Spatio-temporal variation of skeletal Mg-calcite in Antarctic marine calcifiers in a global change scenario

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Abstract

Human driven changes such as increases in oceanic CO_2, global warming and pollution may negatively affect the ability of marine calcifiers to build their skeletons/shells, especially in polar regions. Here we address, for the first time, spatio-temporal variability of skeletal Mg-calcite using bryozoan and serpulid species as models in a recruitment experiment of settlement tiles in East Antarctica. Mineralogies were determined using X-ray diffractometry for 754 specimens belonging to six bryozoan species (four cheilostome and two cyclostome species) and two serpulid species from around Casey Station. All species had calcitic skeletons. Intra- and interspecific variability in wt% $MgCO_3$ in calcite among most species contributed to the biggest source of variation overall. Therefore, biological processes seem to be the main factor controlling the skeletal Mg-calcite in these taxa. However, spatial variability found in wt% $MgCO_3$ in calcite could also reflect local impacts such as freshwater input and contaminated sediments. The vulnerability of these species to global change is also examined and those species with high-Mg calcite skeletons and low thermal tolerance (e.g. *Beania erecta*) could be particularly sensitive to near-future global ocean chemistry changes.

KEY WORDS: Skeletal chemistry· Magnesium calcite· Ocean acidification· Bryozoa· Serpulids· Casey Station· Benthic communities

Introduction

Increases in oceanic CO_2, global warming (GW) and pollution will lead to dramatic changes in global ocean chemistry, particularly the carbonate system, in the near future. Some of the expected consequences of the increase in oceanic CO_2 are a reduction in seawater pH of 0.3-0.5 pH units by 2100 (ocean acidification; OA), a decrease in the carbonate saturation state (Ω) [1] and metal speciation in seawater [2]. In particular, polar regions are acidifying at a faster rate than elsewhere [3]. Temperature increases will also affect the stability of $CaCO_3$ [4], although the combined effects of OA and GW remain poorly constrained.

These human driven changes might negatively affect the ability of marine calcifiers to build their calcified skeletons/shells. Marine calcifiers produce a variety of mineralogical forms (polymorphs) including aragonite, calcite and calcite minerals containing a range of magnesium (Mg) content. Organisms with high-Mg calcite structures are predicted to be more vulnerable to OA as the solubility of calcite increases with its Mg-calcite content [5]. The Mg-calcite in echinoderm skeletons, for example, is expected to increase with GW [6], although this increase may be limited and solubility may not increase dramatically at higher ocean temperatures. Organisms at high latitudes may also be more susceptible to contamination (e.g. trace metals and Persistent Organic Pollutants (POPs)) compared to other regions due to their slower metabolism, growth and larval development and consequent slower detoxification processes, and slower colonisation rates [5,7–10]. Synergistic effects of anthropogenic driven environmental change could mean a significant degradation or even loss of calcareous reef habitats and sediment deposits used as shelter, substrate and food for many benthic communities [5,11,12].

Although seawater carbonate saturation state, pH and temperature play an

important role in the incorporation of Mg into calcified structures, other environmental

(e.g. salinity and Mg/Ca ratio in seawater) and biological factors (e.g. skeletal growth

rate) also mediate this process [13–15]. However, the factors that explain spatial and

temporal variations in skeletal Mg-calcite, especially in calcifying organisms inhabiting

Antarctic waters, remain unclear.

Direct effects of different stressors on particular species of marine calcifiers can

be assessed in laboratory experiments. For example, laboratory studies have

demonstrated that OA reduces growth and calcification in a wide range of marine

calcifiers [16,17]. However, experiments in the field provide a better understanding of

how the effects of environmental change will manifest on calcified organisms living in

complex ecosystems [18]. Such studies allow simultaneous exploration of different

physical, biological and anthropogenic factors (e.g. depth, species interactions and

pollution) which likely interact in their resulting effects on organisms.

In a changing world, ecosystem engineers such as bryozoans and serpulid

polychaetes contribute to habitat resilience, especially in Antarctica where these taxa

are widespread and diverse [19–21], through their wide roles such as creating reef

frameworks that prevent storm damage and enhance biodiversity [22,23]. Serpulid

polychaetes are amongst the most prominent early colonizers in Antarctica but the

$CaCO_3$ biomineralization of their calcareous tube is relatively unknown [9,24,25]. This

study investigates the spatio-temporal variability in skeletal Mg-calcite in Antarctic

bryozoans and serpulid polychaetes that secrete variable-Mg calcite skeletons [24,26–

28]. Locations around Casey Station in East Antarctica were chosen for this study as

they represent excellent opportunities to evaluate skeletal Mg-calcite variations in

undisturbed and human-impacted areas in an Antarctic region. Objectives were to: (1)

explore spatial variability in skeletal Mg-calcite across locations and water depths; (2)

investigate short-term temporal variability (at 3 and 9 y) in skeletal Mg-calcite and (3)

test whether or not there would be differences in mineralogy between impacted

(sediments contaminated with petroleum hydrocarbons and heavy metals) and non-

impacted areas around Casey Station.

Materials and Methods

Study area

The study area is located around Casey Station in the Windmill Islands, on the

coast of Wilkes Land, East Antarctica (Fig 1). The shallow marine benthic environment

is in places muddy sand, gravel, cobbles and boulders [29]. The experiment was

conducted at five locations: Brown Bay outer and Brown Bay inner, O'Brien Bay-1 and

O'Brien Bay-2 and Shannon Bay. These bays are covered by sea ice (1.2−2 m thick)

for most of the year and are ice free for 1−2 months of the year in Brown Bay and 3−4

months in the other bays. Sea ice reduces physical disturbance of the seabed by icebergs

and from wave or wind induced turbulence.

