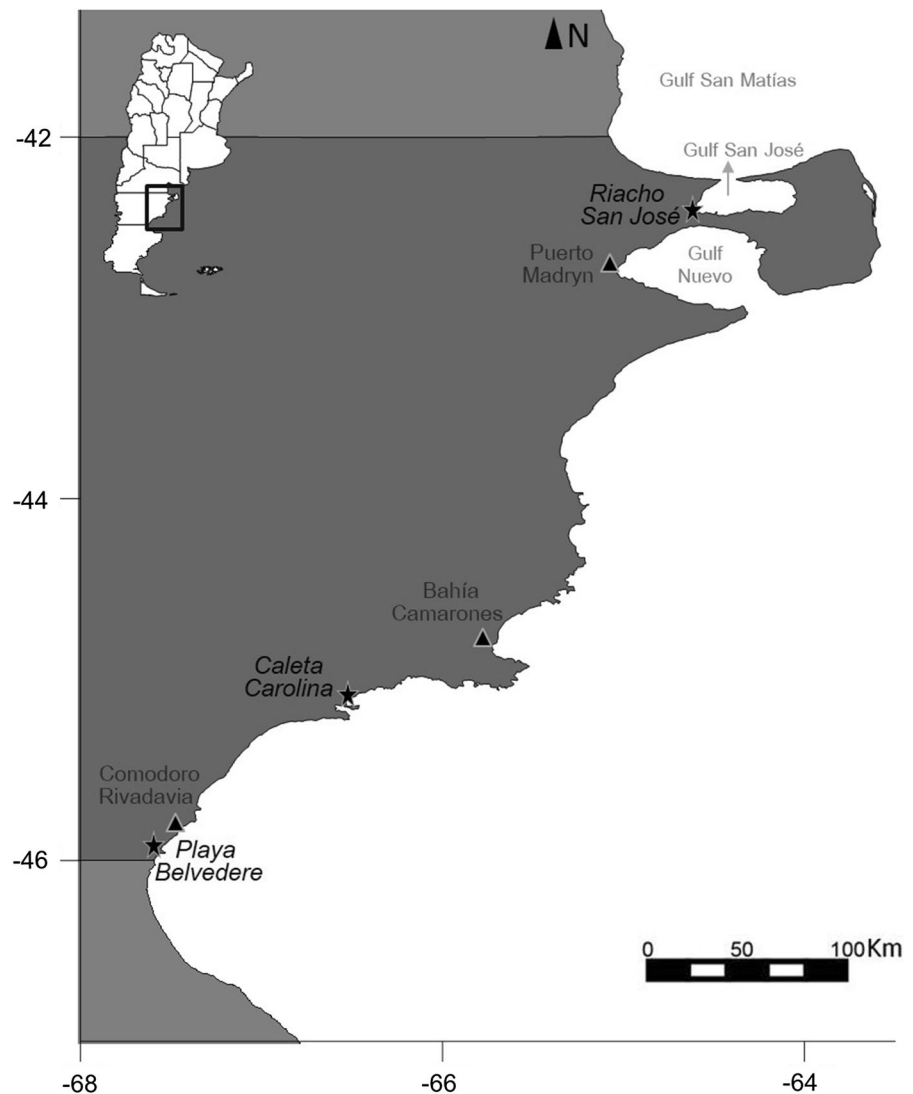
superimposed by Generalized Procrustes Analysis (Rohlf & Slice, 1990; Slice et al., 1996). Given that the right and left valves are pairs of separated structures (Savriama & Klingenberg, 2011) we based our analysis on matching symmetry. Therefore, to study the left–right asymmetry, reflection was removed by transforming all configurations from one body side to their mirror images (Klingenberg & 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 & McIntyre, 1998; Klingenberg et al., 2002).

Data analysis

Since the allometry (shape changes correlated with size) can affect all the parts of the organism, it can interfere in the modularity analysis, hiding a possible modular structure (Klingenberg, 2009). Therefore, in the first place the allometry was estimated through a multivariate regression of shape (Procrustes coordinates used as the dependent variables) on size (average centroid size between right and left valves used as the independent variable; Bookstein, 1991; Monteiro, 1999). After removing the influence of allometry, the covariance matrices of the residuals was used to analyze modularity between two hypothesized

(a)

