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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 microcosm experiment in which numerous, 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 (1.4 mol % $MgCO_3$ increase from low [mean = 17 lux] to high light conditions [mean = 450 lux]) than at higher (10°C) temperature (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 never 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).

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 the algae 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

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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.

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).

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*, drawn from the same wild source environment, superficially scarred to allow following wound repair, and reared in an experimental microcosm over 11 months. A range of light, temperature and time treatments was established 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. 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).

Year-long temperature and light data (lux) were measured with in situ HOBO loggers (HOBO Pendant; Onset Computer Corporation) at a depth of 12m 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 (Fig. 5). Both sources of instrumental data were used to establish the temperature and light parameters of the microcosm complex.

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. 6). 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. 7). 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 1000 h and off at 1500 h. 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 was 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. 7-9). 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. 7). Mean light levels in October were: High light = 450 lux, medium light = 142 lux, low light = 17 lux (Fig. 7). 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 microcosm

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, oven dried for 48 hours at 40°C and shipped to the Smithsonian Institution National Museum of Natural History (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 microcosms 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. 10A).

Each experimental microcosm 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 (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 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.

3 Results

3.1 Sample growth during experiment

3.1.1 Artificial and natural scars

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. 10D. As the SEM image shows, development of characteristic wound tissue from sub-scar pre-existing perithallial tissue occurred to a depth of approximately 400 µm. After the formation of several primordial wound repair cells, a new intercalary meristem forms, gradually returning the wound tissues to normal perithallial tissue. Occasionally when the scars were deeper, lateral secondary hypothallial growth developed to partially refill the base of the wound (Fig. 10D). 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. 11F, 10A-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. 12).

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. 11F). 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 (Fig. 13A and B), as well as signs of grazing, through the epithallium and overlying conceptacle roof tissue, into the conceptacle cavities (Fig. 13C). 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 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. 11A-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. 10C, 11D).

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. 10). 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. 11B, 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. 11E) has not developed in these dark specimens (Fig. 13A). 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

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tank (Table 4). 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 $MgCO_3$

When all of the light treatments were averaged together the relationship between $MgCO_3$ and temperature was strongest (ANOVA: variation within the samples ($F_{(1,78)}$=22.23, $p<0.001$) (Fig. 14A), but the $MgCO_3$ of samples from high light treatments had the most significant correlation with temperature ($F_{(1,,38)}$=13.75, $p<0.001$; Fig. 14B and C) 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). It should be noted that 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.

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. 15). 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. 14C). 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

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 12, 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 one 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 with wild 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.

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. 16). 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 large, 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. 14C). 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).

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 microcosms, 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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Figure captions

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Figure 10. 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 11. 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. 10 (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 12. Total perithallial growth during experiment of samples harvested at end of experiment; multiple transects from individuals were averaged.

Figure 13. (a) Sub-mature asexual conceptacle in *C. compactum* mound collected from high-light section of 4°C tank on December 1, 2012. Although depressed from lack of new production, epithallial cap over conceptacle has yet to break out (ClEx 121 4hl). (b) Surface view, at level of meristem, with much of epithallium broken off, showing two submature asexual conceptacles. Collected December 1, 2012 from high light section of 7°C tank (ClEx 121 7hl). (c) Grazed-out conceptacles in *C. compactum* from low light section of 4°C tank collected on January 4, 2013. Scale bars, 100 µm (a, b), and 200 µm (c).

Figure 14. (a) Electron microprobe derived $MgCO_3$ values of each temperature treatment group after averaging all light levels. (b) Electron microprobe derived $MgCO_3$ values of individual *C. compactum* samples separated into temperature/light treatment groups. (c) Electron microprobe derived $MgCO_3$ values of each temperature/light treatment group.

Figure 15. $MgCO_3$ levels at each experimental light level (all temperatures combined).

Figure 16. 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).

