Dear Dr. Kitazato,

We are pleased to have received positive reviews on our manuscript "Effects of light and temperature on Mg uptake, growth, and calcification in the proxy climate archive *Clathromorphum compactum*" for publication in Biogeosciences, Manuscript #:bg-2018-128. We thank the reviewers and editors for their helpful reviews and comments. Below we have outlined in detail how we have addressed each of the reviewers' comments. Please let us know if you need additional clarification.

Regards,

Siobhan Williams

Siobhan Williams and Co-authors

Dear Dr. Williams and co-authors,

I am pleased to inform you that your article will be proceeding to BG Discussion. Please enjoy to discuss with audiences and reviewers.

There is one comment from a reviewer.

Lux was measured rather than PAR to assess the influence of light. Is lux representative of light differences that affect the algae?

I think you should consider how to answer this question. Answer should be done when you shall have made revision according to reviewers and audiences discussions.

Best wishes,

Hiroshi Kitazato

The issue of LUX has been addressed in the reviewers' comments below.

Anonymous Referee #1
Received and published: 12 June 2018

This is an interesting study on the influencing variables within the calcification process of *Clathromorphum compactum*, a coralline red alga that is well known for its usability as a temperature proxy from various studies. Because of its longevity, it appears as a very suitable organism for the reconstruction of the past climate, even extending historical times. Moreover, the organism was also successfully used for the reconstruction of sea ice cover.

Against this background, it is very important that the function as a proxy of *C. compactum* is really reliable. In this regard, the study is a very good approach in evaluating the different effects of light and temperature and also the combination of these variables. The methods are scientifically sound and I like the circumstance that the experiment was carried out over a comparatively long time interval and under quite realistic conditions, including the presence of the grazing chitons.

I consider the methods and results substantial and also the discussion of the future implications for the use of *C. compactum* as a temperature proxy is reflected and constructive. However, I miss the discussion of the results in view of the former studies which use *C. compactum* as a temperature and sea ice proxy, especially the (cited) studies by Halfar et al and Hetzinger et al. Are the results of these studies still sound? I think this should be discussed in greater detail.

We have added a paragraph to our Implications for proxy section addressing this:

While the findings of this experiment should inform the use of replication and calibration of individual samples to improve the use of C. compactum as a climate proxy, it does not discount the results of past environmental reconstructions using this species. For example, Williams et al. (2014) used Clathromorphum sp. to reconstruct past temperature and tested the calibration between Mg/Ca and instrumental temperature of each sampling location. Also, Halfar et al. (2013) combined annual growth and Mg/Ca concentrations from C. compactum to reconstruct sea ice conditions, based on the assumption that these records respond to both

light and temperature. While the influence of both light and temperature on growth rates of several species of corallines has already been demonstrated (Adey, 1970), their influences on Mg have now also been confirmed. Based on our findings, the results of past studies would only be untrustworthy if a calibration from another study or location was used to convert Mg/Ca to temperature without confirming this relationship.

Additionally to this, I made some specific comments throughout the manuscript, which is attached to this review. Especially, I would be interested in more information of the production of starch also in the wound tissue, just as I mention it within my remarks.

We have addressed this in the general remarks.

Altogether, I consider this study a valuable contribution to our understanding of *C. compactum* as a climate archive and recommend it for publication in Biogeosciences after a minor revision.

Details:
P5 L29: this sounds like 1,000 or 1,500 hours, respectively, but I presume it means time of day. Maybe you choose another format to avoid misunderstanding.

We have changed the formatting to 10:00 and 15:00.

P6 L31: does this mean one specimen per zone per tank? Please do also specify how the "random" collection was carried out.

We specified that the collection was haphazard rather than random, and further explained our collection here:

On the first of each month, beginning with October 1, individual specimens were haphazardly collected from each light zone of each tank with the intention of leaving the remaining specimens evenly distributed over the zone space so as not to bias in-zone distribution with time.

P8 L27-29: However, grazing as an external factor can also not be excluded for the other, "successfully" growing specimens. Therefore, I do not fully understand why the zeros have been excluded

We did not extensively monitor and tally grazer presence on each specimen. This is not a quantitative element of the experiment, so the zeros were excluded.

P10 L1-2: Is there any possibility that, additional to the difference of light and dark ambience, wound repair was slower in the second (dark) part of the experiment because the re-scarred specimens where somehow weakened by the previous treatment?

We have explained in P10 L26-32 that it is unlikely that the scarring affected growth rates or wound repair here:

In addition, it is unlikely that the experimental scarring affected later growth because the typical specimen used in the study was about 4 cm in diameter, while the scars were about 1

mm wide and 4 mm long. The entire photosynthetic surface of the typical specimen was about 1200 mm^2 and the scar less than 4 mm^2, about 1/400th of the surface. Since lateral translocation in the shallow coralline crust is widespread (Adey et al, 2018), it seems further unlikely that the scar would have affected growth following wound repair growth. In addition, the second scarring, typically placed about one cm from the initial scar, took place 6-9 months after the first, making it unlikely that the first scarring affected growth in the second, dark experiment.

P11 L10: remove "with wild growth"

We have removed this phrase.

P19 Figure 1: I would tissue refilling old conceptacles not call "wound tissue"

We have changed this label to say "tissue refilling old conceptacles".

P21 Figure 3: Dashed and black curves are interchanged within the figure legend

We have changed the labeling of the figure legend.

P22 Figure 5: percentages should not be in brackets

We have removed the brackets around "%".

P26 Figure 10: It is very interesting to see that whithin the light experiment, the wound tissue itself contains starch grains. From my point of view, this means that the alga is capable of producing new tissue and storing photosynthates at the same time. Starch grains are also evident in Fig. 11d, which is a dark experiment, but by far not as many as in the light experiment. It would be great if you could add a paragraph on this topic, also amended by your own observations from other SEM samples.

The production of starch as a storage food resulting from photosynthesis and its relationship to growth and calcification is certainly an interesting question. However, as we have discussed (above), growth and calcification are not directly connected, and their determination would require a far more complex experiment. We did not formally log the abundance of starch granules in our analysis, but we have added a mention of starch to section 3.1.1 "After the formation of several primordial wound repair cells, a new intercalary meristem forms, gradually returning the wound tissues to normal perithallial tissue, and starch grains as stored food are present in all stages of the experiment including in samples grown in the dark."

P30 Figure 14: Maybe you could add the standard errors to the data points

We have added standard error to the data points in this figure, and as suggested by the second reviewer, we have also removed panel b.

Anonymous Referee #2
Received and published: 24 July 2018

It is important to understand how different environment parameters affect proxy organism responses as this allows improvements in determining what signals the proxy is recording. This study investigates how light and temperature affect the growth and mineralogy of the coralline alga, *C. compactum*, which is used as a marine environmental proxy.

General comments: PAR vs lux: as alga will be responding mostly to the PAR, it is worth including some context of the relationships between lux and PAR in the context of this study. Maybe some PAR data are available that could be included from the field.

> *We don't have any PAR data from the field. We could have taken PAR more or less easily (with the right equipment) in the lab. In the field, it would have been difficult and costly. In any case, only measuring action spectra would have improved on our lux HOBO loggers, and that would be largely irrelevant to the current experiment.*

Wound recovery vs Mg-light relationships: of course, both of these are important, however, in the context of the title, at points they seem to overlap during the manuscript making it a little tricky to work out which question is being answered and if there is an interaction between the two (in terms of the algal responses). I wonder, if having two separate sections on these in the Results-Discussion would help with this. It will be important to clarify if the wound recovery material was also the material used for the Mg analyses.

> *We have addressed the interaction (statistically) between light and temperature, described in the details. We have also added a "Temperature" and a "Light" section to section 3.2 Effects of light and temperature on algal $MgCO_3$. In this way we have separated out some of the temperature and light results.*
>
> *We have added a clarification that wound recovery material was not analysed in section 2.4 "These high-resolution composite images were used to identify the first annual growth increment, and to select linear transects for geochemical analysis across the annual growth increment, encompassing the length of the experiment and avoiding the wound area of the samples (for details see Hetzinger et al., 2009)." We have also renamed two sections to also help clarify the separation between wound recovery material and Mg; "3.1.1 Wound recovery from scars as indicator for algal growth characteristics/cellular structure" and "4.1 Algal growth characteristics determined from wound recovery".*

Figures**:** there are probably too many figures and the MS would benefit from combining some and moving others to a supp material. This will allow the reader to focus in on the key results being presented. For example: Fig 5 is valuable to explain sampling strategy but could go in supp material also, are fig 10, 11 and 13 needed in the main manuscript or could portions of the three be combined? Results section: good to see detailed results, however, in some cases (see below) I think there is a little too much detail and the key points being made are lost.

> *As suggested, we have moved Figs 5 and 13 to the supplementary materials, but feel that the images shown in figures 10 and 11 (now 9 and 10) are important for the reader's understanding.*

Details:
P2 L9, I would call these mesocosms, applies throughout.

We have changed "microcosm" to "mesocosm" throughout the text.

P2 L9, replace word numerous with the exact number

We have replaced "numerous" with "123" in this sentence.

P2 L13-15, probably better as two sentences

We have split this sentence into two as such:

For specimens grown at low temperature (2°C), the effects of light are smaller, with a 1.4 mol % $MgCO_3$ increase from low [mean = 17 lux] to high light conditions [mean = 450 lux]. At higher (10°C) temperature there was a 1.8 mol% $MgCO_3$ increase from low to high light.

P2 L31, not yet been fully established

We have made this change so this sentence now reads "The precise mode of calcification in coralline algae has been long debated (Adey, 1998; Nash et al 2018, in press), and the role of photosynthesis in influencing calcification has not yet been fully established."

Paragraphs at P2 L30 and P4 L15, seem like they should be next to each other

We have moved the paragraph that used to be at P4 L15 to P3 L5, so those two paragraphs are now next to each other.