(b)
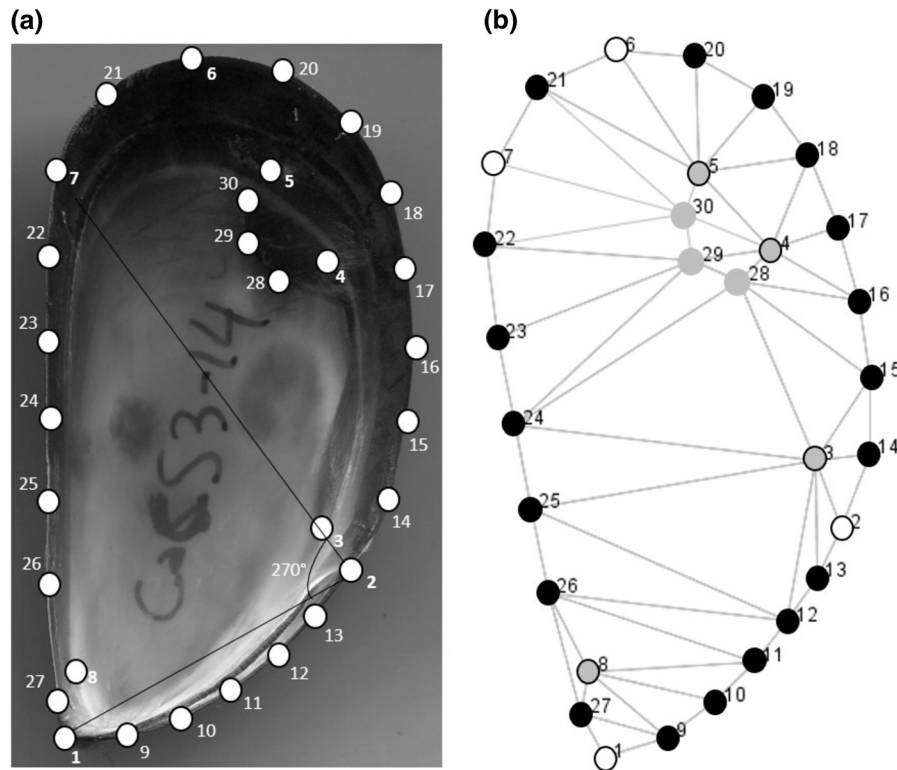
Fig. 2 Landmarks configuration a) Landmark configuration on the right shell (it is the same in the left shell). Position of 8 landmarks: (1) umbo, (2) ligament, (3) pedal retractor muscle scar, (4) posterior adductor muscle scar, anterior part, (5) posterior adductor muscle scar, posterior part, (6) posterior border, (7) projection, (8) anterior adductor muscle scar. For a more precise identification, all landmarks were painted with small indelible marker points on the shell. b) Partition of the configuration into corresponding subsets according to their location in the shell, internal (4 landmarks: gray dots with black borders; 3 semilandmarks: gray dots) and external (4 landmarks: white dots with black borders; 19 semilandmarks: black dots). The gray lines represent the adjacency graph used to define spatially contiguous partitions of reference points in the modularity test (Klingenberg, 2009)

modules: the external configuration composed of 4 landmarks (1, 2, 6 and 7) and 19 semilandmarks (9–13, 14–20, 21 and 22–27), and the internal configuration comprising 4 landmarks (3, 4, 5 and 8) and 3 semilandmarks (28–30) (Fig. 2b). The hypothesis that the internal and external configurations are separate modules was tested using the Escoufier's RV coefficient (Escoufier, 1973) as a measure of covariation between two sets of landmarks (Klingenberg, 2009). The RV term was introduced by Klingenberg (2009) as a scalar measure of the strength of association between the coordinates of two sets of landmarks and presents a new generalization of this measure for multiple sets of landmarks. To test the covariation between internal and external configuration, the MorphoJ software used a permutation test against the null hypothesis of total independence (Fig. 2b;

Good, 2000; Manly, 2007; Klingenberg & Marugán-Lobón, 2013). This test simulates the null hypothesis by randomly reshuffling observations separately within the blocks of landmark coordinates for the two configurations raised a priori and uses the RV coefficient as the statistic test. The RV takes values from 0 to 1, wherein a lower value indicates a weak covariation.

Next, sexual dimorphism and the different asymmetries were examined separately in each configuration. The sexual dimorphism in shell shape was tested by a discriminant analysis; statistical significance of pairwise differences in mean shapes was assessed with permutation tests using Procrustes distance and the T-square test statistic (1,000 permutations per test). Traditional analyses of FA have long used a two-factor, mixed-effect ANOVA (Leamy, 1984; Palmer

& Strobeck, 1986) with individuals and sides as the two factors. Goodall (1991) established the use of ANOVA designs in the context of Procrustes Methods. Procrustes ANOVA uses the landmark coordinates after a joint Procrustes superimposition of all the data (all individuals, both sides, all replicate measurements; Dryden & Mardia, 1998; Klingenberg & McIntyre, 1998; Klingenberg, 2015). Procrustes ANOVA also provides a simple means to gauge the possible effects of measurement error (ME) on estimates of FA (Klingenberg, 2015).

In the present study, the analysis of asymmetry was based on the Procrustes ANOVA (Klingenberg & McIntyre, 1998), where the deviations from the consensus were decomposed into a component of variation among individuals and a component of left–right asymmetry (Klingenberg, 2015). For Procrustes ANOVA, in the framework of matching symmetry, the units of analysis were the repeated parts. The main effects were individuals (representing the inter-individual variation), sides (representing the DA), individuals-by-side (representing the FA), and measurement error (replicate measurements) (Savriama & Klingenberg, 2011; Klingenberg, 2015). Also, the variable site habitat was included in the model as an additional main effect (where sites are Bahía Camarones/Comodoro Rivadavia, and habitats are wild—intertidal/cultured—subtidal). Individual and valve side were considered as random and fixed effects, respectively.

