**Review Article**

# The impact of environmental acidification on the microstructure and mechanical integrity of marine invertebrate skeletons

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Ocean acidification (OA), from seawater uptake of anthropogenic $CO_2$, has a suite of negative effects on the ability of marine invertebrates to produce and maintain their skeletons. Increased organism $pCO_2$ causes hypercapnia, an energetically costly physiological stress. OA alters seawater carbonate chemistry, limiting the carbonate available to form the calcium carbonate ($CaCO_3$) minerals used to build skeletons. The reduced saturation state of $CaCO_3$ also causes corrosion of $CaCO_3$ structures. Global change is also accelerating coastal acidification driven by land-run off (e.g. acid soil leachates, tannic acid). Building and maintaining marine biomaterials in the face of changing climate will depend on the balance between calcification and dissolution. Overall, in response to environmental acidification, many calcifiers produce less biomineral and so have smaller body size. Studies of skeleton development in echinoderms and molluscs across life stages show the stunting effect of OA. For corals, linear extension may be maintained, but at the expense of less dense biomineral. Conventional metrics used to quantify growth and calcification need to be augmented by characterisation of the changes to biomineral structure and mechanical integrity caused by environmental acidification. Scanning electron microscopy and microcomputed tomography of corals, tube worms and sea urchins exposed to experimental (laboratory) and natural (vents, coastal run off) acidification show a less dense biomineral with greater porosity and a larger void space. For bivalves, $CaCO_3$ crystal deposition is more chaotic in response to both ocean and coastal acidification. Biomechanics tests reveal that these changes result in weaker, more fragile skeletons, compromising their vital protective roles. Vulnerabilities differ among taxa and depend on acidification level. Climate warming has the potential to ameliorate some of the negative effects of acidification but may also make matters worse. The integrative morphology-ecomechanics approach is key to understanding how marine biominerals will perform in the face of changing climate.

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## Introduction

Ocean acidification (OA) resulting from increased ocean uptake of $CO_2$, driven by the increase in anthropogenic greenhouse gas emissions, is unprecedented on geological time scales (Zeebe et al., 2016). This uptake is causing major changes to ocean chemistry with a suite of negative effects on the ability of marine invertebrates to produce and maintain their skeletons. Firstly, increased organism $pCO_2$ causes hypercapnia, a stress that can affect many physiological processes. Hypercapnia can be energetically costly reducing the resources that animals need to calcify because maintenance of essential metabolic processes (e.g. acid-base homeostasis) takes priority over diverting energy to growth and calcification (Stumpp et al., 2012; Pan et al., 2015). The production and maintenance of calcium carbonate ($CaCO_3$) structures has been shown to be modulated by energy acquisition (Melzner et al., 2011). Secondly, OA reduces the saturation state of $CaCO_3$ minerals, limiting the carbonate available for calcification (IPCC, 2014). These minerals are the building blocks of biocalcification and include aragonite and calcite. Thirdly, the direct corrosive effect of OA can cause pitting and erosion of $CaCO_3$ structures, as reported for calcifiers that are resident in low pH $CO_2$ seep environments (Harvey et al., 2018). Calcite is less susceptible to dissolution at lower pH values than aragonite, unless it contains high levels of magnesium (Ries et al., 2009; Chan et al., 2012). However, calcite is more brittle compared to aragonite, making it mechanically weaker (Fitzer et al., 2015a; Meng et al., 2018).

Due to sea-level rise and changing weather patterns, global change is exacerbating coastal acidification in areas where freshwater runoff results in reduced pH due to leachate from acid sulphate soils and humic acids and tannic acids from groundwater (Amaral et al., 2011, 2012; Duarte et al., 2013; Jiang et al., 2017; Fitzer et al., 2018). Environmental acidification in a changing ocean is being caused by both ocean and coastal acidification. These two forms of acidification ($CO_2$ and land run off) differ in chemistry mechanisms (Fig. 1). Environmental acidification also occurs at $CO_2$ seeps (Foo et al. 2018; González-Delago and Hernández, 2018).

Marine invertebrate skeletons are complex and ancient structures (Wilkinson, 1979) produced through the superb biocontrol of mineral production, resulting in a great diversity of taxon-specific structures (Wilbur, 1972; Cavey and Markel, 1994; Von Euw et al., 2017). For a broad range of marine invertebrates (e.g. corals, molluscs, echinoderms) experimental acidification in laboratory studies and environmental acidification at natural $CO_2$ seeps and in low pH coastal waters (Amaral et al., 2011, 2012; Duarte et al., 2013; Jiang et al., 2017; Fitzer et al., 2018) show the negative effect of acidification on biomineralisation. Faced with these challenges, building and maintaining calcified structures by marine invertebrates will depend on the balance between calcification and dissolution.

Calcified structures are expensive to produce (Comeau et al., 2017) and, in response to the physiological challenges presented by OA, many calcifiers produce smaller skeletons and so have smaller body sizes. This is seen in the stunting effect of OA at the larval and adult stage of molluscs and echinoderms (Parker et al., 2012, 2013; Byrne et al., 2013; Garilli et al., 2015; Dworjanyn and Byrne, 2018). Vulnerabilities with respect to the amount of biomineral produced, its mechanical properties and propensity for dissolution or etching in OA conditions differ greatly among taxa, even within the same phylum that have similar calcification mechanisms (Table 1). Most laboratory studies have involved translocation of animals that that have calcified (i.e. 'grown up') in control conditions to OA conditions for short- or long-term exposure, an approach that shows the effects of OA on established skeleton and the ability to make new skeleton. For insight into the inherent differences of the production of biomineral in normal ambient and acidification conditions, many studies have reared larvae and juveniles under OA (Parker et al., 2012, 2013; Byrne et al., 2013; Dworjanyn and Byrne, 2018) or have availed of naturally low pH habitats (e.g. $CO_2$ seeps or low pH coastal waters) where biomineralisation has occurred through life (Crook et al., 2013; Garilli et al., 2015; Fitzer et al., 2018; Foo et al., 2018; González-Delago and Hernández, 2018; Migliaccio et al., 2019).

The impact of OA on calcification depends on level of $pCO_2$, with near future projections (e.g. $pH_T$ 7.8) having milder impacts than far future ones (e.g. $pH_T$ 7.6 and lower) and also depends on the duration of exposure (see Table 1). For instance, many calcifiers can reside at a mean $pH_T$ 7.7–7.9 at low pH vent sites zones through their life, but are absent at zones with a mean $pH \leq 7.6$ (Calosi et al., 2013; Kroeker et al., 2013; Foo et al., 2018). The impact of environmental acidification also varies among regions and habitats with some species or populations appearing to be adapted or phenotypically adjusted to environmental acidification. This is seen in the resilience of sea urchins living in low pH upwelling zones and vent sites (Calosi et al., 2013; Hofmann et al. 2014; Migliaccio et al., 2019). Some molluscs can alter the amount of the form of $CaCO_3$ produced (e.g. aragonite or calcite) to a more favourable form making them more resilient to acidification (Fitzer et al., 2014a, 2016; Langer et al., 2014; Rühl et al., 2017).

In calcifying marine invertebrates, environmental carbon for calcification such as dissolved inorganic carbon (DIC) can be sourced in the form of $CO_3^{2-}$ or hydrogen carbonate ($HCO_3^-$) from ambient seawater. Under $CO_2$-driven acidification, this may impair shell growth as pH and $CaCO_3$ saturation are lowered resulting in reduced carbonate available for biomineralisation (Doney et al., 2009). Therefore, under OA, where $CO_3^{2-}$ becomes less available from seawater limited shell growth and increased abnormalities become a coping mechanism for continued biomineralisation (Wittmann and Pörtner, 2013). However, respiratory $CO_2$ can also be a source of carbon for calcification where it is used to form

**Table 1:** Studies that have investigated the impacts of environmental acidification on the microstrucure and/or mechanics of marine invertebrate skeletons and methods used. pH levels are listed as published on the total (pHT) or NBS (pHNBS) scales or no scale (pH∗) if not indicated

| Phylum/ species | Time | pH or Ω levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| **Cnidaria** | | | | | | | |
| *Balanophyllia europaea* | Resident | pHT 7.7, 7.9, 8.1; Vent | μCT, SEM | pHT 7.7, 21-31% ↑ in porosity & 7% ↓ in bulk density | Nanoindentation | pHT 7.7, hardness - no effect of pH; ↓ in stiffness | Fantazzini *et al.,* (2015) |
| *Favia fragum* | 8 d to juvenile** | Ω Ar 0.22-3.71; Lab | SEM | Ω Ar ≤ 1; change in crystal size, shape & orientation, thin septa | - | - | Cohen *et al.,* (2009) |
| *Porites astreoides* | Resident | Ω Ar < 1-3.71; Coast | μCT | Ar < 2; ↓ density | - | - | Crook *et al.,* (2013) |
| *Stylophora pistillata* | 12 mo | pHT 7.2, 7.4, 7.8, 8.0; Lab | μCT | pHT 7.2-7.4, ↑ in porosity & ↓ in bulk density, thinner skeleton, no change in crystallography | - | - | Tambutté *et al.,* (2015) |
| *Stylophora pistillata* | 14 mo | pH NBS 7.3, 7.6, 8.2; Lab | SEM, EBSD | pH NBS 7.3, 7.6, crystallographic changes, shorter less round fibre bundles | - | - | Coronado *et al.,* (2019) |
| **Brachiopoda** | | | | | | | |
| *Magellania venosa* | 335 d | pH∗ 7.35-8.15 Lab, ASW | SEM | pH∗ 7.35-7.6, ↑ pore size; ↑ thickness primary layer; ↓ thickness secondary layer, more organic rich shell | - | - | Ye *et al.,* (2018) |
| **Annelida** | | | | | | | |
| *Hydroides elegans* | 9-18 d** | pHNBS 7.4, 7.6, 7.8,7.9, 8.0; Lab | μCT, SEM | pHNBS 7.4-7.8, ↑ porosity, irregular layers, surface pitting & erosion, thinner; change in mineral layers & crystallography | Nanoindentation, crushing test | pHNBS 7.4-7.8, ↓ hardness, elasticity & 62% ↓ in crushing force | Chan *et al.,* (2012); Li *et al.,* (2014; 2016) |
| **Mollusca** | | | | | | | |
| **Gastropoda** | | | | | | | |
| *Austrocochlea constricta* | 4 mo | pHNBS 7.8, 8.1; Lab | - | - | Vickers hardness, Elastic modulus | pHNBS 7.8, ↑ shell hardness, ↑ stiffness | Leung *et al.,* (2017) |
| *Austrocochlea odontis* | 4 mo | pHNBS 7.86, 8.0; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung *et al.,* (2017) |

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**Table 1:** Continued

| Phylum/species | Time | pH or $\Omega$ levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| Austrocochlea porcata | 95 d | $pH_{NBS}$ 7.7, 7.9, 8.1; Lab | - | No effect of pH | Crushing test | $pH_{NBS}$ 7.7, ↓ shell strength by 28% | Coleman et al., (2014) |
| Bulla quoyii | 4 mo | $pH_{NBS}$ 7.9, 8.1; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung et al., (2017) |
| Charonia lampas | Resident | pHT 7.8, 8.1; Vent | μCT | pHT 7.8, Two-fold ↓ in density and thickness | - | - | Harvey et al., (2018) |
| Columbella rustica | 3 mo | $pH_{NBS}$ 7.6, 8.1, Lab | μCT | $pH_{NBS}$ 7.6, 0.8-8% ↓ in density depending on shell region | - | - | Chatzinikolaou et al., (2017) |
| Littorina littorea | 5 mo (small and large) | $pH_{NBS}$ 7.8, 8.0; Lab | - | - | Crushing test | $pH_{NBS}$ 7.8, Large snails, ↓ in crushing force, Small snails, no effect of pH | Landes and Zimmer, (2012) |
| Nassarius nitidus | 3 mo | $pH_{NBS}$ 7.6, 8.1, Lab | μCT | $pH_{NBS}$ 7.6, 38-51% ↓ in density depending on shell region | - | - | Chatzinikolaou et al., (2017) |
| Nerita atramentosa | 4 mo | $pH_{NBS}$ 7.8, 8.1; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung et al., (2017) |
| Nucella lapillus | 14 mo (juveniles) | pHT 7.8, 7.9, 8.0; Lab | μCT, SEM | pHT 7.8, 20-30% ↓ in density; surface erosion, lack of layering in shell, younger shells tinner, older shells thicker | - | - | Queirós et al., (2015); Rühl et al., (2017) |
| Nucella ostrina | 6 mo. | pHT ∼ 7.6, 8.1; Lab | - | - | Crushing test | 10% reduction in strength | Barclay et al., (2019) |
| Phasianella australis | 4 mo | $pH_{NBS}$ 7.9, 8.1; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung et al., (2017) |
| Phorcus sauciatus | Resident | pHNBS 7.52-8.2 Vent | - | Vent site – corrosion and cracks in shell | Crushing test | Weaker shells | Viotti et al., (2019) |
| Subninella undulata | 65 d | $pH_{NBS}$ 7.7, 7.9, 8.1; Lab | - | $pH_{NBS}$ 7.7, ↓ shell thickness | Crushing test | No effect of pH | Coleman et al., (2014) |
| Tegula funebralis | 6 mo. | pHT 7.4-8.1; Lab | - | - | Crushing test | 50% reduction in strength | Barclay et al., (2019) |
| Thalotia conica | 4 mo | $pH_{NBS}$ 7.9, 8.1; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung et al., (2017) |
| Turbo undulatus | 4 mo | $pH_{NBS}$ 7.86, 8.0; Lab | - | - | Vickers hardness, Elastic modulus | No effect of pH | Leung et al., (2017) |