P4 L28, maybe summarise this paragraph to the key questions being asked, a little too much detail at present

We have removed detail from this paragraph, so it now reads:

It is clear that a better understanding of the effects of temperature and light (or lack thereof) on C. compactum growth, calcification, and elemental composition is necessary to fully understand C. compactum biology and ecology, and the use of this species as a climate archive. In this study, we examine multiple specimens of C. compactum, monitored at a range of light, temperature and time treatments in a suite of tanks having the same open coast source water supply. Post experiment, multiple samples were analyzed for their anatomical and cellular changes, growth, and $MgCO_3$ composition relative to the various treatments.

P4 L31, is there evidence that the scarring affected their mineralogic responses?

We did not geochemically analyse the samples near the region of the scars, but did not find a noticeable difference in our geochemical data at the beginning of the experiment, when the scars were made.

P5 L7, describe how the specimens were identified

We now describe the identification of samples here:

Clathromorphum species are highly distinctive and easily separated from other coralline species in the region by their surface features and deep intercalary meristem (Adey, 1965; Adey et al., 2015b). C. circumscriptum, the only other species of the genus in the northwestern Atlantic occurs primarily in shallow water, rarely reaching to the depth of specimen collection in this study, and at maturity is morphologically quite distinctive (Adey, 1965; Adey et al., 2013). As each collected specimen was delivered by divers to the dive boat for selection and initial scarring, it was identified aboard by co-author WHA. During the experiment, each individual specimen, as selected for analysis, was tagged with a number, after removal from the tanks, for tracking through analysis; the specimens are stored in the Smithsonian Institution National Museum of Natural History (NMNH) with those identification tags for future reference.

P5 L11, lux can be different to PAR, is there a difference at this site? Algae will respond more closely to PAR than lux so this should be expanded.

We have added a paragraph explaining of why we used lux rather than PAR here:

There were several reasons for not measuring photosynthetically active radiation (PAR) in this study. (1) Considerable pre-existing field evidence suggested that calcification in Clathromorphum (and other corallines) is not directly related to photosynthesis (Nash and Adey, 2017a), but rather only to the availability of stored photosynthate energy. (2) Being red algae, and having the accessory pigments phycocyanin and phycoerythrin that supplement chlorophyll, corallines have quite different action spectra from green algae or higher plants. Also, since these algae reach a peak of cover at about 20m, we were working with available light spectra that would be very different from that at the water surface. Using PAR sensors would have raised as many questions as it solved. (3) Since C. compactum occurs over a wide depth range (5-30 m), the level of photosynthesis varies widely and previous studies have not indicated major changes in growth and calcification over that range. Thus we would not have expected a strong relationship between our parameters of interest and light spectra; (4) Adding PAR, with or instead of Lux, especially since action spectra responses would have been an essential component requiring individual chambers for each specimen, would have significantly increased the difficulty and cost of carrying out the experiment.

P5 L25-28, please also give the PAR (if possible) gradient in the tanks including how this compares to the field.

Unfortunately, as described in the previous comment, we do not have PAR data.

P6 L10, this is reasonable.

P8 Section 3.1.1: good to see all the details, but I do think the main points are lost a little in all the information. I think the key results would stand out a little more if the section was shortened.

We have moved "Specimens lacking apparent recovery (zeros) were not included in the summation, since the possibility of grazing, as a factor external to growth, cannot be excluded." from this section to the caption of Table 4. We have also removed the sentences "Post-experiment specimen fracturing, not always easily controllable, was not successful in providing a useable scar groove in some cases. Of the 65 specimens providing sections with good cellular level views of the scar area, 41 specimens showed measurable regrowth after

scarring (Table 2. April 1, 2013). An SEM section of one of the occasionally deeper scars (~ 400 µm), taken after two months in the experimental tank, is shown in Fig. 9D. ", "This compares with the expected mean monthly growth rate of specimens at the Bay Bulls site of approximately 24 µm/month (Halfar et al., 2011b). Total normal perithallial growth during the experiment of the subjects harvested in July ranged from 58 µm to 182 µm (Fig. 11). " and "However, with no predators (especially small starfish) in the experimental system, chitons may well have been more active than in the wild."

P8 L22-25, could be moved to the discussion

We believe that this explanation is required here to give the reader context for the following sentences.

P10 L10, comment regarding how this was handled statistically in the methods section

We address this at the end of the Methods section:

There was only data for 3 temperature treatments for the medium light conditions, instead of the 4 temperature treatments for high and low light conditions. This was addressed through the degrees of freedom associated with specific ANOVA tests used to determine each p-value.

P10 section 3.2, maybe I misunderstood, but could multiple factors (light and temp) be included in the same analysis to account for any interactions? This may help frame the role of light more succinctly using interaction terms.

We have added the results of a two-way ANOVA test in this section:

In addition, there was no statistically significant interaction between light and temperature (tested with a 2-way ANOVA; $F_{(6,68)}=0.95$, $p=0.5$).

P30 & 31 fig legends, concentrations rather than values?

We have changed "values" or "levels" to "concentrations" in these legends.

P30 fig legend, could b and c be combined and present as mean +- SD?

We have combined B and C, and show standard error of the points as suggested by the first reviewer.

Effects of light and temperature on Mg uptake, growth, and calcification in the proxy climate archive *Clathromorphum compactum*

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Abstract. The shallow–marine benthic coralline alga *Clathromorphum compactum* is an important annual to sub-annual resolution archive of Arctic and Subarctic environmental conditions, allowing reconstructions going back >600 years. Both Mg content, in the high Mg-calcitic cell walls, and annual algal growth increments have been used as a proxy for past temperatures and sea ice conditions. The process of calcification in coralline algae has been debated widely, with no definitive conclusion about the role of light and photosynthesis in growth and calcification. Light received by algal specimens can vary with latitude, water depth, sea ice conditions, water turbidity, and shading. Furthermore, field calibration studies of *Clathromorphum* sp. have yielded geographically disparate correlations between $MgCO_3$ and sea surface temperature. The influence of other environmental controls, such as light, on Mg uptake and calcification has received little attention. We present results from an 11-month mesocosm experiment in which 123, wild-collected *C. compactum* specimens were grown in conditions simulating their natural habitat. Specimens grown for periods of one and two months in complete darkness show that the typical complex of anatomy and cell wall calcification develops in new tissue without the presence of light, demonstrating that calcification is metabolically-driven and not a side effect of photosynthesis. Also, we show that both light and temperature significantly affect $MgCO_3$ in *C. compactum* cell walls. For specimens grown at low temperature (2°C), the effects of light are smaller, with a 1.4 mol % $MgCO_3$ increase from low [mean = 17 lux] to high light conditions [mean = 450 lux]. At higher (10°C) temperature there was a 1.8 mol% $MgCO_3$ increase from low to high light. It is therefore concluded that site, and possibly specimen-specific, temperature calibrations must be applied, to account for effects of light when generating *Clathromorphum*-derived temperature calibrations.

1 Introduction

The coralline alga *Clathromorphum compactum* exhibits well-defined annual growth increments, and often produces seasonal conceptacles (seasonal autumn/winter reproductive structures) within those increments (Adey and Hayek, 2011; Halfar et al., 2008). The ovoid conceptacles are embedded within a considerably larger vegetative matrix consisting of a plethora of tissues (Adey, 1965, Adey et al., 2013) within calcified cell walls. The calcified cell walls additionally show a variety of well-defined micro-structures in their high-magnesium calcitic skeleton (Adey et al., 2005; Nash and Adey, 2017a, 2017b). Owing to *C. compactum* longevity, multicentennial chronologies that have the potential to provide data necessary for accurately calibrating climate models have been constructed (Adey, 1965, Adey et al., 2015a, 2015b; Halfar et al., 2013). *C. compactum* grows in Subarctic to high Arctic oceans in the benthic mid photic zone, and its distribution becomes severely limited at temperatures above 11-13°C (Adey, 1965).

The precise mode of calcification in coralline algae has been long debated (Adey, 1998; Nash et al 2018, in press), and the role of photosynthesis in influencing calcification has not yet been fully established. The shape of Mg content curves from Subarctic/Arctic *Clathromorphum* sp. suggests that they continue to grow and calcify for at least part of the winter in darkness at -1.8°C (Halfar et al., 2013). A recent model of coralline calcification (Nash and Adey, 2017b) shows fibrous cellulosic extrusions from the cell membranes into the cell wall environment that provide molecular initiation centres for

calcium and magnesium, but does not demonstrate a process for cellular injection of Ca and Mg. Their model indicates Mg control by temperature in a complex subset of calcification modes, but does not include a light component (other than as a basic photosynthetic requirement).

Production of *C. compactum*'s high magnesium calcite skeleton occurs in an intercalary meristem (as in the cambium of higher plants) and is concurrent with cellular and tissue growth (Fig. 1). The intercalary meristem, in addition to producing perithallium, the primary body of the calcified crust, also generates the thin upper layer of the epithallium, the primary photosynthetic tissue of these plants (Adey, 1965). The perithallial tissue below the meristem preserves the annual growth increments and the remains of the yearly conceptacles, which are ovoid conceptacle cavities (Fig. 1). The primary hypothallium is a multicellular tissue forming the base of each individual and provides attachment to the substratum (Adey, 1965). Since it shows a modified form of calcification with higher Mg levels than the perithallium (Nash and Adey, 2018), it is not utilized in climate archiving. Wound tissue and secondary hypothallia develop to repair physical damage, inflicted by wave tools or grazers to the algal thallus (Fig. 1). While occasionally grazing can damage the meristem and perithallus, most grazing is restricted to the epithallus. A "symbiotic" association between chiton and limpet grazing and *Clathromorphum* sp. has been demonstrated and moderate grazing of surficial epithallium is required to keep the meristem active (Adey 1973; Steneck, 1982).

Skeletal elemental composition (Mg/Ca) of *Clathromorphum* sp. has been shown to correspond to temperature controls (Gamboa et al., 2010; Halfar et al., 2008; Hetzinger et al., 2018; Williams et al., 2018) and displays seasonal cyclicity (Adey et al., 2013). However, there is evidence that light is also influencing Mg incorporation (Moberly, 1968). For example, in the Newfoundland shallow benthos, *C. compactum* Mg/Ca ratios begin to increase in the spring before temperatures increased from the winter lows (Gamboa et al., 2010). In addition, several studies have noted inter-sample variability in Mg/Ca (e.g. Chan et al., 2011) and published *Clathromorphum* sp. Mg/Ca – temperature calibrations have been site specific (Hetzinger et al., 2009, 2018; Williams et al., 2014). It has been hypothesized that because algae collected at the same site are experiencing similar temperatures, inter-sample variability may be caused by differences in shading or orientation relative to the sea surface (Williams et al., 2014). Differences among calibrations might also result from collections at different depths or different water clarity between sites of similar depths.

