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Comparing Disease Prevalence in Hard Corals at Four Different Reefs near the Island of Narganá in the Guna Yala Comarca of Panamá

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Abstract

Coral reefs harbor much of the world's known marine biodiversity. For a number of reasons, coral reefs are becoming increasingly threatened. Large portions of the world's reefs have already been lost, and the number of degrading reefs is constantly on the rise. One cause for the destruction of coral reefs are coral diseases, ultimately causing coral mortality. With the death of corals, a key species is lost, endangering the entire reef ecosystem. Documenting the presence of such diseases could be useful in assessing current reef health and ameliorating the growing threat of coral diseases. In this project, 18 $40m^2$ belt transects were conducted at each of four reefs near the island of Narganá in the Guna Yala Comarca of Panamá. A total of 72 transects were conducted, investigating a $2880m^2$ area. Living corals present were counted and classified. Corals showing evidence of disease were noted and disease was classified. Using statistical tests, the four sites were compared to one another to determine health of the reefs. The Narganá reefs, located nearest to the inhabited island, had the highest percentage of sick coral. At this site, 29.91% of all corals present showed signs of compromised health. The lowest percentage of sick corals was found at the Ordud sample site, with 4.20%. This site appeared to have the lowest human influence. These findings suggest that coral disease prevalence may be linked to human influence. Human waste runoff, pollution, and overfishing have all been attributed to deteriorating reef health and the spread of coral diseases. Areas closer to inhabited islands are often more heavily subjected to these influences. As proximity to human inhabitance increases, the prevalence of coral diseases may also increase.

Acknowledgments

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Introduction

Area of Study

Panamá is the most southern country of Central America, situated between Colombia and Costa Rica. This country is bordered by the Caribbean Sea and the Pacific Ocean, to the north and south, respectively. In addition to trade winds and global sea currents, these waters play a major role in defining the climate of the country. From May to November, Panamá is in the wet season, characterized by high amounts of rainfall and cooler temperatures. The remainder of the year brings much less rainfall and slightly warmer temperatures as strong winds blow drier air across the country. This project was conducted just at the end of the wet season during the second and third week of November.

Panamá is divided into nine different territories, including the comarca of Guna Yala. Located on the Caribbean side, Guna Yala extends east of the Panama Canal all the way to the Colombian border to the southeast. The Guna Yala comarca is a protected land owned and inhabited by the Kuna people. This indigenous group attained political autonomy in 1953. Though the area remains under some influence from the Panamanian government and is subject to national law and police, the Kuna people have their own structured government and lawmaking body. Much of the protected comarca area covers the sea, in which lie hundreds of islands, both inhabited and uninhabited. The designation of this comarca has prohibited industrial fishing from the area, only allowing the Kuna people to use these waters for both subsistence and exportation uses. Further, laws imposed by both the Panamanian and Kuna governments have worked to set further restrictions on actions within this area.

For the better part of the past few decades, this area has seldom been visited by tourists, mainly due to the difficulty of traveling to the region. Though tourism in the comarca has risen with the development of better infrastructure in recent years, the region still experiences much less tourism than more established tourism sites such as the Bocas del Toro territory. For this reason, as well as its protection as a comarca, much of the aquatic life has been preserved. Comparatively, Guna Yala waters have not suffered as drastic a level of degradation as other areas throughout the Panamanian sea shore. Narganá serves as one of the main hubs of tourism in Guna Yala. The island is more developed than many of the other islands and is home to one of the biggest communities in the entire comarca. With better accessibility to accommodations and with its proximity to other points of interests, this island has many visitors throughout the year.

Coral Reefs

The comarca of Guna Yala is home to a vast number of coral reefs. These ecosystems are some of the most diverse environments in the world, teeming with innumerable species of plants, invertebrates, and marine organisms. These environments are crucial to the proper function of many other ecosystems, as well as a large number of human industries. For example, coral reefs act as maturation grounds for many species of fish that are important links in higher food chains. Without these lower species, the higher predators will lose a major source of food. Lacking a substantial food source these predators may be driven to different waters or die. In either situation, the food chain loses a top predator, having immense trickle-down effects on the rest of the ecosystem. Apply this scenario to an economically important fish species such as snapper and the immense effects of losing such species are easy to imagine. Overall, coral reefs are delicate ecosystems that are susceptible to even slight changes in environmental conditions.

The structural base of coral reefs is built primarily by corals. Coral is an invertebrate classified in the phylum Cnidaria. Coral is made up of many small individual poylps. Within the individual polyps live a type of photosynthetic algae called zooxanthellae. The coral polyps and algae exhibit a symbiotic relationship. The algae are able to gain nutrients from the coral emitted as waste; in return, the coral is able to obtain oxygen and other nutrients produced by the algae. The algae, living within the polyps, give coral its color. As the coral polyps grow, they produce a calcium carbonate exoskeleton. After years of growth, this exoskeleton provides the physical structure needed for other organisms to develop and live within and around the coral. Reefs are defined by the interaction of these different organisms with the living coral.

