Fall 2018

The effects of seawater temperature on photosynthesis in Crustose Coralline Algae

Alexander Carlson

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The effects of seawater temperature on photosynthesis in Crustose Coralline Algae

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Major: Economics

Submitted in partial fulfillment of the requirements for Australia: Rainforest, Reef, and Cultural Ecology, SIT Study Abroad, Fall 2018
ISP Ethics Review

(Note: Each AD must complete, sign, and submit this form for every student’s ISP.)

The ISP paper by Alexander Carlson (student) **does**/does _not_* conform to the Human Subjects Review approval from the Local Review Board, the ethical standards of the local community, and the ethical and academic standards outlined in the SIT student and faculty handbooks.

*This paper **does not** conform to standards for the following reasons:

Completed by: Tony Cummings

Academic Director: Tony Cummings

Signature: [Signature]

Program: ASE- Australia: Rainforest, Reef, and Cultural Ecology

Date: 30th November, 2018
Abstract:

Crustose Coralline Algae (CCA) are prolific reef builders and primary producers that play a key role in maintaining the structural integrity of coral reef systems (Littler and Littler, 2013). They are also highly important in increasing reef resilience by serving as a substrate for the recruitment and metamorphosis of coral larvae (Chisholm, 2000). Little is known about the way in which increasing seawater temperatures due to climate change might affect metabolic rates like photosynthesis in CCA. Therefore, a study was carried out in November, 2018, in the lab at Lizard Island Research Station to explore the photosynthetic performance of a species of CCA across a range of temperatures.

Photosynthesis and respiration rates for *Porolithon* sp., a particularly ubiquitous species of CCA among the coral reefs surrounding Lizard Island, were determined at six distinct incubation temperatures (22°C, 24°C, 26°C, 28°C, 30°C, and 32°C). Net photosynthesis was found to be the highest at 26°C, close to the 27°C annual average water temperature at Lizard Island (Daily Average Ocean Water Temperatures, 2018). Net respiration was found to be greatest at 28°C and gross photosynthesis was found to be greatest at 26°C. A 26% decrease in net photosynthesis and a 24% decrease in gross photosynthesis were observed from the determined optimal temperature (26°C) to the upper limit of the study (32°C). With increasing temperature (1°C/hr.), net photosynthesis was found to decrease as temperature increased, reducing to a value of zero μmol O₂ cm⁻² hr⁻¹ at 34°C. The significant decrease in primary productivity of *Porolithon* sp. observed in both assays suggests that projected elevation in seawater temperatures will likely negatively affect the primary productivity and fitness of CCA, which may have serious implications for its surrounding community.

Keywords: Crustose Coralline Algae, *Porolithon* sp., Photosynthesis, Respiration, Thermal Stress
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Acknowledgements:

First and foremost, I would like to thank Tessa Page and Ellie Bergstrom for their time, energy, and support as my ISP advisors. Their expertise and guidance made this study not only fruitful but also enjoyable. I would also like to thank Anne Hogget and Lyle Vail for their logistical assistance and for allowing me to conduct research at Lizard Island Research Station. I would like to thank Tony Cummings for his help in finding advisors and leading the program that made this ISP possible in the first place. Finally, I would like to thank my parents for supporting me in everything I do and making this semester abroad possible.
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1. Introduction

According to the business as usual carbon dioxide emission scenario, atmospheric concentrations of carbon dioxide are set to double between now and 2100 due to anthropogenic activity (Kuffner et al., 2007). The projected rise in atmospheric carbon dioxide concentrations for this coming century poses a major threat to coral reefs worldwide. One of the main challenges that coral reefs face as a result of increasing atmospheric carbon dioxide levels is the projected rise in average seawater temperatures of 1.8°C to 4°C by 2100 (Webster et al., 2010). Significant degradation of coral reefs as a result of more frequent and intense heat waves is likely to occur unless rapid progress is made in the Paris Climate Change Agreement. If action is not taken soon, most warm-water coral reefs along with their economic, social, and cultural benefits are projected to be eliminated by 2050 (Hoegh-Guldberg, 2017).

