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Rapid dissolution of shells of weakly calcified Antarctic benthic macroorganisms indicates high vulnerability to ocean acidification

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Abstract: Antarctic calcified macroorganisms are particularly vulnerable to ocean acidification because many are weakly calcified, the dissolution rates of calcium carbonate are inversely related to temperature, and high latitude seas are predicted to become undersaturated in aragonite by the year 2100. We examined the post-mortem dissolution rates of aragonitic and calcitic shells from four species of Antarctic benthic marine invertebrates (two bivalves, one limpet, one brachiopod) and the thallus of a limpet shell-encrusting coralline alga exposed to acidified pH (7.4) or non-acidified pH (8.2) seawater at a constant temperature of 4°C. Within a period of only 14–35 days, shells of all four species held in pH 7.4 seawater had suffered significant dissolution. Despite calcite being 35% less soluble in seawater than aragonite, there was surprisingly, no consistent pattern of calcitic shells having slower dissolution rates than aragonitic shells. Outer surfaces of shells held in pH 7.4 seawater exhibited deterioration by day 35, and by day 56 there was exposure of aragonitic or calcitic prisms within the shell architecture of three of the macroinvertebrate species. Dissolution of coralline algae was confirmed by differences in weight loss in limpet shells with and without coralline algae. By day 56, thalli of the coralline alga held in pH 7.4 displayed a loss of definition of the conceptacle pores and cracking was evident at the zone of interface with limpet shells. Experimental studies are needed to evaluate whether there are adequate compensatory mechanisms in these and other calcified Antarctic benthic macroorganisms to cope with anticipated ocean acidification. In their absence, these organisms, and the communities they comprise, are likely to be among the first to experience the cascading impacts of ocean acidification.

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Key words: Antarctica, aragonite, brachiopod, calcite, coralline alga, mollusc

Introduction

A significant consequence of increasing atmospheric CO_2 is the acidification of the world's oceans (Pearson & Palmer 2000). To date studies have focused exclusively on the negative effects of ocean acidification on calcification and other biological processes in temperate and tropical benthic marine invertebrates and algae (Kurihara & Shirayama 2004, Kurihara *et al*. 2004, Kuffner *et al*. 2008, Hoegh-Guldberg *et al*. 2007, Fabry *et al*. 2008, Kurihara 2008, Dupont *et al*. 2008, McDonald *et al*. 2009). In contrast, while studies are currently under way on the impacts of ocean acidification on pelagic Antarctic shelled pteropods (V.J. Fabry and colleagues), we know of no studies on the effects of increased acidification on calcified marine invertebrates and algae that comprise the rich benthic communities of Antarctica (Arntz *et al*. 1994). This is a serious knowledge gap.

High latitude seas surrounding Antarctica are arguably among the most vulnerable regions of our planet with respect to prospective biotic impacts of ocean acidification (Fabry *et al*. 2008). This is because there is a preponderance of species with thin, weak, shells (Nicol 1967, Vermeij 1978), a result of either calcium ions being difficult to extract from seawater at low temperature (Graus 1974, Harper 2000, but see Aronson *et al*. 2007) or a protracted evolutionary history that has selected against robust shell calcification due to an absence of shell-crushing invertebrates, such as crabs and heavily jawed fish (Vermeij 1978, Thatje *et al*. 2005, Aronson *et al*. 2007, McClintock *et al*. 2008). Furthermore, there is an inverse relationship between the solubility of calcium carbonate and temperature (Revelle & Fairbridge 1957), a high probability the Southern Ocean will begin to become undersaturated with respect to aragonite (a polymorph of calcium carbonate) in the next few decades (Caldeira & Wickett 2005, Fabry *et al*. 2008, Guinotte & Fabry 2008, McNeil & Matear 2008), and a high incidence of benthic species with slow growth, delayed reproduction, and a long life span (Pearse *et al*. 1991).

