Regional thermal optimization of *Astrangia poculata* and *Oculina arbuscula*.

Ved Ahuja, Gaby Bussiere, Jeremy Feng, and Natalia Karadimitriou

Abstract

Climate change poses an imminent threat to the marine environment and is predicted to cause thermal extremes. We aim to understand if there is a correlation between adaptations of *Astrangia poculata* and *Oculina arbuscula* populations and their thermal performances under cold temperature stressors. This study wants to determine how colonies vary in thermal performance based on different geographic locations. The dark respiration and net photosynthesis of the corals were measured in a cold ramp using oxygen probes in respiration chambers, and we used photo analysis to assess coral status before and after the experiment. Our results showed that different populations of the corals had different respiration and net photosynthesis at different temperatures, and that the thermal performance curve of each population did not have a significantly different overall shape. Specifically, corals at higher latitudes had higher net photosynthesis at lower temperatures. By better understanding the regional differences in thermal performance, we can better focus our conservational efforts on coral communities in less variable climates in order to mitigate the effects of climate change in these more vulnerable populations.

1. Introduction

1.1 Climate change and the Effects of Changing Temperature on Coral Bleaching

Human activity in the past century has tremendously increased the concentration of greenhouse gases being released into the atmosphere, causing significant warming of the planet (Shaftel, 2021). The ocean has absorbed about 90% of the heat of anthropogenic global warming, and as a result we have seen devastating impacts on its organisms and ecosystems (Laura et al., 2021). There are now consistent marine heatwaves, which is when ocean temperatures are extremely warm for an extended period of time (Wernberg et al., 2021). Marine heatwaves are a large cause of the degradation and mortality of distressed coral reefs; these heat wave events can trigger mass coral bleaching (Anton et al., 2020). Coral bleaching describes the loss of some or all of a coral's symbiotic algae and photosynthetic pigments. Corals have a history of bleaching in response to environmental stressors, but recent ocean warming has caused consistent and large-scale bleaching events (van Oppen & Lough, 2009). Species have evolved different mechanisms to cope with spatial and temporal temperature variability (Jurriaans & Hoogenboom, 2019), but the role of ocean warming in relation to Scleractinian corals and their algal endosymbionts has not been studied enough (Anton et al., 2020).

1.2 Coral Symbiosis

A main reason why reef-building corals are so sensitive to temperature is due to their endosymbiotic relationship with dinoflagellates (Silbiger et al., 2019).

Scleractinian corals construct calcium carbonate skeletons, and symbiotic dinoflagellate algae will then integrate itself into their skeleton, establishing a zooxanthellae symbiosis (van Oppen et al., 2009). Under stressful conditions, the corals tend to lose their symbionts through bleaching, by undergoing expulsion of the zooxanthellae (Brennan et al., 2021). Most coral species exhibit obligate symbiosis (where they need symbionts to survive) but there are some temperate corals that maintain facultative symbiosis, resulting in a natural variability in their symbiont density (Dimond & Carrington, 2008). This form of symbiosis means that each colony can be symbiotic, aposymbiotic (without the zooxanthellae), or a mixture of both (Brennan et al., 2021). These types of corals provide researchers with the opportunity to examine zooxanthellae division and expulsion at a range of temperatures and symbiont densities (from no symbionts to a high density of them), giving us insight into their behavior and health at certain levels (Dimond & Carrington, 2008).

1.3 *Astrangia Poculata* and *Oculina arbuscula*

Astrangia poculata and *Oculina arbuscula* are both scleractinian corals that maintain facultative symbiosis. *A. poculata* is a coral species that has a broad distribution in waters along the northwestern Atlantic (Dimond & Carrington, 2008). Due to its latitudinal range, it is extremely resilient and can naturally withstand a wide range of water temperatures (Brennan et al., 2021). *Astrangia poculata* are model organisms due to their tolerance to both cold and warm water and its facultative relationship with their dinoflagellate algae (Brennan et al., 2021). Understanding how the coral, with and without symbionts, can tolerate a wide range of temperatures helps us understand what will happen in the future as climate becomes more variable and the world experiences both "higher high temperatures and colder cold temperatures" (Brennan et al., 2021). Variations in environment characteristics can cause adaptive differentiation across a species' range of habitats (Aichelman et al., 2019), but it is still unknown if species with these broad geographical distributions have subpopulations of locally adapted thermal specialists, or are thermal generalists that can perform well across a range of temperature (Jurriaans & Hoogenboom, 2019). *Astrangia poculata* and *Oculina arbuscula* are temperate scleractinian corals that offer us a unique opportunity to observe the role of environmental adaptation in coral physiological tolerance limits (Aichelman et al., 2019).

1.4 Comparing thermal performance of coral populations through TPCs

An effective way to understand how different populations will respond to increasing temperatures and potentially predict their response to climate change is through thermal performance curves (TPC). Thermal performance curves show how temperature may impact performance of organisms whose body temperature regulation depends on external sources (Rezende & Bozinovic, 2019). This is accomplished by quantifying the relationship between environmental temperature and the biological rates of performance of these organisms (i.e.,

respiration, photosynthetic activity, or growth) (Silbiger et al., 2019). These performance curves tend to present the same shape, where performance increases with temperature until it reaches a thermal optimum (T_{opt}), and then abruptly decreases (Aichelman et al., 2019). This increase and then sharp decrease is represented by the slope and rate of activation energy (Anton et al., 2020).

