Reference: *Biol. Bull.* **226:** 223–236. (June 2014)

The Skeleton of Postmetamorphic Echinoderms in a Changing World

PHILIPPE DUBOIS

Laboratoire de Biologie marine CP160/15, Université Libre de Bruxelles, av F.D. Roosevelt, 50, B-1050 Bruxelles, Belgium

Abstract. Available evidence on the impact of acidification and its interaction with warming on the skeleton of postmetamorphic (juvenile and adult) echinoderms is reviewed. Data are available on sea urchins, starfish, and brittle stars in 33 studies. Skeleton growth of juveniles of all sea urchin species studied so far is affected from pH 7.8 to 7.6 in seawater, values that are expected to be reached during the 21st century. Growth in adult sea urchins (six species studied) is apparently only marginally affected at seawater pH relevant to this century. The interacting effect of temperature differed according to studies. Juvenile starfish as well as adults seem to be either not impacted or even boosted by acidification. Brittle stars show moderate effects at pH below or equal to 7.4. Dissolution of the body wall skeleton is unlikely to be a major threat to sea urchins. Spines, however, due to their exposed position, are more prone to this threat, but their regeneration abilities can probably ensure their maintenance, although this could have an energetic cost and induce changes in resource allocation. No information is available on skeleton dissolution in starfish, and the situation in brittle stars needs further assessment. Very preliminary evidence indicates that mechanical properties in sea urchins could be affected. So, although the impact of ocean acidification on the skeleton of echinoderms has been considered as a major threat from the first studies, we need a better understanding of the induced changes, in particular the functional consequences of growth modifications and dissolution related to mechanical properties. It is suggested to focus studies on these aspects.

Received 18 November 2013; accepted 5 May 2014.

* To whom correspondence should be addressed. E-mail: phdubois@ulb.ac.be

Abbreviations: ACC, amorphous calcium carbonate; CF, coelomic fluid; OA, ocean acidification.

Introduction

Due to anthropogenic emissions, the atmospheric CO_2 concentration has increased from the beginning of the Industrial Revolution to the current value of 400 ppm. This value is expected to rise to approximately 1000 ppm by the end of this century (Caldeira and Wickett, 2003; IPCC, 2007). In the oceans, this is resulting in increased sea-surface temperature and pCO_2. The former has increased by about 0.8 °C in the past 150 years and is predicted to rise a further 2–4.5 °C by the end of this century (IPCC, 2007). The latter will result in a decrease of seawater pH of 0.3–0.4 units by 2100 and a further 0.7 by 2300 (Caldeira and Wickett, 2003; Orr *et al.,* 2005; IPCC, 2007). Ocean uptake of CO_2 also induces a modification of the carbonate system equilibrium toward less saturated conditions with respect to the different calcium carbonate polymorphs. Reduced pH and modified carbonate equilibrium are merged under the term ocean acidification (OA). Warming and acidification may affect numerous physiological processes such as calcification, nutrition, metabolism, and reproduction (Pörtner, 2008; Melzner *et al.,* 2009). Responses of organisms to these stressors greatly differ according to taxa (*e.g.,* Byrne and Prezlawsky, 2013; Wittmann and Pörtner, 2013).

Postmetamorphic (juvenile and adult) echinoderms are benthic organisms including starfish (asteroids), sea urchins (echinoids), sea cucumbers (holothuroids), brittle stars (ophiuroids), and comatulids and sea lilies (crinoids). Members of this phylum play an important ecological role in controlling community structure in numerous ecosystems such as kelp beds, coral reefs, rocky intertidal shores, or Antarctic soft sediment beds (*e.g.,* Lockart and Jones, 2008; Steneck, 2013). Due to their low metabolism and poor regulation abilities, they are considered to be particularly sensitive to OA (Pörtner *et al.,* 2004; Melzner *et al.,* 2009). Furthermore, larvae of sea urchins and brittle stars and

adults of all classes produce a high-magnesium calcite skeleton, a form of calcium carbonate unstable in abiotic conditions and therefore considered to be particularly vulnerable to dissolution under OA conditions (Andersson *et al.,* 2008). The impact of OA and its interaction with temperature in, respectively, echinoderm and sea urchin larvae, including spicule development, has been recently reviewed (Byrne, 2011; Byrne *et al.,* 2013). Effects of the same stressors have also been reviewed in adult echinoderms, including by numerical meta-analysis which found that a large fraction of the studied species are negatively affected. Negative effects are principally seen as reduced somatic and gonadal growth, reflecting a shift in resource allocation from growth to acid-base regulation (Dupont and Thorndyke, 2013; Wittman and Pörtner, 2013). However, these meta-analyses did not assess the interpretations of the studies included in the reviews. In this regard, it is important to note that several recent studies on the impact of acidification and temperature on the skeleton of postmetamorphic echinoderms have reported contrasting results (*e.g.,* sea urchins: Shirayama and Thornton, 2005, *versus* Kurihara *et al.,* 2013; starfish: Gooding *et al.,* 2009, *versus* Appelhans *et al.,* 2012). Thus, a review of these studies appears timely to establish the state of knowledge on the impact of these stressors and to identify further research directions. Methodological issues are also raised that could usefully be taken into account in future studies. Accordingly, this synthesis reviews the outcomes of 33 papers published until the end of 2013 on the effects of increased temperature and acidification on growth, dissolution, or mechanical properties of the postmetamorphic echinoderm skeleton.

The Skeleton of Postmetamorphic Echinoderms

All postmetamorphic echinoderms (except a few holothuroids) have skeletal elements in their body wall (Fig. 1). Echinoids also have a complex chewing apparatus, the Aristotle's lantern. All skeletal elements, including spines and other outer appendages, are of mesodermal origin and located in a connective tissue separated from the external medium by the epidermis. On the inner side, the body wall skeleton is separated from the general body cavity (coelom) by a mesothelium. This body cavity is the largest compartment containing extracellular fluid, the coelomic fluid (CF). The body wall skeleton of all postmetamorphic echinoderms consists of single elements—the ossicles—bound together by connective and/or muscular fibers. Each ossicle consists of a tridimensional network of trabeculae (called stereom) that delimits an internal and complementary network filled by a connective tissue (the stroma) (Fig. 2). (In most holothuroid taxa, the ossicles are reduced to spicules.) The stereom is designed in such a way as to be adaptable to different shapes and functions (Smith, 1980). Calcification occurs in membrane-bound compartments of cells from mesodermal origin, both in larvae and postmetamorphic echinoderms (see reviews by Dubois and Chen, 1989; Killian and Wilt, 2008).

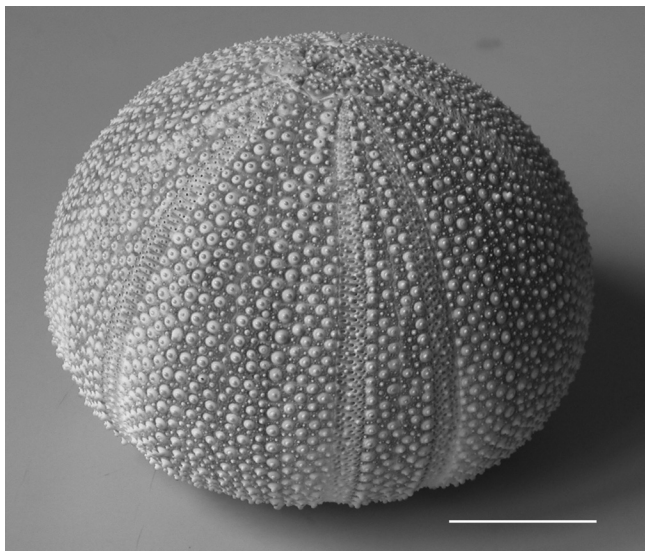

Figure 1. Test of the sea urchin *Echinus esculentus* cleaned of soft tissues and with spines removed, proxy photography (scale bar: 2.5 cm).

