MarMic Lab Rotation I: From 6 March, 2017 to 28 April, 2017
Department: Microsensors

Title/Topic: *EXAMINING THE IMPACT OF PROLONGED DARKNESS ON THE SURFACE PH OF LITHOTHAMNION GLACIALE*

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ABSTRACT

Performing both photosynthesis and calcification, coralline algae present an interesting case regarding the biological carbon pump, as photosynthesis sequesters carbon and calcification locally increases carbon dioxide levels. Coralline algal calcification has been proposed to be driven by either a local increase in pH due to photosynthesis or an ion pump through intracellular membranes. However, the former hypothesis would require photosynthesis to occur for calcification, which is not the case for algae living in the Arctic when exposed to continuous darkness during the winter. A recent, unpublished study has measured an elevated pH on the surface of the Arctic coralline algae of the rhodolith form, *Lithothaminon glaciale* compared to the water column after exposure to continuous darkness for twenty-four hours. This study further investigates this unexpected elevated pH by exposing *L. glaciale* individuals to continuous darkness for twenty days and comparing the differences between the algal surface pH and the pH of the water column using pH microsensors.

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INTRODUCTION

In March 2015, the average concentration of carbon dioxide in Earth's atmosphere surpassed a global record of 400ppm, and it continues to rise faster than ever in Earth's history (Dlugokencky and Tans 2017, Doney et al 2016). While the rapid increase of this greenhouse gas has elevated global temperatures, the acidity of the ocean has also risen (Caldiera and Wickett 2003). Approximately 40% of anthropogenic carbon dioxide emissions dissolve into the ocean, forming carbonic acid (Doney et al 2016). Understanding the dynamics of the carbonate cycle in the ocean is crucial to understanding the consequences of and developing strategies for facing these global changes (Lauvset et al 2015, Orr et al 2005). Calcification, performed by organisms such as coral, coccolithophores, and coralline algae, greatly influences the carbonate cycle (Bates, N. R. 2017; Doney et al 2016; Schmitz et al 2014).

Calcification is the biological assimilation of carbonate ions to build skeletons and other structures of calcium carbonate. When calcification occurs, some carbon is stored in the carbonate structure and some carbon is released as carbon dioxide. Increased levels of carbon dioxide dissolved into the ocean can disturb the equilibrium which drives calcification (Frankignoulle et al 1994). Despite the local decrease in pH due to calcification, coralline algae have been calculated as net sinks for carbon dioxide (van der Heijden et al 2015). This study investigates the impact of prolonged darkness on the calcification rates of the Arctic coralline algae, *Lithothamnion glaciale* (Figure 1).

Figure 1 *Rhodolith in a tank (personal photo; Weinheimer, A., 2017)*

Coralline algae are photosynthetic, multicellular organisms which deposit a calcium carbonate skeleton. Coralline algae may form crusts, referred to as Crustose Coralline Algae, or grow as free-living structures, referred to as Rhodoliths (Boesence, D. W. J, 1991). Coralline algae with a rhodolith morphology typically consist of red algal cells. Many form vast beds on the seafloor, which provide a habitat for other marine organisms, including boring bivalves and worms. These algae are found at all latitudes and various depths within the photic zone, including the Arctic Ocean (Boesence, D. W. J., 1983; Foster, M. S., 2001).

In the sub-tropics, calcification by the coralline algae belonging to the genus *Halimeda* has been found to be induced by photosynthesis. The photosynthetic removal of carbon dioxide increases the local pH, enabling the precipitation of calcium carbonate (Ries 2010). This coupling of photosynthesis with calcification would suggest that coralline algae do not calcify in the dark. However, rhodoliths in the Arctic Ocean of the genus *Clathromorphum*, have been observed to calcify during the dark periods of winter (Adey et al 2013). This study proposes that a proton pump drives the building of inner cell walls

embedded with the calcite crystals. The exact biochemistry of this mechanism is not fully understood.