Tables

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

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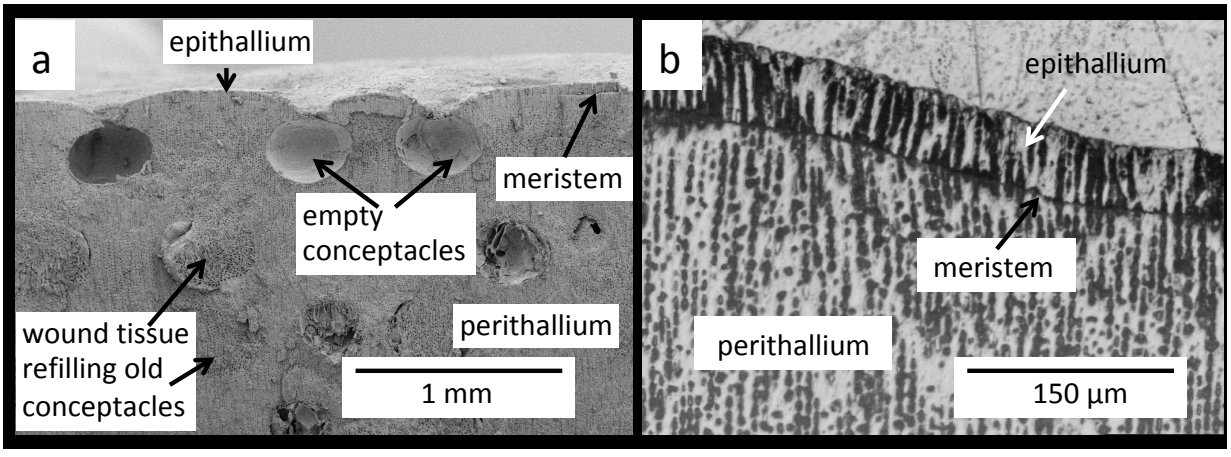
Table 2. *C. compactum* growth experiment (ClEx) at Logy Bay Newfoundland. Specimens collected in mid August, 2012 and brought to experimental temperature through September, 2012. High light (hl) was <400 lux, medium light (ml) was <160 lux, and low light (ll) was <17 lux.

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

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 +.

Figures



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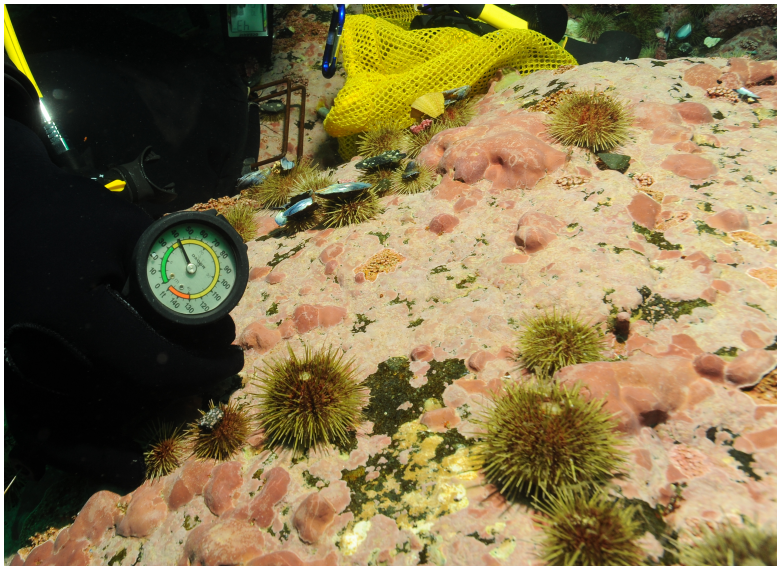

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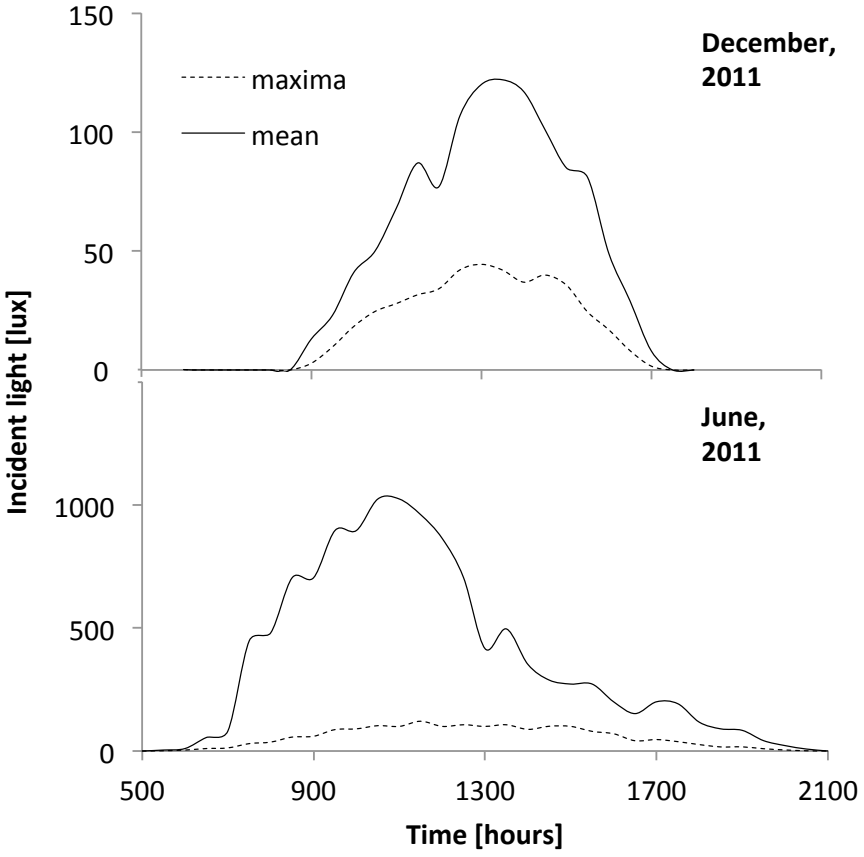

