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ARE CORALS IN THE SOUTH PACIFIC BEING HELPED OR HARMED FROM NUTRIENT RUNOFF? ANSWER: IT'S COMPLICATED

By: Emma Holm-Olsen

Crystal blue waters off of Moorea, French Polynesia.

In the back storage room of the Ocean Recoveries Lab at the University of California, Santa Barbara (UCSB), sit half a dozen industrial-sized refrigerators and freezers. Almost every day for five months, I opened these refrigerators - careful not to confuse them with the ones housing our lunches - to find rows and rows of tiny plastic tubes full of tissue samples from corals grown thousands of miles away in one of the most isolated places on the planet. From January to June 2023, these tubes were my world, and also often the bane of my existence.

A little over a year ago, I joined professor Deron Burkepile's laboratory on campus which, along with professor Adrian Stier's Ocean Recoveries Lab, was studying the relationship between corals and their symbiotic algae in Moorea, French Polynesia. While I never got the chance to actually go to Moorea, many of my colleagues, including my graduate student mentor and research advisor Julianna Renzi, had been working there for several years, and shared incredible stories about the beauty of the island and the surrounding reef.

I've always been interested in coral reefs, but until recently never truly understood many of the issues they face. During my time as a student at UCSB these last four years, I've been lucky to have many opportunities to learn more about these fascinating ecosystems, from taking courses on coral reef ecology and fish biology, to being involved in this project through which I've been able to actually contribute to the current body of knowledge regarding their function and ecological importance.

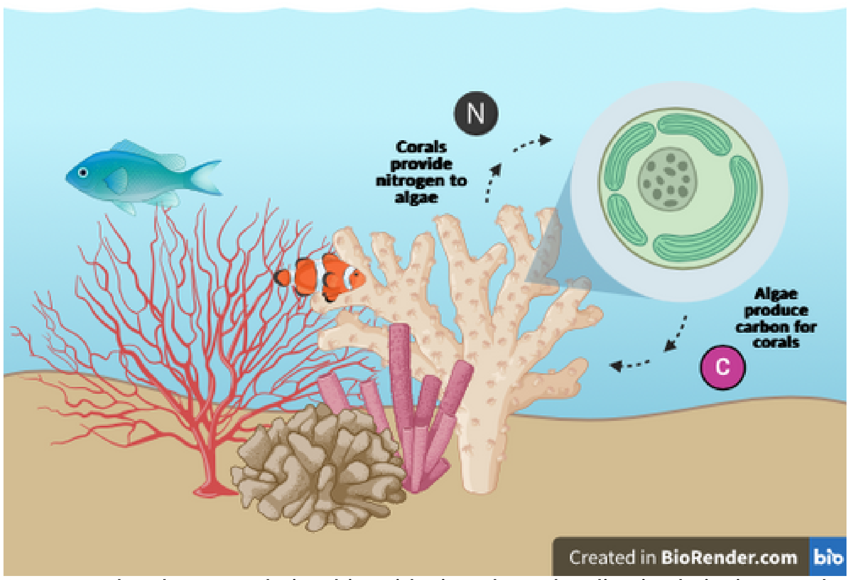
WHAT IS A CORAL REEF?

Coral reefs are definitely one of the more well-known and beloved habitats and ecosystems; they're visually stunning to look at (and so get all the photo and video coverage,) and, being marine systems, are often featured repeatedly in the news as being one of the biggest victims of rising temperatures. Reefs are vastly diverse marine ecosystems, providing shelter and sanctuary for thousands of species of fish, coral, and invertebrates - more than any other marine habitat. They exist all over the world, in both warm and cold waters, and can span hundreds of miles long. In fact, the Great Barrier Reef, the largest of all, spans about 1,600 miles and is considered the largest living organism on Earth; it's so large, it can even be seen from space. That's right. When I say the Great Barrier Reef is the largest living thing on Earth, I really mean it. Not the largest collection of living things, but *the largest living thing*. You see, corals are animals. Not plants, not protists (like algae,) but animals. And just like more charismatic species like the rhinoceros or the orangutan, their existence is being threatened by our own actions.