CCA are a ubiquitous macroalgae that occupy the entire photosynthetically viable depth range and occur in latitudes from polar to tropical regions (Chisholm, 2000). They are often dominant components of tropical coral reef systems where they play an integral role in promoting reef stability, resilience, and colonization by facilitating the recruitment and survival of coral larvae (Littler and Littler, 2013). A study carried out by Sargent and Austin in 1954 also demonstrates their importance in contributing to primary productivity on coral reefs. They found that the rate of oxygen production of *Porolithon* sp., the CCA species of interest in this study, was in a similar range to that of corals that generally receive the most credit for primary productivity on reefs (Marsh, 1970).

Previous studies have explored the effects of changing seawater temperature on various biological processes in CCA. A study performed by Webster, et al., in 2010 investigated the effects of elevated seawater temperature on microbial biofilm communities living on CCA and
the implications a shift in their structural compositions would have on coral larvae recruitment. After being kept at 32°C for 7 days, the CCA showed clear signs of bleaching and the makeup of biofilm communities on their surfaces had significantly changed. A 50% reduction in the ability for coral larvae to settle on these altered surfaces was observed at 32°C. Another study from 2016 explored ways in which high seawater temperatures affect calcification rates of CCA (Tanaka et al., 2016). The study found that calcification rates were unchanged at 30°C, but drastically decreased to negative values, or dissolution, at 32°C. A third study found that CCA are more sensitive to photodamage under elevated temperatures (Vásquez-Elizondo and Enríquez, 2016). Increased temperature left the samples in this experiment even more vulnerable to photodamage than did increased acidity in their aquaria. Although, it has been recorded that CCA are sensitive to changes in temperature, specifically increases in temperature, there have been no studies defining the upper, lower, and optimal photosynthetic temperatures for tropical CCA. Additionally, no studies have examined the effect of steadily increasing temperature on CCA, mimicking that of a day on a reef flat under current and predicted climate change scenarios. However, studies examining temperature and photosynthesis have been done on terrestrial plants, finding that photosynthesis in pea plants is particularly sensitive to variations in temperature and decreases in response to extreme temperatures (Sharkey 2005).

To explore the effects of temperature on the photosynthetic abilities of CCA, a two-part study was conducted in November of 2018 at the Lizard Island Research Station, Lizard Island, Great Barrier Reef, Australia. The study consisted of a point-temperature test and a temperature ramp test. Both methods involved the incubation of four samples of Porolithon sp. at different temperatures in a controlled lab environment. Porolithon sp. was chosen as the focus species of this study because it is the most abundant species of CCA on shallow offshore reefs like the ones
surrounding Lizard Island (Harrington, 2004). Average monthly seawater temperatures at Lizard Island range from 24°C to 28°C. However, temperatures of 31-32°C can be characteristic of extreme warming events that often result in marine organism bleaching (Daily Average Ocean Water Temperatures, 2018). Considering this data, the temperatures chosen to be tested in this study ranged from 22°C to 32°C for the first method and from 26°C to 34°C for the second method. The temperature ramp was carried out from 26°C to 34°C to get a more detailed look at the decrease in photosynthesis caused by heat stress. Oxygen production and consumption were recorded during incubations so that photosynthesis and respiration in *Porolithon* sp. could be compared across a range of temperatures. Photosynthesis is one of the most important biological processes in promoting fitness and primary production in CCA, so the way in which photosynthesis is affected by heat stress is important to understand as it could have significant implications on the future health of our marine ecosystems (Comeau et al., 2016). We believe that *Porolithon* sp. will photosynthesize optimally at a temperature close to the average annual water temperature at Lizard Island (27°C), and that above this temperature photosynthesis will decline.
2. Materials and Methods:

*Biological samples*

Eight individual crusts of *Porolithon* sp. were collected from a reef located between South Island and Palfrey Island, Lizard Island National Park (14°41’48.2”S; 145°27’03.2”E). Samples were collected at 2.5 meters on November 7th, 2018, 48 hours before the commencement of the experiment. The hammer-and-chisel technique was employed to break algal crusts from the reef substrate (Marsh, 1970). Samples were transported to the laboratory at Lizard Island Research Station where they were cut and cleaned to remove all unwanted epibionts and excess substrate. Once the organisms were isolated, they were placed outside in a plastic aquaria with a constant flow of ambient seawater drawn directly from the ocean until they were needed for incubations.

