Summer and winter $MgCO_3$ levels in skeleton of Arctic bryozoans

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Abstract

In the Arctic, seasonal patterns in seawater biochemical conditions are shaped by physical, chemical, and biological processes related to the alternation of polar night and midnight sun periods. During spring-summer, CO_2 is consumed by photosynthetic activity of autotrophs which elevates the seawater pH and, thus, carbonate availability and saturation state. Under winter darkness, primary production is reduced to near zero and respiration processes lower pH levels, which consequently decreases carbonate saturation. As most of these processes are taking place in the euphotic zone, there should also be a carbonate saturation gradient associated with depth. We tested whether the changes in seawater chemistry due to light-driven activities of marine biota can influence the uptake of Mg into calcified skeletons of Arctic Bryozoa. Mg content in biogenic carbonate skeletons helps determine solubility of the skeleton. This study found no differences between summer and winter levels of skeletal $MgCO_3$ in five bryozoan species in spite of differences in carbonate saturation state between these two seasons. Furthermore, we could not detect any depth-related differences in $MgCO_3$ content in skeletons of studied bryozoans. This result may indicate that Arctic bryozoans are able to regulate $MgCO_3$ skeletal concentrations biologically. Characteristic levels of $MgCO_3$ for particular species seems to confirm biological control. On the other hand, Arctic seawater is

still well-saturated in spite of observed variability, even during the high pCO_2 winter season and/or greater depths, which may have led to the observed pattern of no differences in $MgCO_3$ uptake. Yet recorded location-related variability in $MgCO_3$ content in skeletons from stations exhibiting different seawater parameters suggest that environmental factors can also, to some extent, shape the skeletal chemistry of Arctic bryozoans. Overall results of this study show complexity of factors which may control $MgCO_3$.

Key words: polar night, polar day, Mg-calcite, marine calcifier, light-driven changes, ocean acidification

Introduction

Ocean acidification and its associated decrease in carbonate saturation state currently have raised serious concerns about possible consequences for precipitation of calcareous skeletons and the biology of marine calcifiers. Decreasing availability of carbonates in seawater can hinder the biomineralization process and accelerate dissolution of marine organisms' skeleton (e.g., Andersson et al. 2008; Feely et al. 2009; Mollica et al. 2018), with potential impacts on growth rates, competitive abilities, and survival of calcifying organisms.

Decrease in carbonate saturation seems to be particularly pronounced in high latitudes of Arctic and Antarctic ecosystems (e.g., Andersson et al. 2008). CO_2 has higher solubility in cold water, leading to higher uptake than in warmer waters. As a result, the seawater carbonate saturation state is lower in polar areas than in temperate and tropical seas. Therefore, carbonates ($CaCO_3$) and skeletons built of that material at high latitudes are at higher risk of being exposed to unfavourable conditions, especially concerning those carbonates containing high-Mg calcite (e.g., Andersson et al. 2008). Magnesium is a common component of biogenic carbonates influencing chemical and mechanical properties of invertebrates' skeletons. Since the solubility of skeletal carbonate increases with increasing content of $MgCO_3$ (e.g., Morse et al. 2006; Andersson et al. 2009), understanding factors controlling level of Mg in biogenic carbonates in the light of currently rapidly changing carbonate saturation state in marine systems is an important issue.

In the polar regions during summer, the sun stays above the horizon all season (polar day). This results in a continuous primary production with distinct peaks of phytoplankton blooms (e.g., Piwosz et al. 2009; Shadwick et al. 2011). Biological carbon uptake (CO_2) due to the intensive photosynthesis during spring-summer leads to increase of seawater pH and $CaCO_3$ saturation state (Ω) (e.g., Fransson et al. 2016). The elevated productivity is observed

Commented [PER2]: In Mg? CO2?

Commented [PER3]: Uptake? Production?

Commented [PER4]: Is this from this study?

Commented [PER5]: From the atmosphere or by organisms?

Commented [PER6]: Do you wnat to cite papers indicating that Mg content in calcitic organisms ranges form #-#, and within a single taxon can vary from #-#...? At some point yuou need to document that there is variability, otherwise it is difficult to justify why you studied this problem.

Commented [PER7]: Provided nutrients are available

Commented [PER8]: By how much?

primarily in the upper layer of the water column (euphotic zone), which creates clear stratification in oxygen production with depth (e.g., Fransson et al. 2016). Moreover, in Arctic fjords, the spring melt of sea ice contributes to surface water stratification. As primary production in the Arctic is limited to <40 m depth (Hill and Cota 2005), we should observe different O-CO_2 proportion below that zone. At greater depths, organic matter degradation releases CO_2 which consequently leads to pH decrease that influences saturation of $CaCO_3$ (e.g., Andersson et al. 2008; Chierici et al. 2011). Thus, during polar day in the Arctic we can observe stratification between shallow water masses characterized by high carbonate saturation state, and deep-sea areas showing lower saturation levels of carbonates.

On the other hand, during polar night there is no sunlight and the primary production is significantly reduced or not taking place (e.g., Smetacek and Nicol 2005; Piepenburg 2005). Thus, for the months of polar night, the system is in a heterotrophic state, dominated by oxygen consumption and CO_2 production processes, which consequently causes the decrease in seawater pH and Ω (e.g., Chierici et al. 2011). This effect is enhanced by local CO_2- enriched subsurface waters and remineralization of organic matter (e.g., Chierici et al. 2011; Fransson et al. 2013). Therefore, during the dark season the CO_2 levels should be rather homogenous across depth as there is no stratification nor primary production taking place during that period.

