

## Shifts in shell mineralogy and metabolism of *Concholepas concholepas* juveniles along the Chilean coast

Laura Ramajo<sup>A,B,F</sup>, Alejandro B. Rodríguez-Navarro<sup>C</sup>, Carlos M. Duarte<sup>A,D</sup>, Marco A. Lardies<sup>E</sup> and Nelson A. Lagos<sup>B</sup>

<sup>A</sup>Global Change Department, Instituto Mediterráneo de Estudios Avanzados (IMEDEA, CSIC-UIB), C/ Miquel Marqués 21, E-07190 Esporles, Islas Baleares, Spain.

<sup>B</sup>Centro de Investigación e Innovación para el Cambio Climático (CiiCC), Universidad Santo Tomás, Avenida Ejército 146, 8370003 Santiago, Chile.

<sup>C</sup>Departamento de Mineralogía y Petrología, Facultad de Ciencias, Universidad de Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain.

<sup>D</sup>Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal 23955-6900, Kingdom of Saudi Arabia.

<sup>E</sup>Facultad de Artes Liberales e Ingeniería y Ciencias, Universidad Adolfo Ibañez, Avenida Diagonal Las Torres 2640, 7041169 Santiago, Chile.

<sup>F</sup>Corresponding author. Laura Ramajo. Email: lramajo@imedea.uib-csic.es

**Abstract.** Along the west coast of South America, from the tropical zone to the Patagonian waters, there is a significant latitudinal gradient in seawater temperature, salinity and carbonate chemistry. These physical–chemical changes in seawater induce morphological and physiological responses in calcifying organisms, which may alter their energy budget and calcification processes. In this study, we study the organism energy maintenance (i.e. metabolic rate) and mineralogical composition of the shell of the juvenile marine snails *Concholepas concholepas* (Gastropoda: Muricidae), collected from benthic populations located ~2000 km apart, varies across geographic regions along the Chilean coast. We found that in juvenile snails, the *calcite*:*aragonite* ratio in the pallial shell margin (i.e. newly deposited shell) increase significantly from northern to southern populations and this increase in calcite precipitation in the shell of juveniles snails was associated with a decrease in oxygen consumption rates in these populations. Our result suggests that calcite secretion may be favoured when metabolic rates are lowered, as this carbonate mineral phase might be less energetically costly for the organism to precipitate. This result is discussed in relation to the natural process such as coastal upwelling and freshwater inputs that promote geographic variation in levels of pH and carbonate saturation state in seawater along the Chilean coast.

**Additional keywords:** calcium carbonate, metabolism, ocean acidification, temperature.

Received 5 August 2014, accepted 12 January 2015, published online 7 May 2015

### Introduction

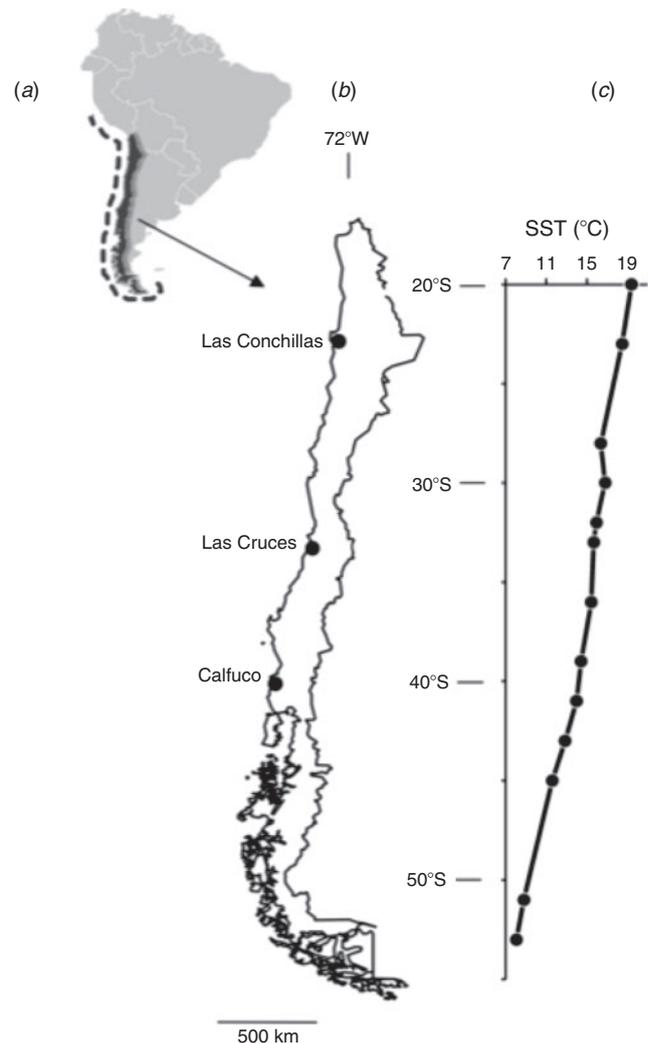
Molluscs exert close control on shell calcification resulting in species specific structural organisation and mineralogical composition (Bøggild 1930; Taylor *et al.* 1969). Shell calcification is mainly biologically controlled (Watabe and Wilbur 1960; Addadi and Weiner 1992; Belcher *et al.* 1996) as shell mineralisation occurs at the extrapallial fluid, which is a gellified media supersaturated with respect to calcium carbonate relevant phases (Saleuddin and Kunigelis 1984; Simkiss and Wilbur 1989; Putnis *et al.* 1995). However, biological and environmental factors such as food supply, water chemistry, salinity,  $p\text{CO}_2$  and temperature can also affect the chemical and mineral composition of mollusc shells (e.g. Hubbard *et al.* 1981; Schifano 1982; Cohen and Branch 1992; Goffredo *et al.* 2014). Earlier studies (Lowenstam 1954a, 1954b; Dodd 1964, 1966; Eisma 1966; Schifano 1982)

have suggested how changes in seawater properties (e.g. temperature, salinity, Mg/Ca) modify mollusc shell thickness and its mineralogical composition (i.e. *aragonite*:*calcite* ratio). Recent studies have shown that low carbonate saturation states in seawater has negative effects on larval development of molluscs, manifested as corrosion and shell dissolution leading to reduced growth rates, which ultimately compromise larval survival and development (Comeau *et al.* 2009; Hale *et al.* 2011). Thus, current understanding points at environmental factors as a major driver of calcification in some marine molluscs, particularly relying on the pH variations that reduce the carbonate saturation state (Waldbusser *et al.* 2010, 2013).

Understanding environmentally induced changes on shell calcification and the potential energetic or metabolic trade-offs that determine shell growth rates and the secretion of alternative

calcium carbonate polymorphs is especially relevant to predict the response of molluscs to current ocean acidification (OA) process (Fabry et al. 2008; Doney et al. 2009; Byrne 2011). In general, OA studies have revealed both negative and neutral effects on calcifying marine organisms mainly over organismal processes such as metabolism, growth and calcification rates and bio-mineralisation processes (Fabry et al. 2008; Hendriks et al. 2010; Byrne 2011; Ries 2011; Kroeker et al. 2013). In particular, increased dissolved CO<sub>2</sub> leads to lower pH values and reduces the saturation states of CaCO<sub>3</sub> minerals ( $\Omega$ ) affecting the calcification and growth of marine calcifiers (Salisbury et al. 2008; Yamamoto-Kawai et al. 2009; Ries 2011; Waldbusser et al. 2013). However, OA experiments present several limitations that compromise their use to issue predictions on the performance of marine calcifiers in the field (Hendriks et al. 2010). An alternative approach involves the examination of shell mineral composition of marine calcifiers across contrasting environmental conditions, but such studies are scarce (Hautmann 2006). Moreover, comparative analyses across environmental gradients in coastal habitats are particularly important, as coastal ecosystems, where most vulnerable species to OA occur, present complex regulation of pH and conditions for calcification that are not solely driven by OA processes (Duarte et al. 2013).

