Thicker shells compensate dissolution in brachiopods under future ocean acidification and warming

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ABSTRACT

Organisms with long generation times require phenotypic plasticity to survive in changing environments until genetic adaptation can be achieved. Marine calcifiers are particularly vulnerable to ocean acidification due to a reduction in shell-building carbonate ions. Long-term experiments assess organisms’ abilities to acclimatise or even adapt to environmental change. Here we present an unexpected compensatory response to extensive shell dissolution in a highly calcium-carbonate-dependent organism after long-term culture in predicted end-century acidification and warming conditions. Substantial shell dissolution with decreasing pH posed a threat to both a polar (*Liothyrella uva*) and a temperate (*Calloria inconspicua*) brachiopod after 7 months and 3 months exposure, respectively, with more extensive dissolution present in *L. uva.* This impact was reflected in decreased outer primary layer thickness in *L. uva*. A compensatory response of increasing inner secondary layer thickness, and thereby producing a thicker shell was exhibited by *L. uva.* Less extensive dissolution in *C. inconspicua* with decreasing pH did not affect shell thickness. Increased temperature did not impact shell dissolution or thickness. Brachiopod ability to produce a thicker shell when extensive shell dissolution occurs suggests this marine calcifier has great plasticity in calcification providing insights into how similar species might cope under future environmental change.

INTRODUCTION

Changing environments pose serious risks to organisms that cannot shift their geographic range, physiologically acclimatise or genetically adapt[1](#_ENREF_1). Current understanding of the biological impacts of ocean acidification and warming is largely based on short- (days) to medium-term (weeks) laboratory and field experiments that have revealed mixed responses in many species[2-5](#_ENREF_2). More recently, however, there has been an increase in long-term (many months to years) studies that demonstrate surprising abilities of marine organisms to acclimate[6-10](#_ENREF_6), or even adapt in organisms with short generation times[11-13](#_ENREF_11) to decreased pH and increased temperature. Compensatory mechanisms could be paramount to maintain overall performance of organisms that have limited capacities to alter their geographic range under future changed conditions and subsequently sustain their key ecological functions in our oceans[14](#_ENREF_14).

Marine calcifiers are considered the most vulnerable organisms to ocean acidification due to the reduction in carbonate ions making shell production more difficult and energetically expensive[2](#_ENREF_2),[15](#_ENREF_15),[16](#_ENREF_16). The external skeleton is crucial for protecting animal tissue in shell-bearing organisms against predation, infection and loss of bodily fluids[17](#_ENREF_17),[18](#_ENREF_18). Any negative impacts to shell integrity, therefore, could compromise its protective function and potentially prove fatal. Shell integrity may be affected by erosion from natural scour or attack from shell-boring organisms as well as dissolution. The calcified shell of all shell-bearing organisms is protected by an outer organic layer, the periostracum[19](#_ENREF_19),[20](#_ENREF_20). Abrasion of this protective layer and subsequently inner shell layers naturally occurs through abrasion from suspended inorganic particulate material, the movement of individuals against each other, and with other calcified biota or substrata. Shell dissolution also poses a threat depending on the solubility of the biomineral, the chemical characteristics of the surrounding seawater and metabolic by-products released by the adhering biofilm[21](#_ENREF_21),[22](#_ENREF_22). Predicted environmental conditions for 2100 will shift surface seawater carbonate chemistry to favour CaCO3 dissolution, which could exacerbate the loss of shell integrity of marine calcifiers.

Compensatory mechanisms may counteract deleterious ocean acidification and warming effects on organisms. For these to succeed, the compensatory mechanism must occur at a faster rate than that of the deleterious effect to provide successful protection. Phenotypic plasticity of shell morphology has been reported in shelled organisms in response to the presence of predators[23](#_ENREF_23) and changing environmental conditions[14](#_ENREF_14),[24-26](#_ENREF_24). These include shell thickening, production of a more rotund shell and increased shell growth rates through plasticity in producing different calcium carbonate polymorphs[14](#_ENREF_14),[23-29](#_ENREF_23). Production of a thicker periostracum could also withstand more wear and deter dissolution[30](#_ENREF_30). Periostracum loss or shell dissolution at the external surface far away from the secretory tissue cannot be directly repaired by the organism. Compensatory mechanisms such as induced thickening, however, could counteract this potentially fatal effect of ocean acidification.

Brachiopods are one of the most calcium-carbonate-dependent groups of marine animals because their calcareous skeleton and other support structures make up > 90% of their dry mass[31](#_ENREF_31),[32](#_ENREF_32). Rhynchonelliform brachiopods possess a low-magnesium calcite shell consisting of the periostracum underlain by two biomineralised inner layers; the thin nanocrystalline primary layer and the generally much thicker fibrous secondary layer[33](#_ENREF_33),[34](#_ENREF_34). In previously published work we showed that shell growth rates of the polar brachiopod *L. uva* and temperate brachiopod *C. inconspicua* were not impacted by predicted end-century seawater pH’s[6](#_ENREF_6),[7](#_ENREF_7). Another study demonstrated increased dissolution in the Antarctic *L. uva* in pH 7.4 conditions after only 14 days[35](#_ENREF_35), however, only empty dried valves were used so the brachiopods ability to compensate shell dissolution remains unknown. This study, therefore, investigated dissolution effects and potential compensatory mechanisms of a polar and a temperate brachiopod living under acidified and warming conditions. Specifically, the extent of dissolution and thickness of whole valves and individual shell layers were assessed under predicted end-century pH levels in *L. uva* and *C. inconspicua* and also under increased temperature in *L. uva.*

MATERIALS AND METHODS

**Sampling collection.** *L. uva* (Broderip, 1833) were hand collected by SCUBA divers from Trolval Island, Ryder Bay, Antarctica (67° 35.44’ S, 68° 12.44’ W) at 15-25 m depth in May 2012. Environmental conditions in Ryder Bay at 15-25 m depth consist of seawater temperatures that range from -1.8 to +1.5°C, however, temperatures rarely exceed +1.0°C and salinity is 33.0-34.0[36](#_ENREF_36) and the pH range is 8.04-8.10[37](#_ENREF_37). Brachiopods were kept in recirculating aquaria (0.0 ± 0.5°C) whilst being transported back to the British Antarctic Survey, Cambridge, UK where the polar experiment was conducted.