The surface seawater and euphotic zone in polar areas are characterised by higher variability of pH and $CaCO_3$ saturation state than deeper areas below euphotic zone. Major factor driving that pattern for the shallows is seasonal variability in abundance and species occurrence of primary producers therefore photosynthetic uptake of CO_2 (e.g., Hegseth 1998; Hodal et al. 2012). In deeper water masses below the euphotic zone, where there is no seasonal photosynthetic uptake of CO_2, the $CaCO_3$ saturation state should be more stable throughout the year. This depth difference in seasonality in pH and carbonate saturation state creates a suitable model system to study influence of these variables on skeletal structure including concentration of magnesium. We predict that greater fluctuations in CO_2 and O_2 will lead to greater variability in Mg skeletal content in organisms from the shallow, euphotic zone. This study uses bryozoans as model organisms to investigate whether Mg level in the calcitic skeleton is controlled by currently occurring pH and $CaCO_3$ saturation state variability in an Arctic system.

Bryozoans are colonial, suspension feeders occurring worldwide at broad depth ranges from abyssal to intertidal zone. Because of their high abundance and diversity (>300 species; Kluge 1975), bryozoans are considered important components of the Arctic ecosystem and significant carbonate producers in this area. The Arctic bryozoans' colonies are often composed of thousands of units (zooids) in most cases composed of calcite with variable amount of

Commented [PER9]: So? Is this an indicator of something relevant to saturation state?

Commented [PER10]: Not really studied in that paper. Maybe Berge et al. 2015 Current Biol is better

Commented [PER11]: Does this belong in the para above?

Commented [PER12]: The link between O2 and Mg content has not been made, except indirectly

$MgCO_3$ ranging from 0 to 8.8 mol% (Kukliński and Taylor 2009). Environmental parameters including temperature and seawater chemistry are believed to control the uptake of Mg into bryozoan skeletons, although it is still not fully understood to what degree (Kukliński and Taylor 2009). As the bryozoan colony has continuous growth, each zooid is a potential archive of ambient environmental conditions during the period of growth. If skeletal chemical composition (Mg content) in marine calcifiers, in this case bryozoans, is controlled by environmental parameter differences in carbonates and pH related to polar night and day it should be reflected in their skeletons by different concentration of magnesium. If no differences are exhibited in skeletal magnesium level, in spite of carbonate and pH differences in the water, we assume organisms can control their skeletal composition biologically or that difference in water mass chemical parameters of winter and polar periods are not large enough to drive those changes.

The presented study aims to answer following questions (1) Is there a difference in Mg content between skeletons which have grown during polar night and polar day? (2) Is Mg content changing with depth and if that is the case does it follow the same pattern during polar day and polar night period? Answering those question will allow us to verify following hypothesis: (1) there is a difference in Mg content in carbonate skeletons which grew during polar day and polar night, which indicates environmental control of skeletal parameters, and (2) as the water column will be stratified when it comes to carbonates during the polar day, there will be a difference in Mg content in skeletons which grew in that period.

Commented [HH13]: You need to simplify these, e.g. Enrivornmental vs. Intrinsic control (e.g., compensatory ion pump) of skeletal parameters in bryozoans.

Material and methods

Study area

The investigation was conducted in a high Arctic location – Kongsfjorden and its close vicinity. The area is situated on the northwest coast of Spitsbergen (Svalbard Archipelago) between 78°50’ and 79°04’N, and 11°10’ and 12°30’E (Fig. 1). The fjord itself is 26 km long and on average 8 km wide. The maximum depth of the fjord is 428 m with mean depth ≈140 m.

The hydrological conditions of the area are shaped by interactions of two water masses. The shelf waters of Kongsfjorden are influenced by relatively cold and fresh Arctic water masses, that originate from the east coast of Spitsbergen, rounding the Sørkapp (southern tip of Spitsbergen) and then flowing along the shelf of the west Spitsbergen coast. Warmer and more saline Atlantic waters, originating from the West Spitsbergen Current, transport a great amount of heat northward and thus keep the shelf waters of the fjord ice-free. In the inner part

Commented [PER14]: And local processes…glacial melt (and rivers?) are critical here and likely responsible for the reduced omega in sfc waters compared to deeper waters. Reading below it isn't clear what two masses you mean: the shelf water which is a mix plus glacial melt or Arctic and Atlantic?

Commented [PER15]: Ok but not just heat. More saline, what about CO2 content/alkalinity?

of Kongsfjorden three tidal glaciers are present (Kongsbreen, Kronebreen and Kongsvegen). In spring, the seawater conditions are shaped by melt water discharge contributing to the subsequent stratification of surface waters during summer (Svendsen et al. 2002; Halbach et al. 2019).

Primary production typically starts between April and May and the highest chlorophyll *a* concentrations are observed in the inner parts of the fjord (Hodal et al. 2012; Hegseth and Tverberg 2013), although suspended sediments at the glacier front cause light limitation that reduces primary production in July (Lydersen et al. 2014; Halbach et al. 2019).

In winter 2014, the seawater pH varied from 8.11 in the middle part to 8.13 in the inner part of Kongsfjorden, whereas calcite saturation states (Ω_c) were 2.73 and 2.61, respectively (Fransson et al. 2016). Summer values in 2014 differed in both, pH and Ω_c, showing pH at 8.28 and Ω_c at 4.00 in the middle part of the fjord, and 8.26 and 3.68 in the inner part of Kongsfjorden (Iglikowska et al. 2017).

