Ocean acidification does not impact shell growth or repair of the Antarctic brachiopod *Liothyrella uva* (Broderip, 1833)

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Keywords: Climate change, calcification, CO_2, pH, carbonate saturation.

Abstract

Marine calcifiers are amongst the most vulnerable organisms to ocean acidification due to reduction in the availability of carbonate ions for skeletal/shell deposition. However, there are limited long-term studies on the possible impacts of increased pCO_2 on these taxa. A 7 month CO_2 perturbation experiment was performed on one of the most calcium carbonate dependent species, the Antarctic brachiopod *Liothyrella uva,* which inhabits the Southern Ocean where carbonate ion saturation levels are amongst the lowest on Earth. The effects of the predicted environmental conditions in 2050 and 2100 on the growth rate and ability to repair shell in *L.uva* were tested with four treatments; a low temperature control (0°C, pH7.98), a pH control (2°C, pH8.05), mid-century scenario (2°C, pH7.75) and end-century scenario (2°C, pH7.54). Environmental change impacts on shell repair are rarely studied, but here repair was not affected by either acidified conditions or temperature. Growth rate was also not impacted by low pH. Elevated temperature did, however, increase growth rates. The ability of *L.uva* to continue, and even increase shell production in warmer and acidified seawater suggests that this species can acclimate to these combined stressors and generate suitable conditions for shell growth at the site of calcification.

1. Introduction

Increasing CO_2 levels from anthropogenic activities over the past 250 years has altered our oceans through warming and also acidification (Caldeira and Wickett, 2003, 2005; IPCC, 2013; Orr et al., 2005). This latter process has received much

attention recently with the chemical implications now being fairly well described, although the biological and ecological consequences are less well described (Gattuso et al., 2013; Wittmann and Pörtner, 2013). However, there is a consensus that marine calcifying organisms are the most susceptible animal group to ocean acidification because the predicted reduction in the availability of carbonate ions will make it more difficult and more energetically expensive for shell production (Byrne, 2011; Byrne and Przeslawski, 2013; Doney et al., 2009; Watson et al., 2012). To date studies have reported varied responses of calcifying organisms to future predicted pH levels with an increasing number of studies indicating that some species are tolerant (Havenhand and Schlegel, 2009; Parker et al., 2012; Ries et al., 2009). However, it must be recognised that the majority of experiments have been conducted on relatively short time scales so the ability of organisms to acclimate or adapt is largely unknown (Byrne, 2011; Byrne and Przeslawski, 2013; Gattuso et al., 2013; Wittmann and Pörtner, 2013). Longer term studies are increasing though, which are providing insights into how organisms are coping with acidifying oceans (Form and Riebesell, 2012; Kelly et al., 2013; Pandolfi et al., 2011; Pespeni et al., 2013).

The fastest rates of change in carbonate chemistry are expected in the Southern Ocean (Caldeira and Wickett, 2005; McNeil and Matear, 2008). CO_2 is more soluble in cold water (Revelle and Fairbridge, 1957) resulting in naturally low carbonate ion saturation levels compared to temperate and tropical regions. Acid-base coefficients are also more sensitive in cold temperatures making this high latitude region a forerunner of biological ocean acidification impacts for other oceans (Fabry et al., 2009). Furthermore, the absence of shell-crushing predators, such as

crabs, lobsters and heavily jawed fish (Aronson et al., 2007) and the difficulty of extracting Ca^{2+} from seawater at low temperature (Aronson et al., 2007; Harper, 2000) has resulted in Antarctic species generally having thin, weakly calcified shells (Vermeij, 1978; Watson et al., 2012). This, added to the low physiological rates of Antarctic marine species (Peck et al., 2007), especially low metabolic rates (Peck and Conway, 2000), slow growth rates (Arntz et al., 1994), delayed reproduction (Meidlinger et al., 1998) and high longevity (Pearse et al., 1991), indicates that these organisms are likely to be amongst the most vulnerable species worldwide to acidifying oceans. Although there are several studies on the potential impacts of this aspect of climate change on the larval stage of Antarctic calcifying organisms (see review by Byrne (2011)), there are limited studies on adults (Cummings et al., 2011; McClintock et al., 2009), and the longest of these lasted 4 months.