There is also evidence for the influence of light and temperature on growth rates of coralline algae. Adey (1970) demonstrated that growth of many boreal-subarctic coralline algal genera exhibited a strong relationship with light at high temperatures, and a weak relationship with light at low temperatures. These patterns suggest growth is limited by photosynthesis in water temperatures above 4-5°C, while respiration and other growth processes likely limit growth at lower temperatures (Adey, 1970). Similarly, Halfar et al. (2011a) found a positive correlation between water temperature and *Clathromorphum* sp. growth increment width in the North Atlantic where winter sea surface temperatures (SSTs) are below

0°C, whereas *Clathromorphum* sp. growth in the Bering Sea (winter SST >3°C) was unrelated to temperature, yet was positively correlated with light (Halfar et al., 2011b). This supports Adey's (1970) finding that growth is limited by photosynthetic production in warmer water, whereas it is temperature controlled in colder water. Adey et al. (2013) modeled the relative control of temperature and light in algal systems, showing that over a broad range of temperatures and light, temperature had a somewhat larger effect on productivity than light. Since both temperature and light are limiting factors, the most limiting will be controlling productivity. However, in winter subarctic conditions, both factors are at or near limiting conditions. Similarly, cloud cover, another light proxy, has been linked to the summer calcification of the rhodolith-forming coralline alga, *Lithothamnion glaciale* (Burdett et al., 2011). In that study, lower light levels caused by winter cloud cover reduced summer carbonate density (Burdett et al., 2011). Additionally, Teichert and Freiwald (2014) found light to be the most important, and mean annual temperature to be the second most important physical parameters limiting calcium carbonate production of coralline algae on the Svalbard shelf (Teichert and Freiwald, 2014). Furthermore, Halfar et al. (2013) used the influence of light on both growth rates and Mg/Ca to reconstruct sea ice cover in the Arctic. Sea ice cover constrains growth by limiting photosynthates that the algae produce (Halfar et al., 2013). Also, bottom temperatures remain relatively constant below sea ice and more ice-free days allow for higher temperatures, which are recorded in the Mg/Ca of the algae (Halfar et al., 2013). In summary, both light and temperature have demonstrated effects on coralline algal calcification and Mg/Ca.

Contrary to the majority of photosynthetic calcifiers, *C. compactum* can thrive in the absence of light for over half of the year. For example, *C. compactum* is found in abundance in Arctic Bay, Nunavut, Canada at 73°N, where sea ice cover causes near darkness at the sea floor for up to 9 months of the year (Halfar et al., 2013). Regardless of ice, *C. compactum* has been described from Novaya Zemlya (Adey et al., 2015b), an archipelago in the Russian Arctic where there is less than 1 h of sunlight per day for 4 mo. of the year. These relationships prevail in other distantly-related coralline genera from high latitude warmer (Boreal) waters as shown by the ability of *Phymatolithon borealis*, *P. investiens, P. tenue,* and *L. glaciale* to develop extensive crusts and mobile rhodoliths in the far north of Norway where long winter darkness also occurs (Adey et al., 2018; Teichert et al., 2013). Even though growth rate is controlled by light and temperature, the chemistry of the associated calcification does not rely directly on photosynthesis but rather on total quantity of photosynthates.

Siobhan Williams 2018-7-24 1:04 PM
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It is clear that a better understanding of the effects of temperature and light (or lack thereof) on *C. compactum* growth, calcification, and elemental composition is necessary to fully understand *C. compactum* biology and ecology, and the use of this species as a climate archive. In this study, we examine multiple specimens of *C. compactum*, monitored at a range of light, temperature and time treatments in a suite of tanks having the same open coast source water supply. Post experiment, multiple samples were analyzed for their anatomical and cellular changes, growth, and $MgCO_3$ composition relative to the various treatments.

Siobhan Williams 2018-7-24 1:06 PM
Deleted: drawn from the same wild source environment, superficially scarred to allow following wound repair, and reared in an experimental microcosm over 11 months. A

Siobhan Williams 2018-7-24 1:07 PM
Deleted: The examination of $MgCO_3$ content allowed for the calculation of separate Mg-temperature relationships for each of the three light levels examined in this study. We also demonstrate wound repair processes and grazing induced damage in *C. compactum* and show that growth and calcification can take place for at least a two-month period of darkness.

2 Materials and Methods

2.1 Test subjects

One hundred and twenty-three living specimens of *Clathromorphum compactum* were chipped off rocky surfaces by divers in August 2012 at 10-12 m depths in Bread and Cheese Cove, Bay Bulls, Newfoundland (47º 18.57' N; 52º 46.98 W). The *C. compactum* specimens were dome-shaped, 3-6 cm in diameter, and 1-3 cm thick, a common size in Newfoundland and Southern Labrador (Fig. 2). *Clathromorphum* species are highly distinctive and easily separated from other coralline species in the region by their surface features and deep intercalary meristem (Adey, 1965; Adey et al., 2015b). *C. circumscriptum*, the only other species of the genus in the northwestern Atlantic occurs primarily in shallow water, rarely reaching to the depth of specimen collection in this study, and at maturity is morphologically quite distinctive (Adey, 1965; Adey et al., 2013). As each collected specimen was delivered by divers to the dive boat for selection and initial scarring, it was identified aboard by co-author WHA. During the experiment, each individual specimen, as selected for analysis, was tagged with a number, after removal from the tanks, for tracking through analysis; the specimens are stored in the Smithsonian Institution National Museum of Natural History (NMNH) with those identification tags for future reference.

Year-long temperature and light data (lux) were measured with in situ HOBO loggers (HOBO Pendant; Onset Computer Corporation) at a depth of 12 m at Bread and Cheese Cove (Figs. 3 and 4). The calibration of the HOBO light loggers used in the experiment was confirmed against the earlier field data by installing the same sensors at the field site for a single day. This provided values similar to the previous time series. In addition, temperature-depth profiles were obtained from Adey (1966) for four exposed stations on the east and northeast coast of Newfoundland, along with a relative abundance-depth profile of *C. compactum* at those sites (Supplementary materials Fig. 1). Both sources of instrumental data were used to establish the temperature and light parameters of the mesocosm complex.

There were several reasons for not measuring photosynthetically active radiation (PAR) in this study. (1) Considerable pre-existing field evidence suggested that calcification in *Clathromorphum* (and other corallines) is not directly related to photosynthesis (Nash and Adey, 2017a), but rather only to the availability of stored photosynthate energy. (2) Being red algae, and having the accessory pigments phycocyanin and phycoerythrin that supplement chlorophyll, corallines have quite

different action spectra from green algae or higher plants. Also, since these algae reach a peak of cover at about 20m, we were working with available light spectra that would be very different from that at the water surface. Using PAR sensors would have raised as many questions as it solved. (3) Since *C. compactum* occurs over a wide depth range (5-30 m), the level of photosynthesis varies widely and previous studies have not indicated major changes in growth and calcification over that range. Thus we would not have expected a strong relationship between our parameters of interest and light spectra; (4) Adding PAR, with or instead of Lux, especially since action spectra responses would have been an essential component requiring individual chambers for each specimen, would have significantly increased the difficulty and cost of carrying out the experiment.

2.2 Experimental setup

The experiment was carried out at the Ocean Sciences Center (OSC) of Memorial University of Newfoundland from September 2012 to July 2013. Sea water, pumped in from a depth of 5 m in the adjacent embayment, Logy Bay, was provided through a constant flow system at 1 L min^{-1} to each tank. Four 180-L glass tanks were placed so that natural light from large rounded windows was provided at one end of each pair of tanks, with the opposite ends of the tanks shaded with black plastic sheets (Fig. 5). Sixty cm long, 20 watt Hagen Marine Glo, T8 fluorescent tubes were positioned over the window-lighted end of each tank so as to provide a significant light gradient (Fig. 6). The light covered one half of each tank (the high light section). The immediate darker quarter of each tank was the mid light section and the darkest quarter the low light section. The fluorescent tubes were automatically switched on at 10:00 and off at 15:00. Day length and morning and evening light intensity were supplied by natural sunlight from the north facing windows. Experimental temperatures were 2°C, 4°C, 7°C, and 10°C. All tanks were supplied with 4°C seawater from a master chiller at a constant flow rate of one l/ min. Temperatures in the 7°C and 10°C tanks were obtained with immersion heaters (Hagen, Fluval M300). Temperatures in the 2°C tank were obtained with two immersion probe coolers (Polyscience, IP 35RCC). September was a month of gradual temperature change in each tank from roughly 12°C in-coming sea water to each experimental value.

HOBO data loggers were placed in each tank at high and low light positions to quantify light and temperature at 5 minute intervals throughout the experiment (Figs. 6-8). A pre-experimental trial with data loggers in all three sections produced a light value as a proportion of that in the high light tank for the remainder of the experiment (Fig. 6). Mean light levels in October were: High light = 450 lux, medium light = 142 lux, low light = 17 lux (Fig. 6). The monthly mean temperatures at each light level in each tank are shown in Table 1. Occasional changes in flow rates of the sea water supply as well as heater function required manual system adjustments to bring temperature to desired values. Due to the limitations of the laboratory and available equipment, the -1.5 to 1°C temperature levels representing winter temperatures in coastal Newfoundland were not achieved with 2°C being the lowest temperature attained for the long-term experiment.

2.3 Placement and harvest of *C. compactum* specimens in mesocosm

To mark the beginning of the experiment (September 2012) the specimens of *C. compactum* were placed in a tank containing approximately 85 mg Alizarin red dye per liter of seawater for 48 h. Alizarin red is incorporated into the living algal tissue and it leaves a permanent red stain line (Kamenos et al., 2008). However, the stain was not incorporated in the tissues, likely, because the test subjects did not grow sufficiently during the staining process, so staining information was not part of this study.

