Reference: *Biol. Bull.* **227:** 221–231. (December 2014)

Geographical Variation of Shell Thickness in the Mussel *Perumytilus purpuratus* Along the Southeast Pacific Coast

CAROLINA BRIONES[1,2]*, MARCELO M. RIVADENEIRA[3,4], MIRIAM FERNÁNDEZ[4], AND RICARDO GUIÑEZ[1]

[1]*Instituto de Ciencias Naturales Alexander von Humboldt, Facultad de Ciencias del Mar y de Recursos Biológicos, Universidad de Antofagasta, P.O. Box 170, Antofagasta, Chile;* [2]*Programa Doctorado en Ciencias Aplicadas mención Sistemas Marinos Costeros, Universidad de Antofagasta, Antofagasta, Chile;* [3]*Centro de Estudios Avanzados en Zonas Áridas (CEAZA), Universidad Católica del Norte, Coquimbo, Chile; and* [4]*Centro de Estudios Avanzados en Ecología & Biodiversidad, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Casilla 114-D, Santiago, Chile*

Abstract. At broad geographical scales, the variation in bivalve shell thickness can be modulated by environmental factors that vary with latitude, such as sea surface temperature (SST), seawater pH, or calcium carbonate availability. Mussels usually form multilayered beds, and shell thickness is also expected to be affected by density and layering due to intraspecific competition. In this work, we explored the geographical variation of shell thickness in the intertidal mussel *Perumytilus purpuratus* between 18° and 42°S along the southeastern Pacific coast. We tested the hypothesis that there was a positive relationship between shell thickness and SST, and then we explored other variables that could have an effect on thickness, such as density, number of layers, and others environmental variables (pH and calcite concentration). The expected positive linear relationship between shell thickness and sea surface temperature was not found, but when the other population variables were included in the analysis, an unexpected inverse SST-thickness relationships appeared as significant, probably because this species could be adapted to colder and more acid seawater as are those of the tips of South America. Thickness was also negatively affected by density, which was expected for a gregarious species showing high intraspecific competition. Finally, our results highlight the importance of including density and crowding effects when macroscale patterns are explored, particularly in gregarious species, since these patterns could also be modulated by density-dependent processes, which might then override latitudinal trends of shell thickness when they are not included in the analyses.

Received 22 May 2012; accepted 8 July 2014.
* To whom correspondence should be addressed. E-mail: carolina.briones@uantof.cl
Abbreviations: ThI, shell thickness index; SST, sea surface temperature; *L*, number of layers; and *N*, density.

Introduction

In gastropods and bivalves, shell shape and thickness may be plastic or adaptive traits (Boulding and Hay, 1993; Parsons, 1997; Beadman *et al.*, 2003; Ubukata, 2003; Nagarajan *et al.*, 2006). On a local scale, shell thickness in bivalves can be affected by a variety of forces (Seed and Richardson, 1999). For example, increased shell thickness can reduce successful predation (Reimer and Tedengren, 1996; Leonard *et al.*, 1999; Smith and Jennings, 2000; Caro and Castilla, 2004), protect individuals from the destructive effects of intense wave action (Guiñez, 1996; Steffani and Branch, 2003), and provide mechanical support to protect mussels from the effects of density and aggregation in mussel beds or matrices (Guiñez, 1996). In filter-feeders such as mussels, which usually form crowded and multilayered matrices (beds), the intraspecific competition is one of the consequences of this lifestyle (Okamura, 1986), and their intensity increases with the increased packing (Guiñez and Castilla, 1999; Guiñez *et al.*, 2005); shell thickness is also expected to be affected by density and layering (Guiñez, 1996).

On a macroscale, the environmental effects of sea surface temperature (SST), which decreases toward high latitudes, can affect several properties of the calcium carbonate ($CaCO_3$) shells of molluscs. Water temperature can influence both the micro- and macroscopic properties of $CaCO_3$-based shells (Palmer, 1992; Vermeij, 1993; Trussell, 2000; Trussell and Etter, 2001). The energetic costs of both deposition and maintenance should increase with lower water temperatures because $CaCO_3$ is less saturated and more soluble with decreasing water temperature. Thus, shells of bivalve molluscs that inhabit cold waters should be thinner than those from warm waters (Vermeij, 1993). Several recent studies have documented a positive relationship between shell thickness and temperature (or a negative relationship with latitude) in invertebrates (Trussell, 2000; Trussell and Smith, 2000; Trussell and Etter, 2001; Sepúlveda and Ibáñez, 2012; Watson *et al.*, 2012). Along the Chilean coast, Sepúlveda and Ibáñez (2012) found that the gastropod *Acanthina monodon* displayed decreasing lip thickness southward of 41°S, whereas Valladares *et al.* (2010) failed to find a significant relationship between shell thickness and latitude in the mussel *Mytilus chilensis*. However, in this last case, density effects, so typical in mussels, were not measured and thus it was not possible to show whether thickness was affected by density and crowding.

Mussels are an interesting taxon to study for geographic variation in shell thickness and their relationship with density or layering because they are abundant and dominant, are usually overcrowded in coastal environments, and have extensive geographical ranges. For example, *Perumytilus purpuratus* (Lamarck, 1819), has a wide distribution range across 56 degrees of latitude along the southeastern Pacific coast (Zagal and Hermosilla, 2001), spanning three biogeographic regions on the Chilean coast: the Peruvian Province northward of 30°S, the Intermediate Area between 30° and 42°S, and the Magellan Province between 43° and 56°S (Camus, 2001).

P. purpuratus is a dominant competitor, a community-structuring species, and a bioengineer that forms extensive banks in the rocky intertidal zone (Guiñez and Castilla, 1999; Prado and Castilla, 2006) with multi-stratified matrices of individuals where intense intraspecific competition occurs (Guiñez and Castilla, 1999; Guiñez *et al.*, 2005). In this study, we describe the pattern of variation in valve thickness in *P. purpuratus* in a latitudinal gradient along 3000 km of the Chilean coastline using two thickness indexes. The main aims were to test whether geographic variation in shell thickness was related to SST or other population (such as density and number of layers) and environmental (pH and calcite concentration) variables that covary across 24° of latitude along the western coast of South America.

Materials and Methods

Sampling localities

Samples of *Perumytilus purpuratus* were collected from 25 sites distributed between 18° and 42°S latitude along the Southeast Pacific coast (Fig. 1). At each site, three samples (*i.e.,* replicates) were taken at random from quadrats (100 cm^2) in matrices with 100% cover, from plane platform (0°–10° of slope), and defined as exposed sites according to the criteria of Castilla *et al.* (1998) and Guiñez and Pacheco (1999). The samples were obtained in two periods: 1998–2004 and the summer of 2009. The first period included 18 sampling sites (see sites marked with as asterisk in Fig. 1), and the second one included 7 new sampling sites (Fig. 1) to cover the geographic range between 25° and 28°S, which was not sampled in the first period. All the individuals sampled were counted and measured for maximum length (l: maximum length of the anterior-posterior axis), maximum height (h: maximum length along the dorsoventral axis), and maximum width (w: maximum length along the latero-lateral axis) using a Mitutoyo digital caliper (0.01 mm ± 0.005 mm). The weight (0.001 g precision using a Precisa 505 M-2020 C-DRSCS scale) was estimated for a subsample of 20 individuals by replicate and site, selecting the largest and smallest mussels and randomly selecting the remaining 18 individuals. We also estimated the shell surface of each valve using two methods that we named as PLANE and CURV.