The purpose of the present study was to examine the effects of seawater acidification on the dissolution rates of

representatives of both aragonitic and calcitic shells of a range of common Antarctic benthic macroorganisms including the bivalves *Laternula elliptica* (King & Broderip) and *Yoldia eightsi* (Courthouy), the gastropod limpet *Nacella concinna* (Strebel), and the brachiopod *Liothyrella uva* Broderip. Moreover, the calcified thallus of a coralline alga that frequently coats the shells of the limpet *N. concinna* was also examined. As in the majority of studies that have examined the impacts of ocean acidification on calcified temperate and tropical marine invertebrates, we chose to employ an experimental pH of 7.4 (similar to 23 of 30 studies cited in table 1 of Kurihara 2008). More importantly, this level of ocean acidity is predicted to occur in shallow coastal waters by the year 2300 (Caldeira & Wickett 2003), a time period unlikely to be sufficient for evolutionary processes to select for more robust shells or physiological mechanisms to adequately protect calcified body parts or enhance calcification. Moreover, pH 7.4 is conservative if atmospheric emissions are modelled on the high end of their predicated range (cumulative atmospheric emission of 20 000 Pg C), where global surface seawater pH reductions could be as high as 1.4 units (pH 6.7) by the year 2300 (Caldeira & Wickett 2005).

Materials and methods

Fresh, intact, empty shells of the bivalves *Laternula elliptica* (70–110 mm long diameter) and *Yoldia eightsi* (30–36 mm long diameter), and living individuals of the gastropod limpet *Nacella concinna* (30–40 mm long diameter) with and without the outer shell encrusted with a coralline alga, and the brachiopod *Liothyrella uva* (30–36 mm long diameter) were collected in April–June 2008 by hand using SCUBA from depths of 5–30 m in the near vicinity of Palmer Station, Anvers Island, off the western Antarctic Peninsula (64°46.5'S; 64°03.3'W). Shells of these species vary in their composition of isomorphs of calcium carbonate. The shells of the bivalves *Laternula elliptica* and *Yoldia eightsi* are aragonitic (Barrera *et al*. 1994, Marshall *et al*. 1996), while the shells of the gastropod limpet *N. concinna* and the brachiopod *L. uva* are both calcitic (Carter 1990, Marshall *et al*. 1996). *Nacella concinna* is often encrusted with a coralline alga comprised of magnesium-calcite (Guinotte & Fabry 2008). It was not possible to completely protect the outer organic periostracal shell layers in the fresh field-collected post-mortem shells of the bivalves *L. elliptica* and *Y. eightsi* (although shells of recently dead marine invertebrates still contain a large amount of organic matter associated with the periostracum or other organic coating, Marshall *et al*. 1996). The shells of the limpet *N. concinna* and the brachiopod *L. uva* were gently cleaned of living tissue to minimize damage to the outer periostracum of limpet shells and the thin, outer, protective nanocrystalline layer of the brachiopod shells. Shells were air dried slowly at room temperature, individually wrapped, and shipped to the University of Alabama at Birmingham, USA. For all shells, there was no contact with preservation agents that might alter the nature of an organic matrix.

Intact shells were cut lengthwise into two approximately equal-sized halves using a variable speed rotary tool (Foredom Electric Company, Bethel, CT) equipped with a 0.4 mm thick abrasive cut-off disk (25 mm diameter). Five shells and their respective paired half were placed into each of five one-litre beakers containing artificial seawater (Instant Ocean^{TM}, Aquarium Systems, Inc. Mentor, OH) made up at 35 ppt salinity and held in a constant cold temperature room at 4°C. This temperature is within 2°C of the upper range of seawater temperatures that occur at Palmer Station (Barnes *et al*. 2006). Control beakers were aerated with atmospheric air driven by an aquarium pump. Experimental beakers received a mixture of air from the pump and USP medical grade 100% by volume CO_2 from a cylinder (Airgas, Inc., Radnor, PA). The gases were mixed using a gas proportioning rotameter (Omega Engineering, Inc., Stamford, CT). The gas mixture in the experimental treatment was adjusted to achieve a pH of 7.4, while the ambient seawater control treatment was pH 8.2. The pH levels of seawater in beakers in both treatments were measured every two days using a Fisher Model AB15 pH/mV/°C/F Meter to ensure stability. The meter displayed pH to two decimal places. In order to ensure that, with dissolution, calcium ion levels did not rise and buffer the pH, calcium ion levels in the seawater of both experimental and control treatments were measured weekly using a calcium ion-specific ion electrode (Thermo Scientific, Beverly, MA). In addition, 25% of the seawater was replaced each week in both treatments to ensure seawater quality stability. It was not necessary to equilibrate the replacement seawater prior to its addition, as it equilibrated within 1–2 hr when added to the experimental beakers.