The Chilean coast is particularly suitable for the analysis of the performance of marine calcifiers across environmental gradients, as it presents a gradual decrease in SST from north to southern coastal locations, with a corresponding strong environmental gradient in upwelling intensity, and an increase in freshwater inputs, resulting in a salinity gradient, with increasing latitude (Strub et al. 1998; Torres et al. 2011; Mayol et al. 2012). These strong gradients offer an opportunity to examine the relationship between the performances of calcifiers and environmental conditions. We do so by focusing on the benthic snail, the keystone predator, *Concholepas concholepas* ('loco'), a muricid gastropod that is also bear socio-economic value along the exposed rocky shores of the South Eastern Pacific coastal ecosystems of Chile and Peru (Fig. 1a). Two layers that have different mineral composition and micro-structural organisation construct its shell: the outer portion of the shell consists of a prismatic layer of calcite, whereas the inner portion of the shell is a cross-lamellar layer of aragonite (Dauphin et al. 2003; Guzmán 2004; Guzmán et al. 2009). *C. concholepas* is an interesting model organism to study the effect of water chemistry on shell calcification across the gradient present in their extended geographic range along the Chilean coast. Recently, we demonstrated that the shell morphology in juveniles of *C. concholepas* is sensitive to latitudinal variations in pCO<sub>2</sub> and SST (Ramajo et al. 2013). However, it still remains unresolved if these changes in morphology emerge from variations in precipitation of carbonate phases induced by physical-chemical gradients existing along their geographic range. In this study, we examine the shell mineral composition, characterised by X-ray diffraction (XRD), in three local populations of the juvenile snails of *C. concholepas* along the latitudinal gradient of the Chilean coast (between 23°S to 40°S). In addition, we explore, using scanning electron microscopy (SEM) and thermogravimetry (TGA) analysis, the existence of geographic changes in microstructure and shell organic composition of the *C. concholepas* shells. Lastly, we also evaluated the



**Fig. 1.** *Concholepas concholepas*. (a) Distribution along the coast (dashed line) of the south-eastern Pacific ocean (b) Location of sampling sites in the Northern (Las Conchillas), Central (Las Cruces) and Southern (Calfuco) regions of the Chilean coast (c) Gradient in sea surface temperature (SST) along the Chilean coast (historical dataset available at <https://podaac.jpl.nasa.gov/>, accessed 6 May 2009).

variations in organismal metabolism (measured as oxygen consumption) with the aim of examining their potential relationship with observed mineralogical changes in the shells of *C. concholepas* juvenile snails.

## Materials and methods

### Study region and environmental variability

Juveniles of *C. concholepas*, of similar size and in the same lifespan stage (see Manríquez et al. 2008), were collected from exposed intertidal shores during December 2009 and January 2010 at three local populations distributed along the main regions of the Chilean coast: Northern (Las Conchillas, Antofagasta, Chile, 23°31'S), Central (Las Cruces, Valparaíso, Chile, 32°43'S); and Southern (Calfuco, Valdivia, Chile, 39°46'S) regions (Fig. 1a, b). The Chilean coast has a gradual

**Table 1. Latitude of study sites, historical SST averages (mean  $\pm$  s.d.) and summary of carbonate systems parameters measured *in situ***  
S, salinity; SST, sea surface temperature; pH levels, total alkalinity, partial pressure of CO<sub>2</sub> and aragonite and calcite saturation state ( $\Omega$ ). Historical SSTs are available at <http://www.podacc.gov>

Region	Site	Latitude (S)	SST (°C) historical	SST (°C)	S (psu)	pH	A <sub>T</sub> ( $\mu\text{mol kg}^{-1}$ )	pCO <sub>2</sub> (ppm)	$\Omega$ calcite	$\Omega$ aragonite
Northern	Las Conchillas	23°31'	18.70 $\pm$ 0.17	18.73	34.53	8.0–8.1 <sup>A</sup>	2292.58 <sup>B</sup>	329.10	4.798	3.107
Central	Las Cruces	32°43'	15.54 $\pm$ 0.07	12.20	34.40	7.596 $\pm$ 0.04	2306.81 $\pm$ 2.4	725.98	2.159	1.377
Southern	Calhuco	39°46'	13.60 $\pm$ 0.10	13.09	32.80	7.796 $\pm$ 0.11	2161.89 $\pm$ 6.6	371.71	3.289	2.095

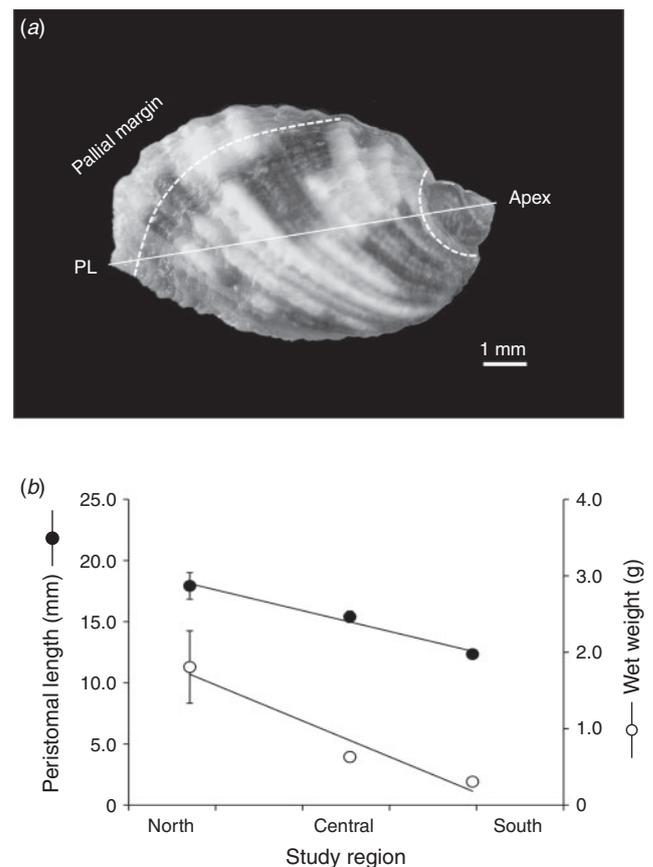
<sup>A</sup>Taken from Torres *et al.* (2002).

<sup>B</sup>Total alkalinity was assumed to be proportional to salinity using the empirical relationship provided by Lefèvre *et al.* (2002).

decrease in SST with increasing latitude (Fig. 1c) and a corresponding strong environmental gradient in upwelling intensity that modulates geographic variations in SST (Strub *et al.* 1998; Lagos *et al.* 2005, 2008). Upwelling and freshwater input into the coastal ocean also interact with geographic variations in air–sea CO<sub>2</sub> fluxes along the Chilean coast, leading to low levels of the saturation state ( $\Omega$ ) of calcite–aragonite minerals (Torres *et al.* 2011; Mayol *et al.* 2012). The Chilean coast also presents a latitudinal salinity gradient (Strub *et al.* 1998; Torres *et al.* 2011; Mayol *et al.* 2012) mainly due to an increase in river discharge from the arid, northern region to the rainy southern areas (Dávila *et al.* 2002; Ramajo *et al.* 2013). Hence, the northern coastal zone presents the highest temperature and salinity, which generally decrease towards the south (Ramajo *et al.* 2013). Northern region also exhibits higher but variable pH, A<sub>T</sub>, and  $\Omega$  values for aragonite and calcite as compared with the central and southern populations (Torres *et al.* 2011; Mayol *et al.* 2012; Ramajo *et al.* 2013). Whereas variability between regions is important, central (30–35°S) and southern (37–40°S) regions present fairly similar values of carbonate saturation parameters due the prevalence of upwelling and freshwater inputs in these regions (Torres *et al.* 2011; Ramajo *et al.* 2013) (Table 1).

#### Shell microstructure and mineralogical analysis

The microstructure of two different sections of the shell: the apex area representing the shell precipitated during early larval stages, and the pallial margin that represents the newly precipitated shell previous to the collection of the individuals (see Fig. 2a; Ramajo *et al.* 2013) were observed through SEM. Organic material of the shell samples was partially removed with 5% sodium hypochlorite for 30 min at room temperature. Samples were carbon-coated (Hitachi UHS evaporator, Hitachi, Tokyo, Japan) and observed with high resolution SEM (Leo Gemini 1530, Berlin, Germany, or Zeiss Auriga, Jena, Berlin). SEM observations were carried out both in intact and fractured (along specific orientation) shells of *C. concholepas*. Intact pieces were used to observe the outer and inner shell surfaces and fractured samples were used to observe the different structural units and layers of the shell. We used XRD analysis to characterise the mineralogical composition (proportion of calcite and aragonite carbonate phases) along shell sections. Approximately 20 mg of shell material were gently ground for 30 s into a fine and homogenous powder using an agate mortar. The powder samples were analysed with an Xpert Pro X-ray powder diffractometer (PANalytical, Almelo, Netherlands) using Cu Ka radiation and a zero-background Si holder.



**Fig. 2.** (a) Outer view of the shell of *Concholepas concholepas* showing the two shell sections (apex and pallial margin) used in X-ray diffraction (XRD) and thermogravimetry (TGA) analysis (separated by dashed lines); the apex section include a remnant of the larval protoconch and the pallial margin section is referred to the newly produced shell during post-settlement stages. The continuous line indicates the peristomal length (PL) used as measured of shell size (b). Geographic variability in the PL (black dots) and wet weight (white dots) in juvenile individuals of *C. concholepas* (mean  $\pm$  1 s.e.) used for metabolism measurements.