The PLANE valve surface was estimated as the area of the corresponding plane projection from digital photographs using the contours of each valve (without considering the shell depth or width). The valves were placed, facing downward, on a horizontally fixed upward-facing camera, including a rounded object of known area and diameter to calibrate the measures. The photographs were then analyzed using the UTHSCSA ImageTool image analysis software (University of Texas, Health Science Center, San Antonio, UTHSCSA; Image Tool for Windows, ver. 3.0, USA). For each individual, the PLANE shell surface area (in mm^2) was calculated as the average area of both valves.

The CURV valve surface incorporated the possible effect of the valve curvature (using shell depth or width as a proxy) on the surface estimation, and was based on the formula of shell surface (A′) proposed by Reimer and Tedengren (1996): $A' = l\,(h^2 + w^2)^{0.5}\,\pi/2$, where l, h, and w were the maximum shell length, height, and width respectively. This formula describes a figure that resembles an ellipsoid and should give estimates relatively close to the true shell surface area (Reimer and Tedengren, 1996; Beadman *et al.*, 2003). Finally, to be equivalent with the PLANE shell surface that was estimated for each individual using the average area of both valves, A′ was divided by two, as $CURV = A'/2$.

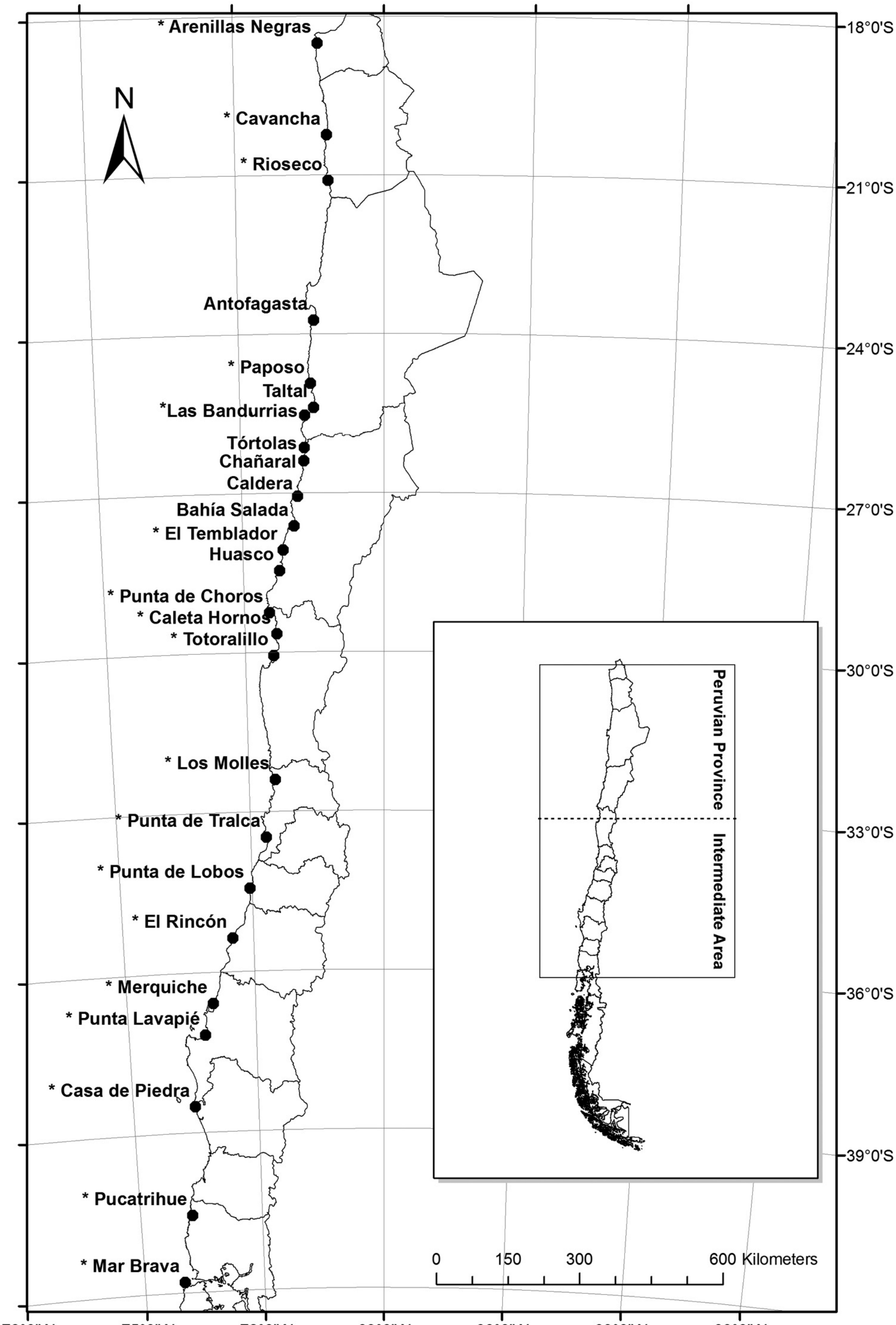

Figure 1. Sampling locations of *Perumytilus purpuratus*, distributed between 18° and 42°S latitude, along the rocky intertidal zone of the southeast Pacific coast. The asterisks indicate locations sampled during the first period (1998–2004).

Shell thickness estimates

We estimated the adjusted shell thickness as an index using the relationship between shell weight and shell surface area following Raubenheimer and Cook (1990), Guiñez (1996), Lord and Whitlatch (2012), and Lord and Whitlatch (2013). The index was estimated with an analysis of covariance (ANCOVA) using the least square means (LSMEANS from PROC GLM of SAS, ver. 9.0) to compare means for shell weight corrected for shell surface by site. Because we used two methods for estimating the shell surface, two indexes were calculated (ThI-1 and ThI-2). We used the PLANE (see above) shell surface estimation for the ThI-1 index, and the CURV shell surface estimation for the ThI-2 index. In this way, both ThI-1 and ThI-2 indexes permit us to compare the possible effect of shell curvature on the thickness estimates and results.

Log-log transformations were applied to linearize the relationship between shell weight and shell surface areas (PLANE and CURV). Consequently, the ANCOVA model applied for each shell surface was

$$\text{log shell weight}_{lkji} = \mu + \text{log shell surface}_i + \text{site}_j$$

$$+ \text{replicates (site)}_{k(j)} + \text{log shell surface} \times \text{site}_{(i*j)} + \varepsilon_{l(ijk)},$$

where log shell surface was the covariate, site and the interaction term (log shell surface × site) were considered fixed factors, and replicates (site) a random factor. The interaction term was used to test for heterogeneity of slopes among the sites.

