Environmental Processes

Thicker shells compensate extensive dissolution in brachiopods under future ocean acidification

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Just Accepted

1 # Thicker shells compensate extensive dissolution in

2 # brachiopods under future ocean acidification

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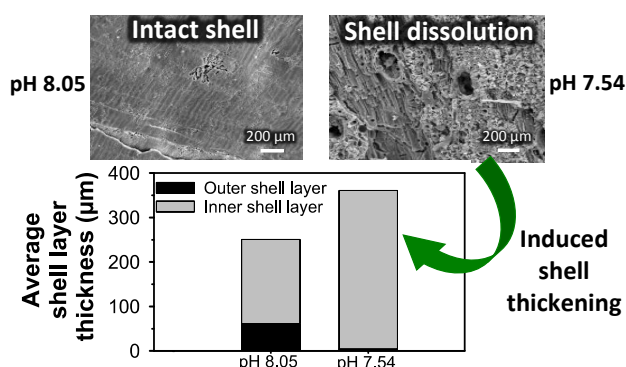
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14   ABSTRACT

15   Organisms with long generation

16   times require phenotypic plasticity to

17   survive in changing environments

18   until genetic adaptation can be

19   achieved. Marine calcifiers are

20   particularly vulnerable to ocean acidification due to dissolution and a reduction in

21   shell-building carbonate ions. Long-term experiments assess organisms' abilities to

22   acclimatise or even adapt to environmental change. Here we present an unexpected

23   compensatory response to extensive shell dissolution in a highly calcium-carbonate-

24   dependent organism after long-term culture in predicted end-century acidification

25   and warming conditions. Substantial shell dissolution with decreasing pH posed a

26   threat to both a polar (*Liothyrella uva*) and a temperate (*Calloria inconspicua*)

27   brachiopod after 7 months and 3 months exposure, respectively, with more extensive

28   dissolution in the polar species. This impact was reflected in decreased outer

29   primary layer thickness in the polar brachiopod. A compensatory response of

30   increasing inner secondary layer thickness, and thereby producing a thicker shell

31   was exhibited by the polar species. Less extensive dissolution in the temperate

32   brachiopod did not affect shell thickness. Increased temperature did not impact shell

33   dissolution or thickness. Brachiopod ability to produce a thicker shell when

34   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.

38   INTRODUCTION

39   Changing environments pose serious risks to organisms that cannot shift their

40   geographic range, physiologically acclimatise or genetically adapt[1]. Current

41   understanding of the biological impacts of ocean acidification and warming is

42   largely based on short- (days) to medium-term (weeks) laboratory and field

43   experiments that have revealed mixed responses in many species[2-5]. More recently,

44   however, there has been an increase in long-term (many months to years) studies

45   that demonstrate surprising capacities of marine organisms to acclimate[6-10], or even

46   adapt in organisms with short generation times[11-13] to decreased pH and increased

47   temperature. Compensatory mechanisms could be paramount to maintain overall

48   performance of organisms that have limited capacities to alter their geographic range

49   under future changed conditions and subsequently sustain their key ecological

50   functions in our oceans[14].

51   Marine calcifiers are considered the most vulnerable organisms to ocean

52   acidification due to the combination of dissolution and the reduction in carbonate

53   ions making shell production more difficult and energetically expensive[2,15,16]. The

54   Southern Ocean has naturally low carbonate ion saturation levels compared to

55   temperate and tropical regions due to carbon dioxide being more soluble in cold

56   water[17]. Acid-base coefficients are also more sensitive in cold temperatures making

57   this high latitude region a forerunner of biological ocean acidification impacts for

58   other oceans[18]. The external skeleton is crucial for protecting animal tissue in shell-

59   bearing organisms against predation, infection and loss of bodily fluids[19,20]. Any

60   negative impacts to shell integrity, therefore, could compromise its protective

61   function and potentially prove fatal. Shell integrity may be affected by erosion from

62   natural scour or attack from shell-boring organisms as well as dissolution. The

63   calcified shell of all shell-bearing organisms is protected by an outer organic layer,

64   the periostracum[21,22]. Abrasion of this protective layer and subsequently inner shell

65   layers naturally occurs through abrasion from suspended inorganic particulate

66   material, the movement of individuals against each other, and with other calcified

67   biota or substrata. Shell dissolution also poses a threat depending on the solubility of

68   the biomineral, the chemical characteristics of the surrounding seawater and

69   metabolic by-products released by the adhering biofilm[23,24]. Predicted environmental

70   conditions for 2100 will shift surface seawater carbonate chemistry to favour $CaCO_3$

71   dissolution, which could exacerbate the loss of shell integrity of marine calcifiers.

72   Compensatory mechanisms may counteract deleterious ocean acidification and

73   warming effects on organisms. For these to succeed, the compensatory mechanism

74   must occur at a faster rate than that of the deleterious effect to provide successful

75   protection. Phenotypic plasticity of shell morphology has been reported in shelled

76   organisms in response to the presence of predators[25] and changing environmental

77   conditions[14,26-28]. These include shell thickening, production of a more rotund shell

78   and increased shell growth rates through plasticity in producing different calcium

79   carbonate polymorphs[14,25-32]. Production of a thicker periostracum could also

80   withstand more wear and deter dissolution[33]. Periostracum loss or shell dissolution

81   at the external surface far away from the secretory tissue cannot be directly repaired

82   by the organism. Compensatory mechanisms such as induced thickening, however,

83   could counteract this potentially fatal effect of ocean acidification.

84   Brachiopods are one of the most calcium-carbonate-dependent groups of marine

85   animals because their calcareous skeleton and other support structures make up >

86   90% of their dry mass[34,35]. Rhynchonelliform brachiopods possess a low-magnesium

87   calcite shell consisting of the periostracum underlain by two biomineralised inner

88   layers; the thin nanocrystalline primary layer and the generally much thicker fibrous

89   secondary layer[36,37]. In previously published work we showed that shell growth

90   rates of *L. uva* Broderip, 1833 (which we refer to as "polar brachiopod") and *C.*

91   *inconspicua* Sowerby, 1846 (which we refer to as "temperate brachiopod") were not

92   impacted by predicted end-century seawater pH's[6,7]. Another study demonstrated

93   increased dissolution in the polar brachiopod in pH 7.4 conditions after 14 days[38],

94   however, empty dried valves were used so the brachiopods ability to compensate

95   shell dissolution remains unknown. This study, therefore, investigated dissolution

96   effects and potential compensatory mechanisms of a polar and a temperate

97   brachiopod living under acidified and warming conditions. Specifically, the extent of

98   dissolution and thickness of whole valves and individual shell layers were assessed

99   under predicted end-century pH levels in both brachiopods and also under

100   increased temperature in the polar brachiopod.

101

102

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

125   on the IPCC 'business-as-usual' scenario of the predicted end-century reduction of

126   0.3-0.5 pH units from the present day average of pH 8.1 in surface oceanic seawater

127   by 2100[43] (Table 1). The third was a pH control where the seawater remained at

128   ambient pH (pH 8.05 ± 0.03). All these three treatments were maintained at 2°C

129   throughout the experiment due to the concurrent 2°C increase in sea surface

130   temperature (SST) expected to occur alongside these predicted decreased pH levels

131   by the end of the century[44]. The fourth treatment was a temperature control which

132   was held at the present-day average conditions for Ryder Bay[45] (SST: 0°C, pH: 7.98 ±

133   0.02). The pH of the acidified treatments was controlled by intermittently bubbling

134   CO_2 gas into a header tank. Seawater was then gravity fed into the experimental

135   tanks[6]. The pH control treatment had a similar set up but without the pH

136   manipulation system. The temperature control treatment was situated separately in

137   the main BAS aquarium. Seawater temperature of all treatments was manipulated

138   by controlling the air temperature in temperature-controlled laboratories.

139   Seawater temperatures (°C, Digital Testo 106) and pH_{NIST} (Aquamedic pH

140   controlled computer and electrode system) were monitored and recorded daily.

