



Antarctic urchin *Ctenocidaris speciosa* spines: lessons from the deep

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Abstract: Ocean acidification is leading to changes in the oceanic carbonate system. As a result, calcium carbonate saturation horizon is shallowing, especially at high latitudes. Biogenic high magnesium-calcites could be particularly vulnerable, since their solubility is either similar or greater than that of aragonite. Cidaroid urchins have magnesium-calcite spines covered by a polycrystalline cortex which becomes exposed to seawater when mature (not covered by an epidermis). However, deep species live at low calcium carbonate saturation states, especially at high latitudes. We describe here the morphology and the magnesium content of *Ctenocidaris speciosa* spines collected at different depths from the Weddell Sea (Antarctica) and relate the features with seawater calcium carbonate saturation. We observed that the spines cortex of *C. speciosa* presented a thicker inner cortex layer and a lower [Mg²⁺] below the aragonite saturation horizon. We suggest that the cortex of cidaroid spines is able to resist to low calcium carbonate saturation state.

Résumé : Les épines de l'oursin antarctique *Ctenocidaris speciosa* : leçons venues des profondeurs. L'acidification des océans apporte des changements dans le système des carbonates de l'eau de mer. En conséquence, l'horizon de saturation du carbonate de calcium est moins profond, en particulier aux hautes latitudes. Les calcites magnésiennes biogènes pourraient être particulièrement vulnérables car leur solubilité est soit similaire soit supérieure à celle de l'aragonite. Les oursins cidaroides ont des piquants formés de calcite magnésienne et sont couverts par un cortex polycristallin qui se trouve exposé à l'eau de mer quand les piquants sont matures (non recouverts par un épiderme). Cependant, les espèces profondes vivent à de faibles états de saturation du carbonate de calcium, en particulier aux hautes latitudes. Nous présentons ici la morphologie et la concentration en magnésium des piquants de *Ctenocidaris speciosa* prélevés à différentes profondeurs dans la mer de Weddell (Antarctique), en relation avec les caractéristiques de saturation en carbonate de calcium de l'eau de mer. Nous avons observé que le cortex interne des piquants de *C. speciosa* est plus épais et montre une concentration plus faible en magnésium en-dessous de l'horizon de saturation de l'aragonite. Nous émettons l'hypothèse que le cortex des piquants des cidaroides est capable de résister aux faibles états de saturation du carbonate de calcium.

Keywords: Ocean Acidification • Antarctica • *Ctenocidaris speciosa* • Cidaroid spines • Calcium carbonate saturation state

Introduction

Anthropogenic carbon dioxide (CO₂) emissions to the atmosphere are being taken up by the oceans, modifying the seawater carbonate equilibrium and resulting in reduced pH and carbonate ion concentration, decreasing in turn the saturation state of calcium carbonate minerals (Orr et al., 2005). This phenomenon, referred to as ocean acidification, implies a decrease of 0.3–0.4 pH units expected by the end of the 21st century if atmospheric CO₂ emissions reach concentrations around 800 ppmv (Caldeira & Wickett, 2005). Subsequently, the surface waters may become undersaturated towards the mineral forms more soluble than calcite, such as aragonite and magnesium calcite (Mg-calcite), whose solubility increases with Mg²⁺ content (Andersson et al., 2008; Zeebe & Wolf-Gladrow, 2001), within the 21st century, especially at the poles (Caldeira & Wickett, 2005; Orr et al., 2005; Andersson et al., 2008).

Several authors have studied the link between the magnesium to calcium ratio (Mg/Ca) in biogenic magnesium calcites and the pH or carbonate concentrations of seawater. In studies where CO₂ was bubbled and TA (total alkalinity) remained constant no relationship in foraminifera magnesium content was observed neither with the carbonate ion saturation ($\Delta[\text{CO}_3^{2-}]$, i.e. the difference between $[\text{CO}_3^{2-}]$ measured in situ and $[\text{CO}_3^{2-}]$ saturation) nor with $[\text{CO}_3^{2-}]$ (Dissard et al., 2010). Using field foraminifera specimens, Rathmann & Kuhnert (2008) did not see a clear influence of $[\text{CO}_3^{2-}]$ on skeleton Mg/Ca, while Yu & Elderfield (2008) showed that Mg/Ca increased with carbonate ion saturation $\Delta[\text{CO}_3^{2-}]$, and that it lowered below a threshold value in one of the two studied species (but a dissolution effect was discarded). Furthermore, in a study where 14 species, including sea urchins, crustaceans, mollusks, corals, among others, were reared at different calcite and aragonite saturation states, the Mg/Ca ratio of the newly formed shells and skeletons did not differ between control values and low saturation states except for a coralline red algae where it decreased and for a serpulid worm tube where it increased (Ries, 2011), but with no link to calcification rate observed.

