

Variability of Mg-calcite in Antarctic bryozoan skeletons across spatial scales

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ABSTRACT: Bryozoans exhibit a highly variable chemistry within their calcium carbonate skeletons. Previous studies have shown that the level of Mg-calcite in skeletons increases with increasing seawater temperature. For high-latitude regions such as the Antarctic, which have a low range of annual sea-temperature variation, there have been no studies on bryozoan skeletons with replicated sampling approaches suitable for statistical testing. Our aim was to conduct high-replicate, multi-site sampling to determine the variability in skeletal mineralogy of bryozoans from a site in Antarctica. During an expedition in January 2012, a total of 584 specimens representing 4 bryozoan species were collected from 8 sites at Adelaide Island, West Antarctic Peninsula, by SCUBA diving. All specimens were sampled within a 3 wk period and were selected to be of similar size, age and breeding status. We compared the variability in the wt% MgCO₃ in calcite of skeletons among species and investigated the relative influence of environmental and biological factors on skeleton chemistry. The results of X-ray diffraction analysis showed that the wt% MgCO₃ in calcite in bryozoan skeletons was statistically different among sites for all study species. The difference in wt% MgCO₃ among sites may be explained by habitat fragmentation driving directional adaptation of isolated populations to local environmental conditions. The relationship between Mg-calcite and temperature was inconsistent among species, and the predicted positive correlation between seawater temperature and Mg-calcite was not exhibited in any of the species examined. On this basis, we suggest that Antarctic bryozoan Mg-calcite should not be considered a reliable indicator of paleo-temperature.

KEY WORDS: Antarctica · Bryozoans · Mineralogy · Mg-calcite · Paleo-temperature · Magnesium

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INTRODUCTION

A relationship between seawater temperature and the skeletal mineralogy of marine animals was first suggested by Clarke & Wheeler (1922). The concentration of magnesium (Mg) incorporated into calcite was subsequently recognised to increase with seawater temperature and was proposed as a paleoclimatic proxy by Chave (1954). Inorganic precipitation

studies demonstrate that the partition coefficient, D_{Mg} , a measure of the difference in solubility between Mg in seawater and in calcite, increases with temperature, resulting in preferential incorporation of Mg into the calcite lattice under warmer temperatures (Chilingar 1962, Katz 1973, Burton & Walter 1987, Mucci 1987, Oomori et al. 1987). Thermodynamic control of biogenic Mg-calcite deposition has been 'calibrated' in some phyla by controlled culture

experiments on Foraminifera (Nürnberg et al. 1996, Lea et al. 1999) and coccoliths (Ra et al. 2010). An observed positive correlation between wt% MgCO_3 in calcite deposition and environmental temperature has subsequently been demonstrated in a number of phyla, including planktonic Foraminifera (Barker et al. 2005, Martínez-Botí et al. 2011), benthic Foraminifera (Rosenthal et al. 1997, Bohaty et al. 2012), ostracods (Decrouy et al. 2012), coccoliths (Ra et al. 2010), gastropods (Cohen & Branch 1992) and corals (Chang et al. 2004), leading to their use as paleothermometers.

Globally, the temperature regime of our oceans is changing (Kerr 2013) at a faster rate than has been seen in at least the past 420 000 yr (Hoegh-Guldberg et al. 2007). The West Antarctic Peninsula is one of the fastest-warming regions on Earth (Meredith & King 2005). With the increases in seawater temperatures, it is anticipated that the level of Mg-calcite deposition in shells and skeletons will increase (Hermans et al. 2010). Mg is incorporated within the calcium carbonate lattice by the replacement of a calcium ion; Mg^{2+} is a smaller cation than Ca^{2+} , with an ionic radius of 0.65 Å vs. 0.99 Å in sixfold coordination (Railsback 2003), and the resultant Mg-calcite ($(\text{Ca,Mg})\text{CO}_3$) is a denser and harder material than CaCO_3 , although it is also less thermodynamically stable (Morse et al. 2007). The Southern Ocean is at a higher risk of ocean acidification than other oceans due to its naturally low saturation levels of carbonate (McNeil & Matear 2008) with predicted negative effects for calcifying organisms (Orr et al. 2005). Ocean acidification studies on Antarctic marine invertebrates have shown a reduction in calcification rates (McClintock et al. 2009) and an increase in calcium carbonate dissolution (McClintock et al. 2009) under acidified conditions. Mg-calcite is more susceptible to dissolution than pure calcite (Anderson & Crerer 1993, Morse et al. 2007, Andersson et al. 2008), so, as a consequence of combined ocean warming and acidification, animals with higher Mg-calcite skeletons may be less well represented in marine communities in the future (Hermans et al. 2010).

Knowledge of the changes in the skeleton chemistry of marine organisms in response to warming seas/increased acidification may enable us to predict which species will survive and how the composition of assemblages will change in the future. Fabry et al. (2009) predict that marine animals that vary their skeletal chemistry in response to environmental change may be useful as indicators of the effects of climate change and ocean acidification.

