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Warming Influences Mg^{2+} Content, While Warming and Acidification Influence Calcification and Test Strength of a Sea Urchin

Maria Byrne,[†] Abigail M. Smith,[‡] Samantha West,[§] Marie Collard,[∥,⊥] Philippe Dubois,[∥] Alexia Graba-landry,[§] and Symon A Dworjanyn*[,§]

[†]Schools of Medical and Biological Science, University of Sydney, Sydney, New South Wales 2006, Australia

[‡]Department of Marine Science, University of Otago, P.O. Box 56, Dunedin 9054, New Zealand

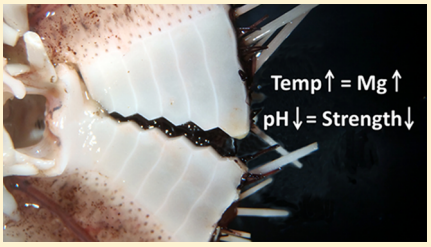
[§]National Marine Science Centre, Southern Cross University, P.O. Box 4321, Coffs Harbour, New South Wales 2450, Australia

[∥]Laboratoire de Biologie Marine, Université Libre de Bruxelles, CP 160/15, 1050, Brussels, Belgium

[⊥]Laboratory for Analytical, Environmental and Geo-Chemistry, Earth Systems Science Research Group, Vrije Universiteit, 1050, Brussels, Belgium

Supporting Information

ABSTRACT: We examined the long-term effects of near-future changes in temperature and acidification on skeletal mineralogy, thickness, and strength in the sea urchin *Tripneustes gratilla* reared in all combinations of three pH (pH 8.1, 7.8, 7.6) and three temperatures (22 °C, 25 °C, 28 °C) from the early juvenile to adult, over 146 days. As the high-magnesium calcite of the echinoderm skeleton is a biomineral form highly sensitive to acidification, and influenced by temperature, we documented the $MgCO_3$ content of the spines, test plates, and teeth. The percentage of $MgCO_3$ varied systematically, with more Mg^{2+} in the test and spines. The percentage of $MgCO_3$ in the test and teeth, but not the spines increased with temperature. Acidification did not change the percentage $MgCO_3$. Test thickness increased with warming and decreased at pH 7.6, with no interaction between these factors. In crushing tests live urchins mostly ruptured at sutures between the plates. The force required to crush a live urchin was reduced in animals reared in low pH conditions but increased in those reared in warm conditions, a result driven by differences in urchin size. It appears that the interactive effects of warming and acidification on the Mg^{2+} content and protective function of the sea urchin skeleton will play out in a complex way as global climatic change unfolds.



INTRODUCTION

Marine environments worldwide are experiencing unprecedented change due to anthropogenic impacts of increased greenhouse gases released into the atmosphere altering the physical and chemical properties of the oceans.[1] Uptake of CO_2 has decreased ocean pH by 0.1 units with a further decrease by 0.3 to 0.4 units by 2100 projected, alongside sea surface warming of ∼2 °C by 2100 ("business as usual scenario", RCP8.5).[1,2] For coastal regions these changes are being exacerbated by local aerial warming and changes in currents.[3,4] Ocean acidification reduces the solubility constant of calcium carbonate, which, for many marine invertebrates, makes production and maintenance of calcified structures more difficult.[5] Marine calcifiers, as calcifying larvae in the plankton and as shelled adults in the benthos are vulnerable to reduced mineral saturation. Taxa, such as echinoderms and molluscs that calcify in both life stages are particularly vulnerable to ocean acidification.[6−8] The stunting effect of ocean acidification on growth of marine calcifiers is a major outcome of global change.[5−9]

Concurrent warming and acidification can have strong interactive or additive effects on marine biota.[10] Depending on the process (e.g., genetic, whole organism) and species (e.g., calcifiers, noncalcifiers), temperature and/or acidification can be deleterious and/or beneficial, demonstrating how important it is to understand the combined effects of these stressors to disentangle complex responses.[10] For several sea urchin species warming (+3 °C) mitigates the negative effect of pH on growth of the larval skeleton.[11−13] For the species investigated here, *Tripneustes gratilla*, there is a significant antagonistic interactive effect, with warming countering the stunting effect of acidification on growth of the larval skeleton.[11] This is likely due to the stimulatory effect of warming on physiological processes, particularly calcification.