141   Salinity (Tropical Marine Centre V2 Handheld refractometer), TCO_2 (mmol L^{-1}; Ciba

142   Corning TCO_2 Analyzer 965, Olympic Analytical. UK) and nutrient content (silicate

143   and phosphate) of each treatment were measured weekly. Other carbonate system

144   parameters, including the partial pressure of CO_2 (pCO_2) and the saturation values

145   for calcite (Ω_C) and aragonite (Ω_A), were modelled from applying TCO_2 and pH_{NIST}

146   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.

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

170   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

171   Values for pCO_2, Ω calcite and Ω aragonite were calculated from CO2SYS[46] with
172   refitted constants[47,48].

173

174   *Shell condition index.* Shell lengths were measured at the start and end of each

175   experiment using Vernier calipers (± 0.1 mm) to determine shell laid down in the

176   natural environment that thickens from the internal surface as brachiopods grow

177   (which we refer to as "thickening shell") and shell growth extension during the

178   experiments (which we refer to as "growing shell"). Mean lengths (± S.E.) of these

179   two shell regions from each treatment are reported in Table S1. Scanning Electron

180   Microscopes (JEOL 820 for the polar brachiopod and FEI QEMSCAN 650F for the

181   temperate brachiopod; both operated using an accelerating voltage of 20 kV) were

182   used to image gold-coated outer surfaces of five ventral valves of adult specimens

183   from each treatment of both species to determine shell condition. Five types of shell

184   condition were present: intact shell (IS; intact periostracum with pitted layer),

185   minimal wear (W1; periostracum without pitted layer), extensive wear (W2; wear

186   but no dissolution), partial shell dissolution (SD1; dissolution in the inner primary

187   layer) and extensive shell dissolution (SD2; dissolution exposing the innermost

188   secondary layer). Full descriptions and examples of each type of shell condition for

189   both species are presented in Table S2. Micrographs (1 mm x 1 mm) were collected at

190   five standardised areas in thickening shell (areas located from umbo region towards

191   anterior margin as detailed in Fig. S1A) and five standardised areas in growing shell

192   (areas evenly spread in anterior margin as detailed in Fig. S1A). Percentage areas of

193   each type of shell condition from each SEM micrograph were calculated/measured in

194   ImageJ (Fig. S1B). Each shell region was analysed separately to determine whether

195   treatment and/or the location of shell analysed (which we refer to as "shell position")

196   affected shell that had already been potentially subjected to substantial wear

197   (thickening shell) and newly produced shell with less time subjected to wear

198   (growing shell).

199   ***Shell thickness.*** Longitudinal cross sections of five dorsal valves of adult

200   specimens from each treatment of both species were finely polished to 3 μm using

201   Kemet met papers (P400, P800, P2500 and P4000) followed by MetPrep diamond

202   solutions (6 μm and 3 μm). Acetate peels from polished cross sections of the brachial

203   valves of both species were made according to a previous study[53]. Thickness

204   measurements (± 0.1 mm) of the primary layer, secondary layer and total shell were

205   then measured from three areas of thickening shell (umbo region, middle of the shell

206　and nearer experimental growth as detailed in Fig. S2) and three areas of growing

207　shell (oldest experimental growth to newest experimental growth in the anterior

208　margin as detailed in Fig. S2) on a Swift monocular petrological microscope with

209　fitted micrometer.

210　　*Statistical analyses.* Shell condition index data were non-normally distributed

211　due to the presence of zeros in the dataset. Non-parametric Kruskal-Wallis tests

212　were, therefore, used to determine whether treatment and/or shell position affected

213　the median percentage area of each type of shell condition. When significant

214　differences occurred, post-hoc Dunn's tests were conducted to identify which

215　treatments and shell positions were statistically different from each other. As shell

216　condition and shell thickness measurements were conducted at several points within

217　an individual, Kruskal-Wallis tests were also used to determine if individual number

218　affected each shell condition. Linear mixed effects models were computed to

219　determine if treatment, shell position (fixed effects) and/or individual number

220　(random effect) impacted primary layer, secondary layer and total shell thickness:

221

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

223　　　　　　　　　　　　　　　　　+ error

224

225　　Likelihood ratio tests were used to determine p values (p < 0.05) between the full

226　model with the effect in question against the reduced model without the effect in

227　question. When the ratio tests identified significant differences, post-hoc Tukey tests

228   were performed to determine which treatments or shell positions were responsible.

229   Shell thickness data were checked for variance homogeneity and normality using

230   Levene's and Shapiro-Wilk tests ($p < 0.05$), respectively. Each shell region was

231   analysed separately for both shell condition index and shell thickness to determine

232   whether treatment and/or shell position affected shell maintenance (thickening shell)

233   and shell production (growing shell). Statistical analyses were computed using R[54]

234   with the *FSA* package[55] used for the Kruskal-Wallis and post-hoc Dunn's tests, the

235   *lme4* package[56] for the linear mixed effects models and the *emmeans* package[57] for the

236   post-hoc Tukey tests.

237

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

260   decreasing pH (Figure 1c & Figure 3; Kruskal-Wallis: H = 53.72, p < 0.001) in growing

261   shell in the temperate brachiopod. Shell dissolution in this temperate species was

262   less extensive than for the polar species (Figure 2 & Figure 3) as the secondary layer

263   was not exposed (SD2) in any individual in any treatment. Shell position or

264   individual number did not affect any shell condition in the thickening shell of both

265   species (Table S3).

266   *Growing shell.* Growing shell in both species was mainly characterised by intact

267   shell (IS) in all treatments (Figure 1b, d; polar brachiopod: > 63.9 ± 4.7%; temperate

268   brachiopod: > 83.2 ± 1.8%). Less intact shell occurred in the most acidified conditions

269   compared to all other treatments in both species (Figure 1b, d; Kruskal-Wallis: polar

270   brachiopod - H = 41.81, p < 0.001; temperate brachiopod – H = 20.96, p < 0.001).

271   Partial shell dissolution (SD1) increased with increasing acidity in the experimental

272   growth of the polar brachiopod (Figure 1b & Figure 2; Kruskal-Wallis: polar

273   brachiopod - H = 63.08, p < 0.001). This shell dissolution, however, occurred at a

274   much lower level (3.2 ± 1.0% in moderate pH and 35.9 ± 4.7% in low pH) in the

275   growing shell than in the thickening shell in this species. Temperature had no effect

276   on partial shell dissolution (Dunn's Test: Temperature control vs pH control: T = -

277   0.22, p = 0.829). Partial shell dissolution (SD1) only occurred in the most acidified

278   treatment in the temperate brachiopod (Figure 1d; 11.1 ± 1.5%), also in lower levels

279   than in the thickening shell (28.3 ± 3.2%) in this species. Extensive shell dissolution

280   (SD2) was absent in the growing shell in both species across all treatments. Minimal