Sea urchins and other echinoderms have a well-developed high-magnesium calcite endoskeleton whose precursor is a transient amorphous calcium carbonate (ACC) phase, a CaCO₃ form 30 times more soluble than calcite (Politi et al., 2004). In fact, they are considered to be particularly vulnerable to ocean acidification effects (Kurihara, 2008). Interestingly, many sea urchin species reach depths under the saturation horizon of aragonite, and most likely of high Mg-calcite (David et al., 2005; Orr et al., 2005). Diverse responses to low pH and calcium carbonate saturation states have been observed in sea

urchins as their growth, calcification rate and survival can be either impaired or not (Shirayama & Thornton, 2005; Ries et al., 2009).

Sea urchin spines are essential structures that play an important role in locomotion, defense, food gathering and inter and intra-specific interactions (H  tierier et al., 2008; Moureaux et al., 2010). Furthermore, they possess the ability to regenerate once broken or removed (Moureaux et al., 2010). The cidaroid primary spines have particular characteristics when compared with those of other sea urchins (“euechinoids”). First, they are characterized by the presence of a monocrystalline stereom surrounded by a polycrystalline cortex (M  rkel et al., 1971). Moreover, an epithelium covers the shaft only until the cortex has been deposited and when the spine becomes mature the epithelium disappears (Prouho, 1885; M  rkel & R  ser, 1983), leaving the cortex exposed to environmental physical and chemical conditions. Subsequently, the shaft becomes heavily colonized by epibionts making these sea urchins islands of biodiversity, especially in deep sea where muddy substrate predominates just as in the Southern Ocean (> 60° S) (H  tierier et al., 2008). As most models of ocean-carbon cycle predict that the shallowing of the CaCO₃ saturation horizons due to increased anthropogenic CO₂ emissions will be more important at higher latitudes (Anderson et al., 2008), the effects of acidification on the cidaroid spines will be relevant to the ecology of this region.

In this study we described the morphology and the magnesium content (along depth) of cidaroid spines from field specimens collected in the Weddell Sea (Antarctica), complementing works such as the ones from M  rkel et al. (1971) and David et al. (2009). We also evaluated these differences in an ocean acidification context, according to calcite and aragonite saturation states of seawater as proxies for Mg-calcite.

Material and Methods

Field samples and data

Eleven primary aboral and ten oral spines from the species *Ctenocidaris speciosa* (Mortensen, 1910) were studied. They were provided by the Paris Natural History Museum (France) and Laboratory of Marine Biology (Brussels University, Belgium) (Table 1). The specimens were trawled in the Weddell Sea (Antarctica) during EPOS 3 (in 1989) and ANT XV/3 (in 1998) campaigns (research ship R/V Polarsten, Alfred Wegener Institute for Polar and Marine Research, Germany). The sampling depths were 237, 602 and 810 m for EPOS 3 and 1286–1681 m for ANT XV/3 (Table 1). All specimens (1 individual from 237 m, 2

Table 1. *Ctenocidaris speciosa*. Origin of sampled specimens (Weddell Sea, Antarctica) from the Paris Natural History Museum (MNHN), France, and the Brussels University (ULB), Belgium.

Expedition	Date of Collection	Depth (m)	Latitude	Longitude	Collection	Museum reference	No. aboral spines	No. oral spines
EPOS 3	18 January 1989	237	60°37.6'S	46°58.1'W	MNHN	EcEs 9310	3	2
EPOS 3	3 February 1989	602	74°39.9'S	29°31.3'W	MNHN	EcEs 9308	2	3
EPOS 3	4 February 1989	810	75°32.4'S	29°53.0'W	MNHN	EcEs 9235	3	2
ANT XV/3	4 February 1998	1286-1681	73°28.05'S- 73°28.04'S	22°30.0'W- 22°40.05'W	ULB	—	3	3

for 810 m and 3 for 602 m) from EPOS 3 were stored dried, while those (3 individuals) coming from the ANT XV/3 campaign were stored in ethanol. Further museological samples were not possible since the applied study methods were destructive.