Jurrians & Hoogenboom (2019) used TPCs to understand the thermal performance of other species of scleractinian corals (*Porites cylindrica* and *Acropora* spp.) to characterize how their latitudinal distributions might affect the photosynthetic efficiency of their algal symbionts. They measured photosynthetic rates and respiration rates to determine how well these corals performed when exposed to acute temperature changes, 5℃ above and below their local average temperature. They observed geographical variation but no correlation between the optimal performance temperatures (T_{opt}) and the local average temperature of the sites where corals were collected. However, they did conclude that symbiotic corals generally had a T_{opt} more similar to their experienced local average temperature, suggesting that symbionts play a strong role in a coral's plasticity to thermal stress. In our study, the thermal performance curves of *A. poculata* and *O. arbuscula* were used to compare respiration, photosynthesis activity, and their overall thermal performances between the different populations.

1.5 Point of Study

This study aims to answer two important questions: Do locationally different populations of *Astrangia poculata* and *Oculina arbuscula* have different thermal performance curves (TPC)? And if so, do the northern populations have a better performance at specific temperatures? This study will measure the metabolic rates (respiration and photosynthesis) of symbiotic and aposymbiotic *A. poculata* and *O. arbuscula* populations. This study is a continuation of PhD Candidate James Fifer's heat ramp experiment on *Astrangia poculata* at various temperatures. The goal is to now compare the thermal performances of *A. poculata* and *O. arbuscula* from different geographical sites under a cold ramp experiment to test if their resiliency and thermal performance is due to local environmental adaptations. In this experiment, the thermal performance curves of *Astrangia poculata* and *Oculina arbuscula* were measured and compared between the corals of different populations. Specifically, oxygen levels during dark respiration and net photosynthesis were measured; additionally, pigmentation changes from before and ~24 hr after the study, as well as the living surface area of each coral, were recorded with photo analysis.

Past studies have found that the thermal limits of corals are linked to their geographic location: Aichelman et al. (2019) wanted to determine if there was adaptation in the thermal physiology of *A. poculata* due to their geographical location, and if that would lead to "population-specific differences" in thermal performance. We are looking at *Astrangia poculata* and *Oculina arbuscula* because of their wide geographic

range and resiliency to temperature changes. This study is curious to see if their resiliency is due to adaptation to their local sites, or if they are naturally resilient despite geographic location. We hypothesize that populations of *Astrangia poculata* and *Oculina arbuscula* show regional differences in thermal performance, and that more northern populations will have a higher thermal performance at lower temperatures due to regional adaptations. Our null is that populations of *Astrangia poculata* and *Oculina arbuscula* do not show regional differences in thermal performance. Similar to Aichelman et al. (2019), when comparing TPCs from multiple populations of the same species across a thermal gradient, we expect that the thermal optima could be adapted from the local thermal environment, meaning that populations from colder sites will display an elevated phenotype (elevated performance, depending on the phenotype measured) compared with populations from warmer sites at cooler temperatures.

By studying the respiration and photosynthesis of *Astrangia poculata* and *Oculina arbuscula* from different latitudinal habitats at different temperature norms, we will be able to identify if latitudinal variation influences thermal performance. It is imperative to understand how a species may respond to future environmental change at large spatial scales, and to see if corals from warmer sites would still thrive the same if placed in a cooler region. All of this research holds promise to guide conservational efforts for these corals.

2. Materials and Methods

2.1 Study sites, coral collections, and coral health

Live *Astrangia poculata* and *Oculina arbuscula* were collected along the East Coast and Gulf Coast of the United States (Figure 1). The present work was done on *A. poculata* colonies that reside in six different locations: Texas, Florida, North Carolina, North Carolina Radio Island, Rhode Island, and Woods Hole, Massachusetts (Table 1). A site for *O. arbuscula* was chosen and collected from North Carolina Radio Island for this experiment. The sample method collection for the corals varied according to each site. The corals from Florida and North Carolina Radio Island were dredged, while the corals at every other site were collected by SCUBA divers using a hammer and chisel (Aichelman et al., 2019). All coral samples that were shipped to Boston University were either dry and wrapped with saltwater-soaked paper towels, or placed in plastic bags filled with oxygenated sea water. The samples were then turned into coral fragments and placed into a holding tank for at least a month before experimentation. The holding tank environment was maintained at a salinity of 35 ppt and a temperature of 18°C. Lights above the tank were at 30 units of photosynthetically active radiation (PAR). During the seven day experimentation period, the selected *A. poculata* and *O. arbuscula* fragments were not fed to standardize metabolic response during trials.