Each specimen was also laterally scarred (incised) with a fine metal file to a depth of 200-400 µm aboard the dive skiff immediately following collection (Fig. 2). The incisions, when sectioned by vertical fracturing, allowed for a Scanning Electron Microscope (SEM)-based estimate of rate of wound tissue growth during the experiment, as well as study of the process of wound repair. Electron microprobe examination was separately applied to sections of normal (unscarred) perithallial tissue and used to estimate the beginning of the experiment (shown by the cessation of seasonal Mg fluctuation). The annual maximum Mg/Ca was used to denote the beginning of the experiment for electron microprobe data, since this represents highest temperatures annually at the collection site, which occur in August, at the time of collection.

The 123 *Clathromorphum compactum* specimens from Bread and Cheese Cove were distributed evenly within each of the four experimental tanks, with ~10 specimens in each light zone (for a total of 30 subjects in each tank). On the first of each month, beginning with October 1, individual specimens were haphazardly collected from each light zone of each tank with the intention of leaving the remaining specimens evenly distributed over the zone space so as not to bias in-zone distribution with time. After collection, specimens were oven dried for 48 hours at 40°C and shipped to the NMNH for sectioning and anatomical analysis with SEM (Table 2). Using SEM, the progress of regrowth of the scarred tissue, as well as the status of the meristem and epithallial and hypothallial tissues was determined. Of the 123 subjects, three were lost to "white patch disease" which is occasionally seen in the wild (Adey et al, 2013a), 85 were vertically fractured in an attempt to both cut across the scars made immediately after collection, and to section the peak of the mound. The remaining 35 specimens, mostly collected on July 1, 2013, at the close of the experiment, were also sent to the NMNH coralline herbarium, as the ClEx Collection, for examination and voucher storage.

Anatomy, growth, and calcification in the dark (Adey et al 2013; 2015) were also monitored and measured, since *C. compactum* is primarily an Arctic species and is seasonally exposed to periods of up to nine months without sunlight. On February 1, 2013, and repeated on May 1, 2013, 16 specimens from the low light areas of each of the experimental mesocosms were re-scarred (position of scar several mm removed from the original scar) and placed in in-situ dark

chambers. These specimens were subsequently collected at one to two month intervals (Table 2) and examined in SEM to determine the extent of wound recovery in the dark (Fig. 9A).

Each experimental mesocosm had chitons (*Tonicella* spp.), collected at Bay Bulls, added in roughly equal numbers to the number of *C. compactum* mounds present in each tank. The chitons, normally having home sites on or near *C. compactum* in the wild, established home sites beneath the experimental specimens and because the chitons might not travel over glass from specimen to specimen we provided one each *C. compactum* mound. The reason for including chitons in the experiment is the above described symbiotic relationship between grazing and *Clathromorphum* sp.

2.4 Sample analyses

All specimens were examined at the NMNH Imaging Laboratory at a range of magnifications from 50 X to 5000 X on a Leica Stereoscan 440 SEM operated at 10 kV, a 13-15 mm working distance, and a sample current of 101 pA. Specimens harvested in July, at the end of the experiment, were sectioned vertically and polished using diamond suspension to a grit size of 1 µm. The software geo.TS (Olympus Soft Imaging Systems) was utilized with an automated sampling stage on a reflected light microscope to produce two-dimensional maps of the experimental subjects' polished surfaces. These high-resolution composite images were used to identify the first annual growth increment, and to select linear transects for geochemical analysis across the annual growth increment, encompassing the length of the experiment and avoiding the wound area of the samples (for details see Hetzinger et al., 2009). One to three parallel electron microprobe line transects were analysed for algal $MgCO_3$ composition on each subject (Table 3) from the meristem to the first growth line at the University of Göttingen, Germany using a JEOL JXA 8900 RL electron microprobe. An acceleration voltage of 10 kV, a spot diameter of 7 µm, and a beam current of 12 nA were used. Along transects samples were obtained at intervals of 10 µm, and to avoid unsuitable areas such as uncalcified cell interiors, the location of analyses were manually chosen no more than 20 µm laterally from the transect line. Further details of the method are described in Halfar et al. (2013).

Using R version 3.3.2 (The R Foundation for Statistical Computing) one-way and two-way analysis of variance (ANOVA) tests were performed to determine the variance between light and temperature conditions. Assumptions for ANOVA were met and normality was verified using the Shapiro-Wilks test, and equal variance was verified with the Bartlett test. Linear regressions were used to determine temperature-$MgCO_3$ relationships. There was only data for 3 temperature treatments for the medium light conditions, instead of the 4 temperature treatments for high and low light conditions. This was addressed through the degrees of freedom associated with specific ANOVA tests used to determine each p-value.

3 Results

3.1 Sample growth during experiment

3.1.1 Wound recovery from scars as indicator for algal growth characteristics/cellular structure

Development of characteristic wound tissue from sub-scar pre-existing perithallial tissue occurred to a depth of approximately 400 µm (Fig. 9D). After the formation of several primordial wound repair cells, a new intercalary meristem forms, gradually returning the wound tissues to normal perithallial tissue, and starch grains as stored food are present in all stages of the experiment including in samples grown in the dark. Occasionally when the scars were deeper, lateral secondary hypothallial growth developed to partially refill the base of the wound (Fig. 9D). Direct perithallial regrowth of scar tissue in almost all cases, both marginally and vertically, was typically initiated by one or several very large primordial cells, with massive calcified cell walls (Figs. 10F, 9A-C).

Until the meristem and sufficient photosynthetic epithallial tissue is formed, development and growth must be sustained by transfer of photosynthate from elsewhere in the crust, either as storage food from old perithallial tissue lying below or newly produced photosynthate from undamaged epithallial tissues beyond the wound. Therefore growth and calcification in these newly formed tissues must be metabolically-driven. The mean growth of scar tissue during the course of this experiment from subjects at all temperature and all-light conditions combined was 42 µm/month (Table 4). Specimens lacking apparent recovery (zeros) were not included in the summation, since the possibility of grazing, as a factor external to growth, cannot be excluded. This compares with the expected mean monthly growth rate of specimens at the Bay Bulls site of approximately 24 µm/month (Halfar et al., 2011b). Total normal perithallial growth during the experiment of the subjects harvested in July ranged from 58 µm to 182 µm (Fig. 11).

In addition to artificial scarring, chiton inflicted grazing deeper than epithallium and apparently also into the recovering wound tissue and developing conceptacles was encountered during the experiment (Table 4). Generally, chitons took up daytime residence beneath each subject and came out to graze the surface at night. The graze marks of these animals are seen in appropriately oriented specimens (Fig. 10F). The development of asexual conceptacles in the experiment, beginning in the autumn and reaching maturity by mid-winter, just as in the wild (Adey, 1965) occurred within the experimental system. Mature and maturing conceptacles were widely spread over the temperature and light range employed, and were commonly found in these experimental specimens (Supplementary materials Fig. 2A and B), as well as signs of grazing, through the epithallium and overlying conceptacle roof tissue, into the conceptacle cavities (Supplementary materials Fig. 2C). Two thirds of the noted significant grazing, including both wound tissue and conceptacles, occurred in the 10°C tank (Table 4), suggesting that at higher temperatures, out of phase with conceptacle development (normally Autumn and Winter), chiton grazing could significantly affect reproduction. The high dominance of observed chiton grazing occurring in the 10°C tank

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suggests that temperature control of chiton activity is important. However, with no predators (especially small starfish) in the experimental system, chitons may well have been more active than in the wild.

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3.1.2 Dark growth and calcification

Four specimens from each of the experimental tanks, all from the low light sections of the tanks, were re-scarred, near the earlier collection scar and placed in dark chambers at the opaque back of each tank on February 1, 2013 and May 1, 2013. Of the 16 subjects placed in the dark, after collection 9 were sectioned and examined in SEM for new growth in the dark. Of those SEM samples, 3 were covered with detritus in the crucial scar section and SEM observations of potential new growth could not be carried out, 3 showed no apparent regrowth (although chiton grazing might have removed such growth), and 3 presented a good section and images in SEM and were capable of reliably providing a recording of dark growth. Specimens from the 4°C tank placed in the dark and collected after one month were sectioned and examined with SEM. Two of these specimens presented the distinctive scar (wound) regrowth (Fig. 10A-C). An additional specimen, taken from the low light level of the 4°C tank, placed in a dark chamber for two months, and manipulated similarly to the samples left in the dark for one month, also showed distinctive scar regrowth (Figs. 9C, 10D).

Wound recovery, both in the light and dark, was initiated with large primordial cells and several typical wound repair cells, followed by the development of typical anatomical tissue with the formation of perithallium, meristem cells and epithallium (Fig. 9). Calcification in the newly formed tissues, with both radial inner wall calcite and large, diagonal interfilament crystals is quite similar to that in lighted, wound regrowth tissue and in normal tissue (Fig. 10B, C, E). The full thickness of interfilament calcite that is often viewed in mature summer growth of normal vegetative tissues of *C. compactum*, and seen in lighted scar regrowth (Fig. 10E) has not developed in these dark specimens (Supplementary materials Fig. 2A). All three specimens that showed regrowth in the dark came from the 4°C experimental tank. The average rate of wound regrowth measured in these dark specimens, at 30 µm/month, is considerably less than the 51 µm/month mean found in all lighted wound regrowth in the 4°C tank (Table 4). In addition, it is unlikely that the experimental scarring affected later growth because the typical specimen used in the study was about 4 cm in diameter, while the scars were about 1 mm wide and 4 mm long. The entire photosynthetic surface of the typical specimen was about 1200 mm^2 and the scar less than 4 mm^2, about $1/400^{th}$ of the surface. Since lateral translocation in the shallow coralline crust is widespread (Adey et al., 2018), it seems further unlikely that the scar would have affected growth following wound repair growth. In addition, the second scarring, typically placed about one cm from the initial scar, took place 6-9 months after the first, making it unlikely that the first scarring affected growth in the second, dark experiment. In short, while dark wound regrowth occurs, and appears to have all the anatomical and calcified cell wall features as normal wound repair tissues, growth rates, at the same temperature, appear to be slower.