Population and environmental variables

Population structure was estimated using two parameters: (i) density (N, individuals m^{-2}) and (ii) the level of aggregation, represented by the number of layers (L, area/area) as defined by Guiñez and Castilla (1999). Accordingly, L was estimated as $L = Ae\,As^{-1}$, where Ae is the effective area, or total projected substrate area, calculated as the sum of the individual area projected to the substrate (Si), with Si = $\text{height}_i\ \text{width}_i$ (Guiñez and Castilla, 1999, 2001), and As is the sampling area. Si was calculated assuming that the maximum length (l) is perpendicular to the substrate and the mean spatial volume of mussels could be described as a rectangular parallelepiped. Thus, the mean width (w) and mean height (h) are related to mean maximum length (l), as $w = c_w\, l^w$ and $h = c_h\, l^h$; and the average area projected to the substrate (Si) is hw (for details, see Guiñez and Castilla, 1999).

The environmental variables included in the analyses were mean sea surface temperature (SST, °C), seawater pH (unitless), and mean calcite concentration ($CaCO_3$ mol/m^3). The database covered the 2002–2009 period (Tyberghein *et al.*, 2011) and was downloaded from the Bio-ORACLE site (Tyberghein *et al.,* 2012). For our analyses we used georeferenced data collected along the shoreline from the nearest latitude and longitude for each site.

Data analyses

Boxplots and probability plots of the residuals were used to check for normality, and plots of standardized residuals against each adjusted thickness index were used to detect heterogeneity of variance. Preliminary analyses showed that some subsets of the predictor variables were highly correlated. As a consequence, high levels of multicollinearity were detected, as indicated by the high values of the condition index and the variance inflation factor (VIF) (Quinn and Keough, 2002). To avoid this multicollinearity, some variables were not included in the final regression models. In this respect, collinearity between SST and latitude has been long recognized (*e.g.*, Broitman *et al.*, 2001), suggesting that statistical analyses with latitude were likely to produce results similar to those with SST. In fact, SST was negatively related to latitude across the geographical range examined (see Results). Because of this, we decided to explore the geographical trends of each variable by means of simple linear and nonlinear (second-order polynomial) regression analyses (SAS, 1996), using SST instead of latitude as an independent variable and each variable (adjusted shell thicknesses, ThI-1 and ThI-2; number of layers, L; density, N; calcite concentration; and seawater pH) as dependent variables. The statistical significance of the inclusion of a quadratic term as appropriate was determined according to an extra sum-of-squares F test, as implemented with the *test* options of PROC REG (SAS, 1996).

A multiple regression analysis with type III sum of squares (SAS, 1996) was used to determine the relative importance of the different explanatory variables (SST, L, N, calcite, and pH) on each thickness index (ThI-1 and ThI-2). To assess whether the linearity assumption was tenable, we examined plots of the residuals *versus* each of the explanatory variables, looking for curvatures. When there was a very pronounced nonlinearity in some of them, we transformed the corresponding explanatory variable to linearize the relationship. The adjusted coefficients of determination (R^2) were used as criteria to compare the best fit of the model for each shell thickness estimate (ThI-1 and ThI-2) (Quinn and Keough, 2002; Ranasinghe *et al.*, 2013).

Multivariate regression analyses using the *mtest* statement of PROC REG (SAS, 1996) were also applied to determine whether there were joint linear effects of a set of explanatory variables on a set of response variables. We tested the multivariate hypothesis that all coefficients of models (except the intercept) were zero, and then for each explanatory variable we tested the hypothesis that the corresponding regression coefficient was zero for the response variables.

Statistical Analysis System (SAS, 1996) was used for statistical analyses and Minitab® ver. 16.2.3.0 and OriginPro ver. 8.0724 were used for data exploration analyses and graphics.

Results

A total of 8360 mussels were collected and used for population analyses, and 1398 were used for shell thickness indexes estimation. According to exploratory data analysis,

Table 1

Mixed two-way analysis of covariance (ANCOVA) for shell thickness, using shell weight as dependent variable and shell surface area (PLANE and CURVE) as covariable

Variable	Source	df	MS	F
Plane	Covariable	1	449.654	124982
	Site	24	0.051	14.23
	Replicates (site)	50	0.024	6.79
	Log shell surface × site	24	0.041	11.43
	Error	1298	0.004	
	R^2 = 0.99			
Curve	Covariable	1	451.930	120590
	Site	24	0.034	8.97
	Replicates (Site)	50	0.015	4.09
	Log shell surface × site	24	0.027	7.17
	Error	1298	0.004	
	R^2 = 0.99			

Site was considered as a fixed factor and replicates (site) a random factor; log shell surface × site was the crossed term for heterogeneity slopes evaluation. The determination coefficients (R^2) are also reported. P value was <0.001 in all cases.

the site Chañaral was considered an outlier and was not incorporated in the analyses.

Shell thickness indexes

The effects of each log shell surface area (PLANE and CURV), site, and replicate nested within site on log shell weight were significant (ANCOVA; $P < 0.001$; Table 1A, B), and the slopes among sites were heterogeneous, as the interaction terms of log shell surface × site were significantly different (ANCOVA; $P < 0.001$; Table 1A, B). The single linear regression of the thickness index ThI-1 on ThI-2 was significant (Slope = 0.632; lower CI = 0.476; upper CI = 0.789; R^2 = 0.761; $F_{[1, 22]}$ = 69.93; $P <$ 0.0001).

Table 2

Simple regression of type III sum of squares with sea surface temperature (SST) as independent variable and the adjusted shell thicknesses indexes (ThI-1, ThI-2), latitude (LAT), number of layers (L)*, density* (N)*, calcite concentration, and seawater pH as dependent variables; quadratic relationships are also reported*

Variable	Source	df	MS	F	P	b	R^2
ThI-1	SST	1	0.0030	3.17	0.089	−0.0059	0.13
	Error	22	0.0012				
ThI-2	SST	1	0.0023	4.88	**0.038**	−0.0052	0.18
	Error	22	0.0006				
LAT	SST	1	875.29	307.9	**<0.001**	−3.1959	0.93
	Error	22	2.84				
L	SST	1	0.8839	5.48	**0.029**	1.7821	0.25
	SST^2	1	0.8304	5.15	**0.034**	−0.0549	
	Error	21	0.1612				
N	SST	1	6.92 E^{+8}	30.67	**<0.001**	−49863.7	0.60
	SST^2	1	6.74 E^{+8}	29.87	**<0.001**	1563.6	
	Error	21	2.2 E^{+7}				
Calcite	SST	1	3.97 E^{-6}	3.76	0.065	−0.0002	0.15
	Error	22	1.06 E^{-6}				
Seawater pH	SST	1	0.0132	56.36	**<0.001**	0.2180	0.89
	SST^2	1	0.0111	45.44	**<0.001**	−0.0064	
	Error	21	0.0002				

P values in bold type are statistically significant.