*C. inconspicua* (Sowerby, 1846) were hand collected at low tide from under rocks in Portobello Bay, Otago Harbour, New Zealand (45° 82.00’S, 170° 70.00’E) in January 2013. Environmental conditions in Otago Harbour are surface seawater temperatures of 6.4-16.0°C[38](#_ENREF_38),[39](#_ENREF_39), pH range of 8.10-8.21 (K. Currie, pers. comm.) and salinity is 32.5-34.8[39](#_ENREF_39). Brachiopods were kept in seawater during the short transportation to Portobello Marine Laboratory, Otago Harbour, New Zealand where the temperate experiment was performed.

**Experimental Design.**

***Polar experiment.***The polar experiment was conducted in a temperature-controlled recirculating CO2 microcosm with four treatments[6](#_ENREF_6). Two were acidified treatments (“Moderate pH” – pH 7.75 ± 0.03 and “Low pH” – pH 7.54 ± 0.03) based on the IPCC ‘business-as-usual’ scenario of the predicted end-century reduction of 0.3-0.5 pH units from the present day average of pH 8.1 in surface oceanic seawater by 2100[40](#_ENREF_40) (Table 1). The third was a pH control where the seawater remained at ambient pH (pH 8.05 ± 0.03). All these three treatments were maintained at 2°C throughout the experiment due to the concurrent 2°C increase in sea surface temperature (SST) expected to occur alongside these predicted decreased pH levels by the end of the century[41](#_ENREF_41). The fourth treatment was a temperature control which was held at the present-day average conditions for Ryder Bay[42](#_ENREF_42) (SST: 0°C, pH: 7.98 ± 0.02). The pH of the acidified treatments was controlled by intermittently bubbling CO2 gas into a header tank. Seawater was then gravity fed into the experimental tanks[6](#_ENREF_6). The pH control treatment had a similar set up but without the pH manipulation system. The temperature control treatment was situated separately in the main BAS aquarium. Seawater temperature of all treatments was manipulated by controlling the air temperature in temperature-controlled laboratories.

Seawater temperatures (°C, Digital Testo 106) and pHNIST (Aquamedic pH controlled computer and electrode system) were monitored and recorded daily. Salinity (Tropical Marine Centre V2 Handheld refractometer), TCO2 (mmol L-1; Ciba Corning TCO2 Analyzer 965, Olympic Analytical. UK) and nutrient content (silicate and phosphate) of each treatment were measured weekly. Other carbonate system parameters, including the partial pressure of CO2 (*p*CO2) and the saturation values for calcite (ΩC) and aragonite (ΩA), were modelled from applying TCO2 and pHNIST data to the program CO2SYS[43](#_ENREF_43) with refitted constants[44](#_ENREF_44),[45](#_ENREF_45). Brachiopods in each treatment were fed weekly with microalgal concentrate of approximately 331 x 104 cells L-1, which is within the natural range of phytoplankton cell abundance along the west Antarctic Peninsula (62–1150 x 104 cells L-1) [46](#_ENREF_46),[47](#_ENREF_47).

***Temperate experiment.*** The temperate experiment was conducted in a flow-through CO2 perturbation system with three treatments[7](#_ENREF_7). Two were acidified treatments (“Moderate pH” – pH 7.79 ± 0.06 and “Low pH” – pH 7.62 ± 0.05) and the third was a pH control (8.16 ± 0.03). The pH of the acidified treatments was lowered in header tanks by intermittently bubbling CO2 gas before being gravity fed into the replicate experimental tanks[7](#_ENREF_7). The pH control system had an identical set up except that it lacked CO2 injection, and air was injected into the header tank. Seawater temperature was not manipulated and was ambient for Otago Harbour.

Seawater temperatures (°C, Digital Testo 106) and pHNIST were measured three times a day and salinity (YSI data logger) was measured once a week. Dissolved inorganic carbon (DIC) and total alkalinity (AT) were analysed at the beginning, middle and end of the experiment by a Single Operator Multi-parameter Metabolic Analyser (SOMMA) and closed-cell potentiometric titration, respectively[48](#_ENREF_48). Other carbonate system parameters, including the partial pressure of CO2 (*p*CO2) and the saturation values for calcite (ΩC) and aragonite (ΩA) were calculated using CO2SYS[43](#_ENREF_43) with CO2 equilibrium constants[44](#_ENREF_44),[45](#_ENREF_45),[49](#_ENREF_49). Brachiopods were fed three times a week with microalgal concentrate of approximately 397 x 104 cells mL-1 of *Tetraselmis* spp., which is within the natural range of phytoplankton cell abundance in Otago Harbour.