The chemical characteristics of the artificial seawater were as follows: total alkalinity (determined by titration): 2558 μmol kg^{-1} (experimental), 2678 μmol kg^{-1} (control), Ω_{Ar} 0.47 (experimental), Ω_{Ar} 2.66 (control), Ω_{Ca} 0.74 (experimental), Ω_{Ca} 4.22 (control). The Ω values were calculated using the Microsoft Excel spreadsheet "co2sys.xls" v.14 by G. Pelletier, E. Lewis and D. Wallace (http://www.ecy.wa.gov/programs/eap/models.html).

Shells were removed from their respective beakers weekly, dried at room temperature under circulating air in an exhaust hood for *c*. 4 hr and then weighed to the nearest 0.1 mg on a top-loading Mettler AJ100 balance (Mettler-Toledo Inc., Columbus, OH). To ensure that shells were completely dry prior to each weighing, several shells were spot checked by further drying them under a hair dryer and re-weighing them to confirm that weights did not decline with further drying.

After a period of 35 days, a stereo microscope (Leica model MZ6 stereo microscope) equipped with a Polaroid

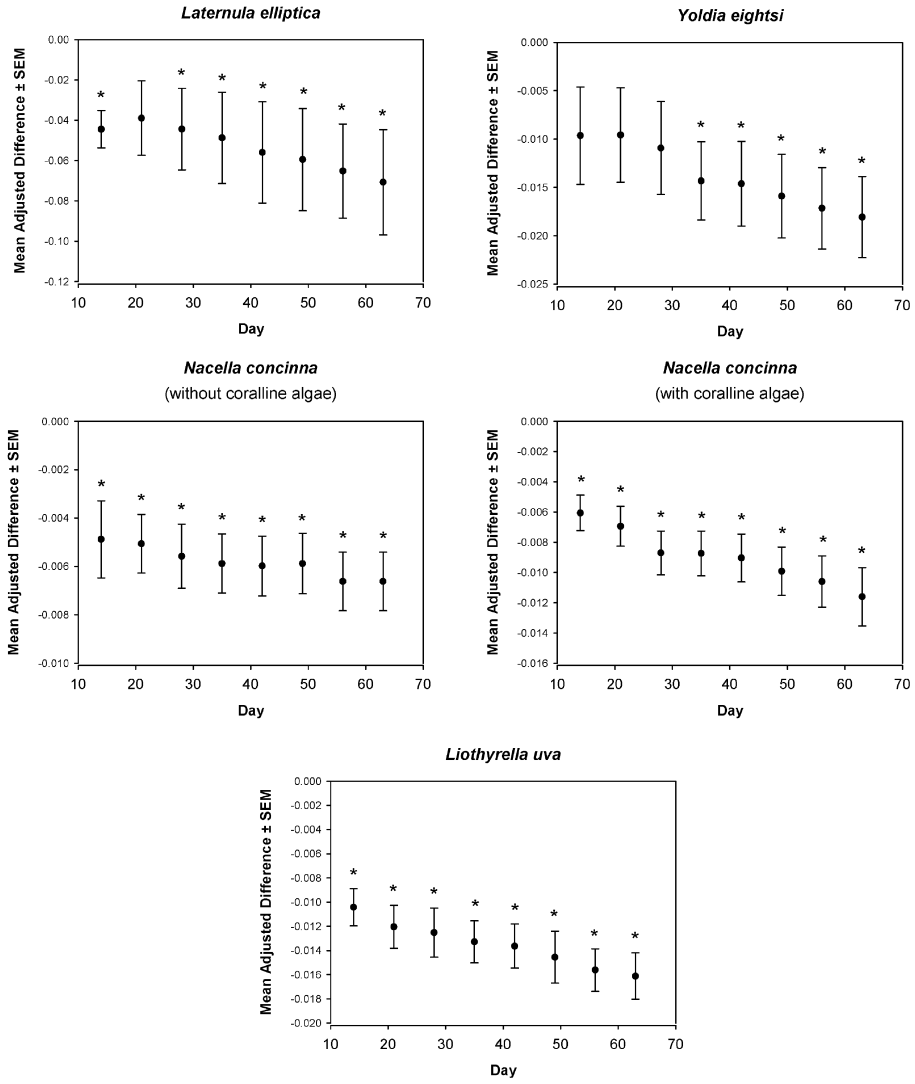

Fig. 1. Mean differences in weight between shell halves exposed to seawater at pH 8.2 and pH 7.4 for various amounts of time. Adjusted differences were calculated as (C - E at day 0) – (C - E at day #), where C is "control" (pH 8.2) and E is "experimental" (pH 7.4). When the E shells lose more weight than the C shells, the adjusted difference is negative. Means shown with an asterisk are significantly less than 0 (paired *t*-test, one-tailed).

Model DMC 1E digital camera was employed to take digital photographs of the outer surfaces of bivalve, limpet and brachiopod shells, as well as the outer surfaces of the coralline alga encrusting the shells of limpets.

Shell and thallus outer surface morphologies were also imaged after 56 days using a Topometrix "Explorer" Atomic Force Microscope (AFM) in contact imaging mode. The cantilevers used were non-conductive triangular silicon nitride with nominal spring stiffness of 0.05 N/m. The nominal tip radius was 20 nm. The images obtained were processed by Topometrix SPM Lab NT Version 5.0 software, and consisted of a first-order levelling of the surface and a left shading of the image.

A modified paired *t*-test was used to compare the mean weights of the shell halves from the two treatments at each seven days during the 56 day exposure period. For each pair of shell halves, the starting weight difference was not 0. So, an "adjusted" difference for each shell was calculated as:

