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Thicker shells compensate extensive dissolution in brachiopods under future ocean acidification

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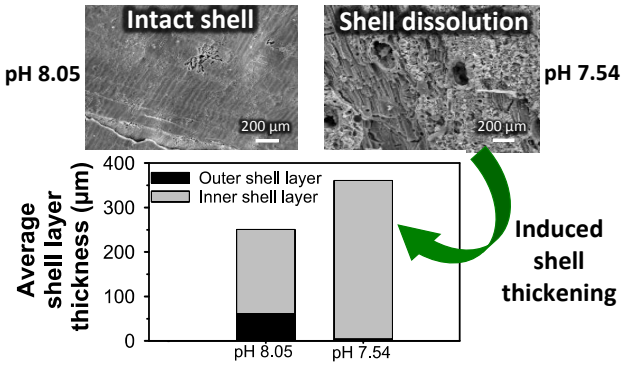
KEYWORDS: climate change, global warming, shell dissolution, shell thickness, compensatory mechanisms, phenotypic plasticity, *Calloria inconspicua*, *Liothyrella uva*, Terebratulide

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 dissolution and 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 in the polar species. This impact was reflected in decreased outer primary layer thickness in the polar brachiopod. A compensatory response of increasing inner secondary layer thickness, and thereby producing a thicker shell was exhibited by the polar species. Less extensive dissolution in the temperate brachiopod 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

35 calcification providing insights into how similar species might cope under future
36 environmental change.

INTRODUCTION

Changing environments pose serious risks to organisms that cannot shift their geographic range, physiologically acclimatise or genetically adapt[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]. More recently, however, there has been an increase in long-term (many months to years) studies that demonstrate surprising capacities of marine organisms to acclimate[6-10], or even adapt in organisms with short generation times[11-13] 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].

Marine calcifiers are considered the most vulnerable organisms to ocean acidification due to the combination of dissolution and the reduction in carbonate ions making shell production more difficult and energetically expensive[2,15,16]. The Southern Ocean has naturally low carbonate ion saturation levels compared to temperate and tropical regions due to carbon dioxide being more soluble in cold water[17]. Acid-base coefficients are also more sensitive in cold temperatures making this high latitude region a forerunner of biological ocean acidification impacts for other oceans[18]. The external skeleton is crucial for protecting animal tissue in shell-bearing organisms against predation, infection and loss of bodily fluids[19,20]. 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[21,22]. 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[23,24]. Predicted environmental conditions for 2100 will shift surface seawater carbonate chemistry to favour $CaCO_3$ 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[25] and changing environmental conditions[14,26-28]. These include shell thickening, production of a more rotund shell and increased shell growth rates through plasticity in producing different calcium carbonate polymorphs[14,25-32]. Production of a thicker periostracum could also withstand more wear and deter dissolution[33]. 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[34,35]. 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[36,37]. In previously published work we showed that shell growth rates of *L. uva* Broderip, 1833 (which we refer to as "polar brachiopod") and *C. inconspicua* Sowerby, 1846 (which we refer to as "temperate brachiopod") were not impacted by predicted end-century seawater pH's[6,7]. Another study demonstrated increased dissolution in the polar brachiopod in pH 7.4 conditions after 14 days[38], however, 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 both brachiopods and also under increased temperature in the polar brachiopod.

103 MATERIALS AND METHODS

104

105 **Sampling collection.** Specimens of the polar brachiopod were hand collected by
106 SCUBA divers from Trolval Island, Ryder Bay, Antarctica (67° 35.44' S, 68° 12.44' W)
107 at 15-25 m depth in May 2012. Environmental conditions in Ryder Bay at 15-25 m
108 depth consist of seawater temperatures that range from -1.8 to +1.5°C, however,
109 temperatures rarely exceed +1.0°C and salinity is 33.0-34.0[39] and the pH range is
110 8.04-8.10[40]. Brachiopods were kept in recirculating aquaria (0.0 ± 0.5°C) whilst being
111 transported by ship back to the British Antarctic Survey, Cambridge, UK where the
112 polar experiment was conducted.
113 Individuals of the temperate brachiopod were hand collected at low tide from
114 under rocks in Portobello Bay, Otago Harbour, New Zealand (45° 82.00'S, 170°
115 70.00'E) in January 2013. Environmental conditions in Otago Harbour are surface
116 seawater temperatures of 6.4-16.0°C[41,42], pH range of 8.10-8.21 (K. Currie, pers.
117 comm.) and salinity is 32.5-34.8[42]. Brachiopods were kept in seawater during the
118 short transportation to Portobello Marine Laboratory, Otago Harbour, New Zealand
119 where the temperate experiment was performed.