Fig 1. Location of study site.

a) Location of Windmill Islands (WI) in East Antarctica; b) the Windmill Islands area

with inset c) Newcomb and O'Brien Bays showing the position of the stations with

inset d) Casey station, and Shannon and Brown Bays.

Brown Bay is in the southwestern corner of Newcombe Bay. The southern shore

is composed of ice cliffs and its western and northern shores are an ice bank at the end

of Thala Valley, the location of a former waste disposal site [30]. During summer, a

melt stream flows down Thala Valley into Brown Bay. O'Brien Bay is on the southern

side of Bailey Peninsula and the shore consists of ice cliffs, 2−30 m high [29]. Shannon

Bay is bordered by ice cliffs 2−15 m high and has a wastewater outfall discharging into

the bay. Brown Bay and Shannon Bay are adjacent to sources of contamination (~50 m

from the waste disposal site and ~100 m from the sewage outfall, respectively) and

petroleum hydrocarbons and heavy metals have been detected in sediments [31].

Sampling design

Two tiles were attached 10 cm apart to the top edge of a trough made from one-

half of a 40 cm long, 15 cm diameter stormwater pipe (see the detailed experimental

design in [9]). Tiles were deployed at five locations: two human impacted (Brown Bay

Inner (BBShall), adjacent to an abandoned waste disposal site, and Shannon Bay (SB),

adjacent to a sewage outfall) and three controls (Brown Bay Outer (BBDeep) (500 m

from the former waste disposal site), O'Brien Bay-2 (OB2) and O'Brien Bay-3 (OB3)).

Tiles were deployed at two depth ranges at each location shallow (6 to 12 m) and deep

(15 to 25 m).   BBShall had no deep site and BBDeep had no shallow site, due to

bathymetry at these locations. At each depth, tiles were deployed at two plots ~20 m

apart in groups of 8 tiles (4 troughs ~1 to 2 m apart), with a total of 32 tiles at each

location. However, at BBShall and BBDeep, two groups of 8 tiles (in plots of 4 troughs)

were placed ~50 m apart to examine small-scale spatial variations. Tiles were deployed

between 15 November and 31 December 1997. Tiles were collected after 3 y (2001)

and 9 y (2006). One tile was collected from each of two randomly selected pipes at each

site/depth at each sampling time (a total of eight per location).

Collection and identification

Collection and identification

Divers collected the tiles which were then preserved in 95% ethanol (Fig 2).

Bryozoan colonies were identified to species level in the laboratory using an optical

microscope and guide [32]. In subtidal benthic communities, serpulid polychaetes and

bryozoans are characteristic early colonists and dominant species until they are

outcompeted by other taxa, particularly by sponges and ascidians (Clarke 1996). Six

abundant and widely distributed Antarctic bryozoan species selected for this study were

the cheilostomes *Arachnopusia decipiens* Hayward & Thorpe, 1988, *Inversiula nutrix*

Jullien, 1888, *Beania erecta* Waters, 1904, *Ellisina antarctica* Hastings, 1945, and the

cyclostomes *Disporella* cf. and *Idmidronea* cf. Two abundant serpulid polychaete

species were also chosen: one species which forms closely coiled tubes (serpulid sp.1)

and another species with tusk-shaped tubes (serpulid sp. 2).

Fig 2. Images of the tiles recovered after 6 (A-B) and 9 (C-E) years.

A) A cyclostome colony of *Disporella* cf.; B) serpulid species which form closely

coiled tubes (referred here as serpulid sp.1); C) a cyclostome colony of *Idmidronea* cf.

and the serpulid species which form tusk-shaped tubes (top right; serpulid sp. 2); D)

colonies of the cheilostome *Beania erecta*. E) entire tile colonized by bryozoan

colonies, serpulid polychaetes and different species of sponges.

Mineralogical analyses

165 **Mineralogical analyses**

166 Wherever possible, three specimens from each location, each depth, each time

167 and each species were selected for mineralogy analyses. Samples were dissected from

168 54 tiles with a scalpel blade and a piece (2 mm^2) from the growing edge of the bryozoan

colonies and entire serpulid polychaete tubes were included. After being air-dried, epibionts were removed to avoid mineralogical contamination.

Samples were powdered using an agate mortar and pestle and placed on a Si-crystal low background holder for analysis. X-ray diffractograms were collected from 5 to 90° 2θ with a Panalytical X'Pert Pro MPD diffractometer, using tube conditions of 45 kV, 40 mA, CuK$_\alpha$ radiation, X'Celerator detector, Bragg Brentano geometry, and a slew rate of 5° 2θ per minute. Diffractometer performance was constrained by measurement of silver behenate and a single silicon crystal. Limits of detection vary from 0.1 to ~2 wt% depending on the crystallinity of the phase. Identification of minerals, including poorly ordered phases, was conducted using Panalytical's Highscore Plus software v2.2.4, with ICDD PDF2 and PAN-ICSD databases. Mg-Ca substitution in calcite was quantified via linear interpolation of the angle of the 104 reflection from calcite ($CaCO_3$) (0 % Mg-calcite at XX° 2θ) to magnesite ($MgCO_3$) (100 % Mg-calcite at YY° 2θ) [33].

Skeletons were divided into low-Mg calcite (LMC; 0-4 wt% $MgCO_3$), intermediate-Mg calcite (IMC; 4–8 wt% $MgCO_3$) and high-Mg calcite (HMC; >8 wt% $MgCO_3$) categories, following a common classification [34].