To further understand calcification in the dark, this study examined the impact a prolonged period of darkness on the surface pH of the Arctic rhodolith *Lithothamnion glaciale*. When these algae are exposed to light, the pH on their surface increases largely due to the occurrence of photosynthesis, implying calcification may also be occurring (Ries 2010). In contrast, surface pH of coralline algae in the dark is usually slightly lower than the bulk seawater due to respiration (Hofmann et al. 2016). Previous measurements of pH at the surface of Greenlandic *Lithothamnion glaciale* exposed to darkness for periods of minutes to hours showed that the surface pH remained elevated compared to bulk seawater pH (Hofmann in prep). We therefore hypothesized that during the Arctic winter, when Greenlandic L. glaciale are exposed to complete darkness for half the year, the surface pH remains higher than bulk seawater pH. To test this hypothesis, we exposed Greenlandic L. glaciale to continuous darkness for 20 days and measured the surface pH using microsensors.

METHODS

Sampling and Culturing

Coralline algae of the species *L. glaciale* were collected from the Akia fjord off the coast of southern Greenland at 64.193210, -51.908612 via SCUBA. The algae were transported to the Max Planck Institute of Marine Microbiology in Bremen, Germany and kept in a cold room (4ºC) in natural seawater (salinity 34 units). Algae were exposed to light of 10 µmol photons m^{-2} sec^{-1} with a daily cycle of 18 hours of light and 4 hours of darkness. A current was maintained with a pump.

For 20 days, four individuals were kept in continuous darkness, simulating light conditions of the high Arctic winter, and four individuals were kept in continuous light of 10 µmol photons m^{-2} $second^{-1}$, simulating light conditions of the high Arctic summer. In the cold room, each alga was maintained in a separate, labeled Tupperware container (350mL), on a shaker table to simulate a current. The seawater was changed 3-4 times per week with seawater from the North Sea.

pH Measurements

The pH profiles of the rhodoliths were measured using pH microsensors assembled at the MPIMM. The pH microsensors utilize liquid ion exchange (LIX) to detect the electrical signal read on a voltmeter. This signal was recorded on a laptop computer with the software program Profix (Pyro-Science). Prior to use, the microsensors were calibrated using buffers of pH 7 and pH 9. To generate profiles, pH measurements were taken every 50µm from the surface of the rhodolith until 1000µm above it. A stereomicroscope was used to position of the sensor tip on the surface of the algae. The movement of the microsensor was controlled by a micromanipulator (Pyro-Science GmbH, Aachen, Germany).

Four to six pH profiles were taken both under the conditions of light saturation (100 µmol photons m^{-2} sec^{-1}) and darkness. Light saturation was determined by generating a light curve from measuring the pH profiles of a rhodolith at 25, 50, 100, and 200 µmol photons m^{-2} sec^{-1} (Figure 2). During the initial measurements, the profiles were measured under light saturation conditions first, followed by the measurements under dark conditions. Once the surface pH appeared to stabilize in the dark, the dark profiles measurements began. After the darkness treatment, the first profiles of the experimental rhodoliths were measured under dark conditions followed by profiles under light saturation, once the surface pH appeared to be stable. As the rhodoliths adjusted to the light, the surface pH was recorded.