Total dissolved inorganic carbon (DIC) and total alkalinity (TA) data from the sampling sites were obtained using the data base from the WOCE Southern Ocean Atlas (Orsi & Whitworth, 2004). Therefore, it was possible to calculate the saturation state values (Ω) of calcite and aragonite using the software CO2SYS (Pierrot et al., 2006), dissociation constants refitted by Dickson & Millero (1987) and K (SO₄) by Dickson (1990). It was not possible to estimate the saturation state of Mg-calcite, since there is no correction for the stoichiometric saturation (K) at pressures different from the atmospheric one, i.e. at different depths (Andersson et al., 2008).

Specimen analysis

Spines were sectioned at the shaft base and cleaned with a solution of 3.3% v/v NaClO aqueous solution (Loda, Belgium) for 1 h. They were mounted on aluminum stubs in positions parallel and perpendicular to the long axis of the spine and coated with gold (JFC-1100E ion sputter, JEOL, Japan). They were observed using a scanning electron microscope (SEM - JEOL JSM 6100, Japan) with secondary electron image acquisition by the software 3.0 SemAfore Jeol 1993-1997 (J. Rimppi Oy, Finland) (Moureaux et al., 2010). The thickness of the different cortical layers was measured on spine cross sections using Image J. 1.33u (Wayne Rasband, National Institutes of Health, USA).

To quantify the magnesium content on different spine layers, energy dispersive X-ray analysis (EDX) was done on cross sections. Segments of the shaft were cut and embedded in polyester resin (MI 42, Mida Composites, Belgium) with 2% hardener-MEC. Cross sections of c.a 6 mm were cut from each one with a low speed saw (Isomet, 11-1180, Buehler, USA). The sections surface was polished using wet sandpapers of decreasing granulometry (P80; P150; P400; P600) and finally mirror polished with a non-

aqueous 1 μ m diamond suspension (1PS-1MIC, ESCIL, France). The polished slices were carbon-coated (rotary evaporator Balzers MED-010, Liechtenstein). Dispersive X-ray analysis spectra were obtained in an environmental SEM (XL30 ESEM-FEG-FEI Philips, The Netherlands) operating at 10kV and at a working distance of 10 mm. The analyses were repeated three times for each region on each spine. They were performed on mineral zones of about 1-10 μ m² in size with a normalized acquisition time of 100 s. The standardless quantitative analysis software calculated the elemental composition in weight percent and atomic percent using an automatic background subtraction function and a ZAF correction matrix. The composition was thereafter corrected by subtraction of the carbon-coating evaluated on the carbon-coated aluminium stub. The MgCO₃ molar percentage was calculated from the elemental composition in atomic percent (Moureaux et al., 2010).

Data analysis

Data analyses were done using ANOVA tests, followed by the mean multiple comparison Tukey test whenever necessary. The significance level (α) was set at 0.05. Values expressed as proportions were arcsine transformed prior to the analysis in order to achieve data normality whenever necessary. Graphs were plotted using untransformed data. The following dependent variables were studied using a nested model III ANOVA with the fixed factor "depth" and the random factor "spine" nested in "depth": total cortex thickness, outer cortex thickness, inner cortex thickness, MgCO₃ content in the outer cortex, MgCO₃ proportion in the inner cortex, MgCO₃ proportion in the central stereom.