120

121 **Experimental Design.**

122 ***Polar experiment.*** The polar experiment was conducted in a temperature-
123 controlled recirculating CO_2 microcosm with four treatments[6]. Two were acidified
124 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[43] (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[44]. The fourth treatment was a temperature control which was held at the present-day average conditions for Ryder Bay[45] (SST: 0°C, pH: 7.98 ± 0.02). The pH of the acidified treatments was controlled by intermittently bubbling CO_2 gas into a header tank. Seawater was then gravity fed into the experimental tanks[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 pH_{NIST} (Aquamedic pH controlled computer and electrode system) were monitored and recorded daily. Salinity (Tropical Marine Centre V2 Handheld refractometer), TCO_2 (mmol L^{-1}; Ciba Corning TCO_2 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 CO_2 (pCO_2) and the saturation values for calcite (Ω_C) and aragonite (Ω_A), were modelled from applying TCO_2 and pH_{NIST} data to the program CO2SYS[46] with refitted constants[47,48]. Brachiopods in each

147 treatment were fed weekly with microalgal concentrate of approximately 331 x 10^4
148 cells L^{-1}, which is within the natural seasonal range of phytoplankton cell abundance
149 along the west Antarctic Peninsula (62–1150 x 10^4 cells L^{-1}) [49,50].

150 ***Temperate experiment.*** The temperate experiment was conducted in a flow-
151 through CO_2 perturbation system with three treatments[7]. Two were acidified
152 treatments ("Moderate pH" – pH 7.79 ± 0.06 and "Low pH" – pH 7.62 ± 0.05) and the
153 third was a pH control (8.16 ± 0.03). The pH of the acidified treatments was lowered
154 in header tanks by intermittently bubbling CO_2 gas before being gravity fed into the
155 replicate experimental tanks[7]. The pH control system had an identical set up except
156 that it lacked CO_2 injection, and air was injected into the header tank. Seawater
157 temperature was not manipulated and was ambient for Otago Harbour.

158 Seawater temperatures (°C, Digital Testo 106) and pH_{NIST} were measured three
159 times a day and salinity (YSI data logger) was measured once a week. Dissolved
160 inorganic carbon (DIC) and total alkalinity (A_T) were analysed at the beginning,
161 middle and end of the experiment by a Single Operator Multi-parameter Metabolic
162 Analyser (SOMMA) and closed-cell potentiometric titration, respectively[51]. Other
163 carbonate system parameters, including the partial pressure of CO_2 (pCO_2) and the
164 saturation values for calcite (Ω_C) and aragonite (Ω_A) were calculated using CO2SYS[46]
165 with CO_2 equilibrium constants[47,48,52]. Brachiopods were fed three times a week with
166 microalgal concentrate of approximately 397 x 10^4 cells mL^{-1} of *Tetraselmis* spp.,
167 which is within the natural summer range of phytoplankton cell abundance in Otago
168 Harbour.

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

Experiment	Treatment	pH_{NIST}	Temperature (°C)	Salinity	pCO_2 (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 pCO_2, Ω calcite and Ω aragonite were calculated from CO2SYS[46] with refitted constants[47,48].

Shell condition index. Shell lengths were measured at the start and end of each experiment using Vernier calipers (± 0.1 mm) to determine shell laid down in the natural environment that thickens from the internal surface as brachiopods grow (which we refer to as "thickening shell") and shell growth extension during the experiments (which we refer to as "growing shell"). Mean lengths (± S.E.) of these two shell regions from each treatment are reported in Table S1. Scanning Electron Microscopes (JEOL 820 for the polar brachiopod and FEI QEMSCAN 650F for the temperate brachiopod; 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 treatment of both species to 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 S2. Micrographs (1 mm x 1 mm) were collected at five standardised areas in thickening shell (areas located from umbo region towards anterior margin as detailed in Fig. S1A) and five standardised areas in growing shell (areas evenly spread in anterior margin as detailed in Fig. S1A). 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[53]. Thickness measurements (± 0.1 mm) of the primary layer, secondary layer and total shell were then measured from three areas of thickening shell (umbo region, middle of the shell

and nearer experimental growth as detailed in Fig. S2) and three areas of growing shell (oldest experimental growth to newest experimental growth in the anterior margin as detailed in 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:

$$\text{Thickness measurement} = \text{Treatment} + \text{Shell Position} + (1 \mid \text{Individual Number}) + \text{error}$$

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[54] with the *FSA* package[55] used for the Kruskal-Wallis and post-hoc Dunn's tests, the *lme4* package[56] for the linear mixed effects models and the *emmeans* package[57] for the post-hoc Tukey tests.