Statistical analyses

To test for differences among species, year (age of colony), depth and site, a 4 factor PERMANOVA [35–38] was used. The design included the fixed factors (species, year and depth) and site nested in the combination of species, year and depth. Where significant differences were found, post hoc pairwise PERMANOVA tests were performed. PERMANOVA was done on untransformed skeletal Mg-calcite using a Euclidean distance similarity matrix, which is directly analogous to a univariate

194 ANOVA [39] but is well suited to unbalanced designs, such as is the case in this study

195 (e.g. for depth). The program PRIMER7 (V7.0.13, PRIMER-e, Quest Research

196 Limited) was used for these analyses and the graphical displays were produced using R

197 version 3.5.0 [40]. The package Lattice [41] was called to perform some of the analysis.

Results

Intra- and interspecific variation in skeletal Mg-calcite

Mineralogies were determined for 754 specimens belonging to six bryozoan

species (four cheilostome and two cyclostome species) and two serpulid polychaete

species (Fig 3-6). All species were entirely calcitic.

Arachnopusia decipiens (n = 53), *Ellisina antarctica* (n = 28), *Idmidronea* cf. (n

= 72) and *Disporella* cf. (n = 111), serpulid sp. 1 (n = 136) and sp. 2 (n = 117) had

skeletons of IMC (4–8 wt% $MgCO_3$ in calcite) (Fig 3). Of the remaining species, *Beania*

erecta (n = 109) consisted of HMC (>8 wt% $MgCO_3$ in calcite) and *Inversiula nutrix*

(n = 128), of LMC (2–4 wt% $MgCO_3$ in calcite).

Fig 3. Mean values (±standard error) of wt% $MgCO_3$ in calcite in the eight

Antarctic species.

Boxes depict standard deviation around mean (mid-line); tail indicates range. Colours

indicate high-Mg calcite (red), intermediate-Mg calcite (orange) and low-Mg (yellow).

Letters indicate results of PERMANOVA tests and show significant differences

between species, as indicated by different letters. Species that share the same letter are

not significantly different.

Fig 4. Mean values (±standard error) of wt% MgCO₃ in calcite in the four

Antarctic cheilostome species among different sites and depths.

Boxes depict standard deviation around mean (mid-line); tail indicates range. Colours

indicate deep and shallow waters.

Brown Bay = BB, OB2 = O'Brien Bay -2, OB3 = O'Brien Bay-3, SB = Shannon Bay. Deep = Deep,

shallow = Shall.

Fig 5. Mean values (±standard error) of wt% MgCO₃ in calcite in the two Antarctic

cyclostome species among different sites and depths.

Boxes depict standard deviation around mean (mid-line); tail indicates range. Colours

indicate deep and shallow waters.

Brown Bay = BB, OB2 = O'Brien Bay -2, OB3 = O'Brien Bay-3, SB = Shannon Bay. Deep = Deep,

shallow = Shall.

Fig 6. Mean values (±standard error) of wt% MgCO₃ in calcite in the two Antarctic

serpulid species among different sites and depths.

Boxes depict standard deviation around mean (mid-line); tail indicates range. Colours

indicate deep and shallow waters.

Brown Bay = BB, OB2 = O'Brien Bay -2, OB3 = O'Brien Bay-3, SB = Shannon Bay. Deep = Deep,

shallow = Shall.

The pairwise tests for species showed that there is a significant difference in the

mean wt% $MgCO_3$ in calcite among most species except *Ellisina antarctica* versus

serpulid sp. 2 (PERMANOVA pairwise test: $t = 1.94$, $P = 0.067$) and *Arachnopusia*

decipiens ($t = 2.15$, $P = 0.051$), serpulid sp. 2 versus *A. decipiens* ($t = 0.87$, $P = 0.402$),

serpulid sp. 1 versus *Beania erecta* ($t = 1.48$, $P = 0.154$), *Idmidronea* cf. versus

Disporella cf. ($t = 2.23$, $P = 0.051$) (Fig 3; Table 1).

Table 1. Post hoc pairwise permutational multivariate analysis of variance

(PERMANOVA) tests for differences in Mg content between species.

	I. nutrix	A. decipiens	Disporella cf.	Idmidronea cf.	B. erecta	serpulid sp. 1	serpulid sp. 2
E. antarctica	*17.25	2.15	**7.70**	**4.12**	**5.33**	**7.71**	1.94
serpulid 2	**24.95**	0.87	**9.49**	**6.01**	**6.03**	**6.12**	
serpulid 1	**35.76**	**6.76**	**19.02**	**13.34**	1.48		
B.erecta	**27.55**	**5.20**	**14.17**	**10.38**			
Idmidronea cf.	**17.32**	***6.03**	2.23				
Disporella cf.	**18.66**	**10.37**					
A. decipiens	**21.29**						

Bold type indicates significant difference at p = 0.001, except where *p<0.01.

Spatio-temporal analysis in skeletal Mg-calcite

There was a strong significant difference in the mean wt% $MgCO_3$ in calcite among species (PERMANOVA pseudo-F = 226.9, p = 0.001) and sites (pseudo-F = 2.04, p = 0.001) (Fig 3-6; Table 2). There was a significant difference among depths (pseudo-F = 5.08, p = 0.029). There were no significant interactions between species, year or depth, indicating differences between species and depth were consistent. There was no effect of year (age of colony).

260 **Table 2. PERMANOVA testing for the effects of species, year and depth on the**

261 **Mg content in calcite.**

Source	Df	SS	MS	Pseudo- F	P	% var
Species	**7**	**2140.1**	**305.73**	**226.9**	**0.001**	62.43
Year	1	0.84573	0.84573	0.66618	0.412	0
Depth	**1**	**6.4576**	**6.4576**	**5.0867**	**0.029**	4.65
SpxYe	7	3.8223	0.54604	0.40524	0.888	0
Spxde	7	8.3086	1.1869	0.88088	0.532	0
YexDe	1	0.26294	0.26294	0.20712	0.632	0
SpxYexDe	7	8.1498	1.1643	0.86405	0.513	0
Site (SpxYexDe)	**53**	**79.796**	**1.5056**	**2.0489**	**0.001**	8.87
Residual	669	491.6	0.73483			24.05
Total	753	3725.3				

% var = Estimates of components of variation of Mg content in calcite for each factor

in the analysis.