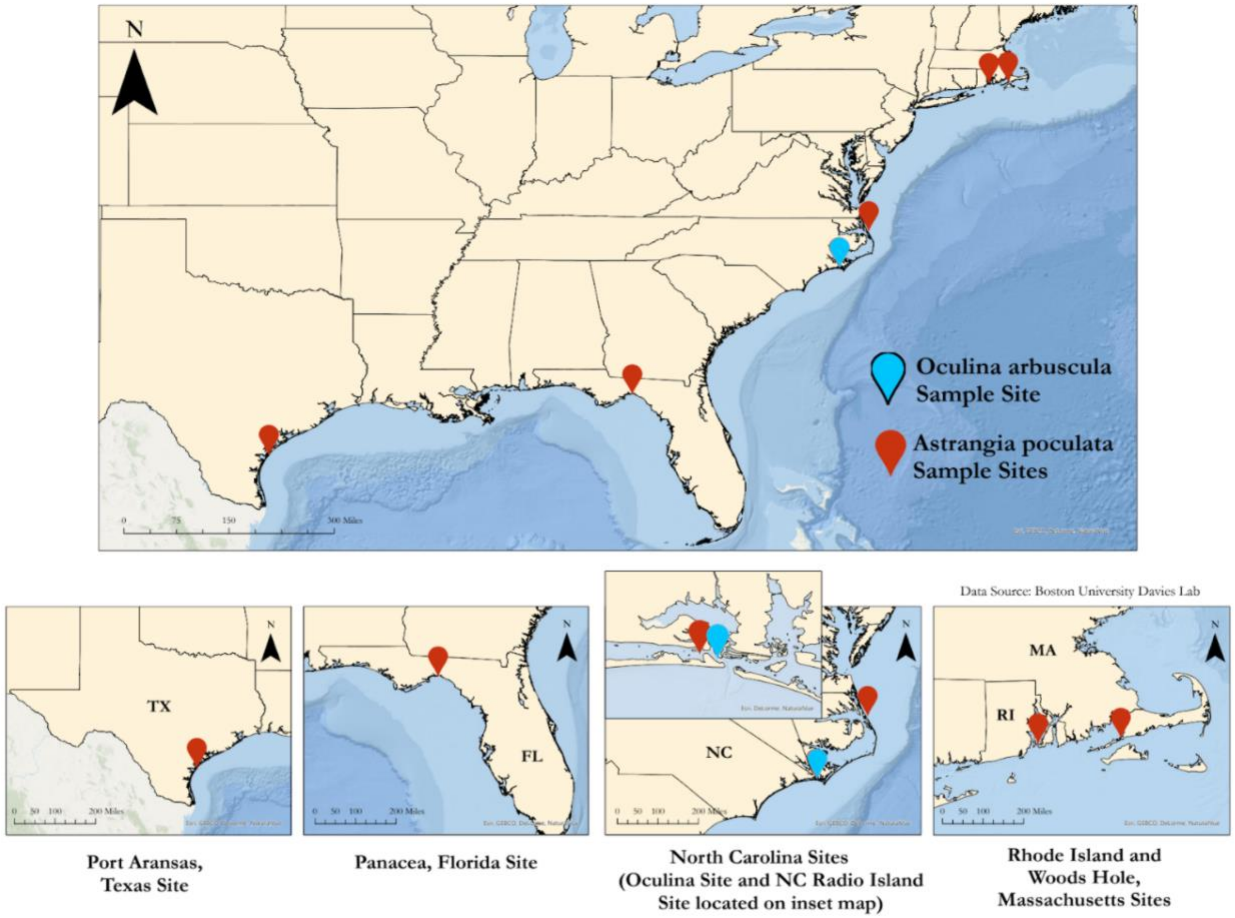

Figure 1: Collection sites for *Astrangia poculata* and *Oculina arbuscula.* Corals that were used in the experiment were collected from sites all over the Eastern Coast of the United States. States where *A. poculata* came from include: Texas, Florida, North Carolina, Rhode Island, and Massachusetts. One site from North Carolina collected a sample of *O. arbuscula*.

Site	Location
Great Harbor, southeast of Woods Hole, Massachusetts	41°31'28.5"N 70°40'22.0"W
Southeast of Jamestown, Rhode Island	41°28'32.6"N 71°21'34.1"W
Shipwrecks northeast of Kill Devil Hills, North Carolina	36°02'20.0"N 75°38'40.5"W
Money Island Bay, west of Radio Island, North Carolina	34°42'35.7"N 76°42'27.2"W
Morehead City Channel, south of Radio Island, North Carolina (*Oculina*)	34°42'15.9"N 76°41'04.8"W
Apalachee Bay, southeast of Panacea, Florida	30°02'56.7"N 84°04'48.5"W
Southeast of Port Aransas, Texas	27°49'45.2"N 97°02'35.6"W

Table 1: GPS Coordinates of the seven study sites where the corals were collected. One site in North Carolina collected *Oculina arbuscula,* while the other six sites collected *Astrangia poculata*.

2.2 Coral Sample Selection

Coral fragments were randomly selected from a large holding tank; nine fragments were selected from each site. Eight fragments from each site were used in the experiment with the ninth fragment being used as an extra in case of experimental errors. Each coral fragment was placed on a newly engraved tile and the coral fragments were then randomized into seven groups (each day for the seven day trial period), with 8 fragments in each respective group. For every experimental day, there was at least one coral fragment from each site along with an extra coral fragment in a fashion where all 8 coral fragments from each site were run over the course of seven days.