Coral Reef Health Threats

In times of increased abiotic stresses, such as higher water temperatures, coral will expel the zooxanthellae as conditions deteriorate. When the coral gets rid of the algae, the coral becomes white in appearance as the calcium carbonate skeleton shows through the transparent tissue of the polyps. Though the corals are still alive at this point, they do not receive the benefits provided by the algae. However, given time and assuming the water is restored back to its natural state, the corals can recover from this condition. The threat in bleaching often lies in mass bleaching events where prolonged stress causes bleaching on a large majority of a reef. In these conditions, coral mortality is often much higher than in low-scale bleaching instances.

Another serious threat to coral reefs is the spread of coral disease. These sicknesses can be caused by bacteria, fungi, or viruses. Additionally, substantial evidence points towards human activity and anthropogenic based environmental changes as other proponents of coral disease. As the disease spreads across the live coral, the coral tissue is often "consumed," exposing the skeleton below. Many of these diseases are transferable among individuals. This may be problematic in areas of high coral density and could lead to faster rates of coral disease transmittance. In killing the living tissue, coral diseases cause permanent mortality within the coral. Aside from killing the coral, this can cause population shifts in corallivores and have far reaching ecological impacts on other species and the environment as a whole.

Project Overview

In this project, certain species of hard corals will be investigated for signs of compromised health at different locations. Four different reefs will be sampled. At each location, the number of coral present will be counted and corals with health disorders will be documented. These sites will then be compared to one another to evaluate differences in coral health among sites. Along with comparing the total percent of corals that are effected at each site, the health of specific coral species will be juxtaposed. Further, the Shannon-Wiener diversity index will be calculated and used as another means for investigating the status of the reefs to be examined. I predict that there will be no significant difference in diversity among sites, but that there will be a significant difference in disease occurrence among sites.

Justification

Much research has been done regarding the degradation of coral reefs throughout the world. Existing research examines the worsening of many factors, ranging from animal diversity to water conditions and coral health. Data such as this have been very useful in creating regulations and pushing the movement for coral reef protection. While work has also been done focusing on specific coral diseases, not much research has been conducted on documenting overall presence of coral diseases, especially within the Guna Yala territory. Recording the distribution of coral diseases is important for coral preservation. Recognizing which areas may be more prone to such diseases may allow for changes to be made, such as limiting human use, to ameliorate the problem. Before any steps can be taken, clear information must first be obtained, this being one of the goals of this research.

Literature Review

Importance of Coral Reefs

Coral reefs are just one of the many types of marine ecosystems. Though they cover just a small percentage of the world's waters, less than 1%, these areas provide immense benefits and services. This miniscule area hosts almost a third of all known fish species and is the source of about 10% of all fish consumed by humans around the world. The list of human derived goods from coral reefs is endless. Extensive research has gone into the pharmaceutical use of compounds derived from soft corals, anemones, sponges, and mollusks for treatment of cancer, AIDS, and other diseases. Coral skeletons have even been used for bone graft operations. In some places, corals have been used for construction and the production of cement. But past these human goods, coral reefs play a much more important ecological role. The structure of coral reefs is important for maintaining and promoting biodiversity. Because of the three-dimensionality established by coral structure and growth, animals are able to create micro-niches, facilitating further specialization and promoting diversification leading to the possible evolution of new species (Moberg and Folke, 1999).

Considering the ecological importance of coral reefs, other ecosystems, such as seagrass beds and mangroves, cannot be overlooked. All three of these ecosystems are highly connected. This means that the different ecosystems interact with one another and are often dependent on the others for proper function. This is highly apparent when considering fish populations. Many fish use seagrass beds as maturation grounds for juveniles. The seagrass offers protection from predators, while also providing bountiful food resources for growth and development. Also, when in close proximity, mangroves are commonly used as feeding grounds for reef fish. As well as benefiting the fish, this predation helps the mangrove in that it controls sub-species populations. This includes limiting the number of herbivorous organisms that, if left to reproduce unchecked, could have harmful consequences on the whole environment in terms of over-grazing (Unsworth et al., 2008). Other influences that exist between these three ecosystems are still being investigated. Considering these findings, the importance of conserving each ecosystem is clear to see. This is why the dilapidation of the health of these ecosystems throughout the world is such a serious threat to the function and survival of these environments.

What are Coral Diseases?

Throughout seas around the world, coral reefs have undergone dramatic change in health and are suffering physical degradation. One of the greatest dangers to coral health is the development of infectious diseases. These diseases result in the progression of abnormal physical conditions which impede normal organismal functions (ICRI/UNEP-WCMC, 2010). There have been a wide variety of coral diseases documented in waters around the world. Some of these diseases are genus specific and are only found in certain species of corals. Other diseases are far reaching and can affect a vast number of different coral species. One such disease is Black Band Disease. The name of this disease comes from the characteristic dark line observed in afflicted corals. Caused by a number of different bacteria working in conjunction, this disease destroys coral tissue by altering the chemical composition of the area surrounding the living polyps. The disease creates a microenvironment that slowly eats away at the living polyps. By producing high concentrations of sulphide at the base of the band, the living tissue is killed due to lack of oxygen, compounded by the high level of toxins (Rosenberg & Ben-Haim, 2002). This disease is one of the most prevalent within the Caribbean and is one of the most easily recognizable. Other common diseases include Dark Spot Disease, White Band Syndrome, and White Plague. In a similar fashion, these diseases also cause death by chemical responses induced by the presence of the disease causing pathogen.