Bryozoans are aquatic, colony-forming invertebrates, comprising tens to thousands of clonal modules, termed zooids. Bryozoa exhibit a wide geochemical spectrum in their skeletons (Smith et al. 2006, Lombardi et al. 2008) with a wide range of wt% MgCO_3 in calcite deposited (Smith et al. 2006, Kuklinski & Taylor 2009). Bryozoa are important components of Antarctic benthic communities (Barnes & Conlan 2007) and are often the most abundant suspension-feeding animals on hard substrates (Koubbi et al. 2010). Three previous studies have been conducted on the mineralogy of Antarctic bryozoans, covering a total of 45 species (number of specimens = 99) (Borisenko & Gontar 1991, Taylor et al. 2009, Loxton et al. 2012). These previous analyses represent an estimated 15% of Antarctic bryozoans (Kuklinski 2013) with the majority remaining unstudied. Within the global bryozoan literature, there is much debate about the relative influence of biological and environmental controls on mineralogy but little conclusive evidence. Biological control mechanisms may include astogeny (Kuklinski & Taylor 2009, Smith & Girvan 2010), growth rate (Smith et al. 1998, Barnes et al. 2007, Smith 2007, Kuklinski & Taylor 2008), food availability (Bone & James 1993), physiological 'wellness' (Stanley & Hardie 1998) and directed adaptation to ecological niches in isolated populations (Cheetham et al. 1994). Environmental control suggests that skeletal mineralogy is driven by the seawater within which the bryozoan lives with little or no physiological involvement from the animal itself. Environmental factors influencing mineralogy may include salinity (Bohaty et al. 2012) and carbonate chemistry (Müller et al. 2014) in addition to temperature; however, it is assumed that temperature is the primary control when using wt% MgCO_3 in calcite as a paleoclimatic proxy (Weiner & Dove 2001). Strong biological control has the potential to confound environmental signals, compromising the usefulness of a species or phylum as a recorder of paleo-temperature (Weiner & Dove 2001). Mineralogical studies on bryozoans to date have been heavily constrained by the mixed origin and limited availability of samples and associated metadata on environmental conditions, and as a consequence, the accuracy of the Bryozoa as palaeoenvironmental recorders has not yet been clearly established.

The overall aim of this study is to conduct a highly replicated, multi-site study to determine the skeletal mineralogy of bryozoans from a single location in the Southern Ocean. Antarctica is often referred to as a thermally constant environment, with seawater tem-

perature varying over approximately 3°C a year (Barnes & Conlan 2007, Morley et al. 2012) compared to an annual range of 15°C or more in temperate regions (Pörtner et al. 2000). Given the limited range of temperature variability, and the observed links between Mg-calcite and temperature (Chave 1954), we could predict a correspondingly low variability of mineralogy in Antarctic aquatic invertebrates and a positive correlation between wt% MgCO₃ in calcite and temperature. The high replicate and multi-site design of the study will allow consideration of the reliability of mineralogical patterns within and between species.

To investigate this, multiple samples were examined from 8 sites in Ryder Bay, Adelaide Island, West Antarctic Peninsula, over local (>10 m, <10 km) and close proximity (<10 m) spatial scales. The environmental parameters of seawater temperature and salinity were recorded at the same time. The resulting dataset was interrogated to (1) compare the mineralogy and range of skeletal Mg-calcite variability across 4 bryozoan species, (2) investigate the relationship between bryozoan wt% MgCO₃ in calcite, seawater temperature and salinity and (3) identify potential biological factors influencing wt% MgCO₃ in calcite in Antarctic bryozoans. The outcomes of this research will establish a mineralogical baseline for the comparison of future studies.

MATERIALS AND METHODS

Study area

The study region was Ryder Bay, southeast of Adelaide Island, on the West Antarctic Peninsula (68° S, 68° W) (Fig. 1A). Ryder Bay is characterised by seasonal coverage of fast-ice (frozen sea surface), and the shallow benthos is subject to extensive ice-scour from floating icebergs during open-water periods (Barnes & Souster 2011).

Sampling and specimen processing protocol

Six local (>10 m, <10 km apart) collection sites (Sites 1, 2a, 3, 4, 5 and 6) were selected within Ryder Bay, with similar depths—(mean ± SD) 8.5 ± 2.5 m—and substrata (boulders/cobbles) (Fig. 1B). Sites were an average of 3.85 km apart (ranging from 425 m to 8 km distant from each other) (Table 1). All samples were collected using SCUBA within a 3 wk period (January to February 2012) in the austral summer.

Close-proximity (<10 m scale) collection sites (Sites 2a, 2b and 2c) were located in Lagoon Back Bay (Fig. 1B,C) and were of similar depth—7 m (± 1 m)—and substrate (boulders/cobbles on silt). Sites