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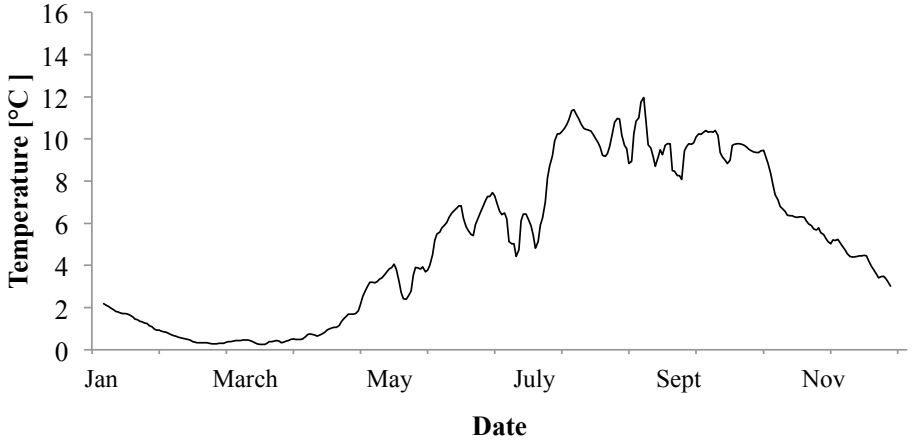

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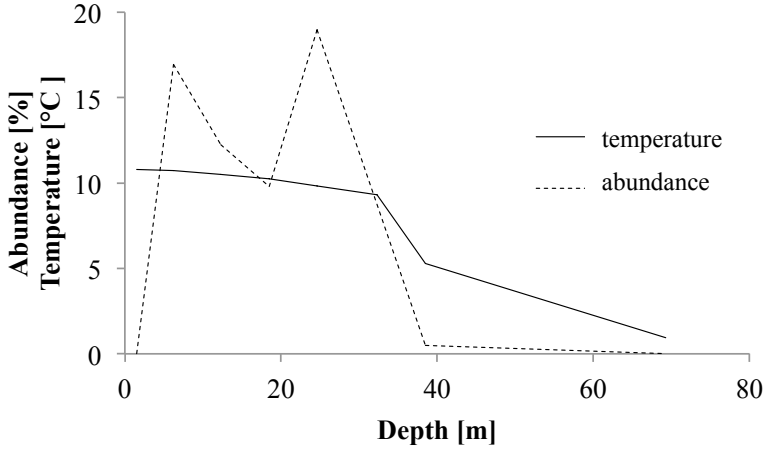