2.3 Preparation of Respiration Chamber

To house the corals during the experiment, a 10 slot chamber stand (Figure 2) in a large respiration chamber tank filled with seawater was used in the dark. Salinity was maintained between 32 and 34ppt throughout the experiment while temperature was monitored and adjusted using Neptune Systems' chiller, heater, and temperature probe which were connected to an Apex controller system. The apex system's temperature probe was calibrated to a glass thermometer (+/- 0.2 degrees). In order to measure the photosynthetic rate of corals, a Viparspectra AquaLight was hung above the respiration chamber and any external sources of light should be covered using garbage bags or an equivalent cover. The light was set to 60 units of photosynthetically active radiation (PAR).

2.4 Respiration and Photosynthesis Measurements

Corals selected for each experimental day were first placed onto chamber mounts and then into the chamber stand itself with stir bars; slots 5 and 10 of the chamber stand were always reserved for blanks (with the exception of day 1 where slot 6 was used as the blank), which were empty chambers with mounts used as controls for oxygen levels. Each experimental day involved rotating new groups of fragments on a site basis in a clockwise manner based on the order of the previous experimental day to account for placement within the chamber system. During setup, any air bubbles found within chamber stands were promptly removed to ensure accurate oxygen readings. PreSens oxygen and temperature probes were then inserted and secured into each chamber. The PreSens oxygen probes are fiber optic contactless O_2 sensors that analyze oxygen distributions and can follow a gradient development over time (*Optical Oxygen Sensors*, 2021). It is a pressure-resistant and polarization-free system that allows for contactless measurements of coral respiration. Corals respiration and photosynthetic rates were measured at four temperatures during a cold ramp: 18°C, 16°C, 14°C, and 12°C sequentially. At each temperature value, all lights were turned off so that the corals could acclimate to dark conditions for 20 minutes. Dark respiration rates were then measured over the course of 20 minutes using the PreSens program. Another 20 minute trial was run after this period, but with the lights above the tank turned on to stimulate photosynthesis. Coral respiration rates were measured while they

were photosynthesizing at each temperature value. The temperature of the tank was then changed to the next value using the Apex system. During this temperature transition interval, coral chambers were unscrewed to reestablish equal oxygen levels to all corals during the dark acclimation period. When the temperature of the tank reached the experimental temperature value, chambers were resealed and the process was repeated again, beginning with dark acclimation.

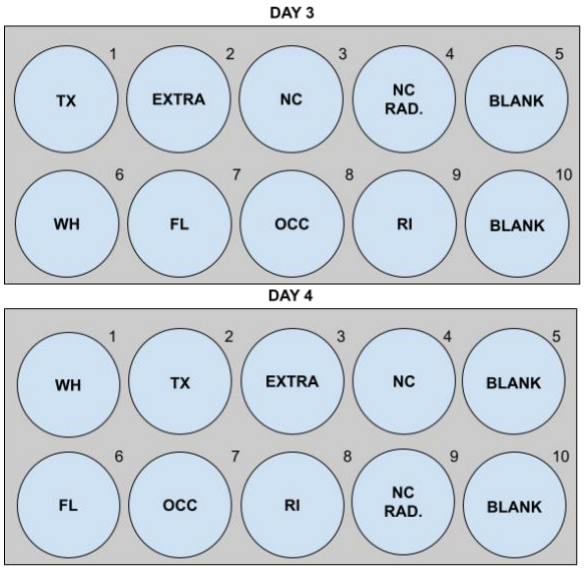

Figure 2: The experimental design set up and rotation scheme of the 10 slot chamber stand. Days 3 and 4 are displayed as an example. To minimize bias in data collection, chambers with corals were rotated each day in a clockwise fashion with respect to population.

2.5 Analysis of *A. poculata* and *O. arbuscula* Color

The full range of coral health was determined through photo analysis. For each experimental day, corals to be tested for that day were photographed prior to experimentation and corals tested the day prior had images taken about 24 hours following the end of their experimentation. These photos are designated as before and after photos respectively. Each photo was taken on a coral health rating chart. Images were taken using an Olympus waterproof camera mounted on a tripod under consistent lighting conditions. Once the photos were collected, a photo analysis was conducted; Photoshop was used to standardize light levels and a custom Davies Lab Matlab script was used to determine the average coral color intensity of corals before and after experimental treatment over 10 points. Differences in coral pigmentation were used as a proxy for shifts in coral health and symbiotic status.

