



No ocean acidification effects on shell growth and repair in the New Zealand brachiopod *Calloria inconspicua* (Sowerby, 1846)

Emma L. Cross^{1,2*}, Lloyd S. Peck¹, Miles D. Lamare³, and Elizabeth M. Harper²

¹British Antarctic Survey, Natural Environment Research Council, High Cross, Madingley Road, Cambridge CB3 0ET, UK

²Department of Earth Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EQ, UK

³Department of Marine Science, University of Otago, 310 Castle Street, Dunedin 9016, New Zealand

*Corresponding author: tel: +44 1223333493; e-mail: emmoss@bas.ac.uk

Cross, E. L., Peck, L. S., Lamare, M. D., and Harper, E. M. No ocean acidification effects on shell growth and repair in the New Zealand brachiopod *Calloria inconspicua* (Sowerby, 1846). – ICES Journal of Marine Science, doi: 10.1093/icesjms/fsv031.

Received 24 October 2014; revised 23 January 2015; accepted 7 February 2015.

Surface seawaters are becoming more acidic due to the absorption of rising anthropogenic CO₂. Marine calcifiers are considered to be the most vulnerable organisms to ocean acidification due to the reduction in the availability of carbonate ions for shell or skeletal production. Rhychonelliform brachiopods are potentially one of the most calcium carbonate-dependent groups of marine organisms because of their large skeletal content. Little is known, however, about the effects of lowered pH on these taxa. A CO₂ perturbation experiment was performed on the New Zealand terebratulide brachiopod *Calloria inconspicua* to investigate the effects of pH conditions predicted for 2050 and 2100 on the growth rate and ability to repair shell. Three treatments were used: an ambient pH control (pH 8.16), a mid-century scenario (pH 7.79), and an end-century scenario (pH 7.62). The ability to repair shell was not affected by acidified conditions with >80% of all damaged individuals at the start of the experiment completing shell repair after 12 weeks. Growth rates in undamaged individuals >3 mm in length were also not affected by lowered pH conditions, whereas undamaged individuals <3 mm grew faster at pH 7.62 than the control. The capability of *C. inconspicua* to continue shell production and repair under acidified conditions suggests that this species has a robust control over the calcification process, where suitable conditions at the site of calcification can be generated across a range of pH conditions.

Keywords: calcification, carbonate saturation, climate change, CO₂, pH.

Introduction

Rising levels of anthropogenic CO₂ are affecting the carbonate chemistry of surface seawaters and causing our oceans to acidify (Caldeira and Wickett, 2003, 2005; Orr *et al.*, 2005; Gattuso and Hansson, 2011; IPCC, 2013). Excess atmospheric CO₂ since the industrial revolution has already caused a 0.1 pH unit decline and the rate of this change is predicted to increase considerably with a further decrease of 0.3–0.5 pH units by 2100 (Caldeira and Wickett, 2005; Orr *et al.*, 2005). Marine calcifying organisms such as corals, coccolithophores, molluscs, and brachiopods are considered to be the most susceptible to ocean acidification due to the predicted reduction in the availability of carbonate ions that is required for shell or skeletal production (Doney *et al.*, 2009; Byrne, 2011; Watson *et al.*, 2012; Byrne and Przeslawski, 2013; Kroeker *et al.*,

2013). Consequently, shell production and maintenance will likely become more difficult and more energetically expensive. Studies have shown variable responses of calcifying organisms to future predicted pH conditions with an increasing number of studies demonstrating tolerant species (Havenhand and Schlegel, 2009; Ries *et al.*, 2009; Parker *et al.*, 2012; Suckling *et al.*, 2014; Cross *et al.*, 2015).

Rhychonelliform brachiopods inhabit all the world's oceans from the intertidal to hadal depths (James *et al.*, 1992; Peck, 2001a). They are important organisms in shallow water communities as they provide a habitat for a broad range of epifauna, including other brachiopods, sponges, bryozoans, and algae, and may act as a significant carbon sink (Barnes and Peck, 1996; Rodland *et al.*, 2004). They have also been characterised as one of the most

© International Council for the Exploration of the Sea 2015.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted reuse, distribution, and reproduction in any medium, provided the original work is properly cited.

calcium carbonate-dependent groups of marine organisms due to the very large proportion of their dry mass (>90%) accounted for by their calcareous skeleton and support structures (Peck, 1993, 2008). Most brachiopod research has focused on extinct taxa as they are one of the few phyla to be represented as fossils extensively throughout the last 500 million years (Richardson, 1986; James et al., 1992; Pennington and Stricker, 2001). Despite a relatively recent increase in the literature on the distribution, ecology, and biology of extant brachiopods (Peck et al., 1997, 2005; Peck, 2005; Harper et al., 2009; Lee et al., 2010; Peck and Harper, 2010), only two studies have investigated ocean acidification impacts (McClintock et al., 2009; Cross et al., 2015), both of which used the Antarctic brachiopod, *Liothyrella uva*, as their study species. McClintock et al. (2009) found significant shell dissolution after only 14 d in pH 7.4 conditions. Only empty valves, however, were used; so, the ability of *L. uva* to compensate any acidification impacts on their shells was not investigated. Cross et al. (2015) extended this research further by investigating future pH conditions on shell growth and repair in living *L. uva* and found no impact on calcification. The target taxon in the current research is the New Zealand brachiopod *Calloria inconspicua* (Sowerby, 1846), which is in the same order (Terebratulida) as *L. uva*, therefore providing a temperate comparison to the Antarctic species studied by Cross et al. (2015).