Results

All the *Ctenocidaris speciosa* aboral spines presented a well developed cortex and an internal stereom core (Fig. 1a). In samples collected at 237 m and 602 m the cortex was made of two layers, an outer (oc) and an inner (ic) one, separated by an intermediate "pillar" layer. However, in

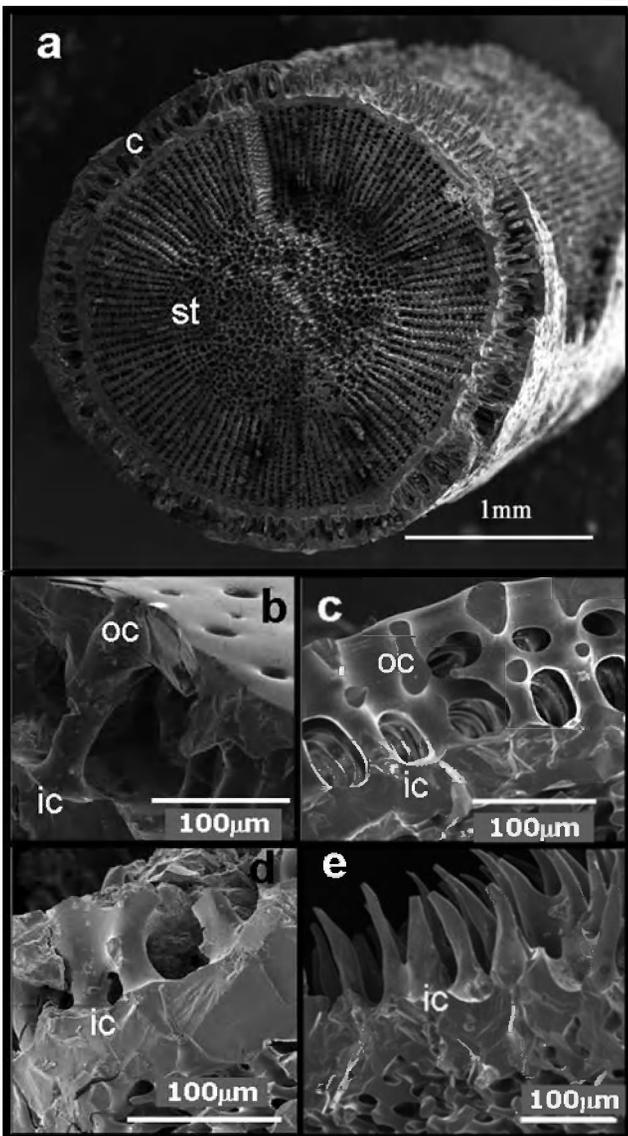


Figure 1. *Ctenocidaris speciosa*. Scanning electron microscopy images of transverse sections of *Ctenocidaris speciosa* aboral primary spines: (a) entire cross-section of a spine from a specimen collected at 237 m (c- cortex, st-stereom); (b, c, d, e) details of the cortex in spines from specimens collected at 237 m, 602 m, 810 m and 1236-1681 m respectively (oc- outer cortex, ic- inner cortex).

samples from deeper waters the outer layer was missing (Fig. 1). The total thickness of the cortex of both types of spines did not differ according to depth ($p_{ANOVA} > 0.07$). The thickness of the outer cortex in aboral spines did not differ between 602 m and 237 m ($p_{ANOVA} = 0.29$), whereas the inner cortex was significantly thicker in deeper specimens ($p_{ANOVA} = 0.012$) (Fig. 2). The magnesium concentration of the outer cortex did not decrease significantly with depth ($p_{ANOVA} = 0.1$), whilst the one from the

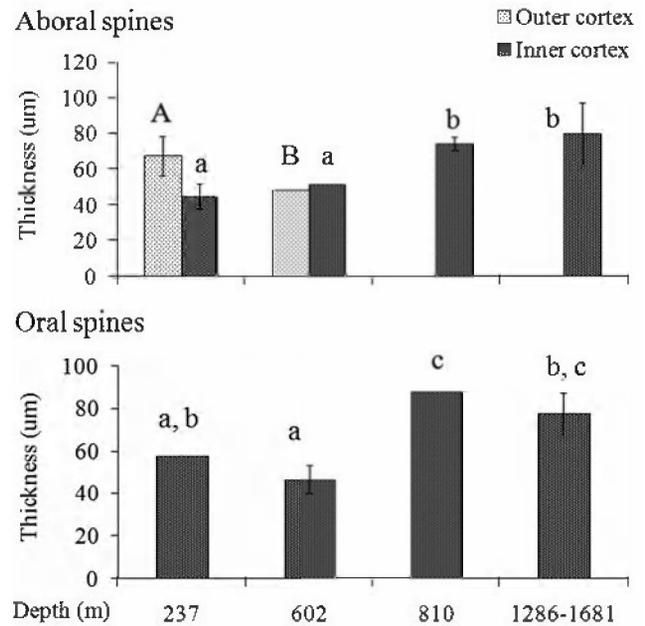


Figure 2. *Ctenocidaris speciosa*. Thickness (mean \pm SD, n = 2 or 3) of the outer and inner cortex layers of *Ctenocidaris speciosa* aboral and oral spines according to depth. Bars sharing the same superscript did not differ significantly ($\alpha = 0.05$).

inner one did ($p_{ANOVA} = 10^{-6}$). The concentration in the stereom did not differ significantly ($p_{ANOVA} = 0.5$) (Fig. 3).