The matching symmetry analysis was conducted in MorphoJ, version 1.06d software (Klingenberg, 2011) which, as part of the Procrustes fit, yielded separate components for symmetry and asymmetry. Since the interest of this study is on asymmetry, the components of symmetric variation were disregarded. The patterns of shape variation of FA were visualized with principal components analysis of the respective matrix (Individual*Side). The FA levels were computed using the Procrustes distances (from Procrustes ANOVA, Shape FA Scores), given that the straightforward choice to measure the amount of overall asymmetry is the Procrustes distance (or squared Procrustes distance) between left and right sides (Smith et al., 1997; Klingenberg & McIntyre, 1998; Klingenberg, 2015). Since the assumptions for parametric tests were not met, statistical significance of the differences between FA group scores was evaluated by Kruskal–Wallis test (Zar, 1999). According to Conover (1999), for significant results ($P < 0.05$) pairwise comparisons test of subgroups was applied. The mean shape FA scores were used to illustrate shape differences among sampling sites and habitat.

Results

The multivariate regression of shape on centroid size showed that allometry is statistically significant (permutation test with 10,000 random permutations, $P < 0.0001$), and accounted for 2.12% of the total amount of shape variation. Therefore, subsequent analyses were performed with the residuals of the regression.

The hypothesis that the internal and external configurations are separate modules was supported (the covariation between the internal and external configuration for the hypothesis of modularity was weaker than for all of the alternative partitions). The RV coefficient between the internal and external set, with a value of 0.29, is near to the lower extreme (left tail) of the distribution of RV coefficients for all 10,000 partitions of the landmarks into subset (none of the 10,000 permutation runs achieved the strength of covariation found in the original data). In fact, no partition showed RV less than or equal to the a priori hypothesis (Fig. 1 supplementary material). Hence, internal and external configurations were considered as independent modules.

For each module, in the discriminant function analysis comparisons for sex, the difference between means was not significant (External: $T^2 = 59.24$, $P = 0.0723$ P values for permutation tests; Internal: $T^2 = 13.61$, $P = 0.2$ P values for permutation tests). Thus, all the subsequent analyses were performed without dimorphism corrections. Concerning density, the results of Kruskal–Wallis tests within habitats between sites and within sites between habitats were not significant ($P > 0.05$).

In each module, the Procrustes ANOVA of shape variation showed that all effects of the model were statistically significant (Table 1). The sample presented both DA (main effect of side in Table 1) and FA (main effect of individual*side in Table 1). Measurement error (ME) was negligible in relation to individual*side effect since the mean square of FA largely exceeded ME (Klingenberg, 2015). The Procrustes FA scores frequency histogram for the left–

Table 1 Procrustes ANOVA results for shape asymmetry for each configuration

| Shape (Procrustes ANOVA) | Configuration | | | | | |
| | Internal | | | External | | |
	MS	DF	P	MS	DS	P
Site-Habitat	0.043783778	30	< 0.0001	0.012844727	126	< 0.0001
Individual	0.000548149	3860	< 0.0001	0.000179613	16338	< 0.0001
Side	0.003651373	10	< 0.0001	0.005082192	42	< 0.0001
Individual*Side	0.000264705	3898	< 0.0001	1.28221E-05	16464	< 0.0001
Measurement	5.28083E-05	1160		6.7795E-06	4956	

The table lists Procrustes mean squares (MS) and degrees of freedom (DF) for all effects, as well as parametric P values

right differences (Fig. 2 supplementary material) showed that they were distributed around a mean that is different from zero (deflected from ideal FA), indicating the presence of directional and fluctuating asymmetry together and absence of antisymmetry, since no platykurtosis or binomial distributions were present. Directional asymmetry of external modules was mainly related to an umbo shift, as a dorsal and posterior expansion and as an anterior restriction of the right valve regarding to the left valve. On the other hand, DA of internal module was related with a displacement in the area of the ligament and a small shift of the adductor muscle (Fig. 3 supplementary material). Principal component (PC) analysis was used to identify and display the patterns of individual shape variation regarding FA. The analysis of FA variation showed that the PC1 in each configuration takes up more than half of the variation (Internal: 52.77%; external: 53.09%). In the internal configuration, the dominant features of variation (PC1) related to FA of the positive extreme were associated with the position of the posterior adductor muscle scar. The PC2 was associated with a posterior expansion of the pedal retractor muscle scar, and the third PC axis was related to the anterior retraction of the dorsal part of the pedal retractor muscle scar. In the external configuration, most shape variation of the PC1 positive extreme was associated with the elongation of the posterior region of the shell and the curvature of the antero-dorsal region. The positive extreme of the second and third PC axes was related to the retraction of the ventral region and to a more elongate shell shape, respectively (Fig. 3).

The Procrustes FA scores for each configuration differed significantly between site habitat (External: Kruskal–Wallis test: $H = 39.21$, $P < 0.0001$; Internal: Kruskal–Wallis test: $H = 42.89$; $P < 0.0001$). For both configurations, the mean individual FA scores from the two sites presented the same general pattern: intertidal rocky shore populations with higher FA scores than subtidal longlines (Table 2, Fig. 4). However, the relationship between wild samples from two sites was different for each configuration.

Discussion

In this work, we provide compelling graphical and analytical evidence of the shell shape variations between two different habitats (intertidal vs. subtidal) related to the development of fluctuating asymmetry (FA) in the *Mytilus platensis*. We hypothesized that the FA reflects developmental instability as a product of environmental stress. In fact, Procrustes FA scores differed significantly between habitats: intertidal populations presented higher FA scores than subtidal, cultured ones. The same pattern was registered on two different regions of south-western American coast.