Calloria inconspicua is a small (maximum length reported is 28 mm; Stewart, 1981), epifaunal, sessile, suspension-feeding terebratulid brachiopod endemic to New Zealand (Doherty, 1979). It has a widespread distribution throughout New Zealand and is highly abundant in the intertidal and shallow subtidal regions, with reported densities of over 1000 ind. m⁻² (Doherty, 1979). This species also has a broad temperature tolerance of 8–18°C (Lee, 1991). *Calloria inconspicua* is usually found individually or in conspecific clumps attached to hard substrata such as rock, other brachiopods, bivalves, bryozoans, gastropods, and corals (Lee, 1991). An early study reported slow growth in this species which is unevenly distributed throughout life; fastest during the first 4 years (up to 10 mm in length) and decreases after reproduction commences (Rickwood, 1977).

Growth is an indicator of an animal's well being in a particular environment as it represents the collective responses of physiological, cellular, and biochemical processes within the organism (Riisgård and Randløv, 1981). Another essential process to the existence of the vast majority of marine, shelled organisms is shell repair. Brachiopods become damaged in their natural environment due to impacts from a variety of causes including impacts from saltating clasts and predator attack (Harper et al., 2009). Such damage requires quick shell repair to prevent the loss of body fluids, protect against predators, and prevent encounters with harmful substances (Harper et al., 2012). Shell repair frequencies in *C. inconspicua* are variable between different localities with a maximum recorded frequency of 0.355 (number of damaged individuals/total number of individuals; E. M. Harper and L. S. Peck, unpublished data). Given the importance of maintaining shell production and repair, in addition to the limited research on ocean acidification effects on brachiopods, the aims of this study were to establish how shell growth and the ability to repair shell in *C. inconspicua* were affected by forecasted future pH conditions and to compare results for this temperate species with those for the Antarctic *L. uva* (Cross et al., 2015). Growth rates and the frequency of shell repair following damage were measured in control conditions and predicted mid- and end-century pH levels.

Material and methods

Sample collection

Specimens of *C. inconspicua* were hand collected at low tide from under rocks in Portobello Bay, Otago Harbour, New Zealand (45° 82.000'S, 170° 70.00'E) in January 2013. Samples remained in their conspecific clumps and, to minimise disturbance, were only collected if they were attached to removable substratum to ensure that no pedicles were cut. Environmental conditions in Otago Harbour are surface seawater temperatures of 6.4–16.0°C (Roper and Jillett, 1981; Greig et al., 1988), pH range of 8.10–8.21 (K. Currie, pers. comm.), and salinity is 32.5–34.8 (Roper and Jillett, 1981). Brachiopods were kept in seawater during the short transportation from the sampling site to Portobello Marine Laboratory, Otago Peninsula. Specimens were then immediately placed in the experimental system.

Experimental design

This study was conducted in a flow-through CO₂ perturbation system where seawater pumped from the harbour passed through sand filters (50 µm) and a finer cartridge filter (5–10 µm) before entering the system. Three treatments were used; a control at the average local current p_{H,NIST} (8.1), the predicted oceanic pH by 2050 (pH 7.8), and the predicted pH by 2100 (pH 7.6) according to the IPCC “business-as-usual” scenario of the forecasted reduction of 0.3–0.5 pH units (IPCC, 2013) with three replicate 10 l tanks for each treatment. The pH of the acidified treatments was lowered in header tanks by intermittently bubbling CO₂ gas through a ceramic diffuser to maintain the pH at predetermined pH levels via a solenoid valve connected to a TUNZE 7070/2pH-controlled computer and electrode system. The experimental pH control system had an identical set up except that it lacked CO₂ injection. A circulating pump in each mixing header tank ensured a constant pH. This set up was shown to provide stable pH conditions over >200 d (Cunningham et al., 2015). Seawater was gravity fed from each header tank at a rate of 1.05 ± 0.05 l min⁻¹ into the experimental tanks.

Seawater temperature was not manipulated and was ambient for Otago Harbour. It was measured up to three times a day (°C, Digital Testo 106) with only small differences (<0.5°C) between treatments (Table 1) and no variation between replicate tanks in each treatment. Flow rate was also checked three times per day as was the computer-controlled pH. p_{H,NIST} was measured in each treatment tank accurately twice weekly with a EUTECH instruments pH 5–10 pH/mV/°C meter and calibrated with pH buffers of pH 4.0, 7.0,

Table 1. Mean (± s.d.) seawater parameters in all three treatments during the 12-week experiment which follow the format recommended by Barry et al. (2010).

Seawater parameter	pH control	pH 7.8	pH 7.6
p _{H,NIST}	8.16 ± 0.03	7.79 ± 0.06	7.62 ± 0.05
DIC (µmol kg ⁻¹)	2082.8 ± 22.0	2211.4 ± 8.3	2252.4 ± 25.3
Alkalinity (µmol kg ⁻¹)	2278.5 ± 18.8	2269.7 ± 9.2	2271.9 ± 6.5
pCO ₂ (µatm)	464.8 ± 82.8	1130.2 ± 11.8	1535.6 ± 234.8
Ω calcite	3.5 ± 0.5	1.6 ± 0.0	1.3 ± 0.2
Ω aragonite	2.2 ± 0.3	1.0 ± 0.0	0.8 ± 0.1
Temperature (°C)	16.5 ± 1.7	16.9 ± 1.7	16.6 ± 1.7
Salinity	33.9 ± 0.2	33.9 ± 0.2	33.9 ± 0.2

Values for pCO₂, Ω calcite, and Ω aragonite were calculated using CO₂calc (Robbins et al., 2010) with refitted constants (Mehrbach et al., 1973; Dickson and Millero, 1987) as recommended by Wanninkhof et al. (1999).

and 9.2 (Pro-analys, Biolab, New Zealand). Salinity was measured once a week using a YSI data logger. A water sample from each treatment was fixed with saturated mercuric chloride (HgCl_2) at the beginning, middle, and end of the experiment. Dissolved inorganic carbon and total alkalinity were later determined by a Single Operator Multi-parameter Metabolic Analyser (SOMMA) and closed-cell potentiometric titration, respectively (Dickson *et al.*, 2007). Other carbonate system parameters, including the partial pressure of CO_2 ($p\text{CO}_2$) and the saturation values for calcite (Ω_{C}) and aragonite (Ω_{A}), were calculated using CO_2calc (Robbins *et al.*, 2010). Seawater properties were determined using CO_2 equilibrium constants from Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987) as recommended by Wanninkhof *et al.* (1999).