$$\text{Adjusted difference} = (\text{C - E at day 0}) - (\text{C - E at day \#})$$

where C is "control" (pH 8.2) and E is "experimental" (pH 7.4). Since the pH 7.4 shells were hypothesized to dissolve at a greater rate than at pH 8.2, the mean adjusted difference was expected to be negative and the *t*-test test was done one-tailed. For all statistical comparisons, the cut-off for significance was $P \leq 0.05$.

The mean percentage of the original weight that was lost by the shells after 63 days was also calculated for both the pH 7.4 and pH 8.2 treatments for each species.

Results

Within 14 days, the outer surfaces of shells held in acidified pH 7.4 seawater were visibly less lustrous than outer

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Table I. Mean ± 1 s.e. percent weight loss in paired half shells of benthic Antarctic macroinvertebrates over a nine week exposure to seawater at either 7.4 or 8.2 pH.

Species	pH 8.2	pH 7.4
Laternula elliptica	0.313 ± 0.240	2.767 ± 0.607
Yoldia eightsi	0.000 ± 0.169	3.705 ± 0.912
Nacella concinna (w/out coralline)	0.278 ± 0.060	1.755 ± 0.332
Nacella concinna (with coralline)	1.061 ± 0.095	4.037 ± 2.390
Liothyrella uva	0.410 ± 0.119	3.127 ± 0.533

surfaces of shells held in ambient 8.2 pH seawater. At 14 days, the rate of shell dissolution (shell mass) was significantly greater in the experimental treatment (pH = 7.4), compared to the control treatment (pH = 8.2) for the brachiopod *L. uva* and for the shells of the limpet *N. concinna*, both with and without coralline algae (Fig. 1). The difference became significant for the bivalves *L. elliptica* after 28 days, and for *Y. eightsi* after 35 days (Fig. 1). In one instance, a shell of *L. elliptica* in the pH 7.4 treatment became so fragile that it fragmented into multiple pieces while the paired shell half held in ambient pH 8.2 seawater remained intact. Shells of the limpet *N. concinna* encrusted with the thalli of a coralline alga exposed to pH 7.4 lost a higher percentage of initial weight than those limpet shells lacking coralline algae (Fig. 1). By day 63, coralline-encrusted limpets had lost 2.3 times the weight of non-encrusted limpets (Table I). Thus, thalli of the coralline algae also experienced dissolution in seawater at pH 7.4. Mean percent weigh losses over 63 days for all shells in both pH treatments are presented in Table I.