Due to a multitude of reasons, a large number of coral reefs have experienced an increase in mortality and greater spread of diseases, ultimately leading to the destruction of reefs across the globe. Gardner et al. (2003), showed that within the Caribbean, coral reef cover has averaged a 40% drop in the past four decades. While, in a related study conducted on Dominican reefs, an increase in both new mortality and disease growth was documented in comparative investigations just one year apart (Weems, 2011). Among the most widely accepted theories for the cause of these devastating transformations are consequences of anthropogenic actions; this includes climate change and overfishing. The danger in overfishing is that natural herbivores are removed from the reefs. With lower herbivory, algae are allowed to grow unchecked. The increased amount of algae overgrows coral and ultimately contributes to disease development through added stress.

Relatedly, higher temperatures have been proven to spur the increased spread of disease between individuals, possibly by reducing the coral's natural ability to fight off pathogens (Harvell, et al., 2007). Further research discovered that this is due to two factors: the virulence of the pathogens, and the host coral's resistance. It was found that both of these factors actually increase with water temperature. However, the pathogens' ability to survive and colonize increases to its maximum at a cooler temperature than does the coral's resistance. The pathogens' prime temperature was found to be just under 30˚ C, while the coral's ability to fight these pathogens is optimized at 31˚ C. This means that the pathogens are able to colonize and begin reproducing before the coral is truly able to ward off these harmful microbes (Ward et al., 2007). Other studies have also cited nutrient enrichment as a reason for increases in the severity of coral diseases within reef communities. Chemicals such as nitrogen and phosphorous, often found in commercial fertilizers, have been closely studied for their link to coral diseases. While the exact mechanism is still unknown, proof has been found linking the presence of these compounds in water with increased spread of coral disease (Vega Thurber, et al., 2013).

Threats of Losing Coral Reefs

With the ever constant threat of climate change, ocean waters have seen an increase in water temperature and a heightened degree of ocean acidification. If these trends continue in the future, coral reefs will be among the most heavily affected ecosystems. On top of promoting disease growth and increasing coral mortality, studies propose that the continued increase of water temperature will reduce coral diversity. The belief is that many corals will die off, leaving only those more tolerable of higher temperatures behind. As temperatures continue to rise, these effects will only become more severe. This could completely alter the composition of coral reefs and have effects on fish populations as well. The die off of commonly predated corals would drive herbivorous fish from the reef. With these primary consumers gone, secondary consumers would soon follow, either dying off or fleeing to other areas. The degradation of water conditions could also spur the growth of macroalgae and promote higher frequency of algae blooms. A sudden explosion of free-floating algae, algae blooms block light from reaching organisms below the water's surface. When considering corals, this would prevent the zooxanthellae from carrying out photosynthesis and providing the coral with nutrients. This could kill off the few remaining coral species, turning reefs into fields of algae that would not permit future coral growth.

The breakdown of reefs would eliminate the three-dimensionality that is so vital to preserving reef fish diversity. As living coral dies and coral heads crumble to coral rubble, the micro-niches would be destroyed. Without their specialized niche, fish would be forced to compete for the dwindling resources. This competition could lead to a mass die off of fish species, reducing marine diversity. In terms of human impacts, this would also lead to the decrease in fishing productivity and revenue. For developing areas or people dependent on the sea for food, the loss of available fish would have disastrous results and could lead to famine or force a change in lifestyle that might not be as productive and efficient (Hoegh-Guldberg et al., 2007).

Monitoring Coral Diseases

With coral diseases being so populous in waters around the world and the effects being so detrimental to marine health, it is important that research be conducted to ameliorate the current state of reefs worldwide. Some research has already been conducted looking into controlling the spread of diseases between individual coral organisms. Efrony, et al., (2006), found that phage therapy may work to kill certain pathogens associated with specific coral diseases. In this experiment, bacteriophage were treated to allow for adherence of a DNA sequence that would prevent bacterial infection. This was done in un-supplemented seawater, as well as seawater treated with compounds to assist in this process by cutting the DNA sequence of the original bacteriophage to allow for easier adherence. The experiment was successful in preventing coral infection by the bacterial pathogen. Further, this affect was continued and appeared to prevent further spread of coral that were already diseased. However, this research is in the early stages and is far from being applied to actual oceanic conditions. Even if a "cure" were found effective, first, the exact extent of coral diseases would need to be documented with high precision. Some work has been done with remote sensing technology, though this primarily serves to monitor coral bleaching (Maynard, et al., 2008). Although it assists in the monitoring of our world's oceans, it does not apply in the same fashion to recording coral diseases. In the matter of tracking the progress of coral diseases, the most reliable method is direct observation and notation. But with such a high diversity of coral, the different diseases associated with coral reefs are also quite numerable. Many of these diseases are specific to certain species of corals and have varying physical affects dependent on species. Reputable guides have been created for the sole purpose of identifying these diseases and documenting their presence (GCDD, 2008). By means of in the field observation and notation, coral disease presence can be monitored and tracked in a way that provides clear data. This method may even be more accurate than remote sensing for disease classification. Using techniques such as belt transects, which can study a relatively large region quickly and easily, has proven effective in many research projects and is cited as a reputable method for investigating such diseases (NPS, 1994).