Rhychonelliform brachiopods are potentially the most calcium carbonate dependent group of marine animals because their calcareous skeleton and other support structures makes up over 90% of their dry mass (Peck, 1993, 2008). They have locally also been important organisms in shallow water communities, providing a habitat for a diverse range of epifauna including encrusting sponges and algae (Barnes and Peck, 1996), for the last 500 million years surviving several geological periods where the pH has fluctuated. Ocean pH has declined in the past 250 years at a rate of at least an order of magnitude faster than has occurred for millions of years (Doney and Schimel, 2007; Doney et al., 2009). Despite this, only two studies have addressed the potential climate change impacts on extant brachiopods (McClintock et al., 2009; Peck, 2008) and only the former investigated ocean acidification effects where significant dissolution in *Liothyrella uva* (our target taxon) valves was found

after only 14 days in pH 7.4 conditions. However, only empty valves were used so the biological response and ability of *L.uva* to compensate for the impacts of future pH conditions therefore remain to be investigated.

Liothyrella uva (Broderip, 1833) is a large (maximum recorded length is 55 mm), epifaunal, sessile, suspension-feeding terebratulide brachiopod with a circumpolar distribution (Peck et al., 2001). It is found down to 300 metres and is highly abundant in habitats protected from anchor ice and ice scour with reported densities up to 3000 individuals per m^2 (Foster, 1974; Peck et al., 2001). *L.uva* is typically found attached singly or in clumps to vertical and overhanging rocks around the South Orkney Islands, the Antarctic Peninsula and Peter I Island (Foster, 1974). Previous growth studies on *L.uva* have recorded slower growth rates than temperate rhychonelliform brachiopods and it can live for over 55 years (Peck and Brey, 1996). It also has a limited tolerance to elevated temperature, surviving up to 4.5°C (Peck et al., 2001).

The shell is essential to the existence of *L.uva,* providing protection from predators and preventing any encounters with harmful substances and the loss of body fluids (Harper et al., 2012). Any environmental insult negatively impacting the production, maintenance and/or repair of their shell could thus prove fatal. *L.uva* also becomes naturally damaged and their shells require repairing in the environment (Harper et al., 2009). Given this, and the current focus on ocean acidification, the aims of this study were to determine how shell growth rates and the frequency of shell repair following damage in *L.uva* were affected in a 7 month experiment using predicted mid and end century pH levels.

2. Materials and methods

2.1. Sampling collection

Specimens of *Liothyrella uva* (Broderip, 1833) were hand collected by SCUBA divers from Trolval Island, Ryder Bay, Antarctica (67° 35.44' S, 68° 12.44' W) at 15-25 m depth in May 2012. Animals remained in their conspecific clumps with only the pedicle of the central brachiopod attached to the cliff face being cut ensuring that the majority of specimens were not damaged during collection. Environmental conditions in Ryder Bay at 15-25m depth consist of seawater temperatures that range from -1.8 to +1.0°C, however, temperatures rarely exceed +0.5°C (Clarke et al., 2008), the pH range is 8.04-8.10 (McNeil and Matear, 2008) and salinity is 33.0-34.0 (Clarke et al., 2008). Brachiopods were kept underwater during the short transportation from the sampling site to the marine laboratory in Rothera and in recirculating aquaria (0.0 ± 0.5°C) whilst being transported back to the UK. Specimens remained in an ambient recirculating seawater system in the UK in similar conditions for a further two weeks to habituate to aquarium conditions before the experiment began.