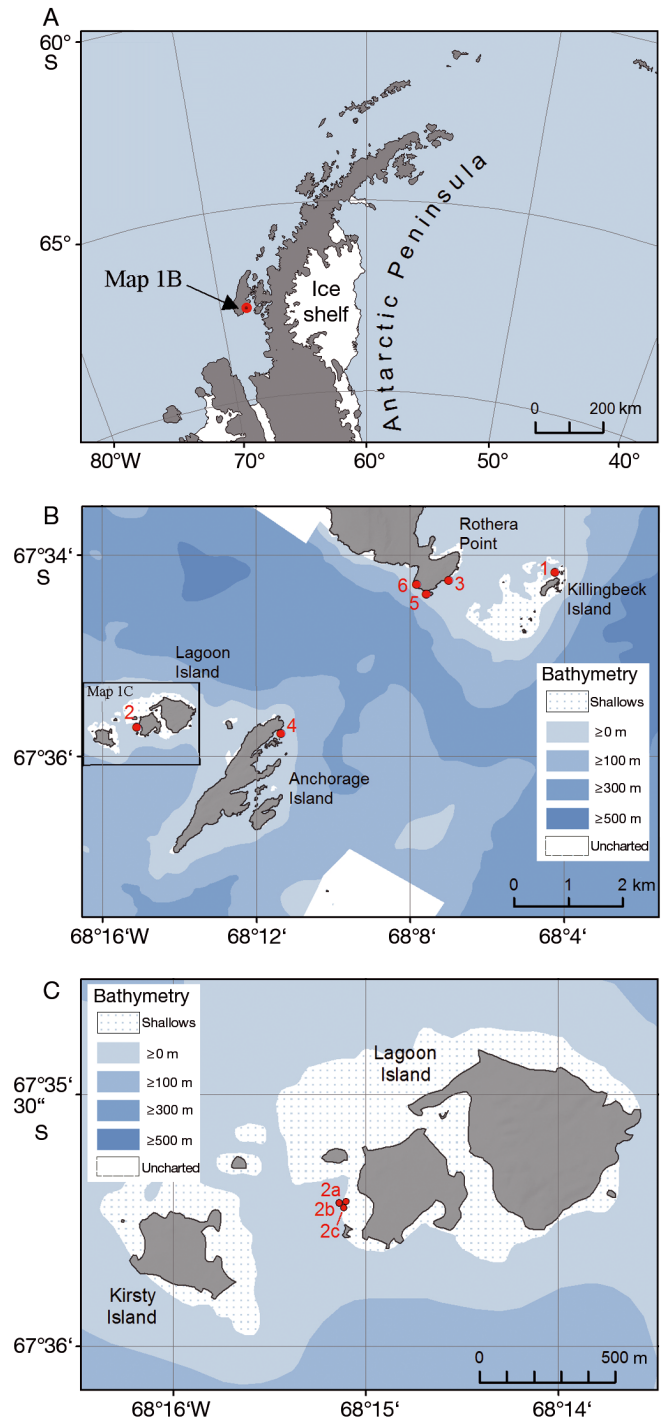


Fig. 1. Location of study sites in Antarctica: (A) location (arrow) of Ryder Bay on the West Antarctic Peninsula, (B) location of local study sites (>10 m, <10 km apart) in Ryder Bay, (C) detail of Lagoon Island, the location of the close-proximity sites (<10 m apart) in Back Bay

Table 1. Matrix showing direct distance in metres between sites

Site name	Site no.	2a	2b	2c	3	4	5	6
Killingbeck Island	1	8008	8008	8008	1980	5920	2350	2550
Lagoon Back Bay	2a	–	7.3	6.1	6220	2500	5790	5650
Lagoon Back Bay	2b		–	1.6	6220	2500	5790	5650
Lagoon Back Bay	2c			–	6220	2500	5790	5650
East Beach	3				–	4274	425	600
Anchorage Island	4					–	3840	3790
Cheshire Island	5						–	292
Honeybucket	6							–

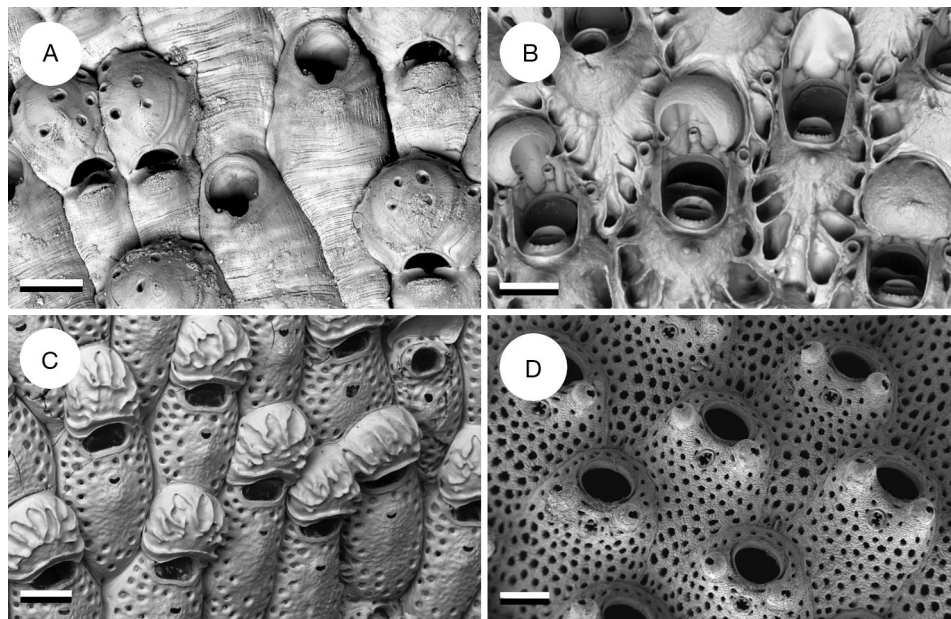


Fig. 2. Scanning electron microscope images of the study species. (A) *Antarctothoa antarctica*, scale bar = 200 µm. (B) *Hippadenella inerma*, scale bar = 100 µm. (C) *Fenestulina rugula*, scale bar = 100 µm. (D) *Inversiula nutrix*, scale bar = 200 µm

were an average of 5 m (ranging from 1.6 to 7.3 m) distant from one another (Table 1). All close-proximity samples were collected using SCUBA on a single dive in January 2012.

