Habitat-modulated shell shape and spatial segregation in a Patagonian false limpet (*Siphonaria lessonii*)

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ABSTRACT: Environmental stressors that characterize intertidal habitats, such as exposure to air, thermal amplitude and wave action, influence biological patterns of distribution. The effects of these stressors on intertidal organisms are often associated with their shape and size. Rocky intertidal shores in Patagonia are particularly stressful environments in which a species of false limpet occurs abundantly in 2 contrasting habitats. Here we describe size and shape segregation of the false limpet *Siphonaria lessonii* across multiple shores on 2 contrasting shore levels where limpets are abundant, namely the high (HT) and mid (MT) intertidal. We tested for differences in resistance to desiccation, and recovery from heat stress, and were able to define 2 ecomorphs using classic and geometric morphometrics analysis. Individuals from the HT lost significantly less water under stressful heat conditions, and showed higher recovery rates than those from the MT. Limpets from the HT had a broad aperture, flatter margin and larger size. In contrast, individuals from the MT had a narrow aperture, arched margin and smaller size. Together these results suggest the existence of 2 ecomorphs modulated by each contrasting habitat. The observed small-scale spatial segregation of the 2 ecomorphs may provide ecological advantages by allowing the exploitation of resources from 2 different habitats. The 2 ecomorphs may provide *S. lessonii* with a broad thermal tolerance breadth, potentially improving resistance of the species to extreme climatic events.

KEY WORDS: Intertidal · Rocky shores · *Siphonaria* · Heat stress

INTRODUCTION

Temperate marine intertidal shores are physically stressful habitats for flora and fauna to develop. Environmental stressors that characterize intertidal habitats, such as exposure to air, thermal amplitude and wave action, often influence biological patterns of distribution (Underwood 1986, Menge 1992). Tolerance to stressors and specialization of coping strategies tend to determine the positioning of species along environmental gradients (Legendre et al. 1997, Thomas et al. 2001). Vertical zonation is a relatively common pattern on rocky intertidal habitats, and resident species have attained physiological, behav-

ioural and morphological strategies to cope with the local environmental stressors. Furthermore, local adaptation can occur on small spatial scales where post-settlement selection processes can structure tolerance limits (Sanford & Kelly 2011).

The shape and size of marine organisms may determine functional limits in organism responses to environmental factors. The shape of marine molluscs may be determined, among other factors, by biological processes such as ontogenetic changes (Vermeij 1980, Kemp & Bertness 1984, Márquez et al. 2010, 2018), adaptation to an environment (Grahame et al. 1990, Johannesson & Johannesson 1996, Johannesson et al. 1997, Denny & Wethey 2000, Márquez et al. 2015,

2017) or long-term evolutionary processes (Serb et al. 2011, Trovant et al. 2018). On intertidal marine shores, exposure to waves, substrate type, thermal amplitude and desiccation are some of the factors that have been associated with the shape and size of organisms (Atkinson & Sibly 1997, Keough et al. 1997, Alfaro & Carpenter 1999, Carvajal-Rodríguez et al. 2005, Márquez et al. 2015). In turn, the amalgamation of physiological, behavioural and morphological responses to the previously mentioned factors will determine species distribution boundaries across environmental gradients (Brown 1984). Hence, the study of shapes in biological organisms can help provide an explanation for the observed ecological patterns on rocky shores (Johannesson 2016).

Intraspecific competition has been described for limpets on several temperate rocky coasts, with contrasting findings. On temperate eastern Australian shores, Marshall & Keough (1994) showed that small limpets had a stronger effect on large conspecifics than the inverse relationship. Keough et al. (1997) later supported this finding and added that competitive superiority of small limpets was spatially variable and potentially related to the texture of the rock surface. On Portuguese rocky shores, Boaventura et al. (2003) found that large size classes of limpets had a stronger effect on small size classes than the reverse, and that the former may modulate the abundance of the latter on rocky shores. Hence, competition can lead to lower abundance of the weaker competitor, but it can also lead to spatial segregation, which reduces competitive interactions. The spatial segregation of size classes within a species reduces intraspecific competition through a differential use of available resources, and adaptation to variable habitats. Furthermore, intraspecific variation in gastropods can lead to 'morphs' that differentially use the limited resources (Johannesson & Johannesson 1996). For example, *Nacella concinna* in Antarctic waters displays 2 morphs: an intertidal–shallow subtidal (upper littoral to 4 m depth) morph and a deep subtidal (>4 m to 110 m) morph (Davenport 1988). Different ecomorphs of *Littorina saxatilis* and *N. lapillus* have been described for contrasting shore levels and exposure (Carvajal-Rodríguez et al. 2005, Conde-Padín et al. 2007, Guerra-Varela et al. 2009).