Commented [PER16]: Yes, but some outer stations may be affected by Krossfjorden glaciers (or even PKF glaciers?)

Commented [PER17]: And also characteristics of these two layers

Commented [PER18]: The Hegseth et all chapter in Hakon's Kongsfjorden book will tell you when the bloom took place inthe relevant years. From that paper it seems there is no 'typical' year.

Commented [PER19]: Both what? Variabiles or locations in Kongsfjorden

Species

For the purpose of this study, five encrusting cheilostomatous bryozoan species were selected that are abundant and widely distributed inhabitants of Arctic seas, including the study area. These are *Escharelloides spinulifera* (Hincks, 1889) (Fig. 2A and B); *Microporella klugei* Kuklinski & Taylor, 2008 (Fig. 2C and D); *Myriozella plana* Dawson, 1859; *Stomacrustula pachystega* (Kluge, 1929) (Fig. 2G and H); *Smittina bella* (Busk, 1860) (Fig. 2I and J). In the study area these species occur across the investigated depth range (Kuklinski 2002, Kuklinski et al. 2005). The species mostly occur on rocks, yet they also grow on living and dead mollusc shells (Kluge, 1975; Kuklinski and Barnes 2005).

Sampling, mineralogical analysis and environmental data

Samples of the five targeted bryozoan species were collected in Kongsfjorden using a bottom dredge during winter cruise of RV *Helmer Hanssen* and summer cruise of RV *Oceania*. Sampling was carried out in January 2014, August 2014 and January 2015 (Table 1, Fig. 1).

On-board the vessel, samples were sorted and then transported dry to the laboratory of Institute of Oceanology in Poland. In the laboratory, bryozoan colonies were identified with use of stereomicroscope to species level. To confirm taxonomic identification selected species were bleached in sodium hypochlorite and then examined using scanning electron microscope at the Natural History Museum in London. All studied specimens were dissected off rocks and shells using scalpel and care was taken to separate the bryozoan from the substrate and

epibionts. Nevertheless, in some calcitic bryozoan species, aragonite contamination was detected likely originating from aragonitic material of molluscs' shell substrate. Only 5 mm of the colony edge of each individual was taken for mineralogical analysis in order to ensure that the most recent growth was sampled.

Mineralogical analyses were conducted using high-precision Enraf-Nonius XRD with a position-sensitive detector (PSD). Operating conditions of a cobalt X-ray source were 40 kV and 40 mA. During each measurement, the sample was rotated to improve the grain orientations randomness in the X-ray beam. All bryozoan skeletons were powdered using an agate mortar and pestle and then affixed with a drop of acetone on a single quartz crystal substrate. Subsequently, samples were analysed to confirm their calcitic mineralogy and to determine the content of magnesium in the form of $MgCO_3$ (mol%). Concentration of $MgCO_3$ in skeletal calcite was calculated by measuring the d_{104} peak position. It is assumed that there is a linear correlation between d_{104} and $MgCO_3$ content existing between 0 and 17 mol% $MgCO_3$ in calcite (e.g., Mackenzie et al. 1983), and all records in this study fall within this range.

Seawater calcite saturation states for all the sampled stations during winter and summer were gathered from literature (Fransson et al. 2016; Iglikowska et al. 2017), as listed in the Table 1, but also expressed as mean values ± standard error along sampled seasons and depths in Figures.

Commented [PER20]: Do all species grow in winter such that you know that the outer 5mm in summer is from summer growth and the outer 5mm in winter is from winter growth?

Statistics and data analysis

We examined variation in skeletal $MgCO_3$ content using permutational multivariate analysis of variance (PERMANOVA) carried out with 9999 random permutations. The univariate PERMANOVA design was performed based on untransformed data and Euclidean resemblance matrix. We used 'season' and 'depth' as two fixed factors, with two levels (winter and summer) and three levels (50, 100 and 150 m), respectively. Because we found statistically significant differences in skeletal $MgCO_3$ between studied species (Kruskal-Wallis ANOVA and Dunn post-hoc test) the PERMANOVA analysis was conducted for each species separately. All graphical displays and numerical analyses were carried out using STATISTICA 12 (Statsoft Inc. 2014), PRIMER (version 6.1.13) and PERMANOVA (version 1.0.3) software packages.

Commented [PER21]: Why permanova? This is a more or less univariate analysis withthe response variable being Mg content. Couldn't a regular ANOVA be used? And inTable 2 it says you used log-transformed data. Why? Did it do what it was meant to do?

Results

Commented [PER22]: The repetitive format of the 5 paragraphs on each species is boring to read and does not add much (except your attempts to slightly change words here and there).Are max and min values all that interesting (really no context to say whether there is somehting interesting inthe ranges). I thtink these can be condensed into 1-2 paragraphs.

The content of $MgCO_3$ determined for 232 bryozoan specimens in five selected species ranged from 4.7 to 12.4 mol%, with mean at 7.0 mol% ± 1.19 (SD). The lowest $MgCO_3$ value

was observed in *Myriozella plana* specimen collected in January from the station 1W at 50 m depth, whereas the highest content was found in *Stomacrustula pachystega* taken in January from 10W station at 100 m depth. *Escharelloides spinulifera*, *Microporella klugei* and *M. plana* comprised mean $MgCO_3$ mol% skeletal values of 7.3 ± 0.55 (± SD), 6.7 ± 0.71 and 5.7 ± 0.57, respectively. Skeletons of remaining species, *S. pachystega* and *Smittina bella*, consisted mean mol% $MgCO_3$ concentration of 8.1 ± 0.90 and 8.4 ± 0.79, respectively.