Conductivity and temperature were measured during collection from both local and close-proximity sites using a HOBO U24-002 salt water conductivity logger; instrument accuracy is reported to be 0.1°C for temperature (measurable range –2 to 36°C) and 3% of conductivity reading (HOBO 2013). Conductivity was converted to Practical Salinity Units, as defined on the Practical Salinity Scale of 1978 (McDougall et al. 2010) using the Gibbs function of seawater (Table 2). British Antarctic Survey (BAS) long-term logger data was provided from the Rothera Time Series (RaTS) dataset (H. Venebles unpubl. data), collected from Cheshire Island using the protocol described by Clarke et al. (2008).

In total, 584 specimens of 4 bryozoan species were collected for mineralogical examination. The species selected for analysis from the study location were the cheilostomatous bryozoans *Antarctothoa antarctica* Moyano & Gordon, 1980, *Hippadenella inerma* (Calvet, 1909), *Fenestulina rugula* Hayward & Ryland, 1990 and *Inversiula nutrix* Jullien, 1888.

The study species were selected on the basis of being well-known, common and abundant Antarctic species with distinctive features for identification (Fig. 2). Specimens were identified to species level under a dissection (stereo) microscope in accordance with Hayward (1995). Scanning electron microscope (SEM) images of each species were taken on a small selection of specimens to confirm identification (Fig. 2).

All specimens were alive when collected and were subsequently rinsed in fresh water and air-

Table 2. Physical and environmental characteristics of the study sites at the time of collection

Site no.	Name	Latitude (°S)	Longitude (°W)	Temperature (°C)	Salinity (psu)	Depth (m)
1	Killingbeck Island	67° 34' 10.60'	68° 4' 14.79'	-0.068	31.0	10.5
2a	Lagoon Back Bay	67° 35' 43.02'	68° 15' 7.14'	0.295	31.4	6.0
2b	Lagoon Back Bay	67° 35' 43.14'	68° 15' 7.68'	0.207		7.0
2c	Lagoon Back Bay	67° 35' 43.14'	68° 15' 7.56'	0.155		7.5
3	East Beach	67° 34' 15.36'	68° 7' 0.48'	0.299	30.9	10.0
4	Anchorage Island	67° 35' 48.45'	68° 11' 30.67'	0.093	30.5	11.0
5	Cheshire Island	67° 34' 22.92'	68° 7' 31.05'	-0.146	31.3	10.0
6	Honeybucket	67° 34' 18.44'	68° 7' 50.82'	0.431	-	8.0

dried for a minimum of 1 mo before samples were extracted for analysis. Individual colonies of similar diameter were selected for sampling, and samples were extracted from the growing edge of the colonies, containing the most recently deposited skeletal material (in the last 6 mo); a minimum of 5 zooids was extracted for each sample (approximately the outermost 2 mm from the growing edge of the colony). As far as possible, care was taken to ensure that no substrate (e.g. coralline algae) or epibionts were included within the sample as they could potentially contaminate results through their added mineralogies.

Detailed growth-rate data is available for *A. antarctica* and *F. rugula*, measured from 2001 to 2003 at Rothera Research Station in Ryder Bay (Bowden 2005). During the peak growing months of the austral summer, the species grow at 0.45 and 0.66 mm mo⁻¹, respectively. At this growth rate, the 2 mm extracted sample would have been deposited in the 3 to 5 mo preceding collection. No growth-rate data is available for *H. inerma* or *I. nutrix*, and so this rate of deposition plus a 50% margin for error is taken as the approximation of the time period of deposition for the 4 Antarctic species (i.e. 6 mo). Temperature for this predicted deposition period was extracted from the BAS long-term logger dataset collected from Cheshire Island (Site 5).

Voucher specimens and extracted skeletal material have been lodged in the Bryozoa collections of the Natural History Museum, London (NHM) under registration numbers NHMUK 2013.10.30.1–928.

Analysis techniques

Mineralogical analyses were conducted at the EMMA unit (NHM London) using an Enraf-Nonius X-ray diffractometer (XRD) with an INEL CPS-120

curved position-sensitive detector and a cobalt X-ray source. Tube operation conditions were 40 kV and 40 mA. A primary Germanium 111 monochromator in combination with slit settings of 0.14 × 5 mm were used to confine the X-ray beam to pure Co Ka₁ radiation. Samples were measured in reflection using asymmetric flat-plate geometry. Diffracted intensities were collected simultaneously over a 2-Theta range of 120° without angular movement of tube, sample or detector. The angle between incoming monochromatic beam and sample-holder was kept constant at 5.9°. The sample-holder was rotated to increase the number of crystallites and randomness of their orientations in the X-ray beam. The angular linearity of the position-sensitive detector was calibrated using silver behenate (AgC₂₂H₄₃O₂) and NIST silicon powder (SRM 640) as external standards, and the calibration curve was fitted using a least-squares cubic spline function.

Bryozoan samples were powdered using an agate pestle and mortar and affixed using a drop of acetone to single-quartz crystal substrates. Quantitative XRD analysis was undertaken to determine the Mg content of calcite.

To calculate wt% MgCO₃ in calcite, the position of the d₁₀₄ peak was measured, assuming a linear interpolation between calcite (CaCO₃) and Mg-calcite (MgCO₃). Chave (1952) and Mackenzie et al. (1983) validated the linear relationship between d₁₀₄ and Mg content to apply to the compositional range up to 13.8 and 17.4 wt% MgCO₃ in calcite respectively; above this range, there is non-linear behaviour. Previous measurements of bryozoan Mg-calcite have ranged between 0 and 13.7 wt% (Smith et al. 2006), within the validated linear range. Composition information is considered to be accurate to within 2% on a well-calibrated instrument (Kuklinski & Taylor 2009). Computations were undertaken using WinX-Pow software.