238 RESULTS

239

240 **Shell condition index.**

241 ***Thickening shell.*** Intact shell (IS) was absent from both acidified treatments and
242 only present in < 8.4 ± 4.5% (mean ± SE) of both controls in the thickening shell in the
243 polar brachiopod (Figure 1a & Figure 2). Instead, minimal wear (W1) dominated this
244 region in both controls (Figure 2a; 71.0 ± 6.2% in pH control and 65.1 ± 4.1% in
245 temperature control). Partial shell dissolution (SD1), however, was the most
246 prominent shell condition in both acidified treatments (Figure 1a; 64.3 ± 4.6% in
247 moderate pH and 71.7 ± 4.3% in low pH). With decreasing pH, the percentage area
248 of partial shell dissolution increased (Figure 1a & Figure 2; Kruskal-Wallis: $H = 70.93$,
249 $p < 0.001$). The extent of shell dissolution in the polar brachiopod also increased with
250 decreasing pH (Kruskal-Wallis: $H = 42.38$, $p < 0.001$), with 18.2 ± 4.5% of shell
251 exhibiting exposed secondary layer in the low pH treatment (SD2) compared to 0.9 ±
252 0.4% in the moderate pH treatment and the secondary layer never being exposed in
253 either control. Temperature had no effect on shell dissolution or wear (Figure 1a;
254 Dunn's Test: SD1 – T = 1.16, $p = 0.244$, SD2 – T = 0.26, $p = 0.795$, W1 – T = 0.25, $p =$
255 0.805, W2 – T = 1.60, $p = 0.109$). In contrast to the polar brachiopod, thickening shell
256 of the temperate brachiopod was mainly characterised by intact shell (Figure 1c; IS;
257 56.6 - 82.3%) across all treatments. Amounts of minimal wear (W1) decreased with
258 decreasing pH in this shell region in the temperate brachiopod (Figure 1c; Kruskal-
259 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 the temperate brachiopod. Shell dissolution in this temperate species was less extensive than for the polar species (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 S3).

Growing shell. Growing shell in both species was mainly characterised by intact shell (IS) in all treatments (Figure 1b, d; polar brachiopod: > 63.9 ± 4.7%; temperate brachiopod: > 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: polar brachiopod - $H = 41.81$, $p < 0.001$; temperate brachiopod – $H = 20.96$, $p < 0.001$). Partial shell dissolution (SD1) increased with increasing acidity in the experimental growth of the polar brachiopod (Figure 1b & Figure 2; Kruskal-Wallis: polar brachiopod - $H = 63.08$, $p < 0.001$). 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 the temperate brachiopod (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 the polar

282 brachiopod and was absent from the temperate brachiopod. Extensive wear (W2)
283 was present in higher levels in the control treatments of both species than in the
284 acidified treatments (Figure 1c, d; Kruskal-Wallis; polar brachiopod – $H = 43.98$, $p <$
285 0.001, temperate brachiopod - $H = 10.67$, $p < 0.001$), however, only in low levels (<
286 11.1 ± 1.2%). Neither shell position nor individual number affected any shell
287 condition in the growing shell of both species (Table S3).
288