Echinoderms have high-magnesium calcite skeletons (4−30 wt % $MgCO_3$), the mineral state most vulnerable to dissolution due to CO_2-driven ocean acidification.[6,14−19] Mg^{2+} is substituted for Ca^{2+} during calcification and the Mg^{2+} content of the skeleton varies with seawater Ca^{2+}/Mg^{2+} ratio, carbonate mineral saturation states, and temperature.[19−21] Echinoderm skeletons in warmer latitude species have a higher Mg^{2+} content than those of species from the poles.[15,18] In the single study where the relationship between temperature and skeletal

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mineralogy was investigated in laboratory culture, the Mg^{2+}/Ca^{2+} ratio of the skeleton of *Paracentrotus lividus* was positively related to temperature.[22] Importantly, this study supports the long-held model of a positive relationship between increased temperature and skeletal Mg^{2+} content, without the confounding influence of disparate phylogeny and latitude, indicating a direct effect of temperature on calcification physiology. In addition, Mg^{2+} incorporation into sea urchin calcite can increase skeletal hardness and so temperature-driven variation together with physiological control in Mg^{2+} content is biomechanically important for some skeletal elements (e.g., teeth),[23] but not others (e.g., spines).[24] Thus, ocean warming can be expected to directly affect the mineralogy and potentially also the biomechanics of echinoderm skeletons. With regard to ocean acidification, recent studies indicate that the Ca^{2+}-Mg^{2+} composition of sea urchin skeletons does not differ among populations from areas differing in carbonate chemistry and does not change in adults maintained in low pH conditions for 60 days.[25,26]

Despite the well-established relationship between temperature and the mineralogy of the echinoderm skeleton, how this relationship may be altered in an ocean simultaneously warming and acidifying is not known, particularly for animals growing all their skeleton in these conditions from the early juvenile stage. Here we investigated the effect of increased temperature and acidification on skeletal mineralogy, test thickness and strength in the sea urchin *Tripneustes gratilla* reared in controlled laboratory culture from the very early postmetamorphic juvenile to the adult stage over 146 days. This species is an ecologically important and conspicuous component of tropical reef systems across the Indo-Pacific.[27] Due to rapid development at larval and post settlement stages, *T. gratilla* is also an important aquaculture species.[27,28] As the different skeletal elements of sea urchins (e.g., spines, test plates, teeth) differ in their chemical composition,[14,29] we documented the mineralogy of the different elements in *T. gratilla* reared in different temperature/acidification treatments. Because acidification was expected to weaken the skeleton, while warming may strengthen the skeleton, through stimulation of calcification and Mg^{2+} incorporation, we quantified the crushing strength of the entire live urchin as would occur during predation by fishes in nature.[30] This is as a more ecologically relevant approach than tests of the dried skeleton.[31] Sea urchin spines are particularly vulnerable to environmental conditions[6] and so we also examined spine structure. To our knowledge this is the first study of the mineralogy and crushing strength in urchins reared in warm-low pH conditions from near the outset of the benthic life phase to adult maturation. We tested hypotheses that the mineralogy and strength of the skeleton would vary over this life history transition: (1) that acidification alone would result in a weaker skeleton; (2) that increased temperature would promote calcification and Mg^{2+} incorporation into the skeleton and thereby increase skeletal thickness and strength; and (3) that there would be a significant interaction between these stressors with warming reducing the negative effects of acidification on skeletogenesis as observed for the larval skeleton.[11]

MATERIALS AND METHODS

Larval and Juvenile Rearing. *Tripneustes gratilla* were collected from South Solitary Island, New South Wales (30° 12′ S, 153° 16′ E). Three male and three female sea urchins were induced to spawn by injection of 2−3 mL of 1 M KCl. The resultant embryos were cultured in 300 L larval rearing containers and fed the micro alga *Proteomonas sulcata*. Competent larvae were settled in 60 L tanks containing aerated seawater and preconditioned with naturally derived biofilms, which acted both as a settlement cue and postlarval food. Flow-through seawater was introduced to the tanks after 48 h. At 8 weeks post settlement the juvenile urchins were weaned onto fresh *Sargassum* sp. at which point they were placed in the experiment.

Experimental Setup. The experiments were run in a flow-through seawater system. Experimental treatments were based on projections for near-future changes in ocean surface water in pCO_2/pH and temperature for southeastern Australia based on the CSIRO climate system Model MK3.5[3]. There were three temperature treatments (control = 22 °C, +3 °C = 25 °C, +6 °C = 28 °C) that were crossed in all combinations with three pHNIST treatments (control = 8.1, −0.3 pH units = 7.8, −0.5 pH units = 7.6).