2.2. Experimental Design

This study was conducted in a recirculating CO_2 microcosm adapted from (Suckling et al., in press) at the British Antarctic Survey (BAS), UK. Four treatments were used where two functioned as lowered pH treatments (pH7.75 and pH7.54) based on the IPCC 'business-as-usual' scenario of the predicted reduction of 0.3-0.5 pH units from the present day average of pH8.05 in oceanic surface waters by 2100 (Table 1) (IPCC, 2013). The third was a pH control as the seawater remained at ambient pH (pH8.05). As a concurrent 2°C increase in temperature is expected to

occur alongside this forecasted decrease in pH by the end of the century (Mitchell et al., 1998), these three systems were maintained at 2°C throughout the experiment. The fourth remaining system was a temperature control which was held at the present-day average surface seawater temperature for Ryder Bay, 0°C (Clarke et al., 2008). The average pH of this treatment was pH7.98 which was slightly lower than, but close to, the pH of the pH control treatment probably as a result of the increased solubility of CO_2 and carbonates (CO_3^{2-}) in seawater at the lower temperatures.

The pH of the lowered pH treatments was altered by intermittently bubbling CO_2 gas through a ceramic diffuser to maintain the pH at the predetermined pH levels via a solenoid valve connected to an Aqua Medic pH controlled computer and glass electrode (with plastic shaft) system. The pH control had a similar set up but without the pH control system. An Aqua Medic Ocean Runner power head 2000 circulated the seawater in the mixing tank to ensure a constant pH. Seawater was then gravity fed from each mixing tank at a rate of 0.65 ± 0.03 L min^{-1} into the experimental tank. Seawater temperature was manipulated by the use of temperature-controlled laboratories. Air temperature was maintained at -2.5°C in the laboratory with the pH control and both lowered pH treatments but the lifting pumps (Aqua Medic Ocean Runner 3500) and the mixing power heads (Aqua Medic Ocean Runner 2000) in each treatment's mixing tank caused the seawater temperature to raise to the desired ~2°C, with minimal variability (Table 1). The temperature control treatment was situated in the main BAS aquarium where the air temperature was set at -1.5°C and the absence of lifting and circulating pumps caused the seawater temperature to be maintained at ~0°C.

Seawater temperatures (°C, Digital Testo 106) and pH_{NIST} (Aquamedic pH controlled computer and electrode system) were monitored and recorded daily. pH_{NIST} was also more accurately measured once a week with a temperature compensated HANNA bench top meter pH/ORP 115 v pH21-01). Salinity (Tropical Marine Centre V2 Handheld refractometer), TCO_2 (mmol L^{-1}; Ciba Corning TCO_2 Analyzer 965, Olympic Analytical. UK) and nutrient content (silicate and phosphate; according to methods in Nickell et al. (2003)) of each treatment were also measured weekly. Twice a week, the Aqua Medic pH probes were calibrated with NIST certified pH buffers. Other carbonate system parameters, including the partial pressure of CO_2 (pCO_2) and the saturation values for calcite (Ω_C) and aragonite (Ω_A), were modelled from applying TCO_2 and pH_{NIST} data to the program CO2SYS (Lewis and Wallace, 1998) with refitted constants (Dickson and Millero, 1987; Mehrbach et al., 1973). Brachiopods in each treatment were fed weekly with microalgal concentrate of approximately 331 x 10^4 cells L^{-1} which is within the natural range of phytoplankton cell abundance along the west Antarctic Peninsula (62 – 1150 x 10^4 cells L^{-1}; (Garibotti et al., 2005; Garibotti et al., 2003). Water quality was maintained and alkalinity and other ions replenished through water changes twice a week and weekly siphoning of the aquaria to remove any debris.

2.3. Growth rates

One hundred and eleven specimens of *L.uva* across a wide size range (2.6-40.9 mm in length) and varying number of individuals in each conspecific clump (30 conspecific clumps with 2-15 individuals and 20 single specimens) were used in this experiment. Prior to the beginning of the experiment, shell lengths of each individual in each conspecific clump were measured to the nearest 0.1 mm using Vernier

186 calipers. Conspecific clumps were placed into one of the four treatments ensuring
187 there was a similar size range of specimens and numbers of conspecific clumps and
188 single individuals in each treatment. The experiment began in mid June 2012 and
189 lasted for 7 months. At the mid-way and end of the experiment, the length of each
190 specimen was measured again, recorded and the shell edge photographed. Growth
191 rates were then calculated from the increase in length (µm day^{-1}).