Escharelloides spinulifera was collected from eight winter (1-3W and 10-14W) and five summer stations (5-9S, Table 1, Fig. 1). The minimum $MgCO_3$ content for this species (6.0 mol%, Appendix) was found in winter station 1W at 50 m depth, whereas the maximum value (9.2 mol%) was observed also in winter station (10W), but at a depth 100 m. *E. spinulifera* skeletons did not differ statistically significantly in $MgCO_3$ content between depths or between seasons (Table 2, Fig 3).

Commented [PER23]: What about other depths? How is there a depth comparison between depths as indicated below (...content between depths and between seasons)?

Microporella klugei individuals were sampled from six winter stations (1-2W and 11-14W) and one summer station (8S, Table 1, Fig. 1). The lowest $MgCO_3$ value (5.5 mol%) in skeletons was observed in 1W winter station at 50 m depth, while the highest concentration (8.6 mol%) was found in 13W winter stations at 100 m depth. Similarly to *E. spinulifera*, no statistical differences in $MgCO_3$ concentrations were observed between depths and seasons (Table 2, Fig. 4).

Commented [PER24]: Can you even test for season with one summer station?

Myriozella plana was collected in both, winter (1-3W and 10-14W) and summer stations (4-9S, Table 1, Fig. 1), and the minimum $MgCO_3$ contents (4.8 mol%) were noted in a specimen from 1W winter station at 50 m depth, whereas the maximum concentration (7.4 mol%) was recorded at 14W winter station at 150 m depth. In *M. plana* we did not find statistically significant differences in $MgCO_3$ values between depths and seasons separately, though interactions between those two variables appeared to be statistically significant (Table 2, Fig. 5).

Commented [PER25]: Not tested?

The individuals of *Stomacrustula pachystega* were collected from six winter stations (1-2W, 10-11W and 13-14W) and three summer stations (4-6S, Table 1, Fig. 1). The lowest $MgCO_3$ content (6.3 mol%) was recorded in a specimen from 2W winter station at 50 m depth, and the highest value (12.5 mol%) was observed in *S. pachystega* collected at 10W winter station (100 m depth). No statistical differences in $MgCO_3$ concentrations were found neither between depths nor between seasons (Table 2, Fig 6).

Smittina bella was taken from winter (10-11W and 13W) and summer stations (5-6S and 8S, Table 1, Fig. 1). The $MgCO_3$ content in *S. bella* ranged from 7.1 mol% in an individual collected in 6S summer station to 10.7 mol% observed in a specimen from another summer

station (8S), and both extreme values were noted at 100 m depth. In *S. bella* we could not find any statistically significant differences in $MgCO_3$ concentration between depths and seasons (Table 2, Fig. 7).

Literature data for seawater calcite saturation states (Ω_c) showed higher values for summer season (mean 3.28 ± 0.48 SD) than for winter (2.57 ± 0.07) (Fig. 8) and differences were statistically significant (Mann-Whitney U = 1.00, $p < 0.01$). However, differences in seawater calcite saturation states between studied depths appeared to be insignificant (Kruskal-Wallis H = 0.27, p = 0.87), with mean values for 50, 100 and 150 m at 2.78 (± 0.34), 2.68 (± 0.21) and 2.66 (± 0.18), respectively (Fig. 9).

Commented [PER26]: Since this is the basis for perhaps expecting differences in Mg content, maybe this shoudl appear first in the results.

Commented [HH27R26]: Agree – move to first in Results.

Discussion

Skeletal $MgCO_3$ concentration did not differ significantly between seasons or among depths for any of the five species studied Only one species (*Myriozella plana*) showed a statistically significant interaction effect between season and depth on $MgCO_3$ content (Table 2). Summer calcite saturation state (Ω_c) values showed slightly higher values comparing to winter Ω_c, yet overall both were saturated with respect to $CaCO_3$ during summer and winter. The lack of differences between summer and winter $MgCO_3$ levels in skeletons of bryozoans may indicate that Arctic bryozoans have physiological mechanisms which enable them to regulate $MgCO_3$ content regardless of observed, although small, differences in ambient seawater Ω_c. Other invertebrates, such as corals possess ion pumps controlling precipitation of the carbonate lattice through creation of favorable conditions for crystal nucleation and growth (e.g., Dove et al. 2003; Tambutté et al. 2011; Zoccola et al. 2015). Such compensatory ion pumps allow the organism to maintain the deposition of skeletal calcite even in undersaturated seawater conditions, although this mechanism may consume more energy (e.g., Wood et al. 2008; Dupont et al. 2013). If such mechanism is used by Arctic bryozoans to maintain the certain range of $MgCO_3$ in their skeletons against changing ambient environment it would be clear sign of biological control of that process.

Commented [PER28]: Again, maybe stat with this since it is the premise behind studying MgCO3 content

Commented [PER29]: Well, within the range studied, not completely regardless

Commented [PER30]: Surely something must be known about the mechanisms of calcification in bryozoans

Commented [PER31]: Is that different from adaptation?

Also, genetic adaptation and ecophenotypic response are quite different: one programmed and one quite flexible. What are the consequences of this? What does Mg content do for the bryozoan? Is there a tradeoff of some sort? Higher Mg content seems to place the organism in greater risk of shell disolution? Then why ever have (high) Mg?