The pCO_2/pH of experimental water was manipulated in 60 L header tanks supplied with flow-through seawater maintained at a constant volume using float valves. Seawater pCO_2/pH were manipulated in two of the header tanks (pH 7.8, 7.6) by injection of pure CO_2, which was dissolved using a vortex mixer (Red Sea). The pCO_2/pH was controlled by a pH probe connected to a pH controller, solenoid valve, regulator (Tunze 7070/20) and CO_2 cylinder. A third 60 L header tank was not injected with CO_2 and tracked ambient pCO_2/pH. Air was bubbled into all headers to aid mixing, and maintain dissolved oxygen levels >95%. Seawater from each header tank flowed to three separate 12 L subtanks where temperature was manipulated. The ambient temperature treatments were not manipulated but allowed to track local ambient SSTs, the +3 °C and +6 °C treatments were controlled using 200 w thermostatically controlled aquarium heaters (Jager). Water in the subtanks was supplied to rearing container through agricultural irrigation dripper taps (Antelco).

The header tanks and subtanks contained pH probes/controllers and thermostats respectively, but the water conditions of the treatments were determined and moderated according to average conditions that were measured in the rearing containers. Over the 146 days of the experiment water pH, temperature and salinity were measured on 94 days in 5 haphazardly selected rearing containers per treatment with a WTW Multimeter (MultiLine P4). Water samples were taken over the experiment fixed with saturated HgCl, and filtered (0.2 μm). Total Alkalinity (mean A_T = 2309 ± 5.84, n = 21) of these samples was determined by potentiometric titration and calculated using the Gran method. A_T results were checked against a reference standard.[32] Experimental pCO_2 was determined from A_T, pHNIST and salinity data using CO2SYS[33] using recommended dissociation constants.[34] The water chemistry conditions were stable over the experiment (Supporting Information (SI) Table 1).

Sea urchins were placed in the experimental system at approximately 5 mm test diameter (average 5.17 mm ±0.2 SE, n = 63). At this size they are fully weaned onto seaweed and have a low mortality.[28] There were seven replicates for each temperature by pH treatment combination. Individual sea urchins were initially cultured in rearing containers that consisted of 100 mL sample jars with a window that acted as an overflow that maintained the water volume at 30 mL. Only one urchin was placed in each container and so these containers

