

I was always a nature person and felt connected with the outdoors. There was something therapeutic about being alone by the water- just a wandering mind and the constant breaking of the waves along the shoreline. To me, the raw beauty of the environment is worth preserving because the small moments where we get the chance to truly appreciate nature are the most memorable ones. In a time where economic gains are prioritized above the state of the environment, it is disheartening to see that the environment that took billions of years to form is being dismantled in a span of decades.

As I learned more about the major issues surrounding oceanic life and climate change, I became drawn to corals, who played pivotal roles in the balance of marine ecosystems while maintaining their intrinsic beauty. It was this perfect harmony of symbiosis that has remained unchanged for thousands of years- until now. Increased carbon emissions across the world have led to increased oceanic temperatures. Because corals thrive at an optimal temperature, they are unable to survive when oceanic temperatures rise. As a result, we are seeing massive bleaching events across the world's oceans. In recent years, bleaching events have become more severe, and corals are running out of time. With coral vitality at risk, I knew that part of the solution to improving coral health was the microbiome, whose wide consortium of bacteria may play a part in increasing resistance to heat stress.

One of my biggest influences would be my mentor Dr. Robert Thacker, (Stony Brook University) as he introduced me to the prospect of analyzing bacterial compositions within the microbiome. Because our research interests were very similar, we were able to begin

brainstorming ideas to develop a project. Most of his studies were focused on the sponge microbiome, but I was interested in corals. After discussing the background behind coral bleaching and the role of the coral microbiome in mediating host response, we outlined the goals of the project. By establishing a timeline of the phases of the study as well as a series of weekly goals, I was able to structure the study into two components: microbial analysis and dinoflagellate analysis. Looking back, having a plan was worth investing my time into because it allowed me to remain focused on what I wanted to uncover and progressively work towards it.

As a research student, one of the experiences that I looked forward to the most was working in a lab. However, COVID guidelines at Stony Brook University prevented me from physically coming into the lab, so I had to develop a virtual project. I preferred to engage directly with the environment and utilize a hands-on approach to combat climate change, but I had to put personal interests aside in order to make developments in the field. My research was done from home, and for the entire summer, I spent entire days writing code and reading literature review. The most challenging part of the project was overcoming the learning curve of coding. As someone with little experience in bioinformatics, I was daunted by the thousands of packages R program. Even the slightest mistake in the code meant the difference between a detailed abundance plot and an error message. However, I found assurance knowing that many of the statistical tests and abundance metrics were accessible through functions. Despite being discouraged several times over failed code, the recurring thought that my code had the potential to contribute to an issue of global importance motivated me to work towards making my own contributions to the field.

It is one thing to collect the data, but it is another to effectively communicate it coherently in an order that made sense. While many of the statistical functions were loaded in the R program, I had to learn how the background of the tests that we conducted in order to accurately explain our findings.

The main takeaway that I got from my project was that innovative ideas guide scientific enterprise. Since the beginning of the project, I was able to take the concept of bleaching and transform it into a project with various applications that can be extended off it. As someone who is fervently invested in climate change and mathematics, I used a combined approach to respond to the growing climate crisis. I was able to take initiative in my research project, utilizing the mathematical aspect of alpha diversity tests to compare microbial communities. Overall, it was interesting to find the junction between mathematics and science because they are complementary to each other. Data is at the core of scientific findings, and mathematics is crucial to support your work and its significance. Scientists serve as the bridge between the scientific community and the public and correctly interpreting findings is necessary to convey the importance and impact of your work. Rather than isolated fields of research, multidisciplinary approaches are the norm. I am a mathematician who passionately seeks to unify environmental research with data science. Efforts to address climate change requires scientists to translate the numbers into realized palatable concepts. For example, microbial abundances are better understood with the lens of symbiotic relationships. The numbers alone do not tell the story, but rather the delicate balance of ecosystem interconnectedness.

If you are considering doing research in science and mathematics, it is incredibly manageable because the two are interconnected. Both go hand in hand and math is used to justify your research. In addition, statistics are also important when confirming the significance of your results because significant data contributes to a richer discussion. My biggest piece of advice for future researchers and research students is to be passionate about your research. If you are genuinely interested and motivated, you will be so involved with your work, and it will become second nature to you. Keep an open mind because there are often multiple ways to approach a problem. From experience, I was a bit hesitant to learn a new coding language during my time in the lab. Over time, I became more receptive to writing code, and I became motivated to write more efficient code in order to obtain the data that I needed.