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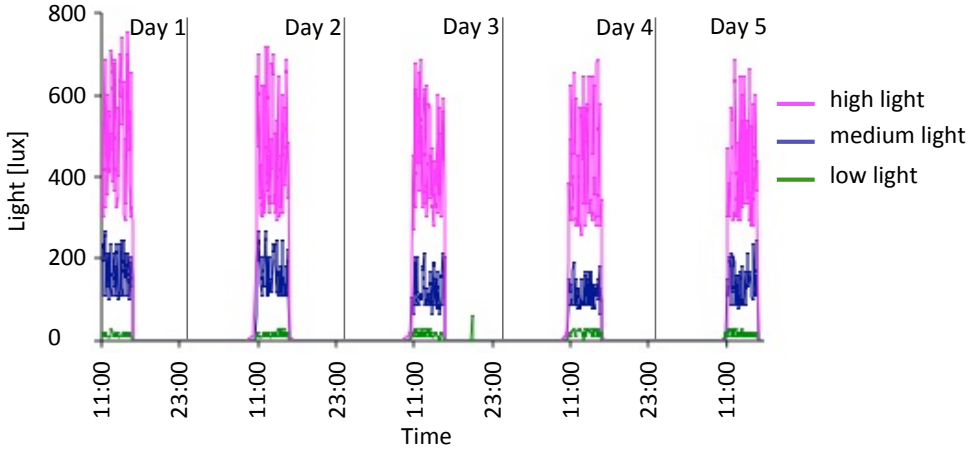

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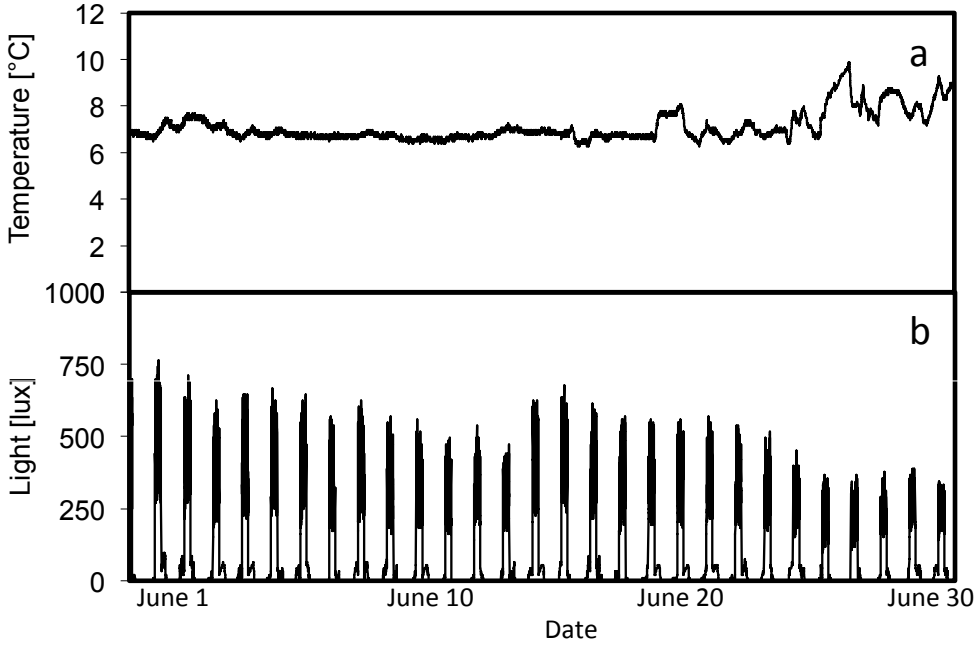

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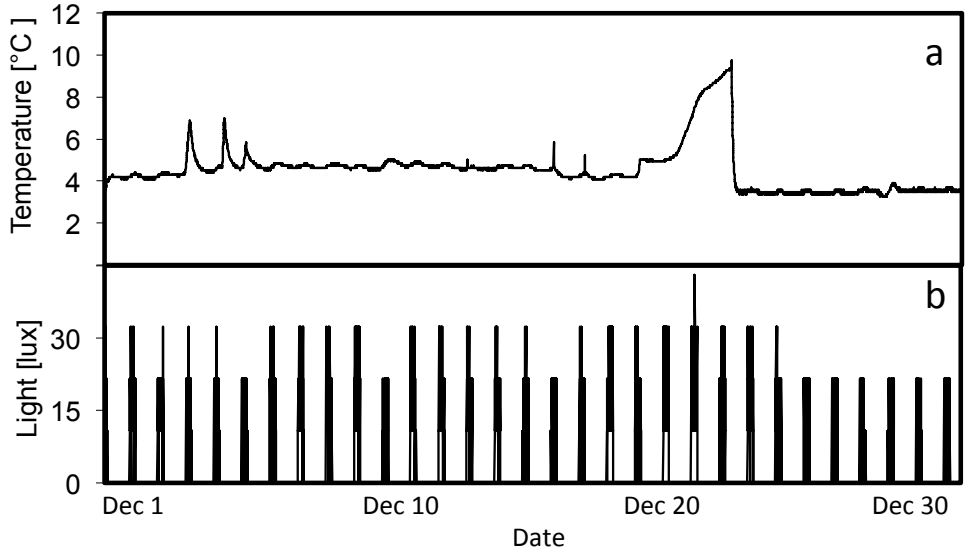