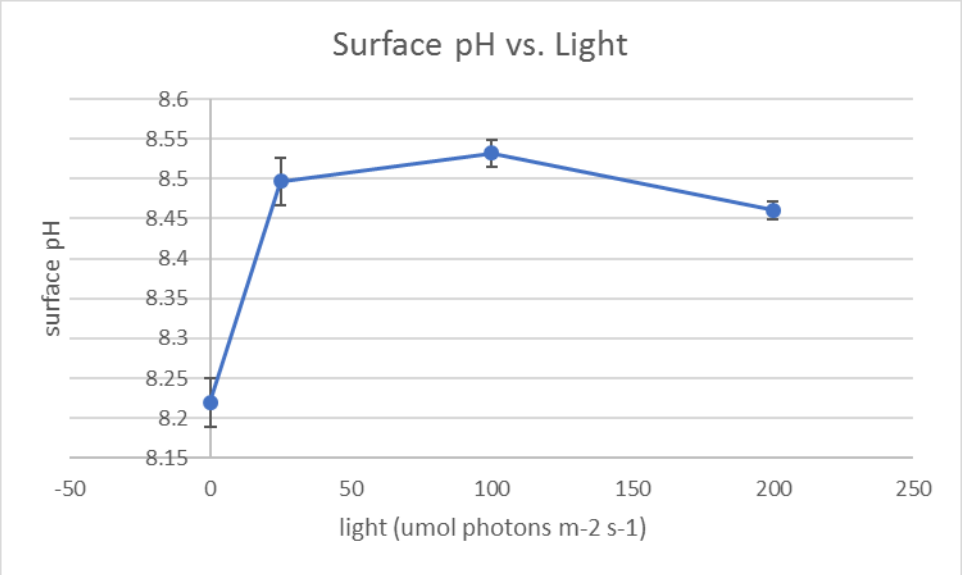

Figure 2 *Surface pH of an algal individual at increasing light intensities with standard error bars.*

A fiber optic halogen lamp (Schott, Mainz, Germany) provided the light source and was measured with Walz Submersible Spherical Micro Quantum Sensor (Heinz Walz, Effeltrich, Germany) attached to an LI-COR light meter (LI-COR Biosciences, Lincoln, Nebraska, USA).

To conduct the pH measurements, the rhodoliths were placed in a plexiglass flow chamber (dimensions). Laminar flow was created with a constant, recirculation of natural seawater from a 4L reservoir through perforated walls at the entrance and exit of the chamber. The water was maintained at 7ºC with a cooling coil and flow rate maintained at roughly 3mL sec^{-1}, measured with a graduated cylinder and timer. No stabilizing support was required to keep rhodoliths in position during measurements.

For each alga, initial pH profiles were measured with both light saturation and darkness conditions. Following completion of all profile measurements, four of the eight alga in Tupperware containers were covered with a light cancelling, black blanket to maintain continuous darkness while the other four remained exposed to 10 µmol photons m^{-2} sec^{-1} on the shaker. After twenty days, the dark and light saturation state pH profiles were measured for each alga. Due to time constraints, all four of the algae exposed to continuous darkness and only one of the algae exposed to continuous light were examined after twenty days.

Data Analysis

The surface pH of each alga under each light condition was compared to the water column. To standardize the measurements, the differences between the surface and water column pH were compared. To examine the rate at which the rhodoliths adjust to the dark, the surface pH in the dark from the initial timepoint was plotted versus time. After the second timepoint, the pH was recorded while the rhodolith adjusted from light saturation to darkness and vice versa.

RESULTS

The initial pH profiles of each alga under light saturation and darkness conditions were similar to those previously measured by Hofmann (in prep). Figure 3 displays a typical pH profile of an individual (Akia03) exposed to darkness and light.

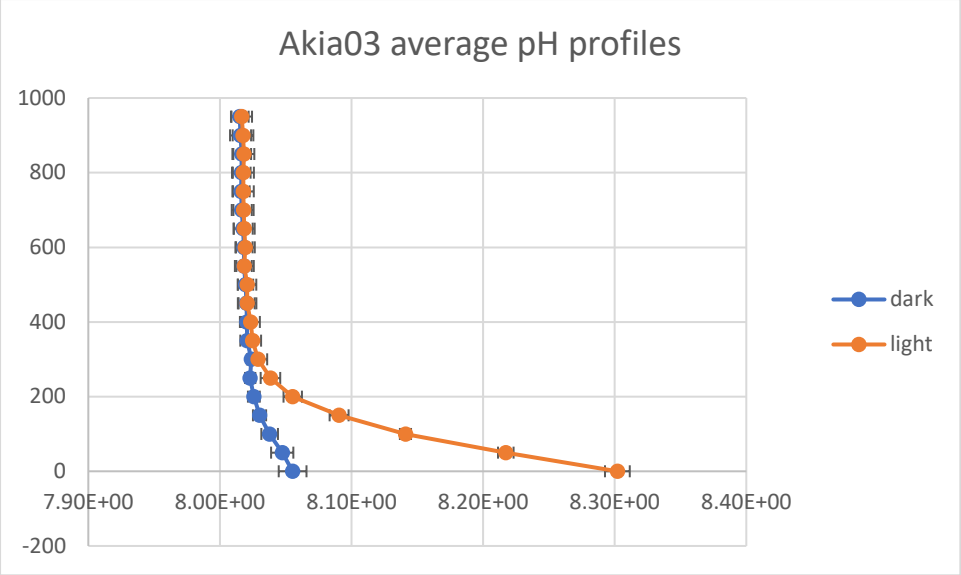

Figure 3 *The average pH measurement at each step in the profile of the individual Akia03 with standard error bars.*

The initial average surface pH of each alga under light was approximately 0.302±0.045 (standard error) higher than the surrounding water column. When exposed to darkness, the average surface pH of each alga was roughly 0.045±0.013 (standard error) higher than the water column (Figure 4).