Differences in skeletal Mg-calcite between species contributed to >62% of the

total variation observed (Fig 3; Table 2). Variation within species (residual variation

among replicates within same depth, year and site) contributed ~24 % to overall

variation. Differences among sites (which are confined to comparisons among the same

species, depths and years as this was a nested factor in the analysis, i.e. site was nested

in species, year and depth) contributed ~9 % of the variance (Table 2, Fig 4-6).

271   There was a small but significant effect of depth, with differences in skeletal

272   Mg-calcite between different depths contributing ~5 % to the estimated total variation

273   (Fig 4-6).

274   There was no significant overall effect of human impact on skeletal Mg-calcite

275   concentrations (Table 3) nor any significant effect on any individual species (species x

276   impact interaction, Table 3).

278   **Table 3. Results of PERMANOVA analysis testing for the effects of human impact**

279   **on the Mg content in calcite, with tests for each species (impact x species).** % var =

280   Estimates of components of variation of Mg content in calcite.

Source	df	MS	Pseudo-F	P(perm)	% var
Impact	1	0.25913	0.17276	0.69	0
Species	7	350.56	166.13	0.001	66.7
Impact x species	6	1.0093	0.50947	0.769	0
Site (Sp. x impact)	20	2.4276	3.1551	0.001	8.4
Residual	719	0.76941			25
Total	753				

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Discussion

This study evaluates the variability of wt% $MgCO_3$ in calcite in the skeletons of a large number of specimens of common bryozoan and serpulid taxa in coastal Antarctic waters, one of the regions expected to be first affected by OA and GW in the near future. To our knowledge, this is the first study addressing the spatio-temporal variability of skeletal Mg-calcite using bryozoan and serpulid polychaete species as models in an experiment of settlement tiles in Antarctica. These two taxonomic groups, and some genera and species studied here (e.g. *Arachnopusia*, *Beania*, *E. antarctica*), are common components of hard-substratum sessile assemblages across a wide range of cold latitudes (e.g. South American Region) [42]. These kinds of studies allow regional and latitudinal comparisons of the effects of changes in global ocean chemistry on marine calcifiers. All species had calcitic skeletons, which is consistent with our expectations from previous research on bryozoans and serpulids from high latitudes [28,43–46]. This trend is possibly due to low temperatures favouring the deposition of calcite over aragonite as the latter is more susceptible to dissolution in cold waters [45].

Inter- and intraspecific variability in skeletal Mg-calcite

Inter-specific differences in the mean wt% $MgCO_3$ in calcite were the largest source of variation overall. Remarkably, there were significant differences in the skeletal Mg-calcite between all bryozoan species except between *E. antarctica* and *A. decipiens* and between the two cyclostome species. There was a wide range of wt% $MgCO_3$ in calcite measurements in the bryozoan and serpulid polychaete species studied here, from 0.1–13.1 wt% $MgCO_3$ in calcite. These values are not surprising for wt% $MgCO_3$ in calcite in bryozoans, which can range from 0 to 14 [43,44], nor for

serpulids, which range from 7 to 15 [24]. Only one species was classified as LMC

(*Inversiula nutrix*); five as IMC (*Arachnopusia decipiens*, *Ellisina antarctica*,

Disporella cf., *Idmidronea* cf. and serpulid sp. 1 and sp. 2); and one as HMC (*Beania*

erecta). Most cheilostome bryozoans secrete IMC [43,44], which is further confirmed

by this study. To our knowledge, this study also represents the first mineralogical

measurements made for the species *A. decipiens*. Our values are within the range of

wt% $MgCO_3$ in calcite obtained by previous studies in Antarctic cheilostome bryozoans

except for *B. erecta,* which has been reported to be IMC [28,47,48]. The skeletons of

B. erecta in our study showed higher mean Mg values even though the range was highly

variable (5.3–13.6 wt% $MgCO_3$ in calcite). This species exhibits a wide geographical

distribution in Antarctica and it could explain the broad range of wt% $MgCO_3$ in calcite

found here. Other studies have reported ICM and LMC skeletons in *E. antarctica* and

I. nutrix, respectively, from King George and Adelaide Islands (Antarctic

Peninsula)[26,28,44]. Significant differences were found in the skeletal Mg-calcite

even between cheilostomes of the same suborder Flustrina, in *B. erecta* and *E.*

antarctica, although they belong to different families (Beaniidae and Ellisinidae,

respectively). Coinciding with our results on the cyclostome genera, *Idmidronea* sp.

and *Disporella* sp. produced LMC and HMC in a previous study [43].

The next biggest source of variation was among individuals of the same species.

Therefore, biological processes, also known as vital effects [49], seem to be the main

factors controlling the skeletal Mg-calcite in these organisms and, in the case of

bryozoans, at intra- and interspecific and intracolonial levels [50]. Other studies provide

corroborative evidence that the variation in the skeletal Mg-calcite in serpulid

polychaete species [51] and Antarctic bryozoans is partially biologically mediated

[26,28].

Potential environmental influence on skeletal Mg-calcite

Differences among sites (within species) contributed less to variation in skeletal

Mg-calcite than among species but was still a significant effect. Several studies have

demonstrated that marine calcifiers such as bryozoans, coccoliths, foraminifera and sea

stars can respond differently to a range of environmental factors (e.g. water

temperature, alkalinity, salinity and Mg/Ca ratio of seawater)[14,26,27,52–54]. The

observed values could reflect a relatively stable environment below ice with constant

low seawater temperatures as these bays are covered by fast sea-ice almost all the year.