Brachiopods were fed three times a week with microalgal concentrate of $\sim 397 \times 10^4$ cells mL^{-1} of *Tetraselmis* spp., which is within the natural range of phytoplankton cell abundance in Otago Harbour. Faeces and other debris were removed twice weekly by siphon.

Growth rates

One hundred and twenty-three specimens of *C. inconspicua* between 0.71 and 14.47 mm length (maximum shell dimension) were used in this experiment. At the start of the experiment, shell lengths of each individual > 3 mm in length were measured to the nearest 0.01 mm using Vernier calipers. For individuals < 3 mm, shell lengths were measured on a graticule eyepiece in a field microscope. The conspecific clumps of specimens were then divided evenly across all tanks ensuring a similar size range of specimens in each treatment. After 6 weeks and at the end of the 12-week experiment, the length of each individual was measured again and the shell edge photographed. Growth rates were calculated from the increase in length (presented as $\mu\text{m d}^{-1}$).

Shell repair frequencies

The largest (> 14 mm in length) 10 or 11 individuals in each treatment were damaged by creating a 1–2 mm deep notch at the valve edge using a metal file. This style of injury replicated very similar damage seen in natural populations of rhychonelliform brachiopods and interpreted as indicative of either predator attacks or abiotic stresses (Harper *et al.*, 2009). Notches of similar size and extent were made and care was taken not to break shells or cause other damage. After 6 and 12 weeks, the damaged section of each shell edge was photographed.

Statistical analyses

All data were analysed using Minitab (Statistical Software™ Version 15). Growth rate data for each treatment were all significantly different from normal (Anderson–Darling test; $p < 0.009$). These data were still not normally distributed after square root, logarithmic, and double logarithmic transformations because of the presence of zeros in the dataset. Non-parametric Kruskal–Wallis tests were

thus used to determine whether treatment affected growth. When significant differences were found in the different datasets, further Kruskal–Wallis multiple comparison tests were used to identify which treatments were different from each other. A χ^2 test was used to establish any treatment effect on the percentage of undamaged individuals that did not grow throughout the experiment. χ^2 tests were also used to determine if treatment affected the percentage of damaged individuals that had completed shell repair at the different time periods.

Results

All seawater parameters in the control were within the ranges reported for shallow surface seawater (Table 1; Barry *et al.*, 2010), although seawater temperature was unusually high for Otago Harbour in January–April 2013 (MDL, pers. obs.) and slightly above the reported range of 6.4–16.0°C (Roper and Jillett, 1981; Greig *et al.*, 1988). Saturation states with respect to calcite and aragonite in both acidified treatments were just below the reported shallow surface seawater values ($\Omega < 1.9$ and < 1.2 , respectively); however, calcite was supersaturated ($\Omega > 1$) in both treatments and aragonite was supersaturated in pH 7.8 but undersaturated ($\Omega < 1$) in the pH 7.6 treatment. No mortality occurred throughout the 12-week experiment in any treatment. In addition, throughout the experiment, no individual demonstrated any signs of stress such as slow snapping responses, remaining closed for extended periods or wide gaping when open, and all specimens responded rapidly to physical stimulation when disturbed (Peck, 2001b; Cross *et al.*, 2015).

Shell repair frequencies

After 6 weeks, all damaged individuals had started to repair their notch and $> 36\%$ of specimens had completed shell repair across all treatments (Table 2). Treatment had no effect on shell repair frequencies ($\chi^2 = 1.714$, $p = 0.424$). After 12 weeks, $> 80\%$ of individuals had fully repaired their notch in every treatment (Table 2; Figure 1) with only three individuals (one specimen in pH 7.8 and two specimens in the pH 7.6 treatments) not completing shell repair. Treatment did not affect overall shell repair frequencies ($\chi^2 = 1.173$, $p = 0.556$). Although the majority of the damaged individuals managed to fully repair their shell, none of the large, notched individuals continued to produce new shell once the repair was complete.

Growth rates

The majority of individuals grew with growth rates ranging to values over $15 \mu\text{m d}^{-1}$ in the pH control and the pH 7.6 treatment and up to $10.70 \mu\text{m d}^{-1}$ in the pH 7.8 treatment (Figure 2). The only apparent ontogenetic trend was a slight decrease in growth rates for individuals > 10 mm in length. Growth rates of undamaged individuals > 3 mm in both acidified conditions were not significantly different from that of the pH control (Kruskal–Wallis, $H = 4.04$, $p = 0.133$).

Table 2. Shell repair frequencies after 6 weeks and after 12 weeks in the stated conditions.