Digital light microphotographs of the upper surfaces of the shells of both species of bivalve and the brachiopod following a period of 35 days revealed a greater irregularity as the more soluble surface regions dissolved on outer surfaces of shells held in the pH 7.4 when compared to the pH 8.2 treatment (see Fig. 2a & b showing the shell of the bivalve *Yoldia eightsi* as a representative example). Moreover, after a similar period the thalli of the coralline algae encrusting shells of limpets exposed to pH 7.4 seawater displayed evidence of dissolution when compared to the pH 8.2 treatment, as seen by an increase in definition of the conceptacle pores (Fig. 2c & d). Three weeks later,

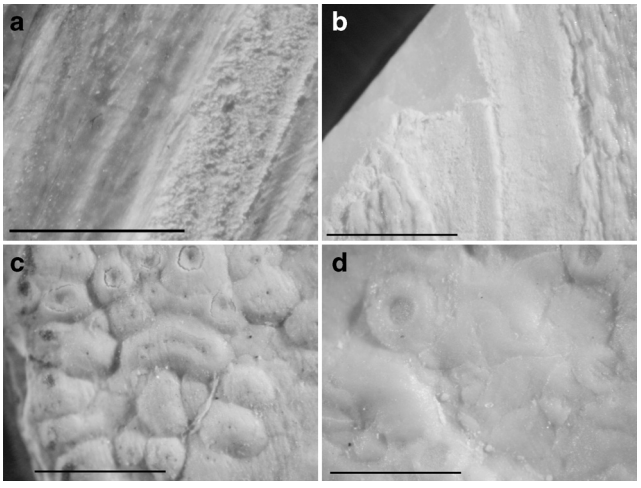

Fig. 2. External shell surfaces of the bivalve *Yoldia eightsi* after 56 days of exposure to seawater at **a.** pH 7.4, and **b.** pH 8.2, and a coralline alga encrusted on the shells of the limpet *Nacella concinna* after 56 days of exposure to seawater at **c.** pH 7.4, and **d.** pH 8.2. In each image, the scale bar is 1 mm.