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**Table 1:** Continued

| Phylum/species | Time | pH or Ω levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| **Bivalvia** | | | | | | | |
| *Adamussium colbecki* | 1 mo | pHT 7.6, 8.1; Lab | SEM | No effect on crystals | Nanoindentation | No effect of pH | Dell'Acqua et al., (2019) |
| *Arctica islandica* | 3 mo | $pH_{NBS}$ 7.7, 7.9, 8.0 | SEM | No effect on shape or size of crystals | - | - | Stemmer et al., (2013) |
| *Cerastoderma edule* | 2 mo | $pH_{NBS}$, 6.4 6.7, 7.0, 7.4, 7.8, Lab | SEM | $pH_{NBS}$ 6.4, 6.7 ↓ net calcification, no change in dissolution | Nanoindentation | No effect of pH | Milano et al., (2016) |
| *Crassostrea virginica* | 20 wk (juveniles) 2 wk (adults) | $pH_{NBS}$ 7.5, 8.2, Lab | FTIR SEM | $pH_{NBS}$ 7.5 40% ↓ in shell mass in juveniles | Microindentation, fracture toughness | $pH_{NBS}$ 7.5 ↓ calcite hardness and fracture toughness in juveniles | Beniash et al., (2010) |
| *Crassostrea virginica* | 11 wk | $pH_{NBS}$ 7.97/8.1 8.11/8.36 Lab | SEM | No difference in shell body mass | Microindentation, fracture toughness | $pH_{NBS}$ 7.9/8.1 ↓ hardness and fracture resistance at salinity | Dickinson et al., (2012) |
| *Magallana angulata* | 35 d early juveniles | $pH_{NBS}$ 7.8, 8.1, Lab | SEM, EBSD µCT, | $pH_{NBS}$ 7.8 ↑ porosity of microstructure, ↓ density | Nanoindentation | $pH_{NBS}$ 7.8 ↓ hardness and stiffness | Meng et al., (2018) |
| *Magallana gigas* | 6 wk | $pH_{NBS}$, 7.7, 8.1, Lab | - | - | Crushing test | $pH_{NBS}$ 7.7 ↓ crushing force | Wright et al., (2018) |
| *Mytilus californianus* | 8 d larva** | $pH_{NBS}$ 7.8, 8.0, 8.1, Lab | SEM | $pH_{NBS}$ 7.8 15% thinner | Crushing test | $pH_{NBS}$ 7.8 15-20% weaker pHNBS 8.0 13-15% weaker | Gaylord et al., (2011) |
| *Mytilus edulis* | 6 mo | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, 7.7, 8.1, 8.2, Lab | SEM, EBSD | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5. 7.7 disorganised crystals & altered layer structure | - | - | Fitzer et al., (2014b) |
| *Mytilus edulis* | 6 mo (juveniles) | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, 7.7, 8.1, 8.2, Lab | SEM, EBSD | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, thinner calcite layer and altered layer structure and crystallography | - | - | Fitzer et al., (2014a) |
| *Mytilus edulis* | 6 mo | pHT 7.65, 8.0; Lab | - | - | Crushing test | pHT 7.65 ↓ flex before failure; Strength not affected | Mackenzie et al. (2014) |
| *Mytilus edulis* | 6 mo | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, 7.7, 8.1, 8.2, Lab | - | - | Microindentation fracture toughness, nanoindentation | $pH_{NBS}$ 7.4, 7.5,7.7 ↑ calcite hardness and ↓ fracture toughness | Fitzer et al., (2014a) |
| *Mytilus edulis* | 2 mo | $pH_{NBS}$ 7.8, 8.1, Lab | SEM | Disordered crystals | Crushing test | $pH_{NBS}$ 7.8, ↓ crushing strength 22-24% | Li et al., (2015) |

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**Table 1:** Continued

| Phylum/species | Time | pH or Ω levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| *Mytilus edulis* | 6 mo | $pH_{NBS}$ 7.4, 8.1, Lab | SEM-EBSD, XPEEM | $pH_{NBS}$ 7.2, altered crystallography structure and more ACC. | - | - | Fitzer et al., (2016) |
| *Mytilus edulis* | 9 months | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, 7.7, 8.1, 8.2, 8.2, 8.1, Lab | Shell shape analysis, shell thickness index (STI) | $pH_{NBS}$ 7.2, 7.3, 7.4, 7.5, 7.7, ↓ shell thickness and rounder flatter shells. | - | - | Fitzer et al., (2015b) |
| *Mytilus edulis* | 7 weeks | $pH_{NBS}$ 7.2, 7.4, 7.7, 8.0, Lab | SEM | $pH_{NBS}$ 7.2, corrosion of internal aragonite layers | - | - | Melzner et al., (2011) |
| *Mytilus edulis* | 6 mo | pHT 7.65, 8.0; Lab | - | - | Crushing test | pHT 7.65 ↓ flex before failure; Strength not affected | Mackenzie et al. (2014) |
| *Mytilus galloprovincialis* | 68 d | pHT 7.25, 8.07; Vent transplant | SEM, EBSD | pHT 7.25, thinner shell, disturbed less ordered structure | - | - | Hahn et al., (2012) |
| *Mytilus galloprovincialis* | 21 d - 5 mo | pHT 6.8, 7.2, 7.8, Vent transplant to lab | SEM | Calcification continued in vents; Isolated shells, dissolution at low pH | - | - | Rodolfo-Metalpa et al., (2011) |
| *Pinctada fucata* | 28 d | $pH_{NBS}$ 7.6, 7.8, 8.1 | SEM | Shell dissolution | Crushing test | $pH_{NBS}$ 7.6, 25.9% and 26.8% weaker shells | Welladsen et al., (2011) |
| *Saccostrea glomerata* | 2 yr | $pH_{NBS}$ 7.7, 8.1 Coast*** | SEM-EBSD | pH 7.7, disordered crystallographic structure | - | - | Fitzer et al., (2018, Fitzer et al., 2019b) |
| *Saccostrea glomerata* | N/A | $pH_{NBS}$ 6.6, 6.8, 6.9, 7.8, 7.9 Coast** | - | - | Crushing test | pH $pH_{NBS}$ 6.6, 6.8, 6.9, Weaker shells | Amaral et al., (2012); Fitzer et al., (2019b) |
| **Cephalopoda** | | | | | | | |
| *Argonauta nodosa* | N/A | pHT 7.35,7.55,7.8,8.0, Lab | SEM, EBSD | Shell only pHT 7.35-7.8, dissolution and etching, altered crystallography and structure | - | - | Wolfe et al., (2012, 2013a) |
| **Echinodermata** | | | | | | | |
| *Diadema africanum* | 100 d, juveniles | pHT 7.6, 8.0, Lab | SEM | pHT 7.6, Test plates thinner, spines, dissolution/etching | Crushing test (whole urchin dried) | pHT 7.6, ↓ crushing force | Rodriguez et al., (2017) |
| *Echinometra* sp. | 343 d | $pH_{NBS}$ 7.7, 8.1, Lab | SEM | Test plates and sines, no dissolution | - | - | Hazan et al., (2014) |

**Table 1:** Continued

| Phylum/species | Time | pH or $\Omega$ levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| *Echinometra mathei* | 13 mo | pHT 7.65, 8.1, Lab | - | - | Ambital and apical plate fracture force and elasticity | No effect of pH | Moulin et al., (2015) |
| *Heliocidaris erythrogramma* | 2 wk, early juveniles | $pH_{NBS}$ 7.4, 7.6, 7.8, 8.1, Lab | SEM | $pH_{NBS}$ 7.4, Spines, ↑ pore size and dissolution | - | - | Wolfe et al., (2013b) |
| *Heliocidaris erythrogramma* | 9 mo | $pH_{NBS}$ 7.6, 8.1, Lab | SEM | $pH_{NBS}$ 7.6, ↑ pore size apical but not ambital plates | Nanoindentation, elasticity | $pH_{NBS}$ 7.6, ↓ hardness and elasticity | Johnson and Byrne (unpublished data) |
| *Lytechinus variegatus* | 3 mo, early juveniles | $pH_{NBS}$ 7.8, 8.0, 8.1, Lab | SEM | $pH_{NBS}$ 7.4, Spines, dissolution and malformation | - | - | Albright et al., (2012) |
| *Lytechinus variegatus* | 59 d | pH* 7.47, 7.7, 7.9, Lab | SEM | pH* 7.47, Spines, reduced barbs | Snap test | pH* 7.47, ↓ snap force | Emerson et al., (2017) |
| *Paracentrotus lividus* | 1 mo (juveniles) | pHT 7.7, 7.8, 8.0, 8.1, Lab | SEM | pHT 7.7, larger pore size in tooth, no change test plate thickness; no spines dissolution/etching | Crushing test (whole urchin dried) Fracture force plate | pHT 7.7, ↓ crushing force; No effect of pH on test plate | Asnaghi et al., (2013), 2014, (2019) |
| *Paracentrotus lividus* | 12 mo | $pH_{NBS}$ 7.8, 7.9, 8.0, 8.1, Lab | - | - | Crushing tests, ambital and apical plate fracture force, nanoindentation, elasticity | No effect of pH | Collard et al., (2016) |
| *Paracentrotus lividus* | Resident | $pH_{NBS}$ 7.8, 8.2, Vent | - | - | Crushing force, ambital and apical plate fracture force, nanoindentation, elasticity | No effect of pH | Collard et al., (2016) |
| *Paracentrotus lividus* | 100 d, juveniles | pHT 7.6, 8.0, Lab | SEM | pHT 7.6, Test plates thinner, Spines, no dissolution/etching | Crushing test (whole urchin dried) | pHT 7.6, ↓ crushing force | Rodriguez et al., (2017) |
| *Tripneustes gratilla* | 146 d juvenile to adult | $pH_{NBS}$ 7.6, 7.8, 8.1 Lab | SEM | $pH_{NBS}$ 7.6, thinner test, spines no dissolution/etching | Crushing test (live whole urchin) | $pH_{NBS}$ 7.6, ↓ crushing force, rupture at sutures not skeleton | Byrne et al., (2014) |

**Table 1:** Continued

| Phylum/species | Time | pH or Ω levels/location | Morph methods | Morph results | Biomechanics methods | Biomechanics results | References |
|---|---|---|---|---|---|---|---|
| *Tripneustes ventricosus* | 5 wk | $pH_T$ 7.4, 7.7, 8.1, Lab | SEM | $pH_T$ 7.4, 7.7, spine etching Test plates, no dissolution/etching | Fracture force spines, two-point bending, elasticity | $pH_T$ 7.4, 7.7, spine more brittle, ↓ fracture force, 35% and 16%, no effect of pH on elasticity | Dery *et al.*, (2017) |
| *Strongylocentrotus droebachiensis* | 45 d | $pH_{NBS}$ 7.2, 7.7, 8.0, Lab | SEM | $pH_{NBS}$ ~ 7.2, test plates pitted; spine dissolution | Fracture force spines | $pH_{NBS}$ ~ 7.2, spines, ↓ fracture force; No effect of pH on test plates | Holtmann *et al.*, (2013) |
| **Crustaceans** | | | | | | | |
| *Amphibalanus amphitrite* | 86 d-20 wk | $pH_{NBS}$ 7.4, 8.2; Lab | - | - | Penetrometry, breaking test | $pH_{NBS}$ 7.4, ↓ force to penetrate shells | McDonald *et al.*, (2009) |
| *Amphibalanus improvisus* | 86d-20 wk | $pH_T$ 7.3-7.9 Lab | SEM | Low pH increasing corrosion | Crushing test | No effect of pH | Panch *et al.*, (2013, 2014) |

Morphology methods: EBSD, electron back scatter defractometry; FTIR, Fourier-transform infrared spectroscopy; μCT, micro-computed tomography; SEM, scanning electron microscopy; XPEEM, photo emission electron microscopy. Biomechanics methods: crushing tests (force of rupture); nanoindentation (hardness); Young's modulus of elasticity—a measure of stiffness). ACC, amorphous calcium carbonate; ASW, artificial seawater; Coast, ***coastal runoff pH gradient; Lab, laboratory; Morph, morphology; Ω, saturation state calcium carbonate minerals; Time, exposure duration; Vent, CO₂ seep pH gradient; –, not investigated; **, larva to juvenile studies, all others are of adults; ***, includes pH flux

$HCO_3^-$ through hydrolysis, a process catalysed by carbonic anhydrase (Wilbur, 1972; Roleda *et al.*, 2012), a highly conserved enzyme that functions in $CO_2$ regulation. Therefore, as DIC sources required for calcification vary between calcifying species, it might also be expected that shell growth responses to OA may be species specific. This is an important consideration in understanding the vulnerability of species and their calcification response in ocean and coastal acidification conditions.