Treatment	Number of individuals damaged at the start of the experiment	Length range of damaged individuals (mm)	Percentage of individuals that had completed repair	
			After 6 weeks	After 12 weeks
pH control	11	14.18–17.79 (mean = 15.59)	36% ($n = 4$)	100% ($n = 11$)
pH 7.8	10	14.14–17.62 (mean = 15.80)	40% ($n = 4$)	90% ($n = 9$)
pH 7.6	10	14.05–17.52 (mean = 15.31)	50% ($n = 5$)	80% ($n = 8$)

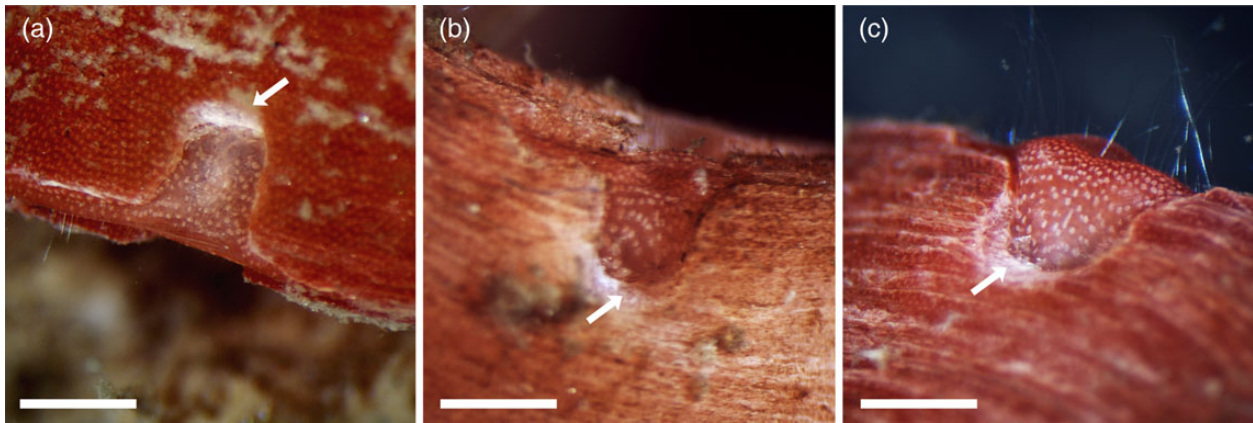


Figure 1. Examples of completed shell repair in damaged individuals after 12 weeks in the (a) pH control, (b) pH 7.8, and (c) pH 7.6 treatment. The arrow indicates the notch created at the start of the experiment. Scale bar = 100 μm .

Whereas growth rates were different among treatments in undamaged individuals <3 mm ($H = 6.23$, $p = 0.044$) and also when all undamaged individuals across the total size range were pooled ($H = 7.90$, $p = 0.019$). A further Kruskal–Wallis multiple comparison test on undamaged individuals <3 mm indicated that growth rates were higher in the most acidified treatment (pH 7.6; $Z = 2.488$, $p = 0.013$) compared with the moderately acidified treatment (pH 7.8) but not compared with the pH control ($Z = 0.826$, $p = 0.409$). Growth rates of undamaged individuals <3 mm in the moderately acidified treatment were not significantly different from the pH control ($Z = 0.746$, $p = 0.456$). A further Kruskal–Wallis Multiple Comparison test on all undamaged individuals indicated that in the most acidified treatment, growth rates were higher than in the pH control ($Z = 2.762$, $p = 0.006$) but not compared with the moderately acidified treatment ($Z = 1.918$, $p = 0.055$). Growth rates of all undamaged individuals in the moderately acidified treatment were also not significantly different from the pH control ($Z = 0.980$, $p = 0.327$). There was also no treatment effect on the proportion of undamaged individuals that did not grow throughout the experiment ($\chi^2 = 1.500$, $p = 0.472$).

Discussion

There were no signs of stress and no mortalities throughout the 12-week experiment, indicating that *C. inconspicua* are able to tolerate predicted mid- and end-century pH conditions. Similarly, mortality rates in other equivalent ocean acidification studies with brachiopods (*L. uva*; Cross et al., 2015) and molluscs (*Arctica islandica*; Hiebenthal et al., 2012) were low (3.9 and 3.3%, respectively). *Calloria inconspicua* will also be able to repair shell damage in the natural environment in the next 100 years as suggested by the ability of damaged individuals to continue shell repair under acidified conditions with $>80\%$ of injured specimens in all treatments completing shell repair after 12 weeks. Shell repair in the gastropod *Subrinella undulata* was also unaffected by ocean acidification; however, the gastropod *Austrocochlea porcata* had a decreased shell repair rate, suggesting a species-specific response of marine shelled organisms in the ability to repair shell (Coleman et al., 2014). Although the majority of the damaged individuals managed to repair their shell fully, none of them continued to produce new shell once the repair was complete. All damaged individuals of *C. inconspicua* in this study were, however, the largest in their treatment, all being above 14 mm. This size coincides with

the reported 14–16 mm size of sexual maturity in *C. inconspicua* (Doherty, 1976; Lee and Wilson, 1979) indicating that growth rates were already low in these individuals due to a transfer of energy and resources from somatic growth to reproduction. Therefore, once the critical process of repair was complete, these individuals were much less likely to grow than the smaller juveniles. It remains unknown how smaller individuals will respond to the additional stress of shell repair in future oceans. Furthermore, shell production in *C. inconspicua* should be unaffected by changing pH levels in the natural environment up to the year 2100, as growth rates in undamaged individuals were either not affected (>3 mm in length) or positively affected (<3 mm in length) by acidified conditions. Shell growth studies in lowered pH conditions on molluscs have shown varied responses (Michaelidis et al., 2005; Berge et al., 2006; Nienhuis et al., 2010; Thomsen et al., 2010; Hiebenthal et al., 2012), which could be due to the short- to medium-term duration (44 d) of some studies or the individual tolerance of different species (Ries et al., 2009). The ability of *C. inconspicua* to continue shell production in low pH conditions suggests that this species has a strong control over their calcification process, similar to molluscs, by being able to generate suitable conditions at the site of calcification against a stronger concentration gradient (Ries, 2011; Gazeau et al., 2013; Wittmann and Pörtner, 2013). Apart from the capability of marine calcifiers to elevate pH in calcifying compartments to facilitate precipitation of calcium carbonate, the mechanisms are poorly known, particularly in less-studied brachiopods.