2.6 Data and Statistical Analyses

The software R (R Core Team, 2021) was used to process and analyze our data into dark respiration, gross photosynthesis, and net photosynthesis, construct respiration and net photosynthesis thermal performance curves for each population, and conduct statistical analyses. The script was written by HM Putnam, adapted and edited by KH Wong, R Ju, and HE Aichelman, and provided by J Fifer. Edits and additions were made as needed to fit our data. Average changes in oxygen for empty chambers were used as baseline oxygen levels for each chamber during that temperature run. Blanks were evaluated before use as an oxygen baseline; successful blank chambers showed no net correlation but rather maintained a stable concentration of oxygen during experimentation. If a blank chamber demonstrated a correlation and/or did not maintain a stable oxygen concentration during a specific run, it was excluded and the

other blank was used alone instead averaging the two blanks. To assess the differences in metabolic activity between populations at the same temperature, we utilized a Wilcoxon signed-rank test with a false discovery rate (fdr) correction. Changes in oxygen concentration in relation to respiration or net photosynthesis were averaged by population and temperature, and a test was conducted for both respiration and net photosynthesis. To characterize and compare the overall shapes of each thermal performance curve, a linear regression was calculated for each individual fragment between temperature and oxygen concentration; the slopes of these regressions were then averaged by population. The differences between these slopes were then analyzed through a one-way ANOVA and grouped through Tukey's honestly significant difference test (HSD). This was also done for both respiration and net photosynthesis.

After photo analysis color intensity data has been collected from Matlab the difference in average R value (Red color value) of after photos and before photos were calculated in an effort to analyze how coral pigmentation is changing. For this study, an issue arose where the lighting conditions changed between days resulting in inconsistent lighting between photos of before and after photos. Shadows and glares which resulted from the inconsistent lighting led to inaccurate color intensity readings. As an alternative we attempted to determine if any of the coral populations' change in color intensity deviated from other sites. To do this, samples are first paired with every other sample whose before and after photos were taken on the same day. Then the difference between the two samples' average before treatment R values are calculated;The same process is repeated to calculate the difference in after treatment R values between the two samples. Using the software R normality and homoscedasticity were first tested for all population comparisons using shapiro tests and pairwise variance tests respectively . A two way ANOVA was then conducted to determine if there was statistical variation in terms of differences in R values between the population comparisons, before and after photos, and the interaction between population comparisons and the before/after status.This data was then visualized through the creation of a grouped box plot.

3. Results

3.1 Metabolic thermal response

The temperature performance curve evaluating respiration rates of the seven different coral populations demonstrate similar respiration rates across all of the populations with the exception of Florida. For the range of temperatures that we tested, the T_{opt} of each population fell between 16℃ and 18℃ for net photosynthetic activity.

Wilcoxon signed-rank tests compared the metabolic activity between populations at each temperature in the cold ramp. These tests revealed ten population pairings in the respiration data that were significantly different from one another and nine pairings in the net photosynthesis data showing statistically significant differences. In the measure of respiration at 12°C, the Florida population was significantly different from all of the other populations respiration rates. Similarly, at 18°C Florida was also statistically different from the respiration

rates of North Carolina, North Carolina Radio, Oculina, and Texas (Figure 3a). In the plot of net photosynthesis rates of the different populations, at 12°C Florida had a statistically different net photosynthetic rate from North Carolina, North Carolina Radio, and Rhode Island. Also at 12°C North Carolina had a significantly different photosynthetic rate from the *Oculina* population just as North Carolina Radio had a significantly different net photosynthetic rate than Texas. At 18°C, North Carolina and North Carolina Radio were both statistically different in net photosynthetic activity in comparison to Texas. North Carolina Radio was also statistically different from the net photosynthetic rate of *Oculina* just as *Oculina* was statistically different from the Texas population photosynthetic rate (Figure 3b).

The one-way ANOVA that compared the average slopes of metabolic activity for each population did not yield a significant difference with an alpha of 0.05 . The results for the respiration and net photosynthesis ANOVAs were df=6, p=0.0795 and p=0.0887 respectively.

3.2 Coral Color Intensity Results

In order to determine if there was any variability between populations' changes in color pigmentation, differences in pairwise population's before and after R color values