The result of the multivariate regression for allometry effect was subtle but statistically significant, because the allometry correction reduces the covariation of the mussel shell and accentuates the relative independence of the internal and external configuration. The first step to apply 2D-GM techniques was to delimit modules between landmark configurations and to evaluate hypotheses about their boundaries (Klingenberg, 2009). Modules are units within which there

Fig. 3 Fluctuating asymmetry shape variation patterns for the internal and external configurations. The figure shows the first three PCs of the covariance matrix for the individual*side interaction effect of Procrustes ANOVA. In gray lines and open dots the symmetric consensus configuration is shown, and in black lines and solid dots a configuration with an arbitrary scale factor of + 0.1 units of Procrustes distance for the respective PC is shown
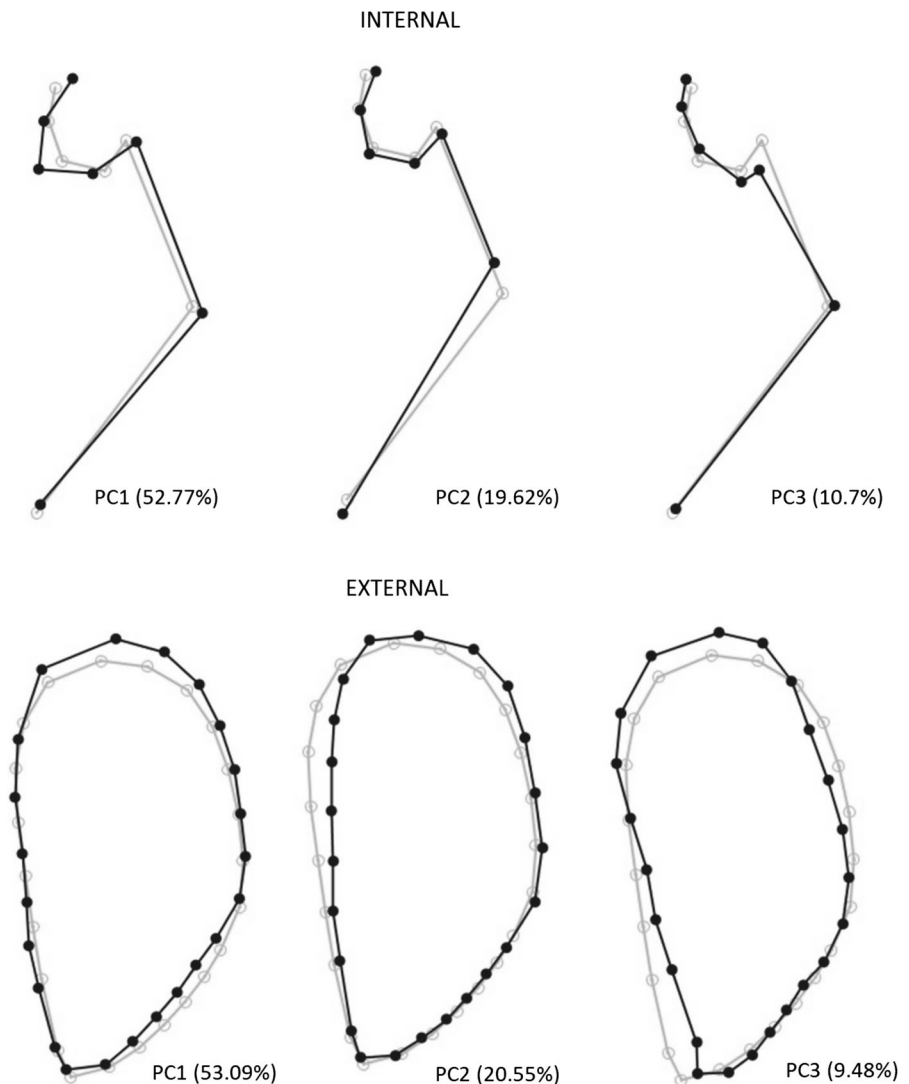
Table 2 Results of the Kruskal–Wallis test for each landmark configuration showing the media ranking in ascending order

Internal			External	
Site-Habitat	Ranks		Ranks	
CaCS	150.35	A	144.04	A
CoCM	166.85	A	180.97	B
CaBN	218.15	B	223.92	C
CoBN	242.98	B	235.25	C

The same letter indicates that there are no significant differences ($P > 0.05$)