Research Question

Does the prevalence of coral diseases in certain species of hard corals differ among four reefs located in and around the waters of Narganá in the Guna Yala territory of Panamá?

Materials and Methods

Site Selection

Each day, the study site was chosen dependent on weather conditions. In nicer weather, sites farther away from Narganá were studied. On days when threats of lightning and thunder did not permit long periods of snorkeling, sites within swimmable distances of Narganá were studied. Upon arriving on the reef to be studied, the examiner swam for approximately five minutes along the fore reef to find a suitable test site. Sites were chosen based on their ability to be studied easily, limiting depth, as well as the presence of live hard corals. Areas with few living corals or high abundances of soft corals were avoided when possible. Sites that did not meet this criteria were noted.

Study Sites

Ordud: This reef was located approximately thirty minutes away from Narganá by motor boat. The site was distinguished by a large sunken ship which stood out of the water. On the first day of sampling here, heavy rains and winds produced very choppy water just prior to arrival. Following sample days were generally a lot calmer. The reef at this location had a high abundance of living hard corals. Many fish species were observed in large numbers. Visibility within the water was high, especially across the reef crest. Transects were primarily run along the edge of the fore reef. As this bordered a large drop off, study depth was steadily between 3 and 4 meters in these areas. Several transects were done on the reef crest, the maximum depth here being 2 meters.

Narganá East Reef: This study site consisted of reefs found off the old landing strip on the island. Reefs were swimmable and various reefs within this area were sampled. The reefs here had notably less hard coral and were primarily dominated by lettuce coral. There was a heavy presence of sea whips and other soft corals here. Hard coral diversity was comparatively low and abundances of hard corals were much lower than at other test sites. Large amounts of algae were noted. Sample depth was fairly consistent, mostly staying within the 2 to 3 meter range. Fewer species and numbers of fish were observed, though this could have been due to the weather conditions of sampling days; this site was mostly studied on stormy days that were inhospitable for travel to farther sites by boat. On the first day of sampling, the water was subject to influxes of cold water carrying trash and organic debris. This water had much lower visibility and created a distinct thermocline. Following sample days were not affected by the incursions of this water.

Dud Sormullu: The reef was located approximately 10 minutes by motor boat from Narganá. The reef encircled a small, uninhabited island that was used solely for coconut harvesting. There were many coral species observed and sampled here, though lettuce coral was the predominant species documented. This reef was the only site tested containing staghorn coral. Visibility was high across the reef but was fairly low in the open water surrounding the reef. Sample depths here were more variable, ranging from 2 meters down to 5 meters. This was due to the large expanse of the reef and its stretch across inconstant depths. The reefs dropped into large open water channels not seen elsewhere.

Digir: This study site was located within a marine protected area constructed around the west end of the inhabited Isla Digir. The structure of the reef, a tall incline that rose close to the surface, prohibited sampling across the reef, limiting transects to the edge of the reef. Depth was unchanging around the greater part of the reef, rarely more than 2 meters deep. However, later test sites around the south side of the reef and farther from the island were much deeper, dropping to depths of 5 or 6 meters. Visibility was high along the reef. A strong current coming in towards the island posed some difficulty during sampling when exposed to the open sea side. Stronger waves were experienced the second day of sampling. All transects were kept within the demarcation buoys marking the protected area. Fish here were noticeably larger than at other sites, possibly due to the protection of the reef. The reef was also characterized by immense numbers of sea anemones. Live coral did not extend to the top of the reef and were only present along the testing areas. Algae covered a large part of the top of the reef.

Sampling Techniques

Once a suitable sample site was found, the transect tape was thrown towards the reef while treading above water. After the transect had sunk completely, it was retrieved and the line was started from the best anchorage point nearest where it landed. A 20 meter line was laid out and depth was kept as consistent as possible. The line was set to follow the natural contour of the reef, often meaning transects were not kept in a straight line. Upon setting the line, the diver swam along the line on one side and returned on the other. Corals that lay within a meter of the transect line on both sides were documented to species and categorized as either healthy or showing signs of disease. To ensure that the diver did not stray beyond one meter, a sewing measuring tape was tied to the transect line at a length of 1 meter. This line was pulled along the transect and used to measure distance from the transect on questionable corals. This method meant that each transect measured a total of $40m^2$. Each site had 18 transects completed, totaling $720m^2$ on each site. Combined for all four sites, 72 transects were conducted and a $2880m^2$ area was sampled.

For corals that did exhibit signs of bad health, a description of the affected area was recorded. If easily distinguishable, the ailment was diagnosed. In times when this was not possible, pictures and notes were compared for later determination back on land. Only corals that were visible from directly above were recorded. Corals that were under reef cliffs and not noticeable from the observation point were ignored, even if they were visible upon diving. The species of corals examined was limited to specific species and excluded all soft corals to narrow the scope of the project. Brain coral (*Diploria sp.*), lettuce coral (*Agaricia agaricites*), massive starlet coral (*Siderastrea siderea*), mustard coral (*Porites astreoides*), elkhorn (*Acropora palmate*) and staghorn coral (*Acropora cervicornis*) were all considered in this project. Star coral was also identified further to mountainous (*Montastraea faveolata*) and lobed (*Montastraea annularis*) when possible. Brain coral was not categorized further due to complications with species determination. Finger coral (*Porites porites*) was not recognized due to the difficulty to distinguish between separate colonies. Further coral species such as golf ball coral (*Favia fragum*) and great star (*Montastraea cavernosa*) were omitted as they were not noted in preliminary transects and doing so in later replicates would skew data. Lastly, fire corals (*Millepora sp.*) of all species were not included due to their true classification as hydrates. For ease of identification and discussion, these corals will be referred to by their common names.