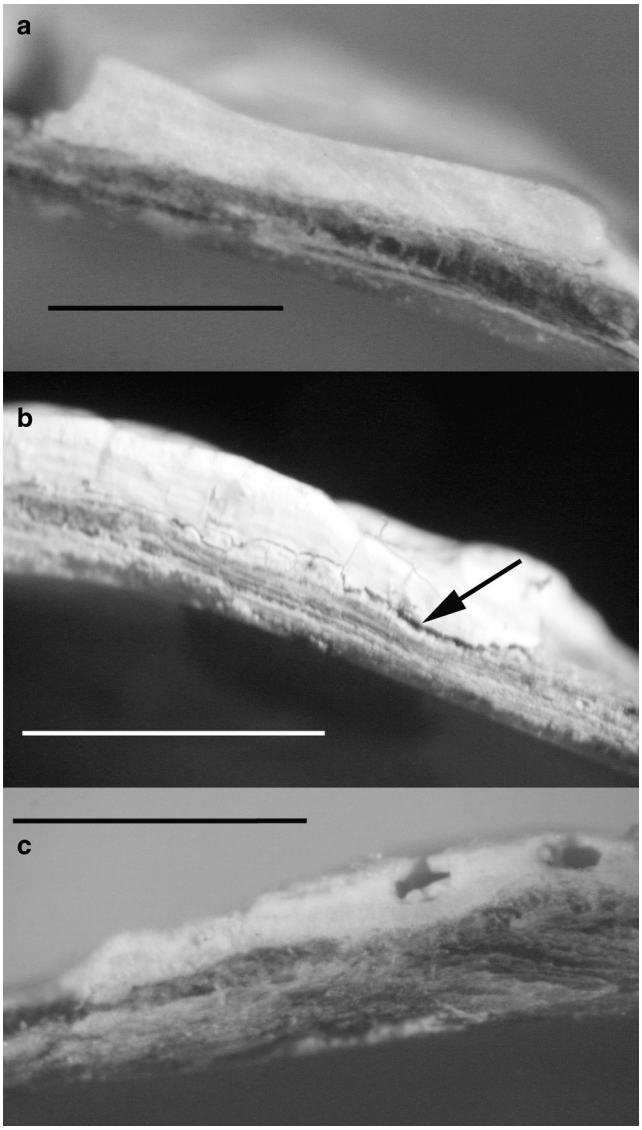

Fig. 3. View of the cut edge of *Nacella concinna* with its surface encrusted with a coralline alga. **a.** is at the beginning of the experiment, **b.** is the same shell after 56 days of exposure to pH 7.4 seawater. The arrow depicts the region of decomposition and probable initial separation from the limpet shell upon which it is encrusted. **c.** is the control half of the same shell after 56 days of exposure to pH 8.2 seawater. In each image, the scale bar is 1 mm.

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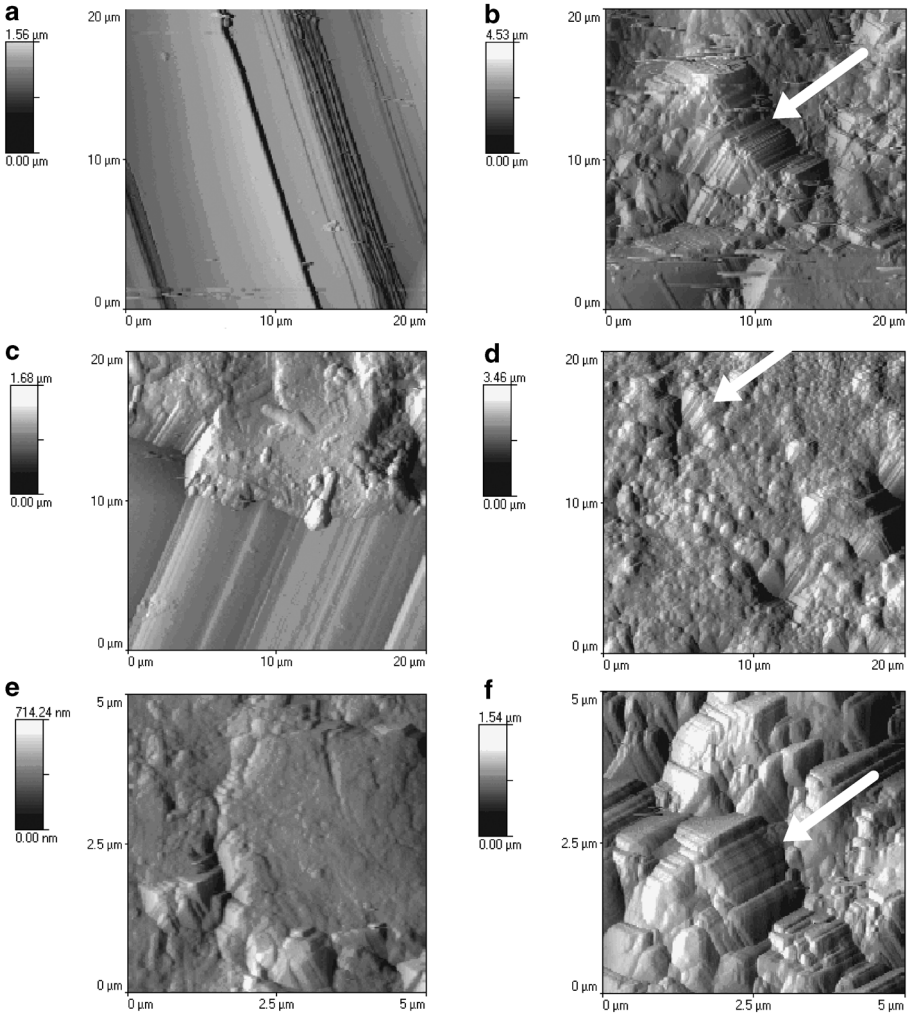