Rocky intertidal environments in the south-western Atlantic (Patagonia) are exposed to unusually harsh physical stressors which strongly structure the intertidal communities (Bertness et al. 2006). There is a marked vertical zonation comprised of 3 zones with contrasting characteristics in which species' tolerance limits are likely responsible for the observed patterns (Wolcott 1973). Predation is scarce, with little effect on intertidal community structure (Hidalgo et al. 2007). The high intertidal zone is dominated by bare rock and barnacles, the mid intertidal zone is covered by a complex matrix of tiny scorched mussels (*Brachidontes rodriguezii* and *Perumytilus purpuratus*), and the low intertidal zone is dominated by articulated coralline algae (Bertness et al. 2006, Raffo et al. 2014). Several gastropod species that are part of the intertidal community tend to be abundant within a particular zone, but are scarce in the others. However, the pulmonate limpet *Siphonaria lessonii* abundantly occurs in the high and mid intertidal zones, despite stark differences between habitats (Olivier & Penchaszadeh 1968, Tablado & López Gappa 2001).

A comprehensive revision of the *Siphonaria* species that occur in southern South America described *S. lessonii* as the only siphonarid to be found north of 47° S on the Atlantic coast (Güller et al. 2016). A recent study of shape and size of *S. lessonii* populations across a broad latitudinal range suggests there may be changes in shape with increased latitude; however, a single intertidal level was sampled, and only large individuals were considered (Soria et al. 2017). Shape and growth of *S. lessonii* have been described as being spatially variable (Tablado et al. 1994, Tablado & López Gappa 2001, Nuñez et al. 2012). Size was the main morphologic variable that differed among habitats in which it was sampled, whilst shore level, nutrient input, intraspecific competition, food availability and pollution were mentioned as environmental factors that may explain the observed differences (Tablado & López Gappa 2001). Shore level has important intrinsic characteristics related to desiccation such as air exposure time, thermal amplitude, microhabitats and community composition that potentially exert limits of limpet distribution within the intertidal.

In this study, we compared shell size of the Patagonian false limpet *S. lessonii* across multiple shores in Golfo Nuevo on 2 contrasting shore levels where limpets are abundant, namely the high (HT) and mid (MT) intertidal levels. Subsequently, we tested the hypothesis that exposure of limpets to elevated temperatures leads to individuals from HT experiencing lower desiccation and higher recovery rates than those from MT. Furthermore, we tested the hypothesis that shell shapes from the HT and MT will differ regardless of size. Such evidence would support the model that limpets develop shapes and physiological tolerances associated with the intertidal level that they inhabit. If correct, this model would help explain shore level distribution of limpets on rocky shores.

MATERIALS AND METHODS

Fieldwork and size segregation

Siphonaria lessonii individuals were collected from 5 intertidal rocky shores during low tides: Cerro Prismático (CP), Casino (CA), Punta Ameghino (PA), Punta Este (PE) and Ambrosetti (AM) within the Golfo Nuevo, Patagonia, Argentina (Fig. 1). Tides are semidiurnal with tidal amplitude of ~4 m. Shore slope was similar at the studied locations, and air exposure time was 8–10 and 6–7 h in the HT and MT levels, respectively. Water and air temperature yearly ranges are 8–18°C and −0.9–41.5°C, respectively (data for 2017; meteorologia.appm.com.ar). All locations have mudstone substrate with the characteristic Patagonian zonation where the HT comprises mainly bare rock and the invasive barnacle *Balanus glandula*. The MT is almost completely covered by a single-layered bed of *Brachidontes rodriguezii* and *Perumytilus purpuratus* mussels, which make up the so-called 'tiny scorched mussel matrix'.