Ailment Identification

For the purpose of this project, predation was not considered an indication of compromised health. Typical bite marks of common coral predators, such as parrot fish and damselfish, were studied to be identified in the field. These marks typically show tissue damage in an oval shaped pattern. These marks also often have some skeletal damage attributed to them. Other coral predators such as worms and snails produce more irregularly shaped tissue loss but most often do not result in any skeletal destruction. These characteristics were looked for in afflicted corals to distinguish between predation and health ailments. Additionally, presence of these animals was used as further evidence to classify the cause of the physical degradation.

Health problems were diagnosed with the aide of several guides (Bruckner, 2009; Weil & Hooten, 2008). In this investigation, compromised health included coral diseases, bleaching, and other signs of recent tissue mortality indicating unhealthy coral. The shape of the lesion was recorded, as well as its distribution: focal, multifocal, diffuse, or linear. Color change or loss was noted. Defined bands that may indicate disease progression were looked for and noted. Corals were recorded as either healthy or as showing signs of coral diseases.

Statistical Analysis

The percent of unhealthy coral was calculated for each coral species found at a site as well as for all corals within a test site. Additionally, the Shannon-Weiner diversity index was calculated for each sample site. Once all values were obtained, ANOVA tests were carried out to compare the values across sites. Tests were run comparing the percent of total corals affected between sites, the diversity index calculated between sites, and the percent of unhealthy lettuce coral and brain coral between sites.

Results

The number of hard corals counted varied between each site. The Narganá test site had the lowest number of corals present with a total of 517 individual colonies documented. The

Digir test site had the highest number of corals with 1584 total corals counted. Ordud and Sormuludub had 785 and 729 total counted, respectively (Figure 1).

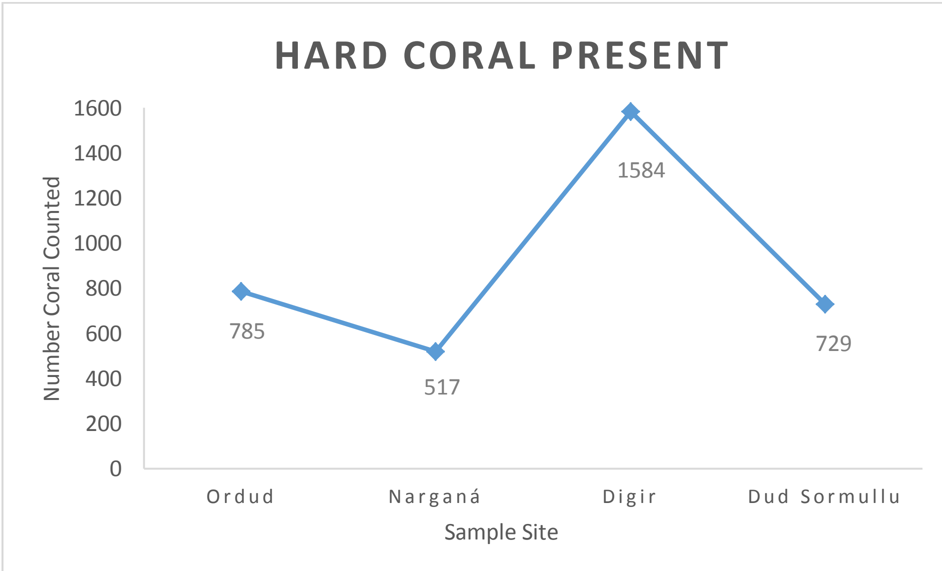

Figure 1. Number of individual coral colonies counted at each sample site. Digir had the largest number of individual colonies counted, with more than double that of the second highest, Ordud. Narganá had the lowest number of corals counted, with less than a third of the amount counted at Digir.

Though the calculated Shannon-Wiener diversity indexes did differ slightly between sample sites, these values were not significant (df=3, n=26, p=0.99). Narganá did prove to have the lowest hard coral diversity, but again, this was not significant compared to any other test sites (Figure 2).