Fig. 4. Atomic force microscopy images of the surfaces of shells exposed for 56 days to control (pH 8.2) or acidified (pH 7.4) seawater. **a.** *Yoldia eightsi* control, **b.** *Y. eightsi* acidified, **c.** *Nacella concinna* control, **d.** *N. concinna* acidified, **e.** *Liothyrella uva* control, and **f.** *L. uva* acidified. Arrows in b, d & f indicate prisms emerging from dissolved shells.

after a period of 56 days, the coralline alga in the 7.4 pH treatment, but not in the control pH 8.2 treatment, had begun to fracture and crack along its zone of interface with limpet shells (Fig. 3).

Atomic force microscopy of the shells and coralline alga confirmed light microscopic observations and provided additional information on the ontogeny of the impacts on calcium carbonate microarchitecture under reduced pH. After a period of 56 days, the outer surface of shells of both bivalves, the limpet, and the outer shell surface of the brachiopod held in the pH 7.4 treatment displayed extensive degradation (see Fig. 4 for representative examples). Evident was the appearance of prisms emerging from the eroded outer shell in both species of bivalves, especially the aragonitic prisms of *Yoldia eightsi* (Fig. 4a & b). Calcitic prisms were less pronounced in the outer shell surface of the gastropod limpet *Nacella concinna* (Fig. 4c & d), while complex calcific prisms were conspicuously visible in the degraded outer shell surface of the brachiopod *Liothyrella uva* (Fig. 4e & f).

Discussion

Our findings indicate that weakly calcified marine macroinvertebrates occurring in Antarctic benthic communities are highly vulnerable to dissolution under predicted conditions of ocean acidification. While shells of the two bivalves, limpet and brachiopod exposed to ambient pH 8.2 seawater remained intact, the outer surfaces of shells exposed to pH 7.4 seawater exhibited evidence of deterioration after a period of only 35 days, and by 56 days it was possible to detect the exposure of aragonitic or calcitic prisms in the outer shell surfaces of the brachiopod *Liothyrella uva* and the bivalve *Yoldia eightsi*. As exposure of prisms occurred in both shells of bivalves and brachiopods, they are similarly vulnerable to the effects of acidification despite differences in their shell architecture. Bivalve shells generally have an inner nacreous layer, a central prismatic layer, and a thin outer organic periostracal layer. In contrast, brachiopod shells, while also structured into layers, have a thin, outer hard

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protective layer (primary layer) comprised of randomly oriented nanocrystalline calcite, which is underlain by a softer secondary layer built of long calcite fibres stacked into parallel blocks. Exposure of shell prisms in both bivalves and brachiopods is indicative of structural disintegration that compromises both shell integrity and strength via a suite of unique microarchitectural properties (Scurr & Eichhorn 2005). Degradation of calcium carbonate was also evident in the thalli of the coralline red algae. By day 35 there was visual evidence of enhanced irregularity on the surface and enhanced definition of the conceptacle pores. We also detected separation (cracking) of the thallus along the zone of interface with the outer limpet shell. This cracking was not due to mechanical damage and cannot be explained by repeated drying as shells with coralline algae were dried similarly in both treatments and cracking only occurred in the pH 7.4 treatment. This observation suggests that encrusting coralline red algae lacking an adequate compensatory response to ocean acidification could become prone to dislodgement from hard surfaces upon which they encrust. The potential dissolution susceptibility of coralline red algae is exacerbated by the fact that magnesium calcite is an important component of coralline red algae, and is known to be even more soluble than aragonite at high latitudes (Andersson *et al*. 2008).