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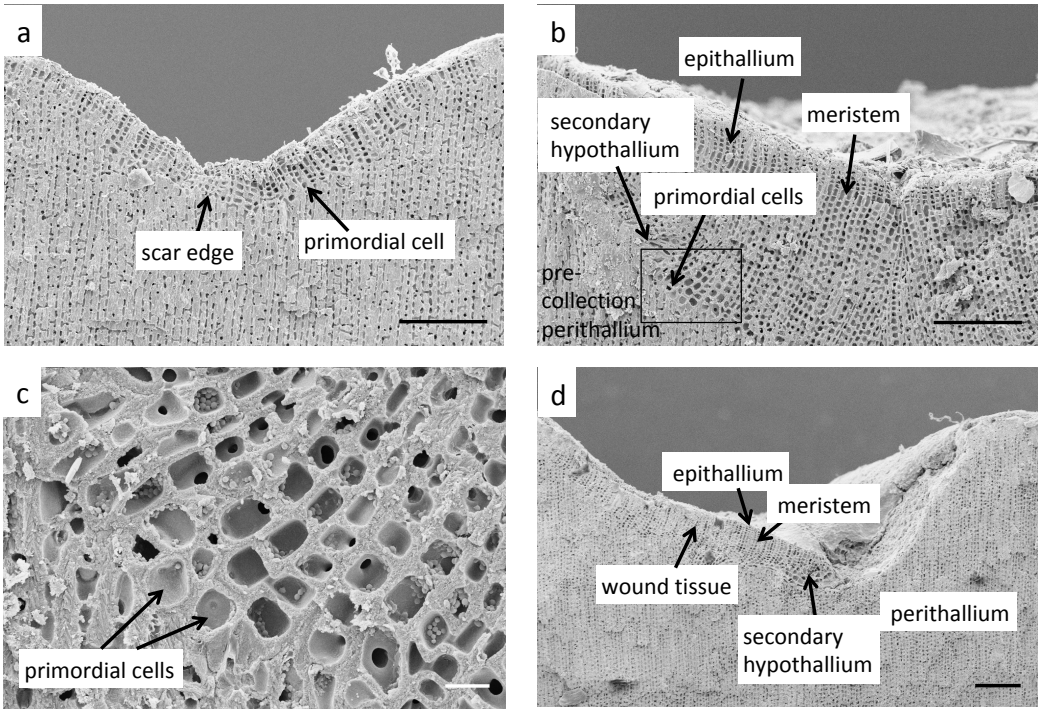