281   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

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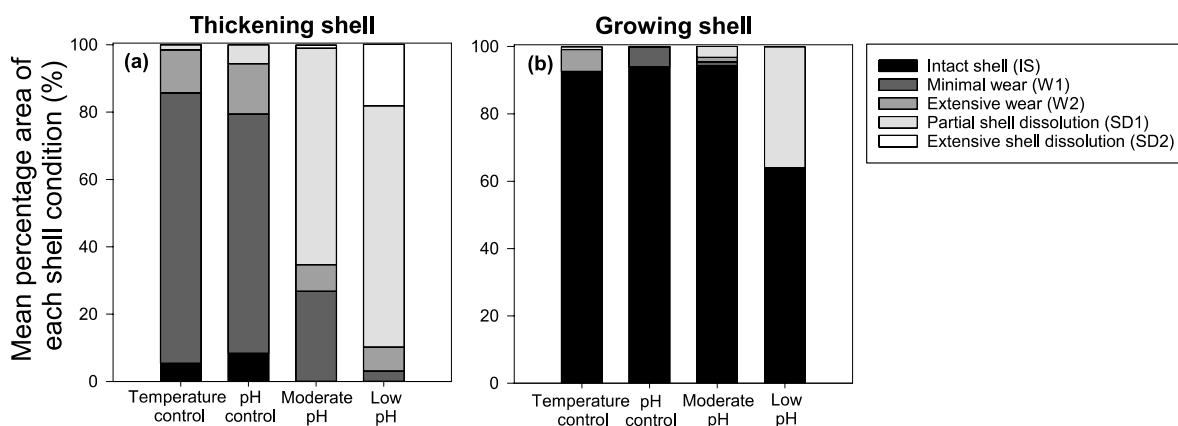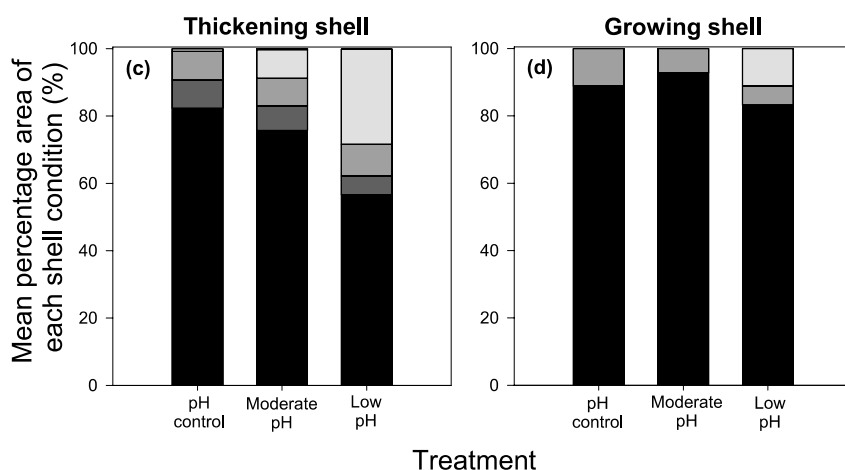
Figure 1. Representative shell condition - Mean percentage area of the different types of shell condition from five standardised areas in thickening shell (a, c) and five standardised areas in growing shell (b, d) in the polar brachiopod (top row, n = 5 per treatment) and in the temperate brachiopod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

Thickening shell

Growing shell

Temperature control

pH control

Moderate pH

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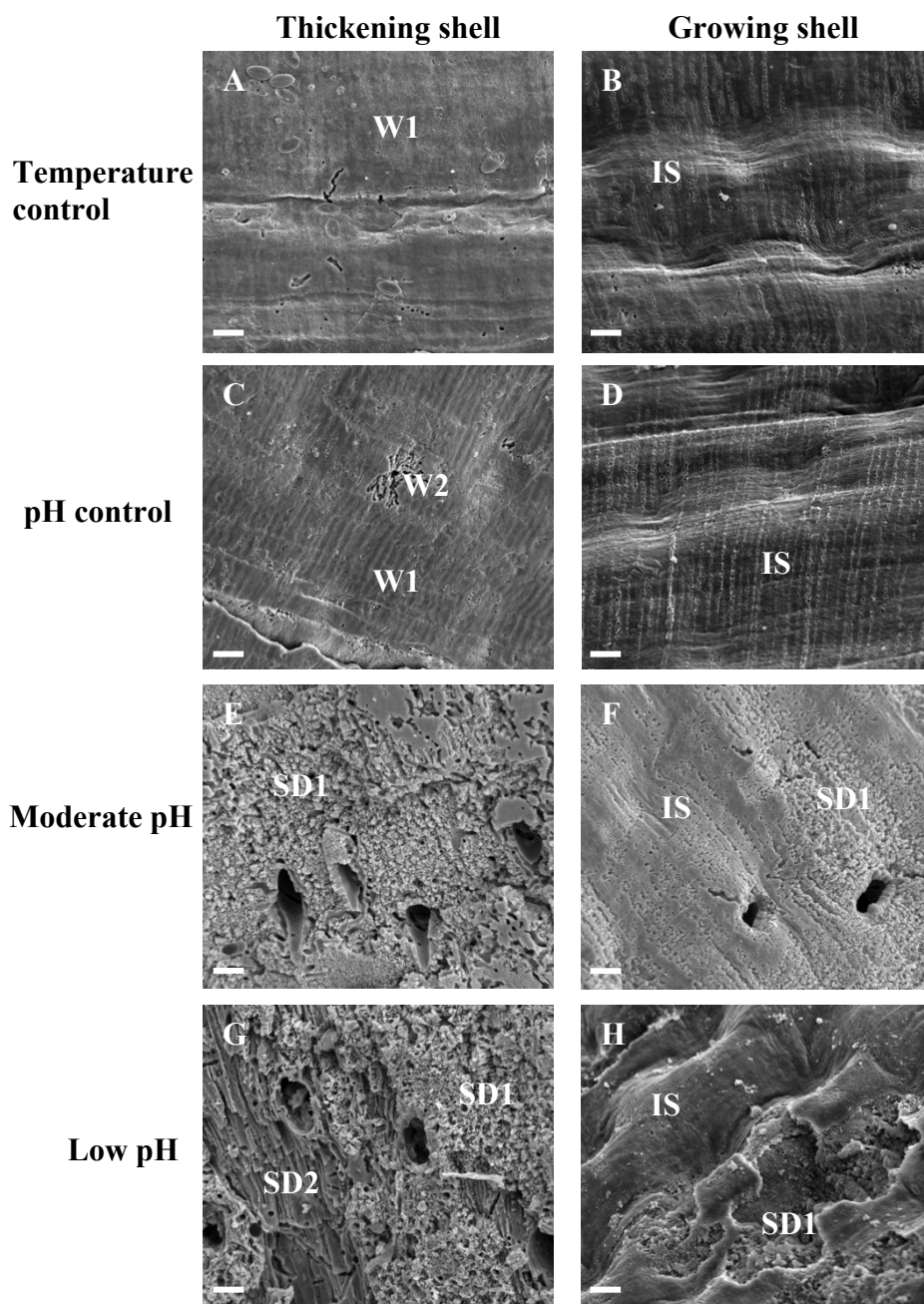
Figure 2. Representative shell condition in the polar brachiopod – Examples of SEM micrographs of shell surfaces of thickening shell (A, C, E, G) and growing shell (B, D, F, H) in temperature control (A, B), pH control (C, D), moderate pH (E, F) and low pH treatment (G, H). IS = intact shell, W1 = minimal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