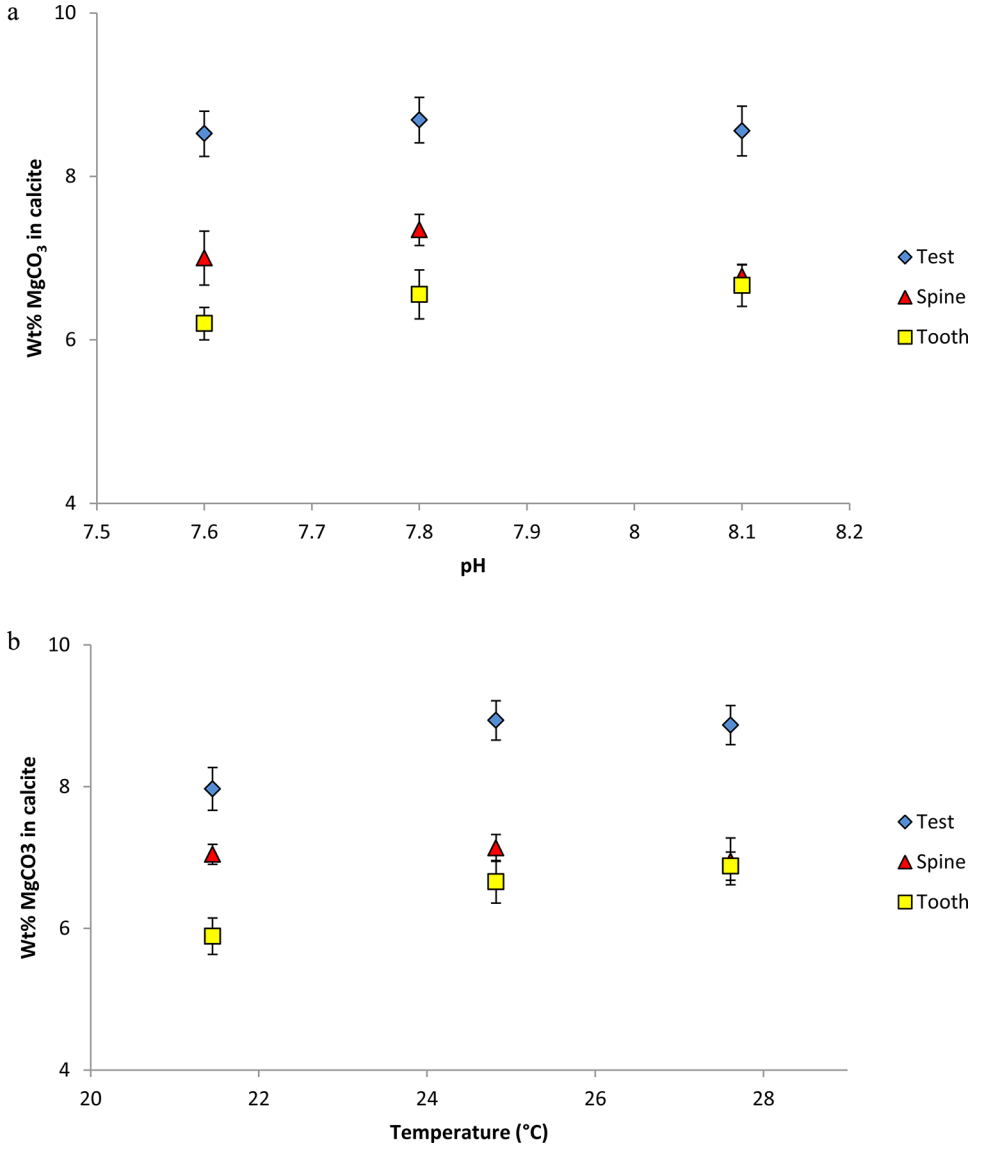

Figure 1. Effect of pH and temperature on weight percentage of $MgCO_3$ in the skeletal elements of *Tripneustes gratilla* after 146 days in culture, in respect to (a) pH treatments and (b) temperature treatments. Post hoc pairwise tests $p > 0.05$, test > spines > teeth; ambient temperature < +3 C° = +6 C°. Blue diamonds, test; red triangles, spine; yellow squares, tooth. All data are means, ± SE; $n = 3$.

(n = 7 per treatment) were the replicates. The rearing containers were supplied with a constant flow of experimental seawater with a turnover rate of greater than 100 container volumes per day. As the urchins grew they were transferred to larger 200 mL and then 500 mL containers. The flow rates were increased with size of container to maintain a water turnover rate of greater than 100 volumes per day. The urchins were fed in excess with fresh *Sargassum* sp. a high preference seaweed for *T. gratilla*.[35] Rearing containers were cleaned daily or as needed. Throughout the experiment there were six mortalities but no more than a single mortality in each treatment. The experiment ran for 146 days by which time the urchins were adults, ca. 60 mm test diameter (56 mm ±2.34 SE, n = 63). At this size *T. gratilla* has gonads (Dworjanyn, Unpublished data).

Mineralogy. X-ray diffractometry (XRD) was used to analyze the mineralogy (% $MgCO_3$) of the experimental animals. At the end of the growth experiment the skeleton was cleaned of internal organs, rinsed with distilled water and dried at 60 °C for 3 days. Three individuals from each temperature by pH treatment were haphazardly selected and a sample of (a) primary spines, (b) test plates, and (c) teeth were collected. The samples were ground to a fine powder with 0.1 g of NaCl, and the carbonate mineralogy was examined following the methods described by Smith et al.[36,37] See Gray and Smith[38] for the calibration equations and a complete description of the process used here.

Crushing Strength. To determine the force needed to crush entire live urchins as might occur in a predation event, 6–7 urchins from each treatment were crushed using a Chatillon DFX-00 digital force gauge attached to an adjustable Chatillon motorized tester. A 100 mm square plate was attached to the motorized tester so that an even force was applied to the urchin. The speed of the tester was set at 100 mm min^{-1}. The maximum compression force applied to the sea urchin at the point that the test collapsed was recorded in Newtons (±0.1 N). The diameter of each urchin was measured prior to testing.

Test Thickness. At the end of the experiment the thickness of the test of the urchins used in the mineralogy and crushing