Global climate change is significantly altering the environmental conditions that coral reefs are facing. From rising water temperatures to ocean acidification - the lowering of water pH to levels dangerous to calciferous organisms like crabs, mussels, and yes, corals - corals are being exposed to things they have never experienced before, and they are suffering.

Stony corals, also known as "reef-building corals" due to their role in building the substrate of reefs upon which other organisms live and depend, are cnidarians (the evolutionary lineage including sea jellies and anemones.) Each individual coral, or "polyp," lives in

Stony Coral + Endosymbiont Mutualism



Stony corals rely on a relationship with tiny algae that live in their tissues. The corals provide the algae with nitrogen in exchange for carbon that they require to produce their food.

colonies with thousands of other polyps. Each polyp secretes its own skeleton of hard calcium carbonate (CaCo3), but is also connected to its neighbors by a thin tissue layer called a "coenosarc." This is what creates the vibrant rock-like formations you would see on a reef floor. To survive, the corals rely on a mutualism - that is, a relationship that is beneficial to both species - with tiny dinoflagellate (a type of single-celled organisms) algae in the family *Symbiodiniaceae*. Also known as endosymbionts, the algae live within (hence, *endo*symbiont) the corals' tissues and create the vibrant colors for which corals are so widely known. Through photosynthesis, the complex process by which plants and algae turn sunlight into nourishment, the algae therefore provide corals with carbon in return for nitrogen. Technically the corals and algae are competitors, as there is a fine line between a mutualistic relationship and a parasitic one, but most of the time it is beneficial for both to help each other out. For example, if environmental conditions are harsh, the algae will want to ensure their corals are healthy enough to continue providing them with nitr--ogen, and therefore will continue to provide the corals with carbon. The algae use this nitrogen to create amino acids, while the corals use their carbon to carry out cellular respiration. Essentially, their endosymbionts are feeding them. And without them, corals will starve. The photographs you may have seen of vast deserts of ghostly white branches are not necessarily dead corals, but very ill ones. "Coral bleaching," as it is called, is the process by which distressed corals expel their tiny algal partners, and subsequently lose their only source of food.

Think of it this way: You probably have a group of close friends that you enjoy hanging out with, be it grabbing a drink on a Friday night or catching a weekday movie. But when you're stressed about work, or not feeling well, you may end up pushing them away. Corals are no different. When water temperatures get too warm, algae struggle to perform their photosynthetic duties and are therefore evicted from their coral homes. However, just as you might without your friends, corals will begin to suffer without their algae, and as starvation sets in, will become that much more susceptible to mortality. This being said, these bleached corals can be nursed back to health, and are not in fact doomed as many sensationalist news stories lead us to believe.

- ***These microscopic algae should not be confused with macroalgae (like seaweed.) While corals are sometimes in competition with their endosymbionts, macroalgae are much more of a concern. From overcrowding to resource monopolization, macroalgae pose a huge threat to corals, and can sometimes take over reefs to the point of total coral exclusion.***

Corals also have mutualisms with fishes and invertebrates ("ectosymbionts.") In our research we looked at mutualisms with shrimps and crabs. *Alpheus lottini* is a species of snapping shrimp that provides several important services to corals, including defending them from predators, removing excess sediment, and even potentially buffering negative effects of ocean acidification. Crabs, such as *Trapezia bidentata* ("TRBIs"), perform similar duties in return for shelter within the corals' branches. A 2018 paper by a team of researchers associated with the Moorea Coral Reef Long Term Ecological Research (LTER) site found that ectosymbionts can actually increase corals' resilience to higher pH and temperature levels. Essentially, in high-stress situations ectosymbionts halt their consumption of coral tissue (the only drawback to corals having these little guys around,) in favor of helping the corals, on whom they rely for food and in which they have a vested interest to ensure remain healthy.

THE EXPERIMENT

When I joined the Stier/Burkepile team in the winter of 2023, Julianna was working on a research project about how nutrient runoff, such as from coastal development projects, might affect these mutualisms between corals and their endosymbionts, as well as with their ectosymbionts. More specifically, she and her colleagues were interested in how elevated levels of nitrogen in the environment affects coral health, measured by the growth and retention of both the algae and invertebrates.