At each location, individuals were randomly collected from the HT and MT levels (n = 100 ind. level^{-1} location^{-1}). All specimens were measured (total length and width) *in situ* with a digital calliper to the nearest 0.1 mm and then returned to their original collection area. Length and width showed a strong linear relationship at all locations (R^2 = 0.9801, p < 0.0001), thus width was excluded from analysis. Length frequency distributions between levels at each location were compared with Student's *t*-test.

Desiccation and heat stress recovery experiments

Maximum temperature during summer days in Puerto Madryn (≤15 km from the sampled shores) can exceed 40°C, and is usually accompanied by strong westerly winds (e.g. maximum temperature: 41.5°C, maximum wind speed: 95 km h^{-1}, 27 January 2017; meteorologia.appm.com.ar). Hence, desiccation and heat stress recovery was tested at 40°C. Individuals from the HT and MT levels were randomly collected at CA and taken for acclimation to aquaria at the CENPAT experimental aquarium, Puerto Madryn. After 3 d of acclimation, individuals were wet-weighed (total weight before heat exposure, TW$_1$) after blotting on tissue, and carefully placed upright into open plastic chambers. The trays with the open chambers were placed in a pre-heated oven at 40°C. Controlled lab experiments present different conditions to those experienced by the individuals in the field and thus cannot be directly extrapolated (Camus & Lima 1995, Petersen et al. 2003). The current experiment did not include the strong dry winds associated with the extreme temperatures replicated in this study. Hence, it is likely our results and interpretations are conservative.

Desiccation experiment

Individuals from the HT and MT levels (n = 50 ind. level^{-1}) were removed from the oven after 2 h of heat exposure, dissected and weighed. Total wet weight (TW$_2$),
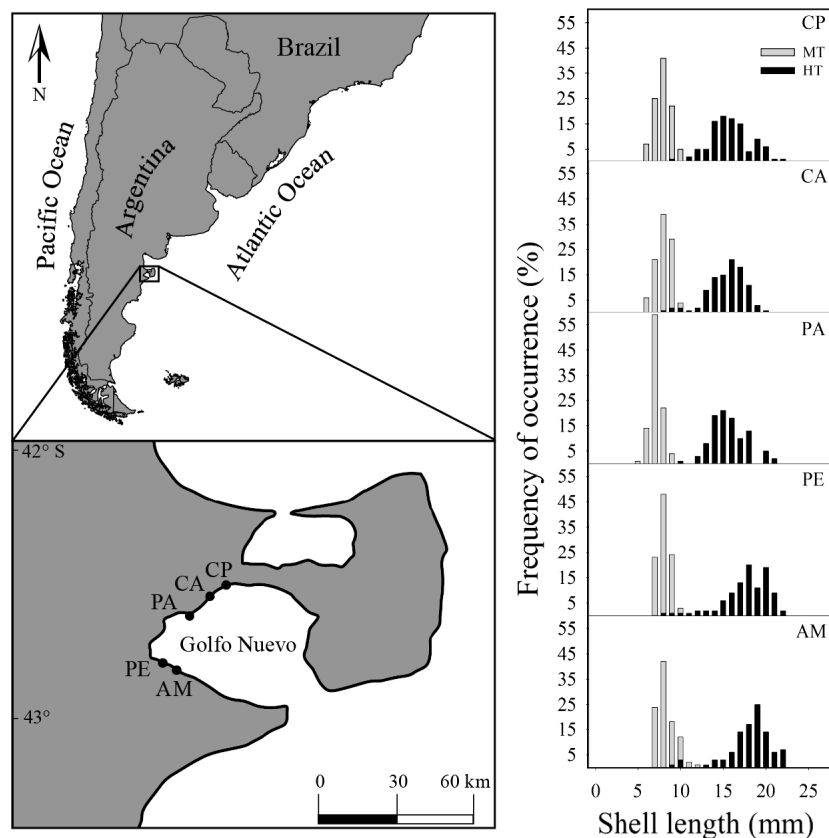