Therefore, environmental variables such as seawater temperature could have little

influence on the skeletal Mg content in calcite in species inhabiting Antarctic waters,

as suggested in previous studies focusing on cold water bryozoans [26,27]. However,

the variation observed could be due to variable temporal local impacts such as

freshwater input from the melt stream that flows into Brown Bay during summer.

Freshwater not only has different chemistries and transports sediment to the bay, but

also affects seawater temperature.

Although depth had the least effect, it was significant, with deeper sites having

slightly higher Mg content in calcite. In particular, the significant higher values of Mg

content in calcite in deeper waters were only found in the case of serpulid sp. 2 from

three control locations (BBDeep, OB2 and OB3). However, other species such as *I.*

nutrix and serpulid sp.1 from shallow control locations exhibited higher values than

deeper samples. Therefore, there was not any clear depth-related pattern. The lack of

influence of depth on mineralogy found is probably due to narrow depth range (6 m)

that was sampled.

Although there was no evidence of significant human impact on skeletal Mg-

calcite concentrations, further studies covering a broader range area and focusing on

other species are needed to evaluate whether or not the Mg content in calcite might be

indirectly influenced by contaminated sediments. Heavy metal toxicity may reduce

growth rates of marine invertebrates such as bryozoans and worms [55,56], due to the

investment in fitness traits (e.g. growth or reproduction) which are compromised by

investment in detoxification mechanisms [57]. Also, organisms with slower growth

rates tend to deposit less wt% $MgCO_3$ in calcite [5]. Therefore, we could expect lower

skeletal Mg content in areas exposed to heavy metals.

Temporal analyses in skeletal Mg-calcite

We document short term temporal analyses in skeletal Mg-calcite (over a 3 year

period) for the first time. Other studies investigated potential temporal variation in

skeletal Mg-calcite for greater periods from 30 years to millions of years [28,44]. In our

study, there was no effect of time (age of colony). Similarly, another study evaluating

temporal variability in skeletal Mg-calcite in the Antarctic bryozoan species *Cellaria*

diversa Livingstone, 1928 and *Antarcticaetos bubeccata* (Rogick, 1955) over the past

30 years did not find significant temporal differences [28]. However, the samples came

from greater depth ranges (107–205 and 100–251 m, respectively) and from a different

location of Admiralty Bay, South Shetland Islands, north of the Antarctic Peninsula,

which lies at 62°10' S (versus Casey at 66°17' S).

Vulnerability of the Antarctic target species in a changing world

Human-induced changes could affect survival and calcification of some particular Antarctic marine calcifiers studied here, particularly those with HMC skeletons and low thermal tolerance [5,11,58]. Serpulid polychaete tubes are mainly composed of the most vulnerable minerals to OA (aragonite, HMC or a mixture of the two) [24]. In our study, *Beania erecta* and serpulid sp. 1 deposited their $CaCO_3$ skeletons containing high proportion of magnesium ions (with mean wt% $MgCO_3$ in calcite ($\pm$ SD) of 8.1 $\pm$ 1.52 and 7.7 $\pm$ 0.69, respectively) although serpulid sp. 1 was classified here as IMC. The solubility of the skeletons of both species is expected to be greater than that of IMC and LMC or even of aragonite. Although some serpulid polychaetes seem to have the ability to vary their tube mineralogy with seawater chemistry changes, such alterations may result in a deterioration of the tube hardness and elasticity [59]. Consequently, these species are highly likely to be threatened under a scenario of global ocean surface pH reductions of 0.3–0.5 units by the year 2100 [1]. Experimental evidence for this is limited, but the Mediterranean bryozoan *Myriapora truncata* (Pallas, 1766), which has a wt% $MgCO_3$ in calcite between 8 and 9.5, has been shown to be vulnerable to ocean acidification at a pH of 7.66, with significant loss of skeleton during a short-term experiment [60].

There is an increase of Mg content in cheilostome bryozoans towards lower latitudes, which is attributed to the warmer seawater [44]. In a recent study, higher values of Mg in two common co-occurring Mediterranean bryozoans *M. truncata* and *Pentapora fascialis* (Pallas, 1766) were also found when the colonies were exposed to high temperatures [61]. Serpulid tubes also show an increase in Mg content with increasing water temperatures [51]. In addition, the interactive effects of multiple

environmental changes (e.g. warming, acidification and pollution) have to be taken in

account as they are occurring together. The interactive effects of temperature and CO_2

concentrations on the widely distributed cheilostome bryozoan *Jellyella tuberculata*

(Bosc, 1802), including from subAntarctic regions [62], was investigated under

controlled conditions [63]. Colonies exposed to reduced pH and increased temperature

showed dissolution of their zooids, possibly due to an increase of skeletal Mg content

at high temperatures which made the skeletons more susceptible to dissolution under

high CO_2 [63]. Therefore, organisms living in Antarctic coastal areas that have

experienced significant warming in recent years (e.g. Western Antarctic Peninsula)

could become more vulnerable to environmental changes if their Mg content increases

with temperature [64], however it is not known whether this is occurring.

Such changes could lead to shifts in community composition of calcareous

marine organisms in future oceans, especially in polar regions [11]. Species with HMC,

such as *B. erecta* and serpulid sp. 1, may be particularly vulnerable to the combined

effects of OA and GW, although variation in species-specific responses may also be

anticipated. Potential subsequent decreases of some species like *B. erecta*, which is

widely distributed and common throughout Antarctica and has colonies which comprise

dense mats and may cover large areas of substratum, could indirectly lead to negative

impacts to other organisms which use their colonies as substrate, food and shelter [32].