The scan range was from 20 to 60° in 2Theta (0.01° step<sup>-1</sup>; 3 s step<sup>-1</sup>) as described in Ramajo *et al.* (2013). For identification and quantification of calcite and aragonite mineral phases, Xpovder software package was used (J. D. Martín-Ramos, see <http://www.xpovder.com/>, accessed 15 December 2011). After XRD analyses, powder samples were recovered and used to

determinate the amount of organic matter in each shell zone by site using thermo-gravimetric analyses (Mettler-Toledo mod, TGA/DSC1, Zurich, Switzerland). For these analyses, owing to the small powder amount of each shell zone explored and technical requirements of equipment, it was necessary to combine powdered samples from three individuals (from the same locality and shell section). For TGA analysis, ~20 mg of pooled shell powder was placed in a ceramic crucible and heated from room temperature up to 900°C (20°C min<sup>-1</sup>). Main weight loss events can be assigned to water loss (20–180°C), inter-crystalline (180–400°C) and intra-crystalline (400–600°C) organic matter loss and emission of CO<sub>2</sub> (>600°C) during carbonate decomposition (Rodríguez-Navarro et al. 2006; Radishi et al. 2012).

#### Oxygen consumption measurements

During July 2010, *C. concholepas* juvenile individuals were collected from the Antofagasta coast ( $n = 11$ ), Las Cruces ( $n = 20$ ) and Valdivia ( $n = 25$ ), transported to the laboratory and then characterised in terms of wet weight (WW) and peristomal length (PL). Before oxygen measurements, all individuals were maintained under inanition condition during 72 h in UV-filtered seawater and natural photoperiod and then acclimated during an additional 4 days at 14°C. Oxygen consumption rates were measured at 14°C controlled by a chiller (BOYU, Model L075, Raoping, Guangdong, China) and using a Hamilton gas-tight syringe as respirometry chamber (Storch et al. 2011). Oxygen consumption rate was measured using a fibre optic oxygen optode connected to a PreSens Microx TX3 temperature compensated oxygen meter (Precision Sensing, GmbH, Regensburg, Germany) with a tip diameter of 140 µm. Prior to measurements, oxygen micro-sensors were calibrated using 0 and 100% oxygen-saturated reference solutions. For 0% oxygen saturation, we used a 5% Na<sub>2</sub>O<sub>3</sub>S solution, whereas a vigorously air-bubbled solution was used for 100% air saturation. Both calibrations were performed at 14°C (see Storch et al. 2011). We ran the measurements for 60 min to obtain several estimates and avoid measurement errors. The first 10 min and the last 5 min of measurements were discarded to allow for possible disturbances associated with stress of the animal when the optic fibre was inserted or removed.

#### Data analysis

Differences between northern, central and southern Chile populations in calcite : aragonite ratio and organic matter content (%) in the shells were tested using two-way ANOVA using populations and shell section as factors. These proportional data were square root transformed to meet ANOVA assumptions (i.e. normality and homocedasticity). As we found significant differences in shell length (ANOVA,  $F_{(2,52)} = 20.14$ ;  $P < 0.001$ ) and WW (ANOVA,  $F_{(2,52)} = 22.84$ ;  $P < 0.001$ ) (Fig. 2b), the inter-population comparison of oxygen consumption rate was evaluated by one-way ANCOVA controlling by snail size and using WW as covariate. Tukey HSD as *post hoc* tests regarding a Bonferroni correction was used to test for differences between study regions. Prior to this statistical analysis, data were log-transformed to satisfy assumptions of normality and homogeneity of variance using Shapiro–Wilk and Levene tests. All of the analyses were performed using corresponding R routines (R Development Core Team 2009).

## Results

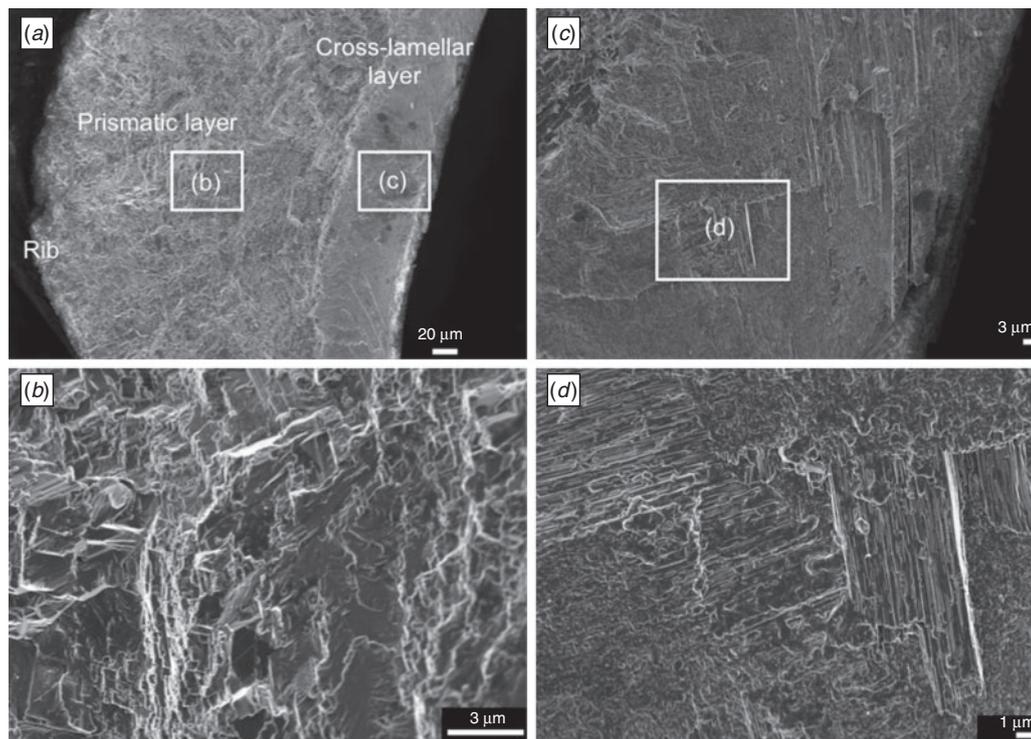
### Shell microstructure

SEM observations for pallial margin of fractured specimens show that *C. concholepas* juveniles (PL = 10.0 ± 1.0 mm,  $n = 3$  from northern; PL = 10.0 ± 2.8 mm,  $n = 3$  from central and PL = 11.0 ± 2.6 mm,  $n = 3$  from southern populations) have a shell constituted by two layers with a different mineralogical composition and microstructure: a thicker (~230 µm) outer calcitic prismatic layer and a thinner (~60 µm) inner aragonitic cross-lamellar layer (Fig. 3a). Fig. 3b shows the microstructure of the calcitic prismatic and inner aragonitic shell layers. The inner cross-lamellar layer of the *C. concholepas* shell is constructed by superimposed lamellae of aragonite fibres (Fig. 3c), which run at angles of ~75° with respect to each other (Fig. 3d).

### Shell mineral composition

*C. concholepas* individuals from the compared populations, assessed using XRD analysis, did not present significant differences in their PL (ANOVA:  $F_{(2,28)} = 1.486$ ;  $P = 0.244$ , Table 2). XRD analysis of shells of *C. concholepas* juveniles demonstrate that calcite was the main mineral phase of the pallial margin of the shell (>70% in all populations, whereas aragonite was the main mineral constituent found at the shell apex (>60% in all populations). These changes in shell mineralogy across the populations were evident when analysing the calcite : aragonite ratio (Fig. 4a). Specifically, a significant difference between *C. concholepas* local populations and shell sections were found in the calcite : aragonite ratio (ANOVA, Interaction R × S;  $P = 0.028$ , Table 3). On the pallial margin of the shell of the juvenile snails shells (i.e. representing the most recent shell mineralisation in post-settlement stages of *C. concholepas*), the calcite : aragonite ratio increases significantly from north to south (Fig. 4a). Calcite percentage (%Ca) increases (and %Ar decreases), an average of 11.9 ± 5.7% ( $n = 10$ ), along the latitudinal gradient. Thus, the shell pallial margin of southern population individuals is notably enriched in calcite (it shows the highest %Ca; 80.7 ± 2.7%,  $n = 10$ ) compared with northern (68.8 ± 3.1%,  $n = 10$ ) and central (78.4 ± 2.5%,  $n = 10$ ) populations of *C. concholepas*. On the contrary, the apex shell sections (i.e. corresponding to the shell grown in pre-settlement stages) do not show a consistent variation with latitude, although the southern population had a small but significant decrease in the calcite : aragonite ratio when compared to the northern and central populations (Fig. 4a). The individuals of the southern population presented the lowest shell apex %Ca (35.0 ± 3.84%,  $n = 11$ ) in comparison with individuals from northern (36.1 ± 2.37%,  $n = 10$ ) and central regions (37.2 ± 2.2%,  $n = 7$ ).