Oral spines did not present systematically two layers in the cortex. Nevertheless the outer cortex was only visible above 602 m in two spines. The inner cortex in oral spines was significantly thicker in deeper waters ($p_{ANOVA} = 0.04$) (Fig. 2).

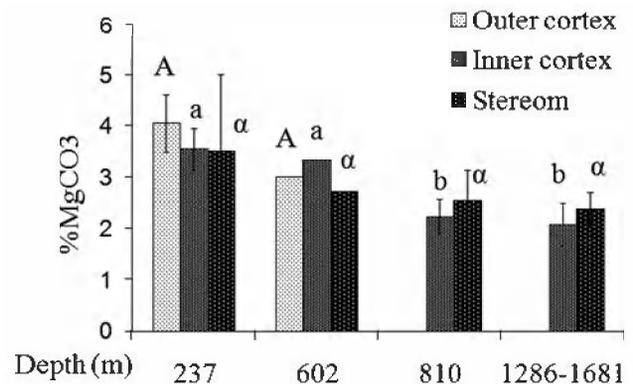


Figure 3. *Ctenocidaris speciosa*. $MgCO_3$ content (mole %, mean \pm SD, n = 2 or 3) of aboral primary spines layers at different depths. Bars sharing the same superscript did not differ significantly ($\alpha = 0.05$).

Discussion

The mature spines of *Ctenocidaris speciosa* collected from the Weddell Sea (Antarctica) presented differences both in their morphology and chemical composition according to depth. We observed the occurrence of an outer cortex differing from an inner one in all aboral spines and in some oral spines from individuals collected at or above 600 m. This morphological feature had already been reported (Fell, 1976 cited by David et al., 2009), but not yet related with depth distribution. The total thickness of the cortex did not differ with depth, most likely thanks to the inner layer thickness increase, compensating the lack of the outer one below 600 m. We propose that these morphological differences can be related to the individual growth rate as deep sea urchins growth is slower than that of their congeners from the upper continental slope or from shallow waters (Gage et al., 1986), but not to dissolution of this structure. Thus, the presence of an outer cortex may be associated with the ability of a faster calcification rate. Slower growth rate in deeper specimens may be linked to both a lower magnesium calcite saturation state dependent on a pressure effect, which is depth associated, and/or to a reduced food availability, in turn also correlated with depth.

Lower $[Mg^{2+}]$ were measured in the cortex of *C. speciosa* collected at higher depths. Similarly, Dissard et al. (2010) mentioned the possibility of a pressure effect responsible for Mg^{2+} content variations along depth in some foraminifera species. Also, Lowenstam (1973) reported an inverse correlation between depth and $MgCO_3$ concentration in the skeleton of the Pacific sea cucumber *Elpidia glacialis* (Théel, 1876), distinct from any temperature effect. Similarly, our observations cannot be attributed to usual environmental factors affecting skeletal magnesium content. Indeed, the seawater Mg/Ca ratio is highly conservative all over the ocean and reported temperatures and salinity for the Weddell Sea at depths between 200-1500 m do not present changes that could explain the variation in spine cortical magnesium content, thus in Mg/Ca ratio (Antonov et al., 2006; Locarnini et al., 2006). Actually, both temperature and salinity slightly increase from 200 m to higher depths. Also, in this case, the observed effects cannot be due to pH/pCO₂, as these remain fairly constant along depth (Table 2). However, it is known that the calcium carbonate saturation state decreases with depth as it is dependent on pressure (Zeebe & Wolf-Gladrow, 2001). Precisely how the calcium carbonate saturation state (Ω_{CaCO_3}) influences the skeletal Mg/Ca is currently unknown. Lower $\Omega_{Mg-calcite}$ could decrease calcification rates, which would give more time to exchange the calcium ions for the magnesium “impurity”, as proposed by Weber (1973), decreasing magnesium content. However, recent experimental data showed that the

Table 2. *Ctenocidaris speciosa*. Seawater physicochemical parameters for the sampling stations. TA stands for total alkalinity, DIC for dissolved inorganic carbon and Ω for saturation state, pH_T in total scale.