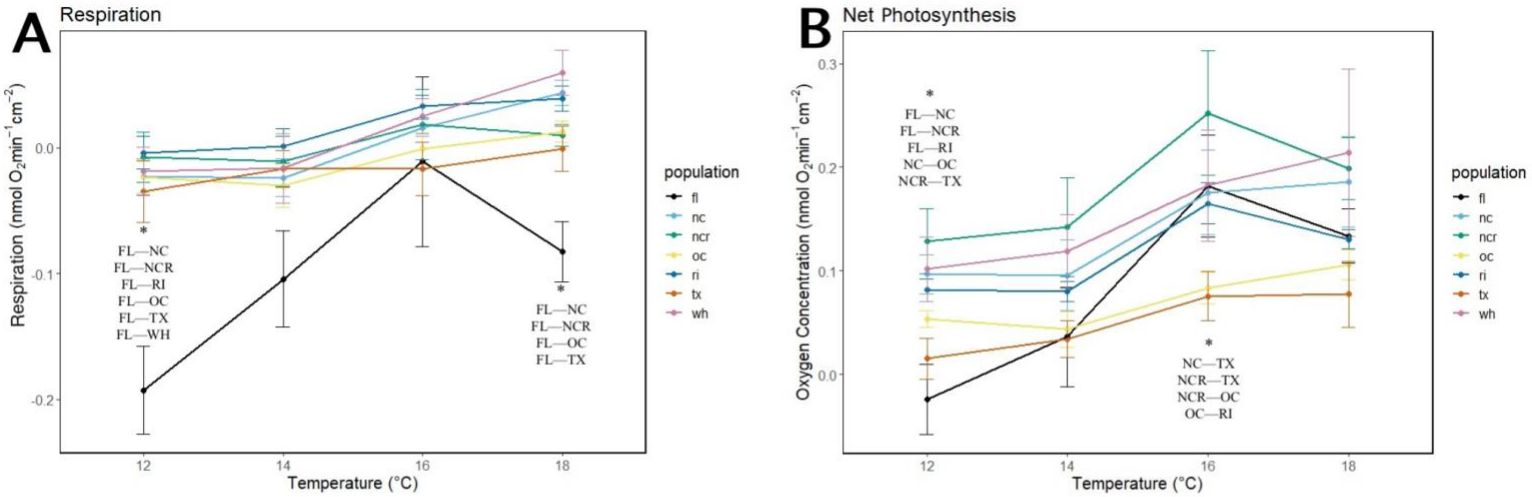

Figure 3: a) Respiration and b) net photosynthesis temperature performance curves created in R Studio of the seven coral populations (six populations of *Astrangia poculata* and one of *Oculina arbuscula)* over a 12-14°C cold ramp. Metabolic activity (y-axis) was measured in nmol O_2 $min^{-1}cm^{-1}$. Coral populations include: Florida "FL"(black), North Carolina "NC" (light blue), North Carolina Radio "NCR" (green), Oculina "OC"(Yellow), Rhode Island "RI" (dark green), Texas "TX" (orange), and Wood Hole "WH" (pink). A Wilcoxon signed-rank test was performed to find a significant difference between a population's metabolic activity at a specific temperature. Statistically significant pairings were found at 12°C and 18°C and those pairs are listed under the * for that temperature.

were calculated and tested using a two way ANOVA. The distribution of differences between pairwise populations can be visualized in Figure 4. After performing pairwise shapiro and variance tests all pairwise site comparisons were found to be both normal and homoskedastic. The results of the ANOVA show no significant variation in R value differences when it comes to comparing before and after photos with pairwise populations considered (df = 20, F = 1.391, p = .123). The ANOVA also showed that there was no significant variation between before and after photos (df = 1, F = 1.292, p = .256), but there was significant variation when only considering population pairs and not before/after status (df = 20, F = 6.004, p = 1.38e-13).