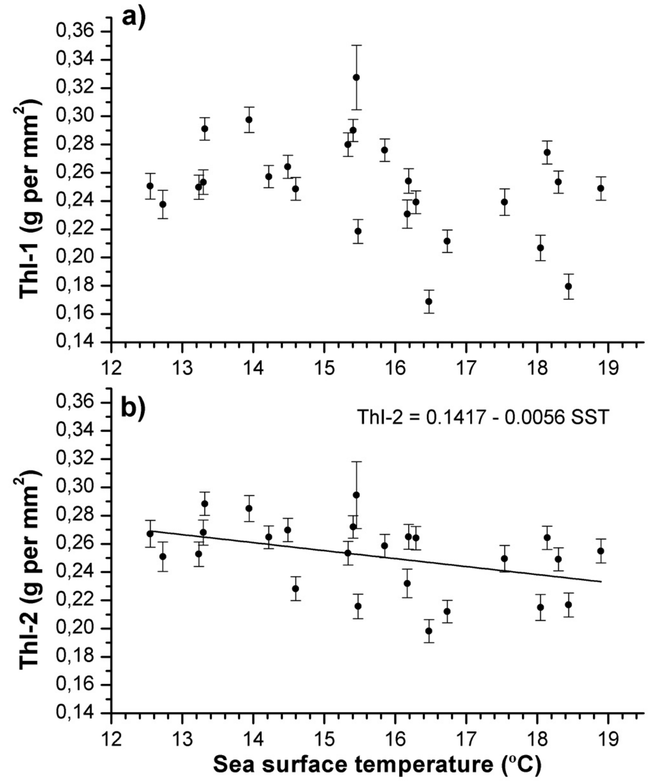

Figure 2. Scatterplot showing the relationship between adjusted shell thicknesses (a) ThI-1 and (b) ThI-2 and sea surface temperature. Error bars represent ± one standard error.

Geographic variation related with SST

The relationships between the shell thickness and SST were significant only for ThI-2 (P = 0.038, Table 2, Fig. 2). However, the multivariate linear regression showed that the overall model of SST effects on both shell thickness indexes (ThI-1 and ThI-2) was not significant (Wilks' Lambda = 0.817; $F_{[2, 21]}$ = 2.35; P = 0.120).

LAT decreased and pH increased monotonically with SST (Fig. 3a, b). In fact, LAT showed a significant linear regression with SST (R^2 = 0.93; $P < 0.001$; Table 2; Fig. 3a), and pH showed a quadratic regression with SST (R^2 = 0.89; $P < 0.001$; Table 2; Fig. 3b). N and L also showed significant quadratic regressions with SST ($P < 0.034$; Table 2), but different patterns with SST (and LAT) were observed. While density showed the minimum values at

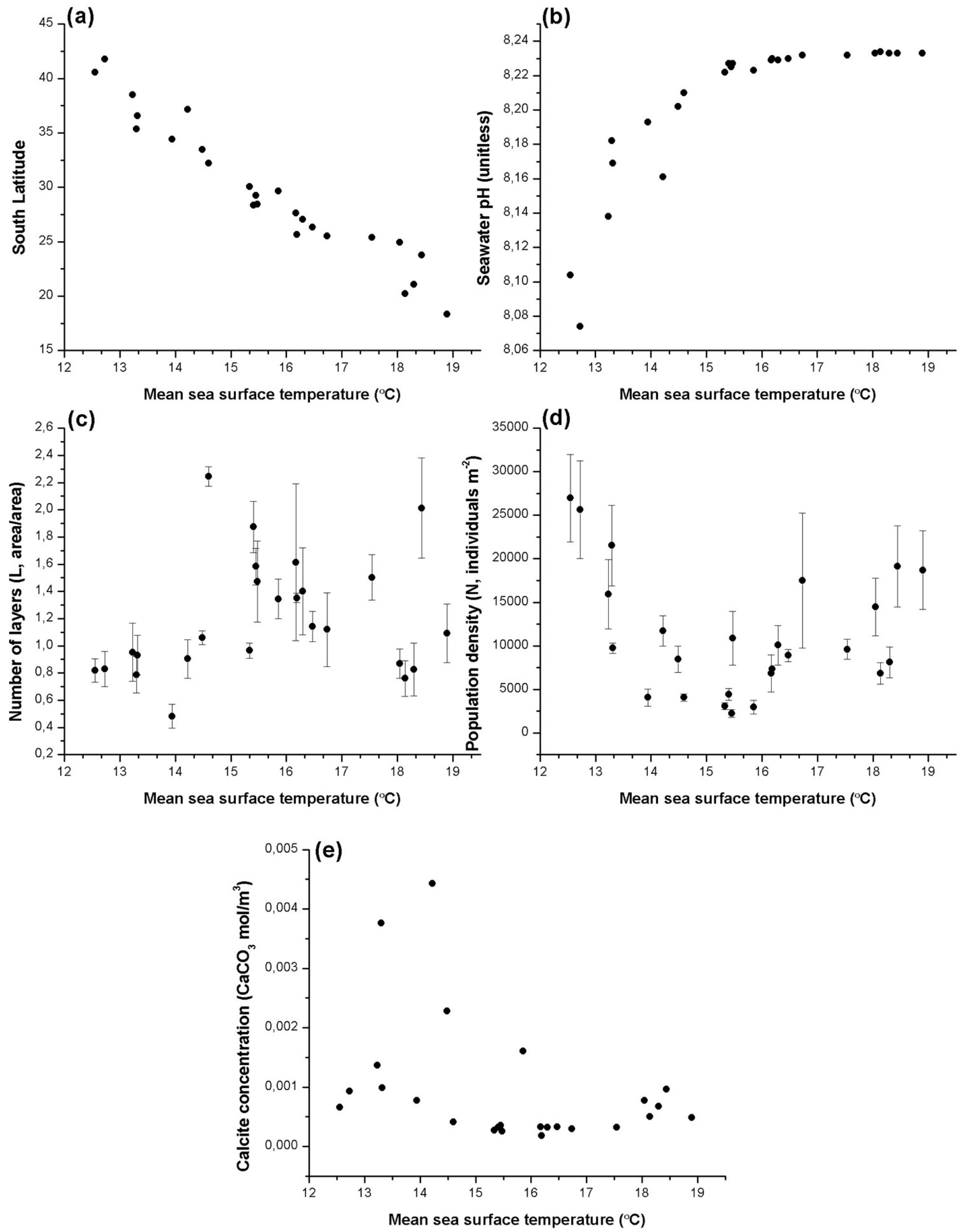

Figure 3. Scatterplot showing the relationship of sea surface temperature with latitude (a), seawater pH (b), number of layers (c), population density (d), and calcite concentration (c). Error bars represent ± one standard error.

intermediate SST (15 °C–16 °C; 28°S–30°S latitude), the number of layers showed the maximum values at those temperatures and latitudes (Fig. 3c, d). Finally, calcite did not show any clear geographic pattern. In fact, a nonsignificant regression was observed between SST and calcite ($P = 0.065$; Table 2 and Fig. 3e).