Very little is known about cell signaling and proteins involved in biomineralization in postmetamorphic echinoderms (Killian and Wilt, 2008; Matranga *et al.,* 2011). In contrast, biomineralization control in sea urchin embryos and larvae has been extensively studied (see reviews by Killian and Wilt, 2008; Matranga *et al.,* 2011, 2013). In embryos and larvae, biomineralization of spicules is carried out by primary mesenchyme cells expressing genes whose transcription is controlled by numerous factors (see Ettensohn, 2009, for a review). Ectoderm cells are also involved in guiding the primary mesenchyme cells and were demonstrated to be involved in the regulation of biomineralization-related genes by the production of growth factors (Matranga *et al.,* 2011). It is not known if the same processes occur in postmetamorphic biomineralization, although the expression of similar genes and the transcription of related proteins is reported in adult skeleton-forming cells (Drager *et al.,* 1989; Livingston *et al.,* 2006; Mann *et al.,* 2008a, b, 2010; Killian *et al.,* 2010). Unfortunately, the functions of these proteins are still mainly unknown. The ion transport systems related to biomineralization in postmetamorphic echinoderms are also poorly undersood. Carbonic anhydrase, an enzyme frequently involved in biomineralization, was localized in the tooth of the sea urchin *Lytechinus variegatus* by ultracytochemistry (Chen and Lawrence, 1986, 1987). The existence of a HCO_3^- transporter has been hypothesized (Holtmann *et al.,* 2013) in the digestive epithelial cells of another sea urchin, *Strongylocentrotus droebachiensis,* and could be involved in the supply of this anion in the CF, facilitating its transport to the calcifying

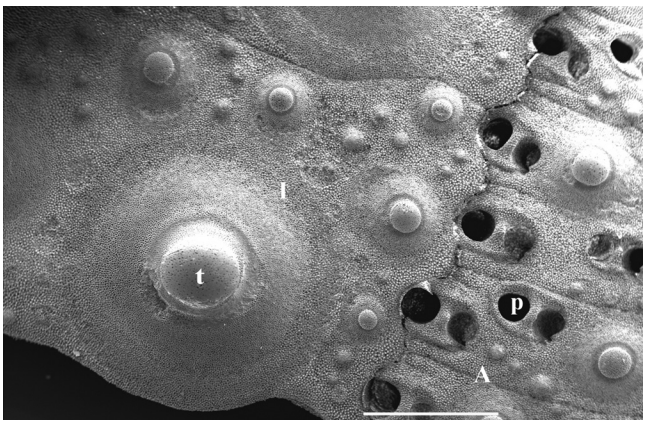

Figure 2. Fragment of the test of *Tripneustes ventricosus* cleaned of soft tissues and with spines removed, showing ambulacral and interambulacral plates (credit: Aurélie Dery); scanning electron microscopy (scale bar: 1 mm). *Abbreviations:* A, ambulacral plate; I, interambulacral plate; p, tube foot pore; t, tubercle.

site. Because dissolved inorganic carbon crosses biological membranes as carbon dioxide or bicarbonate ions and almost never as carbonate ions, reduced saturation states of calcium carbonate linked to OA are probably not a direct threat to calcification in postmetamorphic echinoderms. However, elimination of protons, produced by the precipitation of calcium carbonate, out of the calcifying site (vacuole or membrane-bound extracellular site) could become energetically more expensive (see Ries, 2011a).

The echinoderm skeleton is made of 99.8%–99.9% (w/w) Mg-enriched $CaCO_3$ and of about 0.1%–0.2% intrastereomic organic material (Weiner, 1985). The latter is occluded within the mineral phase and should not be confused with the extracellular matrix of the connective tissue that surrounds the skeletal elements. The mineral phase is mainly composed of magnesium calcite (Chave, 1952) with a $MgCO_3$ content ranging from 3.0 to 43.5 mol% (Schroeder *et al.,* 1969; Weber, 1969; McClintock *et al.,* 2011). An amorphous calcium carbonate (ACC) phase has been reported in regenerating sea urchin spines (Politi *et al.,* 2004). Occurrence of such a phase could be more widespread in echinoderm skeleton, although evidence of this is currently lacking. Most skeletal elements show X-ray diffraction patterns of single magnesium-calcite crystals (Donnay and Pawson, 1969; Donnay, 1975). Fully grown primary spines of the echinoid basal group cidaroids have the peculiarity of being devoid of epidermis, and so the skeleton is in direct contact with seawater (Prouho, 1887; Märkel and Röser, 1983). Cidaroid spines are also unusual in being composed of a central zone of classical monocrystalline stereom and a special outer polycrystalline layer, the so-called cortex; whereas the spines of other sea urchins (euechinoids) are made only of classical stereom (Figs. 3, 4) (Borig, 1933; Märkel *et al.,* 1971).

Solubility of Mg-calcites is still debated (see Morse *et al.,* 2006). In general, biogenic Mg-calcites with 8–12 mol% $MgCO_3$ are more soluble than both calcite and aragonite. At present, high-latitude, tropical, and temperate surface ocean waters are undersaturated with respect to 12, 15, and 18 mol% Mg-calcite (calculations based on the biogenic "minimally prepared" solubility curve) (Andersson *et al.,* 2008). By the year 2100, surface seawaters at all latitudes will be undersaturated or at metastable equilibrium with respect to a 12 mol% Mg-calcite and phases of higher magnesium concentration (Andersson *et al.,* 2008). At high latitude, Mg-calcite with a $MgCO_3$ concentration of 4–5 mol% could become metastable (Andersson *et al.,* 2008). Because the saturation state of Mg-calcites ($\Omega_{\text{Mg-calcite}}$) is not easily calculated and prone to controversy (Morse *et al.,* 2006), the saturation state of aragonite (Ω_{Ar}) is frequently used as a proxy of $\Omega_{\text{Mg-calcite}}$ and the latter is rarely reported. Finally, it is worth mentioning that ACC is 30 times more soluble than calcite (Politi *et al.,* 2004).

Impact of Ocean Acidification on the Skeleton of Postmetamorphic Echinoderms

Data on the impact of ocean acidification on the postmetamorphic skeleton are available for sea urchins, starfish, and brittle stars (see Appendix). Because the skeleton is a major constituent of the body wall of these echinoderms, information on body growth as a proxy of skeleton growth is relevant to this review. Other reported effects deal with skeleton dissolution or etching, magnesium and calcium concentrations, and mechanical properties. Unfortunately many studies have some level of pseudoreplication (*sensu* Hurlbert, 1984) either because a single header tank was used

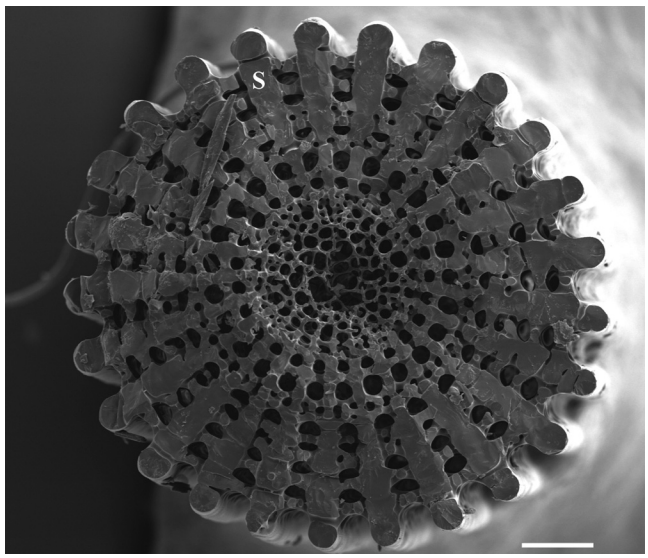

Figure 3. Transverse section through a primary spine of the euechinoid *Tripneustes ventricosus* cleaned of soft tissues (credit: Aurélie Dery); scanning electron microscopy (scale bar: 100 μm). *Abbreviation:* S, septum.

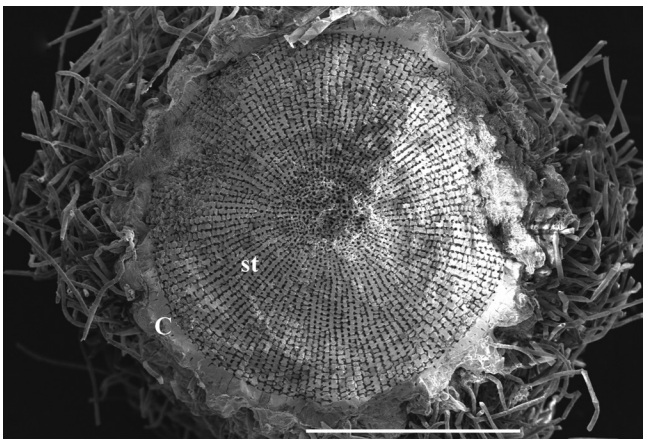

Figure 4. Transverse section through a primary spine of the cidaroid *Phyllacanthus imperialis*, covered with epibionts (credit: Aurélie Dery); scanning electron microscopy (scale bar: 1 mm). *Abbreviations:* C, cortex; st, stereom.

for each experimental pH and temperature condition or because individuals from the same aquarium were considered as independent replicates. In the former case, any artifact in the head tank will be confused with a treatment effect (Hurlbert, 1984). In the latter case, metabolites of one individual might, for instance, affect all the others (Hurlbert, 1984).

Growth

Test and spine growth or weight increase in juveniles of the five sea urchin species studied so far (from temperate and tropical environments) were affected at pH ≤7.8–7.6—that is, at values expected to be reached during the 21st century (Appendix). Interestingly, several of these studies either encompassed the whole larval and juvenile lives or a long (≥ 3 months) period of juvenile life.