The production and maintenance of $CaCO_3$ structures are vital to the success and survival of a vast diversity of marine species as they play essential roles in body support and protection. Corals and oysters provide the structural foundation of reef ecosystems that many species depend on for habitat. Marine calcifiers also provide vital ecosystem services to humanity as species for fisheries and aquaculture and in shoreline protection and are vital to the livelihoods of millions globally (Cooley *et al.*, 2009; 2011; Gattuso *et al.*, 2015). There has been a wealth of studies on the impacts of OA on marine invertebrates with respect to development, physiology and ecology, as detailed in a number of reviews and meta-analyses (Andersson and Gledhill, 2013; Kroeker *et al.*, 2013; Gazeau *et al.*, 2013; Byrne *et al.*, 2013; Wittmann and Pörtner, 2013; Dubois, 2014; Prezeslawski *et al.*, 2015; Foo *et al.*, 2018). The mineralogy of marine skeletons and their vulnerability to OA has also been reviewed (Ries, 2010; Smith *et al.*, 2013, 2016).

Despite concerns for the prospects for marine calcifiers in a changing ocean, the impacts of environmental acidification on the structure of the biomineral itself remains largely underexplored. We need to understand how acidification alters biomineral production and its microstructure and mechanical integrity to address uncertainties on the biological consequences of climate change. This is addressed here in a review of recent research, where advanced microscopy is used to visualize the internal microstructure and crystallography of biomineral in calcifiers that have been exposed to experimental (laboratory) or natural (vents, coastal run off) acidification (Table 1). Changes in microstructure have been revealed by the application of scanning electron microscopy (SEM) to view surface changes and microcomputed tomography (μCT) to generate three-dimensional reconstructions of entire shells and skeletons (Fitzer *et al.*, 2019a).

Several studies have investigated the biomechanical changes to marine skeletons exposed to environmental acidification (Table 1). These studies have largely used crushing tests and nanoindentation. Nanoindentation provides a high-resolution assessment of material hardness and is especially useful on smaller, thinner shell samples using nanoscale indents (depth of 10–100's nm). In addition, nanoindentation can be used to determine the elastic modulus (Young's modulus—*E*) of the surface of the shell (Fitzer *et al.*, 2015a; Milano *et al.*, 2016; Meng *et al.*, 2018). Details of the advanced microscopy and materials science techniques that have been used to investigate

Ocean acidification (OA) CO$_2$-acidification:

Atmospheric CO$_2$

CO$_2$ vents

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+ \leftrightarrow CO_3^{2-} + 2H^+$$

**Other forms of environmental acidification:**

Coastal freshwater runoff

Sulphate soil coastal acidification:

$$FeS_{2(s)} + 7/2\ O_{2(aq)} + H_2O \rightarrow Fe^{2+}_{\ (aq)} + 2\ SO_4^{2-}_{\ (aq)} + 2\ H^+_{\ (aq)}\ (1)$$

$$Fe^{2+}\ (aq) + 1/4\ O_{2(aq)} + 3/2\ H_2O \rightarrow FeO\cdot OH_{(s)} + 2\ H^+_{(aq)}\ (2)$$
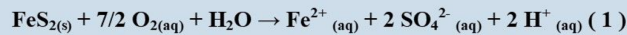
**Figure 1:** Equations for the mechanisms of acidification from CO$_2$-OA and other forms of environmental acidification for example from acid sulphate soil leachates. Modified from Fitzer *et al.* (2018).

marine skeletons in an OA context are reviewed in Fitzer *et al.* (2019a).

The list of studies (Table 1) provides an overview of the impacts of environmental acidification on skeletal structure and biomechanics and the pH levels and scales (e.g. pHT vs pHNBS) used are important to note. In the following text, we review research on the major marine calcifying taxa (tube worms, corals, molluscs, echinoderms) with a focus on those most studied, bivalves and sea urchins. Only a few studies have investigated the impact of habitat acidification and warming, and the interaction of these factors, on the structure and integrity of marine biomineral, and we highlight a few of these.

## Corals

Corals produce an aragonite skeleton that is covered by the tissues of the coral polyps (Von Euw *et al.*, 2017). Several studies have investigated the impact of OA on the microstructure of coral skeletons using SEM or μCT and one study has incorporated mechanical tests (Table 1). The number of studies appears surprisingly limited considering the ecological importance of reef-building corals. For this important taxon more research is needed on the impact of projected near-future acidification on skeletal structure and mechanical integrity and in context with habitat warming.

The temperate solitary cup coral *Balanophyllia europaea* living at a low pH vent site had a more porous and thinner skeleton than those living at nearby control sites (Fantazzini *et al.*, 2015). Skeletal pore size increased and bulk density decreased, with decreasing pH (8.1, 7.9, 7.7). However,

growth, with respect to size, was maintained by these corals along the vent pH gradient. Nanoindentation revealed that the skeleton of *B. europaea* living at low pH (pH 7.7) had a lower hardness and stiffness compared with corals residing at pH 8.1. While this species is able to maintain growth at reduced pH, the deposition of less biomineral is likely to increase susceptibility to physical damage (Fantazzini *et al.*, 2015).

For colonies of the tropical coral, *Stylophora pistilla*, exposed to pH 7.2 for 12 months, SEM and μCT revealed that the biomineral produced had greater porosity and lower bulk density compared with conspecifics maintained at ambient pH (Table 1). Note the caveat that the low pH treatments used are well beyond OA scenarios (IPCC, 2014) (as the case for many of the studies listed in Table 1). Tambutté *et al.*, (2015) suggest that these extreme treatments are useful to help identify trends. While the growth of *Stylophora pistilla*, as linear extension did not change in pH 7.2, the density of the biomineral did, potentially as a trade-off strategy under competition to maintain access to light and space with the compromise of a weaker skeleton (Tambutté *et al.*, 2015). This change in the skeleton also reflects the decline in *S. pistilla* colonies at low pH sites in nature (Tambutté *et al.*, 2015). A recent study of these species maintained at low pH for 14 months showed a change in skeletal chrystallography (Coronado *et al.*, 2019).

Colonies of *Porites astreoides* living in a naturally low pH environment from coastal run off also had a lower density skeleton (Crook *et al.*, 2013) and juvenile *Favia fragum* exposed to CO$_2$ driven under saturation conditions (pH not provided) for 8 days had a thinner skeleton compared with controls (Cohen *et al.*, 2009).

In these coral studies skeletal dissolution was not noted. It appears that the thin veneer of polyp/corallite tissue protects the underlying skeleton from dissolution. Importantly, the most universal measure of coral health and biomineralisation rate (linear extension) is unlikely to be a reliable metric of coral health and growth in the face of OA, with potential production of more fragile phenotypes (Tambutté *et al*., 2015).

## Tube worms

Serpulid polychaetes are a major group of marine worms that produce a calcareous tube that varies in composition from being entirely aragonitic, to entirely high-Mg calcite to mixtures of these two (Smith *et al*., 2013). The serpulid, *Hydroides elegans*, is one of the most important species in tropical biofouling communities and the microstructure and mechanics of its calcareous tube has been investigated in the OA context (Chan *et al*., 2012; Li *et al*., 2014, 2016).

In studies where *H. elegans* was reared in experimental OA conditions across larval development through to tube formation, SEM and µCT revealed that at low pH (7.4–7.8), the tube had increased porosity, decreased thickness and had surface pitting and erosion. There was also a change in the mineral layers and crystallography. These changes reduced the hardness of the tube. The force needed to crush the tube decreased by 62%. The composition of aragonite in the tube is also influenced by OA with *H. elegans* depositing more calcite in the tube at low pH. This species seems to be able to adjust the mineralogy of its tube as a potential adaptation to the changes in water chemistry driven by OA, but this compromises the strength and elasticity of the tube (Li *et al*., 2014, 2016). In comparison, the serpulid, *Spirobranchus triqueter* was similarly fragile when reared under moderate (pH 7.7) and severe (pH 7.4) reductions in pH. Tube fracture toughness was not linearly related to the reduced pH, but was related to changes in porosity as found for *H. elegans*. Larger pores in *S. triqueter* resulted in thinner calcareous layers in the tubes and therefore reduced fracture toughness (Díaz-Castañeda *et al*., 2019).

In experiments where ocean warming was also considered, increased temperature (+6°C) had the opposite effect, increasing skeletal hardness and elasticity (Chan *et al*. 2012). Although the temperature increase used was more extreme than predicted under future climate scenarios, the outcome for this important polychaete indicated that in a future ocean biomineral production may be more dictated by climate warming than acidification.

## Gastropods

Depending on the species and life stage, gastropods produce aragonitic or calcitic shells or a combination of these (Marin *et al*., 2008; Rühl *et al*., 2017). The impact of OA on shell microstructure of several species, maintained in experimental OA for various lengths of time has been investigated (Table 1). For the ecologically important species, *Nucella lapillus* and *Nassarius nitidus*, the reduction in shell density determined using µCT was marked (20–50%), while that for *Columbella rustica* was much less (0.8–8%) (Queirós *et al*., 2015; Chatzinikolaou *et al*., 2017). This shows differences in vulnerability of shells to OA between closely related gastropod species. For the two species greatly affected, the extent of biomineral reduction varied across locations in the shell which would make weaker regions a target for durophagous predators such as crabs. In a study of the mechanical integrity of the shells of *Littorina littorea* maintained in pH 7.8 for 5 months, the shells of larger snails were more vulnerable to the crushing action of a crab predator than those of smaller snails (Landes and Zimmer, 2012). In a study of 7 gastropod species, the mechanical properties of the shells of six of these were not affected by acidification (pH 7.8-7.9) (Leung *et al*., 2017). For *Austrochochlea* constricta, shell hardness and stiffness increased. This species also produced a shell with higher calcite to aragonite and magnesium to calcium ratios under acidification conditions (Leung *et al*., 2017). In this study elevated temperature (+ 2.5-4.0 °C) was the more important factor on shell mechanics. For juvenile *N. lapillus*, grown under OA conditions, a 2°C warming appeared to counter the negative effect of OA, but as this differed between small and large snails, the trend was difficult to interpret (Rühl *et al*., 2017).

Gastropods resident at vents also have thinner, less dense shells as seen in µCT reconstructions (Garilli *et al*. 2015; Harvey *et al*. 2018). The shell surface of *Charonia lampas* exhibited the corrosive effects of low pH water even to the extent that the soft tissue was exposed (Harvey *et al*. 2018). Overall, gastropods living at $CO_2$ vent sites tend to have a smaller adult body size, similar to the Lilliput effect seen in the fossil record for mollusc species that survived past low pH driven extinction events (Garilli *et al*., 2015). It is suggested that smaller body size is a physiological adaptation to environmental acidification in order to maintain calcification as well as to have the energy to repair shell dissolution (Garilli *et al*., 2015). For herbivorous gastropods living at vent sites, the enhanced algal food levels due to high $CO_2$ can buffer, to a variable extent, the negative effect of OA on calcification (Doubleday *et al*., 2019).