**Table 1.** Mean (±SD) seawater parameters during both the polar and temperate experiments.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Experiment | Treatment | pHNIST | Temperature (°C) | Salinity | *p*CO2 (µatm) | Ω Calcite | Ω Aragonite |
| Polar | Temperature control | 7.98 ± 0.02 | -0.3 ± 0.1 | 35 ± 1 | 417 ± 15 | 1.2 ± 0.1 | 0.8 ± 0.1 |
| pH control | 8.05 ± 0.03 | 1.7 ± 0.3 | 35 ± 1 | 365 ± 67 | 1.5 ± 0.2 | 0.9 ± 0.1 |
| Moderate pH | 7.75 ± 0.03 | 1.9 ± 0.4 | 35 ± 1 | 725 ± 133 | 0.8 ± 0.1 | 0.5 ± 0.1 |
| Low pH | 7.54 ± 0.03 | 2.2 ± 0.4 | 35 ± 1 | 1221 ± 179 | 0.5 ± 0.1 | 0.3 ± 0.1 |
| Temperate | pH control | 8.16 ± 0.03 | 16.5 ± 1.7 | 34 ± 1 | 465 ± 83 | 3.5 ± 0.5 | 2.2 ± 0.3 |
| Moderate pH | 7.79 ± 0.06 | 16.9 ± 1.7 | 34 ± 1 | 1130 ± 12 | 1.6 ± 0.0 | 1.0 ± 0.0 |
| Low pH | 7.62 ± 0.05 | 16.6 ± 1.7 | 34 ± 1 | 1536 ± 235 | 1.3 ± 0.2 | 0.8 ± 0.1 |

Values for *p*CO2, Ω calcite and Ω aragonite were calculated from CO2SYS[43](#_ENREF_43) with refitted constants[44](#_ENREF_44),[45](#_ENREF_45).

***Shell condition index.*** Scanning Electron Microscopes (JEOL 820 for *L. uva* and FEI QEMSCAN 650F for *C. inconspicua*; both operated using an accelerating voltage of 20 kV) were used to image gold-coated outer surfaces of five ventral valves of adult specimens from each treatmentof both speciesto determine shell condition. Five types of shell condition were present: intact shell (IS; intact periostracum with pitted layer), minimal wear (W1; periostracum without pitted layer), extensive wear (W2; wear but no dissolution), partial shell dissolution (SD1; dissolution in the inner primary layer) and extensive shell dissolution (SD2; dissolution exposing the innermost secondary layer). Full descriptions and examples of each type of shell condition for both species are presented in Table S1. Micrographs (1 mm x 1 mm) were collected at five standardised areas in shell laid down in the natural environment (Fig. S1A; which we refer to as “thickening shell”) and five standardised areas from growth in the experiments (Fig. S1A; which we refer to as “growing shell”). Percentage areas of each type of shell condition from each SEM micrograph were calculated/measured in ImageJ (Fig. S1B). Each shell region was analysed separately to determine whether treatment and/or the location of shell analysed (which we refer to as “shell position”) affected shell that had already been potentially subjected to substantial wear (thickening shell) and newly produced shell with less time subjected to wear (growing shell).

***Shell thickness.*** Longitudinal cross sections of five dorsal valves of adult specimens from each treatment of both species were finely polished to 3 μm using Kemet met papers (P400, P800, P2500 and P4000) followed by MetPrep diamond solutions (6 µm and 3 µm). Acetate peels from polished cross sections of the brachial valves of both species were made according to a previous study[50](#_ENREF_50).Thickness measurements (± 0.1 mm) of the primary layer, secondary layer and total shell were then measured from three areas of thickening shell and three areas of growing shell (Fig. S2) on a Swift monocular petrological microscope with fitted micrometer.

***Statistical analyses.*** Shell condition index data were non-normally distributed due to the presence of zeros in the dataset. Non-parametric Kruskal-Wallis tests were, therefore, used to determine whether treatment and/or shell position affected the median percentage area of each type of shell condition. When significant differences occurred, post-hoc Dunn’s tests were conducted to identify which treatments and shell positions were statistically different from each other. As shell condition and shell thickness measurements were conducted at several points within an individual, Kruskal-Wallis tests were also used to determine if individual number affected each shell condition. Linear mixed effects models were computed to determine if treatment, shell position (fixed effects) and/or individual number (random effect) impacted primary layer, secondary layer and total shell thickness:

Thickness measurement = Treatment + Shell Position + (1|Individual Number)

+ error

Likelihood ratio tests were used to determine p values (p < 0.05) between the full model with the effect in question against the reduced model without the effect in question. When the ratio tests identified significant differences, post-hoc Tukey tests were performed to determine which treatments or shell positions were responsible. Shell thickness data were checked for variance homogeneity and normality using Levene’s and Shapiro-Wilk tests (p < 0.05), respectively. Each shell region was analysed separately for both shell condition index and shell thickness to determine whether treatment and/or shell position affected shell maintenance (thickening shell) and shell production (growing shell). Statistical analyses were computed using R[51](#_ENREF_51) with the *FSA* package[52](#_ENREF_52) used for the Kruskal-Wallis and post-hoc Dunn’s tests, the *lme4* package[53](#_ENREF_53) for the linear mixed effects models and the *emmeans* package[54](#_ENREF_54) for the post-hoc Tukey tests.