302
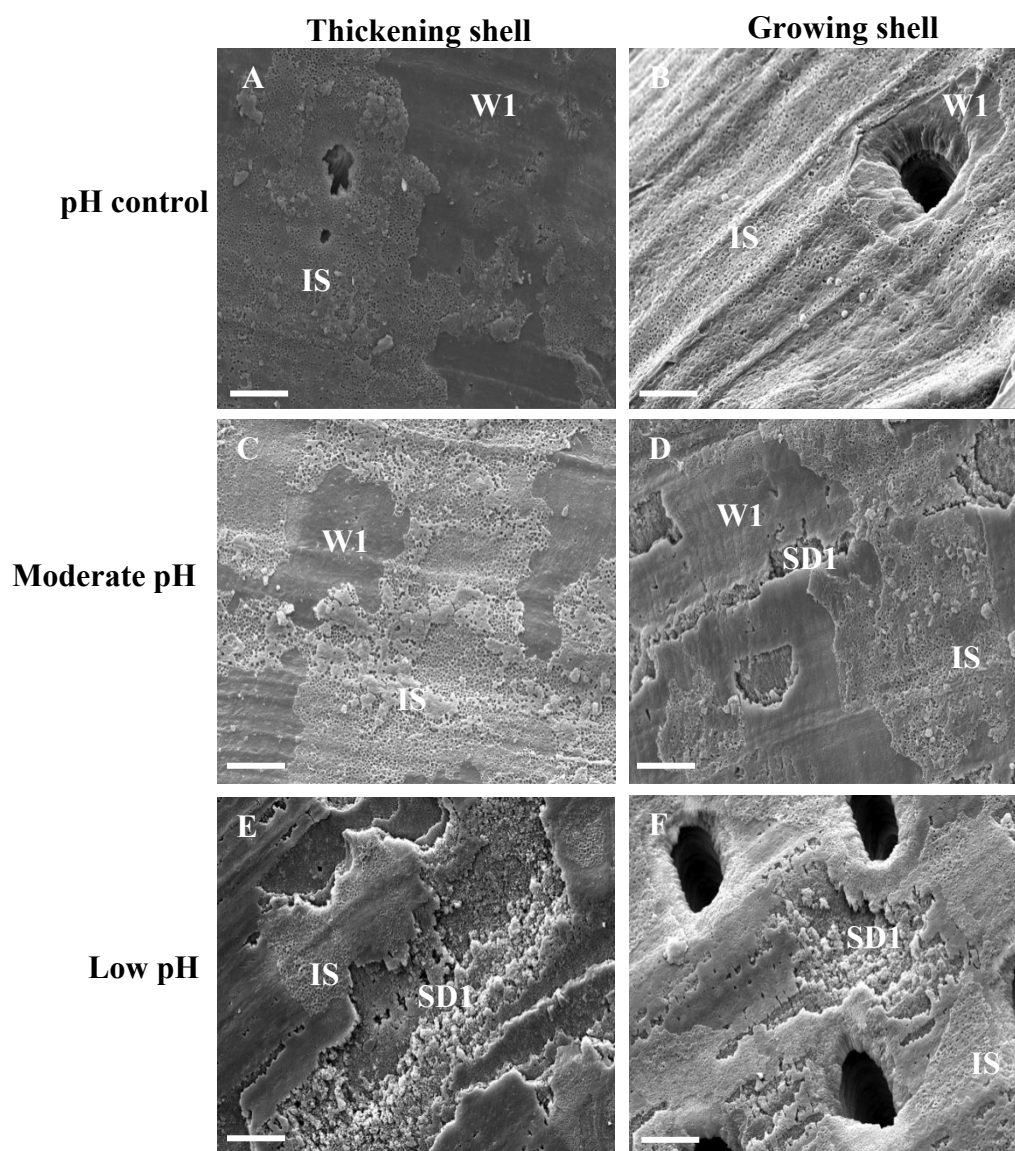
303

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.
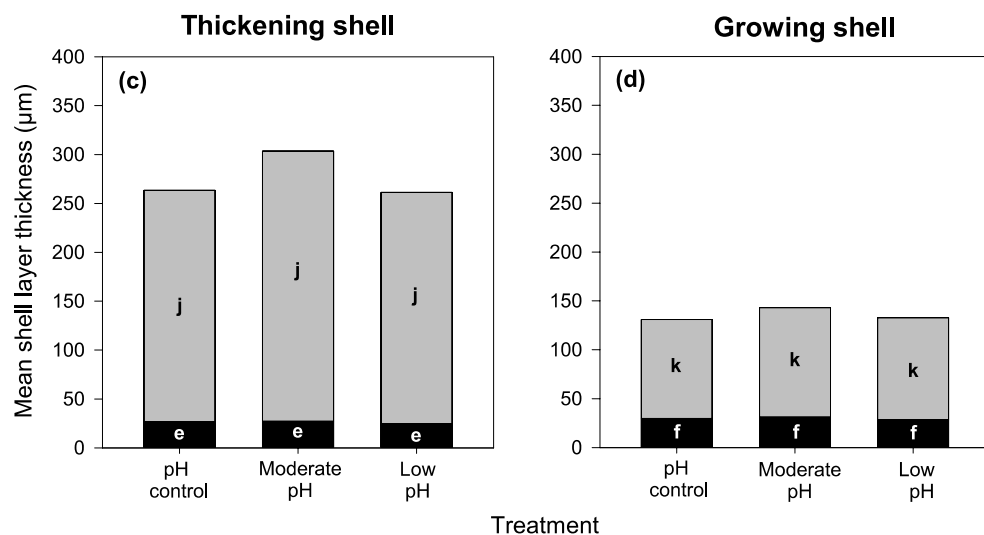
311   **Shell thickness.**

312   *Thickening shell.* The outer primary layer became progressively thinner in

313   thickening shell as pH reduced in the polar brachiopod (Figure 4a; Linear Mixed

314   Effects Model; χ^2 = 79.72, df = 3, p < 0.001). Secondary layer thickened in all

315   treatments as this inner shell layer transitioned from the region of growing shell to

316   thickening shell as the brachiopod grew (larger grey bars in Figure 4a vs smaller

317   grey bars in Figure 4b). This inner secondary layer, and the whole shell, however,

318   were thicker in the most acidified treatment in the thickening shell (Figure 4a; Linear

319   Mixed Effects Model; Secondary Layer - χ^2 = 39.63, df = 3, p < 0.001; Total Shell - χ^2 =

320   18.19, df = 3, p < 0.001). Increased temperature had no effect on primary layer,

321   secondary layer or total shell thickness (Tukey; Primary Layer – T = 1.73, p = 0.319;

322   Secondary Layer – T = -1.20, p = 0.627; Total Shell – T = -0.80, p = 0.855). In contrast,

323   neither individual shell layers nor total shell thickness were affected by lowered pH

324   in the thickening shell in the temperate brachiopod (Figure 4c; Linear Mixed Effects

325   Model; Primary Layer - χ^2 = 4.17, df = 2, p = 0.124; Secondary Layer - χ^2 = 4.80, df = 2,

326   p = 0.091; Total Shell - χ^2 = 4.27, df = 2, p = 0.118). Primary layer was thinnest in the

327   oldest part of the shell, the umbo region, across all treatments in both species (Table

328   S4). Secondary layer and total shell thickness did not differ in different places in the

329   thickening shell in each treatment in both species (Table S4). Individual number also

330   had no effect on individual shell layer and total shell thickness in both species (Table

331   S4).

332   *Growing shell.* Primary layer, secondary layer and total shell thickness were not

333   impacted by lowered pH in either species (Figure 4b & 4d; Linear Mixed Effects

334   Model; polar brachiopod: Primary Layer - χ^2 = 3.62, df = 3, p = 0.306; Secondary Layer

335   - χ^2 = 6.80, df = 3, p = 0.078; Total Shell - χ^2 = 5.26, df = 3, p = 0.154; temperate

336   brachiopod: Primary Layer - χ^2 = 2.63, df = 2, p = 0.268; Secondary Layer - χ^2 = 0.82, df

337   = 2, p = 0.663; Total Shell - χ^2 = 1.12, df = 3, p = 0.572). Increased temperature also had

338   no effect on either individual shell layers or total shell thickness in the polar

339   brachiopod (Figure 4b). Primary layer thickness did not differ indifferent places

340   throughout the growing shell in each treatment in either species (Table S4).

341   Secondary layer and the total shell thickness, however, did get progressively thinner

342   with the direction of growth in each treatment in both species (Table S4). Individual

343   number also had no effect on individual shell layer and total shell thickness in

344   growing shell in either species (Table S4).

Polar brachiopod
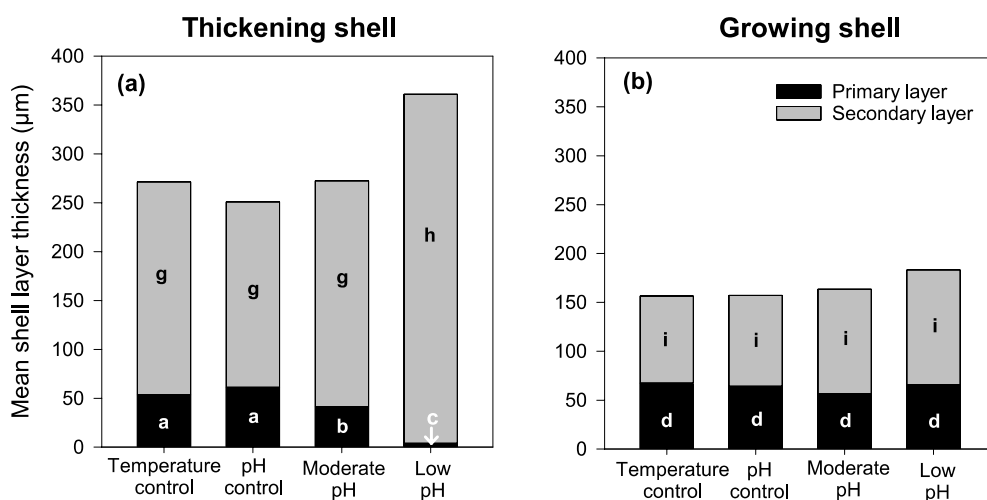
Temperate brachiopod

345

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

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

354   regions or between species.