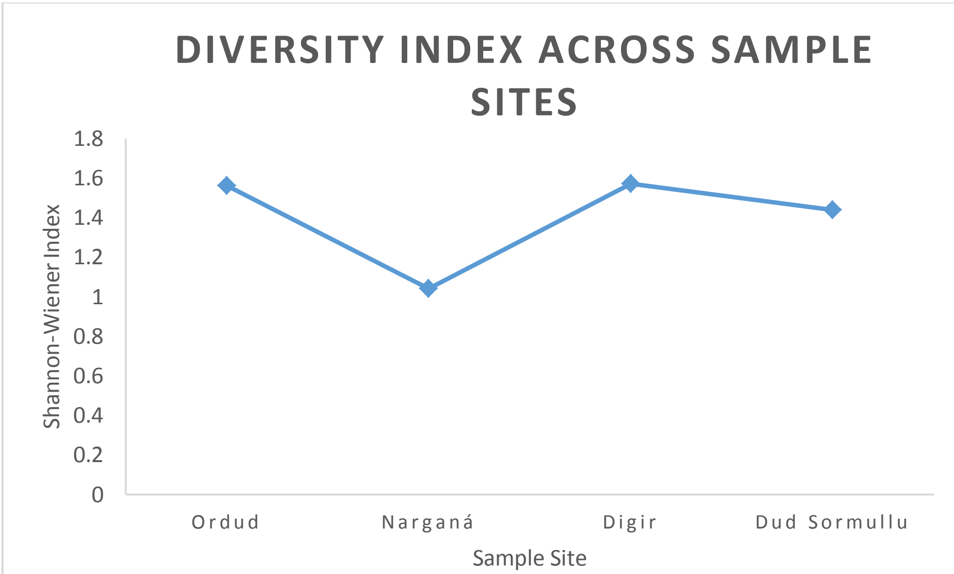

Figure 2. Calculated Shannon-Wiener diversity index for each test site (df=3, p=0.99). Digir had the highest diversity index with 1.57. This was very close to that of Ordud which had a diversity index of 1.56. Dud Sormullu had a calculated value of 1.44 and Narganá had the lowest value of 1.04.

Species distributions and populations varied across the different sample sites. Lettuce coral and mustard coral were consistently among the most prevalent coral species found at each site. The most infrequent species were elkhorn and staghorn coral (Figure 3).

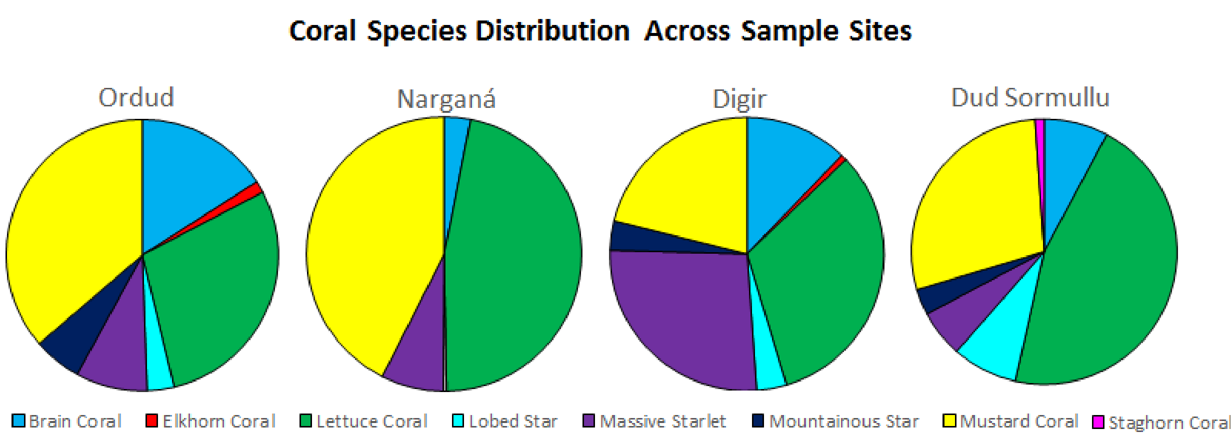

Figure 3. Population distributions of all corals documented at each sample site. A total of eight coral species were investigated and recorded across four different reefs.

Though Narganá had the lowest corals counted, it had the highest prevalence of health problems with 29.21% showing compromised health. Ordud had the lowest amount of coral showing symptoms of compromised health with just 4.20% (Figure 4). These represent significant differences (df=119, n=121, Tukey $p<0.01$). There was also significant difference between Narganá and Digir (df=135, n=137, Tukey $p<0.01$), and between Narganá and Dud

Sormullu (df=123, n=125, Tukey p<0.01). There was no significant difference between any other sample sites.