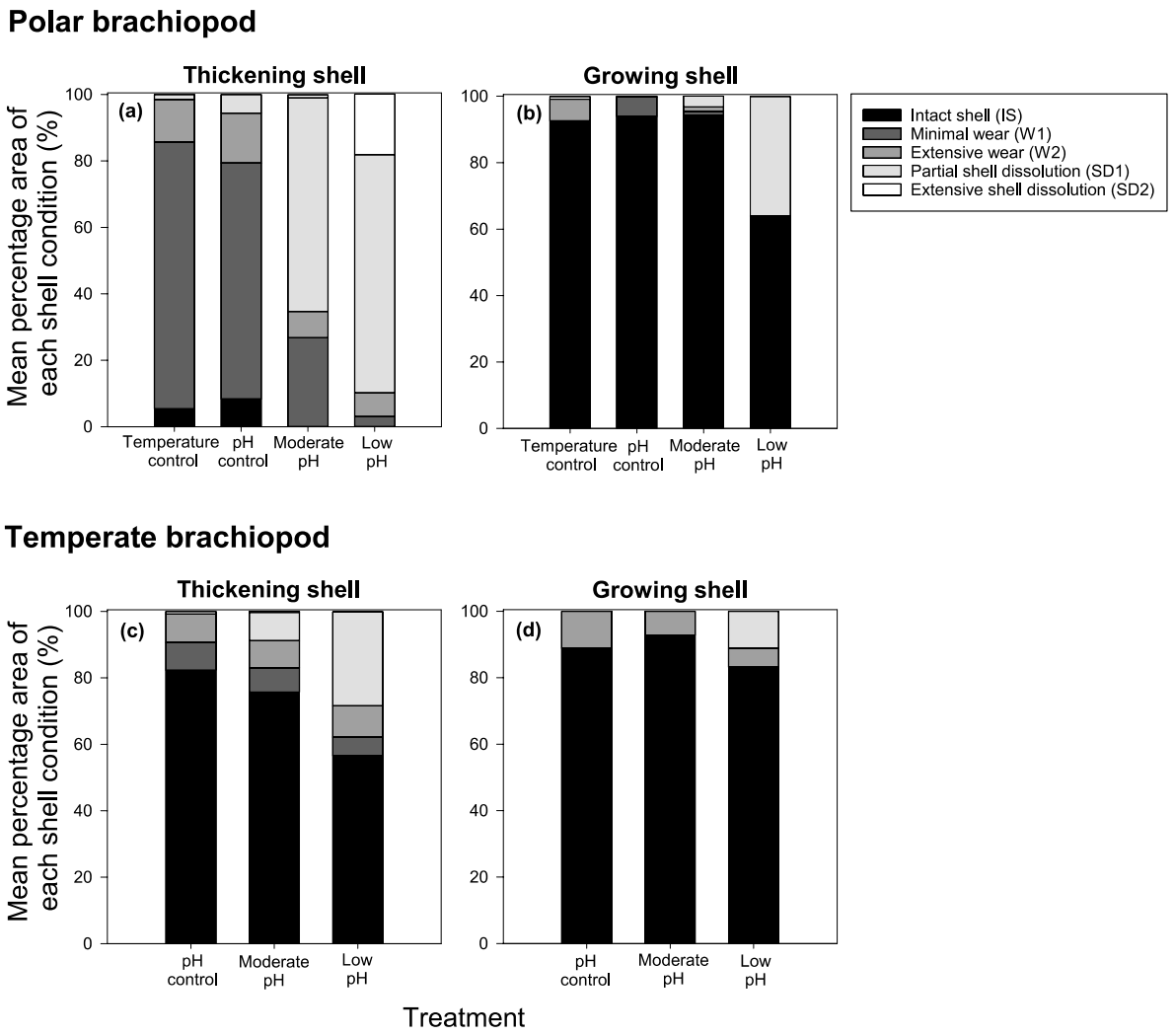
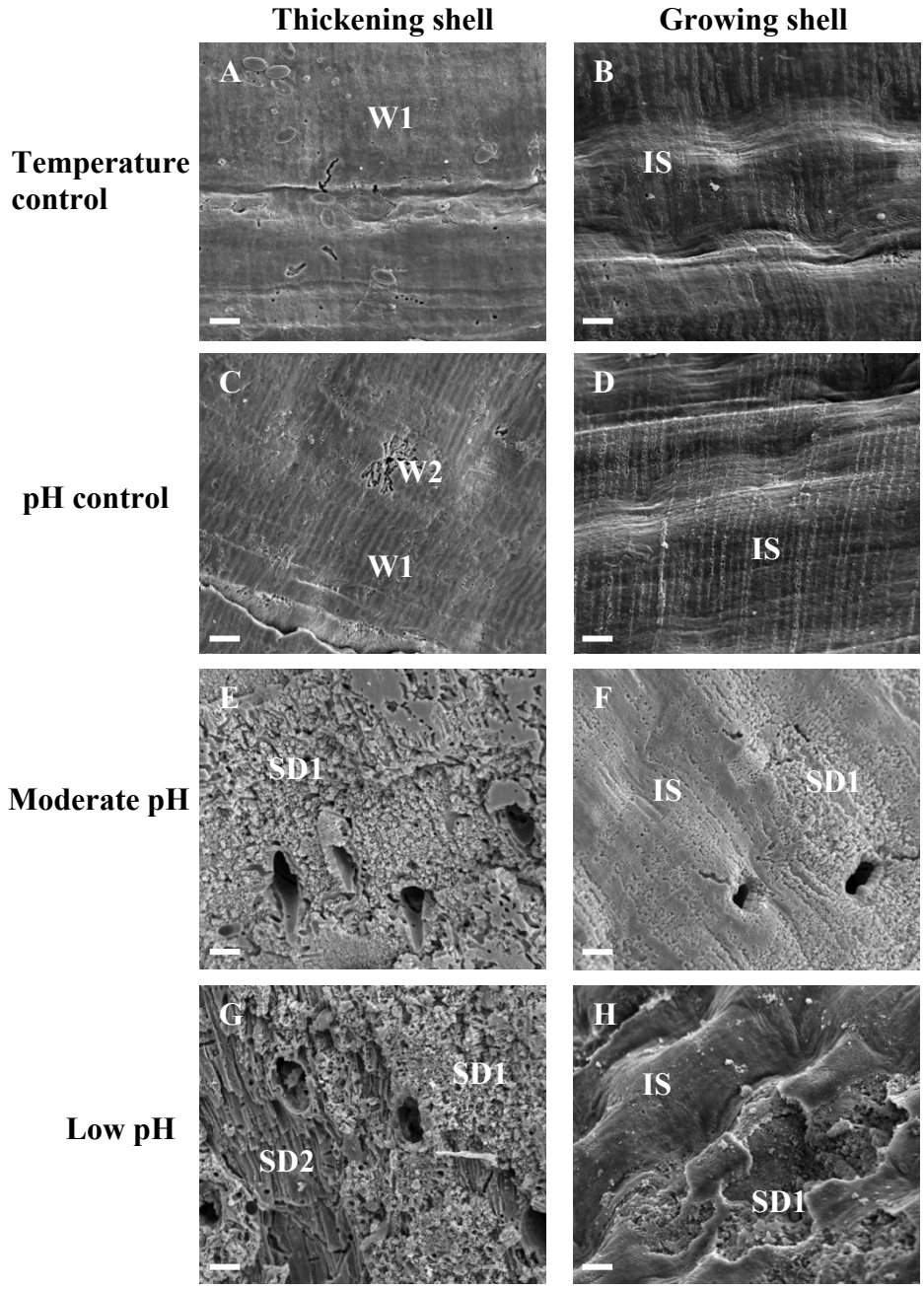