RESULTS

**Shell condition index.**

***Thickening shell.*** Intact shell (IS) was absent from both acidified treatments and only present in < 8.4 ± 4.5% (mean ± SE) of both controls in the thickening shell in *L. uva* (Figure 1a & Figure 2)*.* Instead, minimal wear (W1) dominated this region in both controls (Figure 2a; 71.0 ± 6.2% in pH control and 65.1 ± 4.1% in temperature control). Partial shell dissolution (SD1), however, was the most prominent shell condition in both acidified treatments (Figure 1a; 64.3 ± 4.6% in moderate pH and 71.7 ± 4.3% in low pH). With decreasing pH, the percentage area of partial shell dissolution increased (Figure 1a & Figure 2; Kruskal-Wallis: H = 70.93, p < 0.001). The extent of shell dissolution in *L. uva* alsoincreased with decreasing pH (Kruskal-Wallis: H = 42.38, p < 0.001), with 18.2 ± 4.5% of shell exhibiting exposed secondary layer in the low pH treatment (SD2) compared to 0.9 ± 0.4% in the moderate pH treatment and the secondary layer never being exposed in either control. Temperature had no effect on shell dissolution or wear (Figure 1a; Dunn’s Test: SD1 – T = 1.16, p = 0.244, SD2 – T = 0.26, p = 0.795, W1 – T = 0.25, p = 0.805, W2 – T = 1.60, p = 0.109). In contrast to *L. uva,* thickening shell of *C. inconspicua* was mainly characterised by intact shell (Figure 1c; IS; 56.6 - 82.3%) across all treatments. Amounts of minimal wear (W1) decreased with decreasing pH in this shell region in *C. inconspiuca* (Figure 1c; Kruskal-Wallis: H = 7.92, p = 0.020). Partial shell dissolution (SD1)*,* however, increased with decreasing pH (Figure 1c & Figure 3; Kruskal-Wallis: H = 53.72, p < 0.001) in growing shell in *C. inconspicua*. Shell dissolution in this temperate specieswas less extensive than for the polar species *L. uva* (Figure 2 & Figure 3)as the secondary layer was not exposed (SD2) in any individual in any treatment. Shell position or individual number did not affect any shell condition in the thickening shell of both species (Table S2).

***Growing shell.*** Growing shell in both species was mainly characterised by intact shell (IS) in all treatments (Figure 1b, d; *L. uva*:> 63.9 ± 4.7%; *C. inconspicua*: > 83.2 ± 1.8%). Less intact shell occurred in the most acidified conditions compared to all other treatments in both species (Figure 1b, d; Kruskal-Wallis: *L. uva -* H = 41.81, p < 0.001; *C. inconspicua* – H = 20.96, p < 0.001). Partial shell dissolution (SD1) increased with increasing acidity in the experimental growth of *L. uva* (Figure 1b & Figure 2; Kruskal-Wallis: *L. uva -* H = 63.08, p < 0.001). This shell dissolution, however, occurred at a much lower level (3.2 ± 1.0% in moderate pH and 35.9 ± 4.7% in low pH) in the growing shell than in the thickening shell in this species. Temperature had no effect on partial shell dissolution (Dunn’s Test: Temperature control vs pH control: T = -0.22, p = 0.829). Partial shell dissolution (SD1) only occurred in the most acidified treatment in *C. inconspicua* (Figure 1d; 11.1 ± 1.5%), also in lower levels than in the thickening shell (28.3 ± 3.2%) in this species. Extensive shell dissolution (SD2) was absent in the growing shell in both species across all treatments. Minimal wear (W1) was only present in two individuals across all treatments in *L. uva* and was absent from *C. inconspicua.* Extensive wear (W2) was present in higher levels in the control treatments of both species than in the acidified treatments (Figure 1c, d; Kruskal-Wallis; *L. uva* – H = 43.98, p < 0.001, *C. inconspicua -* H = 10.67, p < 0.001), however, only in low levels (< 11.1 ± 1.2%). Neither shell position nor individual number affected any shell condition in the growing shell of both species (Table S2).

Both shell condition graphs

**Figure 1.** Shell condition - Average percentage area of the different types of shell condition in thickening shell (a, c) and growing shell (b, d) in *L. uva* (top row) and in *C. inconspicua* (bottom row) in all treatments. Lighter grey tones indicate an increase in wear and/or shell dissolution (see legend).