355

356   DISCUSSION

357   Long-term culturing of a polar and a temperate brachiopod under predicted end-

358   century acidified conditions revealed that both species were more susceptible to

359   shell dissolution with increasing acidity. Our two principal findings are significant

360   dissolution and an unexpected compensation of induced thicker shells in the

361   thickening shell.

362   **Dissolution of shell.** Shell loss has been widely reported in several marine

363   calcifiers, however, these have largely been those which use higher solubility

364   polymorphs of calcium carbonate (i.e. aragonite), such as corals[58-60] and

365   molluscs[23,32,61-64], high-magnesium calcite including coralline algae[65,66] and

366   echinoderms[67,68]. Fewer studies have investigated shell dissolution in taxa which are

367   entirely constructed of the lower solubility polymorph, low-magnesium calcite, such

368   as rhynchonelliform brachiopods. Previously, the only other ocean acidification

369   study assessing dissolution in brachiopods was conducted on dead shells[38]. Working

370   on the polar species, they showed deterioration of the primary layer after only 35

371   days exposure to pH 7.4, which after 56 days exposed the fibres of the secondary

372   layer below. This is the same dissolution pattern reported here in experiments

373   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

396   dissolution[31,77-82]. Since periostracum is only formed at the growing edge of the

397   mantle, it cannot be repaired if damaged or lost from the surface of the shell.

398   Thinning or loss of this organic layer through physical or biotic abrasion and

399   epibiont erosion, therefore, restricts protection from corrosive acidified waters. The

400   periostracum in brachiopods is < 1 μm thick[83] and so is very vulnerable to loss.

401   Newly formed growing shell was mainly characterised by intact shell in both

402   species. Partial shell dissolution did occur, however, in the most acidified treatment

403   in both species albeit at a much lower level than in the thickening shell. Damage to

404   the ultrathin periostracum from abrasion of other brachiopods in their conspecific

405   cluster, natural decay of this outer layer or potentially the lowered pH conditions

406   could have either softened the periostracum itself or disrupted the protective

407   function of the periostracum. This latter possibility was suggested for external

408   dissolution reported in newly formed shell in *M. edulis* after 2 months exposure to

409   1400 μatm and 4000 μatm[79]. Disintegration of organic matrix in the shell rather than

410   corrosion of crystals could have caused this shell degradation, as seen in spirorbids

411   after 100-day exposure to pH 7.7 conditions[84].

412   Temperature had no clear effect on shell dissolution or thickness in the polar

413   brachiopod as indicated by the lack of or only minimal primary layer dissolution

414   and no change in any thickness measurement in both thickening and growing shell

415   in the temperature control (held at 0°C – current average Antarctic summer

416   temperatures) and the pH control (kept at the 2°C temperature increase predicted for

417   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

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

441   For compensatory mechanisms to succeed, they must occur at faster rates than the

442   deleterious effect. Thicker basal shells were reported in the barnacle *Amphibalanus*

443   *amphitrite* under lowered pH conditions (pH 7.4), however, this compensation

444   calcification was insufficient as dissolution weakened shells faster than it was

445   deposited[89]. A pteropod specimen collected from the Fram Strait in the Arctic Ocean

446   also produced a shell four times thicker than the original shell in response to

447   mechanical and dissolution damage from undersaturated waters[31].

448   Extensive shell dissolution at low pH in thickening shell of the polar brachiopod

449   led to a drastic decrease in primary layer thickness. The polar species counteracted

450   this chemical attack by laying down more secondary layer on the internal surface of

451   the shell, which resulted in increased overall shell thickness during the experimental

452   period. The less extensive dissolution in the temperate brachiopod was reflected by

453   no clear impact of acidified conditions on either total shell or individual shell layer

454   thicknesses. Our findings appear to contrast with reports of primary layer thickening

455   in the Chilean terebratulide *Magellania venosa* after being cultured in pH 7.35

456   conditions[90], however, their observations appear to be based on only one measured

457   specimen in both the acidified treatment and the control.

458   Compensatory mechanisms must also be sufficient in maintaining an organism's

459   overall performance. The secondary layer of terebratulide brachiopod shells is softer

460   than the harder protective primary layer[37,91] raising the question of whether a shell

461   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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548   REFERENCES

549   (1) Peck, L. S., Organisms and responses to environmental change. *Mar.*
550   *Genom.* **2011,** *4*, 237-243.

551   (2) Doney, S. C.; Fabry, V. J.; Feely, R. A.; Kleypas, J. A., Ocean acidification:
552   the other CO_2 problem. *Ann. Rev. Mar. Sci.* **2009,** *1*, 169-192.

553   (3) Hofmann, G. E.; Barry, J. P.; Edmunds, P. J.; Gates, R. D.; Hutchins, D. A.;
554   Klinger, T.; Sewell, M. A., The effect of ocean acidification on calcifying organisms in
555   marine ecosystems: An organism-to-ecosystem perspective. *Annu Rev Ecol Evol Syst*
556   **2010,** *41*, 127-147.

557   (4) Byrne, M., Impact of ocean warming and ocean acidification on marine
558   invertebrate life history stages: vulnerabilities and potential for persistence in a
559   changing ocean. *Oceanogr Mar Biol Annu Rev* **2011,** *49*, 1-42.

560   (5) Parker, L. M.; Ross, P. M.; O'Connor, W. A.; Pörtner, H. O.; Scanes, E.;
561   Wright, J. M., Predicting the response of molluscs to the impact of ocean
562   acidification. *Biology* **2013,** *2*, 651-92.

563   (6) Cross, E. L.; Peck, L. S.; Harper, E. M., Ocean acidification does not impact
564   shell growth or repair of the Antarctic brachiopod *Liothyrella uva* (Broderip, 1833). *J.*
565   *Exp. Mar. Biol. Ecol.* **2015,** *462*, 29-35.

566   (7) Cross, E. L.; Peck, L. S.; Lamare, M. D.; Harper, E. M., No ocean
567   acidification effects on shell growth and repair in the New Zealand brachiopod
568   *Calloria inconspicua* (Sowerby, 1846). *ICES J. Mar. Sci.* **2016,** *73*, 920-926.

569   (8) Queirós, A. M.; Fernandes, J. A.; Faulwetter, S.; Nunes, J.; Rastrick, S. P.;
570   Mieszkowska, N.; Artioli, Y.; Yool, A.; Calosi, P.; Arvanitidis, C.; Findlay, H. S.;
571   Barange, M.; Cheung, W. W.; Widdicombe, S., Scaling up experimental ocean
572   acidification and warming research: from individuals to the ecosystem. *Global Change*
573   *Biol.* **2015,** *21*, 130-143.

574   (9) Suckling, C. C.; Clark, M. S.; Richard, J.; Morley, S. A.; Thorne, M. A.;
575   Harper, E. M.; Peck, L. S., Adult acclimation to combined temperature and pH

576   stressors significantly enhances reproductive outcomes compared to short-term

577   exposures. *J. Anim. Ecol.* **2014,** *84*, 773-784.

578   (10) Hazan, Y.; Wangensteen, O. S.; Fine, M., Tough as a rock-boring urchin:

579   adult *Echinometra* sp. EE from the Red Sea show high resistance to ocean

580   acidification over long-term exposures. *Mar. Biol.* **2014,** *161*, 2531-2545.