## Bivalves

Bivalve skeletons are comprised of two shells joined at a hinge and vary in their composition of calcite and aragonite (Marin *et al*., 2008; Wehrmeister *et al*., 2011). There has been extensive research on the impacts of OA on these animals as they form the basis of significant aquaculture productivity. They are also ecologically important as being major prey for many species and their filter feeding activity strongly influences water clarity and quality.

Many bivalve studies report reduced shell growth, reduced shell thickness and mechanically weaker shells under OA
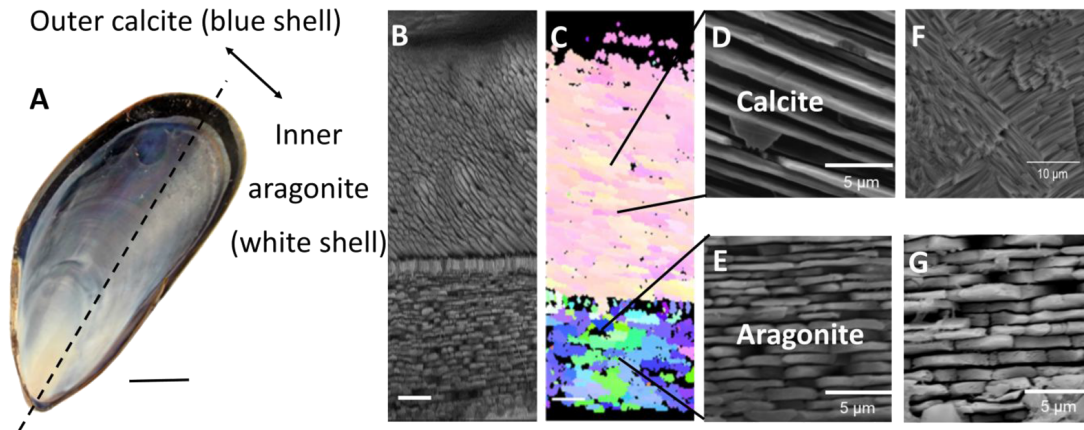
**Figure 2:** A schematic representation of the use of SEM-EBSD as an analytical tool to assess the effects of acidification on shell microstructure. (**A**) *M. edulis* has been a focal study species to understand the impacts of OA on marine biomineral. (**B**) Section imaged using SEM is a cross section through a shell grown under OA (pHNBS 7.5). (**C**) The same section imaged using EBSD and analysed for crystallographic orientation displayed as a crystallographic orientation map. (**D**) Calcite and (**E**) aragonite crystals at higher magnification from the same cross section of the *M. edulis* shell. (**F**) Disordered microstructure of the calcite layer and (**G**) dissolution of the aragonite tablets (edges more rounded compared to panel E and tablets are less tightly packed) of the shells grown in OA. Images adapted from Fitzer *et al.* (2014a).

conditions (Gazeau *et al.*, 2007; Ries *et al.* 2009; Beniash *et al.*, 2010; Dickinson *et al.*, 2012; Fitzer *et al.*, 2015a, b, 2018). Reduced shell growth and thinning of shells under experimental $CO_2$ acidification have been observed using SEM at both the larval (Gazeau *et al.*, 2010; Parker *et al.*, 2012; Fitzer *et al.*, 2014a) and adult stages (Gazeau *et al.*, 2013; Fitzer *et al.*, 2014b). However, no effect on shape or size of crystals was observed in the clam *Arctica islandica* or the scallop *Adamussium colbecki* under elevated $CO_2$ (Stemmer *et al.*, 2013, Dell' Acqua *et al.*, 2019). The abnormalities and changes to shell growth can affect bivalves at the microstructure level of the forming phases of aragonite and calcite (Wehrmeister *et al.*, 2011), and can also impact the amorphous calcium carbonate (ACC) that is an important precursor of crystalline carbonate minerals (Addadi *et al.* 2003).

Microstructural observations of bivalves exposed to acidification conditions have been determined using a range of microscopy techniques including SEM coupled with electron backscatter diffraction (EBSD) (Melzner *et al.*, 2011; Fitzer *et al.*, 2014a, b), Fourier-transform infrared (FTIR) spectroscopy (Beniash *et al.*, 2010) and X-ray photo emission electron microscopy (XPEEM) (Fitzer *et al.*, 2016) to characterize crystallography and the form of calcium carbonate. µCT has been used to examine skeletal density (Meng *et al.*, 2018).

In the mussel *Mytilus edulis*, which contains an outer calcite and an inner aragonite layer (Fig. 2), experimental OA resulted in a thinning of the shell (Fitzer *et al.*, 2015b), with disordered crystallography as revealed by SEM-EBSD as well as a corroded aragonite layer (Melzner *et al.*, 2011; Fitzer *et al.*, 2014a), in favour of a disordered calcite layer (Fitzer *et al.*, 2014a, b). Examination of the shells of *M.*

*edulis* exposed to $CO_2$ acidification with XPEEM revealed that more ACC was produced (Fitzer *et al.*, 2016). This was suggested to aid repair to the disordered calcite layer in the shell (Fitzer *et al.*, 2016).

While *M. edulis* has been a focal case study species (Table 1), many bivalves are similarly affected by OA with commonly observed thinning of the shells and disorder in the crystallography of the calcite layers (Figs 2 and 3). An SEM-EBSD investigation of *Mytilus galloprovincialis* transplanted to a $CO_2$ vent (~pH = 7.2–7.8) revealed that they grew a thinner shell with disordered crystallography (Rodolfo-Metalpa *et al.*, 2011; Hahn *et al.*, 2012). This was also the case the shells of the oysters *Magallana angulata* (pH 7.2, 7.5) and *M. hongkongensis* (pH 7.3) grown in laboratory ($CO_2$ dosing) (Meng *et al.*, 2018, 2019), and *Saccostrea glomerata* farmed in coastal acidified environments (pH 7.6–7.8) (Fitzer *et al.*, 2018, 2019b). The disordered crystallography in *S. glomerata* wild type oysters grown in coastal acidified environments was as a result of altered biomineralisation pathways shown by changing shell carbon isotopes (Fitzer et al., 2019b). Oysters such as the pearl oyster *Pinctada fucata* that have an internal layer of aragonite, dissolution occurred internally under OA conditions (Welladson *et al.*, 2011).

The impacts of OA on the biomechanics of bivalve shells have been studied using a variety of techniques, in particular microindentation of the shell surface to calculate hardness. The resultant cracks propagating from the indents can also be applied to calculate fracture toughness (Mackenzie *et al.*, 2014; Fitzer *et al.*, 2015a). The changes to the shell microstructure in *M. edulis* grown in OA conditions resulted in a mechanically weaker shell as indicated by
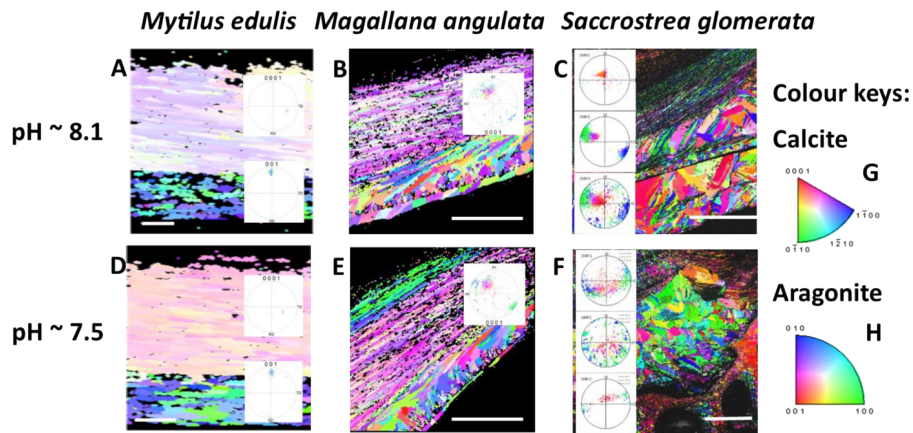
**Figure 3:** Crystallographic orientation maps with accompanying pole figures for the calcite and aragonite shell of *M. edulis* (**A**, **D**), and the calcite shells of *Magallana angulata* (**B**, **E**) and *Saccrostrea glomerata* (**C**, **F**) grown at pH 8.1NBS and pHNBS 7.5 under $CO_2$ acidification and sulphate soil acidification. The figures highlight the similarly altered crystallographic orientation of the mussel and oyster shells with increased disorder at pH 7.5. This is highlighted by the increased range of crystallographic orientation shown by the increased variation of colours. The colours here represent a change in the angle of crystallographic orientation as per the calcite (0001) (**G**) and aragonite (001) (**H**) colour keys. Scale bars represent 5 μm for *M. edulis*, 45 μm for *M. angulata* and 200 μm for *S. glomerata*. Adapted from Fitzer *et al*. (2014a, 2018) and Meng *et al*. (2018).

nanoindentation. The calcite produced was harder and more brittle. Microindentation indicated reduced fracture toughness (Fitzer *et al*., 2015a) and reduced bend/flex before failure (Mackenzie *et al*., 2014).

While OA (pH 7.5) as a single stressor reduced shell hardness and stiffness in *M. edulis*, when this level of acidification is used in combination with moderate warming (+2°C), these negative effects were reduced (Fitzer *et al*., 2015b). Temperature alone did not alter shell size of thickness; however, a combination of +2°C warming with acidification (pH 7.5) reduced the aragonite layer thickness in *M. edulis* (Fitzer *et al*., 2015a). A +4°C warming reduced the maximum crushing load of mussel shells in both ambient and reduced pH (pH 7.6) (Mackenzie *et al*., 2014).

The mechanical properties of bivalve shells that have different forms of $CaCO_3$ differ in response to OA. In the shell of *M. edulis*, which contains both calcite and aragonite, the calcite becomes harder or more brittle when exposed to OA. For the calcitic shell of the oyster *Crassostrea virginica* grown under experimental OA, microindentation revealed that shell hardness is reduced along with a resultant reduced fracture toughness (Beniash *et al*., 2010; Dickinson *et al*., 2012). Similarly, the shells of juvenile *Magallana angulata* grown under experimental OA (pH 7.2, 7.5) had reduced hardness, as a result of increased porosity as visualized by μCT (Meng *et al*., 2018). In the more resistant *M. hongkongensis*, juvenile shells were similarly affected, but at a much lower pH (pH 7.3) (Meng *et al*., 2019). For this species, shell hardness and crystallography remained unchanged at pH 7.6 (Meng *et al*., 2019). Oysters such as *Pinctada fucata* that have an internal layer of aragonite had a weaker shell when grown under experimental OA conditions (Welladsen *et al* 2011) than oysters that have a calcitic internal layer. The shells

of *S. glomerata* growing in estuarine habitats with sulphate soil acidification (pH 6.6, 6.8, 6.9) were also significantly weaker in crushing tests (Amaral *et al*., 2012). Although these pH levels are much lower than predicted OA, this level of acidification occurs in acid sulphate regions during increased rainfall (Amaral *et al*., 2012). Reduced growth of *S. glomerata* during such extreme events may be offset by positive growth in dry periods (Amaral *et al*., 2012).

Overall, OA affects the microstructure of bivalve shells through reduced shell growth with thinner, more porous shells that have reduced fracture toughness. Trends appear similar in mussels and oysters in terms of microstructural responses to OA regardless of whether laboratory $CO_2$ acidification or environmental acidification from $CO_2$ or coastal run off are the source of acidification (Table 1).

## Echinoderms

Echinoderms produce a unique endoskeleton laid down as a three-dimensional mesh-like calcite lattice (Fig. 4), with cells and connective tissue living in the void space (Cavey and Markel, 1994). The impacts of OA on the production and maintenance of the skeleton are extensively investigated for echinoids (sea urchins) across their planktonic and benthic life phases (Byrne *et al*. 2013; Dubois, 2014). These animals are ecologically and economically important grazers across world oceans (Lawrence, 2013). The sea urchin skeleton is composed of Mg-calcite, ~3–16 wt% $MgCO_3$, where $Mg^{2+}$ is substituted for $Ca^{2+}$ during calcification (Chave, 1954; Smith *et al*., 2016). As a result of this chemical composition, the echinoderm skeleton is one of the most soluble forms of $CaCO_3$, and so is vulnerable to dissolution in OA conditions (Andersson *et al*., 2008; McClintock *et al*., 2011; Dubois, 2014).