A 2016 paper by Swiss researcher Nils Rädecker and colleagues outlines the way in which excess environmental nitrogen may negatively affect corals due to them receiving, as a result, less carbon from their endosymbionts. If the algae are receiving their nitrogen from elsewhere, they may not feel as inclined to keep up their trade with the corals, especially as they can then use that extra carbon (that would have gone to the corals to produce more amino acids for themselves.)

- ***Nitrogen is known to induce faster algal growth rates. One extreme example of this is "harmful algal blooms" (HABs), in which excessive growth of microscopic algae in the water column leads to toxic byproducts which pose serious harm to many marine organisms.***
- ***HABs are often the result of sewage spills, and are becoming more frequent with more intense storms and flooding as a result of climate change.***

To answer the question of how nutrient enrichment affects coral mutualisms, the lab developed an experiment in which 40 coral colonies of the genus Pocillopora were grown in Moorea and exposed to a four treatments: a control in which nothing was changed, a treatment in which the corals were exposed to added nitrogen, a treatment in which TRBIs (remember the crabs?) were added to corals, and a treatment in which both nitrogen and crabs were added.

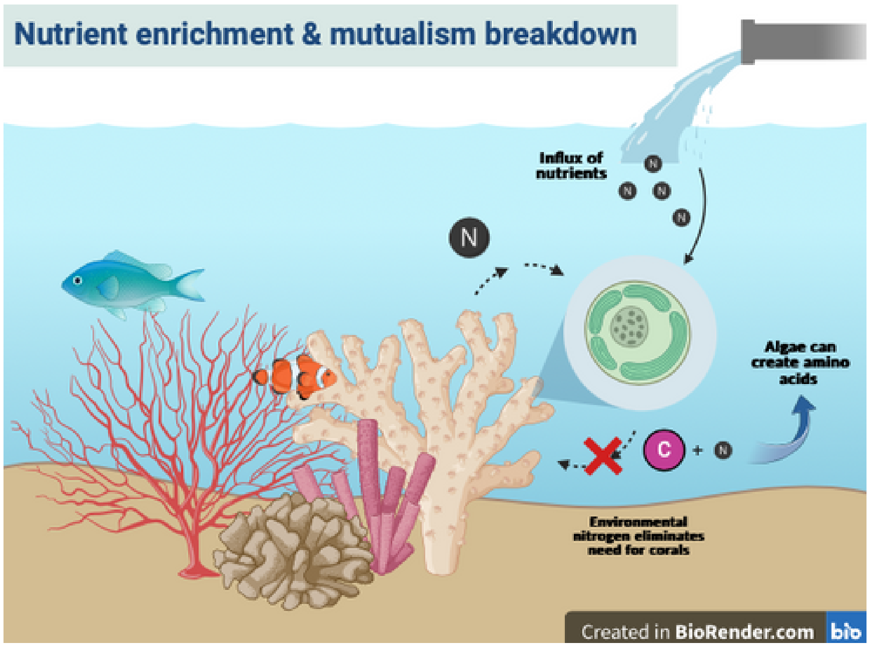
The next part of the experiment is where I came in. I didn't expect to be put in charge of any actual data collection or analysis - in fact I just thought I could help out with some menial labor here and there. To my surprise, and immense gratitude, I was asked to actually lead a portion of the work. Once the samples were flown back to UCSB and prepared for analysis by Julianna, I was put in charge of counting the number of algae cells in each coral colony under each treatment at three different time points: before the treatment was applied, after a month, and after a full year at the end of the experiment.

After having heard so much about UCSB's incredible resources and opportunities for undergraduates to get involved with research on campus, I was so excited to be able to be a part of such an important project. As a third year student, I hadn't really had any prior lab work experience, but I was all in and ready to learn.