Statistics and data analysis

The temperature data was statistically compared to BAS logger data from the same date and time (± 2 h) using a general linear model (GLM) ANOVA and Tukey's post-hoc test.

All mineralogical measurements underwent weighted-average transformation in the context of beta-regression following the methodology of Smithson & Verkuilen (2006). This initial transformation, $[y(n-1) + 0.5]/n$, ensured that no data points equalled 0 or 1. The weighted-average transformation prepared the data for subsequent transformation using the logit function, $\text{logit}(p) = \ln[p/(1-p)]$, which accounts for the mineralogical measurements being proportional data (p) (Warton & Hui 2011).

The mineralogy measurement dataset was tested for normality using Anderson-Darling normality tests and for homogeneity of variance using Levene's test of equal variance. Measurements were normally distributed in most cases but failed Levene's test of equal variance. The criteria for parametric testing were not satisfied for the mineralogical measurements; therefore, the data was analysed using non-parametric tests. Variance was tested using Kruskal 1-way ANOVA with post-hoc pair-wise testing (Mann-Whitney U -test). Correlations with environmental variables were explored using the Kendall correlation method. p -values for all analyses were

calculated based on a probability of error of 0.05. All statistical data analysis was conducted in the R programming environment (R Development Core Team 2013).

RESULTS

Species mineralogy

In this study, the Mg-calcite of 584 specimens from 4 bryozoan species was quantified. Two of the 4 species, *Fenestulina rugula* ($n = 190$) and *Hippadenella inerma* ($n = 145$), had intermediate Mg-calcite (IMC, 4 to 8 wt% MgCO_3 in calcite) with mean wt% MgCO_3 in calcite \pm SD of 4.92 ± 0.772 and 5.02 ± 0.679 , respectively. The remaining species, *Antarctothoa antarctica* ($n = 159$) and *Inversiula nutrix* ($n = 90$), had a low Mg-calcite (LMC, 2 to 4 wt% MgCO_3 in calcite) skeleton with mean wt% \pm SD of 0.60 ± 0.531 and 2.64 ± 0.257 , respectively (Fig. 3).

The ANOVA analysis showed that there is a statistically significant difference in wt% MgCO_3 in calcite among species (Kruskall Wallis: $\chi^2 = 418.6942$, $p < 0.0001$). Post-hoc testing revealed a statistically significant difference in wt% MgCO_3 for all species (Mann-Whitney U -test: $p < 0.0001$) except in the comparison between *F. rugula* and *H. inerma* (Mann-Whitney U -test: $p = 0.553$) (Fig. 3).

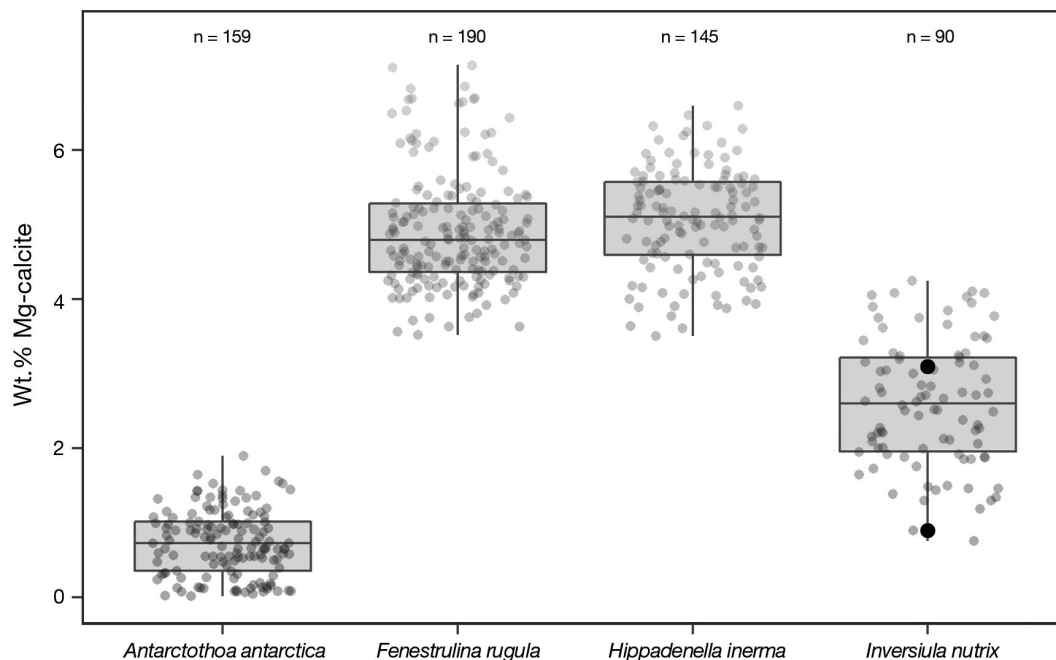


Fig. 3. Box plots showing wt% MgCO_3 in calcite for 4 Antarctic bryozoan species. Box shows standard deviation around mean (mid-line). Tail indicates range. Scatterplot indicates spread of samples (scattered horizontally to prevent excessive overlap). Previous analyses by Taylor et al. (2009) are indicated with black circles

Mineralogical variability among sites

Local sites (>10 m, <10 km apart)

Data was collected for 3 species, *F. rugula*, *A. antarctica* and *H. inerma*, from 6 sites less than 10 km apart (Sites 1, 2a, 3, 4, 5 and 6). *I. nutrix* was only found at the close proximity sites—2a, 2b and 2c—and so was excluded from the local site analysis. A Kruskal Wallis 1-way ANOVA analysis showed that the mean wt% MgCO₃ in calcite was statistically significantly different among sites for all 3 species: *F. rugula* $\chi^2 = 111.3800$, $p < 0.0001$, *A. antarctica* $\chi^2 = 50.4299$, $p < 0.0001$ and *H. inerma* $\chi^2 = 56.9467$, $p < 0.0001$.