This could be expected in colonies living in shallow waters of some Antarctic regions

more exposed to increased temperatures such as the Antarctic Peninsula. However, *B.*

erecta, has a depth range is from 0 to >1500 m [62], and cooler deep waters may form

refugia for this and other species. Also, *B. erecta* is a competitive dominant species,

covering different bryozoan species like *Inversiula nutrix* studied here and other sessile

organisms [65]. Its decrease could favour other less competitive species such as *I.*

432 *nutrix,* which may be more resilient to acidification due to lower Mg content of its

433 skeleton and, consequently, replacing the ecological niche occupied by *B. erecta*.

434  Calcifying marine invertebrates are important components of marine

435 ecosystems and it is clear OA will have a range of effects at the species level, potentially

436 leading to wider ecosystem level effects. Given the range and variation in mineralogy

437 of important calcifying species as observed in this study, some species are likely to be

438 more heavily affected than others. Further work is required to better understand the

439 potential impacts of OA on marine benthic communities, particularly in areas thought

440 to be vulnerable to OA such as Antarctica shallow waters.

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References

452 1. Caldeira K, Wickett ME. Ocean model predictions of chemistry changes from

453  carbon dioxide emissions to the atmosphere and ocean. J Geophys Res.

454  2005;110: 1–12. doi:10.1029/2004JC002671

455 2. Millero F, Woosley R, DiTrolio B, Waters J. Effect of Ocean Acidification on

456  the Speciation of Metals in Seawater. Oceanography. 2009;22: 72–85.

457   doi:10.5670/oceanog.2009.98

458   3.   Fabry V, McClintock J, Mathis J, Grebmeier J. Ocean Acidification at High

459   Latitudes: The Bellwether. Oceanography. 2009;22: 160–171.

460   doi:10.5670/oceanog.2009.105

461   4.   Zeebe R, Wolf-Gladrow D. CO2 in Seawater-Equilibrium, Kinetics, Isotopes.

462   Elsevier Oceanography Series. Elsevier, Amsterdam; 2001. doi:10.1016/S0422-

463   9894(01)80002-7

464   5.   Andersson AJ, Mackenzie FT, Bates NR. Life on the margin: Implications of

465   ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers.

466   Mar Ecol Prog Ser. 2008;373: 265–273. doi:10.3354/meps07639

467   6.   Hermans J, Borremans C, Willenz P, André L, Dubois P. Temperature, salinity

468   and growth rate dependences of Mg/Ca and Sr/Ca ratios of the skeleton of the

469   sea urchin Paracentrotus lividus (Lamarck): An experimental approach. Mar

470   Biol. 2010;157: 1293–1300. doi:10.1007/s00227-010-1409-5

471   7.   Chapman PM, Riddle MJ. Missing and needed: Polar marine ecotoxicology. Mar

472   Pollut Bull. 2003;46: 927–928. doi:10.1016/S0025-326X(03)00252-2

473   8.   Thompson BAW, Goldsworthy PM, Riddle MJ, Snape I, Stark JS.

474   Contamination effects by a "conventional" and a "biodegradable" lubricant oil

475   on infaunal recruitment to Antarctic sediments: A field experiment. J Exp Mar

476   Bio Ecol. 2007;340: 213–226. doi:10.1016/j.jembe.2006.09.010

477   9.   Stark JS. Patterns of higher taxon colonisation and development in sessile marine

478   benthic assemblages at Casey Station, Antarctica, and their use in environmental

479   monitoring. Mar Ecol Prog Ser. 2008;365: 77–89. doi:10.3354/meps07559

480   10.   Bargagli R. Antarctic ecosystems: environmental contamination, climate

481   change, and human impact [Internet]. Ecological studies 175. Berlin: Springer-

Verlag;   2005.   Available: http://agris.fao.org/agris-
search/search.do?recordID=US9182141

11. Orr JC, Fabry VJ, Aumont O, Bopp L, Doney SC, Feely RM, et al.
Anthropogenic ocean acidification over the twenty-first century and its impact
on calcifying organisms. Nature. 2005; 681–686.

12. Ries JB. Skeletal mineralogy in a high-CO2 world. J Exp Mar Bio Ecol.
2011;403: 54–64. doi:10.1016/j.jembe.2011.04.006

13. Davis KJ, Dove PM, De Yoreo JJ. The role of Mg2+ as an impurity in calcite
growth. Science. 2000;290: 1134–1137. doi:10.1126/science.290.5494.1134

14. Borremans C, Hermans J, Baillon S, André L, Dubois P. Salinity effects on the
Mg/Ca and Sr/Ca in starfish skeletons and the echinoderm relevance for
paleoenvironmental   reconstructions.   Geology.   2009;37:   351–354.
doi:10.1130/G25411A.1

15. Chan VBS, Thiyagarajan V, Lu XW, Zhang T, Shih K. Temperature dependent
effects of elevated CO2 on shell composition and mechanical properties of
Hydroides elegans: Insights from a multiple stressor experiment. PLoS One.
2013;8: e78945. doi:10.1371/journal.pone.0078945

16. Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ, Heip CHR. Impact
of elevated CO2 on shellfish calcification. Geophys Res Lett. 2007;34: L07603.
doi:10.1029/2006GL028554

17. Albright R, Mason B, Langdon C. Effect of aragonite saturation state on
settlement and post-settlement growth of *Porites astreoides* larvae. Coral Reefs.
2008;27: 485–490. doi:10.1007/s00338-008-0392-5

18. Kamenos NA, Perna G, Gambi MC, Micheli F, Kroeker KJ. Coralline algae in a
naturally acidified ecosystem persist by maintaining control of skeletal

mineralogy and size. Proc R Soc B Biol Sci. 2016;283: 20161159.

doi:10.1098/rspb.2016.1159

19.   Clarke A, Survey BA, Cross H, Road M, Oet CCB. The distribution of Antarctic

marine benthic communities. Antarct Res Ser. 1996;70: 219–230.