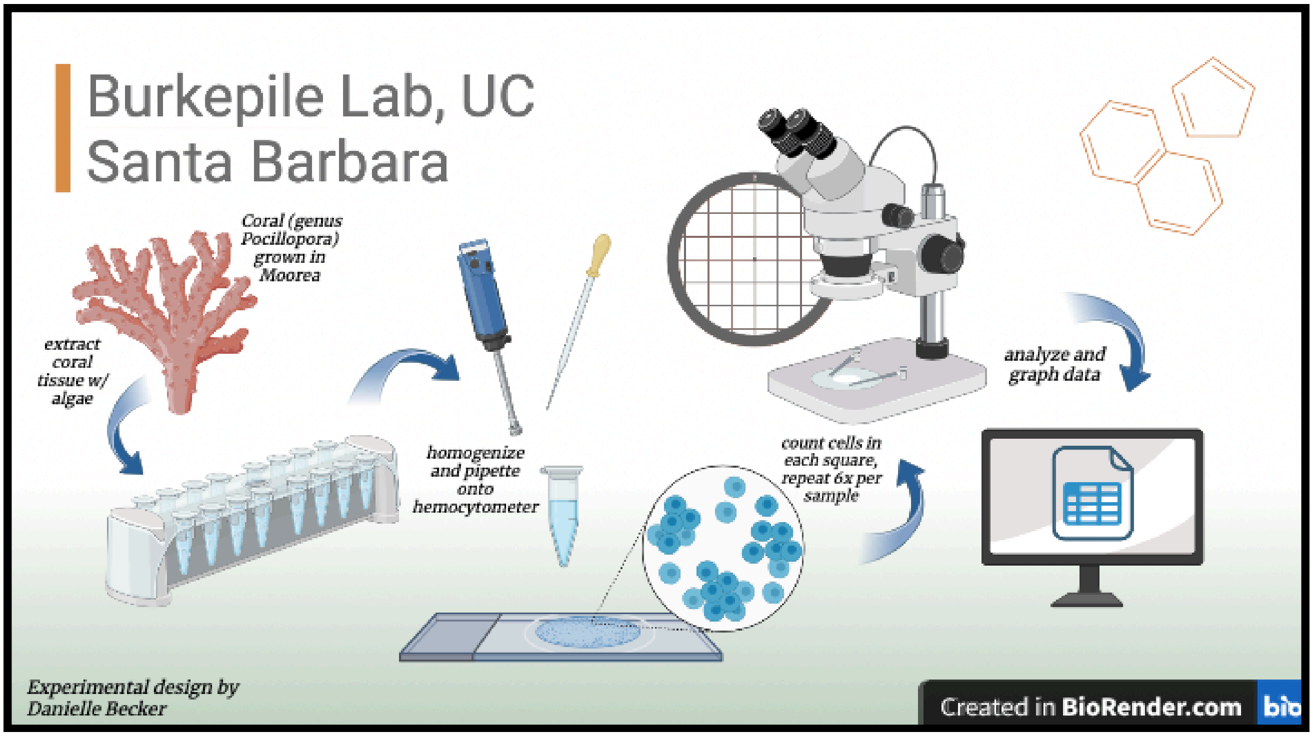
And learn I did...

As I said, I came in with limited knowledge of coral reefs, but I came in with even less experience with data collection and analysis. My work for the first five months or so consisted of counting every individual algae cell in each sample of coral tissue. After six, and sometimes more, repeats of three time steps for 40 samples, I counted over 70,000 cells. Finally, I was ready to start analyzing the data - a terrifying prospect for a beginner such as myself. With Julianna by my side every step of the way, I slowly but surely learned the ropes of using R, a common programming language often used by ecological researchers due to its ability to synthesize and visualize large datasets.

While I had taken a course on the software the previous spring, this was my first introduction to using it in a real, professional environment. We began by "wrangling" the data, what analysts term transforming data into more usable formats for graphing and modeling. For us, this looked like cleaning up our excel files (removing empty cells, condensing columns and combining data sheets, renaming columns etc.) and ensuring all our variables matched up across our files. Once we had everything in order, we were able to begin running analyses on the data to determine what kinds of relationships existed between our variables of interest: endosymbiont density, nutrient enrichment, presence of crabs (and other animals, like shrimp, which happened to be in the area,) and time. As I predicted, there was a steep learning curve with the software, and running and trouble-s



Added nitrogen in the environment has the potential to break down this crucial mutualism; if algae obtain their nitrogen from elsewhere, they no longer require nitrogen from corals, and thus have no incentive to continue providing carbon. They can then use that extra carbon to make amino acids for themselves.



Our experiment consisted of 4 major steps. 1) extracting tissue specimens from every coral in each treatment, 2) taking consistent volumes of samples to view, 3) counting the algae cells in each sample under a microscope, and 4) synthesizing and analyzing the data in RStudio. Experimental design by Danielle Becker.