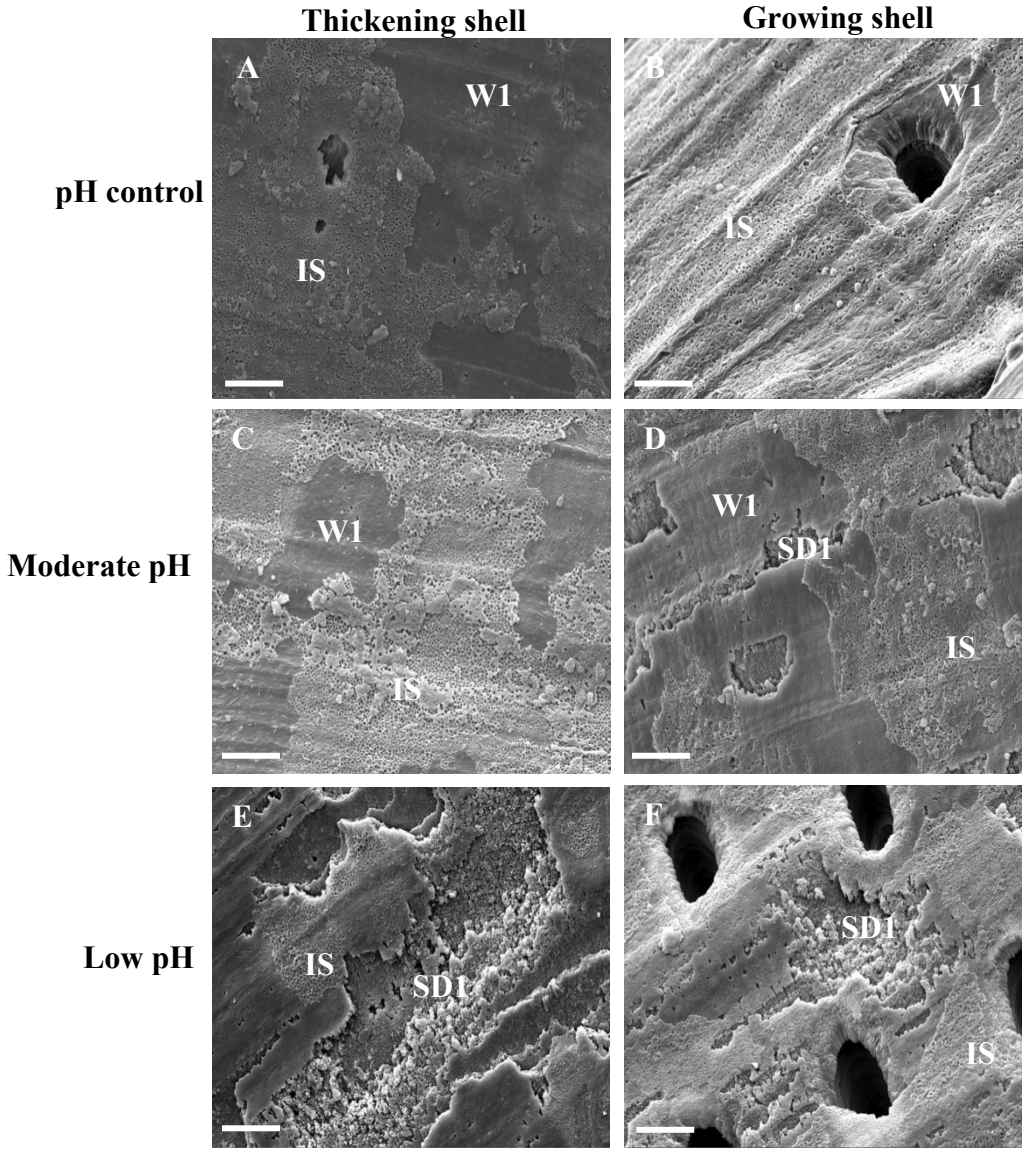
Figure 1. Representative shell condition - Mean percentage area of the different types of shell condition from five standardised areas in thickening shell (a, c) and five standardised areas in growing shell (b, d) in the polar brachiopod (top row, n = 5 per treatment) and in the temperate brachiopod (bottom row, n = 5 per treatment) in all treatments. Lighter grey tones indicate an increase in wear and/or shell dissolution (see legend).



296

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

302



303

304 **Figure 3**. Representative shell condition in the temperate brachiopod – Examples of

305 SEM micrographs of shell surfaces of thickening shell (A, C, E) and growing shell (B,

306 D, F) in pH control (A, B), moderate pH (C, D) and low pH treatment (E, F). IS =

307 intact shell, W1 = minimal wear, W2 = extensive wear and SD1 = partial shell

308 dissolution. SD2 (extensive shell dissolution) was absent in all treatment in this

309 species. Scale bar = 20 μm.

Shell thickness.