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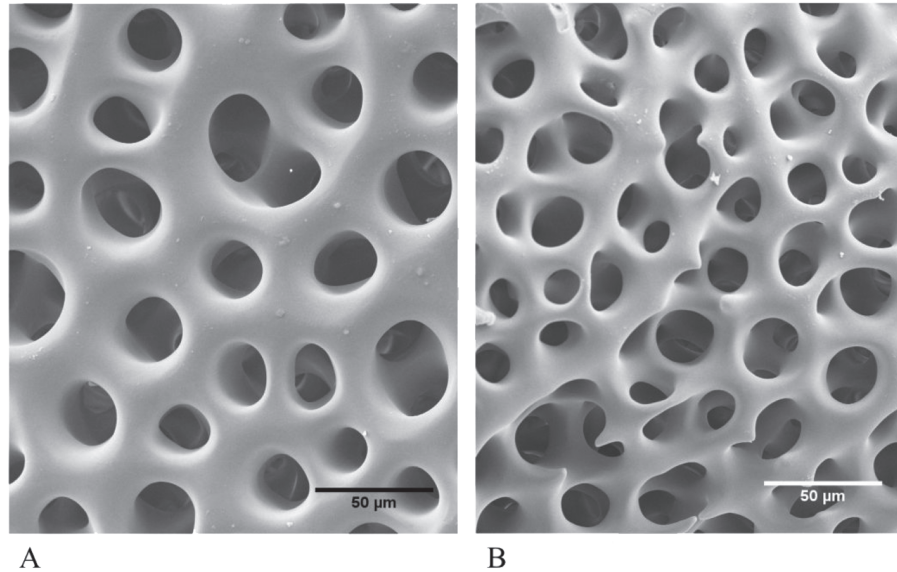
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**Figure 4:** SEM of the surface of the apical test plates of the adult sea urchin, *Heliocidaris erythrogramma* maintained in control (pHNBS 8.1) (**A**) and decreased pH (pHNBS 7.6) (**B**) for 9 months. The skeleton formed in the OA treatment has thinner calcite. Images courtesy of Ms R. Johnson.

Despite the echinoderm skeleton being a form that is vulnerable to dissolution, the epithelium largely protects the living skeleton from direct exposure to low pH conditions (Dubois, 2014). The exceptions are the tips of the spines, potentially due to their thin epithelial cover. Overall, the established sea urchin skeleton does not appear to be vulnerable to direct dissolution under OA conditions (Table 1). Surface pitting of the test has only been reported at very low pH (Holtmann *et al.*, 2013). Interesting, the cidaroids, a group of sea urchins with many species living in deep water below the isocline, produce spines that do not have an epithelial cover, but have a calcitic cortical layer that makes them resistant to dissolution in OA conditions, as shown for 15 species, including in tests where species were maintained in pH 7.2–7.4 for 3–5 weeks (Dery *et al.*, 2014, 2017, 2018).

With respect to production of biomaterial, calcification in sea urchins is constrained by OA. This is shown by the comparatively smaller skeletons of larvae and juveniles (Figs 5 and 6) reared in OA conditions through development and the smaller tests of adult sea urchins (Fig. 7) grown in long-term OA experiments (Byrne *et al.*, 2013, 2014; Dubois, 2014; Dworjanyn and Byrne, 2018). It appears that sea urchins have a reduced ability to deposit biomineral under OA conditions (Figs 4 and 5). This may be due to energetic constraints associated with the higher metabolic costs of life at low pH and the priority to maintain acid-base balance (Stumpp *et al.*, 2012; Carey *et al.*, 2016). The impacts of OA on the skeleton may be mitigated by near future warming. In *Tripneustes gratilla*, a +3°C warming mitigated the negative effects of low pH (pH 7.6, 7.8) on test growth (Fig. 7). Further warming made matters worse (Byrne *et al.*, 2014; Dworjanyn and Byrne, 2018).

In addition to the smaller amount of skeleton produced under OA conditions, skeletal microstructure may also be altered. Several SEM and/or μCT investigations have compared the pore size, the calcitic trabecular connections and the void space of the skeleton of sea urchins exposed to OA with these traits in the skeleton formed under ambient pH (Table 1). Larger pore size is reported for the spines of juvenile *Heliocidaris erythrogramma* (Fig. 6), but only at very low pH (pH 7.4) (Wolfe *et al.*, 2013b) and for the teeth of *Paracentrotus lividus* where the tips were regenerated under OA (pH 7.7) (Asnaghi *et al.*, 2013).

For *H. erythrogramma* maintained on OA conditions for 9 months, the mid body ambital test plates produced prior to exposure to OA exhibited no change in skeletal structure. In contrast, the younger apical plates from the active growth region of the test, that had likely grown over the 9-month exposure at low pH had thinner trabeculae and a greater void space compared to those from control urchins as visualized with SEM (Fig. 4A and B) and μCT (Johnson, 2019).

The μCT study of *Strongylocentrotus fragilis* that had likely grown from the juvenile stage in chronic low pH (pH 7.69–7.57) and oxygen saturation on the Californian continental shelf, showed that that skeletal porosity and pore size is higher than that for conspecifics living at higher pH. For this species, skeletal porosity increased with decreasing pH and oxygen saturation, but it is not known how hypoxia and low pH interact with respect to the differences in the skeleton (Sato *et al.*, 2018).

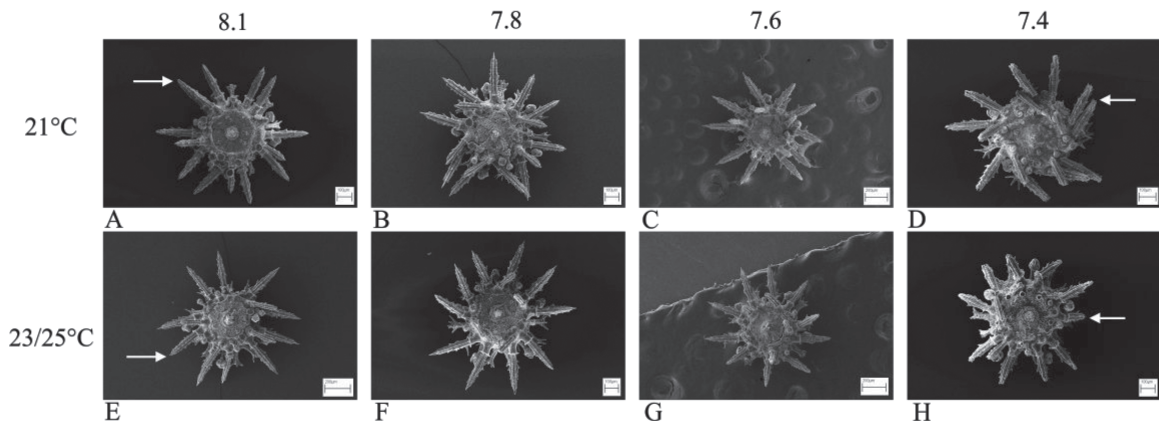The impact of OA on the mechanical properties of the test plates has been documented by nanoindentation and in

**Figure 5:** SEM of juvenile *Heliocidaris erythrogramma* reared in four pH and three temperature levels in all combinations for 14 days. Urchins had shorter spines and smaller tests at pHNBS 7.4 (see Wolfe et al. 2013b). At control pH (**A**, **E**) the arrows point to the terminal spike which is the calcite growing region of the spines compared with the flat-ended spines of juveniles reared in pHNBS 7.4 indicating retarded or no calcification. Images courtesy of Dr K Wolfe.
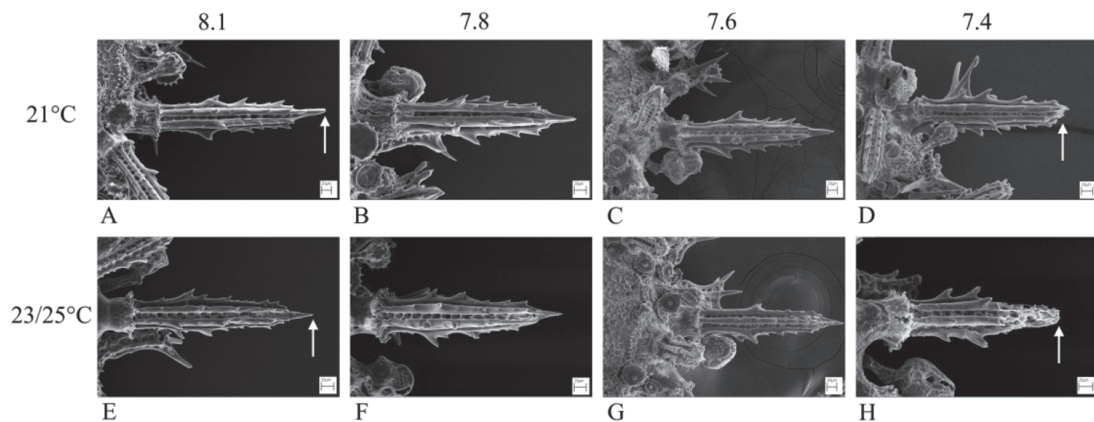


**Figure 6:** SEM of the spines of juvenile *Heliocidaris erythrogramma* reared in four pH and three temperature levels in all combinations for 14 days. The arrows point to the pointed end of the spines in control pHNBS 8.1 (**A**, **E**) at the calcite growing region. At pHNBS 7.4 and at warmer temperature the spines were shorter, more porous, had blunt ends (**D**) and were eroded (**H**) (see Wolfe et al. 2013b). Images courtesy of Dr K Wolfe.

crushing and bending tests (Table 1). In *P. lividus* maintained in OA (pH 7.8, 7.9) for 12 months, there was no effect of pH on the hardness or breaking force of the test plates (Collard *et al.*, 2016), as also the case for *Echinometra mathaei* (Moulin *et al.*, 2014). Exposure to low pH reduced the hardness of the apical plates of *H. erythrogramma* that had likely been produced in OA conditions, but the previously established ambital plates were not affected (Johnson and Byrne, unpublished data). There was also no change in the mechanical properties of the test of *P. lividus* resident at a vent site (~pH 7.7) compared with conspecifics from ambient conditions.

In studies where the whole test was crushed as might occur in an attack by a predatory fish (Guidetti and Mori, 2005), the force required to crush the dried tests of juvenile *P. lividus* and *Diadema africanum* maintained in OA was lower

compared to juveniles from control treatments (Asnaghi *et al.*, 2013; Rodríguez *et al.* 2017). However, as dried tests were used, the ecological relevance of these results is not clear. The force required to crush the test was lower in live *Tripneustes gratilla* reared in OA (pH 7.6) from juvenile to adult in a test designed to mimic the ram force of a predatory fish, but the break occurred at the suture lines, not in the skeleton itself (Byrne *et al.*, 2014). These tests need to be repeated across species with live urchins being most relevant test subjects, to incorporate the elasticity of the ligaments that bind the test plates (Ellers *et al.*, 1998), and with application of forces modelled with respect to the jaw crushing or ramming action of predators, although these forces are poorly characterized.

With respect to the spines of sea urchins maintained at pH 7.4 and 7.7, the thinner, more brittle spines formed in these conditions by *Tripneustes ventricosus* and *Eucidaris*
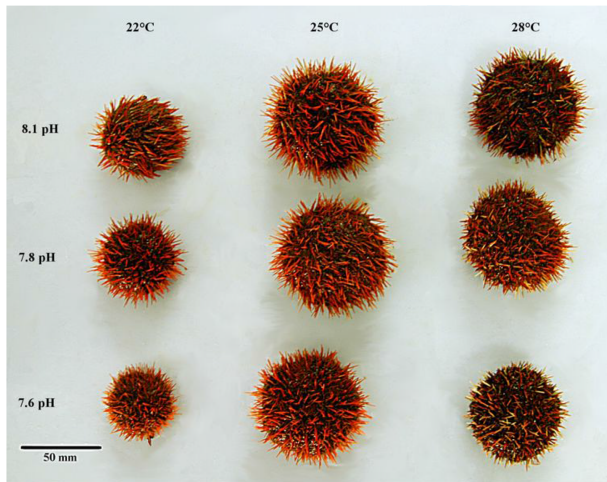
**Figure 7:** *Tripneustes gratilla* reared in three pH and three temperature levels in all combinations from the early juvenile (5.0 mm test diameter) for 146 days. A +3°C warming mitigated the negative effects of low pHNBS (pH 7.6, 7.8), but further warming was deleterious. From Dworjanyn and Byrne (2018).