192 *2.4. Shell repair frequencies*

193 Forty five specimens (8-12 individuals in each treatment) with a range of sizes
194 (5.0-37.0 mm in length) were selected at random and damaged by creating a 1-2 mm
195 deep notch at the valve edge using a metal file. Care was exercised to create
196 notches of equivalent size and severity and not to break shells or cause other
197 damage. After 3.5 and 7 months of the experiment, lengths were measured for each
198 specimen and the damaged region of the shell edge photographed (Figure 1).

199 *2.5. Statistical analyses*

200 All data were analysed using Minitab (Statistical Software™ Version 15). Growth
201 rate data for each treatment were all shown to be significantly different from normal
202 (Anderson-Darling Test; $P<0.008$). These data were still not normally distributed after
203 square root, logarithmic and double logarithmic transformations because of the
204 presence of zeros in the dataset. Non-parametric Kruskal-Wallis tests were thus used
205 to determine whether damage affected growth rate in each treatment and whether
206 treatment affected growth. When a significant difference was found in the growth rate
207 of undamaged individuals, a further Kruskal-Wallis Multiple Comparisons test was
208 used to identify which treatments were statistically different from each other. Chi-

squared tests were used to determine if treatment affected the percentage of individuals that had completed shell repair, the percentage of individuals that had produced shell after fully repairing their notch and the percentage of individuals that did not grow throughout the experiment.

3. Results

Throughout this 7 month experiment, the saturation states of aragonite and calcite were just below the range usually reported for polar shallow coastal seawater (Table 1; Barry et al. (2010)). In both controls, aragonite was slightly undersaturated ($\Omega < 1$) whereas calcite was supersaturated ($\Omega > 1$). Saturation states with respect to aragonite and calcite in every other treatment were undersaturated (Table 1). The order of treatments with decreasing saturation state with respect to calcite was pH control>temperature control >pH7.75>pH7.54. Despite these potentially challenging conditions, mortality across all treatments was low at 3.9% (total N=155) with no differences between treatments (temperature control = 4% (n=3), pH control = 8% (n=2), pH7.75 = 0% (n=0) and pH7.54 = 4% (n=1)). Also, the animals showed none of the previously reported signs of stress (slow snapping responses, remaining closed for extended periods, wide gaped when open; Peck (2001)) and responded rapidly to physical stimulation when disturbed, throughout the study. A few females in each treatment also managed to successfully complete brooding and release larvae with large numbers (up to 100) settling on shells of older brachiopods and continuing development to juveniles (E.C. unpubl. obs.). Survival of these newly settled juveniles between 3 and 7 months was also high (over 90%).

3.1. Shell repair frequencies

After 3 months, all damaged individuals had at least started shell repair with >50% of individuals fully repairing their notch across all treatments (Table 2). Following the completion of shell repair, >33% of specimens had also produced new shell at the anterior margin (Figure 1). After 7 months, >83% of individuals had completed shell repair in every treatment (Table 2) with no significant difference between treatments ($\chi^2 = 0.839$, $p = 0.840$). Punctae, shell perforations, which are part of the shell structure in this species were present in the repaired notches and the new shell growth. A high proportion (>64%) of specimens had also produced new shell at the growth margin in three of the four treatments, including in both acidified treatments. However, only a moderate proportion (38%) of specimens produced growth at the shell margin in the pH control treatment. Treatment appeared to have an effect on the proportion of individuals that had continued shell production after the completion of repair ($\chi^2 = 9.670$, $p = 0.022$), however, a further Chi-squared test on the same dataset but excluding the pH control indicated there was no significant difference ($\chi^2 = 0.815$, $p= 0.665$). All individuals that had completed shell repair and then made new shell growth after 3 months continued to produce new shell after 7 months (Figure 1).