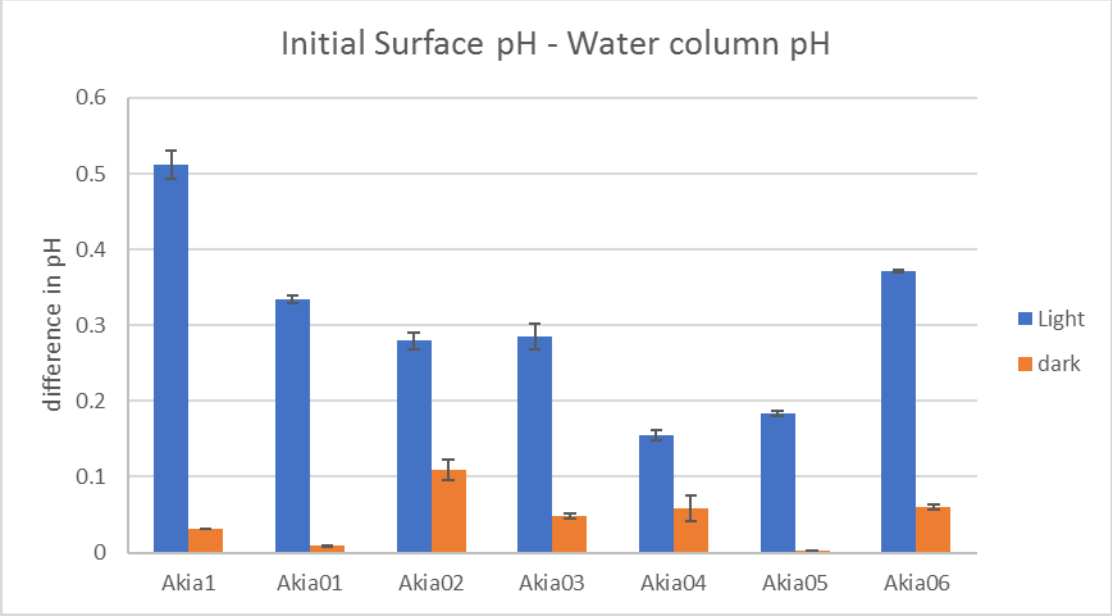

Figure 4 *The difference between the pH on the surface of the rhodolith and the water column in light (orange) and dark (blue) conditions prior to treatment in darkness with standard error bars.*

After exposure to darkness for twenty days, the difference in the pH between the surface of the rhodolith and the water column was 0.071±0.0125 (standard error), much smaller than the difference prior at 0.3. Under dark conditions, the pH on the surface was even lower than the water column, on average, at -0.01±0.005 standard error (Figure 5, 6).