is a high degree of integration from many interactions of different nature (e.g., developing, functional, genetic), but the integration is relatively weak (independent) from other units (Klingenberg, 2009). Therefore, these interactions are presented as strong covariation among units within modules and weak covariation between modules. It was shown that the internal and external landmark configurations in mussels are independent modules; the a priori hypothesis about modularity (internal and external configuration) was evaluated; and a lower covariation for this partition than for any other subdivision of landmarks was obtained. However, low covariation by itself does not imply modularity, but it is a prediction of the
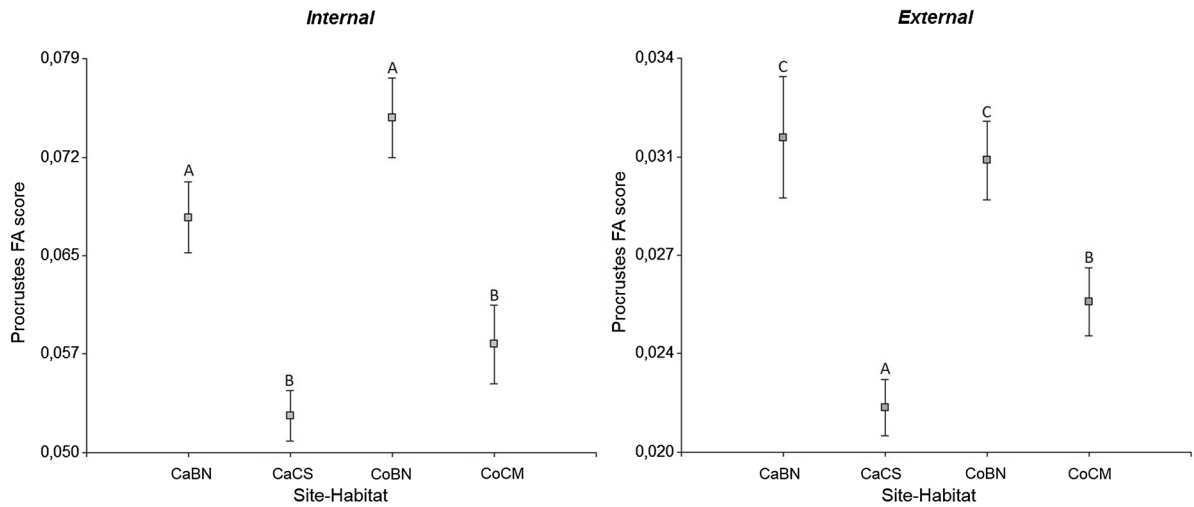
Fig. 4 Individual asymmetry scores mean values and standard deviations (Procrustes FA Scores) of mussels' site and habitat for each configuration. *CaBN* Bahía Camarones/Wild, *CaCS* Bahía Camarones/Cultivated, *CoBN* Comodoro Rivadavia/Wild, *CoCM* Comodoro Rivadavia/Cultivated

modularity hypothesis (Klingenberg, 2009). These two landmark configurations represent different shape traits in the shell: the external configuration focuses only on the shell contour (hard structure), while the internal configuration provides an insight of the soft structure scars, which have diverse variation patterns and are more flexible than the shell. The environmental variables could affect different anatomical features in various ways. Márquez et al. (2010) used landmarks and contour methods to evaluate the differences between internal and external structures over a clam shell, and they found different results.

Methods for the analysis of shape with bilateral symmetry can separate a component of symmetric variation among individuals, that is, variation in the left–right averages of a trait, from the left–right asymmetries within individuals (Klingenberg et al., 2002). In the present study, Procrustes ANOVA with individual and side as the main effect was used to explore the asymmetries in the sample. In addition, the Procrustes ANOVA analysis allows to detect the effect of measurement error (ME) on FA estimates (Klingenberg, 2015). In this paper, ME was negligible in relation to FA, indicating the importance of the exhaustive digitization protocol carried out: shells were digitized twice and randomly by the same person in different days. Also, all items in the Procrustes ANOVA were statistically significant, including directional asymmetry (DA; side); although the

possible functional and adaptive significance of DA is unclear (Klingenberg & McIntyre, 1998; Klingenberg, 2015). The DA is genetic (Palmer, 1994) and generally would be expressed in similar degree for all individuals in a population (Lajus et al., 2015). Anyway, the principal goal in this work was to study FA in *Mytilus platensis* as a measure of stress of living in intertidal wild areas compared to subtidal cultured ones, and the results showed, as predicted, that wild shell shapes (intertidal) showed higher FA levels than cultured ones.

Mussels are characterized by high phenotypic plasticity in response to environmental and ecological factors variation that impact on their shell morphology (Akester & Martel, 2000; Beadman et al., 2003; Steffani & Branch, 2003; Kirk et al., 2007; Funk & Reckendorfer, 2008; Cubillo et al., 2012). Therefore, it is not entirely clear to what extent the shape variation has a direct genetic basis and to what extent it is a consequence of the different environmental factors. According to Klingenberg et al. (2002) a prominent role of phenotypic plasticity may also explain the discrepancy in the patterns of covariation between the symmetric shape variation among individuals and FA. Thus, phenotypic plasticity in response to environmental conditions would cause a qualitative difference between the processes affecting the symmetric and asymmetric components of variation (Klingenberg et al., 2002).