For the multiple regression analyses, LAT and seawater pH were excluded because a multicollinearity was detected, showing a higher condition index (>1401.3), and VIF (= 6.84). Density was root-square transformed to linearize the relationship with the thickness indexes. This analysis showed that the percentage of variance explained was greater for the model including ThI-1 (R^2adj = 61%, Table 3A) than for ThI-2 (R^2adj = 32%, Table 3B). Almost 70% of the variance in ThI-1 was explained significantly by SST, L and √N (P ≤ 0.025; Table 3A), and 20.5% of the variance in ThI-2 was significantly explained only for √N. The multivariate multiple regression showed that the overall model was statistically significant ($P < 0.001$; Table 3C), and only the negative coefficients of SST and were significant ($P \leq 0.047$; Table 3C). A 3D projection of the linear effects of SST and √N on ThI-1 is shown in Figure 4.

Table 3

Multiple regression of type III sum of squares, of (A) shell thickness index ThI-1, (B) shell thickness index ThI-2, and (C) the multivariate multiple regression using both shell thicknesses (ThI-1 and ThI-2) with sea surface temperature (SST), number of layers (L)*, root square of density (√N) and calcite concentration*

	Dependent Variable	Source	df	MS	F	P	b	R^2
A	**ThI-1**	SST	1	0.0029	7.19	**0.015**	−0.00636	11.42
		L	1	0.0024	5.95	**0.025**	−0.02540	9.45
		√N	1	0.0124	30.76	**<0.001**	−0.00073	48.88
		Calcite	1	0.00001	0.03	0.855	−0.80789	0.05
	$R^2_{(adj)} = 0.61$	Error	19	0.0004				30.19
B	**ThI-2**	SST	1	0.0014	3.88	0.064	−0.0045	11.62
		L	1	0.0013	3.51	0.077	−0.0187	10.49
		√N	1	0.0025	6.85	**0.017**	−0.0003	20.50
		Calcite	1	0.00006	0.17	0.682	1.7383	0.52
	$R^2_{(adj)} = 0.32$	Error	19	0.0005				56.87
C	**ThI-1 & ThI-2**	**Hypothesis by Source**	**Wilks' Lambda**	***df***	***F***	***P***		
		Model	0.187	8, 36	5.92	**<0.001**		
		SST	0.712	2, 18	3.65	**0.047**		
		L	0.754	2, 18	2.93	0.079		
		√N	0.274	2, 18	23.81	**<0.001**		
		Calcite	0.932	2, 18	0.65	0.533		

P values in bold type are statistically significant.

Discussion

The main aims of this study were to test whether geographic variation in shell thickness was positively related to sea surface temperature (SST), including other population (such as density and number of layers) and/or environmental (pH and calcite concentration) variables that covary across 24° of latitude along the western coast of South America.

When the relationships of shell thickness indexes and SST were analyzed without incorporating the ecological factors, the multivariate multiple regression model showed that the relationship was not significant (overall model, Wilks' Lambda = 0.817; $F_{[2,\ 21]} = 2.35$; $P = 0.120$). This suggests, as was proposed by Watson *et al.* (2012), that "in some circumstances ecological factors may override latitudinal trends." In fact, when the other population variables were included in the analysis, an inverse SST-thickness relationship appeared as significant (multivariate multiple regression [$P = 0.047$; Table 3C; Fig. 4]). In our case, density overrides the latitudinal trend of SST. In fact, the variances explained by √N in shell thickness were as high as 49% (Table 3A, B) compared to the 11% explained by SST.

The observed shell thickness-density dependence was expected for a mussel such as *Perumytilus purpuratus* that forms multilayered matrices (beds) and shows strong intraspecific competition with increased packing (Alvarado and Castilla, 1996; Guinez, 1996; Briones and Guiñez, 2005; Guiñez *et al.*, 2005). In fact, population density can profoundly influence the population dynamics and phenotypes (Zachar and Neiman, 2013). Density-dependence plays a key role in ecological and evolutionary processes, driving the population dynamics of marine species through intraspecific competition for space or food (Morsan *et al.*, 2011).

Evidences at the microscale and mesoscale of density-dependent regulation, such as the intraspecific competition for limited resources (space or food), affecting growth rates and reproductive effort, have been shown in several bivalve species (*e.g.*, Okamura, 1986; Jarayabhand and Newkirk, 1989; Fréchette and Lefaivre, 1990; Guiñez and Castilla, 1999; Alunno-Bruscia *et al.*, 2000; Morsan *et al.*, 2011; Cubillo *et al.*, 2012a). With respect to gregarious species, it is known that mussel bed properties have an important effect on morphological traits or biological parameters (*e.g.*, Bertness and Grosholz, 1985; Guiñez and Castilla, 1999; Alunno-Bruscia *et al.*, 2000; Cubillo *et al.*, 2012b). For instance, crowding conditions have been shown to have negative impacts, such as shell distortion (Bertness and Grosholz, 1985), growth rate decreases (Fréchette *et al.*, 1992; Alvarado and Castilla, 1996; Guiñez and Castilla, 1999), and self-thinning processes (Fréchette and Lefaivre, 1995; Guiñez, 2005). For example, growth in shell length decreased with increasing population density in the mussel *Mytilus edulis*, where crowding may have caused co-occurrence of exploitative and interference competition (Alunno-Bruscia *et al.*, 2000). This was also observed in *M. galloprovincialis*, where individuals cultured at lower densities had higher growth rates and reached greater weight and length than those cultured at higher densities (Cubillo *et al,*. 2012b).