581   (11) Collins, S.; Rost, B.; Rynearson, T. A., Evolutionary potential of marine

582   phytoplankton under ocean acidification. *Evol. Appl.* **2014,** *7*, 140-155.

583   (12) Kelly, M. W.; Hofmann, G. E., Adaptation and the physiology of ocean

584   acidification. *Funct. Ecol.* **2013,** *27*, 980-990.

585   (13) Sunday, J. M.; Calosi, P.; Dupont, S.; Munday, P. L.; Stillman, J. H.;

586   Reusch, T. B., Evolution in an acidifying ocean. *Trends Ecol. Evol.* **2014,** *29*, 117-125.

587   (14) Leung, J. Y. S.; Russell, B. D.; Connell, S. D., Mineralogical plasticity acts

588   as a compensatory mechanism to the impacts of ocean acidification. *Environ. Sci.*

589   *Technol.* **2017,** *51*, 2652-2659.

590   (15) Byrne, M.; Przeslawski, R., Multistressor impacts of warming and

591   acidification of the ocean on marine invertebrates' life histories. *Integ. Comp. Biol.*

592   **2013,** *53*, 582-596.

593   (16) Watson, S.-A.; Peck, L. S.; Tyler, P. A.; Southgate, P. C.; Tan, K. S.; Day, R.

594   W.; Morley, S. A., Marine invertebrate skeleton size varies with latitude, temperature

595   and carbonate saturation: implications for global change and ocean acidification.

596   *Global Change Biol.* **2012,** *18*, 3026-3038.

597   (17) Revelle, R. R.; Fairbridge, R. W., Carbonates and carbon dioxide. In

598   *Geological Society of America Memoirs*, 1957; Vol. 67, pp 239-296.

599   (18) Fabry, V. J.; McClintock, J. B.; Mathis, J. T.; Grebmeier, J. M., Ocean

600   acidification at high latitudes: the bellwether. *Oceanography* **2009,** *22*, 160-171.

601   (19) Vermeij, G. J., The Mesozoic marine revolution: evidence from snails,

602   predators and grazers. *Paleobiology* **1977,** *3*, 245-258.

603   (20) Harper, E. M.; Clark, M. S.; Hoffman, J. I.; Philipp, E. E.; Peck, L. S.;

604   Morley, S. A., Iceberg scour and shell damage in the Antarctic bivalve *Laternula*

605   *elliptica*. *PLoS ONE* **2012,** *7*, e46341.

606   (21) Harper, E. M., The molluscan periostracum: an important constraint in

607   bivalve evolution. *Palaeonotology* **1997,** *40*, 71-97.

608   (22) Williams, A.; Mackay, S., Secretion and ultrastructure of the periostracum

609   of some terebratulide brachiopods. *Proc. R. Soc. B.* **1978,** *202*, 191-209.

610   (23) Nienhuis, S.; Palmer, A. R.; Harley, C. D., Elevated CO_2 affects shell

611   dissolution rate but not calcification rate in a marine snail. *Proc. R. Soc. B.* **2010,** *277*,

612   2553-2558.

613   (24) Bausch, A. R.; Gallego, M. A.; Harianto, J.; Thibodeau, P.; Bednaršek, N.;

614   Havenhand, J. N.; Klinger, T., Influence of bacteria on shell dissolution in dead

615   gastropod larvae and adult *Limacina helicina* pteropods under ocean acidification

616   conditions. *Mar. Biol.* **2018,** *165*, 40.

617   (25) Freeman, A. S.; Byers, J. E., Divergent induced responses to an invasive

618   predator in marine mussel populations. *Science* **2006,** *313*, 831-834.

619   (26) Peyer, S. M.; Hermanson, J. C.; Lee, C. E., Developmental plasticity of

620   shell morphology of quagga mussels from shallow and deep-water habitats of the

621   Great Lakes. *J. Exp. Biol.* **2010,** *213*, 2602-2609.

622   (27) Fitzer, S. C.; Vittert, L.; Bowman, A.; Kamenos, N. A.; Phoenix, V. R.;

623   Cusack, M., Ocean acidification and temperature increase impact mussel shell shape

624   and thickness: problematic for protection? *Ecol. Evol.* **2015,** *5*, 4875-4884.

625   (28) Telesca, L.; Michalek, K.; Sanders, T.; Peck, L. S.; Thyrring, J.; Harper, E.

626   M., Blue mussel shell shape plasticity and natural environments: a quantitative

627   approach. *Sci. Rep.* **2018,** *8*, 2865.

628   (29) Vermeij, G. J., Phenotypic evolution in a poorly dispersing snail after

629   arrival of a predator. *Nature* **1982,** *299*, 349-350.

630   (30) Fisher, J. A.; Rhile, E. C.; Liu, H.; Petraitis, P. S., An intertidal snail shows

631   a dramatic size increase over the past century. *PNAS* **2009,** *106*, 5209-12.

632   (31) Peck, V. L.; Oakes, R. L.; Harper, E. M.; Manno, C.; Tarling, G. A.,

633   Pteropods counter mechanical damage and dissolution through extensive shell

634   repair. *Nat. Commun.* **2018,** *9*, 264.

635   (32) Duquette, A.; McClintock, J. B.; Amsler, C. D.; Pérez-Huerta, A.; Milazzo,

636   M.; Hall-Spencer, J. M., Effects of ocean acidification on the shells of four

637   Mediterranean gastropod species near a CO_2 seep. *Mar. Pollut. Bull.* **2017**.

638   (33) Telesca, L.; Peck, L. S.; Sanders, T.; Thyrring, J.; Sejr, M. K.; Harper, E. M.,

639   Plasticity and environmental heterogeneity predict geographic resilience patterns of

640   foundation species to future change. *bioRxiv* **2018**.

641   (34) Peck, L. S., The tissues of articulate brachiopods and their value to

642   predators. *Phil. Trans. R. Soc. B.* **1993,** *339*, 17-32.

643   (35) Peck, L. S., Brachiopods and climate change. *Earth Env. Sci. T. R. So.* **2008,**

644   *98*, 451-456.

645   (36) Williams, A.; Brunton, C. H. C.; MacKinnon, D. I., Morphology. In *Treatise*

646   *on Invertebrate Paleontology, Part H, Brachiopods (Revised)*, Kaesler, R. L., Ed. The

647   Geological Society of America and The University of Kansas Press: Boulder,

648   Colorado, and Lawrence, Kansas, 1997; Vol. 1, pp 321-422.

649   (37) Goetz, A. J.; Griesshaber, E.; Neuser, R. D.; Lüter, C.; Hühner, M.; Harper,

650   E. M.; Schmahl, W. W., Calcite morphology, texture and hardness in the distinct

651   layers of rhynchonelliform brachiopod shells. *Eur. J. Mineral.* **2009,** *21*, 303-315.

652   (38) McClintock, J. B.; Angus, R. A.; McDonald, M. R.; Amsler, C. D.;

653   Catledge, S. A.; Vohra, Y. K., Rapid dissolution of shells of weakly calcified Antarctic

654   benthic macroorganisms indicates high vulnerability to ocean acidification. *Antarct.*

655   *Sci.* **2009,** *21*, 449-456.

656   (39) Venables, H. J.; Clarke, A.; Meredith, M. P., Wintertime controls on

657   summer stratification and productivity at the western Antarctic Peninsula. *Limnol.*

658   *Oceanogr.* **2013,** *58*, 1035-1047.

659   (40) McNeil, B. I.; Matear, R. J., Southern Ocean acidification: a tipping point

660   at 450-ppm atmospheric CO_2. *PNAS* **2008,** *105*, 18860-18864.

661   (41) Greig, M. J.; Ridgway, N. M.; Shakespeare, B. S., Sea surface temperature

662   variations at coastal sites around New Zealand. *N. Z. J. Mar. Freshwat. Res.* **1988,** *22*,

663   391-400.