I am missing some ecological background for this minerology and that could be important in evluating why these species appear to exhibit homeostasis in Mg content.

Kukliński and Taylor (2009) suggested that the level of $MgCO_3$ content and skeletal mineralogy in bryozoans should be considered as genetic preadaptation or ecophenotypic response for particular environmental conditions (e.g., given temperature range). Similarly, bryozoan species inhabiting different depths or growing during a particular season were expected to show geochemical variability, in our case different $MgCO_3$ concentration, related to environmental characteristics for that depth or season. However, we found no depth or

Commented [PER32]: Thus?

Commented [PER33]: See comment above. How do you know the last 5mm represent conditions of the season when they were collected?

season related differences in Mg-level of studied Arctic bryozoans. Other studies evaluating correlation between depth and skeletal $MgCO_3$ of bryozoans (Borszcz et al. 2013; Figuerola et al. 2015) also failed to find such relationship, despite examining a greater range of depths therefore greater environmental differences (up to 600 m in Figuerola et al. 2015). Borszcz et al. (2013) suggested that a historically young Arctic bryozoan community could have had too short time to develop a genetic adaptation to variable chemical seawater conditions related to depth. The Arctic is believed to have been colonized by benthic organisms from lower latitudes after the last glaciation ~ 14 000 years ago (Dunton 1992). Glaciation covering vast areas of the shelf likely eradicated the majority of benthic fauna at the time. Yet the potential ancestors of today's Arctic benthos from lower temperate areas or polar areas extended to lower latitudes had to experience the same water temperature variability or higher at that time. Therefore the argument for lack of genetic adaptations to variable chemical seawater conditions related to depth or seasons by species inhabiting Arctic today should be reviewed.

The other possibility which may explain lack of any clear pattern in $MgCO_3$ deposition in skeletons of bryozoans collected during polar summer and winter is that there is too low a difference in Ω_c thus pH, water temperature, salinity which would not lead to generate seasonal differences in $MgCO_3$ concentration. It is believed that factors such as Water temperature influences $MgCO_3$ concentration in bryozoan skeletons; increased water temperature leads to increased $MgCO_3$ concentration (Kuklinski and Tayor 2009; Krzemińska et al. 2016). Such a pattern was observed on the broad latitudinal scale and on the local scale of one sea basin (Kuklinski and Tayor 2009; Krzemińska et al. 2016). In our study area, water temperature during sampling showed an overall range between 0.39-5.20°C (Table 1). Yet the observed temperature variability does not seem to correlate with depth gradient. However, it is worth mentioning that our data were just snapshot measurement and do not exhibit the entire annual trend. Long-term literature data for the study area seem to confirm both seasonal and depth-related differences in water temperature (Tverberg et al. 2019). The difference between surface waters of summer and winter could be in the range of up to 7°C while differences associated with depth between 2 to 3°C (Kwaśniewski et al. 2010; Holmes et al. 2019; Tverberg et al. 2019). Such a limited variability in seawater temperature may simply not affect Mg-levels in their skeletons of Arctic calcifiers. On the other hand, Schäfer and Bader (2008) reported higher concentrations of Mg in skeletons of the temperate bryozoan *Cellaria sinuosa* formed during summer compared to parts of the skeleton formed during winter. Schäfer and Bader (2008) concluded that the likely factors responsible for observed changes were those related to water temperature. The difference in water bottom temperature between summer and winter of study

Commented [PER34]: So what is novel about this study? I miss this somewhere. One coudl argue that if a broader study found no differnces why should we expect there to be differences in this study. Can you address that?

Commented [PER35]: This is unclear. Not clear what you are stating here and int he following sentence

Commented [HH36]: Well, they are there in spite of genetic adaptations, which probably does not exist?

Commented [PER37]: Necessary? Seems ot confuse the meaning here. What leads to Mg content? Omega? pH, salinity? Temp?

Is it just substituted since Mg concentration is much higher than Ca and teh Mg ion fits in the lattice?

Commented [PER38]: ? should it? In what way+ I would think the depth pattern may be differnt in summer vs winter

Commented [PER39]: But Melissa and Agneta have sampled seasonally, haven't they?

Commented [PER40]: unclear

Commented [PER41]: any idea why? Is this a thermodyanmic issue?

area of Schäfer and Bader (2008) was 5.8°C (from 9.6°C to 15.4°C). This temperature range is similar to that which were experienced by the studied Arctic species. It might imply that control of $MgCO_3$ concentration in the skeleton is species-specific. And indeed, the concentration of $MgCO_3$ in skeletons for each of the studied species of bryozoan collected in this study under the same environmental conditions is indication of such specificity.

Some of the examined stations were spaced between each other at least by a few kilometers which resulted in differences in local seawater properties, such as water temperature, carbonate saturation state and/or availability of Mg ions among these sites (Table 1). Locations closer to the open sea (Fig. 1) were characterized by higher water temperature, lower CO_2 and higher CO_3^{2-}, comparing to located inside the fjord and closer to glacier stations (Table 1). Yet even bryozoan skeletal samples from the same depth, collected in close vicinity to each other and influenced by similar environmental conditions exhibited differences in $MgCO_3$ concentration in their skeletons (Figs. 3 to 7). This result indicates a potential complexity of factors influencing $MgCO_3$ incorporation into bryozoan skeletons. The differences in $MgCO_3$ concentration in skeletons among sites differing in physico-chemical parameters of sea water might be a sign of environmental control. While variability in $MgCO_3$ concentration in skeletons within sites characterized by the same environmental conditions indicate physiological control on the process of calcification.