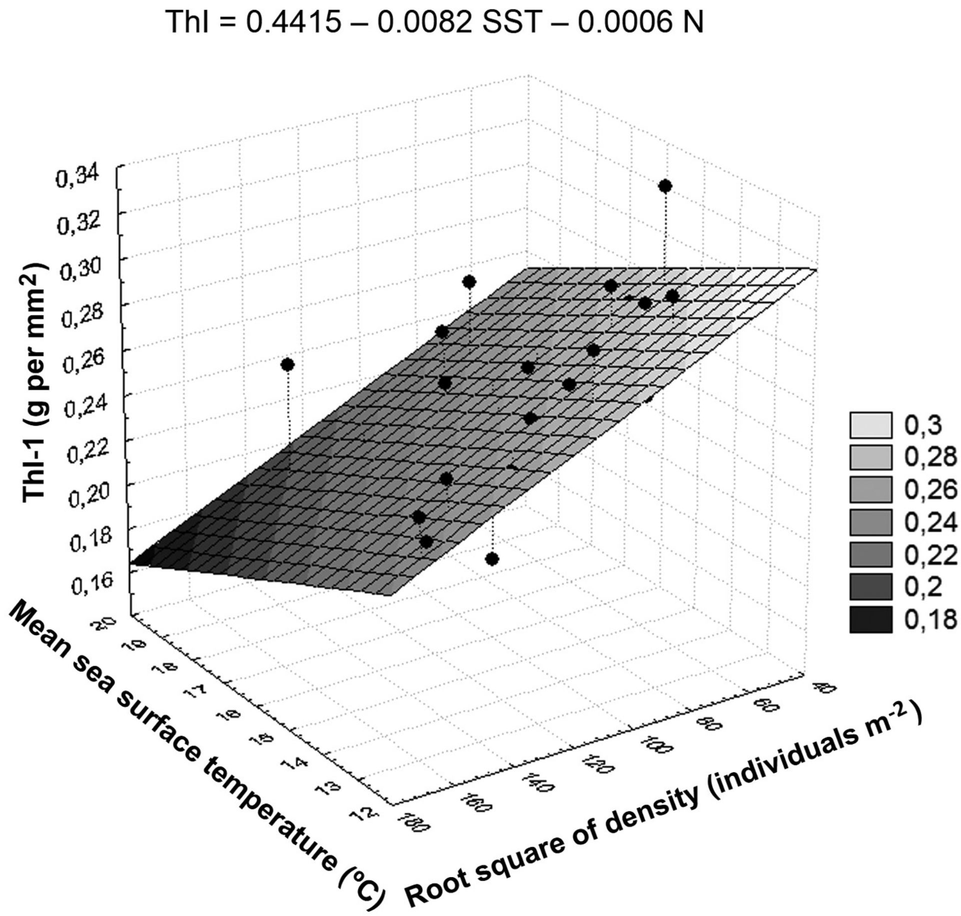

Figure 4. Tridimensional surface scatterplot showing the linear relationship between adjusted shell thickness (ThI-1, *z*-axis) and population root square of density (*x*-axis) and sea surface temperature (*y*-axis).

We are not aware of data from previous studies at the geographic macroscale showing a decreasing shell thickness with increasing density in mussels. This relationship has been documented at the geographic microscale in the barnacles *Semibalanus balanoides* (Bertness, 1989) and *Jehlius cirratus* (Lopez *et al.*, 2007, 2014), where crowded barnacles showed thinner shell or cirral appendages compared with solitary barnacles; and in the gastropod *Cypraea annulus* (Irie, 2006), where juvenile shell size and callus thickness decreased with increasing density. In this context, increased protection from predators associated with higher densities might result in mussels allocating more energy to tissue growth and shell length to escape from predation, at the cost of decreased allocation to shell thickness; hence this hypothesis may explain why mussels at higher densities might have thinner shells. For example, it is possible that the predator abundance explains our results if the abundance of the predators increases towards high latitudes. We explored this hypothesis using data of 19 of the 25 sites we analyzed, where the predator abundance was previously estimated as capture per unit of effort (CPUE), removing all the carnivorous invertebrates (gastropods and carnivorous) present in 5 min of search (M. Rivadeneira, unpubl. results). The $\log_e$ of CPUE was negatively related to latitude (linear regression; $\log_e$CPUE = 0.4010 − 0.007661 LAT; $F_{[1,17]} = 4.91$; $R_2 = 22.4$, $P = 0.041$). And the multiple regression for ThI-1 and ThI-2, including as predictor SST (or LAT), √N and $\log_e$CPUE, did not find any significance of $\log_e$CPUE ($P > 0.356$).

According to our results, the minimum values of density observed were between 15 °C and 16 °C (*i.e.,* between 28° and 30° of south latitude), which coincides with (i) an ecological discontinuity with minimum values observed in cover and recruitment of *P. purpuratus* and other benthic intertidal species, northward 32°S (Broitman *et al.*, 2001; Navarrete *et al.*, 2002, 2005); (ii) an oceanographic coastal transition zone reported at 29°S (Hormazabal *et al.*, 2004); (iii) a biogeographic transition zone at 30°S (Camus, 2001; Thiel *et al.*, 2007); and (iv) a genetic discontinuity observed in *P. purpuratus* at 28°S by us (Briones *et al.*, 2013). It is possible that a biogeographical signal may still be present in our data. To test this hypothesis we applied additional

ANCOVAs comparing the effects of √N and SST on both ThI-1 and ThI-2 thickness indexes at sites with latitudes >30°S (the biogeographic Peruvian Province) *versus* sites ≤30°S (the biogeographic Intermediate Area). The results were similar for both thickness indexes, with no significant effects of the interaction terms (as indicative of different slopes): √N × biogeographic regions and SST × biogeographic regions ($P > 0.1282$), and no significant differences between the adjusted thickness indexes between both biogeographic regions ($P > 0.1951$) were observed. These complementary results indicated that thickness did not show a biogeographical trend, and therefore the biogeographical hypothesis cannot be sustained.

Regarding the relationship between thickness and SST (or latitude), previous research suggested in several species that latitudinal clines of thickness might be caused by the temperature gradient (Trussell, 2000; Trussell and Smith, 2000; Trussell and Etter, 2001; Sepúlveda and Ibáñez, 2012; Watson *et al.*, 2012). For example, in echinoids and buccinid gastropods from the Northern *versus* Southern hemispheres (Watson *et al.*, 2012), and in the gastropod *Littorina obtusata* from the Gulf of Maine (Trussell, 2000), shell thickness decreased with increasing latitude. However, to discuss our results it needs to be taken into account that recently there have been reports of decreasing surface pCO_2 (partial pressure of CO_2) along the Chilean coast, showing predominantly CO_2 supersaturated surface waters north of 37°S and, conversely, pCO_2 being strongly undersaturated south of 37°S (Torres *et al.*, 2011. These reports are consistent with the inverse relation observed between pH and SST (Fig. 3b), and the fact that SST was highly and positively related with pH (Table 2, Fig. 3b). Therefore, thinner shells were expected for *P. purpuratus*, as a marine calcifying organism, not only because seawater acidification leads to a shift in inorganic carbon equilibrium toward higher CO_2 and lower carbonate ion (CO_3^{2-}) concentrations (Gazeau *et al.*, 2007; Navarro *et al.*, 2013), but also because colder water might decrease the calcification rate, as with many other physiological processes (Gazeau *et al.*, 2013). We suggest that if *P. purpuratus* has evolved under colder and more acid seawater like that characterizing the southern tips of South America where this species also dominates (Aguirre *et al.*, 2006), then in such conditions this species may survive better by increasing the rates of both calcification and metabolism, as, for example, has been shown recently in the ophiuroid brittlestar *Amphiura filiformis* (Wood *et al.*, 2008). If this is so, then we can hypothesize that ocean warming may have exacerbated adverse effects on shell thickness of *P. purpuratus* populations.

In conclusion, our results highlight the importance of including density and crowding effects when macroscale patterns are explored, particularly in gregarious species, since these patterns could also be modulated by density-dependent processes, which might then override latitudinal trends of shell thickness.

Acknowledgments

Financial support was provided by the Comisión Nacional de Ciencia y Tecnología CONICYT (*Programas de becas de doctorado nacionales* 2007, and *Beca de apoyo para la realización de tesis doctoral to CB*); concurso *Apoyo a publicación de tesis de grado* 2008 to CB provided by Universidad de Antofagasta; FONDECYT 1101007 and CODEI-UA 31-2010 to RG, FONDECYT 1110582 to MMR, and FONDAP-FONDECYT 1501-0001. Special thanks go to David Campos, Marcelo Flores, and especially to three anonymous referees who reviewed a previous draft and improved the final manuscript.

Literature Cited

Aguirre, M. L., S. I. Perez, and Y. N. Sirch. 2006. Morphological variability of *Brachidontes swainson* (Bivalvia, Mytilidae) in the marine Quaternary of Argentina (SW Atlantic). *Palaeogeogr. Palaeoclimatol. Paleoecol.* **239:** 100–125.