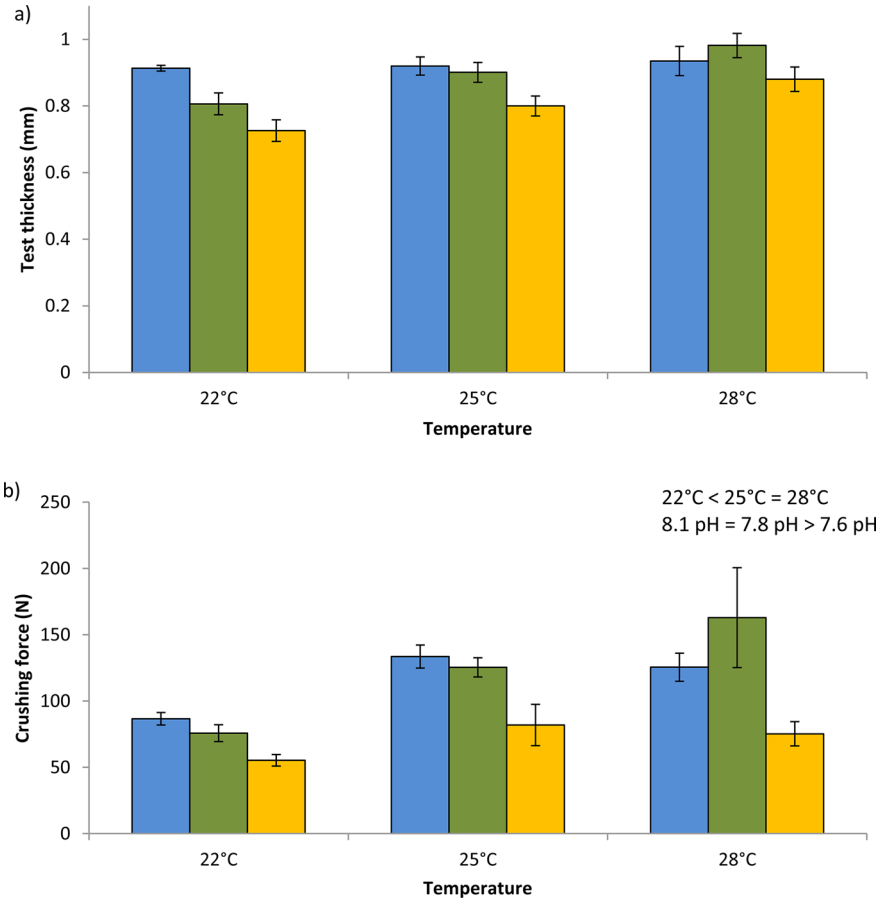

Figure 2. Effect of pH and temperature on a) the test thickness of *Tripneustes gratilla* after 146 days in culture (For statistical analysis see text) b) The force needed to crush live urchins. The symbols (=, >) indicate significant difference of the main factors, temperature and pH (post hoc pair wise tests $p > 0.05$). Bars: blue, pH 8.1; green, pH 7.8; yellow, pH 7.6 pH. All data are means, ± SE; $n = 6–7$.

strength experiment were measured after drying. Five haphazardly selected pieces of test were selected from each individual ($n = 6–7$ per treatment). The thickness (i.e., internal to external width) of these pieces of test was measured with digital callipers to the nearest 0.01 mm and the average of these five measurements used in the analysis.

Scanning Electron Microscopy. Spines collected from the mid body region of 5 specimens from each treatment were processed for scanning electron microscopy (SEM) to examine spine structure and if pore size was altered by warming and/or acidification. The spine tips were examined for evidence of disolution.[39] The surface cuticle and epithelium were removed from the skeleton to visualize the underlying skeleton using a 1% bleach treatment in distilled water (DW) for 30 min.[39] The specimens were then rinsed in DW, dried and mounted on stubs with carbon-based tape, coated with 15 nm gold, and examined and imaged by SEM (Zeiss Intellection Qemscan) using a 15 kV electron beam. The surface area (μm^2) of five pores along the mid spine region was measured in randomly selected spines from five urchins per treatment using ImageJ (NIH). The mean of the data determined for the 5 pores per spine was used as the datum for analysis.

Statistical Analyses. Permutational analysis of variance (PERMANOVA) was used for analyses of the mineralogy, spine pore size and compression force data. Temperature and pH were fixed factors in all analyses. For the mineralogy data the factor body element (i.e., test, spine and teeth) was fixed and initially nested in the factor Individual (i.e., individual sea urchin). The factor individual was not significant with a p greater than 0.25 so it was removed from the final analysis.[40] Pore size data were also nested in the factor Individual. The compression force data were first analyzed using PERMANOVA without correction for size to assess performance in an ecological context and in a second analysis using Permutational analysis of covariance (PERMANCOVA) test diameter was included as a covariate. As test thickness is also likely to vary with urchin size, test diameter was used as a covariate in analysis of test thickness data using PERMANCOVA. PERMANOVA and PERMANCOVA were carried out using Euclidean distance and untransformed data. Post hoc pairwise comparisons were used to test specific hypotheses when significant differences in main analysis involved three levels.

RESULTS

Skeletal Mineralogy. The percentage of $MgCO_3$ in the skeleton of *Tripneustes gratilla* differed among skeletal elements (i.e., tooth, test. spine) and was influenced by temperature (Figure 1, SI Table 2). There was a significantly higher percentage of $MgCO_3$ in the tests (8.6% ± 0.2 SE, n = 3) than the spines (7.0% ± 0.1 SE, $n = 3$) and the lowest percentage of $MgCO_3$ in the teeth (6.5% ± 0.2 SE, n = 3) (Figure 1, SI Table 2; post hoc pairwise test, test > spines > teeth). There was a significantly higher percentage of $MgCO_3$ in the calcite of sea urchins grown in the warmer treatments (25 °C, 28 °C) than at ambient temperature (22 °C), but no difference between the two warmer treatments (Figure 1a, SI Table 2, pot hoc pairwise

test: 22 °C < 25 °C = 28 °C). The increase in percentage of $MgCO_3$ in the test and teeth of urchins reared in 25 and 28 °C, compared with controls, were 12.1% and 10.1%, respectively and 13.0% and 14.9%, respectively. Decreased pH had no effect on the % $MgCO_3$ in the skeleton (Figure 1b, SI Table 2).