The order of weight loss we observed showed no clear pattern of calcitic shells being less resistant to dissolution than those comprised of aragonite, despite calcite being 35% less soluble in seawater than aragonite (Morse *et al*. 1980, Mucci 1983). In the present study the pattern of the most to the least percent shell weight loss over a 63 day period was aragonitic *Yoldia* > calcitic *Liothyrella* > aragonitic > *Laternula* > calcitic *Nacella.* Thus, shell dissolution was greater in the calcitic shells of the brachiopod *Liothyrella uva* than for the aragonitic shells of the bivalve *Laternula elliptica*, despite the lower solubility of calcite. This observation supports the conclusion of Harper (2000) who indicated that the common premise that calcitic shells evolved to retard shell dissolution (e.g. Taylor & Reid 1990, Vermeij 1993) should be treated with caution. Factors outside the scope of the present study including crystal size and morphology, differences in shell chemistry, and differences in the type or structure of organic material surrounding the inorganic matrix of shells, must be considered as potential controlling factors in shell weight loss regardless of whether the shells are comprised of aragonite or calcite (Harper 2000). It has been predicted that shells comprised of aragonite would be especially susceptible to ocean acidification (Caldeira & Wickett 2005, Fabry *et al*. 2008, Guinotte & Fabry 2008) because aragonite is expected to become undersaturated by 2030 (McNeil & Matear 2008). The results of the present study imply that calcitic shells may be as susceptible even though the saturation of calcite may not decrease as rapidly. Our observations also indicate that future studies may need to be cautious about using saturation state as an indicator of when shell dissolution will commence on a large scale.

Glover & Kidwell (1993) and Lockwood & Work (2006) indicate that taphonomic studies show degradation in post-mortem shells is comprised of predictable damage processes that include disarticulation, fine-scale surface alteration, periostracum loss, edge modification, and fragmentation. Degradation in post-mortem shells is similar in some respects to what happens in shell loss in living animals, and Harper (2000) points out that dissolution is not restricted to dead shells and that long-lived shelled macroinvertebrates may be at serious risk of shell-weakening.

Based on current models of atmospheric CO_2 production, the pH level examined in the present study (7.4) is estimated to occur in shallow seas by the year 2300 (Sundquist 1993, Caldeira & Wickett 2003, 2005). As our present study did not examine living individuals, future studies are needed to evaluate the potential ability of calcified Antarctic benthic macroorganisms to compensate for the dissolution effects of acidification on calcification processes and shell dissolution across the range of pH values anticipated to occur under models of ocean acidification. While there have been shown to be varied responses in calcification upon exposure to conditions of ocean acidification in temperate and tropical shelled marine organisms (Fabry 2008), compensation is likely to be challenging for many Antarctic marine macroorganisms. This is because aragonite is expected to become undersaturated in high latitude seas by 2030 (McNeil & Matear 2008) and the difficulties of calcification may be greater in cold Antarctic seas where $CaCO_3$ has a greater solubility at low temperature (Revelle & Fairbridge 1957, but see Aronson *et al*. 2007). Many temperate and tropical benthic marine invertebrates show little or no enhanced calcification in response to seawater acidification (e.g. Kurihara & Shirayama 2004, Kurihara *et al*. 2004, Fine & Tchernov 2007). Furthermore, Antarctic marine benthic macroorganisms, in general, and those examined specifically in the present study, are known to have extremely slow growth and long life span (multiple decades) (Ralph & Maxwell 1977, Peck & Bullough 1993, Arntz *et al*. 1994, Peck *et al*. 1997, Clarke *et al*. 2004). We detected significant shell dissolution within only a few weeks time which constitutes a very small fraction of life expectancy. The differences we detected in dissolution rates, although small, would add up over the years to large differences in shell and thallus strength and integrity.

Calcified marine invertebrates and coralline algae make up a significant component of the rich benthic communities of Antarctica (Clarke & Johnston 2003, Amsler *et al*. 1995). Many of these species play important roles in bioturbation, the provision of hard substrata for epiphytic and epifaunal communities, and community trophodynamics (Arntz *et al*. 1994). Barring a significant reduction in anticipated levels of anthropogenic atmospheric CO_2-induced ocean acidification (Wigley *et al*. 1996), we caution that the benthic marine communities of the Southern Ocean are likely to be the "canaries in the coal mine" when it comes to global

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impacts of ocean acidification on calcified benthic organisms (Hall-Spencer *et al*. 2008). Moreover, if the recent discovery of crushing predators (deep water king crabs) moving up the Antarctic continental slope (Thatje *et al*. 2005) portends their invasion into shallow Antarctic seas, then weakly calcified benthic marine organisms suffering the consequences of ocean acidification may become even more vulnerable.

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