3.2 Effects of temperature and light on algal $MgCO_3$

The $MgCO_3$ of samples from high light treatments had the most significant correlation with temperature (ANOVA: variation within the samples; $F_{(1,,38)}$=13.75, p<0.001; Fig. 12B) and the relationship weakened with diminishing light levels; medium light ($F_{(1,14)}$=5.80, p=0.03) and low light ($F_{(1,22)}$=4.19, p=0.05). In addition, there was no statistically significant interaction between light and temperature (2-way ANOVA; $F_{(6,68)}$=0.95, p=0.5).

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3.2.1 Temperature

When all of the light treatments were averaged together the relationship between $MgCO_3$ and temperature was stronger than any of the individual light levels ($F_{(1,78)}$=22.23, p<0.001) (Fig. 12A). In addition, the standard error of $MgCO_3$ of each of the temperature treatments was smaller than when the light treatments were separated. This was due to an increase in the sample size.

3.2.2 Light

In addition to a significant relationship between $MgCO_3$ and temperature, there was also a significant relationship between light levels and $MgCO_3$ from all temperatures combined ($F_{(1,77)}$=14.95, p<0.001) (Fig. 13). Therefore, the linear regression equation of $MgCO_3$ for each light treatment was different, and the high light samples showed the most temperature sensitivity (steepest slope). In every temperature treatment more light resulted in higher $MgCO_3$ (Fig. 12B). The regressions were:

High light

$MgCO_3$ [mol %] = 0.26 (temperature °C) + 14.5

R^2=0.81

Medium light

$MgCO_3$ [mol %] = 0.22 (temperature °C) + 13.9

R^2=0.55

Low light

$MgCO_3$ [mol %] = 0.16 (temperature °C) + 13.2

R^2=0.63

4 Discussion

4.1 Algal growth characteristics determined from wound recovery

Mean all-temperature, all-light vertical growth of scar (wound) tissue during the course of this experiment was 42 µm/month. This would provide a yearly growth rate of about 500 µm, compared with the expected mean monthly growth rate of specimens at the Bay Bulls site of approximately 287 µm/ year (24 µm/month) (Halfar et al., 2011b). As shown in Fig 11, total perithallial growth during the experiment ranged from about 80 – 270 µm (depending on light level) for an approximate period of eight months (allowing one and a half months for specimen acclimatization), or approximately 120 – 400 µm per year. Thus, the growth rate values found in this study are well within the expected range in the wild. Beginning six months into the experiment, fully grown-in grooves began to be seen. This suggests an even greater differential between wound recovery rate and expected whole crust growth rate. Unfortunately, in this experiment, it was not possible to consistently achieve a tank temperature below 2°C. Most *C. compactum* populations are in localities that reach 0°C or below during the winter. Thus, tank growth could not be precisely compared with wild growth. Clearly, the relatively rapid rate of wound recovery is necessary if an even surface on *C. compactum* mounds is to be maintained and detritus accumulation avoided. Such recovery from wounding and conceptacle break-out is frequently seen in wild-collected specimens (Adey et al, 2013). Chiton grazing is likely a factor in the delay or absence of scar tissue in some plants. However, rapid wound recovery demonstrates that temperature and light do not provide short term controls to growth and calcification rates.

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Growth and calcification are clearly metabolically driven; when a wounded crust can draw upon photosynthates, stored or in photosynthetic production, from other parts of the crust, higher rates than normal vegetative growth under given temperature/light conditions are achievable. Arctic/Subarctic *C. compactum* crusts must store photosynthate for an extended dark season. The growth rate that can be accomplished in the short term, to repair damage using stored energy, is clearly not an option for normal vegetative crust growth. Normal crust vegetative growth, with energy supplied from epithallial photosynthesis, must not only provide for local growth but also for annual reproduction, lateral growth and wound repair. Thus, the wound repair rates seen here are higher than the month to month growth potential of *C. compactum* as controlled by light and temperature and abundantly provided in the literature (Adey et al 2015).

4.2 Dark growth and calcification

This experiment has demonstrated a strong connection between light and $MgCO_3$, while Nash and Adey (2017b) have shown Mg control only by temperature. However, in the latter study, light was not a control, other than as a basic metabolic

requirement. Our experiment demonstrated that growth, with the full complex of cell wall calcification, can occur without simultaneous light, presumably as long as stored photosynthate is available.

Calcification in the dark indicates that photosynthesis is not the direct driver of calcification by altering local chemistry. Rather, with the availability of stored food previously formed by photosynthesis, growth, and calcification is driven metabolically. The initiation of the calcite crystal formation cannot be dependent on photosynthetically-elevated pH, as has been proposed in various studies. *C. compactum* specimens grown in the wild under Arctic conditions of six months of darkness, consistently show a sharp downward spike in Mg content at the equivalent of 0 to -1.8°C water temperature (Fig. 14). Full darkness, often under ice cover, occurs before the temperature reaches its lower limits on the bottom where the plants are growing. To produce carbonate with $MgCO_3$ ratios equivalent to a water temperature of -1°C, growth has to have occurred in dark conditions for some period of time. As we see from this experiment, growth could proceed for at least two months before likely ceasing when sufficient stored photosynthate is exhausted.

4.3 Light and temperature

Results show that both light and temperature significantly affect magnesium in *C. compactum* crusts. At lower temperatures (2°C) the effects of light are relatively small, relating to a 1.4 mol % $MgCO_3$ (corresponding to 8.75°C on the low light curve, and 5.4°C on the high light curve) increase from low to high light. Differences become larger at higher temperatures (10°C) where $MgCO_3$ increases by 1.8 mol % (corresponding to 11.25°C on the low light curve, and 6.9°C on the high light curve) from low to high light levels. Also, at higher light levels R^2 values indicate a stronger correlation between $MgCO_3$ and temperature (Fig. 12B). These observations suggest that light and temperature both result in an increase in $MgCO_3$ and growth, with the effects of light being more significant at higher temperatures as shown previously for other coralline species (Adey, 1970).

4.4 Implications for proxy

Results show that although temperature is a major factor contributing to *C. compactum* $MgCO_3$ incorporation, light must be considered when using *C. compactum* as a proxy. Our findings suggest that a global $MgCO_3$ – temperature calibration cannot be produced for *C. compactum*, because light levels and shading contribute to differences in $MgCO_3$ within individuals or a large sample. The highest correlation between temperature and $MgCO_3$ was found when all experimental samples were combined regardless of light level, suggesting that due to inter- and intra-sample variability replication is very important when generating temperature reconstructions, rather than attempting to collect all samples from similar light conditions. The need for replication caused by inter-specimen differences has also been highlighted in several studies of *Clathromorphum* sp. (Hetzinger et al., 2018; Williams et al., 2014, 2018).

The effects of light on $MgCO_3$ may explain differences in Mg/Ca of samples collected from the same site and water depth found in several *C. compactum* climate reconstruction studies (Chan et al., 2011; Williams et al., 2014). Differential shading can be due to temporary macroalgal overgrowth or the position of the coralline algae, such as under a ledge or orientation with respect to the surface. In these cases, differing light levels would necessitate sample specific calibrations. This would imply calibrating Mg/Ca for each individual sample with known in-situ temperature before averaging all samples to create a record. Applying this method could prevent outlier samples that may have experienced significantly different light levels from having an effect on the proxy reconstruction.

Experiment results also support the use of *C. compactum* as a sea ice proxy, because Mg/Ca is driven by both light and temperature. Changes in light duration on the Arctic seafloor are related to sea ice, and thus to coralline algal Mg/Ca. *C. compactum* is especially suited as a sea-ice proxy because it is the only high resolution shallow marine archive found in seasonally ice-covered regions of the Arctic, including the Greenland coast (Jørgensbye and Halfar, 2016), the Canadian Arctic Archipelago, northern Labrador (Halfar et al., 2013), Novaya Zemlya (Adey et al., 2015b), and Svalbard (Wisshak et al., 2016).

While the findings of this experiment should inform the use of replication and calibration of individual samples to improve the use of *C. compactum* as a climate proxy, it does not discount the results of past environmental reconstructions using this species. For example, Williams et al. (2014) used *Clathromorphum* sp. to reconstruct past temperature and tested the calibration between Mg/Ca and instrumental temperature of each sampling location. Also, Halfar et al. (2013) combined annual growth and Mg/Ca concentrations from *C. compactum* to reconstruct sea ice conditions, based on the assumption that these records respond to both light and temperature. While the influence of both light and temperature on growth rates of several species of corallines has already been demonstrated (Adey, 1970), their influences on Mg have now also been confirmed. Based on our findings, the results of past studies would only be untrustworthy if a calibration from another study or location was used to convert Mg/Ca to temperature without confirming this relationship.

5 Conclusions

C. compactum produces its normal range of tissues with similarly complex high magnesium calcitic wall structures both in the light and in the dark. Growth and calcification occur for at least two months in the dark, presumably following the exhaustion of stored photosynthate. Following the formation of a few distinctive initial primordial and transition cells, wound repair (scar) tissue is similar to normal perithallial tissue, although wound repair tissues grow at significantly greater rates than normal tissue. Since the autumn/winter formation of reproductive structures (conceptacles) requires vegetative tissue growth and calcification of surrounding vegetative tissues, calcification in the dark allows for the survival of *C.*

compactum under sea ice cover and in Arctic winter darkness. Both light and temperature significantly affect the incorporation of $MgCO_3$ in *C. compactum* calcitic cell wall structures. At lower temperatures the effects of light are slightly smaller than at higher temperatures. Also, the correlation between $MgCO_3$ and temperature is stronger at higher light levels than at low light. When generating proxy temperature reconstructions using *Clathromorphum* species, site and possibly specimen specific temperature calibrations need to be applied in order to take into account the effects of light. Corallines, including *Clathromorphum* species, can be successfully grown in the laboratory and used for critical experimentation. However, they are complex organisms with equally complex ecological relationships, and mesocosms, rather than simple aquaria, are required to produce reliable experimental results (Adey and Loveland, 2007; Small and Adey, 2001).