**Thickening shell**

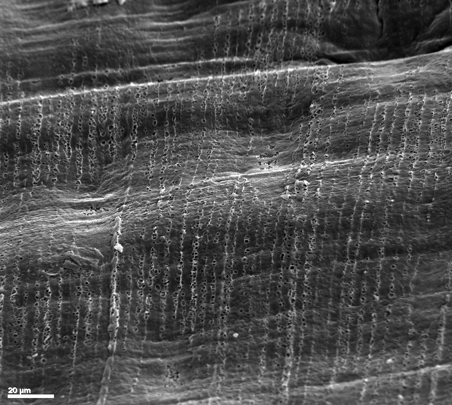
**Growing shell**

**pH control**

**Temperature control**

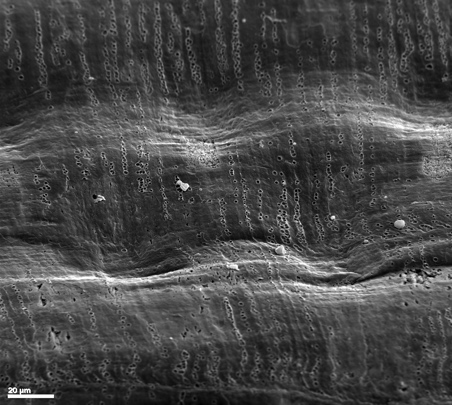
**Moderate pH**

**Low pH**



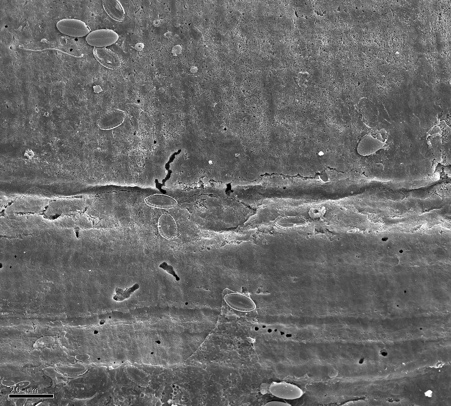
**D**

**IS**



**B**

**IS**



**A**

**W1**



**C**

**W2**

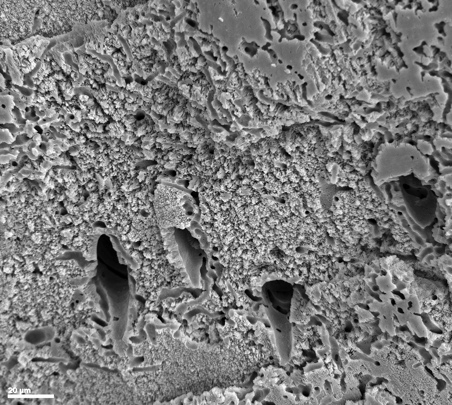
**W1**



**F**

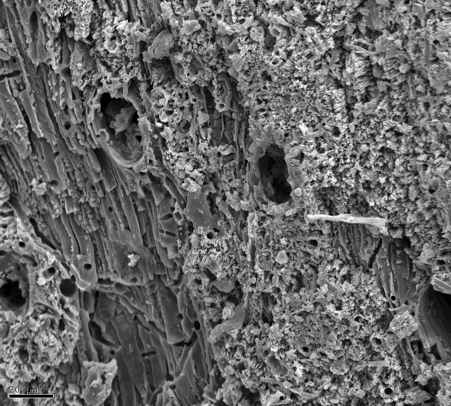
**IS**

**SD1**



**E**

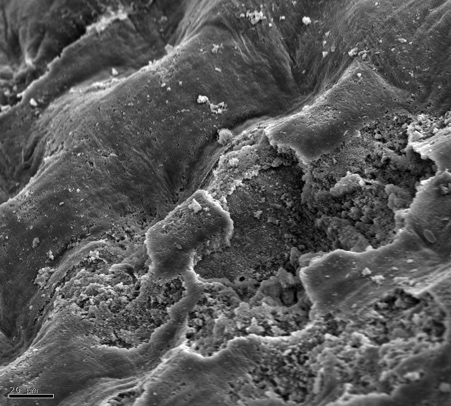
**SD1**



**G**

**SD2**

**SD1**

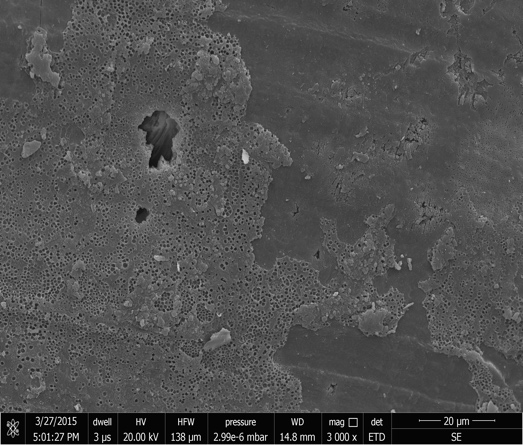
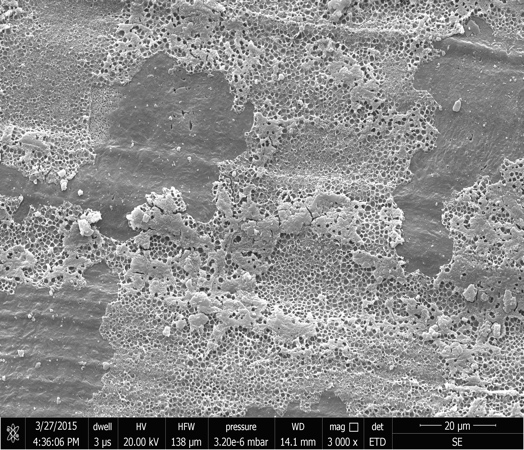


**H**

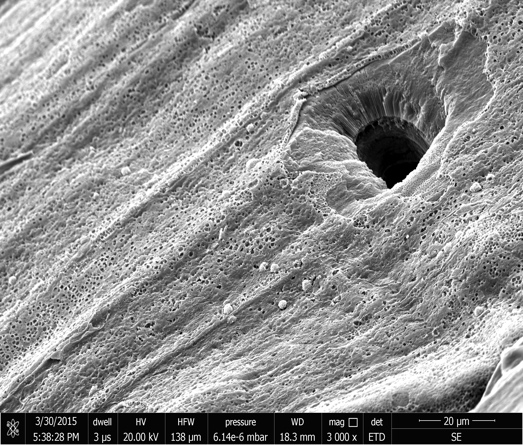
**IS**

**SD1**

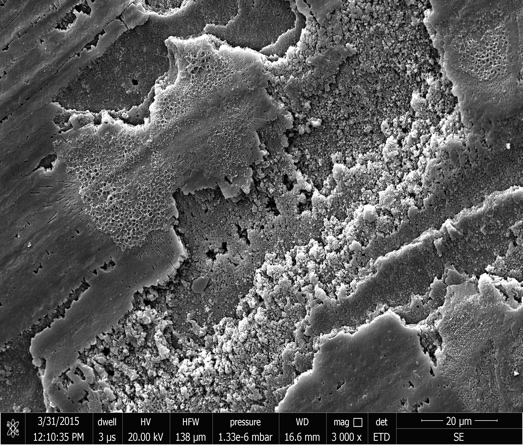
**Figure 2.** Shell condition in *L. uva* – Examples of SEM micrographs of shell surfaces of thickening shell (A, C, E, G) and growing shell (B, D, F, H) in temperature control (A, B), pH control (C, D), moderate pH (E, F) and low pH treatment (G, H). IS = intact shell, W1 = minimal wear, W2 = extensive wear, SD1 = partial shell dissolution and SD2 = extensive shell dissolution. Scale bar = 20 µm.