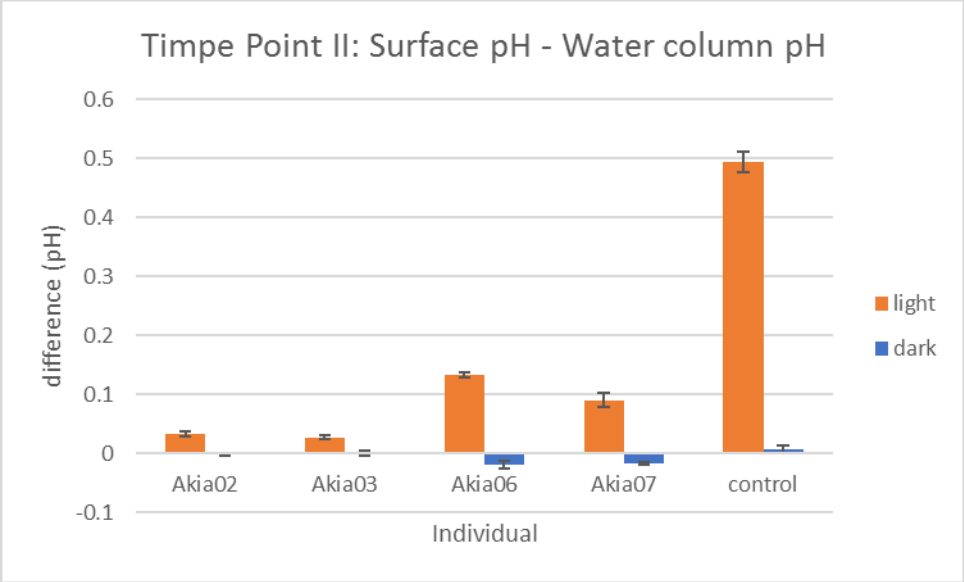

Figure 5*The difference between the pH on the surface of the rhodolith and the water column in light (orange) and dark (blue) conditions after exposure to darkness with standard error bars. The "Control" corresponds to individual Akia04, exposed to continuous light.*

The figure below shows the contrast between the initial differences in pH under the different light conditions and the differences after exposure to darkness for twenty days. The initial difference in pH under light saturation of the control individual, Akia04, (0.155) was much smaller than after exposure to light for twenty days (0.493) (Figure 5, 6).

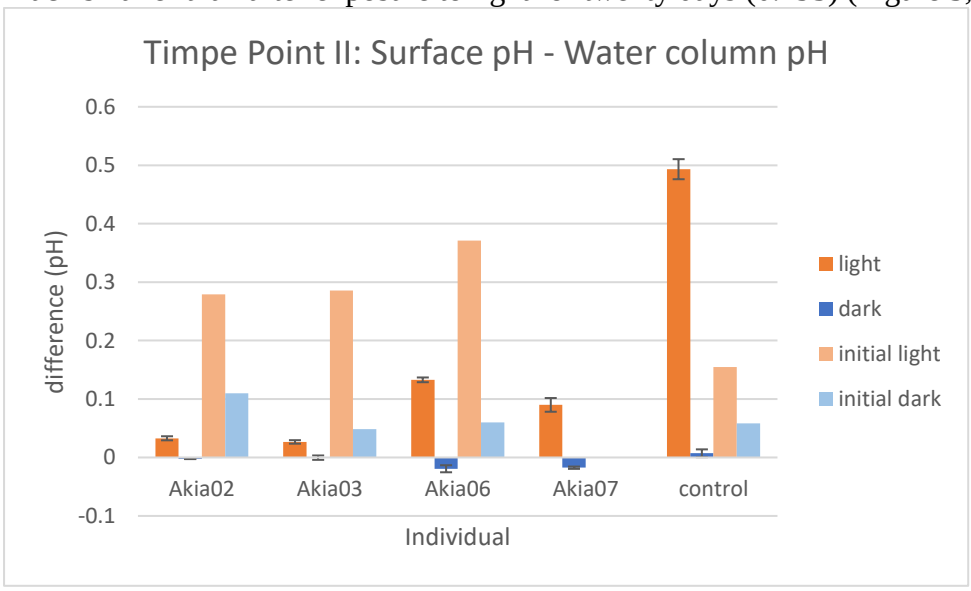

Figure 6 Difference in surface pH and water column pH of individuals after exposure to continuous darkness and the control after exposure to continuous light) and the differences measured before the twenty days. The difference under light saturation is in orange, and the difference under dark conditions is in blue. The initial differences are the lighter shades of the orange and blue.

After exposure to darkness for twenty days, due to time constraints, roughly 20-40 minutes were waited for individuals of the darkness treatment to adjust to light before beginning the light profile measurements. Thus, the recording of the light profiles began before the surface pH fully stabilized. Figure 7 displays the variation in surface pH of the algal individuals Akia02, Akia03, and Akia06, as they adjusted from dark to light conditions after exposure to darkness for twenty days. At an average rate of 0.0001 pH increase per

second, the surface pH of Akia02 appeared to generally increase over time suggesting that the surface pH was not completely stable when the light profiles began (Figure 7, Table 1).