Competing interests

The authors declare that they have no conflict of interest.

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Tables

Table 1. Average tank temperatures, Nov, 2012 - June, 2013.

	Tank section	**10°C**	**7°C**	**4°C**	**2°C**
Nov., 2012	High light	10.2	6.9	3.7	2.4

	Low light	10.4	7	3.8	2
	Average	**10.3**	**6.95**	**3.75**	**2.2**
Dec. 2012	High light	10.1	7.1	4.4	2.2
	Low light	10.2	7.1	4.5	1.8
	Average	**10.15**	**7.1**	**4.45**	**2**
Jan. 2013	High light	9.3	7	4	2.3
	Low light		7	4.4	1.8
	Average	**9.3**	**7**	**4.2**	**2.05**
Feb., 2013	High light	10.39	6.95	3.53	2.03
	Low light	10.47	6.98	3.65	1.52
	Average	**10.43**	**6.97**	**3.59**	**1.78**
March, 2013	High light	10.37	7.63	4.12	2.24
	Low light	10.48	7.66	4.24	1.67
	Average	**10.43**	**7.65**	**4.18**	**1.96**
April, 2013	High light	10.05	7.43	4.22	2.13
	Low light	10.17	7.45	4.37	1.5
	Average	**10.11**	**7.44**	**4.3**	**1.82**
May, 2012	High light	10.91	7.39	4.24	2.31
	Low light	10.95	7.46	4.38	1.74
	Average	**10.93**	**7.43**	**4.31**	**2.03**
June, 2013	High light	10.73	7.09	4.31	2.55
	Low light	10.85	7.19	4.42	1.82
	Average	**10.79**	**7.14**	**4.37**	**2.19**
	Mean	**10.35**	**7.21**	**4.14**	**2**

Table 2. *C. compactum* growth experiment (ClEx) at Logy Bay Newfoundland. Specimens collected in mid August, 2012 and brought to experimental temperature through September, 2012. High light (hl) was <400 lux, medium light (ml) was <160 lux, and low light (ll) was <17 lux.

Sample Date	**Temp. [^{o}C]**	**Light Level**	**Number Harvested**
Oct 1, 2012	10	hl	1

	10	ml	1
	7	hl	1
	7	ml	1
	4	hl	1
	4	ml	1
	2	hl	1
	2	ml	1
Nov 1, 2012	10	hl	1
	10	ll	1
	7	hl	1
	7	ll	1
	4	hl	1
	4	ll	1
	2	hl	1
	2	ll	1
Dec 1, 2012	10	hl	1
	10	ml	1
	10	ll	1
	7	hl	1
	7	ml	1
	7	ll	1
	4	hl	1
	4	ml	1
	4	ll	1
	2	hl	1
	2	ml	1
	2	ll	1
Jan 4, 2013	10	hl	1
	10	ml	1
	10	ll	1
	7	hl	1
	7	ml	1
	7	ll	1

	4	hl	1
	4	ml	1
	4	ll	1
	2	hl	1
	2	ml	1
	2	ll	1
Jan 24, 2013	10	ml	1
	10	ll	1
Feb 1, 2013	10	hl	1
	10	ml	1
	7	hl	1
	7	ml	1
	4	hl	1
	4	ml	1
	2	hl	1
	2	ml	1
March 1, 2013	10	dark one month	1
	10	hl	1
	7	dark one month	1
	7	hl	1
	4	dark one month	1
	4	hl	1
	2	dark one month	1
	2	hl	1
April 1, 2013	10	hl	1
	10	dark one month	1
	7	hl	1
	7	dark one month	1
	4	hl	1
	4	dark one month	1
	2	hl	1
	2	dark one month	1
May 1, 2013	10	hl	1

	10	ml	1
	7	hl	1
	7	ml	1
	4	hl	1
	4	ml	1
	2	hl	1
	2	ml	1
June 2, 2013	10	hl	1
	10	dark one month	1
	7	hl	1
	7	dark one month	1
	4	hl	1
	4	dark one month	1
	2	hl	1
	2	dark one month	1
July 1, 2013	10	hl	4
	10	ml	1
	10	ll	2
	10	dark two months	1
	7	hl	4
	7	ml	2
	7	ll	3
	7	dark two months	1
	4	hl	4
	4	ml	2
	4	ll	3
	4	dark two months	1
	2	hl	4
	2	ml	2
	2	ll	3
	2	dark two months	1

Table 3. Number of transects analysed with electron microprobe from each temperature and light level. Number of samples from each level in brackets.

Temperature [°C]	High light	Medium light	Low light
10	10 (4)	3 (1)	3 (1)
7	10 (4)	1 (1)	7 (3)
4	10 (4)	6 (2)	7 (3)
2	10 (4)	6 (2)	7 (3)

Table 4. ClEx wound (scar) regrowth (thickness of new growth [µm]/time for growth [month]). Extensive chiton grazing of groove marked with *, conceptacles present marked with +. Specimens lacking apparent recovery (zeros) were not included in the summation, since the possibility of grazing, as a factor external to growth, cannot be excluded.

	10°C			7°C			4°C			2°C			Mean (µm)		# specs
Harvest Date (# mos)	hl	ml	ll	hl	ml	ll	hl	ml	ll	hl	ml	ll	total	monthly	examined
November 1, (1.5 mos)	80/53					90/60	0		29/19	0			66	44	8
December 1 (2.5 mos)	100/40	150/60+*	* 0	120/48+	90/36	80/32	175/70+	150/60	150/60		100/40	100/40	122	49	12
January 4 (3.5 mos)	*	150/43	150/43			125/36	225/64	120/34	*** +	150/43		100/29	146	42	10
February 1 (4.5 mos)	37/8	150/33		0			0				125/28	0	104	23	8
March 1 (5.5 mos)	380/69**		200/36	250/45*		110/20	370/67		320/58	320/58		***	279	50	8
April 1 (6.5 mos)	***		***	350/54		250/38	300/46		300/46	220/34		100/15	253	39	8
May 1 (7.5 mos)	** ++	** ++		300/40	300/40						230/31		277	37	8
July 1 (9.5 mos)									375/39			150/16*	263	28	3

													65 specs total
Mean (without 0's)	149/43	150/45	175/40	255/47	195/38	131/37	268/62	135/47	235/44	230/45	152/33	113/25	42/39
mean each tank (without 0's)		158/43			194/41			213/51			165/34		
Chiton grazing	4	2	2	1	0	0	0	0	1	0	0	2	12 specimens with significant chiton grazing
concs present or grazed	1	2	0	1	0	0	1	0	1	0	0	0	6 specimens with developing concepatacle
# specs with regrowth	4	3	2	4	2	5	4	2	5	3	3	4	41 specs with regrowth

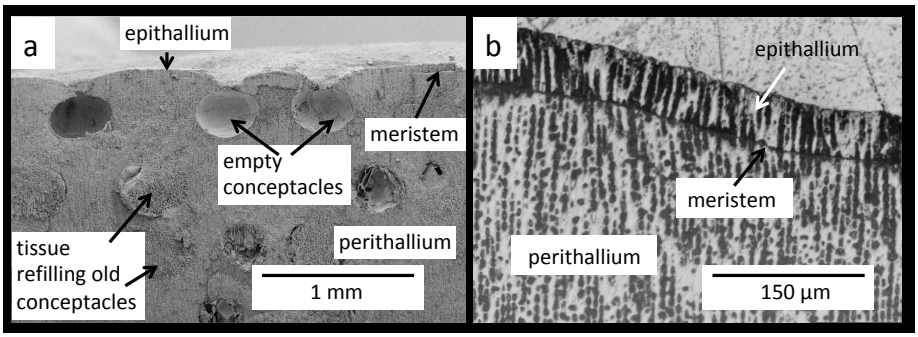

Figure 1. (a) Cross section of *C. compactum* mound indicating major skeletal features. (b) Close up of meristem, epithallium, perithallium cells.

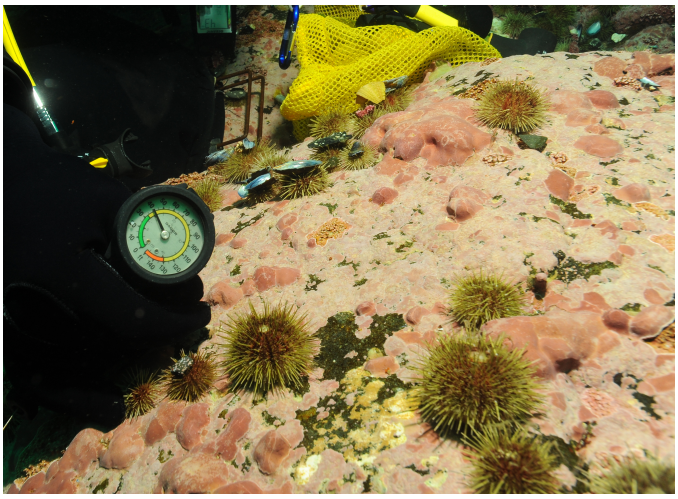

Figure 2. *Clathromorphum compactum* mounds (dark red) up to 3 cm thick in a typical coralline community at 13m depth off Quirpon Island in northernmost Newfoundland. Photo by Nick Caloyianus.