664   (42) Roper, D. S.; Jillett, J. B., Seasonal occurrence and distribution of flatfish

665   (Pisces: Pleuronectiformes) in inlets and shallow water along the Otago coast. *N. Z. J.*

666   *Mar. Freshwat. Res.* **1981,** *15*, 1-13.

667   (43) IPCC, Climate Change 2013: The Physical Science Basis. In *Working Group*

668   *I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate*

669   *Change,* Stocker, T. F.; Qin, D.; Plattner, G.-K.; Tignor, M.; Allen, S. K.; Boschung, J.;

670   Nauels, A.; Xia, Y.; Bex, V.; Midgley, P. M., Eds. Cambridge, United Kingdom and

671   New York, NY, USA, 2013; p 1552.

672   (44) Mitchell, J. F. B.; Senior, C. A.; Johns, T. C., *Transient response to increasing*

673   *greenhouse gases using models with and without flux adjustment*. Metrological Office:

674   1998.

675   (45) Clarke, A.; Meredith, M. P.; Wallace, M. I.; Brandon, M. A.; Thomas, D.

676   N., Seasonal and interannual variability in temperature, chlorophyll and

677   macronutrients in northern Marguerite Bay, Antarctica. *Deep Sea Res. Part II Top.*

678   *Stud. Oceanogr.* **2008,** *55*, 1988-2006.

679   (46) Lewis, E.; Wallace, D. W. R.; Allison, L. J., *Program developed for CO_2 system*

680   *calculations*. Carbon Dioxide Information Analysis Center, Oak Ridge National

681   Laboratory, US Department of Energy, Oak Ridge, Tennessee, US, 1998.

682   (47) Mehrbach, C.; Culberson, C. H.; Hawley, J. E.; Pytkowicxz, R. M.,

683   Measurement of apparent dissociation constants of carbonic acid in seawater at

684   atmospheric pressure. *Limnol. Oceanogr.* **1973,** *18*, 897-907.

685   (48) Dickson, A. G.; Millero, F. J., A comparison of the equilibrium-constants

686   for the dissociation of carbonic-acid in seawater media. *Deep-Sea Res Pt I* **1987,** *34*,

687   1733-1743.

688   (49) Garibotti, I. A.; Vernet, M.; Ferrario, M. E., Annually recurrent

689   phytoplanktonic assemblages during summer in the seasonal ice zone west of the

690   Antarctic Peninsula (Southern Ocean). *Deep-Sea Res Pt I* **2005,** *52*, 1823-1841.

691   (50) Garibotti, I. A.; Vernet, M.; Ferrario, M. a. E.; Smith, R. C.; Ross, R. M.;

692   Quentin, L. B., Phytoplankton spatial distribution patterns along the western

693   Antarctic Peninsula (Southern Ocean). *Mar. Ecol. Prog. Ser.* **2003,** *261*, 21-39.

694   (51) Dickson, A. G.; Sabine, C. L.; Christian, J. R. *Guide to best practices for*

695   *Ocean CO$_2$ Measurements*; 2007.

696   (52) Wanninkhof, R.; Lewis, E.; Feely, R. A.; Millero, F. J., The optimal

697   carbonate dissociation constants for determining surface water *p*CO$_2$ from alkalinity

698   and total inorganic carbon. *Mar. Chem.* **1999,** *65*, 291-301.

699   (53) Richardson, C. A.; Crisp, D. J.; Runham, N. W., Tidally deposited growth

700   bands in the shell of the common cockle, *Cerastoderma edula* (L.). *Malacologia* **1979,** *18*,

701   277-290.

702   (54) R, Core Team, R: A language and environment for statistical computing.

703   R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-

704   project.org/. **2017**.

705   (55) Ogle, D. H., Fisheries Stock Analysis. **2018**, R Package version 0.8.20.

706   (56) Bates, D.; Maechler, M.; Bolker, B.; Walker, S., Fitting linear mixed-effects

707   models using lme4. *J. Stat. Softw.* **2015,** *67*, 1-48.

708   (57) Lenth, R., emmeans: Estimated Marginal Means, aka Least-Squares

709   Means. **2018**, R package version 1.2.3.

710   (58) Comeau, S.; Carpenter, R. C.; Lantz, C. A.; Edmunds, P. J., Ocean

711   acidification accelerates dissolution of experimental coral reef communities.

712   *Biogeosciences* **2015,** *12*, 365-372.

713   (59) Silbiger, N. J.; Donahue, M. J., Secondary calcification and dissolution

714   respond differently to future ocean conditions. *Biogeosciences* **2015,** *12*, 567-578.

715   (60) Andersson, A. J.; Kuffner, I. B.; MacKenzie, F. T.; Jokiel, P. L.; Rodgers, K.

716   S.; Tan, A., Net loss of CaCO$_3$ from a subtropical calcifying community due to

717   seawater acidification: mesocosm-scale experimental evidence. *Biogeosciences* **2009,** *6*,

718   1811-1823.

719   (61) Hall-Spencer, J. M.; Rodolfo-Metalpa, R.; Martin, S.; Ransome, E.; Fine,

720   M.; Turner, S. M.; Rowley, S. J.; Tedesco, D.; Buia, M. C., Volcanic carbon dioxide

721   vents show ecosystem effects of ocean acidification. *Nature* **2008,** *454*, 96-9.

722   (62) Milano, S.; Schöne, B. R.; Wang, S.; Müller, W. E., Impact of high pCO$_2$ on

723   shell structure of the bivalve *Cerastoderma edule*. *Mar. Environ. Res.* **2016,** *119*, 144-155.

724   (63) Bednaršek, N.; Tarling, G. A.; Bakker, D. C. E.; Fielding, S.; Feely, R. A.,

725   Dissolution dominating calcification process in polar pteropods close to the point of

726   aragonite undersaturation. *PLoS ONE* **2014,** *9*, e109183.

727   (64) Harvey, B. P.; Agostini, S.; Wada, S.; Inaba, K.; Hall-Spencer, J. M.,

728   Dissolution: The achilles' heel of the triton shell in an acidifying ocean. *Front. Mar.*

729   *Sci.* **2018,** *5*, 371.

730   (65) Kamenos, N. A.; Burdett, H. L.; Aloisio, E.; Findlay, H. S.; Martin, S.;

731   Longbone, C.; Dunn, J.; Widdicombe, S.; Calosi, P., Coralline algal structure is more

732   sensitive to rate, rather than the magnitude, of ocean acidification. *Global Change Biol.*

733   **2013,** *19*, 3621-3628.

734   (66) Cornwall, C. E.; Boyd, P. W.; McGraw, C. M.; Hepburn, C. D.; Pilditch, C.

735   A.; Morris, J. N.; Smith, A. M.; Hurd, C. L., Diffusion boundary layers ameliorate the

736   negative effects of ocean acidification on the temperate coralline macroalga

737   *Arthrocardia corymbosa*. *PLoS ONE* **2014,** *9*, e97235.

738   (67) Miles, H.; Widdicombe, S.; Spicer, J. I.; Hall-Spencer, J., Effects of

739   anthropogenic seawater acidification on acid-base balance in the sea urchin

740   *Psammechinus miliaris*. *Mar. Pollut. Bull.* **2007,** *54*, 89-96.

741   (68) Dubois, P., The skeleton of postmetamorphic echinoderms in a changing

742   world. *Biol. Bull.* **2014,** *226*, 223-236.

743   (69) Scurr, D. J.; Eichhorn, S. J., Structure/property relationships in seashells.

744   In *Mechanical Properties of Bioinspired Materials* Viney, C.; Katti, K.; Ulm, F.-J.;

745   Hellmich, C., Eds. Materials Research Society Symposium Proceedings: Warrendale,

746   PA, 2005; pp 87–92.

747   (70) Nicol, D., Some characteristics of cold-water marine pelecypods. *J.*

748   *Paleontol.* **1967,** *41*, 1330-1340.

749   (71) Caldeira, K.; Wickett, M. E., Ocean model predictions of chemistry

750   changes from carbon dioxide emissions to the atmosphere and ocean. *J. Geophys. Res.*

751   **2005,** *110*, C09S04.