The effects of OA on growth of adult sea urchins are more ambiguous. All six studies used general indicators of growth like changes in wet or buoyant weight or in test diameter and dry mass. Studies of four species, using these indicators, reported no significant differences between control and acidified conditions, except at very low pH (<7.4) (*Eucidaris tribuloides*, *Strongylocentrotus droebachiensis*, *Echinometra mathaei*, *Hemicentrotus pulcherrimus*). This includes a 9-month experiment (*H. pulcherrimus*; Kurihara *et al.,* 2013). In one study on *Echinometra viridis*, effects on buoyant weight change were reported to be significant at pH 8.1 (control pH was 8.3) (Courtney *et al.,* 2013). A single study investigated the growth of test ossicles using the fluorochrome calcein that labels growing skeleton (*Strongylocentrotus droebachiensis*; Holtmann *et al.,* 2013). This revealed a reduced growth from pH_{SW} 7.67, while growth measured by increase of test diameter did not reveal any effect at that pH. Because growth of echinoderms is usually asymptotic (reviewed by Ebert, 2013), it is more difficult to detect the impact of stressors on growth in large animals with the general indicators. However, in several studies carried out on adult sea urchins, growth was effectively measured but was either not impacted or reduced only at the lowest pH tested. This suggests that growth in adults is only marginally affected by OA. However, more studies using fine markers like calcein would be useful. A particular case is reported by Ries *et al.* (2009). Using quadratic regression analysis, these authors suggested that *Arbacia punctulata* individuals showed a lower increase in buoyant weight at pH 8.0 than at 7.9. However, the sample size was low (3–6), all specimens at a given pH treatment were in the same aquarium, and the water temperature was close to the upper limit for this species. For sea urchins, caution needs to be directed to growth data based on buoyant weight in short-term toxicity tests because decreases in weight or in weight gain may be due to spine loss, a common problem with sea urchins in captivity (pers. obs.). Another problem with this technique occurs in the case of eroding species whose gut may contain significant amounts of calcium carbonate that will be misleadingly considered as skeleton. For these species, fasting for 48 h is necessary to empty the gut (L. Moulin *et al.,* Université Libre de Bruxelles and Université de Mons, unpubl.).

Interestingly, Courtney *et al.* (2013) reported an interaction between the effects of pH and temperature on growth of *Echinometra viridis*, the effect of low pH being more important at the lowest (wintertime) temperature. On the contrary, two studies on juvenile *Heliocidaris erythrogramma* reported worse effects at higher temperature (Wolfe *et al.,* 2013a, b). In general, such interaction studies are very few and their number should be increased, especially when investigating the effects on growth where the two parameters are expected to interfere.

Thus far, there are four studies on the impact of OA on growth of four starfish species (Appendix). These studies reported either an increase in growth at pH ≤7.8–7.7 or no effect on growth or arm regeneration at pH as low as 7.4. The longest study was a 3-mon-long investigation of growth in *Luidia clathrata* (Schram *et al.,* 2011). The body wall of starfish is less calcified than that of sea urchins (60%–70% dry weight of mineral; Lawrence, 2010). So, growth is less directly related to calcification rate than in sea urchins. For instance, Gooding *et al.* (2009) found that the proportion of skeleton (of total wet mass) in *Pisaster ochraceus* was reduced from 11.5% to 10.9% of total wet mass at pH 7.8, although overall wet weight relative growth was increased by 67% at 12 °C and 110% at 15 °C. It should be noted that these data do not allow the inference of a genuine decrease of skeleton proportion in the body wall because the reported decrease could result from an increase in pyloric caeca mass. Indeed, feeding rate increased at pH 7.8 in this

experiment. Furthermore, in another species, *Luidia clathrata*, Schram *et al.* (2011) found no change in ash content of the body wall (a more direct measure of skeleton production) in animals also maitained at pH 7.8.

There are only three studies on the effects of OA on growth in brittle stars (Appendix). In the temperate species *Amphiura filiformis*, the length of regenerating arms was significantly higher in acidified conditions (but threshold pH was not reported) (Wood *et al.,* 2008, reanalyzed in Findlay *et al.,* 2009, 2011). Calcium concentration in arms was higher at the lowest pH tested (6.8). The same studies also analyzed calcium concentrations in arms separated from the disc, frozen and then thawed (so that all tissues were lysed), and maintained for 7 days in seawater. They found that calcium concentrations decreased according to pH. On the basis of this observation, the authors argued that gross calcification increased at all three acidified pH values in living animals and that net calcification remained constant or was reduced due to dissolution. However, all tissues surrounding the ossicles—connective tissue and epidermis—were lysed and decaying, promoting bacterial activity. Such observations clearly cannot be transposed to the living animal, at least in echinoderms (Andersson and Mackenzie, 2012). Furthermore, the use of calcium concentration as a proxy for calcification is debated (see the discussion about the paper by Findlay *et al.,* 2009, reporting, among others, the same data as Wood *et al.,* 2008). In particular, such change in calcium concentration may be due only to a decrease in other non-calcified tissues, which was the case for muscles in this study (Wood *et al.,* 2008). Finally, the feeding status of the brittle stars in this study is not clear, and it cannot be excluded that they were, at least partly, starved. So, further evidence should be provided to conclude that *Amphiura filiformis* actually does increase calcification in response to acidification. In the two other investigated species (the temperate *Ophiura ophiura* and the Arctic *Ophiocten sericeum*), arm regeneration rate was significantly reduced by acidification at pH $\leq$ 7.4 or 7.3 only at the highest tested temperatures (respectively 15 and 8.5 °C) (Appendix; Wood *et al.,* 2010, 2011).

Skeleton etching, dissolution, amorphous calcium carbonate, and magnesium concentration

All published studies that have examined skeleton integrity have focused on sea urchins. Several studies reported etching or surface alteration of the stereom in sea urchin spines at pH $\leq$7.8 (Allbright *et al.,* 2012; Holtmann *et al.,* 2013; Wolfe *et al.,* 2013a). On the contrary, the test stereom was not or only slightly affected (Allbright *et al.,* 2012; Holtmann *et al.,* 2013). This difference could be due to several factors. Spines are more or less continuously in a regeneration state and their apical epidermis is often damaged due to abrasion (Heatfield, 1971; Heatfield and Travis 1975). The level of protection provided to the spine skeleton by the epidermis is also worth questioning because it is very thin (<1 μm, Heatfiled and Travis, 1975). Furthermore, mineral deposition during spine regeneration involves a transient ACC phase, which is much more soluble than magnesium-calcite (Politi *et al.,* 2004). Thus far, no study has looked at the effects of acidified conditions on the youngest test plates (on the adoral side of the test) which could be more prone to etching, although it is not known if ACC is involved in the formation of these plates. Clearly, the occurrence of ACC in postmetamorphic echinoderms should be further researched. Currently, ACC is reported only in early regenerating spines, where it is protected in a membrane-bound space, and is not found in later stages of spine regeneration (Heatfield and Travis, 1975; Politi *et al.,* 2004). So, it is unclear if ACC in postmetamorphic echinoderms is ever in contact with extracellular fluids (except, maybe, in the case of abraded spines). The occurrence of a transient ACC phase is probably very significant for explaining the smooth morphology of the echinoderm stereom as well as the high magnesium concentration in a calcite phase (see Raz *et al.,* 2000; Loste *et al.,* 2003; Politi *et al.,* 2004; Cheng *et al.,* 2007). On the other hand, the ecological relevance of ACC in the context of OA is not clear because, in postmetamorphic echinoderms, it is found only in a compartment whose composition is tightly controlled by the skeleton-forming cells. The situation is clearly different in sea urchin larvae, where the spicules are much more exposed to seawater physicochemical conditions (Stumpp *et al.,* 2012b).

In relation to the possible poor protection of the spine skeleton, it is noteworthy that the spine calcite usually has a lower magnesium concentration than the test of the same individual (*e.g.,* Weber, 1969; Hermans *et al.,* 2011). This may be an adaptative feature because it reduces the solubility of spine calcite. However, it appears not to be efficient in view of the surface alterations of the spine skeleton induced by acidificed seawater (see above).

The observation of limited etching on the inner side of test plates (Allbright *et al.,* 2012; Holtmann *et al.,* 2013) indicates that these are better protected by the surrounding tissues. However, Holtmann *et al.* (2013) showed that the mesothelium covering the inner side of the body wall in *S. droebachiensis* does not form a barrier for small ions, including bicarbonate. Thus it is surprising that some sea urchins (*e.g., Arbacia lixula*, cidaroids) that have naturally low coelomic fluid pH and Ω_{Ar} (*ca.* 7.0 and <1, respectively) (Collard *et al.,* 2013b, 2014; Calosi *et al.*, 2013) have test plates whose inner face is not etched (A. Dery, Université Libre de Bruxelles, Collard, and Dubois, unpubl.). This may point to specific differences of mesothelium permeability or to the occurrence of other protection mechanisms of the skeletal plates.

Care should be taken when analyzing etching results.

Indeed, etching is a regular artifact of skeleton cleaning for electron microscopy, and careful quantification of the etching both in control and treatment conditions should be carried out for ascertaining such effect (see Holtmann *et al.*, 2013). Finally, if spine growth is studied, care should also be taken to compare similar growth stages so that ACC and magnesium levels, which directly influence solubility, are similar (Heatfield, 1971; Davies *et al.,* 1972; Politi *et al.,* 2004). If the stressor induces a growth delay, then different growth stages will be compared and conclusions on skeletal dissolution could be biased.