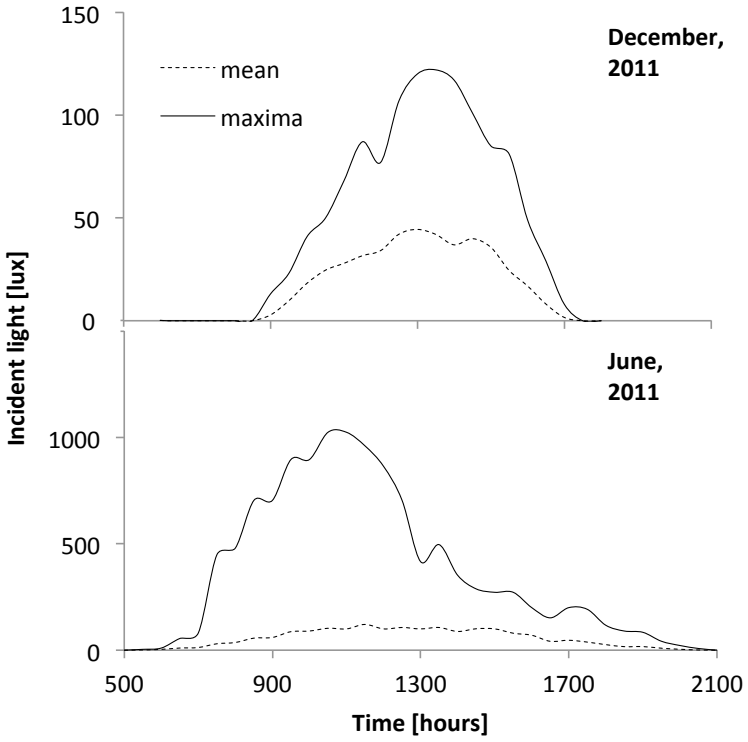

Figure 3. Summer (June) and Winter (December) daily means (dashed curve) and maxima (black curve) of half hour resolution light data from HOBO loggers at the Bay Bulls collection site. HOBO light sensors were oriented vertically for maximum light receipt.

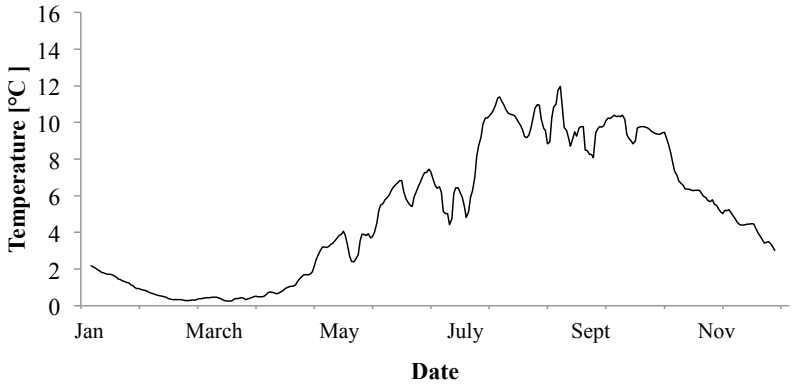

Figure 4. Five-day average temperatures through 2011 at Bay Bulls, Bread and Cheese Cove, at 10 m, taken with HOBO data loggers.

Figure 5. Experimental tank layout in Ocean Sciences Center at Logy Bay, NF. High light right end of each tank is adjacent to large port-like window and has fluorescent lights overlying. Opposite, low light ends of tanks shielded with opaque plastic sheet. Note cooling probes, coated with ice, placed in 2 & 4°C tanks on left. Three light segments (low light, ll; mid light ml; high light hl, left to right) with their emplaced specimens can be seen in right tank.

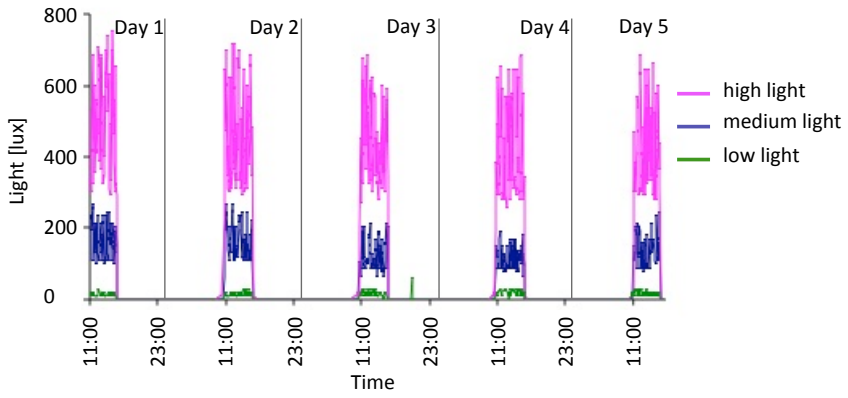

Figure 6. Five-day plot of light levels taken from HOBO loggers in October, 2012 in 10°C tank. Pink is high light, blue is medium light, and green is low light.

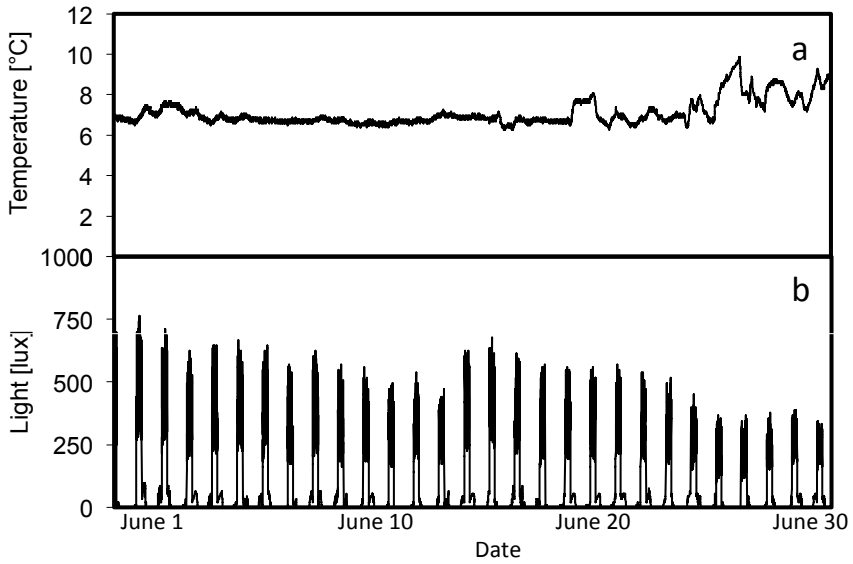

Figure 7 (a) Temperature [°C] and (b) light [lux] during June in high light portion of 7°C tank.

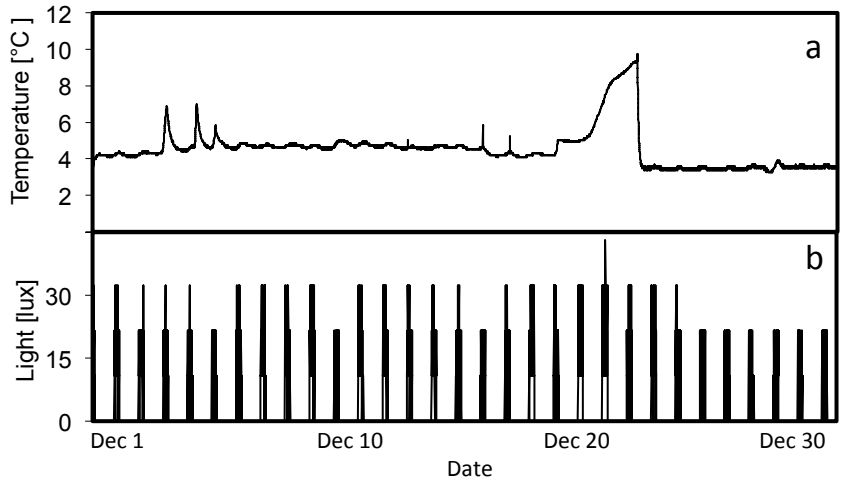

Figure 8 (a) Temperature [°C] and (b) light [lux] during December in high light portion of 7°C tank.

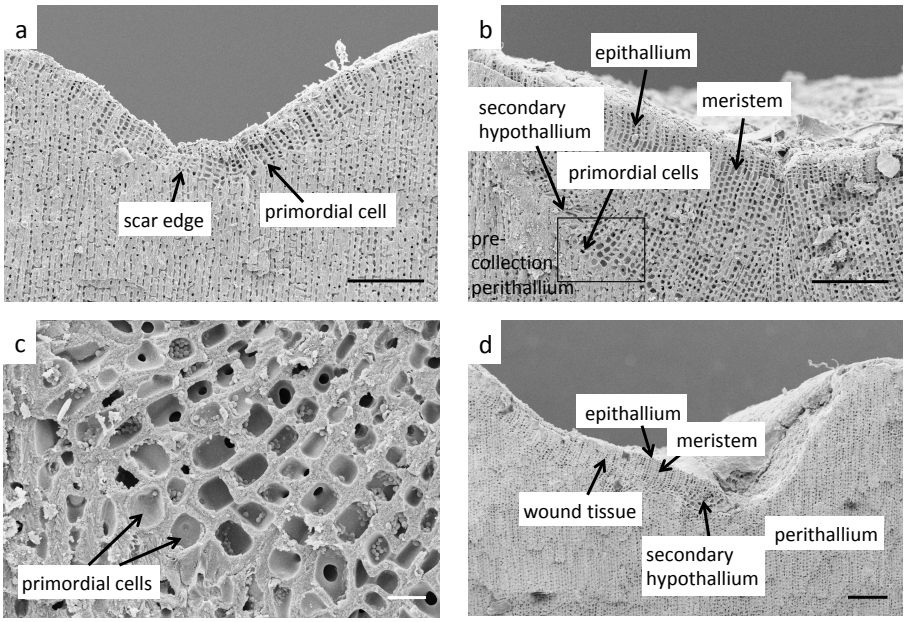

Figure 9. Example of wound recovery of *C. compactum*. (a) Section of *C. compactum* mound through the scar groove, collected from low light, 2°C tank on Dec 1, 2012 after 2 1/2 months of recovery. Wound regrowth was initiated with one or more large primordial cells that gradually transition into normal perithallial cells. Following the production of three to four typical perithallial cells, meristem cells have developed and are beginning to produce epithallial cells. (ClEx 121 211). (b) Scar groove section of *C. compactum* mound from the high light 2°C tank after six months of recovery. Groove has nearly grown in with normal meristem cells and perithallial and epithallial tissue. See blow up of large primordial cells in (c). (c) Primordial cells in lower left of section of (b). These large ovoid to box-shaped cells typically have massive cell walls that appear to be a combination of inner cell wall and interfilament, crystals (ClEx 41 2hl). (d) Partially regrown scar (~ 400 µm at the deepest part) in *C. compactum* retrieved from 10°C high light tank on November 1, 2012. Left side and shallower back portion of scar have formed new perithallium directly from old, pre-scar tissue. However, deeper bottom of scar is being filled by new hypothallium being topped with new perithallium. Allowing for late August and September recovery without growth, new wound tissue represents one to two month's growth. Scale bars, 100 µm (a, b, d), and 10 µm (c).