Post-hoc Mann-Whitney *U*-tests showed that wt% MgCO₃ in calcite was statistically significantly different among sites in 17 of 30 comparisons.

Close proximity sites (<10 m apart)

Data was collected for all 4 species, *F. rugula*, *A. antarctica*, *H. inerma* and *I. nutrix* at 3 sites less than 10 m apart (Sites 2a, 2b and 2c). The mean wt% MgCO₃ in calcite was statistically significantly different among close proximity sites for the species *F. rugula* (Kruskall Wallis: $\chi^2 = 26.5767$, $p < 0.0001$) and *A. antarctica* (Kruskall Wallis: $\chi^2 = 20.2055$, $p < 0.0001$). Post-hoc Mann-Whitney *U*-tests indicated that mean wt% MgCO₃ in calcite from each site was statistically different only for *F. rugula* (Sites 2a and 2c and 2b to 2c) and *A. antarctica* (2a to 2b and 2a to 2c,) as represented in Table 3. There was no statistically significant difference in mean wt% MgCO₃ in

calcite among close proximity sites for *H. inerma* or *I. nutrix*.

Mg-calcite variability among species and all sites is illustrated in Fig. 4. Considering variability across all scales, Site 1, Killingbeck Island, was the most mineralogically distinct, with wt% MgCO₃ in calcite differing statistically in 17 of the 18 species/site combinations (Table 3); the only exception being *A. antarctica* and Site 5, Cheshire Island. Site 5 was the next most mineralogically distinct site, with wt% MgCO₃ in calcite differing significantly from its neighbours in 10 of 12 species/site combinations. The least mineralogically distinct site was Site 4, Anchorage Island, with wt% MgCO₃ in calcite that differed significantly in only 4 of 15 species/site combinations.

The most mineralogically distinct species among sites were *F. rugula* and *A. antarctica*, which both differed statistically in 14 out of 21 site combinations. *H. inerma*, in contrast, showed a statistically distinct mineralogy in just 6 of the 21 site combinations.

Environmental analysis

Overall BAS long-term data was statistically different from collected data (GLM ANOVA: $F = 14.93$, $p < 0.0001$). Post-hoc analysis, however, shows no statistical difference among the BAS long-term logger data and collected data for Site 5, Cheshire Island (Tukey's test: difference of means [DOM] = 0.038, $p = 0.9986$) and Site 1, Killingbeck (Tukey's test: DOM = -0.038, $p = 0.9994$). The comparison between temperature measured during sample collection and the BAS long-term logger data at Site 5, Cheshire Island, enabled 'ground truthing' of the data collected. As

Table 3. Mann-Whitney post hoc analysis comparing wt% MgCO₃ in calcite among sites. Significant differences among sites for each species are indicated by the presence of the following symbols: *Fenestulina rugula*: filled circles, *Antarctothoa antarctica*: empty circles, *Hippadenella inerma*: filled squares, *Inversiula nutrix*: empty squares. (–) no data available for comparison, as insufficient numbers of specimens were found for the species at one or both of the sites

Site no.	1	2a	2b	2c	3	4	5	6
1		● ○ ■ –	● ○ ■ –	● ○ ■ –	● ○ ■ –	● ○ ■ –	● ○ – –	– – ■ –
2a			○	● ○	–	–	● ○ – –	– – – –
2b				● –	○ –	–	● ○	– –
2c			–		● ○ –	● –	○ –	– –
3						–	● ○ – –	– – – –
4						–	● ○ – –	– – – –