Changes in the organic content of *C. concholepas* shells, determined by TGA analyses, showed two marked weight loss events at two different temperatures. At 300°C the principal weight loss can be ascribed to the combustion of inter-crystalline and at 460°C to the combustion of intra-crystalline shell organic matter (Fig. 5a, b). On average, for all studied populations and across shell sections, the inter-crystalline organic matter concentrations were higher (pallial margin: 1.84 ± 0.12%,  $n = 3$ ; apex: 2.15 ± 0.16%,  $n = 3$ ) than intra-crystalline organic



**Fig. 3.** Microstructure of *Concholepas concholepas* shell: (a) cross-section of pallial margin of the shell showing the outer prismatic and inner cross-lamellar layers; (b) detail of the prismatic layer; (c) detail of the cross-lamellar layer; (d) aragonite fibres of superimposed lamellae running parallel to the fracture surface.

**Table 2.** Sample size (*n*) used in mineralogical analyses and peristomal length (PL) of juvenile snails collected at each study region and sites

Region	Site	<i>n</i> samples per shell section		Peristomal length (mm)		
		Apex	Pallial margin	Mean $\pm$ s.e.	Max	Min
Northern	Las Conchillas	10	10	10.15 $\pm$ 0.11	10.9	9.8
Central	Las Cruces	7	10	9.28 $\pm$ 0.69	12.9	6.7
Southern	Calfuco	11	10	9.19 $\pm$ 0.32	11.0	7.9

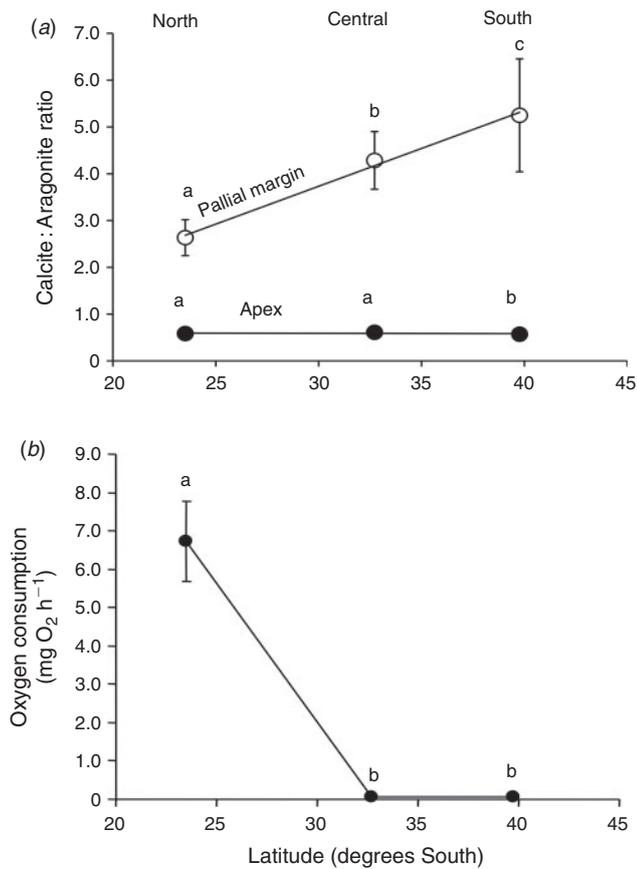
matter concentrations (pallial margin:  $0.52 \pm 0.03\%$ ,  $n = 3$ ; apex:  $0.53 \pm 0.09\%$ ,  $n = 3$ ), however we did not find any significant north–south change in organic matter concentration or differences among shell section (Table 4).

#### Oxygen consumption

The oxygen consumption rate was significantly different between populations (ANCOVA,  $F_{(2,52)} = 115.91$ ;  $P = 0.000$ ). The juveniles snails of *C. concholepas* from northern Chile populations showed much higher oxygen consumption rates ( $6.720 \pm 1.038$  mg O<sub>2</sub> h<sup>-1</sup>,  $n = 11$ ) than those from central ( $0.059 \pm 0.008$  mg O<sub>2</sub> h<sup>-1</sup>,  $n = 20$ ) and southern Chile ( $0.064 \pm 0.009$  mg O<sub>2</sub> h<sup>-1</sup>,  $n = 25$ ). However, the central and southern populations did not have significantly different oxygen consumption rates (Tukey HSD test,  $P = 0.952$ ) (Fig. 4b).

#### Discussion

Our results show that shell mineralogy of *C. concholepas* has significant variations among populations along the Chilean coast. In particular, there is a notable increase in the calcite to aragonite proportion in the pallial shell margin from north to south, which is consistent with previous reports indicating that shell formation processes are highly sensitive to the environmental gradient from north to south along the studied coast (Ramajo *et al.* 2013). It also indicates that, at post-settlement life stages, the calcification process in juvenile individuals of *C. concholepas* may favour the precipitation of calcite (less soluble and thermodynamically more stable than aragonite) in shell. The preferential formation of less soluble calcite would make the shell more resistant against dissolution during events of low carbonate saturation states. Recent studies such as Salisbury *et al.* (2008) have determined that low carbonate

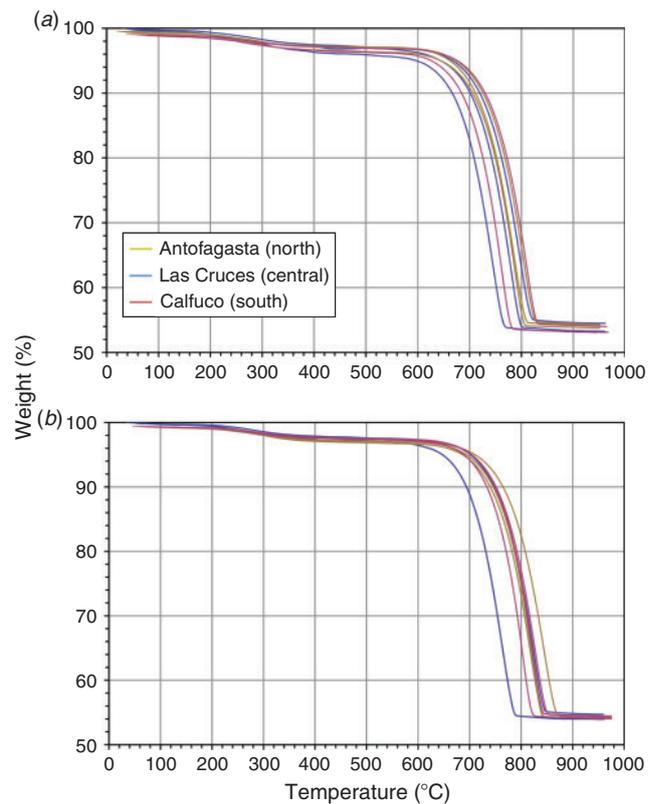


**Fig. 4.** (a) Latitudinal trend (continuous lines) in calcite : aragonite ratio for the apex and pallial margin shell sections in juveniles snails of *Concholepas concholepas*. (b) Mass-specific rate of oxygen consumption ( $\text{mg O}_2 \text{ h}^{-1} \text{ g}^{-1}$ ). Symbols represent the mean  $\pm$  s.e. Different letters indicate significant differences ( $P < 0.05$ ) between regions using Tukey HSD as *post hoc* test.

**Table 3.** *Concholepas concholepas*. Two-way ANOVA results applied to assess the differences in calcite : aragonite ratio between regions (North, Central and Southern Chile) and shell sections (apex and pallial margin)

Response variable	Source of variation	d.f.	MS	F	P-value
Calcite : aragonite ratio	Region (R)	2	0.94	6.52	0.002
	Shell section (S)	1	19.07	132.89	<0.001
	R $\times$ S	2	0.55	3.82	0.028
	Error	52	0.14		

saturation values (not under-saturated) have important effects over the development of shellfish and even in supersaturated conditions occur at significant dissolutions rates (Waldbusser et al. 2011). However, under-saturated carbonate conditions ( $\Omega < 1$ ) have been related to, in some bivalve species, important signs of malformations and dissolutions of the aragonite shell (Green et al. 2004; Welladsen et al. 2010), which might be compromising the shell integrity and strength making them



**Fig. 5.** TGA thermograms of (a) apex and (b) pallial margin of *Concholepas concholepas* shells for the study populations.

more vulnerable to predation (Green et al. 2004; McClintock et al. 2009). Chilean coast presents, to date, calcite and aragonite saturation values over 1 along the entire coast, however an important decrease from northern to south exists (see Table 1, Torres et al. 2011; Mayol et al. 2012).