Test dissolution has also been inferred from increases of magnesium and/or calcium concentrations in the CF (Spicer *et al.,* 1988; Miles *et al.,* 2007), but this has never been correlated with morphological (scanning electron microscopy) investigations. Parallelly, absence of significant changes in concentrations of these ions in the CF during acidification experiments has been interpreted as evidence that the skeleton was not dissolved or that dissolved ions were quickly equilibrated with seawater (Catarino *et al.,* 2012; Calosi *et al.,* 2013; Collard *et al.,* 2013a). These changes in Mg and Ca concentrations are difficult to interpret. Concentrations within extracellular fluids of echinoderms are almost at equilibrium with seawater and, at least for calcium in some species, moderately regulated (for a review, see Russell, 2013). Then, no changes in CF concentrations of these ions should be expected unless the skeleton is continuously dissolved. As discussed by Collard *et al.* (2013a), the Mg concentration increase in the CF would anyway be hard to detect, even if 1% of the skeleton was dissolved. Furthermore, in some studies the CF Mg/Ca ratio increased (Spicer *et al.,* 1988; Calosi *et al.,* 2013) or remained constant (Catarino *et al.,* 2012) when sea urchins were submitted to acidified conditions. This is counter to expectations if changes in concentrations of these ions were due to skeleton dissolution. Indeed, the Mg/Ca ratio of the CF should have decreased, because this ratio is much lower in the skeleton (*ca.* 0.1) than in the CF (*ca.* 4.4–4.8) (Hermans *et al.,* 2010; Catarino *et al.,* 2012). Currently, it is unclear why magnesium and/or calcium concentrations in echinoderm CF changed during some (but not in other) acidification experiments. Furthermore, it is intriguing that such modifications occurred at very different pH and after different periods of time in the different studies (see Appendix). The contrasting results could be linked to the physiological activity of experimental animals, including feeding. Indeed, Catarino *et al.* (2012) reported an increased Mg/Ca ratio in the coelomic fluid of individuals of the sea urchin *P. lividus* maintained at 16 °C in comparison with those maintained at 10 °C. These authors suggested that this was related to an increased transporter activity linked to temperature.

Due to their "naked" mature spines (Fig. 4), cidaroids have been believed to be particularly at risk in the face of ocean acidification. Actually, it seems that the outermost layer of these spines, the polycrystalline cortex, protects them and make them more resistant to acidification (Dery *et al.,* 2014). This resistance seems to be due to the lower magnesium content, higher density, and biofilm cover of the cortex. This is consistent with the bathyal range of cidaroids, which are frequently recorded below the saturation horizon for aragonite (David *et al.,* 2005). Furthermore, magnesium concentration in the spine cortex was reported to be lower in specimens of *Ctenocidaris speciosa* living below the saturation horizon (Catarino *et al.,* 2013). Obviously, further studies on this group would be beneficial.

It has frequently been argued that echinoderms would be particularly at risk from ocean acidification due to the high magnesium content of their skeleton, making the skeletal calcite more soluble, especially at high latitudes where OA will be more pronounced (*e.g.,* Andersson *et al.,* 2008; Sewell and Hofmann, 2011; McClintock *et al.,* 2011). Evidence for this is currently poor. For sea urchin spines, lower magnesium content than in the test (or according to depth) might be taken to suggest an adaptation, indirectly supporting the high-sensitivity hypothesis. The relationship between skeleton mineralogy and depth (and thus calcite saturation), especially at high latitude and in the Pacific Ocean, would be of interest, providing it is carried out in the same species (see Hermans *et al.,* 2011). However, LaVigne *et al.* (2013) did not find any differences in Mg/Ca ratios of spines from adult *Strongylocentrotus purpuratus* collected in locations with contrasting coastal upwelling regimes differing in carbonate saturation. These authors also did not find any difference in the same ratio in the skeleton of newly settled juveniles experimentally exposed to different pH conditions. The same result was obtained for adults (*Arbacia punctulata*, *Eucidaris tribuloides*, Ries, 2011a; *Arbacia lixula*, Calosi *et al.,* 2013). The latter evidence is, however, much less conclusive because the amount of skeleton deposited by adults during the experiment is rather low in comparison with the initial skeleton mass, giving the test a very low level of inference. In this context, it is surprising that Calosi *et al.* (2013) found a change in Mg/Ca skeletal ratio in *Paracentrotus lividus* sea urchins transplanted to a vent site for only 4 days. Furthermore, as mentioned above, the sea urchin test skeleton appears unetched even when the coelomic fluid saturation state is below 1. This suggests that the skeleton is somehow protected, even if extracellular when fully grown. It is noteworthy that the skeleton of all species investigated so far at the transmission electron microscopy level is always covered by a layer of organic material that may stabilize the outermost layer of the trabeculae (Märkel *et al.,* 1986; Dubois and Chen, 1989). Alternatively, acid-base regulation of the dermal connective tissue may also occur, although there are no data on this. Indeed, carbonic anhydrase is known to occur in similar tissue in sea urchins (Chen and Lawrence, 1986, 1987).

Mechanical properties

Very few studies considered the biomechanics of skeleton function with respect to OA, and no information is available on the effects of OA on the sea urchin tooth. Shirayama and Thornton (2005) reported that juvenile *Echinometra mathaei* raised for 26 weeks at pH 7.90 had tests that were more brittle than those of controls raised at 7.94. Unfortunately, this was not quantified. In the same experiment such brittleness was not recorded for tests of juvenile *Hemicentrotus pulcherrimus*. Asnaghi *et al.* (2013) reported that the robustness of whole tests was reduced in juvenile *Paracentrotus lividus* exposed for 30 days to pH 7.7. Unfortunately, this study was carried out on dry tests whose mechanics is deeply altered in comparison with living tests, and the reported results actually illustrate the mechanical properties of the dry ligaments joining the test plates (see Ellers *et al.,* 1998). Holtmann *et al.* (2013) reported breaking forces reduced by 12% for spines of *Strongylocentrotus droebachiensis* exposed for 6 weeks to pH 7.25. The test plates were not affected (perforation test). While it is possible that acidification affects the mechanical properties of sea urchin skeletal elements, more information is needed, especially on specimens subjected to long-term experiments.

Conclusions

Currently, although skeleton integrity and formation were among the first concerns of early acidification studies (*e.g.,* Shirayama and Thornton, 2005), little is known about the impact of acidification and interacting temperature on the skeleton of postmetamorphic echinoderms. This is probably due to the rather heavy logistics required to work with these organisms for the long periods necessary to produce new skeleton in large enough proportions. Studies on starfish and brittle stars are very scarce and more are clearly needed.

Growth of juvenile sea urchins appears more prone to the effects of acidification than that of adults. On the contrary, juvenile starfish as well as adults seem to be either not impacted or even boosted by acidification. This is surprising as all starfish so far studied showed no or very low compensation of coelomic fluid pH under acidification (see Collard *et al.,* 2013a, and references therein). Brittle stars show moderate effects at rather low pH, but reports of increased calcification at reduced pH are inconclusive.

The mechanisms behind the effects on growth are currently unknown. The impact of low pH and increased temperature is difficult to interpret. Indeed, growth is affected by several variables including food consumption rate, digestive abilities, absorption of digestive products, and gametogenesis (Ebert, 2013). Recent papers showed that, in sea urchin larvae, low pH reduced digestive efficiency and increased the energetic costs for acid-base regulation (Stumpp *et al.,* 2012b, 2013). In most experiments with postmetamorphic individuals, food was provided *ad libitum*, a situation rarely encountered in the field and which could mask effects on growth. More experiments should be carried out with realistic food conditions before the "no effect on growth" results can be relied upon. In sea urchins, an indirect effect through the energetic cost of pH compensation in the extracellular fluid, which was reported in several species (see, *e.g.,* Stumpp *et al.,* 2012a; Catarino *et al.,* 2012; Collard *et al.,* 2013b) but not in others (*e.g.,* Miles *et al.,* 2007), could be one of the involved mechanisms. Because homeostasis in the extracellular fluid seems to be rather different in larvae and adults, mechanisms elegantly demonstrated by Stumpp *et al.* (2012b) for sea urchin larvae probably cannot be applied as such to adults. However, similar processes could be involved between the extracellular fluid and seawater. More studies, including energy budgets as done for *S. droebachiensis* by Stumpp *et al.* (2012a), should be carried out.

Dissolution of the body wall skeleton is very probably not a major threat to sea urchins. This is quite surprising in view of the low pH reached in the coelomic fluid by some species. This intriguing question deserves further studies. Due to their exposed position, spines are more vulnerable to this threat, but their regeneration abilities can probably ensure their maintenance. Interestingly, Edwards and Ebert (1991) showed that spine damage induced an increased rate of calcification of the test plates. However, both spine regeneration and increased test calcification could have a significant energetic cost, including changes in resource allocation (Edwards and Ebert, 1991). Unfortunately, no study assessed the cost of skeleton deposition (Lawrence, 2010). No information is available for starfish, and the situation in brittle stars needs further assessment.