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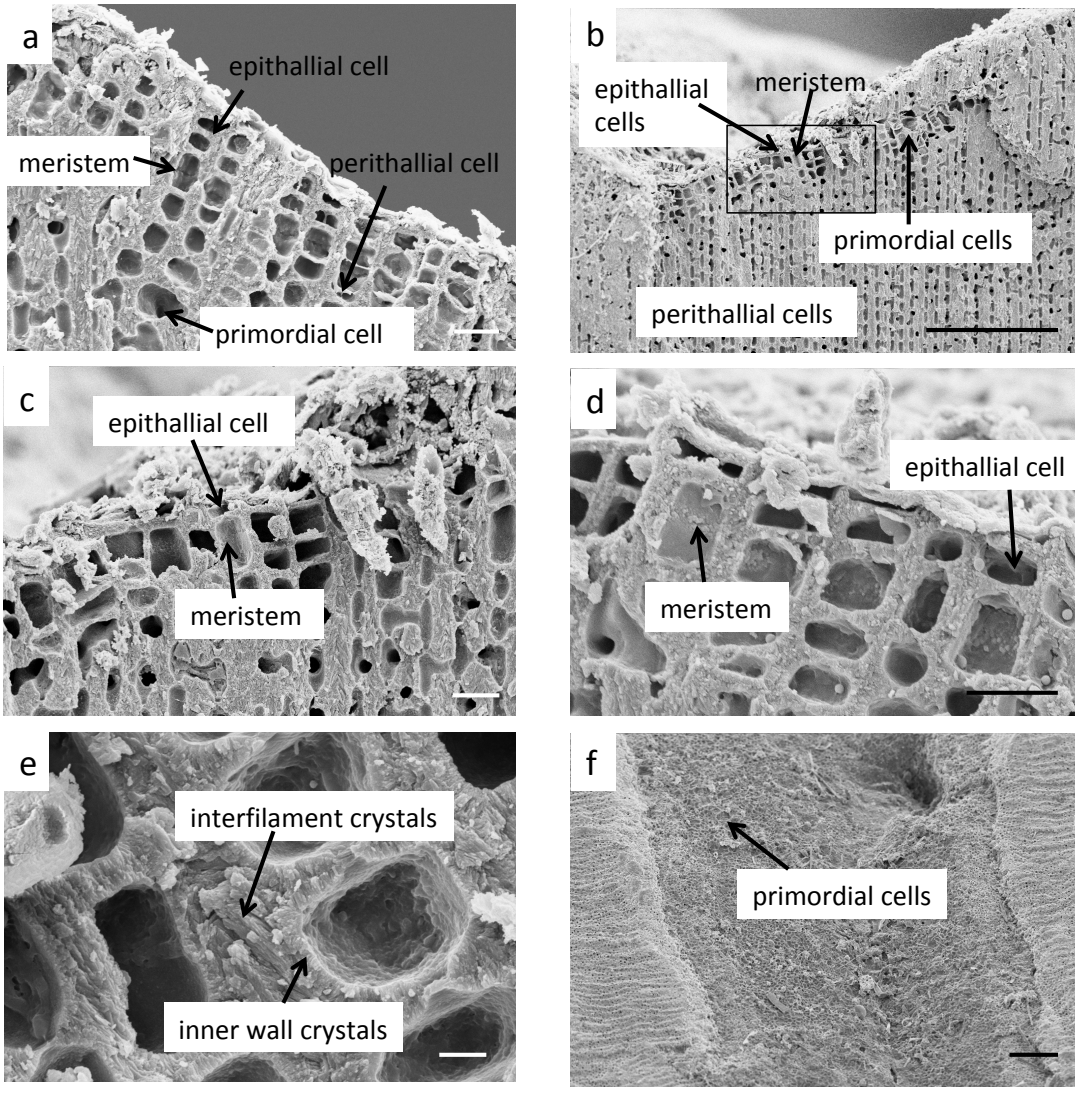

Figure 11. Examples of wound recovery during dark calcification. (a) Wound tissue recovery after two months in dark conditions. Primordial cells, perithallial cells, meristem cells and two to three epithallial cells appear similar to those shown in Fig. 10 (a-c) for lighted wound recovery tissues (ClEx 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

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(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).

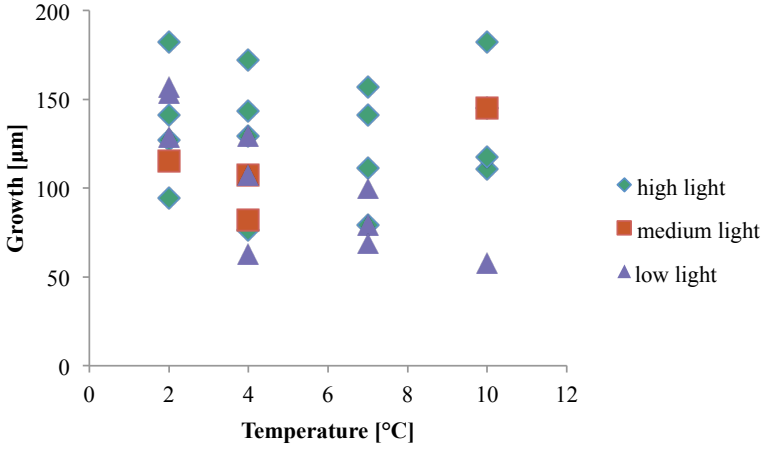

Figure 12. Total perithallial growth during experiment of samples harvested at end of experiment; multiple transects from individuals were averaged.