Early experimental and theoretical studies have tried to determine the role of temperature on the precipitation of calcite or aragonite in shelled organisms (Lowenstam 1954a, 1954b; Burton and Walter 1987, 1991; Mucci 1987; Morse et al. 1997). Schifano (1982) determined in several gastropod species that the proportion of aragonite in the last formed section of the shell is linearly and positively related with SST. Along the Chilean coast, there is an important gradient in temperature as the northern region differs with respect to central and southern regions by more than 5°C in SST (Ramajo et al. 2013). Our mineralogical analysis of *C. concholepas* shells showed that there is a significant increase in calcite : aragonite ratio in the pallial margin of the shell from north to south according to Schifano's (1982) results. Also, calcium carbonate polymorphism selection during inorganic precipitation is greatly influenced by temperature. Many studies such as Walter (1986), Burton and Walter (1987) and Ogino et al. (1987) show that calcite formation is favoured in cold water whereas aragonite precipitation is favoured at higher water temperatures.

Salinity has been reported to affect the mineralogy of a *Mytilus* species beyond the role of temperature (Lowenstam 1954a, 1954b). Dodd (1963) demonstrated an inverse

**Table 4. Organic matter content (%) resulting of thermogravimetry (TGA) analysis (mean  $\pm$  s.e.) by shell section of the juvenile snail collected along the latitudinal gradient of the Chilean coast (results of two-way analysis of variance are presented below)**

Shell sections probabilities are water: (ANOVA,  $F_{(1,11)} = 0.02$ ;  $P = 0.887$ ; inter-crystalline organic matter: ANOVA,  $F_{(1,11)} = 1.29$ ;  $P = 0.280$ ; and intra-crystalline organic matter: ANOVA,  $F_{(1,11)} = 0.02$ ;  $P = 0.891$ ) when comparing across shell sections. Region probabilities are: water: (ANOVA,  $F_{(2,11)} = 0.96$ ;  $P = 0.411$ ); inter-crystalline organic matter (ANOVA,  $F_{(2,11)} = 1.97$ ;  $P = 0.185$ ) and intra-crystalline organic matter (ANOVA,  $F_{(2,11)} = 1.69$ ,  $P = 0.229$ ) among regions (R). The interaction  $R \times S$  was not significant in either case ( $P < 0.05$ )

Shell section	Region	Water (%)	Inter-crystalline organic matter (%)	Intracrystalline organic matter (%)
Pallial margin	North	0.388 $\pm$ 0.002	1.860 $\pm$ 0.040	0.379 $\pm$ 0.142
	Central	0.645 $\pm$ 0.200	2.085 $\pm$ 0.271	0.690 $\pm$ 0.169
	South	0.308 $\pm$ 0.045	1.581 $\pm$ 0.026	0.539 $\pm$ 0.076
Apex	North	0.332 $\pm$ 0.082	2.139 $\pm$ 0.082	0.506 $\pm$ 0.045
	Central	0.650 $\pm$ 0.021	2.543 $\pm$ 0.378	0.564 $\pm$ 0.080
	South	0.372 $\pm$ 0.072	1.779 $\pm$ 0.182	0.485 $\pm$ 0.058

relationship between salinity and shell aragonite content when comparing individual exposed to constant temperature and a salinity variations (ranging from 18 to 32.4‰), and suggested that high salinity environments induce increased calcite precipitation rates (Malone and Dodd 1967), a trend also observed by Zhong and Mucci (1989) in inorganic experiments. The Chilean coast also presents a salinity gradient mainly due to an increase in river discharges into coastal areas located south of  $\sim 37^\circ\text{S}$  latitude (Dávila *et al.* 2002). River discharges add to the system acidity and reduce carbonate saturation stages (Salisbury *et al.* 2008; Waldbusser and Salisbury 2014). Indeed, we found that the lowest aragonite mineral levels in *C. concholepas* shells (for pallial margin sections) occur in southern Chile, a region characterised by a reduced salinity due to freshwater inputs and low SST (Strub *et al.* 1998; Dávila *et al.* 2002). However, according to previous work and considering the coexistence and opposite temperature and salinity gradients along the Chilean coast, our observations suggest that temperature may have a dominant effect on carbonate precipitation, which is in agreement with several inorganic calcium carbonate precipitation studies that established that temperature is the major factor controlling different calcium carbonate polymorphisms (i.e. aragonite: calcite ratio; Zeller and Wray 1956; Ogino *et al.* 1987; Bertram *et al.* 1991; Dickson 2004).

However, the shell organic matrix can play an important protective role against shell mineral dissolution (Green *et al.* 2004) as the intra- and inter-crystalline envelopes the shell mineral. Although studies as Gutowska *et al.* (2010) noted a 30% of reduced incorporation of organic matrix in the calcified structure of cephalopods exposed to corrosive waters, our results do not show any differences in the amount of inter- or intra-crystalline organic matter along the studied geographical populations and shell section studied. Similarly Green *et al.* (2004) and Welladsen *et al.* (2010) did not find any changes in the amount of organic matter in molluscs exposed to under-saturated carbonate solutions, in spite of the significant changes in shell mineralogy.

Geographic variations in temperature are suggested as the main driver of spatial variations in several traits of the marine invertebrates (Lardies and Castilla 2001; Lardies *et al.* 2008; Ramajo *et al.* 2013), indicating that not all populations would be investing the same energy in similar biological processes.

However, few studies have addressed the effect of the geographic variation on respiratory physiology (Vernberg 1959; Vernberg and Vernberg 1964). Broadly, results show that lower latitude populations have more elevated respiration rates than higher latitude conspecifics populations at equal temperatures (Whiteley *et al.* 1997; Osovitz and Hofmann 2007; Lardies *et al.* 2011). Our observations show the same pattern, northern, low latitude population of *C. concholepas* showed a higher metabolic rate compared to central–southern populations. However, temperature may not be the sole environmental driver affecting the metabolic rate of *C. concholepas* juveniles because the gradient of carbonate saturation states along the Chilean coast may play a role, as there is a significant metabolic cost associated with shell mineralisation (Palmer 1992).

OA studies have reported that shallow living species exposed to short periods of reduced pH present different mechanisms to regulate acid–base balance for the intracellular compensation of pH (Langenbuch and Pörtner 2002; Hendriks *et al.* 2015). However, when these mechanisms cannot be activated, a metabolic and protein depression has been observed (Langenbuch and Pörtner 2002; Michaelidis *et al.* 2005; Dupont *et al.* 2010; Stumpp *et al.* 2011) with changes in basal metabolism and reallocation of energy for other biological processes such as reproduction and growth, which in the case of calcifier organisms, imply the precipitation of the shell carbonates (Gattuso and Hansson 2011). Thus, metabolic economy is an important constraint in the bio-mineralisation processes. Palmer (1983, 1992) determined that shell organic matrix formation is more demanding metabolically than the crystallisation of calcium carbonate and Paine (1971) found, for several gastropod species, that calcification costs were equivalent to 75 and 410% of the energy invested in somatic growth and reproduction respectively. However, to date, it is still not clear what energy is necessary for mineral shell formation, as studies have underestimated the energy used for mineralisation by not taking into account aspects of calcification such as the production of enzymes involved in calcium carbonate deposition, transport of Ca and CO<sub>2</sub>, as well as acid–base regulation at the site of CaCO<sub>3</sub> deposition and maintenance shell to avoid dissolution (see Lannig *et al.* 2010).

Based in the type of calcium carbonate precipitated it has been proposed that calcite polymorphism might have evolved

for reason of lower energy expenditure or faster deposition (Taylor and Layman 1972). In addition, Hautmann (2006) determine that calcite secretion was facilitated during short periods of carbonate under-saturation of seawater, because calcite is less soluble than aragonite and requires lower carbonate saturation levels, which would reduce the energy to supply these ions. Thus, aragonite formation in acidic waters with low or under-saturated conditions, must be metabolically more costly than forming calcite (with higher saturation states for the same water solution chemistry as aragonite is more soluble) as the organisms have to pump more calcium and carbonate ions to produce a sufficient increase in the saturation with respect to aragonite for crystals to precipitate and grow. This might explain why more metabolically active organisms (i.e. northern populations) can precipitate shell material with a greater %Ar on the pallial margin of the shell. Hence, our results suggest that the naturally high metabolic rates recorded in juveniles snails collected from northern Chile *C. concholepas* populations could be less affected by increased CO<sub>2</sub> concentrations in seawater as suggested by Bambach et al. (2002). However, Lardies et al. (2014) in an experimental mesocosm approach showed that elevated pCO<sub>2</sub> conditions increase the standard metabolic rates in juveniles of *C. concholepas* collected from northern and southern Chile, likely due to the higher energy cost of homeostasis. However, these authors also highlight the importance of acclimation and tolerance in populations from southern Chile, which showed a lower increment of metabolic rate in the high-pCO<sub>2</sub> conditions.