Alunno-Bruscia, M., P. S. Petraitis, E. Bourget, and M. Fréchette. 2000. Body size-density relationship for *Mytilus edulis* in an experimental food-regulated situation. *Oikos* **90:** 28–42.

Alvarado, J. L., and J. C. Castilla. 1996. Tridimensional matrices of mussels *Perumytilus purpuratus* on intertidal platforms with varying wave forces in central Chile. *Mar. Ecol. Prog. Ser.* **133:** 135–141.

Beadman, H. A., R. W. G. Caldow, M. J. Kaiser, and R. I. Willows. 2003. How to toughen up your mussels: using mussel shell morphological plasticity to reduce predation losses. *Mar. Biol.* **142:** 487–494.

Bertness, M. D. 1989. Intraspecific competition and facilitation in a northern acorn barnacle population. *Ecology* **70:** 257–268.

Bertness, M. D., and E. D. Grosholz. 1985. Population dynamics of the ribbed mussel, *Geukensia demissa:* the costs and benefits of an aggregated distribution. *Oecologia* **67:** 192–204.

Boulding, E. G., and T. K. Hay. 1993. Quantitative genetics of shell form of an intertidal snail: constraints on short-term response to selection. *Evolution* **47:** 576–592.

Briones, C., and R. Guiñez. 2005. Asimetría bilateral de la forma de las valvas y posición espacial en matrices del chorito *Perumytilus purpuratus* (Lamarck, 1819) (Bivalvia: Mytilidae). *Rev. Chil. Hist. Nat.* **78:** 3–14.

Briones, C., P. Presa, M. Pérez, A. Pita, and R. Guiñez. 2013. Genetic connectivity of the ecosystem engineer *Perumytilus purpuratus* north to the 32° S southeast Pacific ecological discontinuity. *Mar. Biol.* **160:** 3143–3156.

Broitman, B. R., S. A. Navarrete, F. Smith, and S. D. Gaines. 2001. Geographic variation of southeastern Pacific intertidal communities. *Mar. Ecol. Prog. Ser.* **224:** 21–34.

Camus, P. 2001. Biogeografía Marina de Chile Continental. *Rev. Chil. Hist. Nat.* **74:** 587–617.

Caro, A. U., and J. C. Castilla. 2004. Predator-inducible defenses and local intrapopulation variability of the intertidal mussel *Semimytilus algosus* in central Chile. *Mar. Ecol. Prog. Ser.* **276:** 115–123.

Castilla, J. C., D. K. Steinmiller, and C. J. Pacheco. 1998. Quantifying wave exposure daily and hourly on the intertidal rocky shore of central Chile. *Rev. Chil. Hist. Nat.* **71:** 19–25.

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

Cubillo, A. M., L. G. Peteiro, M. J. Fernandez-Reiriz, and U. Labarta. 2012b. Density-dependent effects on morphological plasticity of *Mytilus galloprovincialis* in suspended culture. *Aquaculture* **338:** 246–252.

Fréchette, M., and D. Lefaivre. 1990. Discriminating between food and space limitation in benthic suspension feeders using self-thinning relationships. *Mar. Ecol. Prog. Ser.* **65:** 15–23.

Fréchette, M., and D. Lefaivre. 1995. On self-thinning in animals. *Oikos* **73:** 425–428.

Frechette, M., A. E. Aitken, and L. Page. 1992. Interdependence of food and space limitation of a benthic suspension feeder: consequences for self-thinning relationships. *Mar. Ecol. Prog. Ser.* **83:** 55–62.

Gazeau, F., C. Quiblier, J. M. Jansen, J. P. Gattuso, J. J. Middelburg, and C. H. R. Heip. 2007. Impact of elevated CO_2 on shellfish calcification. *Geophys. Res. Lett.* **34:** L07603. doi:10.1029/2006GL028554.

Gazeau, F., L. Parker, S. Comeau, J.-P. Gattuso, W. O'Connor, S. Martin, H.-O. Pörtner, and P. Ross. 2013. Impacts of ocean acidification on marine shelled molluscs. *Mar. Biol.* **160:** 2207–2245.

Guiñez, R. 1996. Dinámica poblacional del chorito maico, *Perumytilus purpuratus* (Lamarck 1819) (Bivalvia: Mytilidae), en gradientes de exposición al oleaje. Ph.D. dissertation, Pontificia Universidad Católica de Chile, Santiago, Chile.

Guiñez, R. 2005. A review on self-thinning in mussels. *Rev. Biol. Mar. Oceanogr.* **40:** 1–6.

Guiñez, R., and J. C. Castilla. 1999. A tridimensional self-thinning model for multilayered intertidal mussels. *Am. Nat.* **154:** 341–357.

Guiñez, R., and J. C. Castilla. 2001. An allometric tridimensional model of self-thinning for a gregarious tunicate. *Ecology* **82:** 2331–2341.

Guiñez, R., and C. J. Pacheco. 1999. Estimaciones de la velocidad máxima del oleaje en Chile central, utilizando un dinamómetro prototipo. *Rev. Chil. Hist. Nat.* **72:** 251-260.

Guiñez, R., P. S. Petraitis, and J. C. Castilla 2005. Layering, the effective density of mussels and mass-density boundary curves. *Oikos* **110:** 186–190.

Hormazabal, S., G. Shaffer, and O. Leth. 2004. Coastal transition zone off Chile. *J. Geophys. Res.* **109:** 1–13.

Irie, T. 2006. Geographic variation of shell morphology in *Cypraea annulus* (Gastropoda: Cypraeidae). *J. Molluscan Stud.* **72:** 31–38.

Jarayabhand, P., and G. F. Newkirk. 1989. Effects of intraspecific competition on growth of the European oyster, *Ostrea edulis* Linnaeus, 1750. *J. Shellfish Res.* **8:** 239–365.

Leonard, G. H., M. D. Bertness, and P. O. Yund. 1999. Crab predation, waterborne cues, and inducible defenses in the blue mussel, *Mytilus edulis. Ecology* **80:** 1–14.

Lopez, B. A., S. Y. Guaitro, and D. A. Lopez. 2007. Density-dependent effects on the cirral structure of the barnacle, *Jehlius cirratus* (Darwin, 1854) (Cirripedia). *Crustaceana* **80:** 793–801.

Lopez, B. A., A. M. Catalan, D. A. Barriga, and D. A. Lopez. 2014. Morphological responses of the exoskeleton in the intertidal barnacle *Jehlius cirratus* (Darwin, 1854) growing at different densities. *J. Crustacean Biol.* **34:** 129–134.

Lord, J., and R. Whitlatch. 2012. Inducible defenses in the eastern oyster *Crassostrea virginica* Gmelin in response to the presence of the predatory oyster drill *Urosalpinx cinerea* Say in Long Island Sound. *Mar. Biol.* **159:** 1177–1182.

Lord, J., and R. Whitlatch. 2013. Impact of temperature and prey shell thickness on feeding of the oyster drill *Urosalpinx cinerea* Say. *J. Exp. Mar. Biol. Ecol.* **448:** 321–326.