Fig. 1. (Right) Study area and sites in the Golfo Nuevo, Patagonia, Argentina. CP: Cerro Prismático, CA: Casino, PA: Punta Ameghino, PE: Punta Este, AM: Ambrosetti. (Left) Histograms show the size frequency distribution of *Siphonaria lessonii* limpets at the 5 study sites in 2 intertidal zones (HT: high intertidal, MT: mid intertidal)

shell weight (SW) and body mass (BM_2) after exposure were recorded. Body mass before exposure was calculated as: $BM_1 = TW_1 - SW$. Water loss percentage was calculated as the difference between body mass before and after heat exposure: $\%WL = BM_2 / BM_1 \times 100$. A Student's *t*-test was used to test for differences in %WL between individuals from HT and MT levels; a square-root transformation of data was necessary to comply with the assumption of homogeneity of variance.

Heat stress experiment

Individuals from the HT and MT levels (n = 30 ind. $level^{-1}$) were removed after 2 h of heat exposure (as above), weighed and immediately placed in trays with seawater at 14°C; the exact position and orientation of each individual was registered at this time (T_0). The thermal shock would be similar to that experienced by individuals on an intertidal platform upon a rising tide in summer. *S. lessonii* individuals were considered to have recovered from heat stress when their position or orientation had changed from that of T_0. The number of individuals recovered from each tidal level was registered at 4 time intervals: 30, 60, 120 and 720 min (T_1, T_2, T_3 and T_4, respectively); after 720 min, the number of individuals that did not recover was also registered. Potential manipulation artefacts were tested for by removing control individuals (n = 10 ind. tidal $level^{-1}$) from acclimation aquaria, wet-weighing and placing individuals in open plastic chambers before they were placed in trays with seawater. All control individuals from both intertidal levels recovered (i.e. changed orientation or position) within 5 min, hence, manipulation artefacts were considered negligible. The difference in recovery time frequency distributions between tidal levels (HT vs. MT) was compared with a chi-squared test.

Shape segregation

Three sites (CP, CA and PA) with similar geophysical characteristics (i.e. orientation, tidal height and substrate) were selected to compare shell shape of *S. lessonii* individuals from the HT and MT levels. Fifty individuals from each level at each location were randomly collected and stored frozen at −18°C for shell geometric morphometric analysis. The shells were dissected, cleaned and dried, and then numbered and photographed in right lateral view. Prior to taking the photographs, shells were fixed to a plas-

ticine base to prevent pitching and rolling effects, because these effects cannot be mathematically standardized, and can therefore influence the dataset (Zeldicht et al. 2004). Shell overall outline shape was captured by Cartesian coordinates of a 2D-configuration of 3 landmarks and 21 semi-landmarks (for anatomical locations of the landmarks, see Fig. 2). The exact locations of semi-landmark coordinates on the curve cannot be identified, and hence was mathematically estimated. Sliding was performed using the algorithm which optimizes the bending energy (Bookstein 1997, Gunz et al. 2005, Gunz & Mitteroecker 2013), to minimize the deformation between each specimen and the consensus shape (Procrustes average of all specimens) in the TPSRelw software (Rohlf 2004). After sliding the semi-landmarks, all 24 landmark configurations were superimposed by a generalized Procrustes analysis to remove non-shape variation (Rohlf & Slice 1990, Slice et al. 1996). This procedure rotates and translates the landmark configurations to a common origin and scales them to unit centroid size. Centroid size (CS) was calculated as a proxy to size (Zelditch et al. 2004), and it is the square root of the sum of the squared distances from the landmarks to the centroid which they define. The presence of allometry (the shape change exclusively explained by variations in size) was tested by a multivariate regression analysis (pooled within levels) for each site, between shell shape scores as a dependent variable (aligned Procrustes coordinates) and size as an independent variable (CS), using MorphoJ v1.06d (Klingenberg 2011). Allometry-free shape variables were analysed with a principal component analysis (PCA) of the variance-covariance matrix, to display the magnitude and direction of shape variation for each site. We then performed a discriminant analysis (DA) to visualize and test the maximum separa-
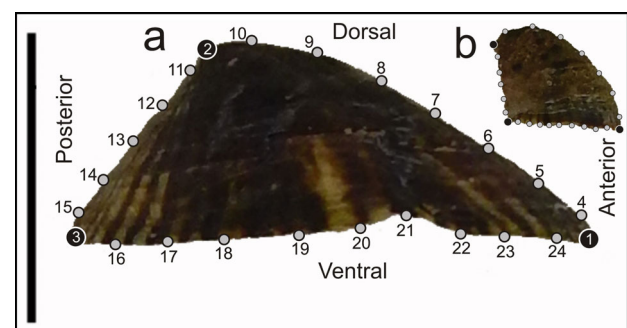