Mechanical properties are linked to both growth and possible dissolution. Indeed, growth rate influences the density of the skeleton (Smith, 1980) and, as a consequence, its mechanical properties. In this context, it is important not to limit the studies to breaking forces but to characterize other important variables like the Young modulus, which expresses the material stiffness or the second moment of area that quantifies the distribution of the material around the neutral fiber (see Burkhardt *et al.,* 1983; Moureaux *et al.,* 2010). In ecotoxicological studies, both these parameters were shown to be affected (Moureaux *et al.,* 2011). Preliminary evidence available indicates that mechanical properties in sea urchins could be affected, but reseach needs to focus on the mechanics of live individuals and relevant skeletal parts (spines, isolated plates) to be more ecologically relevant and provide insights into possible changes in the skeletal material.

It is suggested to dedicate further research to the functional consequences of the impact of ocean acidification on the echinoderm skeleton, principally the mechanical properties, which are key aspects of the skeleton function.

Acknowledgments

The author thanks the editors for offering the opportunity to write this review, Prof. M. Byrne and two anonymous reviewers for fruitful comments and suggestions, M. Collard for help with the literature search, A. Dery for kindly providing SEM micrographs, and V. Desmet for help in editing the manuscript and preparing the figures. Ph. Dubois is a Research Director of the National Fund for Scientific Research (Belgium). Work supported by FRFC contract 2.4587.11.

Literature Cited

Albright, R., C. Bland, P. Gillette, J. E. Serafy, C. Langdon, and T. R. Capo. 2012. Juvenile growth of the tropical sea urchin *Lytechinus variegatus* exposed to near-future ocean acidification scenarios. *J. Exp. Mar. Biol. Ecol.* **426–427:** 12–17.

Andersson, A. J., F. T. Mackenzie, and N. R. Bates. 2008. Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water calcifiers. *Mar. Ecol. Prog. Ser.* **373:** 265–273.

Andersson, A. J., F. T. Mackenzie, and M. Dai. 2012. Revisiting four scientific debates in ocean acidification research. *Biogeosciences* **9:** 893–905.

Appelhans, Y. S., J. Thomsen, C. Pansch, F. Melzner, and M. Wahl. 2012. Sour times: seawater acidification effects on growth, feeding behaviour and acid-base status of *Asterias rubens* and *Carcinus maenas*. *Mar. Ecol. Prog. Ser.* **459:** 85–97.

Asnaghi, V., M. Chiantore, L. Mangialajo, F. Gazeau, P. Francour, S. Alliouane, and J. P. Gattuso. 2013. Cascading effects of ocean acidification in a rocky subtidal community. *PLoS One* **8(4):** e61978. doi:10.1371/journal.pone.0061978.

Asnaghi, V., L. Mangialajo, J. P. Gattuso, P. Francour, D. Privitera, and M. Chiantore. 2014. Effects of ocean acidification and diet on thickness and carbonate elemental composition of the test of juvenile sea urchins. *Mar. Environ. Res.* **93:** 78–84.

Borig, P. 1933. Über Wachstum und Regeneration der Stacheln einiger Seeigle. *Z. Morphol. Ökol. Tiere* **27:** 624–653.

Burkhardt, A., W. Hansmann, K. Märkel, and H. Niemann. 1983. Mechanical design in spines of Diadematoid echinoids (Echinodermata, Echinoidea). *Zoomorphology* **102:** 189–203.

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. *Oceanogr. Mar. Biol. Annu. Rev.* **49:** 1–42.

Byrne, M., and R. Przeslawski. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integr. Comp. Biol.* **53:** 582–596.

Byrne, M., M. Ho, E. Wong, N. A. Soars, P. Selvakumaraswamy, H. Sheppard-Brennand, S. A. Dworjanyn, and A. R. Davis. 2011. Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. *Proc. R. Soc. B* **278:** 2376–2383.

Byrne, M., M. Lamare, D. Winter, S. A. Dworjanyn, and S. Uthicke. 2013. The stunting effect of a high CO_2 ocean on calcification and development in sea urchin larvae, a synthesis from the tropics to the poles. *Philos. Trans. R. Soc. B* **368:** 20120439.

Caldeira, K., and M. Wickett. 2003. Anthropogenic carbon and ocean pH. *Nature* **425:** 365.

Calosi, P., S. P. S. Rastrick, M. Graziano, S. C. Thomas, C. Baggini, H. A. Carter, J. M. Hall-Spencer, M. Milazzo, and J. I. Spicer. 2013. Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. *Mar. Pollut. Bull.* **73:** 470–484.

Catarino, A. I., M. Bauwens, and P. Dubois. 2012. Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environ. Sci. Pollut. Res.* **19:** 2344–2353.

Catarino, A. I., V. Guibourt, C. Moureaux, C. De Ridder, P. Compère, and P. Dubois. 2013. Antarctic urchin *Ctenocidaris speciosa* spines: lessons from the deep. *Cah. Biol. Mar.* **54:** 649–655.

Chave, K. E. 1952. A solid solution between calcite and dolomite. *J. Geol.* **60:** 190–192.

Chen, C.-P., and J. M. Lawrence. 1986. Localization of carbonic anhydrase in the plumula of the tooth of *Lytechinus variegatus* (Echinodermata: Echinoidea). *Acta Zool.* **67:** 27–32.

Chen, C.-P., and J. M. Lawrence. 1987. The role of carbonic anhydrase in facilitating the transport of CO_2 in the tooth of *Lytechinus variegatus* (Echinodermata: Echinoidea) *Comp. Biochem. Physiol.* **87(A):** 327–331.

Cheng, X., P. L. Varona, M. J. Olszta, and L. B. Gower. 2007. Biomimetic synthesis of calcite films by a polymer-induced liquid-precursor (PILP) process—1. Influence and incorporation of magnesium. *J. Cryst. Growth* **307:** 395–404.

Collard, M., A. Catarino, S. Bonnet, P. Flammang, and P. Dubois. 2013a. Effects of CO_2-induced ocean acidification on physiological and mechanical properties of the starfish *Asterias rubens*. *J. Exp. Mar. Biol. Ecol.* **446:** 355–362.

Collard, M., K. Laitat, L. Moulin, A. Catarino, P. Grosjean, and P. Dubois. 2013b. Buffer capacity of the coelomic fluid in echinoderms. *Comp. Biochem. Physiol. A* **166:** 199–206.

Collard, M., A. Dery, F. Dehairs, and P. Dubois. 2014. Euechinoidea and Cidaroidea respond differently to ocean acidification. *Comp. Biochem. Physiol. A* (In press).

Courtney, T., I. Westfield, and J. B. Ries. 2013. CO_2-induced ocean acidification impairs calcification in the tropical urchin *Echinometra viridis*. *J. Exp. Mar. Biol. Ecol.* **440:** 169–175.

David, B., T. Choné, R. Mooi, and C. De Ridder. 2005. *Antarctic Echinoidea. Synopses of the Antarctic Benthos*, Vol. 10. Koeltz Scientific Books, Königstein, Germany. 274 pp.

Davies, T. T., M. A. Crenshaw, and B. M. Heatfield. 1972. The effect of temperature on the chemistry and structure of echinoid spine regeneration. *J. Paleontol.* **46:** 874–883.

Dery, A., V. Guibourt, A. I. Catarino, P. Compère, and P. Dubois. 2014. Properties, morphogenesis and effect of acidification on spines of the cidaroid sea urchin *Phyllacanthus imperialis*. *Invertebr. Biol.* (In press).

Donnay, G. 1975. Biocrystallographic studies. *Biomineralizanon* **8:** 14–20.

Donnay, G., and D. L. Pawson. 1969. X-ray diffraction studies of echinoderm plates. *Science* **166:** 1147–1150.

Drager, B. J., M. A. Harkey, M. Iwata, and A. H. Whiteley. 1989. The expression of embryonic primary mesenchyme genes of the sea urchin, *Strongylocentrotus purpuratus*, in the adult skeletogenic tissues of this and other species of echinoderms. *Dev. Biol.* **133:** 14–23.

Dubois, P. and C. P. Chen. 1989. Calcification in echinoderms. *Echinoderm Stud.* **3:** 109–178.

Dupont, S., and M. Thorndyke. 2013. Direct impacts of near-future ocean acidification on sea urchins. Pp. 449–462 in *Climate Change Perspectives From the Atlantic: Past, Present and Future*, J. M. Fernandez-Palacios, L. de Nascimento, J. C. Hernandez, S. Clemente, A. Gonzalez, and J. P. Diaz-Gonzalez, eds. Servicio de Publicaciones, Universidad de La Laguna, Spain.

Dupont, S., B. Lundve, and M. Thorndyke. 2010. Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. *J. Exp. Zool. B Mol. Dev. Evol.* **314:** 382–389.

Ebert, T. A. 2013. Growth and survival of postsettlement sea urchins.

Pp. 83–117 in *Sea Urchins: Biology and Ecology*, 3rd ed., J. M. Lawrence, ed. Elsevier, Amsterdam.

Edwards, P. B., and T. A. Ebert. 1991. Plastic responses to limited food availability and spine damage in the sea urchin *Strongylocentrotus purpuratus* (Stimpson). *J. Exp. Mar. Biol. Ecol*. **145:** 205–220.