This resilience to predicted future pH levels is also apparent in the Antarctic brachiopod, *L. uva* (Cross et al., 2015). Similar large proportions ($>90\%$) of identically damaged *L. uva* fully repaired their notch in the only other ocean acidification study to involve brachiopods (Cross et al., 2015). The Antarctic study was conducted over 7 months, whereas the current work was a 12-week experiment, suggesting repair mechanisms may be faster in temperate brachiopods than Antarctic species as has been reported for growth (Peck et al., 1997; Baird et al., 2013). The high success rates of shell repair in lowered pH conditions in both a temperate and polar brachiopod suggest these species can maintain the rate of shell repair and regeneration in challenging chemical environments just as had previously been seen in the ophiuroid *Amphiura filiformis* (Wood et al., 2008). Over 63% of all injured specimens in *L. uva* made new shell after repairing their notch (Cross et al., 2015) further demonstrating the tolerance of this species. A wide size

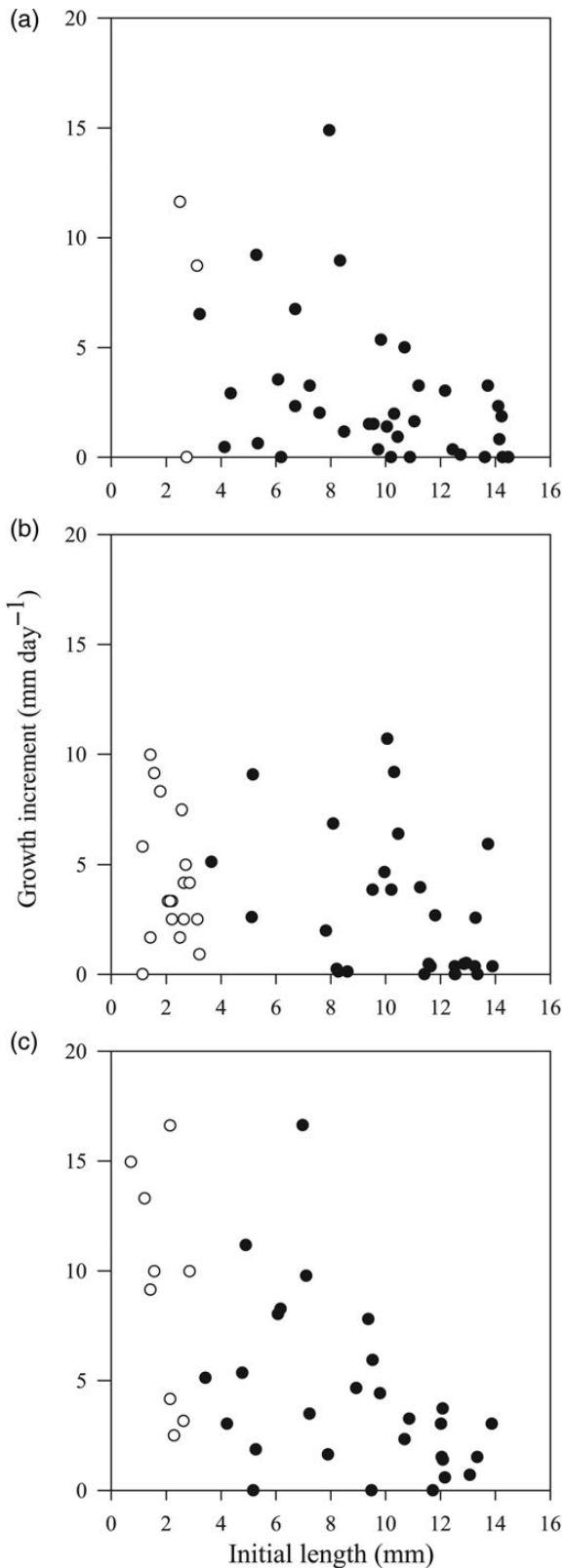


Figure 2. Growth rates of individuals < 3 mm (open circle) and > 3 mm (filled circle) that were left undamaged at the start of the experiment after 12 weeks in (a) the pH control (pH 8.16 ± 0.03), (b) pH 7.8 (pH 7.79 ± 0.06), and (c) pH 7.6 (pH 7.62 ± 0.05) treatments. Different symbols have been used for individuals above and below 3 mm for the two different methods used.

range (5.0–37.0 mm in length) of individuals had been damaged at the start of the Antarctic experiment, though compared with the limited size range used in the current experiment, specimens of all ages were included and therefore more were likely to continue shell deposition after repair. As here, shell growth rates of undamaged individuals of *L. uva* were not affected by acidified conditions. A 2°C increase in temperature, however, positively affected shell growth in *L. uva*.

Multiple stressors are becoming more widely used in ocean acidification studies where parameters such as temperature, food availability, and hypoxia have been shown to have a greater effect on marine organisms than lowered pH (Hiebenthal *et al.*, 2012; Thomsen *et al.*, 2013; Wolfe *et al.*, 2013; Hardy and Byrne, 2014; Hyun *et al.*, 2014; Noisette *et al.*, 2014; Cross *et al.*, 2015; Queiros *et al.*, 2015), although some studies demonstrate an interactive effect (Ericson *et al.*, 2012; Reymond *et al.*, 2013; Gobler *et al.*, 2014; Ko *et al.*, 2014). Research on the mollusc *Mytilus edulis* revealed that acidified conditions did not affect growth rate but rising temperature increased growth up to 20°C, which then sharply declined at 25°C, indicating that 25°C is above this species temperature tolerance limit (Pörtner, 2008; Hiebenthal *et al.*, 2012). Another study on the same species found that an abundant food supply outweighed the effects of ocean acidification on growth and calcification (Thomsen *et al.*, 2013). Oxygen availability, along with temperature but not $p\text{CO}_2$, were also found to be the dominating factors determining metabolic rate reductions in the squid *Dosidicus gigas* (Rosa and Seibel, 2008).