4. Discussion

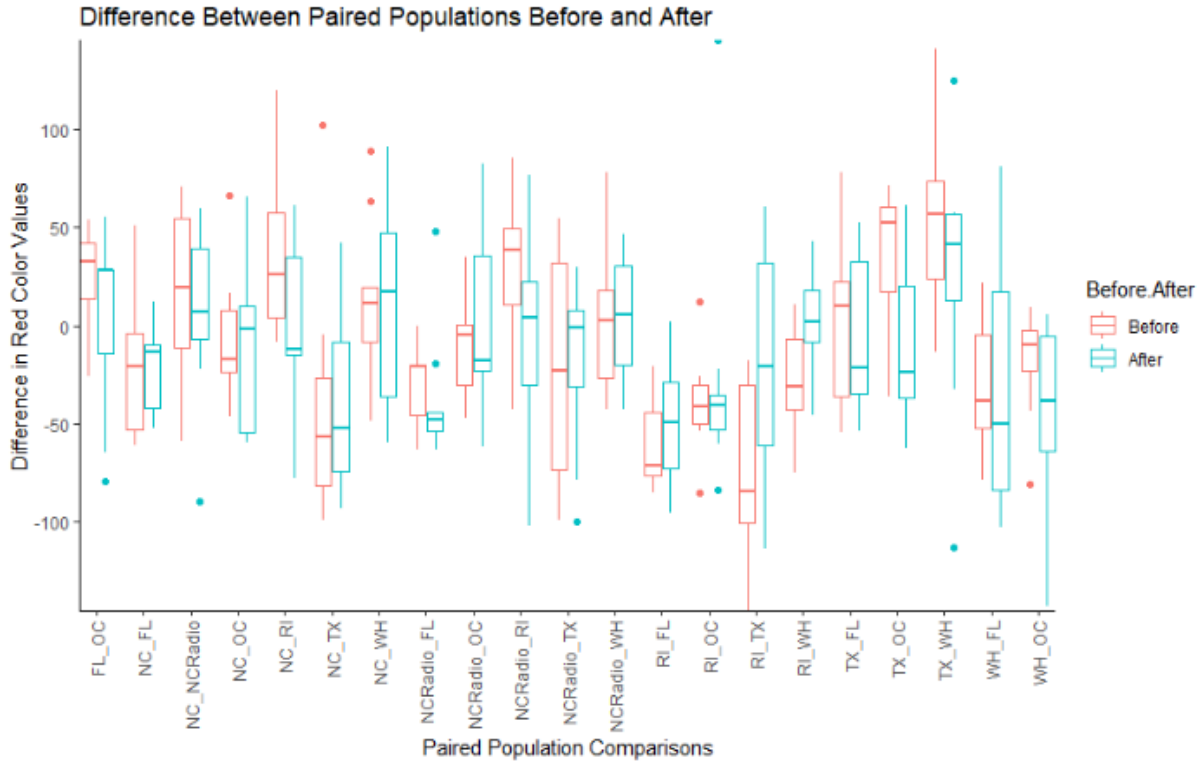

Figure 4: This graph shows the difference in average red color values (R values) between two paired populations' before and after photos. Six populations of *Astrangia poculata* and one population of *Oculina arbuscula* were used. Coral populations include: Florida "FL", North Carolina "NC" , North Carolina Radio "NCRadio" , Oculina "OC", Rhode Island "RI" , Texas "TX" (orange), and Wood Hole "WH". A two way ANOVA was conducted and found that there was no significant variation in difference of R values between paired populations' before and after photos when compared to other paired populations' before and after photos (df = 20, F = 1.391, p = .123). This indicates that there was no variation in how much each individual population changed before and after experimental treatment.

4.1 Motivations for our study:

The results from this experiment will help us better understand how local adaptations may affect a coral's response to thermal stress. In the Aicelman et al. 2019 paper, they discussed how "Adaptation to distinct thermal environments along a species" could prompt "population-level responses to environmental variability and thermal conditions". These potential local adaptations that our study aims to investigate are of interest as more extreme weather and sea surface temperatures (SSTs) are predicted to come as a consequence of climate change.

4.2 Interpretation of Results for Respiration and Net Photosynthesis

Figure 3a demonstrates the lack of significant difference between the respiration rates of the different populations of corals, with the exception of Florida. This points to the idea that the corals respire at around the same rates regardless of the temperature or their original latitudinal location. Figure 3b demonstrates some of the net photosynthetic rates of the different populations of corals across the cold ramp. The most interesting finding from this figure is the significant difference in photosynthetic rates between populations at 12℃. At this temperature we observed a significant difference in the net photosynthetic rates between northeastern populations (North Carolina, North Carolina Radio, and RI) and more Southern Gulf of Mexico locations (Florida and Texas). The southern states demonstrated lower net photosynthetic activity at the lower range of the cold ramp in comparison to the net photosynthetic activity of the north easter populations. This supports our hypothesis of more northern populations being better acclimated to colder temperatures and having higher thermal performances at these temperatures.

Despite not meeting an α of 0.05, the p-values for the ANOVA that compare the slopes of each TPC do indicate a possibility of different overall shapes between them. With greater sampling, and perhaps more robust and fitting statistics for the situation, significant differences in overall shapes of the TPCs can be found.

There were some statistically significant differences between the net photosynthesis rates of the *Oculina* population and North Carolina at 12℃ and with Rhode Island at 16℃. However, due to the small sample size of *Oculina* individuals these results have enough power to truly determine if there is a difference in photosynthetic activity between. species

4.3 Interpretation of Photo Analysis Results

The expulsion of zooxanthellae is a widely documented phenomenon which acts as an active regulatory mechanism in corals. These expulsion rates have been found to be responsive to environmental changes and we hoped through exposing corals, which were collected across a latitudinally diverse range, to cold stress we could see potential differences in zooxanthellae expulsion rate between populations (Dimond & Carrington, 2008). Our results suggest that corals collected from different populations do not show differences in their rate of zooxanthellae expulsion. This can be inferred from our two way ANOVA which demonstrated that the interaction between paired populations and their before/after

differences were not statistically different from other similar interactions. The important aspect of said ANOVA is that it tells us that paired population differences are not changing from before the experiment and after the experiment. Although this test demonstrates that all the coral populations are changing symbiont density at similar rates, it does not tell us how much the corals are changing in general. From this data it is possible that all populations of corals had not changed after the treatment, but at the same time it is also possible that there was significant expulsion of symbionts across all corals. Unfortunately at this moment we are unable to make that determination due to inconsistent lighting across photos, but by knowing that coral populations are expelling symbionts at similar rates we can conclude that these corals do not have local adaptations for managing symbiont density under cold thermal conditions.

4.4 Limitations and Sources of Error:

The drastically different patterns of respiration data for our Floridian corals was our most questionable result, and several explanations are possible. All of our Florida fragments were quite small, despite our standardization by surface area,

this inherently creates a greater margin of error for measurements of oxygen over a set period of time, and may have also resulted in lower overall respiration values. Additionally, sample D37, a Florida coral run on day 1, only had 12 minutes of recorded respiration at 16℃. In our dataset, this yielded a set of NAs for that data point, and may have further increased the error of that average. The greatest overall limitation of our respiration and photosynthesis data was our occasional dysfunctional blanks. Negative values in the respiration TPC mean that blank could have been respiring more than the actual sample. Although we evaluated each blank before using it as a baseline and excluded blanks that seemed dysfunctional, several runs did not have a blank that met our criteria, and we decided to move forward with the blank baseline average regardless in those cases. Another one of our large limitations was the fact that we only had one population of *O. arbuscula*, and future studies focusing on it just as much as *A. poculata* will be fruitful for the understanding of thermal performance for facultative corals.