*Figure 1: CCA collection site circled in red. (Satellite image courtesy of Google Maps)*
Method 1:

24 hours prior to the first incubations, all four organisms were moved indoors to a temperature controlled plastic aquaria with a light intensity of 250 μmol quanta m\(^{-2}\) s\(^{-1}\). This light intensity was kept constant for the organisms throughout the entire experiment, including incubations. Light was measured with a LI-COR LI-1500 light meter. Water in the temperature-controlled aquaria was changed at least once daily to maintain a constant salinity of 35 parts per thousand (ppt).

The first method used to track the photosynthetic change in *Porolithon* sp. was a point-temperature test. In this test, photosynthesis and respiration rates of *Porolithon* sp. were recorded at six temperatures over the course of six days by measuring percent oxygen changes in the light and in the dark. Each day, photosynthesis and respiration of each organism was measured at a different temperature. The incubations on day one were performed at 24°C, day two at 26°C, day three at 28°C, day four at 30°C, day five at 32°C, and day 6 six at 22°C.

Incubations were carried out in a fixed temperature bath fashioned with a Julabo Corio CD heater that maintained a steady water temperature and circulation in the bath. Individuals were placed in acrylic metabolic chambers that remained partially submerged in the water bath for the duration of each incubation. An empty “blank” metabolic chamber was also included to account for any extraneous bacterial or other microbial respiration that could affect dissolved oxygen measurements.

Each metabolic chamber was fitted with a stir bar as well as temperature and oxygen dipping probes. These probes were attached to a 10 channel PreSens oxygen meter and changes in dissolved oxygen could be observed and graphed on PreSens Measurement Studio 2 in real time. Before every incubation, the probes and oxygen meter were calibrated to 100% oxygenated
water as well as anoxic water. The 100% oxygenated water was prepared by bubbling water, and the anoxic water was prepared by dissolving sodium sulfite in water. Chambers containing the samples were sealed with zero head space, avoiding bubbles that might disrupt oxygen or temperature readings. All components except the fiber optic cables attached to each chamber were contained within the water bath.

Photosynthesis was measured by tracking percent oxygen in the water of each metabolic chamber over time. Measurements of temperature and percent oxygen were taken every minute until each individual had increased the oxygen content in its chamber by more than 5%.

After photosynthesis incubations were completed, the system was covered with black tarps and lights were turned out in the laboratory to prepare for respiration incubations. Organisms were then left in the dark for 30 minutes before beginning to record dissolved oxygen change.

Once both incubations were complete, CCA samples were removed from their metabolic chambers and placed back into the temperature controlled tank, which would be heated to the next day’s temperature at that time. This gave the CCA time to acclimatize to the new temperature. Finally, the volume of water held in each chamber with and without the sample was recorded.

Before any analysis was carried out, the first 15 minutes from each data set was removed. Graphs of percent oxygen in the water over time were produced for each sample in the net photosynthesis and net respiration incubations. These graphs had nearly linear slopes allowing for accurate best fit lines to be taken and used to convert percent oxygen in the water to micromoles of oxygen produced or consumed per centimeter squared per hour (µmol O₂ cm⁻² h⁻¹). To do so, the surface area of living tissue on each *Porolithon* sp. sample had to be calculated. This was
done using the foiling technique (Comeau et al., 2016). Aluminum foil was wrapped over the
surface of each sample and pressed to mold into all depressions and features. The foil was
trimmed around the edges to match the shape of the living surface area and then weighed.
Weights produced from this process were then plugged into a function of plotted weights of
known surface areas of the same aluminum foil to convert weight to surface area. Values for the
solubility of oxygen in seawater were also needed and were gathered from the “oxygen solubility
at different temperatures and salinities of seawater” table made by Unisense (Ramsing and
Gunderson, 2018). Furthermore, the slopes of percent dissolved oxygen over time were corrected
with data collected from the “blank” control that was run at every temperature.