Fig. 2. Landmark (black dots) and semi-landmark (grey dots) configurations used to capture the contour of the shell of *Siphonaria lessonii*: (a) high intertidal morph, (b) mid intertidal morph. Scale bar = 1 cm

tions of the shell shapes between HT and MT of each site. We estimated the readability of discrimination, and performed a leave-one-out cross-validation procedure. Finally, to evaluate the difference in shell shapes from all sites simultaneously, we proceeded to a Hotelling's T^2 with permutation test (1000 permutation runs) between HT and MT shell shapes. All statistical analyses related to geometric morphometrics were made in MorphoJ v.1.06d (Klingenberg 2011).

RESULTS

Size segregation

Size frequency distributions of *Siphonaria lessonii* from the HT and MT levels were significantly different at all locations (Table 1). The HT individuals were larger than the MT individuals consistently across all locations (Fig. 1). Length of individuals from the HT was more than double that of the MT (n = 500 level^{-1}; mean ± SD: HT: 16.05 ± 2.65 mm, MT: 7.40 ± 0.95 mm).

Desiccation and heat stress recovery experiments

S. lessonii individuals from the HT lost significantly less water than those from the MT after heat exposure (t = 6.987, p < 0.001, df = 98). Water loss percentage of individuals from the HT was on average 17% less than that of individuals from the MT (HT: 32.23 ± 9.66% [mean ± SD], MT: 49.08 ± 12.61%; n = 50 level^{-1}).

Individuals from the HT recovered faster from heat stress than those from the MT (χ^2 = 13.22, p = 0.0103, df = 4). Furthermore, the percentage of limpets from the MT that had not recovered 12 h after submersion was 3-fold that of the HT (57 vs. 17%).

Table 1. Student's *t*-test results for comparison of (a) size frequency distribution of *Siphonaria lessonii* individuals from high (HT) vs. mid intertidal (MT) levels. CP: Cerro Prismático; CA: Casino; PA: Punta Ameghino; PE: Punta Este; AM: Ambrosetti

Site	t	p	df
CP	31.08	<0.001	198
CA	30.69	<0.001	198
PA	39.34	<0.001	198
PE	34.19	<0.001	198
AM	35.75	<0.001	198

Shape segregation

For CA and PA sites, allometric regression between shape coordinates and CS were significant (p < 0.05), and accounted for 6.94 and 2.76% of the total shape variation, respectively. Thus subsequent statistical analyses were performed with the residuals of the regression, considered as new allometry-free shape variables. The scatterplot for the first 2 PCs and the pattern of shell shape variations for each site are summarized in Fig. 3. The individuals from the 3 sites present the same patterns of ordination in the morphospace with the PC1 axis representing the shell variations between the HT and MT levels. The geometric interpretations of the negative values of the PC1 (mean of 3 sites = 59.43% of the total shell shape variations) were related to higher shells with narrower aperture in lateral view (antero-posterior restriction). The opposite shell change was registered towards positive values of PC1 with a broader aperture in the right lateral view (antero-posterior expansion with depressed shell, Fig. 3). In concordance, the discriminant analysis (DA) between the HT and MT levels showed the same patterns of separation for shell shape (Fig. 4). The mean shell shapes from each HT individual presented depressed (dorsal ventral restriction) and more antero-posterior expanded shells with a channel in the right anterior margin of the shell, whilst in the MT individuals this channel was absent (Fig. 4). Concurrently, the HT shells had a relatively straight right lateral margin, whilst the MT shells presented a slightly arched lateral margin. The cross-validated classification analysis showed that on average for the 3 sites, 98.63% of specimens were correctly classified to the appropriate intertidal level. All mean shell shape pair comparisons between the HT and MT levels were statistically significant (Table 2). Comparison of mean shell shape between HT and MT from all sites simultaneously indicated significant differences in shape between them (Hotelling's T^2: 2958.25, Mahalanobis distance: 6.27, p <0.0001).