Overall it is difficult to determine unequivocally whether lack of $MgCO_3$ concentration differences with season or depth is a result of favorable environmental conditions or an effect of physiological regulation by the organism. On the other hand, it is still under debate whether calcite saturation state is an appropriate measure of conditions enabling deposition of high-Mg calcite skeletons, because it does not account for magnesium concentration of calcitic lattice influencing its solubility. Thus, instead of Ω_c, species-specific $\Omega_{Mg\text{-}c}$ is proposed to reflect more accurately seawater chemical conditions regarding high-Mg calcite deposition (more details in: Lebrato et al. 2016).

In conclusion both of the hypothesis tested by this study: (1) there is a difference in Mg content in organisms' skeleton which grew during polar day and polar night which is indication of environmental control of that skeletal parameters, (2) as water column will be stratified when it comes to carbonates during polar day there will be a difference in Mg content in organisms skeleton which grew in that period which is again indication of environmental control of that skeletal parameters, have been rejected. Our results indicate that environmental effects on skeletal chemistry can be masked by various biological processes. There is little doubt that the level of $MgCO_3$ in skeletons of each of the studied species of bryozoan is an indication of

Commented [HH42]: Probably not, since you looked at 5 species and found the same, non-significant answer. Maybe some latitudinal difference?

Commented [HH43]: Don't think you can conclude this based on these findings.

Commented [PER44]: Yes, the interspecies variability is only about 1(-2)% for each species. This would imply little chance of environmentally driven plasticity (maybe).

I would also at least acknowledge that the env characterisitc measured are in open water whereas the conditions in the boundary layer where the skeleton is califying may be quite different due to restricted diffusion (controlled or not controlled by the bryozoan itself) This has beenshown to be important for bivalve calcification (although the calc process is likely quite different in bivalves as it is quite dependent on mantle fluid characterisitcs)

Commented [PER45]: Do we think this is the case? How variable is that?

Commented [HH46]:

Commented [PER47]: This sounds like a take home message and I htink it should be developed a bit more clearly and logically

Commented [HH48]: Could be an explanation, but it is justified based on these data?

Commented [HH49]: Some of this, on one-hand – on the other hand, can be cut. The Discussion is too long for these non-significant findings. It should probably be shortened substantially (i.e. by at least 30%) and focussed on interpretations of data findings, rather than discussion on how difficult it is to deternine any differences with regard environmental effects.

Commented [HH50]: Was this clear based on the results?

Commented [PER51]: Including relatively inflexible, preprogrammed physiochemical pathways resulting in Mg content across a relatively narrow range per species

biological controlled, genetically programmed processes. Although obtained results on station-related differences suggest that seawater properties can also to some extent affect the level of $MgCO_3$ in skeletal calcite of Arctic bryozoans. Thus, it is likely that observed skeletal chemistry is shaped by both, biological and environmental factors to a different degree, as was also suggested by other authors (e.g., Taylor et al. 2014; Figuerola et al. 2015). This study indicates the complexity of factors influencing incorporation of $MgCO_3$ into biota skeletons, showing that field studies might be not sufficient to pinpoint the particular factors influencing properties of skeletons and detailed experimental studies are needed to shed more light on the subject.

Commented [HH52]: How you know that it is genetically programmed?
Maybe it is just a physiologically-controlled process?

Commented [PER53]: Not a sentence...and the results indicate that this effect is minimal at best (and certainly not significant in your data)

Commented [HH54]: You should focus the discussion around the main findings, and this was not one of them..

Acknowledgments

We would like to thank Mikołaj Mazurkiewicz, Kajetan Deja, Maria Włodarska-Kowalczuk and Joanna Legeżyńska for sample collection during winter champagnes. The funding for this study was received from to the Norwegian Research Council through Marine Night (NRC 226417) project and FRAM-High North Research Centre for Climate and Environment Flagship program Ocean acidification and ecosystem effects in Northern Waters. The study was commenced during the projects funded by PANIC project (grant no. 2016/23/B/ST10/01936) funded by the National Science Centre, Poland and funds from Ministry of Science and Higher Education, Poland (3991/CERD/2018/0).

References

Andersson, A. J., Kuffner, I. B., Mackenzie, F. T., Jokiel, P. L., Rodgers, K. S., Tan, A. 2009. Net loss of $CaCO_3$ from a subtropical calcifying community due to seawater acidification: mesocosm-scale experimental evidence. Biogeosciences, 6: 1811-1823.

Andersson, A. J., Mackenzie, F. T., Bates, N. R. 2008. Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. Marine Ecology Progress Series, 373: 265-273.

Aranha, R., Edinger, E., Layne, G., Piercey, G. 2014. Growth rate variation and potential paleoceanographic proxies in Primnoa pacifica: Insights from high-resolution trace element microanalysis. Deep Sea Research Part II, 99: 213-226.

Borszcz et al. 2013

Chierici, M., Fransson, A., Lansard, B., Miller, L. A., Mucci, A., Shadwick, E., Thomas, H., Tremblay, J.-E., Papakyriakou, T. N. 2011. Impact of biogeochemical processes and environmental factors on the calcium carbonate saturation state in the Circumpolar

Flaw Lead in the Amundsen Gulf, Arctic Ocean. Journal of Geophysical Research: Oceans, 116(C9).