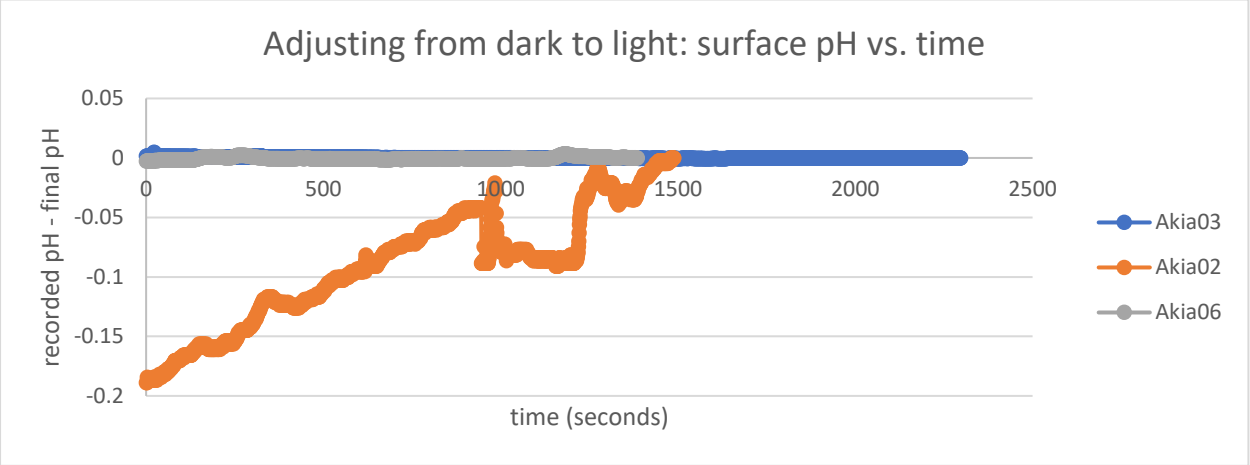

Figure 7 The surface pH of the algal individuals Akia03, Akia02, and Akia06 as they adjusted to light conditions after exposure to darkness for twenty days. The pH measurements were standardized between the algae for comparison purposes by subtracting the final surface pH value before the profiles began from the recorded value.

Table 1. Rate of change in pH over time (seconds) as individuals Akia02, Akia03, and Akia06, adjusted to light saturation after exposure to darkness for twenty days.

Individual	ΔpH s^{-1}
Akia02	0.0001
Akia03	-8.00E-07
Akia06	1.00E-06

Zooming in on the surface pH of Akia03 and Akia06 by plotting without Akia02 reveals that the surface pH of Akia06 increased and decreased, but never fully stabilized. Its rate of change in pH per second recorded was $1x10^{-6}$ increase in pH per second (Table 1). Meanwhile, the surface pH of Akia03 began slightly decreasing and then slowly increased (Figure 8). Its general rate of change in pH per second recorded was negative, albeit very small at $-8x10^{-7}$ decrease in pH per second (Table 1). Perhaps, the surface pH of Akia03 may have continued to increase if a longer waiting period was had before beginning the light profiles. At these very slow rates of change, perhaps days may have been necessary for the surface pH to fully stabilize.