Ellers, O., A. S. Johnson, and P. E. Moberg. 1998. Structural strengthening of urchin skeletons by collagenous sutural ligaments. *Biol. Bull*. **195:** 136–144.

Ettensohn, C. A. 2009. Lessons from a gene regulatory network: echinoderm skeletogenesis provides insights into evolution, plasticity and morphogenesis. *Development* **136:** 11–21.

Findlay, H. S., H. L. Wood, M. A. Kendall, J. I. Spicer, R. J. Twitchett, and S. Widdicombe. 2009. Calcification, a physiological process to be considered in the context of the whole organism. *Biogeosci. Discuss*. **6:** 2267–2284. See discussion [Online]. Available: http://www.biogeosciences-discuss.net/6/2267/2009/bgd-6-2267-2009-discussion.html [2014, 29 May].

Findlay, H. S., H. L. Wood, M. A. Kendall, J. I. Spicer, R. J. Twitchettand, and S. Widdicombe. 2011. Comparing the impact of high CO_2 on calcium carbonate structures in different marine organisms. *Mar. Biol. Res*. **7:** 565–575.

Gooding, R. A., C. Harley, and E. Tang. 2009. Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *Proc. Natl. Acad. Sci*. USA **106:** 9316–9321.

Heatfield, B. M. 1971. Growth of the calcareous skeleton during regeneration of spines of the sea urchin, *Strongylocentrotus purpuratus:* a light and scanning electron microscope study. *J. Morphol*. **134:** 57–90.

Heatfield, B. M., and D. F. Travis. 1975. Ultrastructural studies of regenerating spines of the sea urchin *Strongylocentrotus purpuratus.* 1. Cell types without spherules. *J. Morphol*. **145:** 13–50.

Hermans, J., C. Borremans, P. Wilenz, L. André, and P. Dubois. 2010. Temperature, salinity and growth rate dependences of Mg/Ca and Sr/Ca ratios of the skeleton of the sea urchin *Paracentrotus lividus* (Lamarck): an experimental approach. *Mar. Biol*. **157:** 1293–1300.

Hermans, J., L. André, J. Navez, P. Pernet, and P. Dubois. 2011. Relative influences of solution composition and presence of intracrystalline proteins on magnesium incorporation in calcium carbonate minerals: insight into the vital effects. *J. Geophys Res*. **116:** G01001. doi:10.1029/2010JG001487.

Holtmann, W. C., M. Stumpp, M. Gutowska, S. Syré, N. Himmerkus, F. Melzner, and M. Bleich. 2013. Maintenance of coelomic fluid pH in sea urchins exposed to elevated CO_2: the role of body cavity epithelia and stereom dissolution. *Mar. Biol.* **160:** 2631–2645.

Hurlbert, S. H. 1984. Pseudoreplication and the design of ecological field experiments. *Ecol. Monogr*. **54:** 187–211.

IPCC (Intergovernmental Panel on Climate Change). 2007. *Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change,* S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K. B. Averyt, M. Tignor, and H. L. Miller, eds. Cambridge University Press, Cambridge.

Killian, C. E., and F. H. Wilt. 2008. Molecular aspects of biomineralization of the echinoderm endoskeleton. *Chem. Rev*. **108:** 4463–4474.

Killian, C. E., L. Croker, and F. H. Wilt. 2010. SpSM30 gene family expression patterns in embryonic and adult biomineralized tissues of the sea urchin, *Strongylocentrotus purpuratus. Gene Expr. Patterns* **10:** 135–139.

Kurihara, H., R. Yin, G. N. Nishihara, K. Soyano, and A. Ishimatsu. 2013. Effect of ocean acidification on growth, gonad development and physiology of the sea urchin *Hemicentrotus pulcherrimus. Aquat. Biol*. **18:** 281–292.

La Vigne, M., T. M. Hill, E. Sanford, B. Gaylord, A. D. Russell, E. A. Lenz, J. D. Hosfelt, and M. K. Young. 2013. The elemental composition of purple sea urchin (*Strongylocentrotus purpuratus*) calcite and potential effects of pCO_2 during early life stages. *Biogeosciences* **10:** 3465–3477.

Lawrence, J. M. 2010. Energetic costs of loss and regeneration of arms in stellate echinoderms. *Integr. Comp. Biol*. **50:** 506–514.

Livingston, B. T., C. E. Killian, F. Wilt, A. Cameron, M. J. Landrum, O. Ermolaeva, V. Sapojnikov, D. R. Maglott, A. M. Buchanan, and C. A. Ettensohn. 2006. A genome-wide analysis of biomineralization-related proteins in the sea urchin *Strongylocentrotus purpuratus. Dev. Biol*. **300:** 335–348.

Lockhart, S. J., and C. D. Jones. 2008. Biogeographic patterns of benthic invertebrate megafauna on shelf areas within the Southern Ocean Atlantic sector. *CCAMLR Science* **15:** 167–192.

Loste, E., R. M. Wilson, R. Seshadri, and F. C. Meldrum. 2003. The role of magnesium in stabilising amorphous calcium carbonate and controlling calcite morphologies. *J. Cryst. Growth* **254:** 206–218.

Mann, K., A. J. Poustka, and M. Mann. 2008a. In-depth, high-accuracy proteomics of sea urchin tooth organic matrix. *Proteome Sci*. **6:** 33.

Mann, K., A. J. Poustka, and M. Mann. 2008b. The sea urchin (*Strongylocentrotus purpuratus*) test and spine proteomes. *Proteome Sci*. **6:** 22.

Mann, K., F. H. Wilt, and A. J. Poustka. 2010. Proteomic analysis of sea urchin (*Strongylocentrotus purpuratus*) spicule matrix. *Proteome Sci*. **8:** 33.

Märkel, K., and U. Röser. 1983. The spine tissues in the echinoid *Eucidaris tribuloides*. *Zoomorphology* **103:** 25–41.

Märkel, K., F. Kubanek, and A. Willgallis. 1971. Polykristalliner Calcit bei Seeigeln *(*Echinodermata, Echinoidea). *Z. Zellforsch.* **119:** 355–377.

Märkel, K., U. Röser, U. Mackenstedt, and M. Klostermann. 1986. Ultrastructural investigations of matrix-mediated biomineralization in echinoids (Echinodermata, Echinoidea). *Zoomorphology* **106:** 232–243.

Matranga, V., R. Bonaventura, C. Costa, K. Karakostis, A. Pinsino, R. Russo, and F. Zito. 2011. Echinoderms as blueprints for biocalcification: regulation of skeletogenic genes and matrices. *Prog. Mol. Subcell. Biol*. **52:** 225–248.

Matranga, V., A. Pinsino, R. Bonaventura, C. Costa, K. Karakostis, C. Martino, R. Russo, and F. Zito. 2013. Cellular and molecular bases of biomineralization in sea urchin embryos. *Cah. Biol. Mar*. **54:** 467–478.

McClintock, J. B., M. O. Amsler, R. A. Angus, R. C. Challener, J. B. Schram, C. D. Amsler, C. L. Mah, J. Cuce, and B. J. Baker. 2011. The Mg-Calcite composition of Antarctic echinoderms: important implications for predicting the impacts of ocean acidification. *J. Geol*. **119:** 457–466.

Melzner, F., M. A. Gutowska, M. Langenbuch, S. Dupont, M. Lucassen, M. C. Thorndyke, M. Bleich, and H. O. Pörtner. 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? *Biogeosciences* **6:** 2313–2331.

Miles, H., S. Widdicombe, J. I. Spicer, and J. Hall-Spencer. 2007. Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Mar. Pollut. Bull.* **54:** 89–96.

Morse, J. W., A. J. Andersson, and F. T. Mackenzie. 2006. Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and "ocean acidification": role of high Mg-calcites. *Geochim. Cosmochim. Acta* **70:** 5814–5830.

Moureaux, C., A. Pérez-Huerta, P. Compère, W. Zhu, T. Leloup, M. Cusack, and P. Dubois. 2010. Structure, composition and mechanical relations to function in sea urchin spine. *J. Struct. Biol*. **170:** 41–49.

Moureaux, C., J. Simon, G. Mannaerts, A. I. Catarino, P. Pernet, and P. Dubois. 2011. Effects of field contamination by metals (Cd, Cu, Pb, Zn) on biometry and mechanics of echinoderm ossicles. *Aquat. Toxicol*. **105:** 698–707.

Orr, J. C., V. J. Fabry, O. Aumont, L. Bopp, S. C. Doney, R. A. Feely, A. Gnanadesikan, N. Gruber, A. Ishida, F. Joos *et al.* 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437:** 681–686.

Politi, Y., T. Arad, E. Klein, S. Weiner, and L. Addadi. 2004. Sea urchin spine calcite forms via a transient amorphous calcium carbonate phase. *Science* **306:** 1161–1164.

Pörtner, H. O. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Mar. Ecol. Prog. Ser*. **373:** 203–217.