Overall, studies showing tolerance of marine species to ocean acidification are increasing, especially with the wider use of longer term experiments (Hazan *et al.*, 2014; Suckling *et al.*, 2014; Cross *et al.*, 2015; Queiros *et al.*, 2015). After 3 months, it was apparent that *C. inconspicua* is resilient to lowered pH in terms of shell growth and repair, highlighting the need for longer term ocean acidification studies to allow for acclimation and adaptation to better understand different species capabilities to respond to changing pH conditions. However, perhaps other biological processes could have been impacted by increasing acidity as seen in the ophiuroid *A. filiformis* where muscle wastage was reported in lowered pH treatments (Wood *et al.*, 2008). Further investigation is needed to determine such effects in *C. inconspicua*. Therefore, more environmentally relevant research, including several variables over long-term durations, is crucial to fully understand and predict how organisms will respond to near future changing environmental conditions.

Acknowledgements

The authors would like to thank the science support staff at the Portobello Marine Laboratory, University of Otago, for their help in the set up and maintenance of the ocean acidification experimental system. Thanks also to Kim Currie at National Institute of Water and Atmospheric Research for the DIC and total alkalinity measurements. ELC is supported by the NERC PhD Studentship (NE/T/A/2011).

References

- Baird, M. J., Lee, D. E., and Lamare, M. D. 2013. Reproduction and growth of the terebratulid brachiopod *Liothyrella neozelanica* Thomson, 1918 from Doubtful Sound, New Zealand. *The Biological Bulletin*, 225: 125–136.
- Barnes, D. K. A., and Peck, L. S. 1996. Epibiota and attachment substrata of deep-water brachiopods from Antarctica and New Zealand.