20.   Barnes DKA, Griffiths HJ. Biodiversity and biogeography of southern temperate

and polar bryozoans. Glob Ecol Biogeogr. 2008;17: 84–99. doi:10.1111/j.1466-

8238.2007.00342.x

21.   Figuerola B, Monleón-getino T, Ballesteros M, Avila C. Spatial patterns and

diversity of bryozoan communities from the Southern Ocean: South Shetland

Islands, Bouvet Island and Eastern Weddell Sea. Syst Biodivers. 2012;10: 109–

123. doi:10.1080/14772000.2012.668972

22.   Smith AM. Growth and calcification of marine bryozoans in a changing ocean.

Biol Bull. 2014;226: 203–210.

23.   Wright JT, Gribben PE. Disturbance-mediated facilitation by an intertidal

ecosystem engineer. Ecology. 2017;98: 2425–2436. doi:10.1002/ecy.1932

24.   Smith AM, Riedi MA, Winter DJ. Temperate reefs in a changing ocean: Skeletal

carbonate mineralogy of serpulids. Mar Biol. 2013;160: 2281–2294.

doi:10.1007/s00227-013-2210-z

25.   Vinn O, Ten Hove HA, Mutvei H, Kirsimäe K. Ultrastructure and mineral

composition of serpulid tubes (Polychaeta, Annelida). Zool J Linn Soc.

2008;154: 633–650. doi:10.1111/j.1096-3642.2008.00421.x

26.   Loxton J, Kuklinski P, Barnes DKA, Najorka J, Jones MS, Porter JS. Variability

of Mg-calcite in Antarctic bryozoan skeletons across spatial scales. Mar Ecol

Prog Ser. 2014;507: 169–180. doi:10.3354/meps10826

27.   Figuerola B, Kuklinski P, Taylor P. Depth patterns in Antarctic bryozoan skeletal

Mg-calcite: Can they provide an analogue for future environmental changes?

Mar Ecol Prog Ser. 2015;540: 109–120. doi:10.3354/meps11515

28.   Krzeminska M, Kuklinski P, Najorka J, Iglikowska A. Skeletal Mineralogy

Patterns of Antarctic Bryozoa. J Geol. 2016;124: 000–000. doi:10.1086/685507

29.   Stark JS. The distribution and abundance of soft-sediment macrobenthos around

Casey Station, East Antarctica. Polar Biol. 2000;23: 840–850.

doi:10.1007/s003000000162

30.   Stark JS, Snape I, Riddle MJ. Abandoned Antarctic waste disposal sites:

Monitoring remediation outcomes and limitations at Casey Station. Ecological

Management and Restoration. 2006. p. 7:21-31. doi:10.1111/j.1442-

8903.2006.00243.x

31.   Stark JS, Riddle MJ, Snape I, Scouller RC. Human impacts in Antartic marine

soft-sediment assemblages: Correlations between multivariate biological

patterns and environmental variables at Casey Station. Estuar Coast Shelf Sci.

2003;56: 717–734. doi:10.1016/S0272-7714(02)00291-3

32.   Hayward P. Antarctic Cheilostomatous Bryozoa. Oxford Univ Press Oxford.

1995;

33.   Mackenzie FT, Bischoff WD, Bishop FC, Loijens M, Schoonmaker J, Wollast

R. Magnesian calcites: low-temperature occurrence, solubility and solid-solution

behavior. Carbonates Mineral Chem. 1983; 97–144. Available:

http://www.scopus.com/inward/record.url?eid=2-s2.0-

0021080597&partnerID=40&md5=abf2bbd57957f8cf91519411ddd9d58e

34.   Rucker JB, Carver RE. A Survey of the Carbonate Mineralogy of Cheilostome

Bryozoa. J Paleontol. Paleontological Society; 1969;43: 791–799. Available:

http://www.jstor.org/stable/1302474

35. Anderson MJ. A new method for non parametric multivariate analysis of
variance. Austral Ecol. 2001;26: 32–46. doi:10.1111/j.1442-
9993.2001.01070.pp.x

36. Anderson MJ, Braak CJFTER. Permutation tests for multi-factorial analysis of
variance. J Stat Comput Simul. 2003;73: 85–113. doi:10.1080/00949650215733

37. McArdle BH, Anderson MJ. Fitting multivariate models to community data: A
comment on distance-based redundancy analysis. Ecology. 2001;82: 290–297.
doi:10.1890/0012-9658(2001)082[0290:FMMTCD]2.0.CO;2

38. Anderson MJ. Permutation tests for univariate or multivariate analysis of
variance and regression. Can J Fish Aquat Sci. 2001;58: 626–639.
doi:10.1139/f01-004

39. Anderson MJ, Gorley RN, Clarke KR. PERMANOVA+ for PRIMER: Guide to
Software and Statistical Methods. Plymouth, UK. 2008. pp. 1–214.
doi:10.13564/j.cnki.issn.1672-9382.2013.01.010

40. R Core Team. R: A language and environment for statistical computing. R
Foundation for Statistical Computing. Vienna, Austria. URL https://www.R-
project.org/.; 2018.