For those that are hesitant about the workload: once you are done, you will truly appreciate the amount of dedication that you have put into your research. Do not be afraid to do some exploring because you do not know everything. Ask questions. Your role as a scientist is to pose the questions that nobody is asking and think from new perspectives. The core of scientific enterprise relies on drawing the conclusions from your experiment and effectively communicating it for knowledge to be shared and understood by the general public.

Another piece of advice is to conduct research for the sake of conducting research and making your own contribution to the greater scientific community. It is good to be motivated by the idea of winning science competitions, but at the end of the day, you get more gratification from taking ownership of your work. The impact of your research can be so more far-reaching than any

award that you receive. Enjoy the process of research and the small moments in between when you either write a successful line of code or make an incredible discovery. Take it from me--the research is the fulfilling part. Winning is just the cherry on top.

My final piece of advice is to always expect the unexpected. You may head into a project expecting one outcome, but sometimes your hypothesis may not always be correct. And that is alright because it is part of the experience as a researcher. As my research teacher likes to say: If you confirm your hypothesis, you have made a confirmation of a finding, but if you disprove your hypothesis then you have made a discovery.” Enjoy the journey so that one day, you can look back and appreciate the contributions that you have made, not just for the scientific community, but for humanity.

My Research:

Corals are important to the ocean ecosystem, home to diverse communities of animals. Aside from serving as vibrant habitats, they also have practical applications for humans. They can produce bioactive compounds that treat cancer and arthritis and also support a major part of the fishing industry.

What most people don't realize is that the coral microbiome plays a major role in promoting coral health. Corals are made of tiny animals called polyps, and they are responsible for reef building. The microbiome consists of bacteria, dinoflagellates from the family *Symbiodiniaceae*, and fungi that live in coral tissue. They exhibit mutualism with the host, providing them with amino acids in exchange for photosynthetic material. However, in recent years, coral health has been threatened by increased greenhouse gases, which induce bleaching, a phenomenon in which coral hosts expel members of the microbiome. Without them, corals begin to die as symbiosis breaks down and they lose their main source of nutrients. Because of the sensitivity of corals to temperature, when the constituents in the microbiome are expelled, the coral host is at great risk of tissue necrosis.

The main endosymbiont that my study focused on dinoflagellates from the family Symbiodiniaceae. Several *Symbiodiniaceae* clades exhibit heat tolerant properties, and we wanted to find out if they influenced microbial abundances under heat stress.

My study analyzed and compared the microbiomes of different corals in oceans across the world. The first goal of the study was to identify the key bacteria present under higher temperatures to see if they can help corals survive at higher temperatures in the future. Using potential heat-

resistant bacterial species as a search variable, metadata from five studies were extracted from the GenBank database and 16s rRNA bacterial sequences were processed with *Symbiodiniaceae* ITS2 sequences in the Galaxy platform. Dissimilarities between microbial communities were calculated using the Bray- Curtis Dissimilarity Index and statistically significant treatments were identified using a permutational multivariate analysis of variance. Similarity percentages were calculated to determine the relative contributions of each species toward the variable of interest. Microbiome compositions were investigated with computer code written to search for high abundances of either pathogenic or beneficial heat-resistant bacteria.

Another metric that was used to compare microbiome compositions in addition to relative abundance was Alpha Diversity (Shannon Index and Inverse Simpson Index) Coral hosts that contained a greater number of bacterial species were indicated by higher alpha diversity levels while hosts with a lesser number of bacterial species by lower alpha diversity.

$$H' = - \sum_{i=1}^S p_i \ln p_i$$

where:

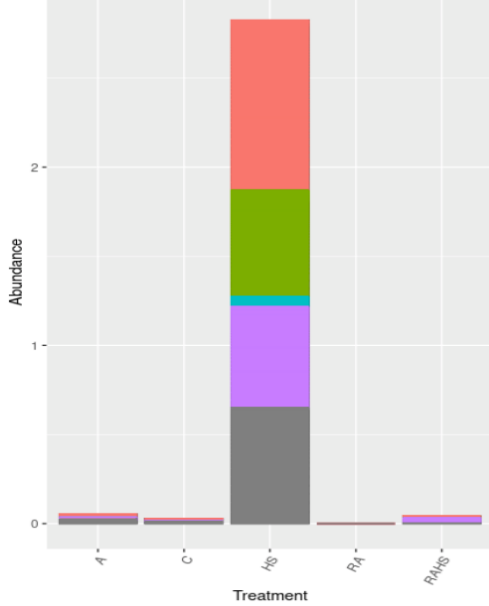
- Σ : A Greek symbol that means “sum”
- \ln : Natural log
- p_i : The proportion of the entire community made up of species i

Shannon Index formula for alpha diversity

It was found that in certain heat-sensitive coral species, there was an increase in pathogenic bacteria abundances under high temperatures (Figure 1). In those microbiomes, alpha diversity was low as the microbiome became dominated by pathogenic bacteria from the genus *Vibrio* that promoted disease when exposed to heat stress (Figures 2, 3). However, there were also potentially beneficial bacteria from genus *Ruegeria* that were found in several coral species to

combat disease, which can aid coral resistance to high temperatures. This was significant because their high abundance under heat stress indicates that they may be able to provide resiliency to their respective coral hosts as oceanic temperatures increase.

Vibrio Abundance Associated with *Acropora Pruinosa* Coral



Treatment Key

A- Acclimated Group 31°C for 2 days

C- Control Group 26°C for 14 days

HS- Heat Stress 33°C for 14 days

RA- Acclimated group with recovery period at 26°C for 14 days

RAHS- RA treatment subsequently exposed to 33°C

Figure 1- Vibrio Abundance in *A. pruinosa* (Treatment $r=.883$ $p=.001$): The heat sensitive coral *A. pruinosa* demonstrated an increased abundance of bacteria belonging to genus *Vibrio* after being subject to heat stress treatment of 33°C for 3 days. *Vibrio* has been found to be pathogenic in other coral hosts. To account for the differentiation according to species, a species breakdown was displayed (Image created by student researcher)

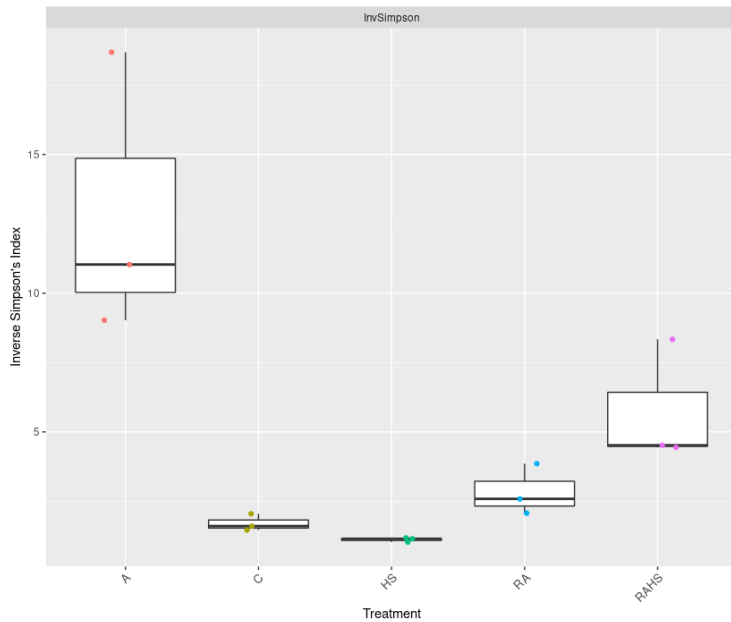


Figure 2- Alpha diversity in the coral *A. pruinosa* decreased under heat stress: An inverse Simpson diversity index found alpha diversity to be lowest in the heat stress treatment (Image created by student researcher)