- Philosophical Transactions of the Royal Society of London Series B: Biological Sciences, 351: 677–687.
- Barry, J. P., Tyrell, T., Hansson, L., Plattner, G. K., and Gattuso, J. P. 2010. Atmospheric CO₂ targets for ocean acidification perturbation experiments. *In* Guide to Best Practices for Ocean Acidification Research and Data Reporting, pp. 53–66. Ed. by U. Riebesell, V. F. Fabry, L. Hansson, and J. P. Gattuso. Publications Office of the European Union.
- Berge, J. A., Bjerkeng, B., Pettersen, O., Schaanning, M. T., and Oxnevad, S. 2006. Effects of increased sea water concentrations of CO₂ on growth of the bivalve *Mytilus edulis* L. *Chemosphere*, 62: 681–687.
- Byrne, M. 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. *Oceanography and Marine Biology: An Annual Review*, 49: 1–42.
- Byrne, M., and Przeslawski, R. 2013. Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. *Integrative and Comparative Biology*, 53: 582–596.
- Caldeira, K., and Wickett, M. E. 2003. Anthropogenic carbon and ocean pH. *Nature*, 425: 365.
- Caldeira, K., and Wickett, M. E. 2005. Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research*, 110: C09S04.
- Coleman, D. W., Byrne, M., and Davis, A. R. 2014. Molluscs on acid: gastropod shell repair and strength in acidifying oceans. *Marine Ecology Progress Series*, 509: 203–211.
- Cross, E. L., Peck, L. S., and Harper, E. M. 2015. Ocean acidification does not impact shell growth or repair of the Antarctic brachiopod *Liothyrella uva* (Broderip, 1833). *Journal of Experimental Marine Biology and Ecology*, 462: 29–35.
- Cunningham, S. C., Smith, A. M., and Lamare, M. D. 2015. The effects of elevated pCO₂ on growth, shell production and metabolism of cultured juvenile abalone, *Haliotis iris*. *Aquaculture Research*, doi:10.1111/are.12684.
- Dickson, A. G., and Millero, F. J. 1987. A comparison of the equilibrium-constants for the dissociation of carbonic-acid in seawater media. *Deep Sea Research A: Oceanographic Research Papers*, 34: 1733–1743.
- Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to Best Practices for Ocean CO₂ Measurements. IOCCP Report No. 8.
- Doherty, P. J. 1976. Aspects of the feeding ecology of the sub-tidal brachiopod *Terebratella inconspicua* (Sowerby, 1846). Unpublished PhD thesis, Zoology Department, University of Auckland.
- Doherty, P. J. 1979. A demographic study of a subtidal population of the New Zealand articulate brachiopod *Terebratella inconspicua*. *Marine Biology*, 52: 331–342.
- Doney, S. C., Fabry, V. J., Feely, R. A., and Kleypas, J. A. 2009. Ocean acidification: the other CO₂ problem. *Annual Review of Marine Science*, 1: 169–192.
- Ericson, J. A., Ho, M. A., Miskelly, A., King, C. K., Virtue, P., Tilbrook, B., and Byrne, M. 2012. Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biology*, 35: 1027–1034.
- Gattuso, J. P., and Hansson, L. 2011. *Ocean Acidification*. Oxford University Press, Oxford.
- Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J-P., O'Connor, W. A., Martin, S., Pörtner, H-O., et al. 2013. Impacts of ocean acidification on marine shelled molluscs. *Marine Biology*, 160: 2207–2245.
- Gobler, C., DePasquale, E. L., Griffith, A. W., and Baumann, H. 2014. Hypoxia and acidification have additive and synergistic negative effects on the growth, survival, and metamorphosis of early life stage bivalves. *PLoS ONE*, 9: e83648.
- Greig, M. J., Ridgway, N. M., and Shakespeare, B. S. 1988. Sea surface temperature variations at coastal sites around New Zealand. *New Zealand Journal of Marine and Freshwater Research*, 22: 391–400.
- Hardy, N. A., and Byrne, M. 2014. Early development of congeneric sea urchins (*Heliocidaris*) with contrasting life history modes in a warming and high CO₂ ocean. *Marine Environmental Research*, 102: 78–87.
- Harper, E. M., Clark, M. S., Hoffman, J. I., Philipp, E. E., Peck, L. S., and Morley, S. A. 2012. Iceberg scour and shell damage in the Antarctic bivalve *Laternula elliptica*. *PLoS ONE*, 7: e46341.
- Harper, E. M., Peck, L. S., and Hendry, K. R. 2009. Patterns of shell repair in articulate brachiopods indicate size constitutes a refuge from predation. *Marine Biology*, 156: 1993–2000.
- Havenhand, J. N., and Schlegel, P. 2009. Near-future levels of ocean acidification do not affect sperm motility and fertilisation kinetics in the oyster *Crassostrea gigas*. *Biogeosciences*, 6: 3009–3015.
- Hazan, Y., Wangensteen, O. S., and Fine, M. 2014. Tough as a rock-boring urchin: adult *Echinometra* sp. EE from the Red Sea show high resistance to ocean acidification over long-term exposures. *Marine Biology*, 161: 2531–2545.
- Hiebenthal, C., Philipp, E. E. R., Eisenhauer, A., and Wahl, M. 2012. Effects of seawater pCO₂ and temperature on shell growth, shell stability, condition and cellular stress of Western Baltic Sea *Mytilus edulis* (L.) and *Arctica islandica* (L.). *Marine Biology*, 160: 2073–2087.
- Hyun, B., Choi, K-H., Jang, P-G., Jang, M-C., Lee, W-J., Moon, C-H., and Shin, K. 2014. Effects of increased CO₂ and temperature on the growth of four diatom species (*Chaetoceros debilis*, *Chaetoceros didymus*, *Skeletonema costatum* and *Thalassiosira nordenskiöldii*) in laboratory experiments. *Journal of Environmental Science International*, 23: 1003–1012.
- IPCC. 2013. Climate change 2013: the physical science basis. *In* Working Group I Contribution to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, p. 1552. Ed. by T. F. Stocker, D. Qin, G-K. Plattner, M. Tignor, S. K. Allen, J. Boschung, A. Nauels, et al. Cambridge, UK and New York, NY, USA.
- James, M. A., Ansell, A. D., Collins, M. J., Curry, G. B., Peck, L. S., and Rhodes, M. C. 1992. Biology of living brachiopods. *Advances in Marine Biology*, 28: 175–387.
- Ko, G. W., Dineshram, R., Campanati, C., Chan, V. B., Havenhand, J., and Thiagarajan, V. 2014. Interactive effects of ocean acidification, elevated temperature, and reduced salinity on early-life stages of the pacific oyster. *Environmental Science and Technology*, 48: 10079–10088.
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., et al. 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Global Change Biology*, 19: 1884–1896.
- Lee, D. E. 1991. Aspects of the ecology and distribution of the living Brachiopoda of New Zealand. *In* Brachiopods Through Time, pp. 273–279. Ed. by D. I. MacKinnon, D. E. Lee, and J. D. Campbell. A.A Balkema Publishers, Rotterdam, The Netherlands.
- Lee, D. E., Robinson, J. H., Witman, J. D., Copeland, S. E., Harper, E. M., Smith, F., and Lamare, M. D. 2010. Observations on recruitment, growth and ecology in a diverse living brachiopod community, Doubtful Sound, Fiordland, New Zealand. *Special Papers in Palaeontology*, 84: 177–191.
- Lee, D. E., and Wilson, J. B. 1979. Cenozoic and recent rhynchonellide brachiopods of New Zealand: Systematics and variation in the genus *Notosaria*. *Journal of the Royal Society of New Zealand*, 9: 437–463.
- McClintock, J. B., Angus, R. A., McDonald, M. R., Amsler, C. D., Catledge, S. A., and Vohra, Y. K. 2009. Rapid dissolution of shells of weakly calcified Antarctic benthic macroorganisms indicates high vulnerability to ocean acidification. *Antarctic Science*, 21: 449.
- Mehrbach, C., Culberson, C. H., Hawley, J. E., and Pytkowicz, R. M. 1973. Measurement of apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, 18: 897–907.