Aragonitic shells are more susceptible to corrosion in coastal waters with low carbonate saturation state with respect to these mineral species due to upwelling and river discharge, which are typically supersaturated in CO<sub>2</sub> (Fabry et al. 2008; Salisbury et al. 2008; Barton et al. 2012). Taylor (1973) and Harper et al. (1997) suggested that evolution to calcite in mollusc shells might represent an adaptation to avoid corrosion, although other posterior studies as Harper (2000) determined that not only carbonate type, but also crystal size and organic content affect the dissolution rates of the shells. In our case, differences in the amount of organic matter and the size of crystals were not observed among studied populations. Therefore, the different properties and formation conditions of calcite and aragonite need to be invoked in order to understand the shifts in shell mineralogy observed along Chilean coast for *C. concholepas* juveniles.

Our results suggest that *C. concholepas* metabolism and in turn its shell mineralisation process are spatially variable and sensitive to changes in environmental parameters. In particular, the observed shift in the shell mineralogy that favours calcite over aragonite formation towards high latitudes could be determined by the latitudinal changes in the physical–chemical conditions along the Chilean coast. However, it is still unclear how the different physical–chemical parameters (i.e. temperature, salinity, carbonate saturation state) could be acting (oppositely, additively or synergistically) and their relative influence over the relevant biological processes associated to shell calcification. Experimental studies including orthogonal design are necessary to shed light on the relative role of each environmental factor separately on shell mineralisation and composition. In general, our result imply that projected increases of pCO<sub>2</sub> in seawater (OA) might have and important effect on the

physiological cost of shell carbonate precipitation, especially in populations already exposed to lowered values of pH and carbonate saturation states as those found in upwelling and river discharge areas.

## Acknowledgements

We thank G. Gonzalez, S. Urrejola, B. Arias and J. Pantoja for their kind support in the field and L. Prado in laboratory procedures. This study was supported by grants FONDECYT 1090624–2009 (TOA-SPACE), ANILLO ACT 132. N. A. Lagos and M. A. Lardies acknowledge the Millennium Nucleus Project NC 1200286 ‘Center for the study of multiple drivers on marine socio-ecological systems (MUSELS)’ from the Ministerio de Economía, Fomento y Turismo. ARN acknowledges financial support through the grant CGL2011-25906 (Ministerio de Ciencia e Innovación, Spain), RNM-179 group (Junta de Andalucía, Spain). L. Ramajo was supported by BECAS CHILE fellowship programme from Comisión Nacional de Investigación Científica y Tecnológica de Chile (CONICYT). Critical insights received from Liz Harper and one anonymous reviewer helped us to improve the final version of this study.

## References

- Addadi, L., and Weiner, S. (1992). Control and design principles in biological mineralization. *Angewandte Chemie* **31**, 153–169. doi:10.1002/ANIE.199201531
- Bambach, R. K., Knoll, A. H., and Sepkoski, J. J. (2002). Anatomical and ecological constraints on Phanerozoic animal diversity in the marine realm. *Proceedings of the National Academy of Sciences of the United States of America* **99**, 6854–6859. doi:10.1073/PNAS.092150999
- Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., and Feely, R. A. (2012). The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: implications for near-term ocean acidification effects. *Limnology and Oceanography* **57**, 698–710. doi:10.4319/LO.2012.57.3.0698
- Belcher, A., Wu, X., Christensen, R., Hansma, P., Stucky, G., and Morse, D. (1996). Control of crystal phase switching and orientation by soluble mollusc shell proteins. *Nature* **381**, 56–58. doi:10.1038/381056A0
- Bertram, M. A., Mackenzie, M. A., Bishop, F. T., and Bischoff, W. D. (1991). Influence of temperature on the stability of magnesian calcite. *The American Mineralogist* **76**, 108–134.
- Bøggild, O. B. (1930). The shell structure of the Mollusks. *Det Kongelige Danske videnskabernes selskabs skrifter, Naturvidenskabelig og mathematisk afdeling* **9**, 231–326.
- Burton, E. A., and Walter, L. M. (1987). Relative precipitation rates of aragonite and Mg-calcite from seawater: temperature or carbonate ion control? *Geology* **15**, 111–114. doi:10.1130/0091-7613(1987)15<111:RPROAA>2.0.CO;2
- Burton, E. A., and Walter, L. M. (1991). The effects of pCO<sub>2</sub> and temperature on magnesium incorporation in calcite in seawater and MgCl<sub>2</sub>–CaCl<sub>2</sub> solutions. *Geochimica et Cosmochimica Acta* **55**, 777–785. doi:10.1016/0016-7037(91)90341-2
- Byrne, M. (2011). Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanography and Marine Biology – an Annual Review* **49**, 1–42.
- Cohen, A. L., and Branch, G. M. (1992). Environmentally controlled variation in the structure and mineralogy of *Patella granularis* shells from the coast of southern Africa: implications for paleotemperature assessments. *Palaeogeography, Palaeoclimatology, Palaeoecology* **91**, 49–57. doi:10.1016/0031-0182(92)90031-Y
- Comeau, S., Gorsky, G., Jeffree, R., Teyssié, J. L., and Gattuso, J. P. (2009). Impact of ocean acidification on a key Arctic pelagic mollusc (*Limacina helicina*). *Biogeosciences* **6**, 1877–1882. doi:10.5194/BG-6-1877-2009