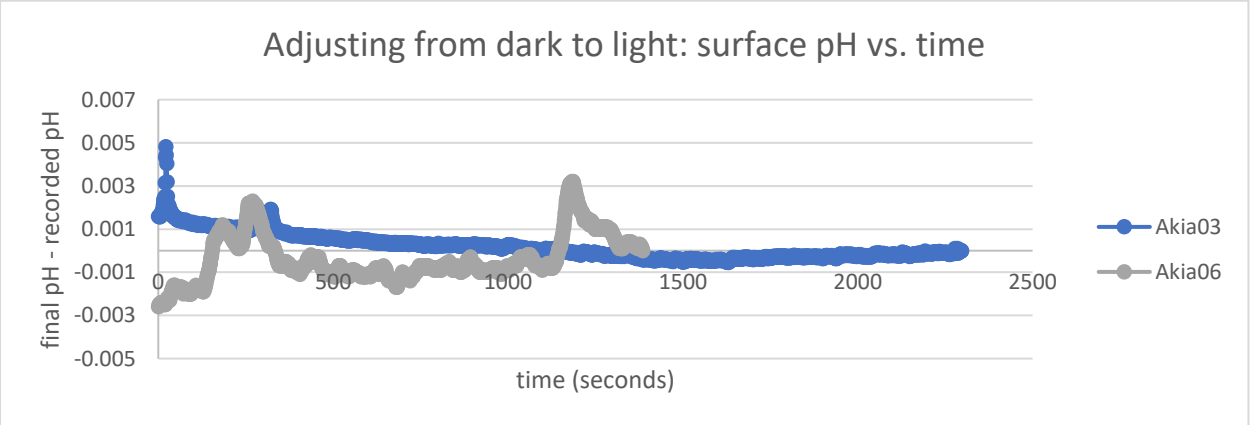

Figure 8 The surface pH of algal individuals Akia03 and Akia06 as they adjusted to light conditions after exposure to continuous darkness for twenty days. The pH measurements were standardized between the algae for comparison purposes by subtracting the surface pH from the final value recorded before beginning the light profiles.

Due to time constraints, the surface pH of the control individual, Akia04, also did not fully stabilize between the light and dark profiles after exposure to continuous light for twenty days. Its surface pH continued to decrease over time decreasing $-1x10^{-5}$ pH units per second (Figure 9, Table 1).

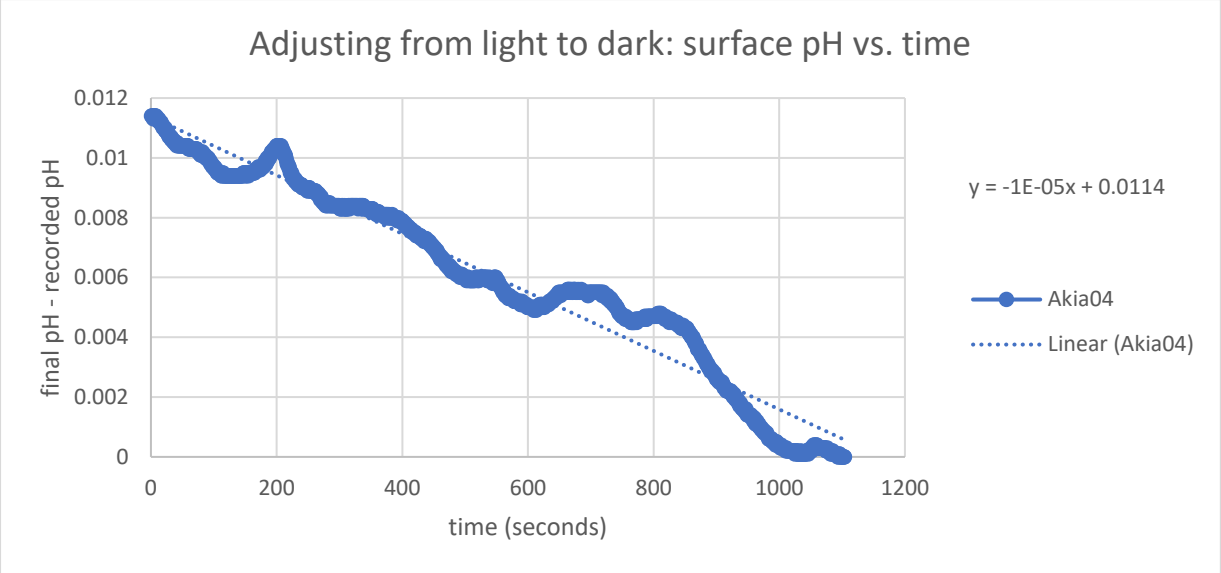

Figure 9 The recorded pH minus the final pH recorded on the surface of the control individual, Akia04 over time, before beginning the dark profile measurements, after exposure to continuous light for twenty days.

DISCUSSION

Overall, the difference between the surface pH of the *Lithothamnion glaciale* individuals and the water column was much lower when the algae were exposed to darkness over 20 days. In fact, under dark conditions, the average surface pH of the algae was -0.01 lower than the water column (Figure 5). In addition to the respiration of an algal itself accounting for this observation, the presence of microbial communities on the surface of the algae could be contributing to this lower pH.