Test Thickness. Increased acidification significantly reduced test thickness, but this difference varied with temperature (Figure 2a, PERMANCOVA see SI Table 3). Examination of the effect of pH within each of the temperatures revealed that at 22 °C, tests were only thinner in sea urchins reared at pH 7.6 compared to pH 7.8 (Figure 2b, Pairwise post hoc test pH 8.1 = 7.8 > 7.6); at 25 °C tests were thinner in sea urchins reared at pH 7.8 and 7.6 compared to controls (Figure 2a, Pairwise post hoc test pH 8.1 > 7.8, 8.1 > 7.6, 7.8 = 7.6) and; at 28 °C tests were thinner in sea urchins reared at pH 7.6 compared to controls (Figure 2b, Pairwise post hoc test pH 8.1 = 7.8, 8.1 > 7.6, 7.8 = 7.6).

Test Strength. Acidification and warming had a significant effect on test strength as measured as crushing force with no interaction between these factors, (Figure 2b, 2-way PERMANOVA; temperature, $F_{2,47}$ = 11.04, P = 0.0002; pH, $F_{2,47}$ = 12.05, P = 0.0001; temp × pH $F_{4,47}$ = 1.65, P = 0.117. There was no difference in test strength between sea urchins reared at control and pH 7.8, but both of these were stronger than those reared at pH 7.6 (Figure 2b, Pairwise post hoc test 8.1 pH = 7.8 pH > 7.6 pH). The tests of urchins reared at 22 °C were weaker than those reared at 25 and 28 °C with no difference in strength between these two (Figure 2b, Pairwise post hoc test 22 °C < 25 °C = 28 °C). When test diameter is used as a covariate neither acidification or warming had an effect on test strength (PERMANCOVA SI Table 3). This indicates that the differences in test strength described above were a function of the effect of the treatments on urchin size. Interestingly, most urchins ruptured along the connective tissue suture line and not in the test plates (Figure 3).

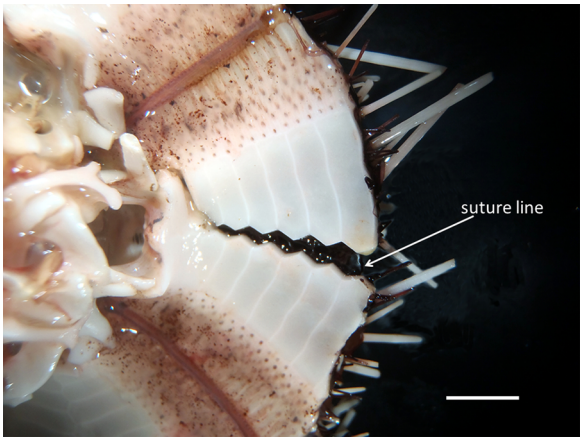

Figure 3. Photograph inside the test of a freshly crushed *Tripneustes gratilla* showing the typical breaking pattern along the suture. Scale bar is 5 mm.

Spine Structure. Electron micrographs of the spines did not reveal any change in stereome organization or dissolution of the spine tips among treatments (Figure 4). There was no effect of treatment on the surface area of the pores (SI Figure 1 and Table 4).

DISCUSSION

In this first cross-factorial study of the effects of warming and acidification on the sea urchin skeleton in specimens reared from the juvenile to the adult stage, we found contrasting effects of these factors. Acidification within levels projected in the near future and beyond for the ocean[1] resulted in a thinner test in *Tripneustes gratilla*. Temperature within levels projected in the near future for the east coast of Australia,[3] had the opposite effect. In the presence of both factors, increased temperature boosted test thickness across all pH levels but, counter to expectations, the interaction between stressors did not consistently result in warming reducing the negative effects of acidification. Temperature also increased test strength and acidification reduced it, a result most likely due to changes in size of *T. gratilla* in the treatments.