We compared mussels FA levels from two sites that are environmentally and ecologically different: rocky intertidal (wild mussels) and subtidal longlines (cultured mussels). The intertidal rocky shores are fluctuating environments characterized by a wide range of physical conditions (Denny & Wethey, 2001), and particularly in the Patagonian coasts they are strongly structured by physical stress (Bertness et al., 2006). The more commonly studied physical factors that affect intertidal organisms are desiccation risk, salinity, high temperatures and wave exposure (Levinton, 2001). Regarding subtidal shallow waters, they present more homogeneous environmental conditions than the intertidal, resulting in less physical stress for the biodiversity (Rechimont, 2011). Another difference between these two environmental systems is that predation is greater in the subtidal (Márquez et al., 2015); however, the aquaculture structures (the culture system used in Chubut coasts are longlines) might act as shelter from sea stars, crabs or other invertebrate predators that cannot access to suspended lines. Differences relation found between intertidal samples of external and internal configuration could also be explained as the outcome of the different developmental instability from both sites subjected to different environmental conditions. While internal configuration focuses only on soft structures scars, external configuration provides an insight of outline shell shape; both modules could respond in a different way to the environmental stress. Individuals from Bahía Camarones were taken from the low intertidal zone with the presence of the green crab (*Carcinus maenas*) and wave protected area, while samples from Comodoro Rivadavia were taken from the middle intertidal zone dominated by other little scorched mussels, *Perumytilus purpuratus*, placed on a wave exposed area, and the presence of sea stars (*Anasteria minuta*). These environmental differences could be producing the inversion on the FA means, between internal and external configurations from wild samples shown in Fig. 3. As regards the patterns of the shell shape variation related to FA, our results showed that the maximum FA shell variation in the internal configuration was observed over the posterior adductor muscle scar, while in the external configuration the highest shape variation was in the elongation of the posterior region and the curvature of the antero-dorsal region. Our results were consistent with those of others studies, and suggest strong differences between

cultured and wild mussels populations. For example, Kirk et al. (2007) reported that shell morphology in *Mytilus* differed between cultivated and wild populations; likewise Valladares et al. (2010) found that diverse physiological conditions were closely related to shell shape variation as well as population habitat (cultured vs. wild). Another example is the one of Arranz et al. (2016) who found physiological differences associated with the habitat: the intertidal mussels put more effort on shell calcification and thickening, while subtidal mussels allocated energy resources preferably into flesh growth. Our results show that in the more stressful site (intertidal rocky shore) FA levels were higher, suggesting that FA levels in mussels shell shape are modulated by environmental differences. Other studies in Mytilids reported a relationship between FA and different environmental factors. For instance, Scalici et al. (2017) observed that individual asymmetry scores in *Mytilus galloprovincialis* showed higher values in polluted sites, and Lajus et al. (2015) reported lower FA values in *Mytilus* mussels from estuarine areas (lower salinity) compared to those from the Barents Sea (with oceanic salinity). In this sense, the close association between the environmental conditions and FA levels could be considered as an evidence of a direct effect of environmental stress on the *Mytilus* shell shape.

Whether FA is in fact a good measure of development instability and whether it correlates with exposure to adverse conditions is still in debate (Klingenberg, 2015). Our results reflect that intertidal wild mussels show higher FA levels, adding evidence that FA is a reliable measure of developmental instability (Graham et al., 1993). Under the paradigm that FA might also reflect indirectly a fitness-related quality, such as individual condition (Møller & Swaddle, 1997; Debat & David, 2001), these individuals are important as a source of variation to preserve biological diversity and should be considered in conservation programs. Moreover, *Mytilus platensis* is a commercially relevant species and the knowledge of the different environmental, ecological and physiological variables that affect its shell shape is vital to assemble the puzzle of their biological diversity.

Compliance with ethical standards

Conflict of interest All scholars immediately involved, MMT, SVdM and FM have approved the manuscript and declared that they have no conflict of interest regarding to this work.

Ethical approval All applicable international, national, and/ or institutional guidelines for the care and use of animals were followed.

References

Akester, R. J. & A. L. Martel, 2000. Shell shape, dysodont tooth morphology, and hinge-ligament thickness in the bay mussel *Mytilus trossulus* correlate with wave exposure. Canadian Journal of Zoology 78(2): 240–253.

Alunno-Bruscia, M., E. Bourget & M. Fréchette, 2001. Shell allometry and length-mass-density relationship for *Mytilus edulis* in an experimental food-regulated situation. Marine Ecology Progress Series 219: 177–188.

Arranz, K., U. Labarta, M. J. Fernández-Reiriz & E. Navarro, 2016. Allometric size-scaling of biometric growth parameters and metabolic and excretion rates. A comparative study of intertidal and subtidal populations of mussels (*Mytilus galloprovincialis*). Hydrobiologia 772(1): 261–275.

Astorga, M. P., L. Cardenas & J. Vargas, 2015. Phylogenetic approaches to delimit genetic lineages of the *Mytilus* complex of South America: how many species are there? Journal of Shellfish Research 34(3): 919–930.

Beadman, H., R. Caldow, M. Kaiser & R. Willows, 2003. How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. Marine Biology 142(3): 487–494.

Beardmore, J. A., 1960. Developmental stability in constant and fluctuating temperatures. Heredity 14(3/4): 411–422.

Bertness, M. D., C. M. Crain, B. R. Silliman, M. C. Bazterrica, M. Reyna, F. Hildago & J. Farina, 2006. The community structure of western Atlantic Patagonian rocky shores. Ecological Monographs 76(3): 439–460.

Bookstein, F. L., 1991. Morphometric Tools for Landmark Data. Cambridge University Press, New York.

Briones, C. & R. Guiñez, 2005. Bilateral asymmetry of shell shape and spatial position in matrices of the mussel *Perumytilus purpuratus* (Lamarck, 1819) (Bivalvia: Mytilidae). Revista Chilena de Historia Natural 78(1): 3–14.

Conover, W. J., 1999. Practical Nonparametric Statistics. Wiley, New York.

Cubillo, A. M., L. G. Peteiro, M. J. Fernández-Reiriz & U. Labarta, 2012. Influence of stocking density on growth of mussels (*Mytilus galloprovincialis*) in suspended culture. Aquaculture 342: 103–111.