41. Sarkar D. Lattice: multivariate data visualization with R. Springer, New York.
Use R! 2008; doi:10.1007/978-0-387-75969-2

42. Figuerola B, Barnes DKA, Brickle P, Brewin PE. Bryozoan diversity around the
Falkland and South Georgia Islands: Overcoming Antarctic barriers. Mar
Environ Res. Elsevier Ltd; 2017;126: 81–94.
doi:10.1016/j.marenvres.2017.02.005

43. Smith AM, Key MM, Gordon DP. Skeletal mineralogy of bryozoans: Taxonomic
and temporal patterns. Earth-Science Rev. 2006;78: 287–306.

doi:10.1016/j.earscirev.2006.06.001

44.   Taylor PD, James NP, Bone Y, Kuklinski P, Kyser TK. Evolving mineralogy of

cheilostome bryozoans. Palaios. 2009;24: 440–452. doi:10.2110/palo.2008.p08

45.   Kuklinski P, Taylor PD. Mineralogy of Arctic bryozoan skeletons in a global

context. Facies. 2009;55: 489–500. doi:10.1007/s10347-009-0179-3

46.   Bornhold BD, Milliman JD. Generic and environmental control of carbonate

mineralogy in serpulid (polychaete) tubes. J Geol. 1973;81: 363–373. Available:

http://www.jstor.org/stable/30084830

47.   Borisenko Y, Gontar VI. Biogeochemistry of skeletons of coldwater Bryozoa.

Biol Morya. 1991;1: 80–90.

48.   Taylor P. Access details : Access Details : [subscription number 789296668]

New species of cheilostomatous Bryozoa from subantarctic Marion and New

species of cheilostomatous Bryozoa from subantarctic Marion and Prince

Edward Islands. 2009. doi:10.1080/00222930500124664

49.   Weiner S, Dove PM. An overview of biomineralization processes and the

problem of the vital effect. Rev Mineral Geochemistry. 2003;54: 1–29.

doi:10.2113/0540001

50.   Taylor PD, Lombardi C, Cocito S. Biomineralization in bryozoans: present, past

and future. Biol Rev. 2015;90: 1118–50. doi:10.1111/brv.12148

51.   Bornhold BD, Milliman JD. Generic and environmental control of carbonate

mineralogy in serpulid (polychaete) tubes. J Geol. 1973;81: 363–373.

52.   Stoll HM, Ruiz Encinar J, Ignacio Garcia Alonso J, Rosenthal Y, Probert I, Klaas

C. A first look at paleotemperature prospects from Mg in coccolith carbonate:

Cleaning techniques and culture measurements. Geochemistry Geophys

Geosystems. 2001;2: 2000GC000144. doi:10.1029/2000GC000144

53.   Russell AD, Hönisch B, Spero HJ, Lea DW. Effects of seawater carbonate ion

concentration and temperature on shell U, Mg, and Sr in cultured planktonic

foraminifera.   Geochim   Cosmochim   Acta.   2004;68:   4347–4361.

doi:10.1016/j.gca.2004.03.013

54.   Stanley SM, Ries JB, Hardie LA. Seawater chemistry, coccolithophore

population growth, and the origin of Cretaceous chalk. Geology. 2005;33: 593.

doi:10.1130/G21405.1

55.   Levinton JS, Suatoni E, Wallace W, Junkins R, Kelaher B, Allen BJ. Rapid loss

of genetically based resistance to metals after the cleanup of a Superfund site.

Proc Natl Acad Sci U S A. 2003;100: 9889–9891. doi:10.1073/pnas.1731446100

56.   Piola RF, Johnston EL. Differential tolerance to metals among populations of the

introduced   bryozoan   Bugula   neritina.   Mar   Biol.   2006;148:   997–1010.

doi:10.1007/s00227-005-0156-5

57.   Sibly RM, Calow P. A life-cycle theory of responses to stress. Biol J Linn Soc.

1989;37: 101–116. doi:10.1111/j.1095-8312.1989.tb02007.x

58.   IPCC. Global Warming of 1.5°C. Summary for Policymakers. Contribution of

Working Groups I, II and III to the 48th Session of the IPCC, Incheon, Republic

of   Korea   [Internet].   2018.   Available:

http://report.ipcc.ch/sr15/pdf/sr15_spm_final.pdf

59.   Chan VBS, Li C, Lane AC, Wang Y, Lu X, Shih K, et al. CO2-driven ocean

acidification alters and weakens integrity of the calcareous tubes produced by

the   serpulid   Tubeworm,   Hydroides   elegans.   PLoS   One.   2012;7:   e42718.

doi:10.1371/journal.pone.0042718

60.   Lombardi C, Rodolfo-metalpa R, Cocito S, Gambi MC, Taylor D. Structural and

geochemical alterations in the Mg calcite bryozoan Myriapora truncata under

elevated seawater p CO2 simulating ocean acidification. Mar Ecol. 2011;32:

211–221. doi:10.1111/j.1439-0485.2010.00426.x

61.   Pagès-Escolà M, Hereu B, Garrabou J, Montero-Serra I, Gori A, Gómez-Gras D,

et al. Divergent responses to warming of two common co-occurring

Mediterranean bryozoans. Sci Rep. 2018;8: 17455. doi:10.1038/s41598-018-

36094-9

62.   GBIF. .org (16 October 2018) GBIF Occurrence Download.

doi:10.15468/dl.1y0m2v

63.   Swezey DS, Bean JR, Ninokawa AT, Hill TM, Gaylord B, Sanford E. Interactive

effects of temperature, food and skeletal mineralogy mediate biological

responses to ocean acidification in a widely distributed bryozoan. Proc R Soc B

Biol Sci. 2017;284: 20162349. doi:10.1098/rspb.2016.2349

64.   Meredith MP, King JC. Rapid climate change in the ocean west of the Antarctic

Peninsula during the second half of the 20th century. Geophys Res Lett. 2005;

doi:10.1029/2005GL024042

65.   Clark GF, Stark JS, Johnston EL. Tolerance rather than competition leads to

spatial dominance of an Antarctic bryozoan. J Exp Mar Bio Ecol. 2017;486: 222–

229. doi:10.1016/j.jembe.2016.10.008

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6