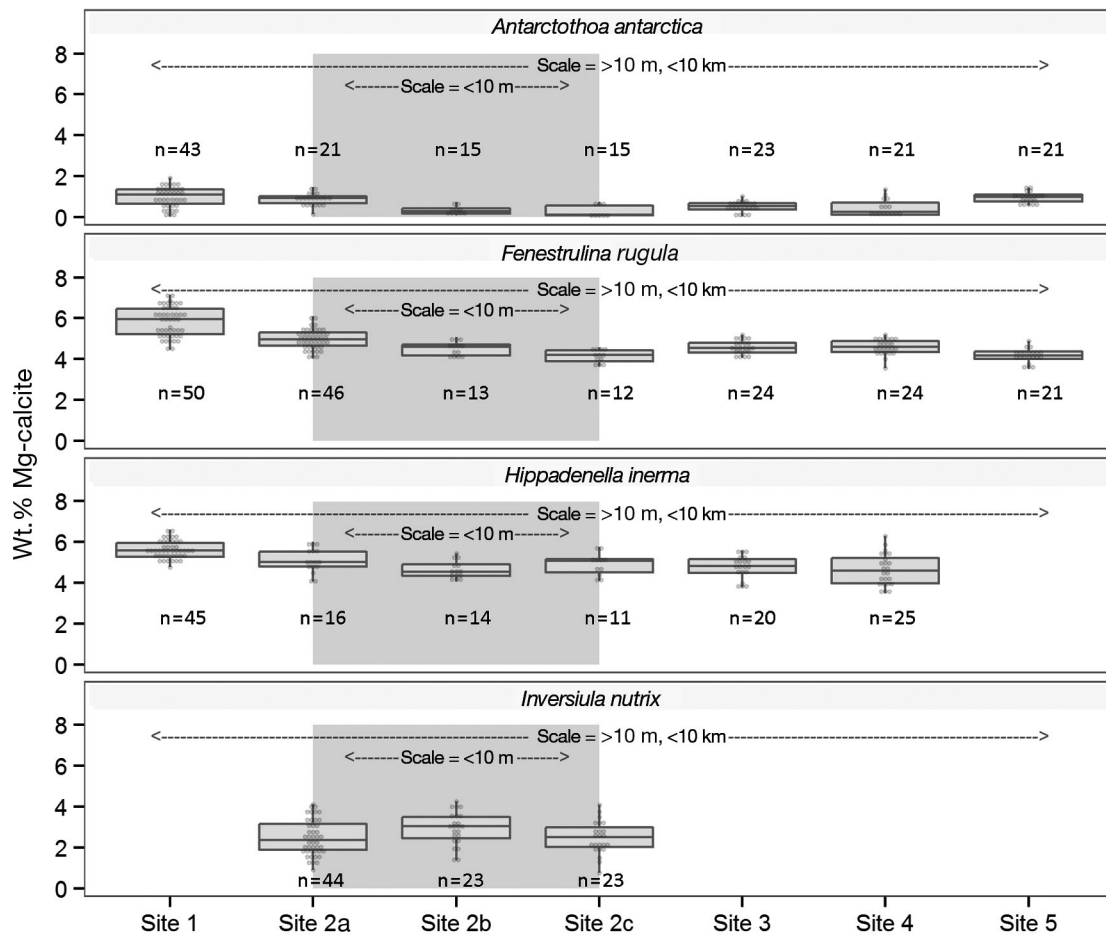


Fig. 4. Box plots showing wt% MgCO₃ in calcite for Antarctic species at different sites. Box shows standard deviation around mean (mid-line), tail indicates range. Individual data points are stacked around the vertical axis to show their distribution. Shaded area indicates close-proximity sites (<10 m apart)

the datasets from our logger and the BAS long-term logger at Cheshire Island were not statistically different, a high degree of accuracy in the HOBO loggers is indicated.

Temperature measured during sample collection (Table 2) was statistically different among sites (GLM ANOVA: $F = 36.48$, $p < 0.0001$). Post-hoc tests illustrate that sites are statistically similar in only 2 of 10 cases (Tukey's test: Sites 2a to 3, $p = 0.4685$; Sites 1 and 5, $p = 0.8918$).

The only statistically significant correlations between environmental conditions and wt% MgCO₃ in calcite for the Antarctic bryozoan species tested are negative correlations exhibited for *H. inerma* (Kendall's correlation: $p < 0.001$, Kendall's tau = -0.322) and *A. antarctica* (Kendall's correlation: $p < 0.001$, Kendall's tau = -0.235). For these 2 species, the wt% Mg-calcite decreases as the seawater temperature increases.

During the estimated period of skeleton deposition, the BAS long-term logger data shows the seawater temperature at Cheshire (Site 5) ranged through 2.47°C (minimum = -1.80°C , maximum = 0.67°C), with the midday mean fluctuating day-to-day by up to 0.95°C . Between August 2011 and January 2012, the mean day-to-day seawater temperature range was 0.09°C .

No statistically significant relationship was detected between wt% MgCO₃ in calcite and salinity for any of the study species.

DISCUSSION

The overall aim of this study was to determine the variability of wt% MgCO₃ in calcite in the skeletons of Bryozoa at an Antarctic location across different spatial scales. The aim was achieved (1) by compar-

ing the mineralogy and range of skeletal wt% MgCO₃ in calcite variability among 4 study species. Then (2), we examined the relationship between bryozoan wt% MgCO₃ in calcite, seawater temperature and salinity before (3) examining biological factors, which may also be influencing Mg-calcite in Antarctic bryozoans. To our knowledge, this study represents the largest multi-replicated dataset generated for any Bryozoa to date.

Our study species had skeletons composed of LMC (*Antarctothoa antarctica* and *Inversiula nutrix*) or IMC (*Fenestrulina rugula* and *Hippadenella inerma*). Taylor et al. (2009) also found 2 specimens of *I. nutrix* from King George Island (North Antarctic Peninsula) to be LMC (0.9 and 3.1 wt% MgCO₃ in calcite). We present the first mineralogical measurements made for the species *F. rugula*, *H. inerma* and *A. antarctica*; our measurements fall within the range of wt% MgCO₃ in calcite recorded for other Antarctic bryozoans by previous workers (Borisenko & Gontar 1991, Taylor et al. 2009).

We investigated the relationships between bryozoan Mg-calcite and some environmental parameters and found that (1) over the small temperature range tested here, the relationship between Mg-calcite and temperature is inconsistent among species, (2) the predicted positive correlation between seawater temperature and Mg-calcite is not exhibited in any of the species examined, and (3) no relationship between wt% MgCO₃ in calcite and salinity occurs in these Antarctic bryozoans.