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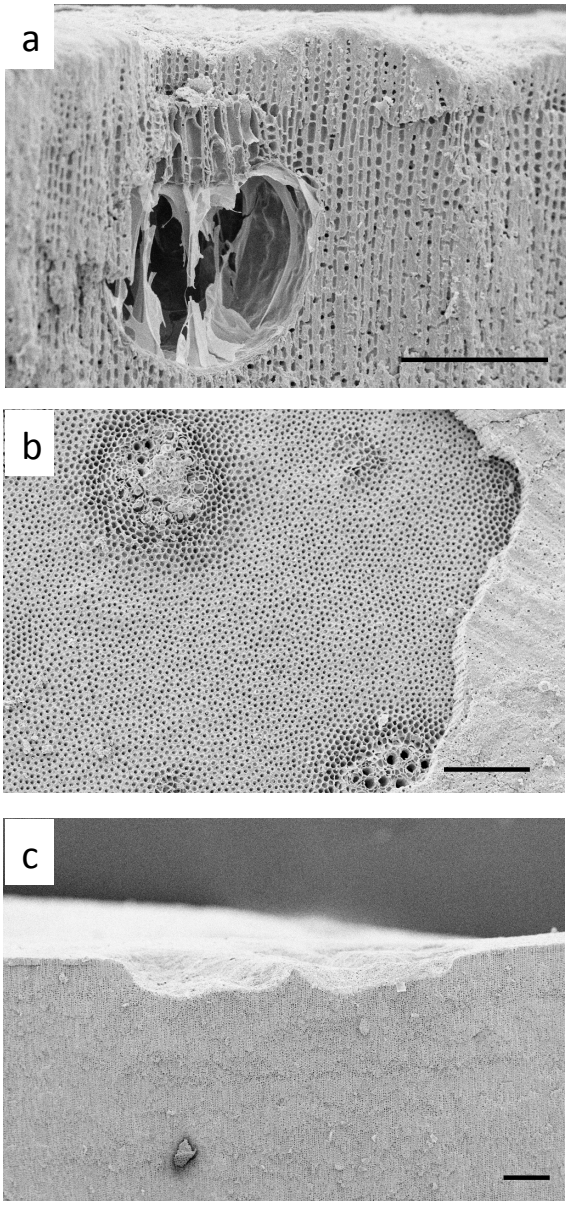

Figure 13. (a) Sub-mature asexual conceptacle in *C. compactum* mound collected from high-light section of 4°C tank on December 1, 2012. Although depressed from lack of new production, epithallial cap over conceptacle has yet to break out (ClEx 121 4hl). (b) Surface view, at level of meristem, with much of epithallium broken off, showing two submature asexual

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conceptacles. Collected December 1, 2012 from high light section of 7°C tank (ClEx 121 7hl). (c) Grazed-out conceptacles in *C. compactum* from low light section of 4°C tank collected on January 4, 2013. Scale bars, 100 µm (a, b), and 200 µm (c).