Gross photosynthesis was then calculated by adding net photosynthesis and net
respiration. This isolated the total amount of oxygen produced by photosynthesis by accounting
for the oxygen that was consumed by respiration in the net photosynthesis incubation.

Method Two:

The second incubation method was a gradual temperature ramp over the course of one
day. Four Porolithon sp. crusts that had not been incubated yet were placed in sealed metabolic
chambers with oxygen and temperature probes and placed into a water bath. A “blank” chamber
was also included in this incubation. Rather than being incubated until a 5% change in dissolved
oxygen was observed, the samples were incubated as the temperature in the water bath increased
from 26°C to 34°C. The temperature was increased by 1°C every 45 minutes, resulting in a 6.5
hour incubation period. Percent dissolved oxygen in the water was measured every minute and
graphed over time for the four samples. When the incubation was over, the volume of each
metabolic chamber was measured with and without samples. Due to the extended duration of this
incubation, oxygen and temperature probes were prone to collecting bubbles as samples produced gasses. Occasionally, probes had to be shaken to remove these bubbles.

Net photosynthesis in method two was calculated by analyzing the slope of the percent oxygen over time graph for each sample at every whole number temperature in the tested range (26°C, 27°C, 28°C, 29°C, 30°C, 31°C, 32°C, 33°C, and 34°C). 10 minutes of data centered around each temperature change was analyzed for best fit slope. Slopes were then converted to oxygen production rates using the previously described technique. Oxygen production rates were averaged and plotted. Lastly, students t-tests were run on all data collected to determine statistical significance.
3. Results:

Method One:

Data for net photosynthesis was compiled, analyzed, and graphed (Figure 2). Net photosynthesis in *Porolithon* sp. increased with temperature until an optimal temperature of 26°C was reached, at which point it began to decrease. At the optimal temperature, the four samples produced an average of 2.56 µmol O₂ cm⁻² h⁻¹ with a standard error (SE) of ± 0.213. The lowest value for net photosynthesis in the tested temperature range was 1.89 µmol O₂ cm⁻² h⁻¹ at 32°C. A 26% decrease in net photosynthesis was observed from the determined optimal temperature (26°C) to the upper temperature limit of the study (32°C). Furthermore, a four-degree warming from the optimal temperature resulted in a 17% decrease in oxygen production whereas a four-degree cooling from the optimal temperature resulted in an 11% decrease in oxygen production. The decrease in net photosynthesis from 26°C to 32°C was calculated to be statistically significant within a 95% confidence interval (p = 0.02). The p-values for other temperature intervals can be seen in Table 1 in the appendix.

Figure 2. Net photosynthesis of *Porolithon* sp. at six temperatures. Points are averages of all individuals (n = 4), and error bars represent standard error.
Net respiration data was compiled, analyzed, and graphed (Figure 3). Net respiration for *Porolithon* sp. increased with temperature until a maximum was reached at 28°C. Above this temperature, respiration began to decrease. At 28°C, the four samples consumed an average of 1.577 µmol O₂ cm⁻² h⁻¹ with a standard error (SE) of ± 0.066. None of the temperature intervals for net respiration were statistically significant within a 95% confidence interval (p < 0.05), however the decrease in respiration from 28°C to 30°C and increase in respiration from 22°C to 28°C were statistically significant within a 90% confidence interval (p < 0.10) with p-values of 0.094 and 0.079 respectively. The p-values for other temperature intervals can be seen in Table 2 in the appendix.