*tribuloides* are weaker in bending tests, but not the spines of *Prionocidaris baculosa* (Dery *et al*., 2017). While the spines are the most vulnerable skeletal element in sea urchins to OA, these structures are designed to break in a predatory attack, and regenerate quickly (Dery *et al*., 2017). But this entails an energetic cost (Haga *et al*., 2016)

Overall, OA affects the material properties of the sea urchin skeleton through changes in growth rate with less biomineral produced. While it appears that there is weakening of the sea urchin skeleton in response to OA, thereby compromising its protective roles, more studies are needed where characterisation of the impacts of OA on biomineral microstructure is investigated in tandem with biomechanical tests and in skeletal elements that have been completely produced at low pH. Finally, the 3D void space of the sea urchin endoskeleton is important for the resident cells and tissues. Any change in the stereotypic arrangement of the skeleton is likely to have impacts for organism function beyond the skeleton itself.

## Crustaceans

The crustacean exoskeleton is made of chitin and calcite and is comparatively tolerant of acidification due to the lower degree of calcification (Whiteley, 2011), the exception being adult barnacles, with several studies on these animals (Table 1). For *Amphibalanus amphitrite*, there were no effects of low pH (to pH 7.4) through the larval stage, but the adult shell was weaker (McDonald *et al*., 2009). Corrosion of the shell of *A. improvises* was evident at low pH (Pansch *et al*., 2014). Increased temperature (+4°C) resulted in an increase in shell strength of this species, but there was no effect of pH (Pansch et al. 2013, 2014). For *Balanus improvises*, low pH

(stable pH 7.7, fluctuating pH 7.5–7.9) reduced the strength of the shell (Eriander *et al*., 2016).

## Discussion

The morphological and biomechanical properties of marine invertebrate skeletons are integral to animal function and individual fitness which in turn affects population dynamics and community structure (Kroeker *et al.,* 2014). In a climate change world, it is important to understand how calcification systems, and the biomineral that is produced, respond to environmental acidification. The combination of detailed morphology using advanced imaging techniques and biomechanics, is emerging as a leading-edge approach to quantify the vulnerability of marine skeletons. This approach is also key to detecting the potential for phenotypic adjustment in biomineralisation as a means to produce and maintain biomineral in the face of environmental acidification.

Although the field of marine climate change has focused on anthropogenic $CO_2$-driven acidification, many important calcifying species live in shallow coastal habitats that are vulnerable to acidification from increasing run off due to pressures from sea level rise and precipitation (Amaral *et al*., 2011; Fitzer *et al*., 2018). The chemistry of 'ocean' and 'coastal' acidification, and how they impact the carbonate system, the basis for calcification, are fundamentally different (Fig. 1). For bivalves, despite these differences, the resulting morphology and biomechanics of the biomineral produced under the two forms of acidification are similar (Beniash *et al*., 2010; Rodolfo-Metalpa *et al*., 2011; Dickinson *et al*., 2012; Hahn *et al*., 2012; Fitzer *et al*., 2018; Meng *et al*., 2018). Coastal waters have a more variable chemistry than the ocean and understanding how this chemistry interacts with $CO_2$-driven acidification, remains a significant challenge. This is especially important for socioeconomically important mollusc resources that are typically fished and cultured in coastal bays and estuaries.

In response to environmental acidification, the biomineral produced by marine invertebrates across many taxa, is more porous and less dense. Increased skeletal porosity in diverse species has been revealed by SEM and/or high-resolution 3D reconstructions generated by μCT. Indeed, some skeletal malformations are only evident through application of these methods (Fitzer *et al*., 2014a, b, 2015a, b, 2018; Rühl *et al*., 2017). There may be a balance between the absolute amount of mineral that can be physiologically deposited in the skeleton in order to maintain its key physiological functions and the energy that can be devoted to this function under OA. For molluscs and echinoderms, constraints in biomineral production result in smaller body size, similar to the Lilliput effect seen in the fossil record when smaller mollusc species survived global warming extinction events (Garilli *et al.,* 2015). In contrast for corals, growth, as measured

by linear extension of the skeleton, the standard metric of coral health appears to be maintained under $CO_2$-driven acidification, but the skeleton produced has a lower density and is thus more vulnerable to physical damage (Tambutté et al., 2015). OA also impacts biomineral directly through dissolution and this is most commonly noted for molluscs that lack an outer protective cover of conchiolin (Ries et al., 2009; Rodolfo-Metalpa et al., 2011; Harvey et al., 2018). Echinoderm skeletons are protected from corrosion by their epithelial cover, and in corals, the polyp tissue protects the skeleton.

Within each of the groups considered here, environmental acidification impacts skeletal microstructure and biomechanics in different ways, reflecting the diverse calcification biology of the species investigated. In bivalves, where skeletal $CaCO_3$ varies in composition of calcite and aragonite, the responses to $CO_2$-driven acidification are often similar within the same family. For example, the mytellids (Mytilus sp) have harder, brittle shells prone to fracture under OA, compared with the Ostreidae (Crassostrea sp.) where the shells have reduced hardness. Despite these different responses, the outcome with regard to the mechanical integrity of the shell of mussels and oysters is similar with reduced fracture toughness of shells formed in OA conditions.

Volcanic $CO_2$ vent systems have been used as natural laboratories to investigate the impacts of near and far future OA (Foo et al., 2018; González-Delago and Hernández, 2018). The $CO_2$ gradient at these sites provides a pH range from very low ∼pH 6 to ambient levels in nearby habitats not affected by $CO_2$ venting. Investigation of marine calcifiers resident along these $CO_2$ gradients where all or most of their skeleton has formed under low pH, has been an important means to understand how acidification impacts calcification. Overall, the results of laboratory and vent studies, at similar pH/acidification levels, are similar with respect to the response of marine invertebrate calcification. For herbivorous species resident at vents, higher levels of algal resources promoted by the $CO_2$ helps to ameliorate the energetic stress caused by acidification (Uthicke et al., 2016).

Biomechanical tests of the biomineral produced under experimental and natural acidification indicate that the shells and skeletons of many species are weaker than those produced in ambient conditions. This has been largely shown for molluscs with some studies of tube worms and corals, although the shells of many gastropod species are not affected by acidification (Leung et al., 2017). For echinoderms, biomechanics tests indicated that the spines are most affected by acidification (Collard et al., 2016; Dery et al., 2017) and that the newly formed apical plates were weaker (Johnson and Byrne, unpublished data). Overall, the shift to producing weaker skeletons with increased porosity decreased density under acidification conditions in many species will compromise the function of these structures in protection against physical stress and defence against predators.

It is key to consider the fitness consequences of observed change. Change to animal size, strength or defence will have effects to marine communities beyond the fitness of the individual. Changes to predator–prey interactions may change predation rates of vulnerable species and thereby alter population dynamics and community structure in the future (Kroeker et al., 2014). Weaker shells change predator–prey dynamics as durophagous predators such as crabs optimize their foraging to preferentially target weaker-shelled individuals or weaker regions of the shell of their molluscan prey (Kroeker et al., 2014). Predatory mollusc and boring epibionts also target the weaker, less dense areas of shells to drill and bore through the biomineral. Many molluscs also exhibit an inducible defence behaviour producing stronger, more calcified shells in response to the presence of a predator or in response to hydrodynamic conditions and the latter also applies to sea urchins (Bibby et al., 2007; Collard et al., 2016). For the gastropod Littorina obtusata, the inducible defence response expressed in the presence of a crab predator was inhibited at low pH, albeit at a rather extreme level (pH 6.6, ∼14 000 µatm $pCO_2$) (Bibby et al., 2007). The thinner shells of the oyster Ostrea lurida reared from the larval stage in OA appears to have made them more vulnerable to predatory gastropods (Sanford et al., 2014). In a study of M. edulis and the drilling gastropod Nucella lapillus maintained in the same acidification conditions, the thinner mussel shells produced in OA conditions made them more vulnerable to predation by the gastropod (Sadler et al., 2018). The outcomes for marine calcifiers in the face of OA will also be influenced by other environmental factors such as food levels. Mussels and barnacles are able to maintain a higher-level calcification in the presence of high food levels (Melzner et al., 2011; Pansch et al., 2014). Sea urchins with a higher level of calcified algae in their diet produce stronger skeletons (Asanaghi et al., 2013). Species adapted to fluctuations in intertidal habitats are also more tolerant of acidification (Wolfe et al., 2013b; Leung et al., 2017).

For several marine invertebrate groups across a variety of biomineralized structures, the lack of studies of the microstructure and mechanics stands out as gaps to address. In particular, the response of the crustacean chitin-calcite exoskeleton to acidification is poorly studied. The impact of acidification on jellyfish calcium sulfate (basanite) statoliths (Mooney and Kingsford, 2016; Sötje et al., 2017) is not known. Understanding the effects of acidification on the organic matrix of marine skeletons is also an important gap in knowledge, especially as shell matrix proteins are essential for crystal nucleation and other key aspects of skeletogenesis (Marin et al., 2008).

Finally, while the integrated morphology-mechanics approach has great potential to characterize the impacts of environmental acidification on marine invertebrate skeletons and advance our understanding of the biological consequences of climate change, this approach has only been applied to a relatively small number of species (Table 1). There is great interest in how calcification and the biomineral

produced will be altered in a changing ocean and it is clear that application of morphology and ecomechanics, in context with use of realistic pH levels, will be particularly informative.

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## References

Addadi L, Raz S, Weiner S (2003) Taking advantage of disorder: amorphous calcium carbonate and its roles in biomineralization. *Adv Mater* 15: 959–970.

Albright R, Bland C, Cillette P, Serafy JE, Kangdon C, Capo T (2012) Juvenile growth of the tropical sea urchin *Lytechinus variegatus* exposed to near-future ocean acidification scenarios. *J Exp Mar Biol Ecol* 426: 12–17.

Amaral V, Cabral H, Bishop MJ (2011) Resistance among wild invertebrate populations to recurrent estuarine acidification. *Estuar Coast Shelf Sci* 9: 460–467.

Amaral V, Cabral H, Bishop MJ (2012) Effects of estuarine acidification on predator-prey interactions. *Mar Ecol Prog Ser* 445: 117–127.

Andersson AJ, Mackenzie FT, Bates NR (2008) Life on the margin: implications of ocean acidification on mg-calcite, high latitude and cold-water marine calcifiers. *Mar Ecol Prog Ser* 373: 265–273.

Andersson AJ, Gledhill D (2013) Ocean acidification and coral reefs: effects on breakdown, dissolution, and net ecosystem calcification. *Ann Rev Mar Sci* 5: 321–348.

Asnaghi V, Chiantore M, Mangialajo L, Gazeau F, Francour P, Alliouane S, Gattuso JP (2013) Cascading effects of ocean acidification in a rocky subtidal community. *PLoS One* 8: e6197.

Asnaghi V, Magialajo L, Gattuso JP, Francour P, Privitera D, Chiantore M (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.

Asnaghi V, Collard M, Mangialajo L, Gattuso JP, Dubois P (2019) Bottom-up effects on biomechanical properties of the skeletal plates of the sea urchin *Paracentrotus lividus* (Lamarck, 1816) in an acidified ocean. *Mar Environ Res* 144: 56–61.

Barclay KM, Gaylord B, Jellison B, Shukla P, Sanford E, Leighton LR (2019) Variation in the effects of ocean acidification on shell growth and strength of two intertidal gastropods. *Mar Ecol Prog Ser* 626: 109–121.

Beniash E, Ivanina A, Lieb NS, Kurochkin I, Sokolova IM (2010) Elevated level of carbon dioxide affects metabolism and shell formation in oysters *Crassostrea virginica*. *Mar Ecol Prog Ser* 419: 95–108.

Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea*. *Biol Lett* 3: 699–701.

Byrne M, Lamare M, Winter D, Dworjanyn SA, Uthicke S (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 Lond B Biol Sci* 368: 20120439.

Byrne M, Smith AM, West S, Collard M, Dubois P, Graba-landry A, Dworjanyn SA (2014) Warming influences $Mg^{2+}$ content, while warming and acidification influence calcification and test strength of a sea urchin. *Environ Sci Technol* 48: 12620–12627.

Calosi P, Rastrick SPS, Graziano M, Thomas SC, Baggini C, Carter HA, Hall-Spencer JM, Milazzo M, Spicer Jl (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.

Carey N, Harianto J, Byrne M (2016) Urchins in a high $CO_2$ world: partitioned effects of body-size, ocean warming and acidification on metabolic rate. *J Exp Biol* 219: 1178–1186.