3.2. Growth rates

In the undamaged individuals, the majority, but not all individuals, grew (Figure 2 and 3). In those that did grow, growth rates ranged from 2.4 to 33.7 μm day^{-1} in all three treatments at the higher temperature (pH control, pH7.75 and pH7.54; Figure 2). However, growth rates in the lower temperature trial (temperature control treatment) were lower ranging from 1.2 to 13.2 μm day^{-1} (Figure 3). The only clear ontogenetic

trend in every treatment was that individuals with initial lengths of >32.0 mm produced no growth. Growth rates of undamaged individuals in both the acidified treatments were not significantly different from the pH control (Kruskal-Wallis, $H = 3.96$, $p = 0.138$). A further Kruskal-Wallis test including the temperature control, showed a significant effect of treatment on the growth rate ($H = 13.06$, $p = 0.005$). A Kruskal-Wallis Multiple Comparisons test indicated that higher temperature increased the growth rate over the temperature control in two treatments (pH control: $Z = 2.20$, $p = 0.028$; pH7.75: $Z = 3.21$, $p = 0.001$), however, growth rate in the lowest pH treatment (pH7.54) was not significantly different from the temperature control treatment ($Z = 0.93$, $p = 0.352$).

In damaged individuals that produced new growth at the shell margin, growth rates ranged from 1.2 to 23.1 µm day^{-1} across all treatments with no ontogenetic trend (Figure 2 and 3), and there was no effect of treatment on growth rate of damaged individuals (Kruskal-Wallis, $H = 1.24$, $P = 0.743$).

Growth rates were not significantly different in damaged and undamaged individuals in any treatment (Temperature control – $H = 0.24$, $P = 0.623$; pH control – $H = 2.82$, $P = 0.093$; pH7.75 – $H = 3.96$, $P = 0.046$; pH7.54 – $H = 0.06$, $P = 0.807$). Treatment also had no detectable effect on the proportion of damaged or undamaged individuals that did not grow throughout the experiment ($\chi^2 = 2.590$, $P = 0.459$).

4. Discussion

Clearly *Liothyrella uva* are able to tolerate predicted 2050 and 2100 pH levels as specimens showed no signs of prolonged stress and mortality was low. A similar

mortality rate was found in a study of growth rates of *Liothyrella uva* in their natural habitat (2% yr^{-1}; Peck et al. (1997)). The ability of the damaged individuals to repair their shells was not affected by acidified conditions or temperature and >90% of all injured specimens had completed shell repair and >63% had continued shell deposition after completion of repair after 7 months. This suggests that *Liothyrella uva* will be able to repair damage to their shells under the predicted pH and warming conditions in the next century. Similarly, shell repair rates in the gastropod *Subninella undulata* were also not affected by ocean acidification, however, the gastropod *Austrocochlea porcata* exhibited a decreased rate (Coleman et al., 2014). This study concluded that different species have varying tolerances and therefore the response is species-specific. Shell repair rates in living brachiopods have only been investigated in purposely damaged specimens of the temperate brachiopod, *Terebratulina retusa,* in ambient seawater where shell repair began after four weeks and caeca developed after eight weeks (Alexander et al., 1992). All notches in the Antarctic brachiopod in this study were made in the anterior margin where new shell is laid down, and so relatively easily repaired. The chemical environments differed between the treatments, but this did not affect the success rate of repairs in *Liothyrella uva,* possibly reflecting an increase in the rate of shell regeneration in the altered pH conditions as seen in some other species (e.g. Wood et al. (2008)). Energetic costs of repairing damaged shells, and whether the repair material has the same shell structure under altered pH conditions is under investigation.