DISCUSSION

Our results support the model that size and shape of *Siphonaria lessonii* false limpets are directly related to the habitat they live in. This was evidenced by the size segregation described and supported by the presence of 2 ecomorphs: the HT morph and the MT morph. The morphometric differences were associated with desiccation tolerance levels and sub-
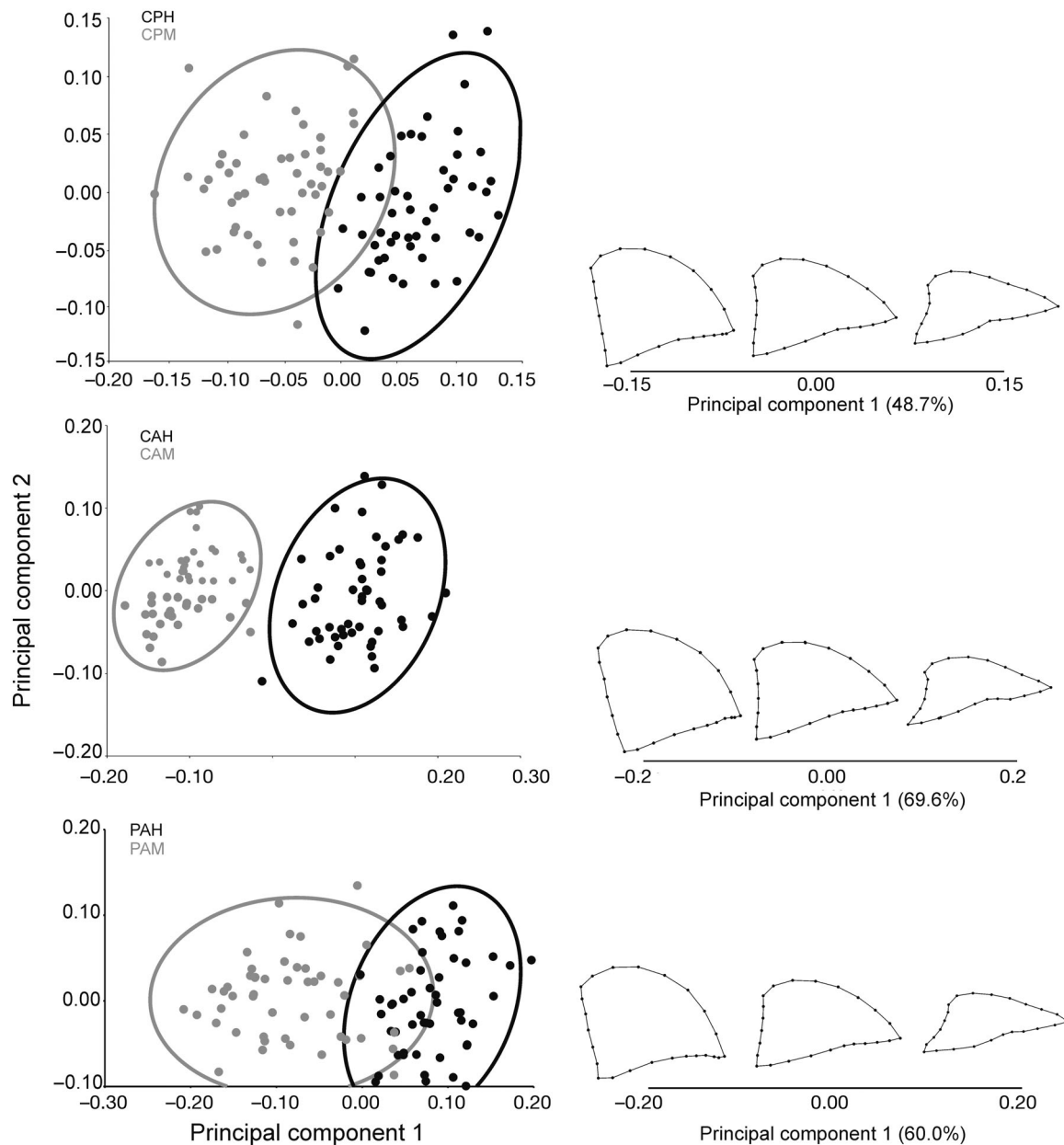
Fig. 3. Left column: principal components (PCs) of the shell shape variations of *Siphonaria lessonii* at 3 study sites (CP: Cerro Prismático, CA: Casino, PA: Punta Ameghino) for different levels of the intertidal (H: high, M: mid intertidal). Right panels: transformation and displacement vectors from the overall mean shape (center) to the positive (high intertidal) and negative (mid intertidal) extreme shape. Percentage variance explained for the first PC axis is given in parentheses