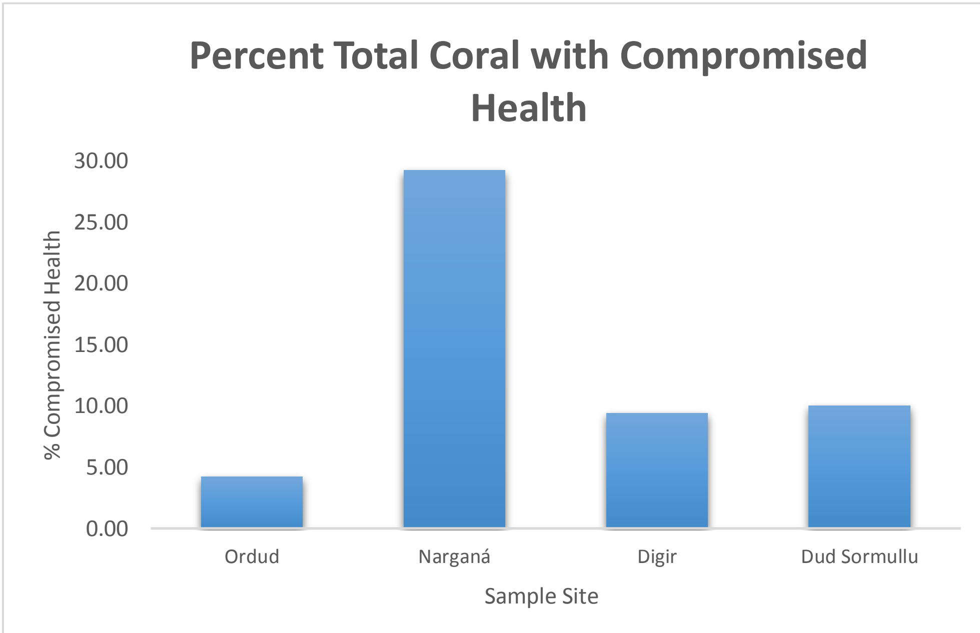

Figure 4. Percent of total coral counted at each site showing signs of compromised health. Significant difference found between Ordud and Narganá (df=119, p<0.01), between Narganá and Digir (df=135, p<0.01) and Narganá and Dud Sormullu (df=123, p<0.01).

Considering specific species of corals, there were differences in percentage of species affected among all sites. The highest percentage of lettuce coral afflicted occurred at Narganá with 57.26% of all colonies evidencing sickness. The lowest percentage of sick lettuce coral was found at Ordud with 3.52%. Digir had 20.23% unhealthy lettuce coral and Dud Sormullu had 11.41% affected. Significant differences exist between sites (ANOVA p<0.001); Ordud and Narganá (Tukey test p<0.01), Ordud and Digir (Tukey test p<0.01), Narganá and Digir (Tukey test p<0.01), Narganá and Dud Sormullu (Tukey test p<0.01) and Digir and Dud Sormullu (Tukey test p<0.05). For affected massive starlet corals, the highest percentage of ill colonies was found at Dud Sormullu with 35.71%. The lowest percentage occurred at Digir with only 8.79% sick. Narganá and Ordud had 10.53% and 19.40% effected, respectively. Significant differences exist between some sites (ANOVA p=0.002); these sites are Narganá and Dud Sormullu (Tukey test p<0.01), and between Digir and Dud Sormullu (Tukey test p<0.01). Diseased brain corals were only found at two of the four sites, Ordud and Digir. There is no significant difference among any of the sites (ANOVA p=0.27) (Figure 5).

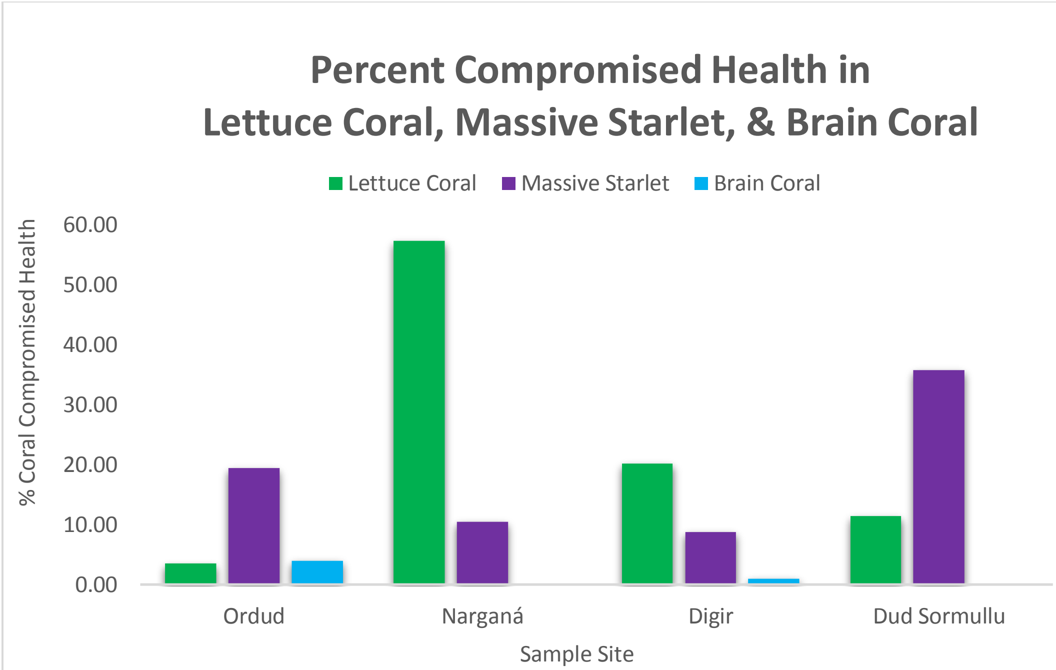

Figure 5. Percentage of three hard coral species affected by coral diseases or showing signs of compromised health at the four sample sites. ANOVA tests showed significant difference in diseased lettuce coral (ANOVA $p<0.001$) and diseased massive starlet (ANOVA $p=0.002$) among the four sites.