- Michaelidis, B., Ouzounis, C., Paleras, A., and Portner, H. O. 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series*, 293: 109–118.
- Nienhuis, S., Palmer, A. R., and Harley, C. D. 2010. Elevated CO₂ affects shell dissolution rate but not calcification rate in a marine snail. *Proceedings of the Royal Society of London Series B: Biological Sciences*, 277: 2553–2558.
- Noisette, F., Richard, J., Le Fur, I., Peck, L. S., Davoult, D., and Martin, S. 2014. Metabolic responses to temperature stress under elevated pCO₂ in *Crepidula fornicata*. *Journal of Molluscan Studies*, doi:10.1093/mollus/eyu084.
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., et al. 2005. Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, 437: 681–686.
- Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., and Pörtner, H. 2012. Adult exposure influences offspring response to ocean acidification in oysters. *Global Change Biology*, 18: 82–92.
- Peck, L. S. 1993. The tissues of articulate brachiopods and their value to predators. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, 339: 17–32.
- Peck, L. S. 2001a. Ecology. In *Brachiopods Ancient and Modern: a Tribute to G. Arthur Cooper*. The Paleontology Society Papers, pp. 171–183. Ed. by S. Carlson, and M. Sandy. Yale University, New Haven, CT.
- Peck, L. S. 2001b. Physiology. In *Brachiopods Ancient and Modern: a Tribute to G. Arthur Cooper*. The Paleontology Society Papers, pp. 89–104. Ed. by S. Carlson, and M. Sandy. Yale University, New Haven, CT.
- Peck, L. S. 2005. Prospects for survival in the Southern Ocean: vulnerability of benthic species to temperature change. *Antarctic Science*, 17: 497.
- Peck, L. S. 2008. Brachiopods and climate change. *Earth and Environmental Science Transactions of the Royal Society of Edinburgh*, 98: 451–456.
- Peck, L. S., Barnes, D. K. A., and Willmott, J. 2005. Responses to extreme seasonality in food supply: diet plasticity in Antarctic brachiopods. *Marine Biology*, 147: 453–463.
- Peck, L. S., Brockington, S., and Brey, T. 1997. Growth and metabolism in the Antarctic brachiopod *Liothyrella uva*. *Philosophical Transactions of the Royal Society of London Series B: Biological Sciences*, 352: 851–858.
- Peck, L. S., and Harper, E. M. 2010. Variation in size of living articulated brachiopods with latitude and depth. *Marine Biology*, 157: 2205–2213.
- Pennington, J. T., and Stricker, S. A. 2001. Phylum Brachiopoda. In *Atlas of Marine Invertebrate Larval Forms*, pp. 441–461. Ed. by C. M. Young. Academic Press, New York.
- Pörtner, H. 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. *Marine Ecology Progress Series*, 373: 203–217.
- Queiros, A. M., Fernandes, J. A., Faulwetter, S., Nunes, J., Rastrick, S. P., Mieszkowska, N., Artioli, Y., et al. 2015. Scaling up experimental ocean acidification and warming research: from individuals to the ecosystem. *Global Change Biology*, 21: 130–143.
- Reymond, C. E., Lloyd, A., Kline, D. I., Dove, S. G., and Pandolfi, J. M. 2013. Decline in growth of foraminifer *Marginopora rossi* under eutrophication and ocean acidification scenarios. *Global Change Biology*, 19: 291–302.
- Richardson, J. R. 1986. Brachiopods. *Scientific American*, 255: 100–106.
- Rickwood, A. E. 1977. Age, growth and shape of the intertidal brachiopod *Waltonia inconspicua* Sowerby, from New Zealand. *American Zoologist*, 17: 63–73.
- Ries, J. B. 2011. A physicochemical framework for interpreting the biological calcification response to CO₂-induced ocean acidification. *Geochimica et Cosmochimica Acta*, 75: 4053–4064.
- Ries, J. B., Cohen, A. L., and McCorkle, D. C. 2009. Marine calcifiers exhibit mixed responses to CO₂-induced ocean acidification. *Geology*, 37: 1131–1134.
- Riisgård, H. U., and Randløv, A. 1981. Energy budgets, growth and filtration rates in *Mytilus edulis* at different algal concentrations. *Marine Biology*, 61: 227–234.
- Robbins, L. L., Hansen, M. E., Kleypas, J. A., and Meylan, S. C. 2010. CO₂calc: a User-friendly Carbon Calculator for Windows, Mac OS X, and iOS (iPhone).
- Rodland, D. L., Kowalewski, M., Carroll, M., and Simões, M. G. 2004. Colonization of a “Lost World”: encrustation patterns in modern subtropical brachiopod assemblages. *Palaios*, 19: 381–395.
- Roper, D. S., and Jillett, J. B. 1981. Seasonal occurrence and distribution of flatfish (Pisces: Pleuronectiformes) in inlets and shallow water along the Otago coast. *New Zealand Journal of Marine and Freshwater Research*, 15: 1–13.
- Rosa, R., and Seibel, B. A. 2008. Synergistic effects of climate-related variables suggest future physiological impairment in a top oceanic predator. *Proceedings of the National Academy of Sciences of the United States of America*, 105: 20776–20780.
- Stewart, I. R. 1981. Population structure of articulate brachiopod species from soft and hard substrates. *New Zealand Journal of Zoology*, 8: 197–207.
- Suckling, C. C., Clark, M. S., Richard, J., Morley, S. A., Thorne, M. A. S., Harper, E. M., and Peck, L. S. 2014. Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. *Journal of Animal Ecology*, doi:10.1111/1365-2656.12316.
- Thomsen, J., Casties, I., Pansch, C., Kortzinger, A., and Melzner, F. 2013. Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology*, 19: 1017–1027.
- Thomsen, J., Gutowska, M. A., Saphörster, J., Heinemann, A., Trübenbach, K., Fietzke, J., Hiebenthal, C., et al. 2010. Calcifying invertebrates succeed in a naturally CO₂-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences*, 7: 3879–3891.
- Wanninkhof, R., Lewis, E., Feely, R. A., and Millero, F. J. 1999. The optimal carbonate dissociation constants for determining surface water pCO₂ from alkalinity and total inorganic carbon. *Marine Chemistry*, 65: 291–301.
- Watson, S.-A., Peck, L. S., Tyler, P. A., Southgate, P. C., Tan, K. S., Day, R. W., and Morley, S. A. 2012. Marine invertebrate skeleton size varies with latitude, temperature and carbonate saturation: implications for global change and ocean acidification. *Global Change Biology*, 18: 3026–3038.
- Wittmann, A. C., and Pörtner, H. 2013. Sensitivities of extant animal taxa to ocean acidification. *Nature Climate Change*, 3: 995–1001.
- Wolfe, K., Dworjanyn, S. A., and Byrne, M. 2013. Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Heliocidaris erythrogramma*). *Global Change Biology*, 19: 2698–2707.
- Wood, H. L., Spicer, J. I., and Widdicombe, S. 2008. Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society of London, Series B: Biological Sciences*, 275: 1767–1773.