- Dauphin, Y., Guzmán, N., Denis, A., Cuif, J. P., and Ortlieb, J. L. (2003). Microstructure, nanostructure and composition of the shell of *Concholepas concholepas* (Gastropoda, Muricidae). *Aquatic Living Resources* **16**, 95–103. doi:10.1016/S0990-7440(03)00022-6
- Dávila, P. M., Figueroa, D., and Müller, E. (2002). Freshwater input into the coastal ocean and its relation with the salinity distribution off austral Chile (35–55°S). *Continental Shelf Research* **22**, 521–534. doi:10.1016/S0278-4343(01)00072-3
- Dickson, J. A. D. (2004). Echinoderm skeletal preservation: calcite–aragonite seas and the Mg/Ca ratio of Phanerozoic oceans. *Journal of Sedimentary Research* **74**, 355–365. doi:10.1306/112203740355
- Dodd, J. R. (1963). Paleocological implications of shell mineralogy in two pelecypod species. *The Journal of Geology* **71**, 1–11. doi:10.1086/626872
- Dodd, J. R. (1964). Environmentally controlled variation in the shell structure of a pelecypod species. *Paleontological Journal* **38**, 1065–1071.
- Dodd, J. R. (1966). The influence of salinity on mollusk shell mineralogy: a discussion. *The Journal of Geology* **74**, 85–89. doi:10.1086/627144
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. (2009). Ocean acidification: the other CO<sub>2</sub> problem. *Annual Review of Marine Science* **1**, 169–192. doi:10.1146/ANNUREV.MARINE.010908.163834
- Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J. A., and McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on marine pH. *Estuaries and Coasts* **36**, 221–236. doi:10.1007/S12237-013-9594-3
- Dupont, S., Ortega-Martinez, O., and Thorndyke, M. (2010). Impact of near-future ocean acidification on echinoderms. *Ecotoxicology (London, England)* **19**, 449–462. doi:10.1007/S10646-010-0463-6
- Eisma, D. (1966). The influence of salinity on mollusk shell mineralogy: a discussion. *The Journal of Geology* **74**, 89–94. doi:10.1086/627145
- Fabry, V. J., Seibel, B. A., Feely, R. S., and Orr, J. C. (2008). Impacts of ocean acidification on marine fauna and ecosystem processes. *Journal of Marine Science* **65**, 414–432.
- Gattuso, J. P., and Hansson, L. (2011). Ocean acidification: background and history. In 'Ocean acidification'. (Eds J. P. Gattuso and L. Hansson.) pp. 1–20. (Oxford University Press: Oxford, UK.)
- Goffredo, S., Prada, F., Caroselli, E., Capaccioni, B., Zaccanti, F., Pasquini, L., Fantazzini, P., Fermani, S., Reggi, M., Levy, O., Fabricius, K. E., Dubinsky, Z., and Falini, G. (2014). Biomineralization control related to population density under ocean acidification. *Nature Climate Change* **4**, 593–597. doi:10.1038/NCLIMATE2241
- Green, M. A., Jones, M. E., Boudreau, C. L., Moore, R. L., and Westman, B. A. (2004). Dissolution mortality of juvenile bivalves in coastal marine deposits. *Limnology and Oceanography* **49**, 727–734. doi:10.4319/LO.2004.49.3.0727
- Gutowska, M. A., Melzner, F., Pörtner, H. O., and Meier, S. (2010). Cuttlebone calcification increases during exposure to elevated seawater pCO<sub>2</sub> in the cephalopod *Sepia officinalis*. *Marine Biology* **157**, 1653–1663. doi:10.1007/S00227-010-1438-0
- Guzmán, N. (2004). Validation d'une approche scléroclimatologique sur la côte du Chili et du Pérou par l'analyse microstructurale et biogéochimique des coquilles du gastéropode *Concholepas concholepas* [Bruguère, 1989]. Ph.D. Thesis, Université de Paris-Sud.
- Guzmán, N., Dauphin, Y., Cuif, J. P., Denis, A., and Ortlieb, L. (2009). Diagenetic changes in *Concholepas concholepas* shells (Gastropoda, Muricidae) in the hyper-arid conditions on Northern Chile – implications for palaeoenvironmental reconstructions. *Biogeosciences* **6**, 197–207. doi:10.5194/BG-6-197-2009
- Hale, R., Calosi, P., McNeill, L., Mieszkowska, N., and Widdicombe, S. (2011). Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. *Oikos* **120**, 661–674. doi:10.1111/J.1600-0706.2010.19469.X
- Harper, E. M. (2000). Are calcitic layers and effective adaptation against shell dissolution in the Bivalvia? *Journal of Zoology* **251**, 179–186. doi:10.1111/J.1469-7998.2000.TB00602.X
- Harper, E. M., Palmer, T. J., and Alphey, J. R. (1997). Evolutionary response by bivalves to changing Phanerozoic sea-water chemistry. *Geological Magazine* **134**, 403–407. doi:10.1017/S0016756897007061
- Hautmann, M. (2006). Shell mineralogical trends in epifaunal Mesozoic bivalves and their relationship to seawater chemistry and atmospheric carbon dioxide concentration. *Facies* **52**, 417–433. doi:10.1007/S10347-005-0029-X
- Hendriks, I. E., Duarte, C. M., and Alvarez, M. (2010). Vulnerability of marine biodiversity to ocean acidification; a meta-analysis. *Estuarine, Coastal and Shelf Science* **86**, 157–164. doi:10.1016/J.ECSS.2009.11.022
- Hendriks, I. E., Duarte, C. M., Olsen, Y. S., Steckbauer, A., Ramajo, L., Moore, T. S., Trotter, J. A., and McCulloch, M. (2015). Biological mechanisms supporting adaptation to ocean acidification in coastal ecosystems. *Estuarine, Coastal and Shelf Science* **152**, A1–A8. doi:10.1016/J.ECSS.2014.07.019
- Hubbard, F., McManus, J., and Al-Dabbas, M. (1981). Environmental influences on the shell mineralogy of *Mytilus edulis*. *Geo-Marine Letters* **1**, 267–269. doi:10.1007/BF02462445
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., and Gattuso, J. P. (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology* **19**, 1884–1896. doi:10.1111/GCB.12179
- Lagos, N. A., Navarrete, S. A., Véliz, F., Masuero, A., and Castilla, J. C. (2005). Meso-scale spatial variation in settlement and recruitment of intertidal barnacles along central Chile. *Marine Ecology Progress Series* **290**, 165–178. doi:10.3354/MEPS290165
- Lagos, N. A., Castilla, J. C., and Broitman, B. R. (2008). Spatial environmental correlates of intertidal recruitment: a test using barnacles in northern Chile. *Ecological Monographs* **78**, 245–261. doi:10.1890/07-0041.1
- Langenbuch, M., and Pörtner, H. O. (2002). Changes in metabolic rate and N excretion in the marine invertebrate *Sipunculus nudus* under conditions of environmental hypercapnia: identifying effective acid–base variables. *The Journal of Experimental Biology* **205**, 1153–1160.
- Lannig, G., Eilers, S., Pörtner, H. O., Sokolova, I. A., and Bock, C. (2010). Impact of ocean acidification on energy metabolism of oyster, *Crassostrea gigas*. Changes in metabolic pathways and thermal response. *Marine Drugs* **8**, 2318–2339. doi:10.3390/MD8082318
- Lardies, M. A., and Castilla, J. C. (2001). Latitudinal variation in the reproductive biology of the commensal crab *Pinnaxodes chilensis* (Decapoda:Pinotheridae) along the Chilean coast. *Marine Biology* **139**, 1125–1133. doi:10.1007/S002270100661
- Lardies, M. A., Naya, D. E., and Bozinovic, F. (2008). The cost of living slowly: metabolism, Q<sub>10</sub> and repeatability in a South American harvestman. *Physiological Entomology* **33**, 193–199. doi:10.1111/J.1365-3032.2008.00621.X
- Lardies, M. A., Muñoz, J. L., Paschke, K. A., and Bozinovic, F. (2011). Latitudinal variation in the aerial/aquatic ratio of oxygen consumption of a supratidal high rocky-shore crab. *Marine Ecology (Berlin)* **32**, 42–51. doi:10.1111/J.1439-0485.2010.00408.X
- Lardies, M. A., Arias, M. B., Poupin, M. J., Manríquez, P. H., Torres, R., Vargas, C. A., Navarro, J. M., and Lagos, N. A. (2014). Differential response to ocean acidification in physiological traits of *Concholepas concholepas* populations. *Journal of Sea Research* **90**, 127–134. doi:10.1016/J.SEARES.2014.03.010
- Lefèvre, N., Aiken, J., Rutllant, J., Daneri, G., Lavender, S., and Smyth, T. (2002). Observations of pCO<sub>2</sub> in the coastal upwelling off Chile: spatial and temporal extrapolation using satellite data. *Journal of Geophysical Research* **107**, 3055. doi:10.1029/2000JC000395