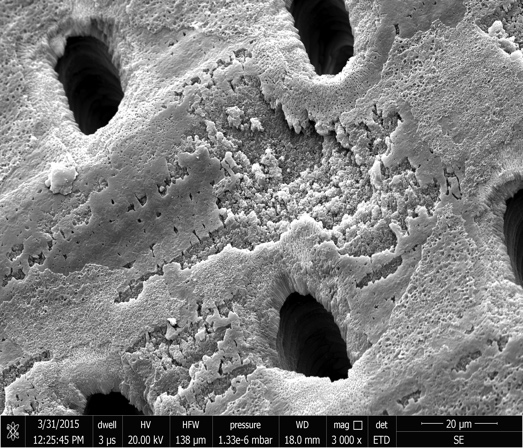
**A**



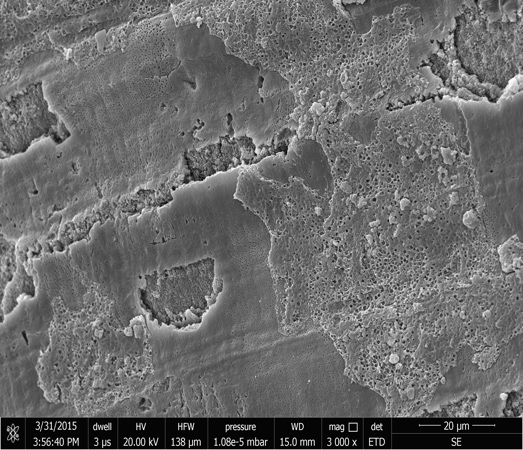
**B**



**E**



**F**



**D**

**C**

**SD1**

**IS**

**IS**

**SD1**

**IS**

**W1**

**W1**

**W1**

**SD1**

**IS**

**IS**

**IS**

**W1**

**Thickening shell**

**Growing shell**

**pH control**

**Moderate pH**

**Low pH**

**Figure 3**. Shell condition in *C. inconspicua* – Examples of SEM micrographs of shell surfaces of thickening shell (A, C, E) and growing shell (B, D, F) in pH control (A, B), moderate pH (C, D) and low pH treatment (E, F). IS = intact shell, W1 = minimal wear, W2 = extensive wear and SD1 = partial shell dissolution. SD2 (extensive shell dissolution) was absent in all treatment in this species. Scale bar = 20 µm.

**Shell thickness.**

***Thickening shell.*** The outer primary layer became progressively thinner in thickening shell as pH reduced in *L. uva* (Figure 4a; Linear Mixed Effects Model; χ2 = 79.72, df = 3, p < 0.001). Simultaneously in this species, the inner secondary layer, and the whole shell, were thicker in the most acidified treatment (Figure 4a; Linear Mixed Effects Model; Secondary Layer - χ2 = 39.63, df = 3, p < 0.001; Total Shell - χ2 = 18.19, df = 3, p < 0.001). Increased temperature had no effect on primary layer, secondary layer or total shell thickness (Tukey; Primary Layer – T = 1.73, p = 0.319; Secondary Layer – T = -1.20, p = 0.627; Total Shell – T = -0.80, p = 0.855). In contrast, neither individual shell layers nor total shell thickness were affected by lowered pH in the thickening shell in *C. inconspicua* (Figure 4c; Linear Mixed Effects Model; Primary Layer - χ2 = 4.17, df = 2, p = 0.124; Secondary Layer - χ2 = 4.80, df = 2, p = 0.091; Total Shell - χ2 = 4.27, df = 2, p = 0.118). Primary layer was thinnest in the oldest part of the shell, the umbo region, across all treatments in both species (Table S3). Secondary layer and total shell thickness did not differ in different places in the thickening shell in each treatment in both species (Table S3). Individual number also had no effect on individual shell layer and total shell thickness in both species (Table S3).

***Growing shell.*** Primary layer, secondary layer and total shell thickness were not impacted by lowered pH in either species (Figure 4b & 4d; Linear Mixed Effects Model; *L. uva*: Primary Layer - χ2 = 3.62, df = 3, p = 0.306; Secondary Layer - χ2 = 6.80, df = 3, p = 0.078; Total Shell - χ2 = 5.26, df = 3, p = 0.154; *C. inconspicua*: Primary Layer - χ2 = 2.63, df = 2, p = 0.268; Secondary Layer - χ2 = 0.82, df = 2, p = 0.663; Total Shell - χ2 = 1.12, df = 3, p = 0.572). Increased temperature also had no effect on either individual shell layers or total shell thickness in *L. uva* (Figure 4b). Primary layer thickness did not differ indifferent places throughout the growing shell in each treatment in either species (Table S3). Secondary layer and the total shell thickness, however, did get progressively thinner with the direction of growth in each treatment in both species (Table S3). Individual number also had no effect on individual shell layer and total shell thickness in growing shell in either species (Table S3).