Sampling depth (m)	237	602	810	1286-1681
Depth (m)	200	600	800	1400
Salinity	34.5	34.5	34.7	34.7
Temperature (°C)	-1.0	0.0	0.5	0.2
TA (mmol kg ⁻¹)	2.34	2.35	2.35	2.35
DIC (mmol kg ⁻¹)	2.26	2.27	2.27	2.26
pH	8.01	7.98	7.97	7.97
pCO ₂ (µatm)	520.2	536.7	549.7	510.6
[CO ₃ ²⁻] (µmol kg ⁻¹)	72.9	73.4	73.1	75.3
$\Omega_{calcite}$	1.68	1.55	1.48	1.35
$\Omega_{aragonite}$	1.06	0.98	0.94	0.86

growth rate by itself does not influence the skeletal Mg/Ca ratio in echinoderms (Borremans et al., 2009; Ries, 2011). A higher saturation state is thought to favor the precipitation of amorphous calcium carbonate (ACC), which in turn favors the incorporation of magnesium into calcium carbonate, including after crystallization into calcite (Loste et al., 2003). The ACC is known to be the initial mineral deposited during sea urchin skeleton formation (Politi et al., 2004). So the Ω_{CaCO_3} could influence the Mg/Ca ratio, i.e. the magnesium content, of *C. speciosa* cortex by affecting ACC formation during initial steps of calcification.

It is noteworthy, however, that the skeletal magnesium content differed significantly according to depth only in *C. speciosa* spines cortex, a polycrystalline structure, and not in the central monocrystalline stereom, discarding a pressure effect on the Mg^{2+} incorporation into this skeleton structure. The cortex is richer in organic matrix (Märkel et al., 1971), in comparison with the very delicate organic matrix which pervades the stereom (accounting for less than 0.1% w/w of the skeleton) (Ameye et al., 2001). It has been recently shown that the sea urchin organic matrix composition affects the Mg/Ca ratio in calcium carbonate precipitation (Hermans et al., 2011). Providing that its composition would change with depth, it could be suggested that the nature of the organic matrix synthesized by the sea urchin would play a role in their magnesium composition. Nevertheless, this idea needs yet to be tested. In such case the nature of the organic matrix would have a protective role against dissolution. An additional factor that can protect the cortex is the biofilm that covers any exposed underwater surface including spines (David et al., 2009). Whatever the actual mechanism, the lower Mg^{2+} content in the cortex of deeper *C. speciosa* is an advantageous feature as it reduces the solubility of the cortex, compensating the

potential $\Omega_{\text{Mg-calcite}}$ decrease, once the epithelium degenerates and the spine becomes directly exposed to ambient seawater.

From the 21 known cidaroid Antarctic species, 16 can currently be found at depths below 750 m (David et al., 2005) below the Mg-calcite saturation horizon (Orr et al., 2005). Whether this fact demonstrates a strong adaptation potential of cidaroid species to seawater lower saturation state conditions, remains yet to be determined. For instance little is known about their early deep sea colonization. Also, it is unknown how geological events where ocean acidification was observed, such as the Paleo-Eocene Thermal Maximum (PETM), affected deep sea cidaroid fauna. Current global changes are occurring at a faster rate than events such as PETM (Ridgwell & Schmidt, 2010). In fact, the aragonite saturation horizon in the Southern Ocean could be found at around 730 m depth in 1994 and is predicted to reach surface waters by 2100 (Orr et al., 2005). Cidaroid sea urchins can have a long life expectancy and whether they will be or not able to cope, i.e. to adjust thanks to their phenotypic plasticity, with the speed at which ocean acidification promotes biogeochemical changes in their environment is unknown. Nonetheless, we have seen for instance that the cortex provides protection towards adverse conditions, minimizing the risk of spine exposure to more corrosive waters.

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