Thickening shell. The outer primary layer became progressively thinner in thickening shell as pH reduced in the polar brachiopod (Figure 4a; Linear Mixed Effects Model; $\chi^2 = 79.72$, df = 3, $p < 0.001$). Secondary layer thickened in all treatments as this inner shell layer transitioned from the region of growing shell to thickening shell as the brachiopod grew (larger grey bars in Figure 4a vs smaller grey bars in Figure 4b). This inner secondary layer, and the whole shell, however, were thicker in the most acidified treatment in the thickening shell (Figure 4a; Linear Mixed Effects Model; Secondary Layer - $\chi^2 = 39.63$, df = 3, $p < 0.001$; Total Shell - $\chi^2 = 18.19$, 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 the temperate brachiopod (Figure 4c; Linear Mixed Effects Model; Primary Layer - $\chi^2 = 4.17$, df = 2, p = 0.124; Secondary Layer - $\chi^2 = 4.80$, df = 2, p = 0.091; Total Shell - $\chi^2 = 4.27$, 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 S4). Secondary layer and total shell thickness did not differ in different places in the thickening shell in each treatment in both species (Table S4). Individual number also had no effect on individual shell layer and total shell thickness in both species (Table S4).

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; polar brachiopod: Primary Layer - $\chi^2 = 3.62$, df = 3, p = 0.306; Secondary Layer - $\chi^2 = 6.80$, df = 3, p = 0.078; Total Shell - $\chi^2 = 5.26$, df = 3, p = 0.154; temperate brachiopod: Primary Layer - $\chi^2 = 2.63$, df = 2, p = 0.268; Secondary Layer - $\chi^2 = 0.82$, df = 2, p = 0.663; Total Shell - $\chi^2 = 1.12$, df = 3, p = 0.572). Increased temperature also had no effect on either individual shell layers or total shell thickness in the polar brachiopod (Figure 4b). Primary layer thickness did not differ indifferent places throughout the growing shell in each treatment in either species (Table S4). Secondary layer and the total shell thickness, however, did get progressively thinner with the direction of growth in each treatment in both species (Table S4). Individual number also had no effect on individual shell layer and total shell thickness in growing shell in either species (Table S4).