Morsan, E. M., P. Pappalardo, and M. D. Doldan. 2011. Growth compensation as a regulatory mechanism of purple clam *Amiantis purpurata* population dynamics in Patagonia. *Mar. Ecol. Prog. Ser.* **443:** 207–216.

Nagarajan, R., S. E. G. Lea, and J. D. Goss-Custard. 2006. Seasonal variations in mussel, *Mytilus edulis* L. shell thickness and strength and their ecological implications. *J. Exp. Mar. Biol. Ecol.* **339:** 241–250.

Navarrete, S. A., B. R. Broitman, E. A. Wieters, G. R. Finke, R. M. Venegas, and A. Sotomayor. 2002. Recruitment of intertidal invertebrates in the southeast Pacific: interannual variability and the 1997-1998 El Niño. *Limnol. Oceanogr.* **47:** 791–802.

Navarrete, S. A., E. A. Wieters, B. R. Broitman, and J. C. Castilla. 2005. Scales of benthic-pelagic coupling and the intensity of species interactions: from recruitment limitation to top-down control. *Proc. Natl. Acad. Sci. USA* **102:** 18046–18051.

Navarro, J. M., R. Torres, K. Acuna, C. Duarte, P. H. Manriquez, M. Lardies, N. A. Lagos, C. Vargas, and V. Aguilera. 2013. Impact of medium-term exposure to elevated pCO_2 levels on the physiological energetics of the mussel *Mytilus chilensis. Chemosphere* **90:** 1242–1248.

Okamura, B. 1986. Group living and effect of spatial position in aggregations of *Mytilus edulis. Oecologia* **69:** 341–347.

Palmer, A. R. 1992. Calcification in marine mollusks: How costly is it?. *Proc. Natl. Acad. Sci. USA* **89:** 1379–1382.

Parsons, K. E. 1997. Contrasting patterns of heritable geographic variation in shell morphology and growth potential in the marine gastropod *Bembicium vittatum:* evidence from field experiments. *Evolution* **51:** 784–796.

Prado, L., and J. C. Castilla 2006. The bioengineer *Perumytilus purpuratus* (Mollusca: Bivalvia) in central Chile: biodiversity, habitat structural complexity and environmental heterogeneity. *J Mar. Biol. Assoc. UK* **86:** 417–421.

Quinn, G. P., and M. J. Keough. 2002. *Experimental Design and Data Analysis for Biologists.* Cambridge University Press, Cambridge.

Ranasinghe, C., P. Gamage, P. Katulanda, N. Andraweera, S. Thilakarathne, and P. Tharanga. 2013. Relationship between Body Mass Index (BMI) and body fat percentage, estimated by bioelectrical impedance, in a group of Sri Lankan adults: a cross sectional study. *BMC Public Health* **13:** 797. doi: 10.1186/1471-2458-13-797.

Raubenheimer, D., and P. Cook. 1990. Effects of exposure to wave action on allocation of resorces to shell and meat growth by subtidal mussel, *Mytilus galloprovincialis. J. Shellfish Res.* **9:** 87–93.

Reimer, O., and M. Tedengren. 1996. Phenotypical improvement of morphological defenses in the mussel *Mytilus edulis* induced by exposure to the predator *Asterias rubens. Oikos* **75:** 383–390.

SAS. 1996. *SAS/STAT User's Guide.* Release 6.3 edition. SAS Institute, Cary, NC.

Seed, R., and C. A. Richardson. 1999. Evolutionary traits in *Perna viridis* (Linnaeus) and *Septifer virgatus* (Wiegmann) (Bivalvia: Mytilidae). *J. Exp. Mar. Biol. Ecol.* **239:** 273–287.

Sepúlveda, R. D., and C. M. Ibáñez. 2012. Clinal variation in the shell morphology of intertidal snail *Acanthina monodon* in the Southeastern Pacific Ocean. *Mar. Biol. Res.* **8:** 363–372.

Smith, L. D., and J. A. Jennings. 2000. Induced defensive responses by the bivalve *Mytilus edulis* to predators with different attack modes. *Mar. Biol.* **136:** 461–469.

Steffani, C. N., and G. M. Branch. 2003. Growth rate, condition, and shell shape of *Mytilus galloprovincialis:* responses to wave exposure. *Mar. Ecol. Prog. Ser.* **246:** 197–209.

Thiel, M., E. C. Macaya, E. Acuña, W. E. Arntz, H. Bastias, K. Brokordt, P. Camus, J. C. Castilla, L. R. Castro, M. Cortés *et al.* 2007. The Humboldt Current System of northern and central Chile: oceanographic processes, ecological interactions and socioeconomic feedback. *Oceanogr. Mar. Biol.* **45:** 195–345.

Torres, R., S. Pantoja, N. Harada, H. E. González, G. Daneri, M. Frangopulos, J. A. Rutllant, C. M. Duarte, S. Rúiz-Halpern, E. Mayol, and M. Fukasawa. 2011. Air-sea CO_2 fluxes along the coast of Chile: From CO_2 outgassing in central northern upwelling waters to

CO_2 uptake in southern Patagonian fjords. *J. Geophys. Res.* **116:** doi:10.1029/2010JC006344.

Trussell, G. C. 2000. Phenotypic clines, plasticity, and morphological trade-offs in an intertidal snail. *Evolution* **54:** 151–166.

Trussell, G. C., and R. J. Etter. 2001. Integrating genetic and environmental forces that shape the evolution of geographic variation in a marine snail. *Genetica* **112-113:** 321–337.

Trussell, G. C., and L. D. Smith. 2000. Induced defenses in response to an invading crab predator: an explanation of historical and geographic phenotypic change. *Proc. Natl. Acad. Sci. USA* **97:** 2123–2127.

Tyberghein, L., H. Verbruggen, K. Pauly, C. Troupin, F. Mineur, and O. De Clerck. 2012. Bio-ORACLE: a global environmental dataset for marine species distribution modelling. *Glob. Ecol. Biogeogr.* **21:** 272–281.

Ubukata, T. 2003. A morphometric study on morphological plasticity of shell form in crevice-dwelling Pterioida (Bivalvia). *Biol. J. Linn. Soc.* **79:** 285–297.

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

Vermeij, G. J. 1993. *A Natural History of Shells.* Princeton University Press, Princeton, NJ.

Watson, S. A., L. S. Peck, P. A. Tyler, P. C. Southgate, K. S. Tan, R. W. Day, and S. A. Morley. 2012. Marine invertebrate skeleton size varies with latitude, temperature and carbonate saturation: implications for global change and ocean acidification. *Glob. Change Biol.* **18:** 3026–3038.

Wood, H. L., J. I. Spicer, and S. Widdicombe. 2008. Ocean acidification may increase calcification rates, but at a cost. *Proc. R. Soc. B Biol. Sci.* **275:** 1767-1773.

Zachar, N., and M. Neiman. 2013. Profound effects of population density on fitness-related traits in an invasive freshwater snail. *PLoS One* **8:** e80067. doi: 10.1371/journal.pone.0080067.

Zagal, C., and C. Hermosilla. 2001. *Guía de Invertebrados Marinos del Litoral Valdiviano.* Quebecor World Chile S.A., Santiago, Chile.