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

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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.

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 (Guíñez, 1996; Steffani and Branch, 2003), and provide mechanical support to protect mussels from the effects of density and aggregation in mussel beds or matrices (Guíñez, 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 (Guíñez and Castilla, 1999; Guíñez et al., 2005); shell thickness is also expected to be affected by density and layering (Guíñez, 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 (CaCO₃) shells of molluscs. Water temperature can influence both the micro- and macroscopic properties of CaCO₃-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 CaCO₃ 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 (Guinéz and Castilla, 1999; Prado and Castilla, 2006) with multi-stratified matrices of individuals where intense intraspecific competition occurs (Guínez and Castilla, 1999; Guínez 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 cm²) 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 Guínez 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 an 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 mm²) 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): 

\[ A' = l \left( h^2 + w^2 \right)^{0.5} \pi / 2, \]

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 = \( A'/2. \)
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

\[
\text{log shell weight}_{ijkl} = \mu + \text{log shell surface}_{i} + \text{site}_{j} + \text{replicates (site)}_{k(i,j)} + \text{log shell surface} \times \text{site}^{(i)j}_{(i,v)} + \epsilon_{ijkl},
\]

where log shell surface was the covariate, site and the interaction term (log shell surface \(\times\) 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 \((N, \text{individuals m}^{-2})\) and (ii) the level of aggregation, represented by the number of layers \((L, \text{area} / \text{area})\) as defined by Guinéz and Castilla (1999). Accordingly, \(L\) was estimated as \(L = A e A s^{-1}\), where \(A e\) is the effective area, or total projected substrate area, calculated as the sum of the individual area projected to the substrate \((Si)\), with \(Si = \text{height} \times \text{width}\) (Guinéz 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 \(w = c_w \, l^2\) and \(h = c_h \, l^2\); and the average area projected to the substrate \((Si)\) is \(hw\) (for details, see Guinéz and Castilla, 1999).

The environmental variables included in the analyses were mean sea surface temperature \((\text{SST, } ^{\circ}\text{C})\), seawater \(\text{pH}\) (unitless), and mean calcite concentration \((\text{CaCO}_3 \text{ mol/m}^3)\). 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 \(\text{pH}\)) 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, \(L\), calcite, and \(\text{pH}\)) 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\textsuperscript{®} ver. 16.2.3.0 and OriginPro ver. 8.0.724 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,
the site Chanarál 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 \( \times \) 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 \( = 0.789 \); \( R^2 = 0.761 \); \( F[1, 22] = 69.93; P < 0.0001 \)).

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

### Table 1

<table>
<thead>
<tr>
<th>Variable Source</th>
<th>df</th>
<th>MS</th>
<th>( F )</th>
<th>( P )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plane Covariable</td>
<td>1</td>
<td>449.654</td>
<td>124982</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>24</td>
<td>0.051</td>
<td>14.23</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Replicates (site)</td>
<td>50</td>
<td>0.024</td>
<td>6.79</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Log shell surface × site</td>
<td>24</td>
<td>0.041</td>
<td>11.43</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1298</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R^2 = 0.99 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Curve Covariable</td>
<td>1</td>
<td>451.930</td>
<td>120590</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site</td>
<td>24</td>
<td>0.034</td>
<td>8.97</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Replicates (Site)</td>
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<td>0.015</td>
<td>4.09</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Log shell surface × site</td>
<td>24</td>
<td>0.027</td>
<td>7.17</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>1298</td>
<td>0.004</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( R^2 = 0.99 )</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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.

### Table 2

<table>
<thead>
<tr>
<th>Variable Source</th>
<th>df</th>
<th>MS</th>
<th>( F )</th>
<th>( P )</th>
<th>( b )</th>
<th>( R^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ThI-1 SST</td>
<td>1</td>
<td>0.0030</td>
<td>3.17</td>
<td>0.089</td>
<td>-0.0059</td>
<td>0.13</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>0.0012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ThI-2 SST</td>
<td>1</td>
<td>0.0023</td>
<td>4.88</td>
<td>( \text{&lt;0.001} )</td>
<td>-0.0052</td>
<td>0.18</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>0.0006</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LAT SST</td>
<td>1</td>
<td>875.29</td>
<td>307.9</td>
<td>( \text{&lt;0.001} )</td>
<td>-3.1959</td>
<td>0.93</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>2.84</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L SST</td>
<td>1</td>
<td>0.8899</td>
<td>5.48</td>
<td>( \text{0.029} )</td>
<td>1.7821</td>
<td>0.25</td>
</tr>
<tr>
<td>SST(^2)</td>
<td>1</td>
<td>0.8304</td>
<td>5.15</td>
<td>( \text{0.034} )</td>
<td>-0.0549</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.1612</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N SST</td>
<td>1</td>
<td>6.92 ( E^{+8} )</td>
<td>30.67</td>
<td>( \text{&lt;0.001} )</td>
<td>-49863.7</td>
<td>0.60</td>
</tr>
<tr>
<td>SST(^2)</td>
<td>1</td>
<td>6.74 ( E^{+8} )</td>
<td>29.87</td>
<td>( \text{&lt;0.001} )</td>
<td>1563.6</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>2.2 ( E^{+7} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcite SST</td>
<td>1</td>
<td>3.97 ( E^{-6} )</td>
<td>3.76</td>
<td>0.065</td>
<td>-0.0002</td>
<td>0.15</td>
</tr>
<tr>
<td>Error</td>
<td>22</td>
<td>1.06 ( E^{-6} )</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seawater pH SST</td>
<td>1</td>
<td>0.0132</td>
<td>56.36</td>
<td>( \text{&lt;0.001} )</td>
<td>0.2180</td>
<td>0.89</td>
</tr>
<tr>
<td>SST(^2)</td>
<td>1</td>
<td>0.0111</td>
<td>45.44</td>
<td>( \text{&lt;0.001} )</td>
<td>-0.0064</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>21</td>
<td>0.0002</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( P \) values in bold type are statistically significant.
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 ($R^2_{adj} = 61\%$, Table 3A) than for ThI-2 ($R^2_{adj} = 32\%$, Table 3B). Almost 70% of the variance in ThI-1 was explained significantly by SST, $L$, and $\sqrt{N}$ ($P = 0.025$; Table 3A), and 20.5% of the variance in ThI-2 was significantly explained only for $\sqrt{N}$. 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 $\sqrt{N}$ were significant ($P \leq 0.047$; Table 3C). A 3D projection of the linear effects of SST and $\sqrt{N}$ on ThI-1 is shown in Figure 4.

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.
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 $R^2_{(adj)} = 0.61$). In our case, density overrides the latitudinal trend of SST. In fact, the variances explained by $\text{\#N}$ 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; Guinéz, 1996; Briones and Guinéz, 2005; Guinéz 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; Guinéz 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; Guinéz 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; Guinéz and Castilla, 1999), and self-thinning processes (Fréchette and Lefaivre, 1995; Guinéz, 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).
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 loge of CPUE was negatively related to latitude (linear regression; logeCPUE = 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), \( \sqrt{N} \) and logeCPUE, did not find any significance of logeCPUE (\( P > 0.356 \)).

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 $\sqrt{N}$ and SST on both Thl-1 and Thl-2 thickness indexes at sites with latitudes $>30^\circ$S (the biogeographic Peruvian Province) versus sites $\leq 30^\circ$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): $\sqrt{N} \times$ biogeographic regions and SST $\times$ 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 bocciid 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 $37^\circ$S and, conversely, $pCO_2$ being strongly undersaturated south of $37^\circ$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 suspect 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). This is if 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.

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**Literature Cited**