Growth rates in both undamaged and damaged individuals were not affected in the pH control or lowered pH, indicating that *L.uva* should be able to continue shell production in the predicted future ocean acidification conditions even after the

disturbance of completing repair to shell damage. Other studies of shell growth in altered pH conditions, which have all been conducted on non-Antarctic molluscs, have demonstrated mixed responses. Early studies showed reduced growth in two congeneric bivalves, *Mytilus galloprovincialis* (Michaelidis et al., 2005) and *M.edulis* (Berge et al., 2006), after medium-term exposure of 90 and 44 days, respectively. However, acidified conditions as low as pH7.3 (Michaelidis et al., 2005) and pH6.7 (Berge et al., 2006) were used and no growth occurred in the latter. The negative effect in *M.edulis* started between pH7.4 and pH7.1 (Berge et al., 2006), a level which is below the range of pH used in the current work but also below the predicted end century acidified oceans. More recently, studies have predominantly used predicted mid and end century pH levels (>pH7.5). However, short or medium-term exposure (49-84 days, or less) is still generally the norm and this may not allow for acclimation and trans-generational effects. Decreased growth rates in some species of gastropods (*Urosalpinx cinerea, Littorina littorea, Nucella lamellosa*) are still seen in these more environmentally relevant experiments (Nienhuis et al., 2010; Ries et al., 2009). Although, lowered pH conditions have also had no impact on growth rates in some molluscs (*Arctica islandica and Mytilus edulis*) (Hiebenthal et al., 2012; Ries et al., 2009; Thomsen et al., 2010) and *Crepidula fornicata* has even demonstrated a positive effect to intermediate *p*CO2 levels (605 and 903 ppm) (Ries et al., 2009). An extensive study on 18 benthic marine calcifiers concluded that the affect of ocean acidification on the calcification process is species-specific (Ries et al., 2009). The ability of *L.uva* to continue shell production in low pH and undersaturated with respect to aragonite and calcite, suggests that this species can generate suitable conditions at the site of calcification (Gazeau et al., 2013; Ries, 2011; Wittmann and Pörtner, 2013). It is believed that marine calcifiers can elevate pH in calcifying

328 compartments to facilitate calcium carbonate precipitation, however, the mechanisms
329 are largely unknown, especially in the less-studied brachiopods. Potential methods
330 are via either proton channelling (McConnaughey and Falk, 1991), Ca^{2+}-activated
331 proton-translocating ATPase (Cohen and McConnaughey, 2003; McConnaughey and
332 Falk, 1991; McConnaughey and Whelan, 1997), transcellular symporter and co-
333 transporter proton-solute shuttling (McConnaughey and Whelan, 1997) or cellular
334 extrusion of hydroxyl ions into the calcifying medium (Ries, 2011; Ries et al., 2009).
335 Further research is needed to determine which mechanism is used in the study
336 species, however, it is apparent that *Liothyrella uva* has a robust control on the
337 calcification process similar to molluscs.

338 Increasing the temperature to the predicted warming conditions alongside
339 ocean acidification positively affected the growth rate of *Liothyrella uva.* This
340 suggests shell deposition could occur at a faster rate in 50-100 years as a
341 consequence of elevated temperatures irrespective of the acidity of the oceans. The
342 long-term upper temperature tolerance limit of the Antarctic brachiopod is 4.5°C
343 (Peck, 1989) and higher shell growth rates have been shown with increasing
344 temperatures in other species (Almada-Vilela et al., 1982; Lewis and Cerrato, 1997;
345 Stor et al., 1982), therefore, warming to 2°C is unlikely to negatively impact shell
346 production in *Liothyrella uva*. Several ocean acidification studies have involved
347 combined effects with other environmental variables, particularly with temperature
348 (Courtney et al., 2013; Findlay et al., 2008; Hiebenthal et al., 2012; Martin and
349 Gattuso, 2009; Reynaud et al., 2003; Rodolfo-Metalpa et al., 2011). Organism
350 responses to the interaction of these variables have been varied where temperature
351 has both increased and decreased the effect size of ocean acidification, which has