For species showing no relationship between temperature and Mg-calcite, it is possible that the temperature variability among sites is too low to cause a significant change in Mg incorporation. Controlled culture experiments, undertaken to 'calibrate' the relationship between Mg-calcite and temperature in Foraminifera, have shown that Mg incorporation within calcite can be expected to increase by approximately 8 to 10% per 1°C (Lea et al. 1999). The temperature differences among sites in this study are fractions of a degree. If a similar range of temperature-induced variability occurs in bryozoans, as has been seen in Foraminifera, then any increase in Mg-calcite caused by temperature may be below the levels of detection. This theory concurs with data published by Rathburn & De Deckker (1997) on benthic Foraminifera; these authors were unable to detect any significant temperature-related pattern of Mg/Ca in Antarctic specimens from -2 to 0.2°C, in contrast to Foraminifera from temperate and tropical regions.

When using wt% MgCO₃ in calcite as a proxy for seawater temperature, we make the assumption that

biomineralization is influenced by environmental conditions alone (Urey et al. 1951). If species mineralogy is deposited asynchronously with environmental parameters then the biomineralization process is considered to be under the influence of the 'vital effect' (Weiner & Dove 2001). If seawater temperature is assumed to be the main controlling factor of mineralogy, then, given the consistently low seawater temperatures in Antarctica, we would predict that the skeletons of endemic bryozoan species would be composed of LMC. In this study, the mean wt% MgCO₃ in calcite differed significantly among species, and 2 species, *H. inerma* and *F. rugula*, were composed of IMC. Taylor et al. (2009) also found that the dominant mineralogical type of Antarctic bryozoans was IMC, with 19 of 22 species featuring this form (Taylor et al. 2009). Within the 4 species studied here, the relationship between wt% MgCO₃ in calcite and environmental temperature was inconsistent. The negative trend of decreasing wt% MgCO₃ in calcite with site temperature, seen in the species *A. antarctica* and *H. inerma*, cannot be explained by thermodynamics. We suggest that the results provide evidence that biological processes are influencing the process of Mg-calcite deposition in these Antarctic bryozoan species.

A population study on the temperate bryozoan *Celleporella hyalina* by Goldson et al. (2001) showed that gene flow in bryozoans can be limited over distances as small as 10 m, due to a combination of the short pelagic phase of lecithotrophic larvae and local physical hydrography. All of the species in this study brood lecithotrophic larvae, and the close proximity sites (Sites 2a, 2b and 2c), which are hydrologically homogeneous, are most likely within swimming distance of each other for larvae and gametes (Wendt 2000). The resulting population connectivity might explain the similarity in Mg-calcite among the sites at the <10 m scale. At the local scale (>10 m, <10 km) physical hydrography is likely to play a much stronger role in population connectivity. The dominant flow in Ryder Bay is from North-East to South-West, although in winter, a shallower current has been documented flowing in the opposite direction, northwards (Beardsley et al. 2004, Wallace et al. 2008). This pattern of flow could be providing a larval and gamete dispersal route between Sites 2, 3 and 4. In our study, the wt% MgCO₃ in calcite in bryozoan skeletons from Sites 2, 3 and 4 is similar, and we suggest that this MgCO₃ similarity may be related to the connectivity of the populations from Sites 2, 3 and 4. We suggest that relative population connectivity strongly contributes to the observed wt% MgCO₃ in calcite

variability among study sites at both the local (>10 m, <10 km) and close-proximity (<10 m) scale. To confirm this hypothesis, population genetics would need to be conducted on bryozoans from the study sites to quantify gene flow and genetic distance among individuals from the different locations. This population connectivity data could then be compared to the observed differences in wt% MgCO₃ in calcite.

This study provides no evidence for the predicted positive correlation between wt% MgCO₃ in calcite and temperature (Chave 1954) in Antarctic bryozoans. The low annual temperature range in Antarctica thermodynamically limits the range of possible wt% MgCO₃ in calcite variability that could be caused by temperature, and any pattern is also further masked or overridden by physiological processes. This leads us to question whether the expectation of a positive relationship between wt% MgCO₃ in calcite and temperature in all bryozoan species from all regions is sensible or realistic. Urey et al. (1951) introduced the concept of 'good and bad' phyla for use as paleo-climatic indicators. Since then, Foraminifera (Rathburn & De Deckker 1997, Martínez-Botí et al. 2011, Bohaty et al. 2012), Echinodermata (McClintock et al. 2011) and coccoliths (Ra et al. 2010), phyla which had previously been considered 'faithful recorders' of temperature in their skeletal Mg:Ca ratios, have been found to show more complex relationships than previously believed (Weiner & Dove 2001). The data presented in this study suggest that the control of mineralogy in bryozoans is also more complex than previously reported, and therefore, it is recommended that the relationship between Mg-calcite and temperature in the Bryozoa should not be assumed, but should be proven for each species and region before it can be reliably used as a paleoclimatic proxy.

CONCLUSIONS

In summary, we found the skeletal Mg-calcite of Antarctic bryozoans to be highly variable among both species and sites over different spatial scales. Among species, differences in mean wt% MgCO₃ in calcite provide evidence for the vital effect in Antarctic bryozoans. We found variability among sites to be greater at the local (>10 m, <10 km) scale than at the close proximity (<10 m) scale and this difference in variability is attributed to the differential influence of physical hydrography on population connectivity. No evidence for the expected positive correlation between temperature and wt% MgCO₃ in calcite in

Antarctic bryozoan skeletons was found, and we suggest that physiological processes may be overriding or masking any environmentally driven pattern. On this basis, we suggest that Antarctic bryozoan Mg-calcite does not provide a reliable indicator of paleo-temperature, and we draw attention to these potential drawbacks in any use of Mg-based paleothermometry in bryozoans.

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