The stunting effect of ocean acidification on skeleton and shell formation in marine invertebrates is a broad response of biocalcification systems to global change.[6−13] Understanding the mechanism(s) underlying this response is a challenge to disentangle because each of the highly correlated parameters (pH, pCO_2/hypercapnia, decreased Ω calcite) can influence biotic responses.[10] While decreased mineral saturation and skeletal dissolution by lower pH is suggested to be particularly important for corals and molluscs,[8,41,42] the weight of evidence for echinoderms suggests that impaired calcification in tests is largely due to hypercapnia-driven alteration in metabolism.[6,12,43,44] For the spines, carbonate saturation and direct dissolution may play a more influential role on calcification.[6] A recent synthesis for sea urchin larvae shows that regardless of phylogeny, latitude or habitat, acidification impedes calcification and regression models indicate that pCO_2 is the most important stressor.[12] This is also the case for the adults.[6] When temperature, within tolerance levels, is an added factor, warming can reduce the negative effect of acidification on calcification.[11] In this study, however, increases in the calcification parameter measured (test thickness) did not offset the effects of acidification at pH 7.6.

We did not see any obvious change in the ultrastructure of the spine skeleton or evidence of dissolution of the spine tips and there was no change in spine pore size. A recent study that examined spine structure in juvenile (0.5 mm test diameter) *Helocidaris erythrogramma* reared in warm-acidification treatments shows that increased temperature (+2−4 °C) and extreme acidification, at levels lower than used here (pH 7.4), disrupts the skeleton with dissolution of the spine tips.[39] As a tropical species *T. gratilla* that experiences the maximum temperature used here (28 °C) in the more tropical parts of its range, this species is likely be comparatively robust to this level of warming above ambient. In a single stressor acidification study of juvenile *Lytechinus variegatus* malformation and/or dissolution of the spine skeleton was evident after 89 days at pH 7.8.[45]

The increase in incorporation of Mg^{2+} into the skeleton with increased temperature seen in *T. gratilla* is typical of sea urchins, as is the difference in the $MgCO_3$ content of the skeletal elements.[14,15,46] A higher Mg^{2+} in the test compared with the spine is similar to that reported for other species.[14] This is likely to make the test stronger than the spines. Sea urchin spines have a decrease in Mg^{2+} content from the base to the tip resulting in a reduction in hardness facilitating brittle failure toward the tip, a property that may serve as a defensive mechanism[20,47] (but see 24). It is not known if the 10−12%

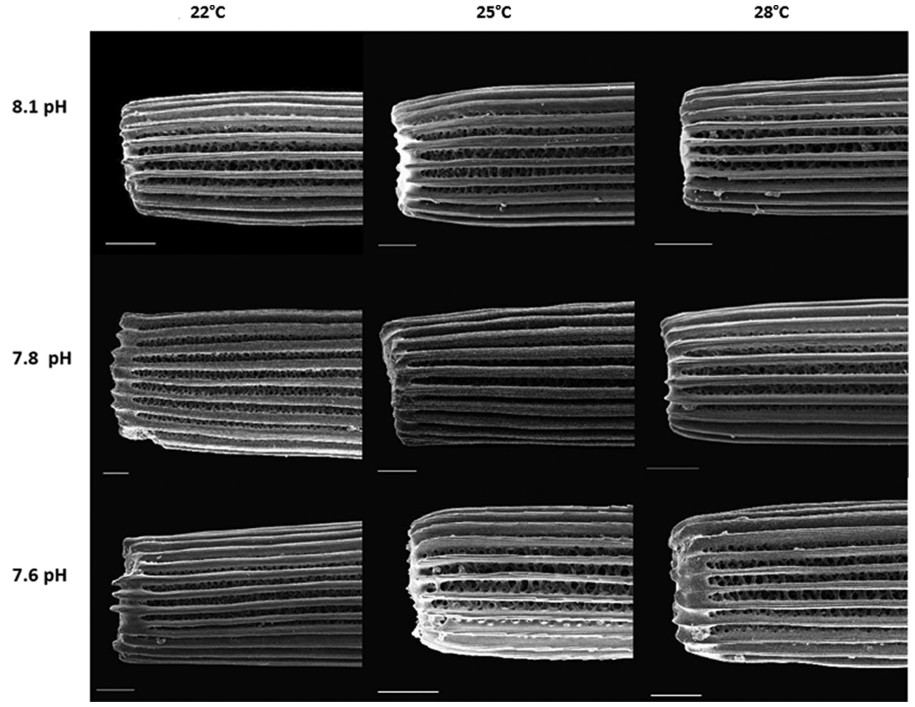

Figure 4. Scanning electron micrographs of the tips of spines of *Tripneustes gratilla* reared in experimental treatments for 146 days. The spines are presented to facilitate observation of the tips and so are not all the same magnification. There was no evidence of dissolution of the tips. Scale bars = 50 μm.

increase in $MgCO_3$ over the warming used here (+4−6 °C) is a significant change with respect to the protective function of the test.