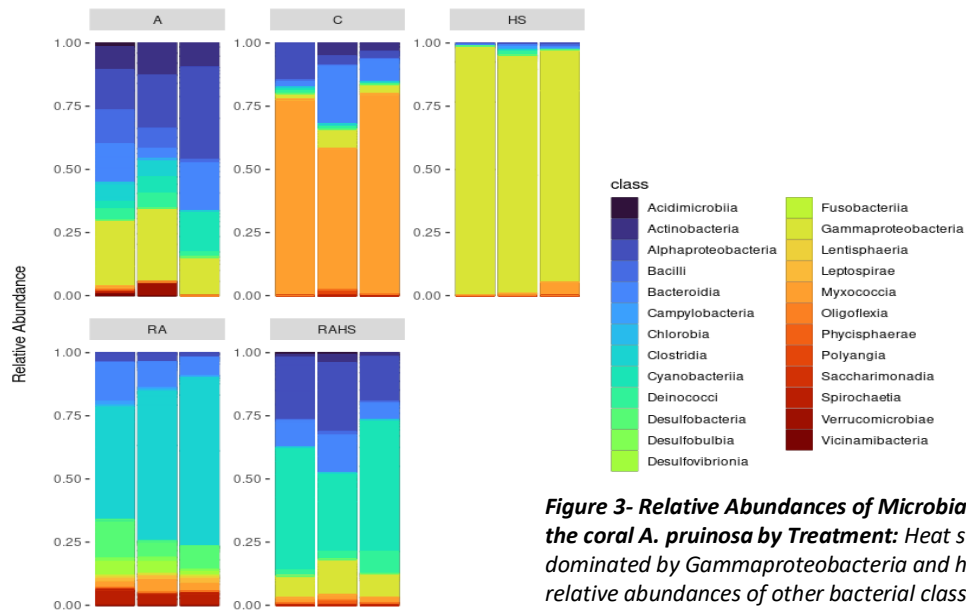


Figure 3- Relative Abundances of Microbial Classes in the coral *A. pruinosa* by Treatment: Heat stress was dominated by Gammaproteobacteria and had lower relative abundances of other bacterial classes while acclimated (A) had the most diverse group of bacterial classes (Image created by student researcher)

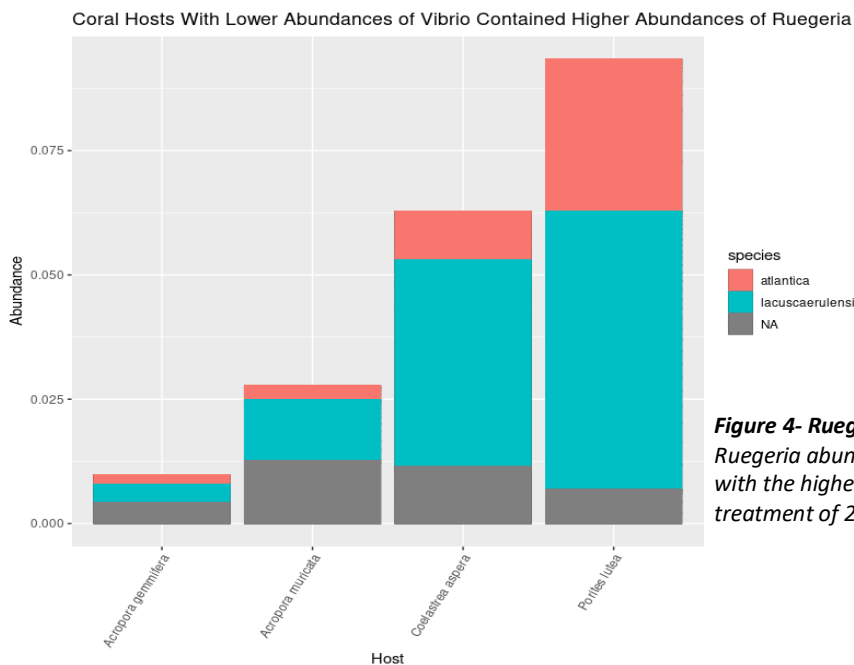


Figure 4- Ruegeria Abundance in the coral *A. tenuis*: Ruegeria abundance increased as temperatures increased, with the highest abundance occurring in the stress_ambient treatment of 29.8°C. (Image created by student researcher)

The second phase of the study was to determine whether there was an association between *Symbiodiniaceae* and the coral microbiome. Using a mantel test, it was found that there was a positive correlation between microbial abundances and *Symbiodiniaceae* clade (r= .7028) Clade C *Symbiodiniaceae* is generally associated with heat-sensitive corals while Clade D *Symbiodiniaceae* associated with heat-resistant corals. Moving forward, it is important to keep this in mind as microbiome engineering is considered to improve coral resilience to increased oceanic temperatures.