752   (72) Orr, J. C.; Fabry, V. J.; Aumont, O.; Bopp, L.; Doney, S. C.; Feely, R. A.;

753   Gnanadesikan, A.; Gruber, N.; Ishida, A.; Joos, F.; Key, R. M.; Lindsay, K.; Maier-

754   Reimer, E.; Matear, R.; Monfray, P.; Mouchet, A.; Najjar, R. G.; Plattner, G. K.;

755   Rodgers, K. B.; Sabine, C. L.; Sarmiento, J. L.; Schlitzer, R.; Slater, R. D.; Totterdell, I.

756   J.; Weirig, M. F.; Yamanaka, Y.; Yool, A., Anthropogenic ocean acidification over the

757   twenty-first century and its impact on calcifying organisms. *Nature* **2005,** *437*, 681-6.

758   (73) Fabry, V. J.; Seibel, B. A.; Feely, R. A.; Orr, J. C., Impacts of ocean

759   acidification on marine fauna and ecosystem processes. *ICES J. Mar. Sci.* **2008,** *65*,

760   414-432.

761   (74) Guinotte, J. M.; Fabry, V. J., Ocean acidification and its potential effects on

762   marine ecosystems. *Ann. N. Y. Acad. Sci.* **2008,** *1134*, 320-42.

763   (75) Peck, L. S.; Brey, T., Bomb signals in old Antarctic brachiopods. *Nature*

764   **1996,** *380*, 207-208.

765   (76) Doherty, P. J., A demographic study of a subtidal population of the New

766   Zealand articulate brachiopod *Terebratella inconspicua. Mar. Biol.* **1979,** *52*, 331-342.

767   (77) Ries, J. B.; Cohen, A. L.; McCorkle, D. C., Marine calcifiers exhibit mixed

768   responses to CO_2-induced ocean acidification. *Geology* **2009,** *37*, 1131-1134.

769   (78) Tunnicliffe, V.; Davies, K. T. A.; Butterfield, D. A.; Embley, R. W.; Rose, J.

770   M.; Chadwick Jr, W. W., Survival of mussels in extremely acidic waters on a

771   submarine volcano. *Nat. Geosci.* **2009,** *2*, 344-348.

772   (79) Thomsen, J.; Gutowska, M. A.; Saphörster, J.; Heinemann, A.;

773   Trübenbach, K.; Fietzke, J.; Hiebenthal, C.; Eisenhauer, A.; Körtzinger, A.; Wahl, M.;

774   Melzner, F., Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat

775   but are threatened by high levels of future acidification. *Biogeosciences* **2010,** *7*, 3879-

776   3891.

777   (80) Rodolfo-Metalpa, R.; Houlbreque, F.; Tambutte, E.; Boisson, F.; Baggini,

778   C.; Patti, F. P.; Jeffree, R.; Fine, M.; Foggo, A.; Gattuso, J.-P.; Hall-Spencer, J. M., Coral

779   and mollusc resistance to ocean acidification adversely affected by warming. *Nature*

780   *Clim. Change* **2011,** *1*, 308-312.

781   (81) Coleman, D. W.; Byrne, M.; Davis, A. R., Molluscs on acid: gastropod

782   shell repair and strength in acidifying oceans. *Mar. Ecol. Prog. Ser.* **2014,** *509*, 203-211.

783   (82) Peck, V. L.; Tarling, G. A.; Manno, C.; Harper, E. M.; Tynan, E., Outer

784   organic layer and internal repair mechanism protects pteropod *Limacina helicina* from

785   ocean acidification. *Deep Sea Res. Part II Top. Stud. Oceanogr.* **2016,** *127*, 41-52.

786   (83) Williams, A.; MacKay, S., Differentiation of the brachiopod periostracum.

787   *Palaeontology* **1979,** *22*, 721-736.

788   (84) Peck, L. S.; Clark, M. S.; Power, D.; Reis, J.; Batista, F. M.; Harper, E. M.,

789   Acidification effects on biofouling communities: winners and losers. *Global Change*

790   *Biol.* **2015,** *21*, 1907-1913.

791   (85) Mackenzie, C. L.; Ormondroyd, G. A.; Curling, S. F.; Ball, R. J.; Whiteley,

792   N. M.; Malham, S. K., Ocean warming, more than acidification, reduces shell

793   strength in a commercial shellfish species during food limitation. *PLoS ONE* **2014,** *9*,

794   e86764.

795   (86) Melzner, F.; Stange, P.; Trubenbach, K.; Thomsen, J.; Casties, I.; Panknin,

796   U.; Gorb, S. N.; Gutowska, M. A., Food supply and seawater pCO_2 impact

797   calcification and internal shell dissolution in the blue mussel *Mytilus edulis*. *PLoS*

798   *ONE* **2011,** *6*, e24223.

799   (87) Day, E. G.; Branch, G. M.; Viljoen, C., How costly is molluscan shell

800   erosion? A comparison of two patellid limpets with contrasting shell structures. *J.*

801   *Exp. Mar. Biol. Ecol.* **2000,** *243*, 185-208.

802   (88) Hoffman, J. I.; Peck, L. S.; Hillyard, G.; Zieritz, A.; Clark, M. S., No

803   evidence for genetic differentiation between Antarctic limpet *Nacella concinna*

804   morphotypes. *Mar. Biol.* **2009,** *157*, 765-778.

805   (89) McDonald, M. R.; McClintock, J. B.; Amsler, C. D.; Rittschof, D.; Angus,

806   R. A.; Orihuela, B.; Lutostanski, K., Effects of ocean acidification over the life history

807   of the barnacle *Amphibalanus amphitrite*. *Mar. Ecol. Prog. Ser.* **2009,** *385*, 179-187.

808   (90) Ye, F.; Jurikova, H.; Angiolini, L.; Brand, U.; Crippa, G.; Henkel, D.;

809   Laudien, J.; Hiebenthal, C.; Šmajgl, D., Variation in brachiopod microstructure and

810   isotope geochemistry under low-pH–ocean acidification conditions. *Biogeosciences*

811   **2019,** *16*, 617-642.

812   (91) Griesshaber, E.; Schmahl, W. W.; Neuser, R.; Pettke, T.; Blum, M.;

813   Mutterlose, J.; Brand, U., Crystallographic texture and microstructure of

814   terebratulide brachiopod shell calcite: An optimized materials design with

815   hierarchical architecture. *Am. Mineral.* **2007,** *92*, 722-734.

816   (92) Pérez-Huerta, A.; Dauphin, Y.; Cusack, M., Biogenic calcite granules--are

817   brachiopods different? *Micron* **2013,** *44*, 395-403.

818   (93) Harper, E. M., Are calcitic layers an effective adaptation against shell

819   dissolution in the Bivalvia? *J. Zool. Lond.* **2000,** *251*, 179-186.

820   (94) Cross, E. L.; Harper, E. M.; Peck, L. S., A 120-year record of resilience to

821   environmental change in brachiopods. *Global Change Biol.* **2018,** *24*, 2262-2271.

822   (95) Palmer, A. R., Calcification in marine molluscs: How costly is it? *PNAS*

823   **1992,** *89*, 1379-1382.

824   (96) Spalding, C.; Finnegan, S.; Fischer, W. W., Energetic costs of calcification

825   under ocean acidification. *Global Biogeochem. Cycles* **2017**.

826   (97) Watson, S.-A.; Morley, S. A.; Peck, L. S., Latitudinal trends in shell

827   production cost from the tropics to the poles. *Sci. Adv.* **2017,** *3*, e1701362.

828   (98) Ghedini, G.; Connell, S. D., Organismal homeostasis buffers the effects of

829   abiotic change on community dynamics. *Ecology* **2016,** *97*, 2671-2679.

830   (99) Connell, S. D.; Ghedini, G., Resisting regime-shifts: the stabilising effect of

831   compensatory processes. *Trends Ecol. Evol.* **2015,** *30*, 513-5.

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833   DISCLOSURES

834   The authors declare no competing financial interest.