Dove et al. 2003

Dunton, K.H. 1992. Arctic biogeography: the paradox of the marine benthic fauna and flora. Trends in Ecology and Evolution, 7: 183–189.

Dupont et al. 2013

Feely, R. A., Doney, S. C., Cooley, S. R. 2009. Ocean acidification: Present conditions and future changes in a high-CO_2 world. Oceanography, 22: 36-47.

Figuerola et al. 2015

Fransson, A., Chierici, M., Hop, H., Findlay, H. S., Kristiansen, S., Wold, A. 2016. Late winter-to-summer change in ocean acidification state in Kongsfjorden, with implications for calcifying organisms. Polar Biology, 39: 1841-1857.

Fransson, A., Chierici, M., Miller, L. A., Carnat, G., Shadwick, E., Thomas, H., Pineault, S., Papakyriakou, T. N. 2013. Impact of sea-ice processes on the carbonate system and ocean acidification at the ice-water interface of the Amundsen Gulf, Arctic Ocean. Journal of Geophysical Research: Oceans, 118: 7001-7023.

Halbach, L., Vihtakari, M., Duarte, P., Everett, A., Granskog, M. A., Hop, H., Kauko, H. M., Kristiansen, S., Myhre, P. I., Pavlov, A. K., Pramanik, A., Tatarek, A., Torsvik, T., Wiktor, J. M., Wold, A., Wulff, A., Steen, H., Assmy, P. 2019. Tidewater glaciers and bedrock characteristics control the phytoplankton growth environment in a fjord in the Arctic. Frontiers in Marine Science, 6.254. doi: 10.3389/fmars.2019.00254Unsp 25410.3389/Fmars.2019.00254

Hegseth, E. N., Tverberg, V. 2013. Effect of Atlantic water inflow on timing of the phytoplankton spring bloom in a high Arctic fjord (Kongsfjorden, Svalbard). Journal of Marine Systems, 113: 94-105.

Hill, V., Cota, G. 2005. Spatial patterns of primary production on the shelf, slope and basin of the Western Arctic in 2002. Deep Sea Research Part II, 52: 3344-3354.

Hodal, H., Falk-Petersen, S., Hop, H., Kristiansen, S., Reigstad, M. 2012. Spring bloom dynamics in Kongsfjorden, Svalbard: nutrients, phytoplankton, protozoans and primary production. Polar Biology, 35: 191-203.

Holmes FA, Kirchner N, Kuttenkeuler J, Krützfeldt J, Noormets R. 2019. Relating ocean temperatures to frontal ablation rates at Svalbard tidewater glaciers: Insights from glacier proximal datasets. Scientific Reports, 9:9442.

Iglikowska, A., Bełdowski, J., Chełchowski, M., Chierici, M., Kędra, M., Przytarska, J., Sowa, A., Kukliński, P. 2017. Chemical composition of two mineralogically contrasting Arctic bivalves' shells and their relationships to environmental variables. Marine Pollution Bulletin, 114: 903-916.

Kluge, G. A. 1975. Bryozoa of the northern seas of the USSR. Amerind Publishing Co, New Delhi.

Krzemińska, M., Kukliński P., Iglikowska A., Najorka J., 2016. Skeletal mineralogy patterns of Antarctic bryozoans. The Journal of Geology, 124:411-422.

Kuklinski, P. 2002. Fauna of bryozoans in Kongsfjorden, West Spitsbergen. Polish Polar Research, 23: 193-206.

Kuklinski, P., Barnes D.K.A. 2005. Microhabitat diversity of Svalbard Bryozoa. Journal of Natural History, 39: 539-554.

Kuklinski, P., Gulliksen, B., Lønne, O.J., Weslawski, J.M. 2005. Composition of bryozoan assemblages related to depth in Svalbard fjords and sounds. Polar Biology, 28: 619-630.

Kuklinski, P., Taylor, P. D. 2008. Arctic species of the cheilostome bryozoan *Microporella*, with a redescription of the type species. Journal of Natural History, 42: 1893-1906.

Kuklinski, P., Taylor, P. D. 2009. Mineralogy of Arctic bryozoan skeletons in a global context. Facies, 55: 489-500.

Kwaśniewski et al. 2010

Lebrato et al. 2016

Lydersen, C., Assmy, P., Falk-Petersen, S., Kohler, J., Kovacs, K. M., Reigstad, M., Steen, H., Strøm, H., Sundfjord, A., Varpe, Ø., Walczowski, W., Weslawski, J.M., Zajaczkowski, M. 2014. The importance of tidewater glaciers for marine mammals and seabirds in Svalbard, Norway. Journal of Marine Systems, 129: 452-471.

Mackenzie, F.T., Bischoff, W.D., Bishop, F.C., Loijens, M., Schoonmaker, J., Wollast, R. 1983. Magnesian calcites; low-temperature occurrence, solubility and solid-solution behavior. Reviews in Mineral Geochemistry 11:97–144

Mollica, N. R., Guo, W., Cohen, A. L., Huang, K. F., Foster, G. L., Donald, H. K., Solow, A. R. 2018. Ocean acidification affects coral growth by reducing skeletal density. Proceedings of the National Academy of Sciences, 115: 1754-1759.

Morse, J. W., Andersson, A. J., Mackenzie, F. T. 2006. Initial responses of carbonate-rich shelf sediments to rising atmospheric pCO_2 and “ocean acidification”: Role of high Mg-calcites. Geochimica et Cosmochimica Acta, 70: 5814-5830.

Piepenburg, D. 2005. Recent research on Arctic benthos: common notions need to be revised. Polar Biology, 28: 733-755.