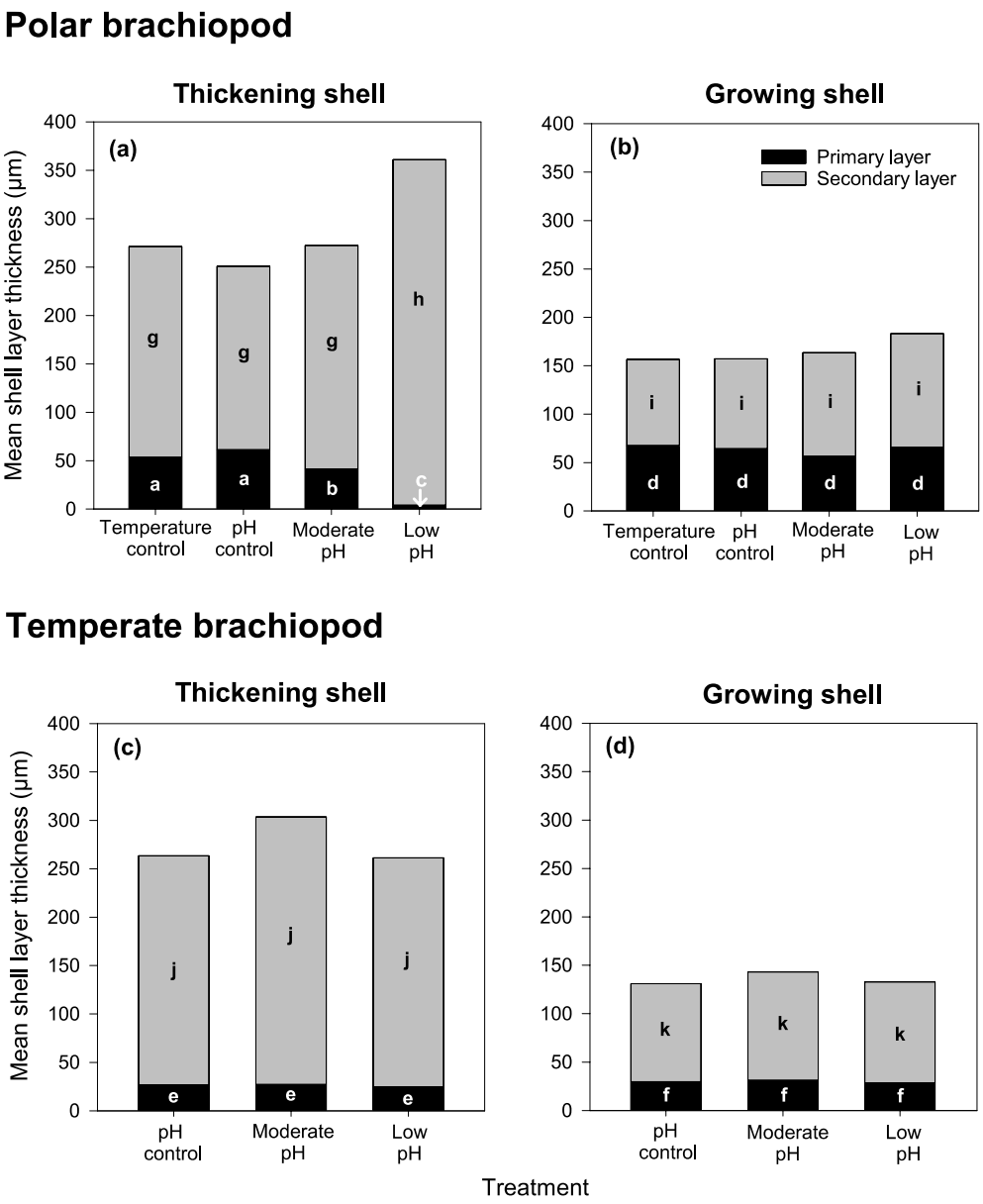


Figure 4. Shell thickness – Mean primary layer (black bar) and secondary layer (grey bar) thicknesses from three areas in the thickening shell (a, b) and from three areas in the growing shell (c, d) in the polar brachiopod (top row, n = 5 per treatment) and in the temperate brachiopod (bottom row, n = 5 per treatment) in all treatments. Whole bars represent total shell thickness. Lowercase letters a-f indicate significant differences in primary layer thickness and g-k represent significant differences in secondary layer and total shell thicknesses between treatments in each shell region

in each species. Comparisons were made only within shell region not between shell regions or between species.

DISCUSSION

Long-term culturing of a polar and a temperate brachiopod under predicted end-century acidified conditions revealed that both species were more susceptible to shell dissolution with increasing acidity. Our two principal findings are significant dissolution and an unexpected compensation of induced thicker shells in the thickening shell.

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[58-60] and molluscs[23,32,61-64], high-magnesium calcite including coralline algae[65,66] and echinoderms[67,68]. 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[38]. Working on the polar species, 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

374 secondary layer calcite fibres may compromise shell integrity and probably strength
375 due to the loss of the hard outer protective primary layer[38,69].

376 Dissolution was more extensive in the polar than in the temperate brachiopod, as
377 indicated by increased deterioration in the primary layer of the polar species
378 compared to the temperate brachiopod in the moderate pH treatment and it was
379 only in the polar species that the secondary layer was exposed in the low pH
380 treatment. Antarctic calcified invertebrates are probably the most vulnerable
381 organisms to ocean acidification for a number of reasons: they tend to be weakly
382 calcified[16,70]; dissolution rates of calcium carbonate are inversely related to
383 temperature[17]; and the polar regions are predicted to become the first to be
384 undersaturated in aragonite by 2050 and calcite by 2100[18,40,71-74]. Both the moderate
385 pH and the low pH treatment in the polar experiment were undersaturated with
386 respect to calcite, however, both the acidified treatments in the temperate
387 experiment were not undersaturated with respect to calcite. This could explain the
388 differences in the extent of dissolution present between both investigated species.
389 The state of the shells could have also influenced these species differences. Wear was
390 more prominent in the thickening shell of the polar brachiopod than in the
391 temperate brachiopod, which was most likely due to the longer lifespan of the polar
392 species (up to 55-60 years)[75] compared to the temperate species (up to 14 years)[76].
393 Thus the shells of the polar brachiopod had been exposed to wear for a longer time
394 in their natural environment before the experiment began. Such wear will have
395 damaged or removed periostracum, which is key in protecting the animal from shell

dissolution[31,77-82]. 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[83] 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[79]. Disintegration of organic matrix in the shell rather than corrosion of crystals could have caused this shell degradation, as seen in spirorbids after 100-day exposure to pH 7.7 conditions[84].

Temperature had no clear effect on shell dissolution or thickness in the polar brachiopod 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.*

418 *edulis* after 6 months exposure to forecasted end-century pH and warming
419 conditions[85]. It was concluded that warming had an indirect effect on shell strength
420 by shifting the energy budget from shell deposition to increased maintenance costs.
421 Food availability was limited throughout the experiment, which would likely have
422 enhanced the temperature effect as low food levels can reduce shell growth and
423 significantly influence the amount of inner shell dissolution in *M. edulis* after 7 weeks
424 exposure to varying pCO_2 levels[86]. This highlights the necessity of using
425 multistressors in ocean acidification research to better understand the abilities of
426 marine calcifiers to maintain shell integrity under future predicted environmental
427 conditions.

428 **Compensation.** Despite the widely reported significant effects of dissolution on
429 marine calcifiers in ocean acidification research, very few studies investigate
430 organisms' abilities to compensate for shell loss. New shell deposited by *M. edulis*
431 after 9 months exposure to 750 μatm and 1000 μatm pCO_2 was rounder and flatter
432 with a thinner aragonite layer than shell produced in ambient conditions of 380
433 μatm[27]. The authors attributed this new shell shape to a compensatory mechanism to
434 enhance protection from predators and changing environments as these mussels
435 were unable to grow thicker shells in high pCO_2 conditions. Shell thickening has
436 occurred in response to biotic shell loss by endoliths and other conspecifics grazing
437 on their external shell in Patellid limpets, *Patella granatina* and *P. argenvillei*[87], and to
438 abiotic shell loss by physical impacts from ice in the Antarctic limpet *Nacella*
439 *concinna*[88]. Decreased shell thickness has also been reported in molluscs in lowered

pH conditions, due to internal dissolution of the highly soluble aragonite layer[27,78,86]. 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[89]. 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[31].