Both species averages

**Figure 4**. Shell thickness *–* Average primary layer (black bar) and secondary layer (grey bar) thicknesses in thickening shell (a, b) and growing shell (c, d) in *L. uva* (top row) and *C. inconspicua* (bottom row) in all treatments. Whole bars represent total shell thickness.

DISCUSSION

Long-term culturing of polar and temperate brachiopod species’ under predicted end-century acidified conditions revealed that although shell dissolution was minimal in ambient conditions, both were more susceptible with increasing acidity. We have two principal findings: there was significant dissolution in the thickening shell and an unexpected compensation of induced thicker shells.

**Dissolution of shell.** Shell loss has been widely reported in several marine calcifiers, however, these have largely been those which use higher solubility polymorphs of calcium carbonate (i.e. aragonite), such as corals[55-57](#_ENREF_55) and molluscs[21](#_ENREF_21),[58-61](#_ENREF_58), high-magnesium calcite including coralline algae[62](#_ENREF_62),[63](#_ENREF_63) and echinoderms[64](#_ENREF_64),[65](#_ENREF_65). Fewer studies have investigated shell dissolution in taxa which are entirely constructed of the lower solubility polymorph, low-magnesium calcite, such as rhynchonelliform brachiopods. Previously, the only other ocean acidification study assessing dissolution in brachiopods was conducted on dead shells[35](#_ENREF_35)*.* Working on *L. uva,* they showed deterioration of the primary layer after only 35 days exposure to pH 7.4, which after 56 days exposed the fibres of the secondary layer below. This is the same dissolution pattern reported here in experiments involving live individuals after 7 months exposure to pH 7.54. Exposure of the secondary layer calcite fibres may compromise shell integrity and probably strength due to the loss of the hard outer protective primary layer[35](#_ENREF_35),[66](#_ENREF_66).

Dissolution was more extensive in the polar *L. uva* than the temperate *C. inconspicua,* as indicated by increased deterioration in the primary layer of *L. uva* compared to *C. inconspicua* in the moderate pH treatment and it was only in *L. uva* that the secondary layer was exposedin the low pH treatment. Antarctic calcified invertebrates are probably the most vulnerable organisms to ocean acidification for a number of reasons: they tend to be weakly calcified[16](#_ENREF_16),[67](#_ENREF_67); dissolution rates of calcium carbonate are inversely related to temperature[68](#_ENREF_68); and the polar regions are predicted to become the first to be undersaturated in aragonite by 2050 and calcite by 2100[37](#_ENREF_37),[69-72](#_ENREF_69). Differences in dissolution between the two investigated species may have also been influenced by the state of the shells. Wear was more prominent in the thickening shell of *L. uva* than in *C. inconspicua,* which was most likely due to the longer lifespan of the polar brachiopod (up to 55-60 years)[73](#_ENREF_73) compared to the temperate brachiopod (up to 14 years)[74](#_ENREF_74). Thus the shells of *L. uva* had been exposed to wear for a longer time in their natural environment before the experiment began. Such wear will have damaged or removed periostracum, which is key in protecting the animal from shell dissolution[29](#_ENREF_29),[75-80](#_ENREF_75). Since periostracum is only formed at the growing edge of the mantle, it cannot be repaired if damaged or lost from the surface of the shell. Thinning or loss of this organic layer through physical or biotic abrasion and epibiont erosion, therefore, restricts protection from corrosive acidified waters. The periostracum in brachiopods is < 1 µm thick[81](#_ENREF_81) and so is very vulnerable to loss.

Newly formed growing shell was mainly characterised by intact shell in both species. Partial shell dissolution did occur, however, in the most acidified treatment in both species albeit at a much lower level than in the thickening shell. Damage to the ultrathin periostracum from abrasion of other brachiopods in their conspecific cluster, natural decay of this outer layer or potentially the lowered pH conditions could have either softened the periostracum itself or disrupted the protective function of the periostracum. This latter possibility was suggested for external dissolution reported in newly formed shell in *M. edulis* after 2 months exposure to 1400 µatm and 4000 µatm[77](#_ENREF_77). Disintegration of organic matrix in the shell rather than corrosion of crystals could have caused this shell degradation, as seen in spirorbirds after 100-day exposure to pH 7.7 conditions[82](#_ENREF_82).

Temperature had no clear effect on shell dissolution or thickness in *L. uva* as indicated by the lack of or only minimal primary layer dissolution and no change in any thickness measurement in both thickening and growing shell in the temperature control (held at 0°C – current average Antarctic summer temperatures) and the pH control (kept at the 2°C temperature increase predicted for 2100). In contrast, temperature and not acidification reduced shell strength in *M. edulis* after 6 months exposure to forecasted end-century pH and warming conditions[83](#_ENREF_83). It was concluded that warming had an indirect effect on shell strength by shifting the energy budget from shell deposition to increased maintenance costs. Food availability was limited throughout the experiment, which would likely have enhanced the temperature effect as low food levels can reduce shell growth and significantly influence the amount of inner shell dissolution in *M. edulis* after 7 weeks exposure to varying *p*CO2 levels[84](#_ENREF_84). This highlights the necessity of using multistressors in ocean acidification research to better understand the abilities of marine calcifiers to maintain shell integrity under future predicted environmental conditions.