Debat, V. & P. David, 2001. Mapping phenotypes: canalization, plasticity and developmental stability. Trends in Ecology & Evolution 16(10): 555–561.

Denny, M. & D. Wethey, 2001. Physical processes that generate patterns in marine communities. In Bertness, M., M. Hay & S. Gaines (eds), Marine community ecology. Sinauer Press, New York.

Dongen, S. V., 2006. Fluctuating asymmetry and developmental instability in evolutionary biology: past, present and future. Journal of Evolutionary Biology 19(6): 1727–1743.

Ducos, M. B. & S. R. Tabugo, 2015. Fluctuating asymmetry as bioindicator of stress and developmental instability in *Gafrarium tumidum* (ribbed venus clam) from coastal areas of Iligan Bay, Mindanao, Philippines. Aquaculture, Aquarium, Conservation & Legislation-International Journal of the Bioflux Society (AACL Bioflux) 8(3): 292–300.

Dryden, I. L. & K. V. Mardia, 1998. Statistical Analysis of Shape. Wiley, New York.

Escoufier, Y., 1973. Le traitement des variables vectorielles. Biometrics 29: 751–760.

Funk, A. & W. Reckendorfer, 2008. Environmental heterogeneity and morphological variability in *Pisidium subtruncatum* (Sphaeriidae, Bivalvia). International Review of Hydrobiology 93(2): 188–199.

Graham, J. H., D. C. Freeman & J. M. Emlen, 1993. Developmental stability: a sensitive indicator of populations under stress. In Landis, Wayne G., Jane S. Hughes & Michael A. Lewis (eds), Environmental toxicology and risk assessment. ASTM STP 1179. American Society for testing and Material, Philadelphia: 136–158.

Graham, J. H., S. Raz, H. Hel-Or & E. Nevo, 2010. Fluctuating asymmetry: methods, theory, and applications. Symmetry 2(2): 466–540.

Good, P., 2000. Permutation Test: A Practical Guide to Resampling Methods for Testing Hypothesis, 2nd ed. Springer, New York.

Goodall, C., 1991. Procrustes methods in the statistical analysis of shape. Journal of the Royal Statistical Society: Series B, Statistical Methodology 53(2): 285–339.

Gunz, P. & P. Mitteroecker, 2013. Semilandmarks: a method for quantifying curves and surfaces. Hystrix 24(1): 103–109.

Hosken, D. J., W. U. Blanckenhorn & P. I. Ward, 2000. Developmental stability in yellow dung flies (*Scathophaga stercoraria*): fluctuating asymmetry, heterozygosity and environmental stress. Journal of Evolutionary Biology 13(6): 919–926.

Kirk, M., D. Esler & W. S. Boyd, 2007. Morphology and density of mussels on natural and aquaculture structure habitats: implications for sea duck predators. Marine Ecology Progress Series 346: 179–187.

Klingenberg, C. P., 2009. Morphometric integration and modularity in configurations of landmarks: tools for evaluating a priori hypotheses. Evolution and development 11(4): 405–421.

Klingenberg C.P., 2011. MorphoJ: an integrated software package for geometric morphometrics. Molecular Ecology Resources 11: 353–357.

Klingenberg, C. P., 2015. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. Symmetry 7(2): 843–934.

Klingenberg, C. P. & G. S. McIntyre, 1998. Geometric morphometrics of developmental instability: analyzing patterns of fluctuating asymmetry with Procrustes methods. Evolution 52(5): 1363–1375.

Klingenberg, C. P., M. Barluenga & A. Meyer, 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. Evolution 56(10): 1909–1920.

Klingenberg, C. P. & J. Marugán-Lobón, 2013. Evolutionary covariation in geometric morphometric data: analyzing integration, modularity, and allometry in a phylogenetic context. Systematic biology 62(4): 591–610.

Lajus, D., M. Katolikova, P. Strelkov & H. Hummel, 2015. Fluctuating and directional asymmetry of the blue mussel (*Mytilus edulis*): improving methods of morphological analysis to explore species performance at the northern border of its range. Symmetry 7(2): 488–514.

Leamy, L., 1984. Morphometric studies in inbred and hybrid house mice. V. Directional and fluctuating asymmetry. The American Naturalist 123(5): 579–593.

Levinton, J. S., 2001. Marine Biology: Function, Biodiversity, Ecology. Oxford University Press, New York.

Manly, B. F. J., 2007. Randomization, Bootstrap and Monte Carlo Methods in Biology. Chapman & Hall/CRC, Boca Raton.

Mardia, K. V., F. L. Bookstei & I. J. Moreton, 2000. Statistical assessment of bilateral symmetry of shapes. Biometrika 87(2): 285–300.

Markow, T. A., 1995. Evolutionary ecology and developmental instability. Annual Review of Entomology 40(1): 105–120.

Márquez, F., J. Robledo, G. E. Peñaloza & S. Van der Molen, 2010. Use of different geometric morphometrics tools for the discrimination of phenotypic stocks of the striped clam *Ameghinomya antiqua* (Veneridae) in north Patagonia, Argentina. Fisheries Research 101(1–2): 127–131.