Figure 3. Net respiration of *Porolithon* sp. at six temperatures. Points are averages of all individuals (n = 4), and error bars represent standard error.
Values for net photosynthesis and net respiration were added and graphed at each temperature to calculate gross photosynthesis (Figure 4). Gross photosynthesis increased with temperature until a maximum was reached at 26°C, after which it decreased with increasing temperature. At 26°C, oxygen production reached a value of 4.13 µmol O₂ cm⁻² h⁻¹ with a standard deviation of 0.44 and a standard error of 0.22. The lowest oxygen production measured occurred at 32°C where it equaled 3.13 µmol O₂ cm⁻² h⁻¹ with a standard error (SE) of ± 0.29. A four-degree warming from the optimal temperature resulted in a decrease in oxygen production by around 14% whereas a four-degree cooling from the optimal temperature resulted in a 12% decrease in oxygen production. Furthermore, the decrease in photosynthesis from 26°C to 32°C was found to be statistically significant with a p-value of 0.001. More temperature intervals were found to be statistically significant and all p-values can be found in Table 3 in the appendix.

Figure 4. Gross photosynthesis of *Porolithon* sp. at six temperatures. Points are averages of all individuals (n = 4), and error bars represent standard error.
Method 2:

Net photosynthesis data from the temperature ramp method was compiled and graphed (Figure 5). A value of 3.12 µmol O₂ cm⁻² h⁻¹ was observed at the previously calculated optimal temperature of 26°C. As temperature increased, net oxygen production decreased until it reached zero at 34°C. Consequently, a 100% decrease in photosynthesis occurred between 26°C and 34°C. The rate at which photosynthesis decreased seemed to fluctuate over the temperature range. The jump between 27°C and 28°C saw the highest rate of decrease across the whole graph. Most temperature intervals in this method were found to be statistically significant. All p-values can be found in Table 4 in the appendix.

![Figure 5. Net photosynthesis of Porolithon sp. from temperature ramp (26°C to 34°C). Points are averages of all individuals (n = 4), and error bars represent standard error.](image-url)
4. Discussion:

Significant decreases in photosynthetic activity were observed in both the temperature ramp and point-temperature incubations as a result of thermal stress. These findings support the study hypothesis as well as the findings of other studies that biological processes in CCA are affected by temperature change (Tanaka et al., 2016). Furthermore, this study aligns with the findings of Webster’s 2010 experiment in which CCA exhibited signs of stress at high temperatures (Webster et al., 2010). A 2017 study by Bach found that CCA under stable thermal conditions were larger and more abundant than those under thermally variable conditions. This suggests that CCA reallocate their resources and energy as a result of thermal stress (Bach, 2017). It is possible that the decrease in photosynthesis observed at high temperatures is a mechanism of this reallocation. Further research could be done to analyze the specific distribution of resources in thermally stressed CCA.

A four-degree warming from the optimal temperature resulted in a decrease in net photosynthesis by around 17% whereas a four-degree cooling from the optimal temperature resulted in an 11% decrease in net photosynthesis. This suggests that seawater warming has a more negative impact on *Porolithon* sp. than does cooling within this tested temperature range.

The results from the temperature ramp incubation suggest that the threshold for maintaining positive oxygen production in CCA lies around 34°C, as this is where net photosynthesis reached a value of zero. If the temperature ramp were to be continued, net photosynthesis would most likely be seen to go negative. When net photosynthesis goes negative, oxygen consumed by respiration becomes greater than oxygen produced by photosynthesis (Pacella et al., 2018). When this happens, more carbon dioxide is produced by the organism than is consumed. A net positive release of carbon dioxide can decrease the pH of a
marine organism’s immediate microenvironment (Pacella et al., 2018). Through this process, an increase in atmospheric carbon dioxide can theoretically cause a positive feedback loop on ocean acidification in marine ecosystems.