There was no change in the $MgCO_3$ content of the skeleton of *T. gratilla* reared in ocean acidification conditions over 146 days. Similarly, adult skeletal $MgCO_3$ of other sea urchins and bivalves maintained for 60 days in similar environmental conditions did not change.[26,48] The element composition of the larvae of *Strongylocentrotus purpuratus* is also insensitive to near future acidification with no change in Mg^{2+} content.[25] This may be because the skeleton of regular urchins is protected by an epithelium. However, the spines of adult cidaroid urchins, which lack an epithelial cover, have lower Mg^{2+} content in deep water species that live below the aragonite saturation horizon.[49] Thus, it seems unlikely that regular urchins will alter the mineralogy of their skeleton to a less soluble form of $CaCO_3$ in response to modern day rapid change in ocean pH conditions. The rational for the increase in trace elements (Mn^{2+}, Zn^{2+}, Sr^{2+}) in the skeleton of urchins living near a vent site (pH 7.5−7.8/pCO_2 1400−2700) is not clear[50] and may be due to an increase in these elements in vent water. For other calcifiers, the Mg^{2+} content of polychaete tubes and gastropod shells varied with pCO_2 levels in the rearing environment, indicating that the mineralogy of some species is sensitive to near-future acidification conditions.[26,48]

Although there is evolutionary potential for sea urchins to alter expression of calcification genes and the pace of skeleton production as adaptive responses to maintain calcification under acidification conditions,[51] mineralogy is not likely to change. On the other hand, ocean warming is a significant contemporary phenomenon in many parts of the world, including the region of this study,[4] and has potential to alter skeletal mineralogy.

The integrity of test and spines is the most important line of defense that sea urchins have against mechanical damage from the physical environment and predator attack.[6,29,52] In a future ocean this defense will be compromised by ocean acidification. Although warming may result in stronger tests, it appears this will not offset the weakening effects of the most extreme acidification treatment tested here (pH 7.6). A recent single stressor acidification study also showed decreased skeletal robustness of the sea urchin test, although the mechanical trials were done on dried tests.[31] Our data and previous studies on the crushing strength of the body of live urchins where the soft tissues that bind the plates act as a jointed membrane to provide flexibility, provide a more ecologically relevant proxy of the performance of the test in response to fish predators.[30,53] In our study test plates often ruptured along the sutures, but it is not known how typical this pattern is among urchin species. The strength of the test structure depends on stress deformation levels of the soft collagenous tissue ligaments that bind the plates, rather than solely the strength of calcareous plates.[53] The mechanical properties of echinoderm connective tissues are affected by pH, but changes are typically only seen at pH levels much lower than used here and in experiments that used mineral acid to lower the pH.[54] It is not known how these tissues will be affected by warming and acidification.[55] Because the mutable connective tissues in the sutures can quickly change their mechanical porperties,[53] the rate at which a stress is applied may influence the outcome of mechanical tests.[56] In the present study, we used an ecologically relevant rate (100 mm/min), commensurate with a fish attack. It is interesting to note that juvenile sea urchins fed a high calcareous diet (coralline algae) had more robust tests than those reared on fleshy macroalgae, indicating a further consideration of the importance of diet as a potential buffer against low pH conditions.[31]

The absence of urchins around some vent sites (pH 7.6–7.8), open systems used as an analogue for future ocean conditions, has been taken to suggest that this level of acidification may prevent survival of sea urchins[57] potentially due to vulnerability of a weaker skeleton to predation.[31] However, this trend differs among sea urchin species as shown in a recent study where *Arbacia lixula* was particularly abundant at vent sites, while *Paracentrotus lividus* was not.[58]

How the interactive effects of warming and acidification will play out in the future with respect to the strength of the sea urchin skeleton and its integrity for protection and defense, is not known. With the sensitivity of calcification to temperature, however, it is likely that strong regional differences in the pace of climatic warming along coasts and in the oceans will have a major influence.

■ ASSOCIATED CONTENT

Supporting Information

Supplementary Table 1 containing water chemistry data Supplementary Table 2–4 containing results of statistical analyses, of the: wt % $MgCO_3$ in the test, spine and tooth (PERMANOVA); test thickness with diameter as a covariate (ANCOVA); crushing force with diameter as a covariate (PERMANCOVA) and; the pore surface are of the spines (PERMANOVA), respectively. Supplementary Figure 1 showing the effect of pH and temperature on the size of pores of the spine. This material is available free of charge via the Internet at http://pubs.acs.org/.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +61 266483909; e-mail: Symon.dworjanyn@scu.edu.au.

Notes

The authors declare no competing financial interest.

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