Cavey MJ, Markel K (1994) Class echinoidea. In Harrison F, Chia FS (eds) *Microanatomy of the Invertebrates*. Vol 14: Echinodermata. Alan R. Liss, New York, NY, p 345–400.

Chan VBS, Li C, Lane AC, Wang Y, Lu X, Shih K, Zhang T, Thiyagarajan V (2012) $CO_2$-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, *Hydroides elegans*. *PLoS One* 7: e42718.

Chatzinikolaou E, Grigoriou P, Keklikoglou K, Faulwetter S, Papageorgiou N (2017) The combined effects of reduced pH and elevated temperature on the shell density of two gastropod species measured using micro-CT imaging. *ICES J Mar Sci* 74: 1135–1149.

Chave KE (1954) Aspects of the biogeochemistry of magnesium 1. Calcareous marine organisms. *J Geol* 62: 266–283.

Cohen AL, McCorkle DC, de Putron S, Gaetani GA, Rose KA (2009) Morphological and compositional changes in the skeletons of new coral recruits reared in acidified water: insights into the biomineralization response to ocen acidification. *Geochem Geophys Geosyst* 10: 7.

Coleman D, Byrne M, Davis A (2014) Molluscs on acid gastropod shell repair and integrity in acidifying oceans. *Mar Ecol Prog Ser* 509: 203–211.

Collard M, Rastrick SPS, Calosi P, Demolder Y, Dille J, Findlay HS, Hall-Spencer JM, Milazzo M, Moulin L, Widdicombe S, Dehairs F (2016) The impact of ocean acidification and warming on the skeletal

mechanical properties of the sea urchin *Paracentrotus lividus* from laboratory and field observations. *ICES J Mar Sci* 73: 727–738.

Comeau S, Tambutté E, Carpenter RC, Edmunds PJ, Evensen NR, Allemand D, Ferrier-Pages C, Tambutté S, Venn AA (2017) Coral calcifying fluid pH is modulated by seawater carbonate chemistry not solely seawater pH. *Proc Roy Soc B* 284: 20161669.

Cooley SR, Doney SC (2009) Anticipating ocean acidification's economic consequences for commercial fisheries. *Environ Res Lett* 4: 024007.

Cooley SR, Lucey N, Kite-Powell H, Doney SC (2011) Nutrition and income from molluscs today imply vulnerability to ocean acidification tomorrow. *Fish Fish* 13: 182–215.

Coronado I, Fine M, Bosellini FR, Stolarski J (2013) Impact of ocean acidification on crystallographic vital effect of the coral skeleton. *Nature communications* 10: 2896.

Crook ED, Cohen AL, Rebolledo-Vieyra M, Hernandez L, Paytam A (2013) Reduced calcification and lack of acclimatization by coral colonies growing in areas of persistant natural acidification. *Proc Natl Acad Sci U S A* 110: 11044–11049.

Dell'Acqua O, Trębala M, Chiantore M, Hannula SP (2019) Robustness of *Adamussium colbecki* shell to ocean acidification in a short-term exposure.. *Mar Environ Res* 149: 90–99.

Dery A, Guibourt V, Catarino AI, Compère P, Dubois P (2014) Properties, morphogenesis and effect of acidification on spines of the cidaroid sea urchin *Phyllacanthus imperialis*. *Invertebr Biol* 133: 188–199.

Dery A, Collard M, Dubois P (2017) Ocean acidification reduced spine mechanical strength on euechinoid but not cidaroid sea urchins. *Environ Sci Technol* 51: 3640–3648.

Dery A, Tran PD, Compère P, Dubois P (2018) Cidaroid spines facing ocean acidification. *Mar Environ Res* 133: 9–18.

Díaz-Castañeda V, Cox TE, Gazeau F, Fitzer S, Delille J, Alliouane S, Gattuso J-P (2019) Ocean acidification affects calcareous tube growth in adult stage and reared offspring of serpulid polychaetes. *J Exp Biol* 222: jeb194543.

Dickinson GH, Ivanina AV, Matoo OB, Pörtner HO, Lannig G, Bocl C, Beniash E, Sokolova IM (2012) Interactive effects of salinity and elevated CO2 levels on juvenile eastern oysters, *Crassostrea virginica*. *J Exp Biol* 215: 29–43.

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: the other CO2 problem. *Ann Rev Mar Sci* 1: 169–192.

Doubleday ZA, Nagelkerken I, Coutts MD, Goldenberg SU, Connell SD (2019) A triple trophic boost: how carbon emissions indirectly change a marine food chain. *Glob Chang Biol* 25: 978–984.

Duarte CM, Hendriks IE, Moore TS, Olsen YS, Steckbauer A, Ramajo L, McCulloch M (2013) Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coast* 36: 221–236.

Dubois P (2014) The skeleton of postmetamorphic echinoderms in a changing world. *Biol Bull* 226: 223–236.

Dworjanyn SA, Byrne M (2018) Impacts of ocean acidification on sea urchin growth across the juvenile to mature adult life-stage transition is mitigated by warming. *Proc Roy Soc B* 285: 20172684.

Ellers O, Johnson AS, Moberg PE (1998) Structural strengthening of urchin skeletons by collagenous sutural ligaments. *Biol Bull* 195: 136–144.

Emerson CE, Reinardy HC, Bates NR, Bodnar AG (2017) Ocean acidification impacts spine integrity but not regenerative capacity of spines and tube feet in adult sea urchins. *R Soc Open Sci* 4: 170140.

Eriander L, Wrange A-L, Havenhand JN (2016) Simulated diurnal pH fluctuations radically increases variance in—but not the mean of—growth in the barnacle *Balanus improvisus*. *ICES J Mar Sci* 73: 596–603.

Fantazzini P, Mengoli S, Pasquini L, Bortolotti V, Brizi L, Mariani M, Di Giosia M, Fermani S, Capaccioni B, Caroselli E, Prada F (2015) Gains and losses of coral skeletal porosity changes with ocean acidification acclimation. *Nat Comm* 6: 7785.

Fitzer SC, Cusack M, Phoenix VR, Kamenos NA (2014a) Ocean acidification reduces the crystallographic control in juvenile mussel shells. *J Struct Biol* 188: 39–45.

Fitzer SC, Phoenix VR, Cusack M, Kamenos NA (2014b) Ocean acidification impacts mussel control on biomineralisation. *Sci Rep* 4: 6218.

Fitzer SC, Zhu W, Tanner KE, Kamenos NA, Phoenix VR, Cusack M (2015a) Ocean acidification alters the material properties of *Mytilus edulis* shells. *J R Soc Interface* 12: 20141227.

Fitzer SC, Vittert L, Bowman A, Kamenos NA, Phoenix VR, Cusack M (2015b) Ocean acidification and temperature increase impact mussel shell shape and thickness: problematic for protection? *Ecol Evol* 5: 4875–4884.

Fitzer S, Chung CP, Maccherozzi F, Dhesi SS, Kamenos NA, Phoenix VR, Cusack M (2016) Biomineral shell formation under ocean acidification: a shift from order to chaos. *Sci Rep* 6: 21076.

Fitzer SC, Torres Gabarda S, Daly L, Hughes B, Dove M, O'Connor W, Potts J, Scanes P, Byrne M (2018) Coastal acidification impacts on shell mineral structure of bivalve mollusks. *Ecol Evol* 8: 8973–8984.

Fitzer SC, Chan VBS, Meng Y, Rajan LC, Suzuki M, Not C, Toyofuko T, Falkenberg L, Byrne M, Harvey BP (2019a) Established and emerging techniques for characterising the formation, structure and performance of calcified structures under ocean acidification. In *Oceanography and Marine Biology: An Annual Review*, 57. CRC Press, Boca Raton pp 89–126.

Fitzer S, McGill RAR, Gabarda ST, Hughes B, Dove M, O'Connor , Byrne (2019b) Selectively bred oysters can alter their biomineralization pathways, promoting resilience to environmental acidification. *Global Change Biology*. doi: 10.1111/gcb.14818.

Foo SA, Byrne M, Ricevuto E, Gambi MC (2018) The carbon dioxide vents of Ischia, Italy, a natural laboratory to assess impacts

of ocean acidification on marine ecosystems: an overview of research and comparisons with other vent systems. *In Oceanography and Marine Biology: An Annual Review*, 56. CRC Press, Boca Raton pp 237–310.

Garilli V, Rodolfo-Metalpa R, Scuderi D, Brusca L, Parrinello D, Rastrick SPS, Foggo A, Twitchett RJ, Hall-Spencer JM, Milazzo M (2015) Physiological advantages of dwarfing in surviving extinctions in high-$CO_2$ oceans. *Nat Clim Chang* 5: 678–682.

Gattuso JP Magnan A, Billé R, Cheung WW, Howes EL, Joos F, Allemand D, Bopp L, Cooley SR, Eakin CM, Hoegh-Guldberg O (2015) Oceanography. Contrasting futures for ocean and society from different anthropogenic $CO_2$ emissions scenarios. *Science* 349: aac4722.

Gaylord B, Hill TM, Sanford E, Lenz EA, Jacobs LA, Sato KN, Hettinger A (2011) Functional impacts of ocean acidification in an ecologically critical foundation species. *J Exp Biol* 214: 2586–2594.

Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ, Heip CHR (2007) Impact of elevated $CO_2$ on shellfish calcification. *Geophys Res Lett* 34: L07603.

Gazeau F, Gattuso JP, Dawber C, Pronker AE, Peene F, Peene J, Middelburg JJ (2010) Effect of ocean acidification on the early life stages of the blue mussel *Mytilus edulis*. *Biogeosciences* 7: 2051–2060.

Gazeau F, Parker LM, Comeau S, Gattuso JP, O'Connor WA, Martin S, Ross PM (2013) Impacts of ocean acidification on marine shelled molluscs. *Mar Biol* 160: 2207–2245.

González-Delgado S, Hernández JC (2018) The importance of natural acidified systems in the study of ocean acidification: what have we learned? *Adv Mar Biol* 80: 57–99.

Guidetti P, Mori M (2005) Morpho-functional defences of Mediterranean sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, against fish predators. *Mar Biol* 147: 797–802.

Haag N, Russell MP, Hernandez JC (2016) Effects of spine damage and microhabitat on resource allocation of the purple sea urchin Strongylocentrotus purpuratus (Stimpson 1857). *J Exp Mar Biol Ecol* 482: 106–117.

Hahn S, Rodolfo-Metalpa R, Griesshaber E, Schmahl WW, Buhl D, Hall-Spencer JM, Baggini C, Fehr KT, Immenhauser A (2012) Marine bivalve shell geochemistry and ultrastructure from modern low pH environments: environmental effect versus experimental bias. *Biogeosciences* 9: 1897–1914.

Harvey BP, Agostini S, Wada S, Inaba K, Hall-Spencer JM (2018) Dissolution: the Achilles' heel of the triton shell in an acidifying ocean. *Front Mar Sci* 5: 371.

Hazan Y, Wangensteen OS, Fine M (2014) Tough as a rock-boring urchin: adult *Echinometra* sp. EE from the Red Sea show high resistance to ocean acidi cation over long-term exposures. *Mar Biol* 161: 2531–2545.

Hofmann GE, Evans TG, Kelly MW, Padilla-Gamiño JL, Blanchette CA, Washburn L, Chan F, McManus MA, Menge BA, Gaylord B, Hill TM (2014) Exploring local adaptation and the ocean acidification

seascape—studies in the California current large marine ecosystem. *Biogeosciences* 11: 1053–1064.

Holtmann WC, Stumpp M, Gutowska MA, Syré S, Himmerkus N, Melzner F, Bleich M (2013) Maintenance of coelomic fluid pH in sea urchins exposed to elevated CO2: the role of body cavity epithelia and stereom dissolution. *Mar Biol* 160: 2631–2645.

IPCC (2014) Climate change 2014: synthesis report. contribution of working groups I, II and III to the fifth assessment report of the intergovernmental panel on climate change. (eds Core Writing Team, Pachauri RK, Meyer LA) 151 pp. IPCC, Geneva, Switzerland.

Jiang T, Kaal J, Liang J, Zhang Y, Wei S, Wang D, Green NW (2017) Composition of dissolved organic matter (DOM) from periodically submerged soils in the three gorges reservoir areas as determined by elemental and optical analysis, infrared spectroscopy, pyrolysis-GC–MS and thermally assisted hydrolysis and methylation. *Sci Total Environ* 603–604: 461–471.