strate types (i.e. bare flat rock and tiny-mussel beds) that define each habitat.

Previous studies on limpet species have found a broad range of morphological responses to vertical intertidal gradients (Branch 1981, Harley et al. 2009, Miller et al. 2009). In many cases, larger species or larger specimens of a single species occupy higher intertidal areas than smaller ones (Branch 1981, 1984).

This size segregation has often been attributed to desiccation tolerance related to size differences, but little information is available on shape variation (but see Lowell 1984). The shape, not only the size, may be exerting an effect that has been overlooked, particularly within the same species. The geometric morphometrics analysis in the current study recognizes and describes the direction and magnitude of shape
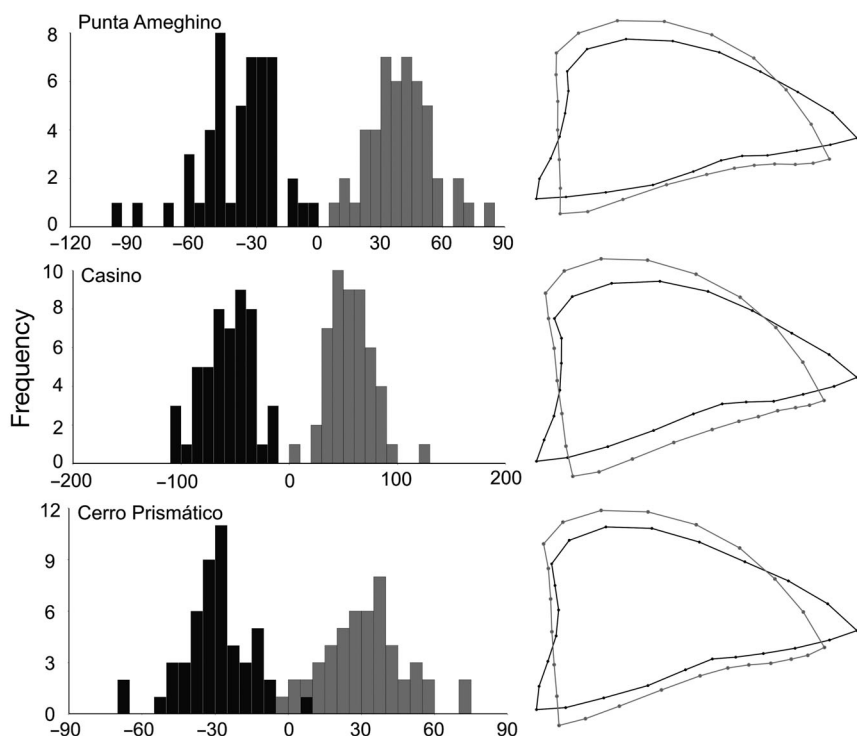
Fig. 4. Discriminant analysis of shell shape differences of *Siphonaria lessonii* between high (black) and mid intertidal (grey) locations at 3 study sites. Left column: discriminant scores predicted by a jackknife (leave-one-out) cross-validation. Right column: representations of the differences between the mean shell shape of *S. lessonii* at high and mid intertidal sites