Pörtner, H. O., M. Langenbuch, and A. Reipschläger. 2004. Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. *J. Oceanogr*. **60:** 705–718.

Prouho, H. 1887. Recherches sur le *Dorocidaris papillata* et quelques autres échinides de la Méditerranée. *Arch. Zool. Exp.* **5:** 214–380.

Raz, S., S. Weiner, and L. Addadi. 2000. Formation of high-magnesium calcites via an amorphous precursor phase: possible biological implications. *Adv. Mater.* **12:** 38–42.

Ries, J. B. 2011a. A physicochemical framework for interpreting the biological calcification response to CO_2-induced ocean acidification. *Geochim. Cosmochim. Acta* **75:** 4053–4064.

Ries, J. B. 2011b. Skeletal mineralogy in a high-CO_2 world. *J. Exp. Mar. Biol. Ecol.* **403:** 54–64.

Ries, J. B., A. L. Cohen, and D. C. McCorkle. 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology* **37:** 1131–1134.

Russell, M. P. 2013. Echinoderm responses to variation in salinity. *Adv. Mar. Biol.* **66:** 171–212.

Schram, J. B., J. B. McClintock, R. A. Angus, and J. M. Lawrence. 2011. Regenerative capacity and biochemical composition of the sea star *Luidia clathrata* (Say) (Echinodermata: Asteroidea) under conditions of near-future ocean acidification. *J. Exp. Mar. Biol. Ecol.* **407:** 266–274.

Schroeder, J. H., E. J. Dwomik, and J. J. Papike. 1969. Primary protodolomite in echinoid skeletons. *Geol. Soc. Am. Bull*. **80:** 1613–1616.

Sewell, M. A., and G. E. Hofmann. 2011. Antarctic echinoids and climate change: a major impact on the brooding forms. *Glob. Change Biol*. **17:** 734–744.

Shirayama, Y., and H. Thornton. 2005. Effect of increased atmospheric CO_2 on shallow water marine benthos. *J. Geophys. Res*. **110:** doi: 10.1029/2004JC002618.

Smith, A. B. 1980. Stereom microstructure of the echinoid test. *Spec. Pap. Palaeontol*. **25:** 1–81.

Spicer J. I., A. C. Taylor, and A. D. Hill. 1988. Acid-base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion. *Mar. Biol*. **99:** 527–534.

Spicer, J. I., S. Widdicombe, H. R. Needham, and J. A. Berge. 2011. Impact of CO_2-acidified seawater on the extracellular acid-base balance of the northern sea urchin *Strongylocentrotus dröebachiensis*. *J. Exp. Mar. Biol. Ecol*. **407:** 19–25.

Steneck, R. S. 2013. Sea urchins as drivers of shallow benthic community structure. Pp. 196–212 in *Sea Urchins: Biology and Ecology*, 3rd ed., J. M. Lawrence, ed. Elsevier, Amsterdam.

Stumpp, M., K. Trübenbach, D. Brennecke, M. Y. Hu, and F. Melzner. 2012a. Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. *Aquat. Toxicol*. **110–111:** 194–207.

Stumpp, M., M. Y. Hu, F. Melzner, A. Gutowska, N. Dorey, N. Himmerkus, W. C. Holtmann, S. T. Dupont, M. C. Thorndyke, and M. Bleich. 2012b. Acidified seawater impacts sea urchin larval pH regulatory systems relevant for calcification. *Proc. Natl. Acad. Sci*. **109:** 18192–18197.

Stumpp, M., M. Hu, I. Casties, R. Saborowski, M. Bleich, F. Melzner, and S. Dupont. 2013. Digestion in sea urchin larvae impaired under ocean acidification. *Nat. Clim. Change* **3:** 1044–1049.

Uthicke, S., N. Soars, S. Foo, and M. Byrne. 2013. Effects of elevated pCO_2 and the effect of parent acclimation on development in the tropical Pacific sea urchin *Echinometra mathaei*. *Mar. Biol*. **160:** 1913–1926.

Wangensteen, O. S., S. Dupont, I. Casties, X. Turon, and C. Palacin. 2013. Some like it hot: temperature and pH modulate larval development and settlement of the sea urchin *Arbacia lixula*. *J. Exp. Mar. Biol. Ecol*. **449:** 304–311.

Weber, J. N. 1969. The incorporation of magnesium into the skeletal calcite of echinoderms. *Am. J. Sci*. **267:** 537–566.

Weiner, S. 1985. Organic matrix-like macromolecules associated with the mineral phase of sea-urchin skeletal plates and teeth. *J. Exp. Zool*. **234:** 7–15.

Wittman, A. C., and H. O. Pörtner. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nat. Clim. Change*. doi: 10.1038/NCLIMATE1982.

Wolfe, K., A. S. Dworjanyn, and M. Byrne. 2013a. Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Heliocidaris erythrogramma*). *Glob. Change Biol*. **19:** 2698–2707.

Wolfe, K., A. S. Dworjanyn, and M. Byrne. 2013b. Thermal and pH/pCO_2 fluctuations in the intertidal habitat of *Heliocidaris erythrogramma*: effects on post-metamorphic juveniles. *Cah. Biol. Mar*. **54:** 657–666.

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

Wood, H. L., J. I. Spicer, D. M. Lowe, and S. Widdicombe. 2010. Interaction of ocean acidification and temperature; the high cost of survival in the brittlestar *Ophiura ophiura*. *Mar. Biol*. **157:** 2001–2013.

Wood, H. L., J. I. Spicer, M. A. Kendall, D. M. Lowe, and S. Widdicombe. 2011. Ocean warming and acidification; implications for the Arctic brittlestar *Ophiocten sericeum*. *Polar Biol.* **34:** 1033–1044.

Appendix

Literature available by the end of 2013 on the effects of acidification and its interaction with temperature on the skeleton of postmetamorphic echinoderms. Effects are reported together with the higher pH at which they are statistically significant at the 0.05 level.

Class	Species	pH_T SW[A]	pH_T CF[B]	Time	ΩAr[C]	Effects on skeleton[D]	Remarks[E]	References
Echinoidea Juveniles	*Hemicentrotus pulcherrimus*	7.94* 7.90*		26 weeks		Lower ww increase 7.90	Effect started at week 14-16 (?)	Shirayama & Thornton 2005
	Echinometra mathaei	7.94* 7.90*		26 weeks		Lower ww reduced ww gain or decreased ww 7.90 Tests became brittle	Effect started at week 14-16 (?)	Shirayama & Thornton 2005
	Heliocidaris erythrogramma Newly settled juveniles	8.2* 7.8* 7.6*		5 d (larval and juvenile)	3.02–3.39 1.56–1.77 1.03–1.17	Number of spines reduced ≤7.8 at 22 °C No effect at 24 °C	3 temp. 22, 24, 26 °C (3)	Byrne *et al.,* 2011
	Heliocidaris erythrogramma Newly settled juveniles	8.13-8.08* 7.80–7.74* 7.65–7.59* 7.47–7.44*		14 d	3.5–3.1 1.7–1.5 1.1–1.0 0.7–0.6	Test diameter 1–4.2% smaller ≤7.6 No effect on spine length Spine length 19–32% shorter 7.4 + interaction with temp (worse impact 7.4–25 °C) Altered spine tip ≤7.8, ≥23 °C	3 temp. 21, 23, 25 °C (3)	Wolfe *et al.,* 2013a
	Heliocidaris erythrogramma Newly settled juveniles	8.17 7.8 7.32 6.99		14 d	3.20 1.69 0.60 0.29	Test diameter smaller ≤ 7.32 Spine number reduced 6.99, temp 25 °C Spine pore size larger ≤7.8, temp 25 °C	2 temp. 21, 25 °C (3)	Wolfe *et al.,* 2013b
	Lytechinus variegatus	8.1 7.96 7.83		89 d	No ΩAr provided	Wet weight increase reduced ≤7.96 Delay in stereom morphogenesis in spines 7.83 Surface alterations of sterom in spines 7.83 No effect on test stereom	(1)	Allbright *et al.,* 2012
	Arbacia lixula Newly settled juveniles	8.09 7.69		52 d (larval and juvenile life)	2.87 1.27	Smaller diameter 7.69	(2)	Wangensteen *et al.,* 2013
	Strongylocentrotus purpuratus Newly settled juveniles	8.02 7.73		48–49 d (whole larval life)	No ΩAr provided	Mg/Ca in skeleton not affected Sr/Ca increased by 8% 7.73 in juveniles from southernmost genitors	Genitors from different populations	LaVigne *et al.,* 2013
	Paracentrotus lividus	8.09 7.98 7.84 7.70		30 d	4.02 3.32 2.58 1.95	Robustness of whole dried test reduced 7.70 Stereom with signs of corrosion in Aristotle's lantern 7.70 – no quantification	(1)	Asnaghi *et al.,* 2013, 2014