352 been found in closely related molluscs, *M.edulis* and *M.galloprovincialis* (Hiebenthal
353 et al., 2012; Rodolfo-Metalpa et al., 2011). Rodolfo-Metalpa et al. (2011) found a
354 negative synergistic effect of low pH (pH_T 7.4) and elevated temperature (25°C) on
355 the gross calcification rate of *M.galloprovincialis* whereas Hiebenthal et al. (2012)
356 found that acidified conditions (*p*CO2 1093.3 ± 123.6 µatm and 1654.5 ± 82.7 µatm)
357 did not have an impact on shell growth of closely related *M.edulis,* however, elevated
358 temperature did. Growth in *M.edulis* increased up to 20°C but then decreased at
359 25°C indicating that 25°C is above this species temperature threshold limit
360 (Hiebenthal et al., 2012; Pörtner, 2008). Growth rates in the temperature control
361 treatment here, which was used to compare the predicted scenarios to current
362 Antarctic conditions, were in the same range (0-15 µm per day) as individuals of the
363 same size (2.5-40.9 mm) in a study of *Liothyrella uva* growth rates in the wild (Peck
364 et al., 1997). No growth occurred in individuals >32.0 mm in the current study. This is
365 the approximate size that this species reaches sexual maturity (Meidlinger et al.,
366 1998; Peck and Holmes, 1989), and our data possibly results from a shift in energy
367 from somatic growth to reproduction.

368 Even though brachiopods arguably have the largest proportion of skeleton to
369 tissue mass of any group and *Liothyrella uva* inhabits the Southern Ocean which has
370 the naturally lowest carbonate ion saturation levels, our study shows that this species
371 can survive, repair shell damage and deposit new shell after 7 months exposure to
372 forecasted 2050 and 2100 pH conditions. However, there may be a cost to this
373 apparent resilience under future climate change scenarios. The ophiuroid brittlestar,
374 *Amphiura filiformis,* increased its metabolism and calcification to compensate for
375 increased seawater acidity, which came as a substantial cost as muscle wastage

was reported to coincide with this increase (Wood et al., 2008). *L.uva* is a low energy species that uses low metabolic rates to survive the long period of low food availability in the Antarctic winter, although it can opportunistically exploit resuspended benthic microalgae when available (Peck et al., 2005). Increased metabolic costs from elevated temperature may, however, compromise abilities to survive periods of low food availability. The current study is a long-term CO_2 perturbation experiment measuring shell growth on a marine organism with a shell permanently exposed to seawater, therefore, providing insights into how these highly calcium carbonate dependent organisms can acclimate in future CO_2 scenarios. The main mechanisms that organisms can use to respond to environmental change are acclimation and genetic adaptation (Peck, 2011; Somero, 2010). Acclimation to altered temperatures can take 6 months in Antarctic marine invertebrates (Peck et al., 2014), and rates of acclimation to altered pH in negatively impacted species remain to be quantified. Comparable long term studies on other species from polar to tropical environments are essential to increase our knowledge of the capability of these integral organisms to succeed under changing environmental conditions.

Acknowledgements

The authors would like to thank the scientific dive team at the British Antarctic Survey Rothera Research Station for collecting specimens and also to Coleen Suckling, Simon Morley and Rebecca Smith for their help in the set up and maintenance of the ocean acidification experimental system. Emma Cross is supported by the NERC PhD Studentship (NE/T/A/2011).

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630 **Figure legends**

631 **Figure 1** – Examples of shell repair and further shell growth in damaged individuals
632 after 3 months (top row) and 7 months (bottom row) in the following four treatments:
633 pH control (a, e), temperature control (b, f), pH7.75 (c, g) and pH7.54 (d, h). The
634 same individual is shown in each treatment at both growth periods where the arrow
635 indicates the notch created at the start of the experiment. Scale bar = 100µm. (Colour
636 on Web only)

637 **Figure 2** – Growth rates of specimens that were left undamaged (●) and were
638 damaged at the start of the experiment (○) after 7 months in the treatments kept at
639 the higher temperature; (a) pH control (pH8.05 ± 0.03, T=1.7°C ± 0.3), (b) pH7.75
640 (pH7.75 ± 0.03, T=1.9°C ± 0.4) and (c) pH7.54 (pH7.54 ± 0.03, T=2.2°C ± 0.4).

641 **Figure 3** - Growth rates of specimens that were left undamaged (●) and were
642 damaged at the start of the experiment (○) after 7 months in the low temperature
643 control (pH7.98 ± 0.02, T=-0.3 °C ± 0.1).