- Lowenstam, H. (1954a). Environmental relations of modification compositions of certain carbonate secreting marine invertebrates. *Proceedings of the National Academy of Sciences of the United States of America* **40**, 39–48. doi:10.1073/PNAS.40.1.39
- Lowenstam, H. (1954b). Factors affecting the aragonite-calcite ratios in carbonate-secreting marine organisms. *The Journal of Geology* **62**, 284–322. doi:10.1086/626163
- Malone, P. G., and Dodd, J. R. (1967). Temperature and salinity effects on calcification rate in *Mytilus edulis* and its paleoecological implications. *Limnology and Oceanography* **12**, 432–436. doi:10.4319/LO.1967.12.3.0432
- Manríquez, P. H., Delgado, A. P., Jara, M. E., and Castilla, J. C. (2008). Field and laboratory experiments with early ontogenetic stages of *Concholepa concholepa* under field and laboratory conditions in Central Chile. *Aquaculture* **279**, 99–107. doi:10.1016/J.AQUACULTURE.2008.03.031
- Mayol, E., Ruiz-Halpern, S., Duarte, C. M., Castilla, J. C., and Pelegrí, J. L. (2012). Coupled CO<sub>2</sub> and O<sub>2</sub>-driven compromises to marine life in summer along the Chilean sector of the Humboldt Current System. *Biogeosciences* **9**, 1183–1194. doi:10.5194/BG-9-1183-2012
- McClintock, J. B., Angus, R. A., McDonald, M. R., Amsler, C. D., Catledge, S. A., and Vohra, Y. K. (2009). Rapid dissolution of shells of weakly calcified Antarctic benthic macroorganisms indicates high vulnerability to ocean acidification. *Antarctic Science* **21**, 449–456. doi:10.1017/S0954102009990198
- Michaelidis, B., Ouzounis, C., Paleras, A., and Pörtner, H. O. (2005). Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series* **293**, 109–118. doi:10.3354/MEPS293109
- Morse, J. W., Wang, Q., and Tsio, M. Y. (1997). Influences of temperature and Mg:Ca ratio on CaCO<sub>3</sub> precipitates from seawater. *Geology* **25**, 85–87. doi:10.1130/0091-7613(1997)025<0085:IOTAMC>2.3.CO;2
- Mucci, A. (1987). Influence of temperature on the composition of magnesium calcite overgrowths precipitated from seawater. *Geochimica et Cosmochimica Acta* **51**, 1977–1984. doi:10.1016/0016-7037(87)90186-4
- Ogino, T., Suzuki, T., and Sawada, K. (1987). The formation and transformation mechanism of calcium carbonate in water. *Geochimica et Cosmochimica Acta* **51**, 2757–2767. doi:10.1016/0016-7037(87)90155-4
- Osovitz, C. J., and Hofmann, G. E. (2007). Marine macrophysiology: studying physiological variation across large spatial scales in marine systems. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **147**, 821–827. doi:10.1016/J.CBPA.2007.02.012
- Paine, R. T. (1971). A short-term experimental investigation of resource partitioning in a New Zealand rocky intertidal habitat. *Ecology* **52**, 1096–1106. doi:10.2307/1933819
- Palmer, A. R. (1983). Relative cost of producing skeletal organic matrix versus calcification: evidence from marine gastropods. *Marine Biology* **75**, 287–292. doi:10.1007/BF00406014
- Palmer, A. R. (1992). Calcification in marine mollusks: how costly is it? *Proceedings of the National Academy of Sciences of the United States of America* **89**, 1379–1382. doi:10.1073/PNAS.89.4.1379
- Putnis, A., Prieto, M., and Fernández-Díaz, L. (1995). Fluid supersaturation and crystallization in porous-media. *Geological Magazine* **132**, 1–13. doi:10.1017/S0016756800011389
- R Development Core Team (2009). R: a language and environment for statistical computing. (R Foundation for Statistical Computing: Vienna, Austria.) Available at <http://www.R-project.org> [Verified 26 June 2012].
- Radishi, N. A., Mohamed, M., and Yusup, S. (2012). The kinetic model of calcination and carbonation of *Anadara Granosa*. *Renewable Energy* **2**, 497–503.
- Ramajo, L., Baltanás, A., Torres, R., Manríquez, P. H., and Lagos, N. A. (2013). Geographic variation in shell morphology, weight and mineralization of juvenile snails of *Concholepa concholepa* (loco) along the Chilean coast. *Journal of the Marine Biological Association of the United Kingdom* **93**, 2167–2176. doi:10.1017/S0025315413000891
- Ries, J. B. (2011). Skeletal mineralogy in a high-CO<sub>2</sub> world. *Journal of Experimental Marine Biology and Ecology* **403**, 54–64. doi:10.1016/J.JEMBE.2011.04.006
- Rodríguez-Navarro, A., CabraldeMelo, C., Batista, N., Morimoto, N., Alvarez-Lloret, P., Ortega-Huertas, M., Fuenzalida, V. M., Arias, J. I., Wiff, J. P., and Arias, J. L. (2006). Microstructure and crystallographic-texture of giant barnacle (*Austrorhynchus psittacus*) shell. *Journal of Structural Biology* **156**, 355–362. doi:10.1016/J.JSB.2006.04.009
- Saleuddin, A. S. M., and Kunigelis, S. C. (1984). Neuroendocrine control mechanism in shell formation. *American Zoologist* **24**, 9111–9116.
- Salisbury, J., Green, M., Hunt, C., and Campbell, J. (2008). Coastal acidification by rivers: a new threat to shellfish. *Eos, Transactions, American Geophysical Union* **89**, 513. doi:10.1029/2008EO500001
- Schifano, G. (1982). Temperature effects on shell mineralogy and morphology in three gastropod species. *Marine Geology* **45**, 79–91. doi:10.1016/0025-3227(82)90181-5
- Simkiss, K., and Wilbur, K. M. (1989). 'Biom mineralization: Cell Biology and Mineral Deposition.' (Academic Press: San Diego, CA.)
- Storch, D., Fernández, M., Navarrete, S., and Pörtner, H. (2011). Thermal tolerance of larval stages of the Chilean kelp crab *Taliepus dentatus*. *Marine Ecology Progress Series* **429**, 157–167. doi:10.3354/MEPS09059
- Strub, T., Mesías, J., Montecino, V., Rutllant, J., and Salinas, S. (1998). Coastal ocean circulation off western South America. Coastal segment. In 'The Sea'. (Eds A. R. Robinson and K. H. Brink.) pp. 273–313. (Wiley: Hoboken, NJ, USA.)
- Stumpp, M., Wren, J., Melzner, F., Thorndyke, M. C., and Dupont, S. T. (2011). Seawater acidification impacts sea urchin larval development. I. Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology* **160**, 331–340. doi:10.1016/J.CBPA.2011.06.022
- Taylor, J. D. (1973). The structural evolution of the bivalve shell. *Palaeontology* **16**, 519–534.
- Taylor, J. D., and Layman, M. A. (1972). The mechanical properties of bivalve (Mollusca) shell structure. *Palaeontology* **15**, 73–87.
- Taylor, J. D., Kennedy, W., and Hall, A. (1969). Shell structure and mineralogy of the Bivalvia. Introduction. *Nuculacae-Trigonacae. Bulletin of the British Museum (Natural History). Zoology* **3**, 1–125.
- Torres, R., Turner, D., Rutllant, J., Sobarzo, M., Antezana, T., and Gonzalez, H. (2002). CO<sub>2</sub> outgassing off Central Chile (31–30°S) and northern Chile (24–23°S) during austral summer 1997: the effect of wind intensity on the upwelling and ventilation of CO<sub>2</sub>-rich waters. *Deep-sea Research. Part I, Oceanographic Research Papers* **49**, 1413–1429. doi:10.1016/S0967-0637(02)00034-1
- Torres, R., Pantoja, S., Harada, N., González, H. E., Daneri, G., Frangopulos, M., Rutllant, J. A., Duarte, C. M., Rúa-Halpern, S., Mayol, E., and Fukasawa, M. (2011). Air-sea CO<sub>2</sub> fluxes along the coast of Chile: from CO<sub>2</sub> outgassing in central-northern upwelling waters to CO<sub>2</sub> sequestering in southern Patagonian fjords. *Journal of Geophysical Research* **116**. doi:10.1029/2010JC006344
- Vernberg, F. J. (1959). Studies on the physiological variation between tropical and temperate zone fiddler crabs of the genus *Uca*. II. Oxygen consumption of whole organisms. *The Biological Bulletin* **117**, 163–184. doi:10.2307/1539048
- Vernberg, F. J., and Vernberg, W. B. (1964). Metabolic adaptation of animals from different latitudes. *Helgoland Marine Research* **9**, 476–487.
- Waldbusser, G. G., and Salisbury, J. E. (2014). Ocean acidification in the coastal zone from an organism's perspective: multiple system parameters, frequency domains, and habitats. *Annual Review of Marine Science* **6**, 221–247. doi:10.1146/ANNUREV-MARINE-121211-172238

- Waldbusser, G. G., Bergschneider, H., and Green, M. A. (2010). Size-dependent pH effect on calcification in post-larval hard clam *Merccenaria* spp. *Marine Ecology Progress Series* **417**, 171–182. doi:[10.3354/MEPS08809](https://doi.org/10.3354/MEPS08809)
- Waldbusser, G. G., Steenson, R. A., and Green, M. A. (2011). Oyster shell dissolution rates in estuarine waters: effects of pH and shell legacy. *Journal of Shellfish Research* **30**, 659–669. doi:[10.2983/035.030.0308](https://doi.org/10.2983/035.030.0308)
- Waldbusser, G. G., Brunner, E. L., Haley, B. A., Hales, B., Langdon, C. J., and Prah, F. G. (2013). A developmental and energetic basis linking larval oyster shell formation to ocean acidification. *Geophysical Research Letters* **40**, 2171–2176. doi:[10.1002/GRL.50449](https://doi.org/10.1002/GRL.50449)
- Walter, L. M. (1986) Relative efficiency of carbonate dissolution and precipitation during diagenesis: a progress report on the role of solution chemistry. In 'Roles of Organic Matter in Sediment Diagenesis'. (Eds D. L. Gautier.) Special Publication 38, pp. 1–11 (Society of Economic Paleontologists and Mineralogists: Tulsa, OK, USA.)
- Watabe, N., and Wilbur, K. (1960). Influence of the organic matrix on crystal type in mollusks. *Nature* **188**, 334. doi:[10.1038/188334A0](https://doi.org/10.1038/188334A0)
- Welladsen, H. M., Southgate, P. C., and Heimann, K. (2010). The effects of exposure to near-future levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia: Pteriidae). *Molluscan Research* **30**, 125–130.
- Whiteley, N. M., Taylor, E. W., and El-Haj, A. J. (1997). Seasonal and latitudinal adaptation to temperature in crustaceans. *Journal of Thermal Biology* **22**, 419–427. doi:[10.1016/S0306-4565\(97\)00061-2](https://doi.org/10.1016/S0306-4565(97)00061-2)
- Yamamoto-Kawai, M., McLaughlin, F., Carmack, E. C., Nishino, S., and Shimada, K. (2009). Aragonite undersaturation in the Arctic Ocean: effects of ocean acidification and sea ice melt. *Science* **326**, 1098–1100. doi:[10.1126/SCIENCE.1174190](https://doi.org/10.1126/SCIENCE.1174190)
- Zeller, E. J., and Wray, J. L. (1956). Factors influencing the precipitation of calcium carbonate. *The American Association of Petroleum Geologists Bulletin* **40**, 140–152.
- Zhong, S., and Mucci, A. (1989). Calcite and aragonite precipitation from seawater solutions of various salinities: precipitation rates and overgrowth compositions. *Chemical Geology* **78**, 283–299. doi:[10.1016/0009-2541\(89\)90064-8](https://doi.org/10.1016/0009-2541(89)90064-8)