Appendix *(Continued)*

Class	Species	pH_T SW[A]	pH_T CF[B]	Time	ΩAr[C]	Effects on skeleton[D]	Remarks[E]	References
						No effect on apical disk or spines Effects increased when fed non-calcified algae No effect on test thickness Mg/Ca in test increase when fed *Corallina* but not when fed *Cystoseira* or *Dictyota* ≤ 7.98		
Echinoidea Adults	*Arbacia punctulata*	8.04* 7.90* 7.77* 7.36*		60 d	2.1 1.6 1.2 0.5	Buoyant weight increase lower 7.36 and 8.04 No effect on Mg/Ca in skeleton	(2)	Ries *et al.,* 2009 Ries 2011b
	Eucidaris tribuloides	8.04* 7.90* 7.77* 7.36*		60 d	2.1 1.6 1.2 0.5	Buoyant weight decrease 7.36 No effect on Mg/Ca in skeleton	(2)	Ries *et al.,* 2009 Ries 2011b
	Strongylocentrotus droebachiensis	7.98 7.67 7.25	7.75* 7.70* 7.45*	41–45 d	1.55 0.79 0.32	Sterom surface alteration ≤ 7.67 Breaking force – test ossicle ns – spine $P = 0.04$ 7.25 (diff: 11.5%) Reduced growth of test ossicle ≤7.67 Test growth – diameter 7.25 – dry mass 7.25	(3)	Holtmann *et al.,* 2013 Stumpp *et al.,* 2012a
	Echinometra mathaei	7.97 7.79 7.61 7.45		45 d	3.11–2.90 2.24–2.03 1.71–1.32 1.39–0.99	No significant effect on wet weight growth (P = 0.075/0.094)	(1,2)	Uthicke *et al.,* 2013
	Echinometra viridis	8.29–8.25 8.10–8.04		60 d	7.2–5.2 5.3–3.8	Buoyant weight decrease at 8.04/20 °C Buoyant weight reduced increase at 8.1/30 °C	2 temp 20, 30 °C Significant mortality (8/44) (?)	Courtney *et al.,* 2013
	Hemicentrotus pulcherrimus	8.10* 7.83*	7.61* 7.03*	9 month	2.4 1.4	No effect on test diameter, body wall and Aristotle's lantern wet weight Mg^{++} in CF lower 7.83 Mg/Ca in coelomic fluid decreased from 5.21 to 4.89	(2)	Kurihara *et al.,* 2013
	Antarctic echinoids			Lifetime		Thin, weakly calcified tests in sea urchins living below calcite saturation horizon	Aragonite saturation horizon is relevant not calcite saturation horizon	Sewell and Hofmann 2011

Appendix *(Continued)*

Class	Species	pH_T SW[A]	pH_T CF[B]	Time	ΩAr[C]	Effects on skeleton[D]	Remarks[E]	References
	Psammechinus miliaris	Emersion	7.12*	2-24h		Test dissolution inferred from Mg^{2+} increase in the coelomic fluid	Mg/Ca in CF increased (?)	Spicer *et al.,* 1988
	Echinus esculentus	Emersion	7.10*	2-24h		Test dissolution inferred from Mg^{2+} increase in the coelomic fluid	Mg/Ca in CF increased	Spicer *et al.,* 1988
	Psammechinus miliaris	7.96* 7.46* 6.63* 6.16*	7.53* ca. 6.9* 6.72* 6.42*	6–7 d		Test dissolution inferred from Mg^{2+} increase in the coelomic fluid 7.44	(2)	Miles *et al.,* 2007
	Strongylocentrotus droebachiensis	7.89* 7.44* 7.16* 6.78*	7.5* 7.3* 7.25* 6.9*	5 d	0.66 0.26 0.14 0.06	Increased Ca^{++} concentration in coelomic fluid ≤7.16	(1)	Spicer *et al.,* 2011
	Paracentrotus lividus	7.90–8.01 7.67–7.70 7.40–7.39	7.56–7.54 7.46–7.51 7.36–7.45	19 d	1.90–2.12 1.06–1.22 0.58–0.70	No effect on Mg^{++} and Ca^{++} nor on Mg/Ca in coelomic fluid	2 temp. 10–16 °C (3)	Catarino *et al.,* 2012
	Paracentrotus lividus	8.05* in situ 7.73* in situ	7.18–7.34 7.18–7.34	4 d	2.70 1.50	Increase of Mg^{++} and Ca^{++} in the test No effect on coelomic fluid concentrations	*In situ* transplantation	Calosi *et al.,* 2013
	Arbacia lixula	8.05* in situ 7.73* in situ	6.80–6.87 6.80–6.87	4 d	2.70 1.50	No effect on Mg^{++}, Ca^{++} and Sr^{++} concentration in calcified tissues No effect on coelomic fluid concentrations	*In situ* transplantation	Calosi *et al.,* 2013
	Paracentrotus lividus	8.06* 7.69*	7.23 6.94	1 d	2.96 1.54	Mg^{++}, Ca^{++}, Sr^{++} in coelomic fluid increase	(2)	Calosi *et al.,* 2013
	Arbacia lixula	8.06* 7.69*	Ca. 6.85 Ca. 6.85	1 d	2.96 1.54	Mg^{++} in coelomic fluid decrease ($P = 0.045$) No effect on Ca^{++} and Sr^{++} in coelomic fluid	(2)	Calosi *et al.,* 2013
	Ctenocidaris speciosa	8.01 7.98 7.97 7.97		Lifetime	1.06 0.98 0.94 0.86	Structural differences in spine cortex 7.97 $MgCO_3$ concentration in cortex lower 7.97	Specimens from 4 depths: 237, 602, 810, 1286–1681m	Catarino *et al.,* 2013
	Strongylocentrotus purpuratus	Environmental gradient of pH along the Pacific coast of the USA		Lifetime		No effect on spine skeleton Mg/Ca and Sr/Ca		LaVigne *et al.,* 2013
	Phyllacanthus imperialis	8.2 7.6 7.2		3 weeks	3.90–2.58[#] 1.29–0.85[#] 0.57–0.40[#]	Spine cortex is less etched than stereom	(3)	Dery *et al.,* 2014

Appendix *(Continued)*

Class	Species	pH_T SW[A]	pH_T CF[B]	Time	ΩAr[C]	Effects on skeleton[D]	Remarks[E]	References
Asteroidea Juveniles	*Pisasterochraceus*	7.85–7.88* 7.79–7.82*		10 weeks		Increase in ww relative growth 7.8: 67% (12 °C), 110% (15 °C) Skeleton proportion in total wet mass reduced from 11.5 to 10.9% 7.8	2 temp: 12, 15 °C (3)	Gooding *et al.,* 2009
	Crossaster papposus	8.1* 7.7*		32d (larval life) + 6d juvenile	2.0 1.0	Growth rate double 7.7	(?)	Dupont *et al.,* 2010
Asteroidea Adults	*Luidia clathrata*	8.2* 7.8*		97d	4.94 1.89	No effect on arm regeneration rate nor on ww increase of whole individual No effect on ash content in body wall	(3)	Schram *et al.,* 2011
	Asterias rubens	8.06* 7.84* 7.36*	7.55* 7.45* 7.28*	10 weeks	0.96 0.53 0.20	No difference in ww growth between control and acidified conditions	(1)	Appelhans *et al.,* 2012
	Asterias rubens	7.90–7.91 7.72–7.73 7.40–7.48	7.31–7.58 7.21–7.50 7.11–7.34	7, 14d	1.8–1.9 1.2 0.6–0.8	No effect on Mg^{++} and Ca^{++} nor on Mg/Ca in coelomic fluid	(3)	Collard *et al.,* 2013a
Ophiuroidea Adults	*Amphiura filiformis*	8.0* 7.7* 7.3* 6.8*		40d	1.61 0.13	Ca concentration higher in acidified condition 6.8 Length of regenerating arms higher in acidified conditions (threshold not mentioned)	(1, 2) but see Findlay *et al.*, 2011)	Wood *et al.,* 2008 Findlay *et al.,* 2009, 2011
	Ophiura ophiura	7.95–7.99* 7.66–7.62* 7.42–7.38*		40d	1.35–1.36 0.77–0.63 0.44–0.38	No difference in arm Ca and Mg concentrations Arm regeneration rate at 15 °C lower 7.4 No effect on regeneration rate at 10.5 °C	2 temp 10.5, 15 (1, 2)	Wood *et al.,* 2010
	Ophiocten sericeum	8.31–8.30* 7.73–7.69* 7.32–7.34*		20d	2.53–2.20 0.78–0.64 0.31–0.29	No difference in arm Ca and Mg concentration No effect on arm regeneration and differentiation at 5 °C At 8 °C lower regeneration and differentiation 7.3	2 temp: 5.0 (ambient), 8.5 °C (1, 2)	Wood *et al.,* 2011

[A] pH_T SW: mean pH of seawater in total scale.

[B] pH_T CF: mean pH of coelomic fluid (CF) in total scale;

[C] ΩAr: saturation state of aragonite in seawater; #: Ω Mg-calcite calculated according to Mg concentration in skeleton.

[D] ww: wet weight.

[E] (1): single head tank per condition; (2): individuals in same aquarium used as true replicates; (3) appropriate statistical model; (?): statistical design cannot be determined.

*pH reported in NBS-NIST scale.