**Compensation.** Despite the widely reported significant effects of dissolution on marine calcifiers in ocean acidification research, very few studies investigate organisms’ abilities to compensate for shell loss. New shell deposited by *M. edulis* after 9 months exposure to 750 *µ*atm and 1000 *µ*atm *p*CO2 was rounder and flatter with a thinner aragonite layer than shell produced in ambient conditions of 380 µatm[25](#_ENREF_25). The authors attributed this new shell shape to a compensatory mechanism to enhance protection from predators and changing environments as these mussels were unable to grow thicker shells in high *p*CO2 conditions. Shell thickening has occurred in response to biotic shell loss by endoliths and other conspecifics grazing on their external shell in Patellid limpets, *Patella granatina* and *P. argenvillei*[85](#_ENREF_85), and to abiotic shell loss by physical impacts from ice in the Antarctic limpet *Nacella concinna*[86](#_ENREF_86). Decreased shell thickness has also been reported in molluscs in lowered pH conditions, due to internal dissolution of the highly soluble aragonite layer[25](#_ENREF_25),[76](#_ENREF_76),[84](#_ENREF_84). For compensatory mechanisms to succeed, they must occur at faster rates than the deleterious effect. Thicker basal shells were reported in the barnacle *Amphibalanus amphitrite* under lowered pH conditions (pH 7.4), however, this compensation calcification was insufficient as dissolution weakened shells faster than it was deposited[87](#_ENREF_87). A pteropod specimen collected from the Fram Strait in the Arctic Ocean also produced a shell four times thicker than the original shell in response to mechanical and dissolution damage from undersaturated waters[29](#_ENREF_29).

Extensive shell dissolution at low pH in thickening shell ofthe polar brachiopod led to a drastic decrease in primary layer thickness. *L. uva* counteracted this chemical attack by laying down more secondary layer on the internal surface of the shell, which resulted in increased overall shell thickness during the experimental period. The less extensive dissolution in *C. inconspicua* was reflected by no clear impact of acidified conditions on either total shell or individual shell layer thicknesses. Our findings appear to contrast with reports of primary layer thickening in the Chilean terebratulide *Magellania venosa* after being cultured in pH 7.35 conditions[88](#_ENREF_88), however, their observations appear to be based on only one measured specimen in both the acidified treatment and the control.

Compensatory mechanisms must also be sufficient in maintaining an organism’s overall performance. The secondary layer of terebratulide brachiopod shells is softer than the harder protective primary layer[34](#_ENREF_34),[89](#_ENREF_89) raising the question of whether a shell made solely out of secondary layer would provide adequate protection to ensure survival. No external dissolution of the exposed secondary layer of *L. uva* was observed perhaps due to protection from the organic matrix shrouding calcite crystals of this innermost fibrous shell layer[90](#_ENREF_90),[91](#_ENREF_91). Primary layer is often missing in older parts of brachiopod shells or in older individuals[88](#_ENREF_88), therefore, a thicker shell consisting of only secondary layer could provide sufficient protection in predicted pH conditions expected by 2100, although this warrants further investigation.

Total shell thickness or individual shell layer thickness of growing shell of *L. uva* and *C. inconspicua* were not affected by predicted end-century acidified conditions. In a previous study, shell thickness in *C. inconspicua* did not vary over the last 120 years despite a 0.1 pH unit decrease and 2°C increase in temperature since the Industrial Revolution[92](#_ENREF_92). The resilience of shell thickness in *L. uva* and *C. inconspicua* to past and predicted environmental change, in addition to their unaffected shell growth rates under end-century pH levels[6](#_ENREF_6),[7](#_ENREF_7), indicates the robust ability of rhynchonelliform brachiopods to construct shell under acidified conditions. The thickness of calcite and aragonite layers in newly formed shell of *M. edulis* were also not affected by elevated *p*CO2[77](#_ENREF_77). This lack of variation in shell thickness to acidified conditions in newly produced shell further demonstrates the increase of shell thickness in the thickening shell is a compensatory response to extensive shell dissolution occurring at the external shell surface, although the mechanisms whereby the brachiopods identify the shell is thinning remain to be elucidated.

The extent of vulnerability of two highly calcium-carbonate-dependent species to dissolution in acidified seawater is concerning. Without any counteracting response, dissolution may compromise shell integrity leading to reduced protection and decreased suitability of brachiopod shells as a habitat for other marine organisms. Physiological acclimatisation is one approach organisms can utilise to cope with such threats in the challenging conditions predicted by 2100. We identified induced shell thickening forming thicker shells in polar *L. uva* as a compensatory mechanism to extensive shell dissolution under lowered pH levels. The less extensive dissolution in *C. inconspicua* was probably a function of higher temperatures in the temperate study and the corresponding lower CaCO3 solubility. This suggests that the level of dissolution in *C. inconspicua* after 3 months exposure to predicted end-century pH conditions did not induce similar compensation. This induced shell thickening could come at an overall cost to the organism as calcification is energetically expensive, involving the accumulation, transportation and precipitation of calcium carbonate as seen in the ophiuroid *Amphiura filiformis*[93](#_ENREF_93)*.* Acidification also significantly increases the proportion of the animal’s energy budget that needs to be devoted to shell production[94](#_ENREF_94), therefore, there may be long-term impacts on life histories and maintenance of populations. Long-term experiments investigating the capacity of organisms to acclimatise and possibly adapt to future change is crucial to further our understanding of how marine organisms will cope with future environmental change.

ASSOCIATED CONTENT

**Supporting Information**

Descriptions and examples of each shell condition (Table S1)

Schematic and example of shell condition index measurements (Figure S1)

Schematic and example of shell thickness measurements (Figure S2)

Shell dissolution additional statistical results (Table S2)

Shell thickness additional statistical results (Table S3)

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DISCLOSURES

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