Piwosz, K., Walkusz, W., Hapter, R., Wieczorek, P., Hop, H., Wiktor, J. 2009. Comparison of productivity and phytoplankton in a warm (Kongsfjorden) and a cold (Hornsund) Spitsbergen fjord in mid-summer 2002. Polar Biology, 32: 549-559.

Schäfer, P., Bader, B. 2008. Geochemical composition and variability in the skeleton of the bryozoan *Cellaria sinuosa* (Hassall): biological versus environmental control. In: Proceedings of the14th International Bryozoology Association conference. Martinsville, Virginia: Virginia Museum of Natural History Publications, pp. 269-279.

Shadwick, E. H., Thomas, H., Chierici, M., Else, B., Fransson, A., Michel, C., Miller, L. A., Mucci, A., Niemi, A., Papakyriakou, T. N., Tremblay, J. É. 2011. Seasonal variability of the inorganic carbon system in the Amundsen Gulf region of the southeastern Beaufort Sea. Limnology and Oceanography, 56: 303-322.

Smetacek, V., Nicol, S. 2005. Polar ocean ecosystems in a changing world. Nature, 437: 362.

Smith, A. M., Key Jr, M. M., Gordon, D. P. 2006. Skeletal mineralogy of bryozoans: taxonomic and temporal patterns. Earth-Science Reviews, 78: 287-306.

Stanley, S. M. 2006. Influence of seawater chemistry on biomineralization throughout Phanerozoic time: Paleontological and experimental evidence. Palaeogeography, Palaeoclimatology, Palaeoecology, 232: 214-236.

STATISTICA software, version 12 (Statsoft Inc. USA) available at: http://www.statsoft.com

Svendsen, H., Beszczynska-Møller, A., Hagen, J. O., Lefauconnier, B., Tverberg, V., Gerland, S., Orbaek, J.B., Bischof, K., Papucci, C., Zajączkowski, M., Azzolini, R., Bruland, O., Wiencke, C., Winther, J.B., Dallmann, W. 2002. The physical environment of Kongsfjorden–Krossfjorden, an Arctic fjord system in Svalbard. Polar Research, 21: 133-166.

Tambutté, S., Holcomb, M., Ferrier-Pagès, C., Reynaud, S., Tambutté, É., Zoccola, D., Allemand, D. 2011. Coral biomineralization: from the gene to the environment. Journal of Experimental Marine Biology and Ecology, 408: 58-78.

Taylor, P. D., James, N. P., Bone, Y., Kuklinski, P., Kyser, T. K. 2009. Evolving mineralogy of cheilostome bryozoans. Palaios, 24: 440-452.

Tverberg V. et al. (2019) The Kongsfjorden Transect: Seasonal and Inter-annual Variability in Hydrography. In: Hop H., Wiencke C. (eds) The Ecosystem of Kongsfjorden, Svalbard. Advances in Polar Ecology, vol 2. Springer, Cham, pp. 49-104.

Wood et al. 2008

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Zoccola, D., Ganot, P., Bertucci, A., Caminiti-Segonds, N., Techer, N., Voolstra, C. R., Aranda, M., Tambutté, E., Allemand, D., Casey, J.R., Tambutté, S. 2015. Bicarbonate transporters in corals point towards a key step in the evolution of cnidarian calcification. Scientific Reports, 5: 9983.

Figure captions

Figure 1. Study area with sampling locations and site names identified.

Figure 2. Scanning electron microscope images of studied cheilostomatous bryozoan species. *Escharelloides spinulifera* A-B, *Microporella klugei* C-D, *Myriozella plana* E-F, *Stomacrustula pachystega* G-H, *Smittina bella* I-J.

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Blomstrandoya = Blomstrandhalvøya
Lovénøyane
Fram Strait, rather than Greenland Sea
Ny-Ålesund

Figure 3. Summer and winter $MgCO_3$ concentrations (mean ± standard error) in skeletal calcite of *Escharelloides spinulifera*, comparison between three examined depths (50, 100 and 150 m) (N – number of individuals analyzed).

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Turn x-axis labels (180)
(same for Figs. 4-7)

Figure 4. Summer and winter $MgCO_3$ concentrations (mean ± standard error) in skeletal calcite of *Microporella klugei*, comparison between three examined depths (50, 100 and 150 m) (N – number of individuals analyzed).

Figure 5. Summer and winter $MgCO_3$ concentrations (mean ± standard error) in skeletal calcite of *Myriozella plana*, comparison between three examined depths (50, 100 and 150 m) (N – number of individuals analyzed).

Figure 6. Summer and winter $MgCO_3$ concentrations (mean ± standard error) in skeletal calcite of *Stomacrustula pachystega*, comparison between three examined depths (50, 100 and 150 m) (N – number of individuals analyzed).

Figure 7. Summer and winter $MgCO_3$ concentrations (mean ± standard error) in skeletal calcite of *Smittina bella*, comparison between two examined depths (100 and 150 m) (N – number of individuals analyzed).

Figure 8. Mean values (± standard error) of seawater calcite saturation states at studied stations – comparison between winter and summer values (data from Fransson et al. 2016 and Iglikowska et al. 2017).

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Figure 9. Mean values (± standard error) of seawater calcite saturation states at studied stations – comparison between depths (data from Fransson et al. 2016 and Iglikowska et al. 2017).

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Depth (m)
You can combine Figs. 9 and 10 within same frame, since they have the same y-axis.