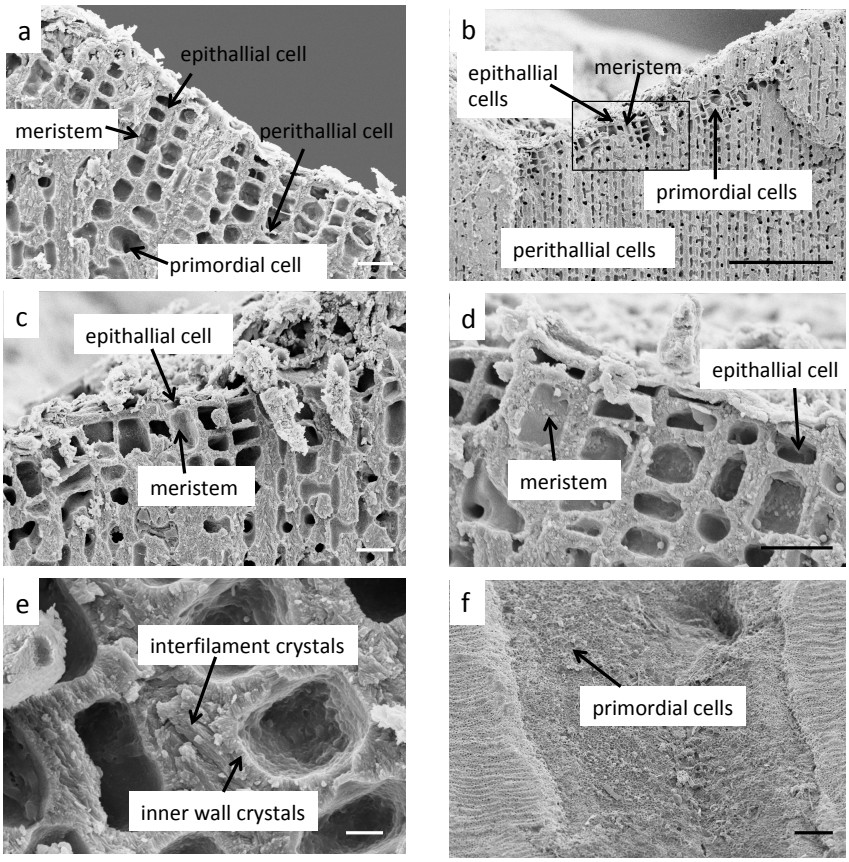

Figure 10. Examples of wound recovery during dark calcification. (a) Wound tissue recovery after two months in dark conditions. Primordial cells, perithallial cells, meristem cells and two to three epithallial cells appear similar to those shown in Fig. 9 (a-c) for lighted wound recovery tissues (ClEx 71 4dk). (b) Similar wound recovery after one month growth in the dark. There are fewer cells, with only few meristem cells apparent as compared to (a), rectangle indicates area magnified in (c) (ClEx 31 4dk). (c) Magnification section of (b) showing three meristem and two new epithallial cells being initiated (ClEx 31 4dk). (d) Section of one month dark groove developing new tissues. Meristem cells are in process of developing along with one or two epithallial cells. Note clear development of interfilament, even at this early stage, along with standard

radial inner wall crystals (ClEx 41 4dk). (e) Normal upper perithallial tissue from 4°C tank after two months dark growth (meristem cell in upper left) showing extensive, normal diagonal interfilament crystals developed in the dark (ClEx 71 4dk). (f) Surface view of part of scar made on May 1, 2013, when specimen was placed in dark chamber of 4°C tank. Collected on July 1, 2013, large cells visible on mid slope lateral surfaces of scar are recovering primordial cells. Lateral to groove, on normal tissue, right and left, overlying epithallial tissue can be seen to be heavily grazed by chitons in form of en-echelon, fine grooves (ClEx 71 4dk). Scale bars, 2 µm (e), 10 µm (a, c, d), 100 µm (b, f).

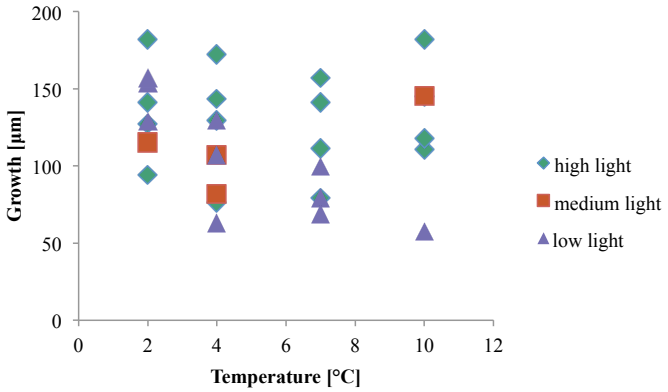

Figure 11. Total perithallial growth during experiment of samples harvested at end of experiment; multiple transects from individuals were averaged.

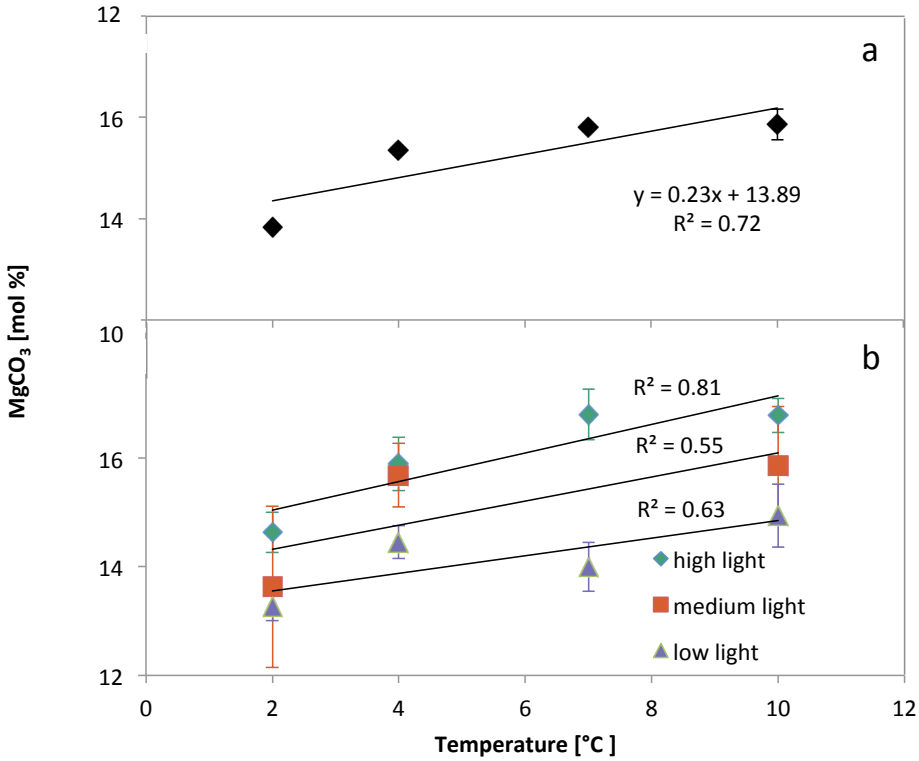

Figure 12. (a) Electron microprobe derived $MgCO_3$ concentrations (± standard error) of each temperature treatment group after averaging all light levels. (b) Electron microprobe derived $MgCO_3$ concentrations (± standard error) of each temperature/light treatment group.

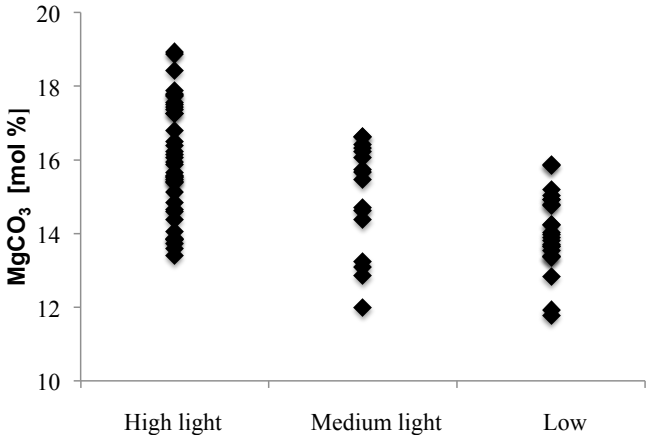

Figure 13. $MgCO_3$ concentrations at each experimental light level (all temperatures combined).

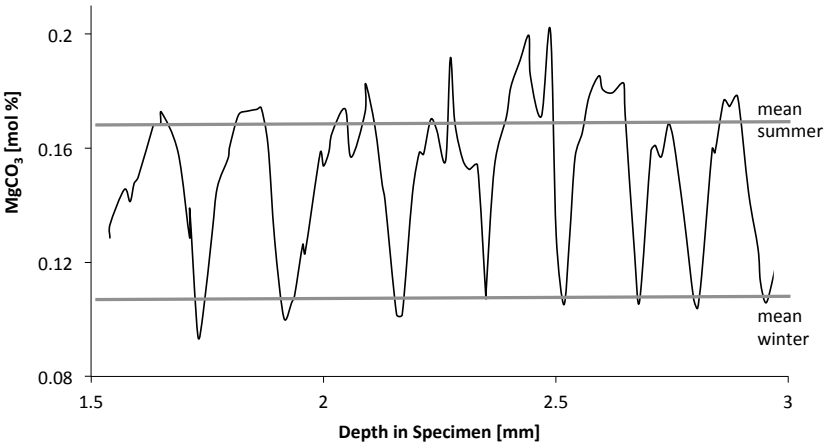

Figure 14. Annual cycles of $MgCO_3$ content of *C. compactum* mound from 15-20 m in the Kingitok Islands in north-central Labrador. Cycles show V-shaped pattern, indicating growth halt during winter. Water temperature at Kingitok below 0°C for over 200 days per year and sea surface is typically frozen from early December to late June (Adey et al, 2015).