Johnson R, Harianto J, Thomson M, Byrne M (2019) The effects of long-term exposure to low pH on the skeletal microstructure of the sea urchin *Heliocidaris erythrogramma*. *J Exp Mar Biol Ecol*.

Kroeker KJ, Kordas RL, Crim R, Hendriks IE, Ramajo J, Singh GS, Duarte CM, Gatusso JP (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob Chang Biol* 19: 1884–1896.

Kroeker KJ, Sanford E, Jellison BM, Gaylord B (2014) Predicting the effects skeleton of ocean acidification on predator-prey interactions: a conceptual framework based on coastal molluscs. *Biol Bull* 226: 211–222.

Landes A, Zimmer M (2012) Acidification and warming affect both a calcifying predator and prey, but not their interaction. *Mar Ecol Prog Ser* 450: 1–10.

Langer G, Nehrke G, Baggini C, Rodolfo-Metalpa R, Hall-Spencer JM, Bijma J (2014) Limpets counteract ocean acidification induced shell corrosion by thickening of aragonitic shell layers. *Biogeosciences* 11: 7363–7368.

Lawrence J (2013) *Edible Sea Urchins: Biology and Ecology*. Academic Press, San Diego, California.

Leung JY, Connell SD, Nagelkerken I, Russell BD (2017) Impacts of near-future ocean acidification and warming on the shell mechanical and geochemical properties of gastropods from intertidal to subtidal zones. *Environ Sci Technol* 51: 12097–12103.

Li C, Chan VBS, He C, Meng Y, Yao H, Shih K, Thiyagarajan V (2014) Weakening mechanisms of the serpulid tube in a high-$CO_2$ world. *Environ Sci Technol* 48: 14158–14167.

Li C, Meng Y, He C, Chan VBS, Yao H, Thiyagarajan V (2016) Mechanical robustness of the calcareous tubeworm *Hydroides elegans*: warming mitigates the adverse effects of ocean acidification. *Biofouling* 32: 191–204.

Li S, Liu C, Huang J, Liu Y, Zheng G, Xie L, Rongqing Zhang R (2015) Interactive effects of seawater acidification and elevated temperature

on biomineralization and amino acid metabolism in the mussel *Mytilus edulis*. *J Exp Biol* 218: 3623–3631.

Mackenzie CL, Ormondroyd GA, Curling SF, Ball RJ, Whiteley NM, Malham SK (2014) Ocean warming, more than acidification, reduces shell strength in a commercial shellfish species during food limitation. *PLoS One* 9: e86764.

Marin F, Luquet G, Marie B, Medakovic M (2008) Molluscan shell proteins: primary structure, origin, and evolution. *Curr Top Dev Biol* 80: 209–276.

McClintock JB, Amsler MO, Angus RA, Challener RC, Schram JB, Amsler CD, Mah CL, Cuce J, Baker BJ (2011) The Mg-calcite composition of Antarctic echinoderms: important implications for predicting the impacts of ocean acidification. *J Geo* 119: 457–466.

McDonald MR, McClintock JB, Amsler CD, Rittschof D, Angus RA, Orihuela B, Lutostanki K (2009) Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. *Mar Ecol Prog Ser* 385: 179–187.

Melzner F, Stange P, Trubenbach K, Thomsen J, Casties I, Panknin U, Gorb SN, Gutowska MA (2011) Food supply and seawater $pCO_2$ impact calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS One* 6: e24223.

Meng Y, Guo Z, Fitzer SC, Upadhyay A, Chan VBS, Li C, Cusack M, Yao H, Yeung KWK, Thiyagarajan V (2018) Ocean acidification reduces hardness and stiffness of the Portuguese oyster shell with impaired microstructure: a hierarchical analysis. *Biogeosciences* 15: 6833–6846.

Meng Y, Guo Z, Yao H, Yeung KWK, Thiyagarajan V (2019) Calcium carbonate unit realignment under acidification: a potential compensatory mechanism in an edible estuarine oyster. *Mar Pollut Bull* 139: 141–149.

Migliaccio O, Pinsino A, Maffioli E, Smith AM, Agnisola C, Matranga V, Nonnis S, Tedeschi G, Byrne M, Gambi MC, Palumbo A (2019) Living in future ocean acidification, physiological adaptive responses of the immune systems of sea urchins resident at a CO2 vent system. *Sci Tot Environ* 672: 938–950.

Milano S, Schöne BR, Wang S, Müller WE (2016) Impact of high $pCO_2$ on shell structure of the bivalve *Cerastoderma edule*. *Mar Environ Res* 119: 144–155.

Mooney CJ, Kingsford MJ (2016) Statolith morphometrics can discriminate among cubozoan jellyfishes. *PLoS One* 11: e0155719.

Moulin L, Grosjean P, Leblud J, Batigny A, Dubois P (2014) Impact of elevated $pCO_2$ on acid-base regulation of the sea urchin *Echinometra mathaei* and its relation to resistance to ocean acidification: a study in mesocosms. *J Exp Mar Biol Ecol* 457: 97–104.

Pan TCF, Applebaum SL, Manahan DT (2015) Experimental ocean acidification alters the allocation of metabolic energy. *Proc Natl Acad Sci U S A* 112: 4696–4701.

Pansch C, Nasrolahi A, Appelhans Y, Whal M (2013) Tolerance of juvenile barnacles (*Amphibalanus improvisus*) to warming and elevated pCO₂. *Mar Biol* 160: 2023–2035.

Pansch C, Schaub I, Havenhand J, Wahl M (2014) Habitat traits and food availability determine the response of marine invertebrates to ocean acidification. *Glob Chang Biol* 20: 765–777.

Parker L, Ross P, O'Connor W, Borysko L, Raftos D, Pörtner H (2012) Adult exposure influences offspring response to ocean acidification in oysters. *Glob Chang Biol* 18: 82–92.

Parker L, Ross P, O'Connor W, Pörtner H, Scanes E, Wright J (2013) Predicting the response of molluscs to the impact of ocean acidification. *Biology* 2: 651.

Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. *Glob Chang Biol* 21: 2122–2140.

Queirós AM, Fernandes JA, Faulwetter S, Nunes J, Rastrick SP, Mieszkowska N, Artioli Y, Yool A, Calosi P, Arvanitidis C, Findlay HS (2015) Scaling up experimental ocean acidification and warming research: from individuals to the ecosystem. *Glob Chang Biol* 21: 130–143.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to $CO_2$-induced ocean acidification. *Geology* 37: 1131–1134.

Ries JB (2010) Review: geological and experimental evidence for secular variation in seawater Mg/Ca (calcite- aragonite seas) and its effects on marine biological calcification. *Biogeosciences* 7: 2795–2849.

Rodolfo-Metalpa R, Houlbrèque F, Tambutté É, Boisson F, Baggini C, Patti FP, Jeffree R, Fine M, Foggo A, Gattuso JP, Hall-Spencer JM (2011) Coral and mollusc resistance to ocean acidification adversely affected by warming. *Nat Clim Change* 1: 308–312.

Rodríguez A, Hernández JC, Brito A, Clemente S (2017) Effects of ocean acidification on juveniles sea urchins: predator-prey interactions. *J Exp Mar Biol Ecol* 493: 31–40.

Roleda MY, Boyd PW, Hurd CL (2012) Before ocean acidification: calcifier chemistry lessons. *J Phycol* 48: 840–843.

Rühl S, Calosi P, Faulwetter S, Keklikoglou K, Widdicombe S, Queirós AM (2017) Long-term exposure to elevated $pCO_2$ more than warming modifies early-life shell growth in a temperate gastropod. *ICES J Mar Sci* 74: 1113–1124.

Sadler DE, Lemasson AJ, Knights AM (2018) The effects of elevated $CO_2$ on shell properties and susceptibility to predation in mussels *Mytilus edulis*. *Mar Environ Res* 139: 162–168.

Sanford E, Gaylord B, Hettinger A, Lenz EA, Meyer K, Hill TM (2014) Ocean acidification increases the vulnerability of native oysters to predation by invasive snails. *Proc R Soc B* 281: 20132681.

Sato KN, Andersson AJ, Day JMD, Taylor JRA, Frank MB, Jung J-Y, McKittrick J, Levin LA (2018) Response of sea urchin fitness traits to environmental gradients across the Southern California oxygen minimum zone. *Front Mar Sci* 5: 258.

Smith AM, Riedl MA, Winter DJ (2013) Temperate reefs in a changing ocean: skeletal carbonate mineralogy of serpulids. *Mar Biol* 160: 2281–2294.

Smith AM, Clark DE, Lamare MD, Winter DJ, Byrne M (2016) Risk and resilience: variations in magnesium in echinoid skeletal calcite. *Mar Ecol Prog Ser* 561: 1–16.

Sötje I, Dishon T, Hoffmann F, Holst S (2017) New methods of morphometric analyses on scyphozoan jellyfish statoliths including the first direct evidence for statolith growth using calcein as a fluorescent marker. *Microsc Microanal* 23: 553–568.

Stemmer K, Nehrke G, Brey T (2013) Elevated $CO_2$ levels do not affect the shell structure of the bivalve *Arctica islandica* from the Western Baltic. *PLoS One* 8: e70106.

Stumpp M, Trubenbach K, Brennecke D, Hu MY, Melzner F (2012) Resource allocation and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis*. *Aquat Toxicol* 110–111: 194–207.

Tambutté E, Venn AA, Holcomb M, Segonds N, Techer N, Zoccola D, Allemand D, Tambutté S (2015) Morphological plasticity of the coral skeleton under $CO_2$-driven seawater acidification. *Nat Commun* 6: 7368.

Uthicke S, Ebert T, Liddy M, Johansson C, Fabricius KE, Lamare M (2016) *Echinometra* sea urchins acclimatized to elevated $p$CO$_2$ at volcanic vents outperform those under present-day $p$CO$_2$ conditions. *Glob Chang Biol* 22: 2451–2461.

Viotti S, Sangil C, Hernández CA, Hernández JC (2019) Effects of long-term exposure to reduced pH conditions on the shell and survival of an intertidal gastropod. DOI: 10.1016/j.marenvres.2019.104789

Von Euw S, Zhang Q, Manichev V, Murali N, Gross J, Feldman LC (2017) Biological control of aragonite formation in stony corals. *Science* 356: 933–938.

Wehrmeister U, Jacob DE, Soldati AL, Loges N, Häger T, Hofmeister W (2011) Amorphous, nanocrystalline and crystalline calcium carbonates in biological materials. *J Raman Spectrosc* 42: 926–935.

Welladson HM, Southgate PC, Heimann K (2011) The effects of exposure to near-future levels of ocean acidification on shell characteristics of *Pinctada fucata* (Bivalvia: Pteriidae). *Molluscan Res* 30: 125–130.

Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. *Mar Ecol Prog Ser* 430: 257–271.

Wilbur KM (1972) Shell formation in molluscs. In Flourkin and Scheer, Chem Zool 103-145. Academic Press, New York.

Wilkinson BH (1979) Biomineralization, paleoceanography, and the evolution of calcareous marine organisms. *Geology* 7: 524–527.

Wittmann AC, Pörtner HO (2013) Sensitivities of extant animal taxa to ocean acidification. *Nat Clim Chang* 3: 995–1001.

Wolfe K, Smith AM, Trimby P, Byrne M (2012) Vulnerability of the paper nautilus (*Argonauta nodosa*) to a climate change ocean, potential for extinction by dissolution. *Biol Bull* 223: 236–244.

Wolfe K, Smith AM, Trimby P, Byrne M (2013a) Microstructure of the paper nautilus (*Argonauta nodosa*) shell and the novel application of electron backscatter diffraction (EBSD) to address effects of ocean acidification. *Mar Biol* 160: 2271–2278.

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

Wright J, Parker L, O'Connor W, Ross P (2018) Ocean acidification affects both the predator and prey to alter interactions between the oyster *Crassostrea gigas* (Thunberg, 1793) and the whelk *Tenguella marginalba* (Blainville, 1832). *Mar Biol* 165: 3.

Ye F, Jurikova H, Angiolini L, Brand U, Crippa G, Henkel D, Smajgl D (2018) Variation in brachiopod microstructure and isotope geochemistry under low pH–ocean acidification–conditions. *Biogeosciences* 16: 617–642.

Zeebe RE, Rigwell A, Zachos J (2016) Anthropogenic carbon release rate unprecedented during the past 66 million years. *Nat Geosci* 9: 325–329.