The average annual seawater temperature at Lizard Island is 27°C (Daily Average Ocean Water Temperatures, 2018) and it was determined in this study that the optimal seawater temperature for the photosynthetic activity of Porolithon sp. is 26°C. This suggests that Porolithon sp. at Lizard Island already photosynthesizes at a temperature above its optimal for a large portion of the year (more than five months). As seawater temperatures around the island are projected only to rise, it is likely that we will see a decrease in the primary productivity and fitness of Porolithon sp. in coming years.

Another species of CCA, Sporolithon sp., was originally included in the incubations of this experiment. Sporolithon sp. is a slightly darker shade of reddish-purple and is generally found at lower depths than Porolithon sp. (Gomez-Lemos and Diaz-Pulido, 2017). This species had to be omitted from the study because the data became unusable for a number of reasons that are worth discussing. Frequently, dissolved oxygen content in the Sporolithon sp. chambers would not change more than 5% during incubations. There were also cases of some Sporolithon sp. consuming more oxygen than they released, creating a downward sloping percent oxygen in water vs. time graphs, while other individuals in the same incubation photosynthesized as expected. The highly variable data collected from Sporolithon sp. could have been a result of human error but was likely not as the data collected from Porolithon sp. was consistent. One possible explanation for this variability is that the Sporolithon sp. samples were significantly more stressed than the Porolithon sp. samples. This would follow as Sporolithon sp. is found in
calmer, cooler microhabitats than *Porolithon* sp. and the shock of being collected and stored in artificial aquaria might have caused them to behave unusually.

This study had a number of limitations. The sample size (n=4) was low and could have been increased to reduce the error and variation found in our results. Additionally, increasing the sample size would make the results more statistically robust and define the trends seen in the data. Although the incubations ran in this experiment provide valuable insight as to how CCA may react to fluxes in seawater temperature, in reality, these increases will happen gradually and not over the course of a few days. It is difficult to perfectly model the time scale at which the increase will occur, but it would be valuable to carry out a longer-term study with more gradual increases in temperature. Another limitation of this study was that temperatures for method 1 were tested in two-degree increments starting from 22°C. Although it was concluded that that the optimal temperature for photosynthesis in *Porolithon* sp. was around 26°C, 27°C and 25°C were not tested and could therefore be the actual optimal temperatures.

5. Conclusion

Two experiments were carried out to better understand the way in which elevating seawater temperatures might affect primary productivity and fitness of CCA. Both the temperature ramp and point-temperature incubations resulted in a significant decrease in photosynthetic activity with increasing water temperature. In method 1, a 26% decrease in net photosynthesis and a 24% decrease in gross photosynthesis were observed from the calculated optimal temperature (26°C) to the upper limit of the study (32°C). In method 2, a 100% decrease in net photosynthesis was observed from 26°C to 34°C. Furthermore, within the temperature range tested, heat stress proved to adversely affect photosynthesis to a greater degree than did stress from cooler
temperatures. *Porolithon* sp. around Lizard Island is already operating at a seawater temperature above its optimum for a large portion of the year. Further increases in seawater temperature as well as the increased frequency and severity of heat wave events will likely cause a decline in photosynthesis and therefore primary productivity in *Porolithon* sp., having serious implications on the health of surrounding coral reef ecosystems.
6. References:


doi:10.4319/lo.2000.45.7.1476


Vásquez-Elizondo, R. M., & Enríquez, S. (2016). Coralline algal physiology is more adversely affected by elevated temperature than reduced pH. *Scientific Reports, 6*(1). doi:10.1038/srep19030


7. Appendix:

Table 1: p-values of temperature intervals for net photosynthesis in Method 1.

<table>
<thead>
<tr>
<th>Interval</th>
<th>p-value</th>
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<tr>
<td>22 vs 24</td>
<td>0.27864409</td>
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<td>24 vs 26</td>
<td>0.28986075</td>
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<td>26 vs 28</td>
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<td>28 vs 30</td>
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Table 2: p-values of temperature intervals for net respiration in Method 1.

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Table 3: p-values of temperature intervals for gross photosynthesis in Method 1.

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Table 4: p-values of temperature intervals for net photosynthesis in Method 2.

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