Márquez, F., R. A. NietoVilela, M. Lozada & G. Bigatti, 2015. Morphological and behavioral differences in the gastropod *Trophon geversianus* associated to distinct environmental conditions, as revealed by a multidisciplinary approach. Journal of Sea Research 95: 239–247.

Márquez, F., M. Adami, B. Trovant, R. A. Nieto Vilela & R. González-José, 2018. Allometric differences on the shell shape of two scorched mussel species along the Atlantic South America coast. Evolutionary Ecology. 32(1): 43–56.

McDonald, J. H., R. Seed & R. K. Koehn, 1991. Allozymes and morphometric characters of three species of *Mytilus* in the Northern and Southern Hemispheres. Marine Biology 111(3): 323–333.

Møller, A. P. & J. P. Swaddle, 1997. Asymmetry, Developmental Stability and Evolution. Oxford University Press, UK.

Monteiro, L. R., 1999. Multivariate regression models and geometric morphometrics: the search for causal factors in the analysis of shape. Systematic Biology 48(1): 192–199.

Monteiro, L. R., J. A. F. Diniz-Filho, S. F. dos Reis & E. D. Araújo, 2002. Geometric estimates of heritability in biological shape. Evolution 56(3): 563–572.

Palmer, A. R., 1994. Fluctuating asymmetry analyses: A primer. In Markow, T. A. (ed.), Developmental Instability: Its Origins and Evolutionary Implications. Kluwer, Dordrecht: 335–364.

Palmer, A. R. & C. Strobeck, 1986. Fluctuating asymmetry: measurement, analysis, patterns. Annual Review of Ecology and Systematics 17(1): 391–421.

Rechimont M. E., 2011. Patrón de distribución y abundancia de invertebrados bentónicos en costas del Golfo Nuevo. Dissertation, Universidad Nacional de la Patagonia San Juan Bosco, Puerto Madryn.

Ricklefs, R. E. & D. B. Miles, 1994. Ecological and evolutionary inferences from morphology: an ecological perspective. In Wainwright, R. C. & S. M. Reilly (eds), Ecological morphology: integrative organismal biology. University of Chicago Press, Chicago: 13–41.

Rohlf F. J., 2017a. TPSDig2, version 2.30. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf F. J., 2017b. TPSUtil, version 1.74. Stony Brook, NY: Department of Ecology and Evolution, State University of New York.

Rohlf F. J., 2017c. TpsRelw v. 1.67. Ecology and Evolution, SUNY, Stony Brook.

Rohlf, F. J. & D. Slice, 1990. Extensions of the Procrustes method for the optimal superimposition of landmarks. Systematic Biology 39(1): 40–59.

Savriama, Y. & C. P. Klingenberg, 2011. Beyond bilateral symmetry: geometric morphometric methods for any type of symmetry. BMC Evolutionary Biology 11(1): 280.

Scalici, M., L. Traversetti, F. Spani, V. Malafoglia, M. Colamartino, T. Persichini & M. Colasanti, 2017. Shell fluctuating asymmetry in the sea-dwelling benthic bivalve *Mytilus galloprovincialis* (Lamarck, 1819) as morphological markers to detect environmental chemical contamination. Ecotoxicology 26(3): 396–404.

Seed, R., 1968. Factors influencing shell shape in the mussel *Mytilus edulis*. Journal of the Marine Biology Association of the United Kingdom 48(03): 561–584.

Slice, D. E., F. L. Bookstein, L. E. Marcus & F. J. Rohlf, 1996. A glossary for geometric morphometrics. In Marcus, L. E., M. Corti, A. Loy, G. J. P. Naylor & D. Slice (eds), Advances in Morphometrics. Plenum Press, New York: 531–552.

Smith, D. R., B. J. Crespi & F. L. Bookstein, 1997. Fluctuating asymmetry in the honey bee, *Apis mellifera*: effects of ploidy and hybridization. Journal of Evolutionary Biology 10(4): 551–574.

Steffani, C. N. & G. M. Branch, 2003. Growth rate, condition, and shell shape of *Mytilus galloprovincialis*: responses to wave exposure. Marine Ecology Progress Series 246: 197–209.

Valladares, A., G. Manríquez & B. A. Suárez-Isla, 2010. Shell shape variation in populations of *Mytilus chilensis* (Hupe 1854) from southern Chile: a geometric morphometric approach. Marine Biology 157(12): 2731–2738.

Van Valen, L., 1962. A study of fluctuating asymmetry. Evolution 16: 125–142.

Westfall, K. M. & J. P. Gardner, 2010. Genetic diversity of Southern hemisphere blue mussels (Bivalvia: Mytilidae) and the identification of non-indigenous taxa. Biological Journal of the Linnean Society of London 101(4): 898–909.

Weyl H., 1989. Symmetry. Reprint of the 1952 original. Princeton Science Library.

Zaixso H. E. & A. Boraso de Zaixso, 2015. Recursos biológicos bentónicos: la Zona Costera Patagónica Argentina. Universitaria de la Patagonia—EDUPA, Comodoro Rivadavia.

Zar, J. H., 1999. Biostatistical Analysis. Prentice, Hill.

Zbawicka, M., M. I. Trucco & R. Wenne, 2018. Single nucleotide polymorphisms in native South American Atlantic coast populations of smooth shelled mussels: hybridization with invasive European *Mytilus galloprovincialis*. Genetic Selection Evolution 50(1): 5.