Extensive shell dissolution at low pH in thickening shell of the polar brachiopod led to a drastic decrease in primary layer thickness. The polar species 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 the temperate brachiopod 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[90], 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[37,91] raising the question of whether a shell made solely out of secondary layer would provide adequate protection to ensure

462 survival. No external dissolution of the exposed secondary layer of the polar
463 brachiopod was observed perhaps due to protection from the organic matrix
464 shrouding calcite crystals of this innermost fibrous shell layer[92,93]. Primary layer is
465 often missing in older parts of brachiopod shells or in older individuals[90], therefore,
466 a thicker shell consisting of only secondary layer could provide sufficient protection
467 in predicted pH conditions expected by 2100. Although, ocean acidification impacts
468 on brachiopod shell strength warrant further investigation.

469 Total shell thickness or individual shell layer thickness of growing shell of both
470 species were not affected by predicted end-century acidified conditions. Shell
471 thickness, therefore, is only impacted by lowered pH when extensive shell
472 dissolution occurs. In a previous study, shell thickness in the temperate brachiopod
473 did not vary over the last 120 years despite a 0.1 pH unit decrease and 2°C increase
474 in temperature since the Industrial Revolution[94]. Forecasted acidified conditions by
475 2100 also did not impact shell growth rates and the ability to shell repair in both the
476 polar and temperate brachiopod[6,7]. The resilience of shell thickness in both the polar
477 and temperate species to past and predicted environmental change, in addition to
478 their unaffected shell growth rates under end-century pH levels[6,7], indicates the
479 robust ability of rhynchonelliform brachiopods to construct shell under acidified
480 conditions. The thickness of calcite and aragonite layers in newly formed shell of *M.*
481 *edulis* were also not affected by elevated pCO_2[79]. This lack of variation in shell
482 thickness to acidified conditions in newly produced shell further demonstrates the
483 increase of shell thickness in the thickening shell is a compensatory response to

484 extensive shell dissolution occurring at the external shell surface, although the
485 mechanisms whereby the brachiopods identify the shell is thinning remain to be
486 elucidated.

487 The extent of vulnerability of two highly calcium-carbonate-dependent species to
488 dissolution in acidified seawater is concerning. Without any counteracting response,
489 dissolution may compromise shell integrity leading to reduced protection and
490 decreased suitability of brachiopod shells as a habitat for other marine organisms.
491 Physiological acclimatisation is one approach organisms can utilise to cope with
492 such threats in the challenging conditions predicted by 2100. We identified induced
493 shell thickening forming thicker shells in the polar brachiopod as a compensatory
494 mechanism to extensive shell dissolution under lowered pH levels. The less
495 extensive dissolution in the temperate species was probably a function of higher
496 temperatures in the temperate study and the corresponding lower $CaCO_3$ solubility.
497 This suggests that the level of dissolution in the temperate brachiopod after 3
498 months exposure to predicted end-century pH conditions did not induce similar
499 compensation. This induced shell thickening could come at an overall cost to the
500 organism as increased shell production is energy-demanding, involving the
501 accumulation, transportation and precipitation of calcium carbonate as well as the
502 production of the organic matrix[95,96]. Acidification also significantly increases the
503 proportion of the animal's energy budget that needs to be devoted to shell
504 production[97], therefore, there may be long-term impacts on life histories and
505 maintenance of populations. Long-term experiments investigating the capacity of

506 organisms to acclimatise and possibly adapt to future change is crucial to further our
507 understanding of how marine organisms will cope with future climate change.
508 Marine organisms may also adjust physiological, behavioural or ecological traits as
509 additional compensatory responses to their changing habitats. As well as direct
510 effects on energy budgets (e.g. induced shell thickening), ocean acidification could
511 also have indirect impacts through the alteration of their resource quality (e.g.
512 energy intake)[98]. To maintain organismal homeostasis in varying environments,
513 individuals may compensate by modifying the quality and quantity of food
514 consumed, which in turn could also stabilise community productivity[99]. Multiple
515 compensatory mechanisms could be paramount to maintain overall performance of
516 organisms and subsequently sustain key community processes under future
517 environmental change.
518
519 ASSOCIATED CONTENT
520 **Supporting Information**

521 Mean lengths (±S.E) of thickening and growing shell regions (Table S1)
522 Descriptions and examples of each shell condition (Table S2)
523 Schematic and example of shell condition index measurements (Figure S1)
524 Schematic and example of shell thickness measurements (Figure S2)
525 Shell dissolution additional statistical results (Table S3)
526 Shell thickness additional statistical results (Table S4)

527

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DISCLOSURES

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