Coralline algae have been found to harbor diverse bacterial communities distinct from the water column and from other species (Cavalcanti et al 2014, Huggett et al 2006, Lewis et al 1985). Many of these microbes found are heterotrophic, including aerobic ammonia-oxidizing betaproteobacteria and dissimilative sulfate-reducing deltaproteobacteria. The release of carbon dioxide from respiration of these microbes and the algae may keep the pH low, despite possible use of an ion pump to drive calcification.

When saturated with light, the surface pH of the algae treated with darkness was higher than the water column, albeit not as high prior to darkness treatment (Figure 6). A possible explanation could be that when sufficient light is present, the algae photosynthesize in combination with calcification at a faster rate than the microbes respire, resulting in a net increase in pH. However, after exposure to darkness for long periods, the increase in surface pH is possibly more gradual than was measured in this experiment of roughly 20-40 minutes until stabilization (Figures 7 & 8). In the dark, the algae may be utilizing a variety of adaptation mechanisms that require time to change. Freiwald and Heinrich (1994) observed the production of starch granules by *L. glaciale* in the summer, suggesting the algae store carbon there in the summer to be used as a carbon source when photosynthesis is inactive in the winter. Additionally, thin, dark bands of growth were observed in the winter, suggesting overall growth is reduced in the winter compared to the summer. Downregulation and

upregulation of particular enzymes and genes may account for this seasonal pattern of growth. Changing the expression of these genes may take longer than the 20-40 minutes waited in this study.

The varying rates of stabilization between the different algal individuals in this study may be due to differences in microbial communities and densities and epibiont communities. Huggett and colleagues found varying bacterial abundance and densities between different coralline algae individuals (2006). Akia02 may have fewer surface microbes or epibionts than Akia03 and Akia06, enabling its surface pH to rise more quickly under light saturation (Figure 7).

However, to fully understand the dynamics of calcification in the dark, other parameters related to coralline algae could be measured. To quantify the changes in the dynamic carbonate system surrounding the algae, the pH and carbonate ion measurements are needed. During calcification, carbonate ion concentrations have been found to be higher on the surface of stromatolites. As the carbonate ions are being used during calcification, the equilibrium of inorganic carbon in the water shifts, resulting in a higher concentration of carbonate on the surface than the water column (de Beer et al 2008). Additionally, one could use calcium concentrations with pH measurements to analyze the occurrence of calcification. de Beer and colleagues (2008) have also found calcium concentration is lower on the surface of calcifying organisms than that of the water column.

Future studies could also include a bacterial analysis of the rhodolithic algae. Some heterotrophic bacteria have been found to induce precipitation of calcium carbonate through non-targeted physiological processes that increase carbonate alkalinity (Knorre and Krumbein 2000). Considering that these algae host specific microbial communities, some members may provide benefits, such as enhancing calcification, possibly in the dark.

In contrast to the darkness treatment, the surface pH of the algal individual Akia04 exposed to continuous light for 20 days was much higher in the water column, as measured in the light saturation profiles (Figure 5). This elevated pH was 0.338 pH units higher than its surface pH prior to the light treatment, when exposed to an 18:4 hours light:dark cycle. This considerable elevation in pH supports the ideas presented by Fankignoulle and Henrich (1994) that the algae produce storage granules of carbon in the continuous light of the high Arctic summer to utilize in the continuous darkness of the high Arctic winter. Additionally, the significantly thicker, whiter bands of calcite they found to be produced by the algae during the summer, suggests calcification rates by the algae are greater in the summer. However, more ion data would be needed to connect the observed increase in surface pH in the light to an increase in calcification.

In summary, pH data alone cannot explain observed calcification of the Arctic rhodolith-forming algae *Lithothamnion glaciale* during the continuous darkness of the high Arctic winter. The surface pH of the individuals exposed to continuous darkness matched the pH of the water column in the dark, which does not support the theory that a decrease in surface pH due to photosynthesis induces algal calcification. Further studies including additional ion sensors and microbial analyses could shed light on the mechanisms driving calcification by Arctic rhodolith-forming algae in the dark.

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