Table 2. Cross-validated classification showing the number of individuals correctly assigned to each intertidal level shape, correct percentages, and differences between means and Mahalanobis distances (D^2) of each site. HT: high intertidal, MT: mid intertidal

Level	HT	MT	Total	Correct percentage	Hotelling's T^2	D^2
Cerro Prismatico					<0.0001	7.85
HT	49	1	50	97.96		
MT	2	48	50	95.83		
Casino					<0.0001	10.80
HT	50	0	50	100.00		
MT	0	50	50	100.00		
Punta Ameghino					<0.0001	8.98
HT	50	0	50	100.00		
MT	1	49	50	97.96		

adaptations of the 2 ecomorphs. Individuals from the HT morph were larger with flat lateral margins. The HT level is a flat smooth mudstone surface with very little cover of algae or invertebrates (Rechimont et al. 2013, Raffo et al. 2014). Flat lateral margins would allow for a fitted seal to the substrate (i.e. flat mud-

stone) during exposure to air and intense winds that are frequent in the study zone. In contrast, shells from the MT morph were small with arched lateral margins. The lack of flat broad shells in the MT level suggests that the aperture shape of MT morphs is likely determined by the contours of the mussels they live on. Matching margins and substrate could allow each ecomorph to obtain a better seal through a meniscus of water created between them consequently reducing desiccation stress (Lowell 1984). Thus, the shape of the lateral margin is likely related to the available substrate in each intertidal zone.

The spatial segregation of ecomorphs and sizes likely reduces intraspecific competition by means of local adaptation to different habitats, and allows each ecomorph to perpetuate within them. The absence of small individuals from all HT levels sampled suggests that settlement may occur in the MT level. Mussel beds that dominate the MT level provide more suitable conditions for settlers than the HT level, such as moisture retention (Underwood 1979), protection from predators (Underwood et al. 1983, Fairweather 1985) and less exposure time to air. If early settlement occurs in the MT level, it is unclear and beyond the scope of this study what process drives migration of some individuals to the HT level. Studies on recruitment, ageing and growth of *S. lessonii* are needed to elucidate these processes on Patagonian rocky shores.

The described ecomorphs, with distinctive thermal tolerances, may have further implications on species distribution in the context of climate change. As extreme climatic events are growing in intensity and frequency (Meehl & Tebaldi 2004, Kerr 2011), ensuing species distribution range changes have been reported (Poloczanska et al. 2013, Wernberg et al. 2013, Bennett et al. 2015). Distribution ranges can be determined by thermal-safety ranges which in turn may be influenced by local adaptation/acclimatization (Bennett et al. 2015). Hence, the susceptibility of organisms to changes in the environment, such as ambient thermal amplitude is of great interest. In this study, one of the ecomorphs was tolerant to extreme temperatures, whilst the other was not. If such tolerance is a result of local adaptation and acclimatization, then the species

would benefit from a broader thermal tolerance breadth potentially improving resistance of the species to extreme climatic events.

We have shown that differences within the same species — such as 2 distinct ecomorphs that have different tolerance to desiccation — may lead to spatial segregation. On a small spatial scale, segregation provides ecological advantages to the species, by allowing it to use a range of habitats and resources with the ensuing reduction of intraspecific competition. Branch (1984) stated that competition between species is often assumed to exist, and that differences between species may facilitate spatial segregation among habitats. This concept may be applied to the current study for a single species on a small spatial scale. Size and shape features of the 2 ecomorphs described here for *S. lessonii* allow limpets to thrive within a range of contrasting habitats, thereby differentially exploiting resources of each habitat and potentially reducing intraspecific competition within the extreme environmental conditions of Patagonian rocky shores.

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