-hooting the code for all of this literally took me months to do. Nonetheless, by early spring of 2024 we had the beginnings of some interesting results.

COMBING THROUGH THE DATA

Based on the background of the topic and our understanding of the mechanisms of coral-algal mutualisms, we had initially approached this project with two main assumptions: that higher levels of endosymbionts are better for corals, and that excess nutrients would be harmful to corals. Our reasoning was that more endosymbionts would mean more carbon for the corals to use in their metabolic processes, but that added environmental nitrogen would cause the mutualism between the corals and their endosymbionts to break down.

Ultimately, we found that the density of endosymbionts found in the coral tissue decreased the least amount under nutrient enrichment, and that the presence of the TRBI crabs on the corals did not really have much of an effect on endosymbiont densities. We also decided to look at the corals' carbohydrate and protein levels and whether there was any interesting relationship with endosymbiont densities. Indeed, we found that there seems to be a "hump-shaped" curve, indicating that there may actually be an intermediate optimal amount of endosymbionts, with higher levels of endosymbionts actually acting as a stress on the corals by taking too much nitrogen without reciprocating with carbon. If this is true, then we may need to backtrack and rethink our methods of analysis. For example, potentially looking at deviations from the optimum might be a better approach than simply quantifying increases or decreases in endosymbiont densities as indicators of coral health.

We did, however, find some clear relationships between endosymbiont densities and the corals' shrimp and crab macro symbionts. It seems as though there s a slight negative correlation between the presence of excess nutrients and ALLO shrimp under the "nutrient" treatment; if we assume that more endosymbionts are relatively bad for corals, that shrimp are potentially beneficial to corals under stress, and high levels of nutrients would qualify as a stressful situation, then it would make sense that there would be more shrimp present on corals exposed to higher nutrient levels. On the other hand, there seems to be a slight positive relationship between shrimp and endosymbionts when there are no added nutrients. A possible explanation for this is that shrimp are actually harmful to corals under ambient conditions because they eat coral tissue, with this leading to more endosymbionts. There also seemed to be more crabs present on corals with more endosymbionts (i.e. the ones exposed to nutrients,) further indicating that more endosymbionts lead to a snowball of stressors on the corals.

Overall, our results imply that elevated nitrogen levels may be beneficial to algae, but not necessarily for corals. However, the conclusions drawn from our data depend heavily on which assumptions we use; therefore more research is needed on the actual mu-

-tualistic relationships of corals before the effects of environmental stressors can be effectively studied. That being said, our research is already informing us about how our own actions are actively affecting c- -oral reefs, and in the future may aid legislation regarding coastal development projects in the South Pacific. I got to share all of this with a group of my peers this past April at our department's Undergraduate Research Symposium, in which I presented on my role in the project and our findings, and was very pleased to see how excited and responsive people were to the research - I must say, it's extremely satisfying to see your work be validated by others you respect and admire.

MOVING FORWARD...

While I likely won't remain involved in the actual research that the lab continues to do, since I'm graduating from UCSB this spring, I am incredibly excited to see what continues to come of this work. And of course, I plan on staying in contact with the team, and in particular with Julianna. In fact, I will be listed as a co-author on her paper which will hopefully be published some time next year, and referenced as a collaborator when she presents at the Ecological Society of America in Los Angeles this August.

With our ever-growing global population and rapidly changing climate conditions, it is becoming increasingly critical to understand the ecological roles and relationships of at-risk species - and this is nowhere more true than in the oceans. The ocean is simultaneously our biggest hope in the fight against climate change, and one of the most threatened habitat systems. And yet, so much of it remains unexplored. If we can dedicate more time and effort to deciphering the secrets of this vast carpet of blue that covers more than two thirds of our planet, we just may find ourselves with a solution to a better future for all.

More to Explore:

"Coral Basics." *Coral Basics | Flower Garden Banks National Marine Sanctuary*, NOAA, flowergarden.noaa.gov/education/coralbasics.html#:~:text=Hundreds%20to%20thousands%20of%20coral,mat%20over%20a%20shared%20skeleton. Accessed 11 June 2024.

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