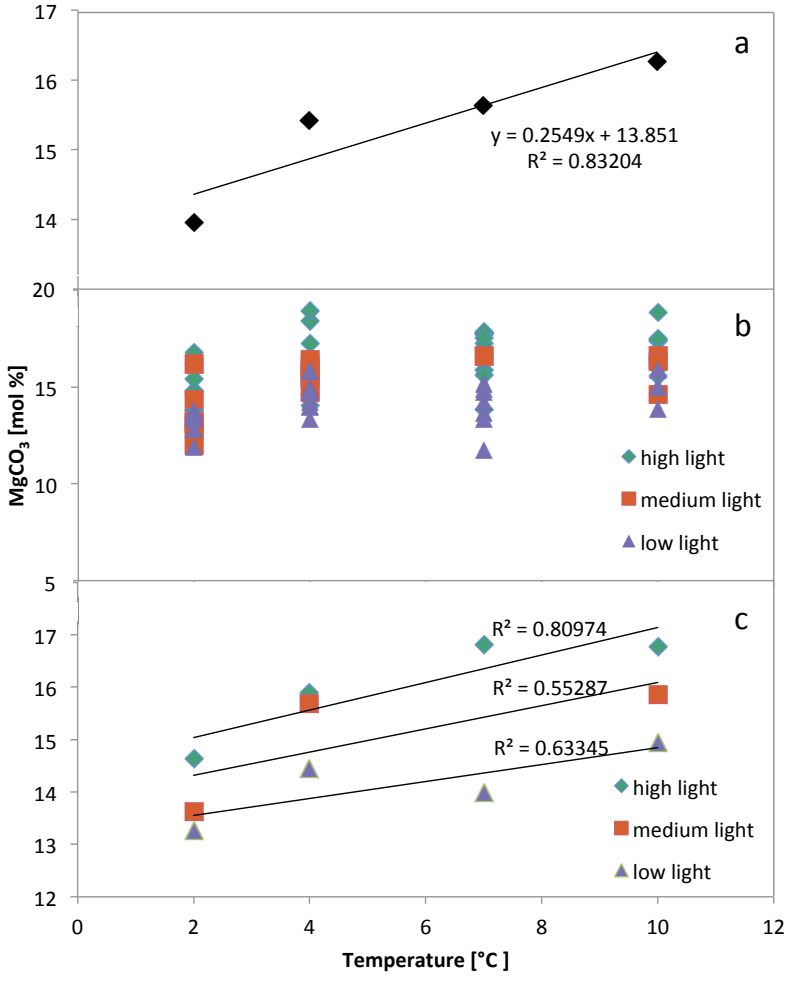

Figure 14. (a) Electron microprobe derived $MgCO_3$ values of each temperature treatment group after averaging all light levels. (b) Electron microprobe derived $MgCO_3$ values of individual *C. compactum* samples separated into temperature/light treatment groups. (c) Electron microprobe derived $MgCO_3$ values of each temperature/light treatment group.

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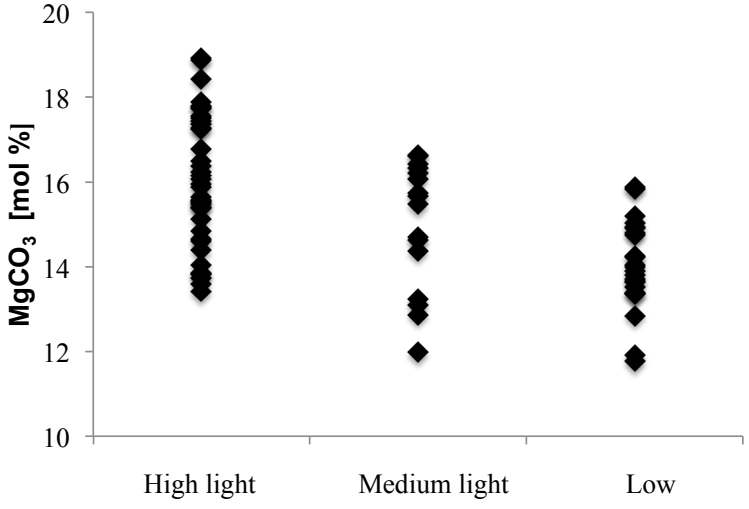

Figure 15. $MgCO_3$ levels at each experimental light level (all temperatures combined).

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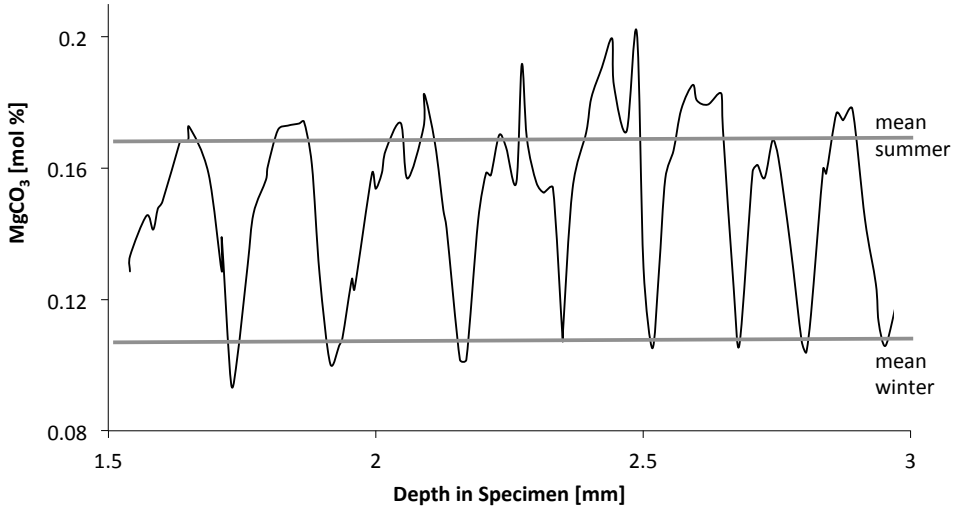

Figure 16. Annual cycles of $MgCO_3$ content of *C. compactum* mound from 15-20 m in the Kingitok Islands in north-central Labrador. Cycles show V-shaped pattern, indicating growth halt during winter. Water temperature at Kingitok below 0°C for over 200 days per year and sea surface is typically frozen from early December to late June (Adey et al, 2015).

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

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	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. [°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)

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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 +.

	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	

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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