In order to determine changes in coral symbiont density, coral photos were taken before and after experimental treatment. These photos were taken one day apart to allow the effects of the cold stress to fully present itself on coral symbiont density. The issue that arose was that although the same camera was used for all photos, the photos were taken under different lighting conditions on each day. As a result of the inconsistent lighting, changes in color intensity which were calculated on Matlab cannot be trusted to be indicative of actual symbiont change. Lighting issues such as shadows or glares consistently muddled results and as a result we were unable to determine the extent of symbiont change for all coral populations.

Acknowledgements

We would like to thank the BUMP Program, specifically Dr. Sarah Davies and James Fifer for their support throughout the research

project and for their help with experimental design and data analysis. We would also like to thank Kimberely Candelario for mentoring us during experiments, along with the Davies Lab for their established protocols in R and MatLab. Finally, we would like to thank Justin Scace and Isabela Trumble for their help and expertise in aquaria system maintenance.

References & Literature Cited

Aichelman, H. E., Zimmerman, R. C., & Barshis, D. J. (2019). Adaptive signatures in thermal performance of the temperate coral Astrangia Poculata. *Journal of Experimental Biology*. https://doi.org/10.1242/jeb.189225

Anton, A., Randle, J. L., Garcia, F. C., Rossbach, S., Ellis, J. I., Weinzierl, M., & Duarte, C. M. (2020). Differential thermal tolerance between algae and corals may trigger the proliferation of algae in coral reefs. *Global Change Biology*, *26*(8), 4316–4327. https://doi.org/10.1111/gcb.15141

Brennan, S., Liesegang, M., Almanzar, A., & Chavez, D. (2021). *Assessing the effects of cold thermal stress on Astrangia poculata quiescence: analyzing photosynthetic efficiency, calcification rate, polyp behavior, and Symbiodinium*. The Davies Marine Population Genomics Lab. Retrieved December 13, 2021, from http://sites.bu.edu/davieslab/files/2018/05/ColdThermalStressAPoculata.pdf

Dimond, J., & Carrington, E. (2008). Symbiosis regulation in a facultatively symbiotic temperate coral: zooxanthellae division and expulsion. *Coral Reefs*, *27*(3), 601–604. https://doi.org/10.1007/s00338-008-0363-x

Jurriaans S, Hoogenboom MO. (2019). Thermal performance of scleractinian corals along a latitudinal gradient on the Great Barrier Reef. *Phil. Trans. R. Soc.* B 374: 20180546. http://dx.doi.org/10.1098/rstb.2018.0546

Laura, A.-M., Ono, R., & Leonard, G. (2021, December 10). *Confronting Climate Change*. Ocean Conservancy. Retrieved December 15, 2021, from https://oceanconservancy.org/climate/?ea.tracking.id=20HPXGJAXX&gclid=CjwKCAiA-9uNBhBTEiwAN3IlNP935nAVZkhIUx8wPEr2eWlZLCZ7vUnPe7uyq5edBZbNvj5eNqjSDRoCB0wQAvD_BwE

Quality Management. (2021). *Optical Oxygen Sensors*. PreSens Precision Sensing. Retrieved December 15, 2021, from https://www.presens.de/products/o2/sensors?gclid=Cj0KCQiAnuGNBhCPARIsACbnLzocDhI9VEasR5oeQbff-Qu6xfIe-noHJSbRMg_9l_ee-w798RvPJsgaAioAEALw_wcB#c1092

Rezende, E. L., & Bozinovic, F. (2019). Thermal performance across levels of biological organization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1778), 20180549. https://doi.org/10.1098/rstb.2018.0549

Shaftel, H. (Ed.). (2021, November 30). *The Causes of Climate Change*. NASA Global Climate Change. Retrieved December 13, 2021, from https://climate.nasa.gov/causes/

Silbiger, N. J., Goodbody-Gringley, G., Bruno, J. F., & Putnam, H. M. (2019, September 17). *Comparative thermal performance of the reef-building Coral Orbicella Franksi at its latitudinal range limits - marine biology*. SpringerLink. Retrieved December 15, 2021, from https://link.springer.com/article/10.1007/s00227-019-3573-6

University of Arizona. (2021, October 20). *Changing ocean currents are driving extreme winter weather*. ScienceDaily. Retrieved December 12, 2021, from https://www.sciencedaily.com/releases/2021/10/211020140042.htm.

van Oppen, M. J. H., & Lough, J. M. (2009). *Coral bleaching: Patterns, processes, causes and consequences* (Vol. 205, Ser. Ecological Studies). Springer.

van Oppen, M. J. H., Lough, J. M., van de Schootbrugge, B., & Stanley, G. D. (2009). Chapter 2: The Evolution of the Coral-Algal Symbiosis. In *Coral bleaching: Patterns, processes, causes and consequences* (Vol. 205, Ser. Ecological Studies, pp. 7–17). essay, Springer.

Wernberg, T., Smale, D., Holbrook, N., Oliver, E., & Hobday, A. (2021). *Marine Heatwaves Explained*. Marine Heatwaves International Working Group. Retrieved December 13, 2021, from http://www.marineheatwaves.org/all-about-mhws.html.

R Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.