Three diseases were classified within massive starlet corals. The prevalence of these diseases varied based on sample site. The highest occurrence of dark spot disease as well as white plague within massive starlet coral was within the Digir sample area. There were 20 corals with physical signs of dark spot disease and 11 that appeared to be suffering from white plague. Black band disease was only found in Ordud and at Digir. Narganá had the lowest incidence of all types of diseases, as the only disease found at this site was dark spot disease. Overall, dark spot disease was the most prevalent disease with 34 total corals affected across all sites. White plague was the second most prevalent disease with 27 sick corals. Black band disease was the least frequently found disease, having only 7 diseased corals (Figure 6).

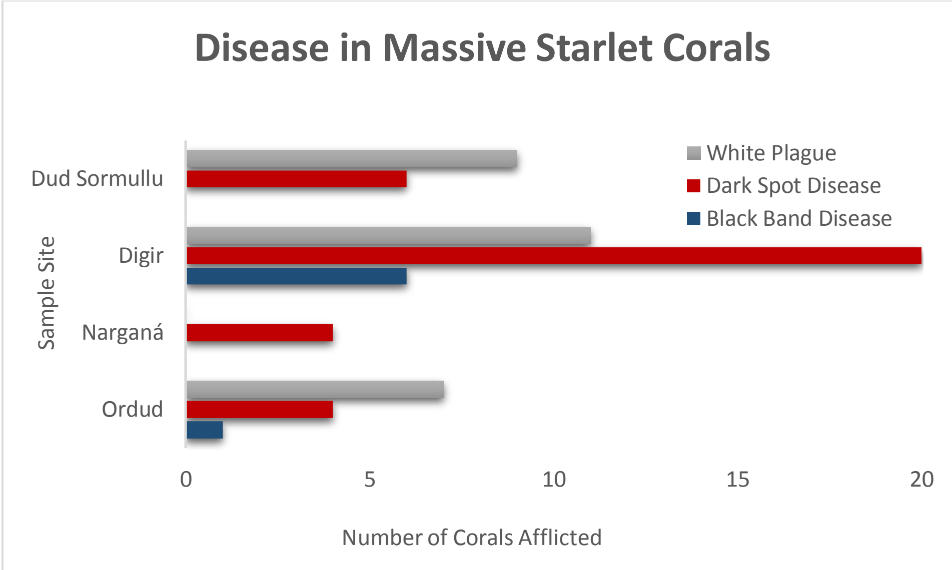

Figure 6. Types and distribution of diseases documented in massive starlet corals at each sample site. The highest number of all diseases was found within the Digir sample site. Narganá had the lowest number of diseased massive starlet corals. Dark spot disease was the most commonly found disease and black band disease was the least commonly encountered disease.

All lettuce corals documented with compromised health were determined to be suffering from white syndrome. The number of sick colonies was highly variable between sample sites. Narganá again had the highest number of sick colonies, 138, and Ordud had the lowest, 12 (Figure 7).

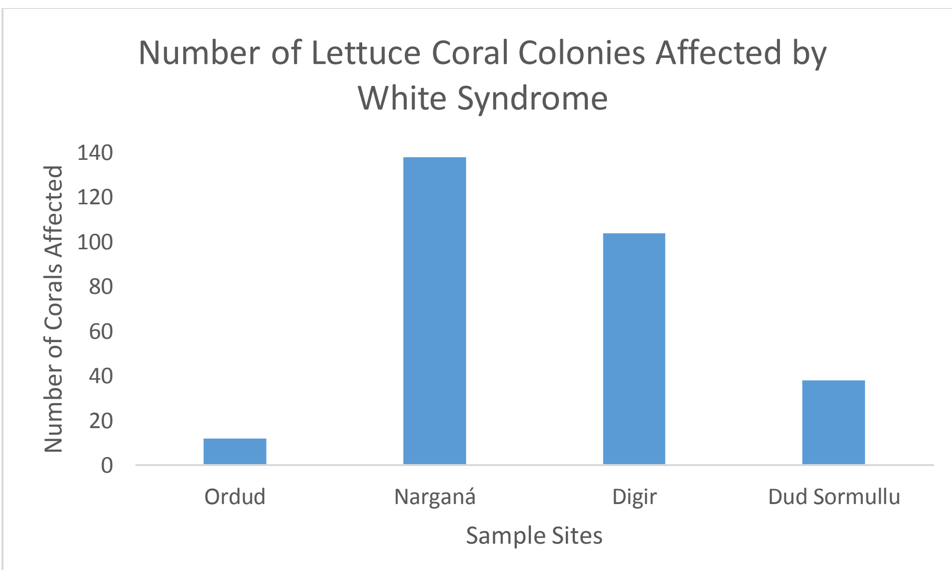

Figure 7. Number of lettuce coral colonies documented to have signs of white syndrome at each of the four sample sites. Narganá had the largest number of sick lettuce coral colonies with a total of 138. Digir had 104 sick colonies. Thirty-eight sick colonies were found at Dud Sormullu. Ordud had the lowest number of sick colonies with just 12 affected.

Other diseases found included white patch disease on elkhorn coral at Ordud. Two brain coral diseases were documented. At Ordud, one brain coral suffered black band disease and 3 were afflicted with white plague. Two brain corals were also found with white plague at Digir. Dud Sormullu had 6 colonies of stag horn coral suffering from white band disease; this was the only site at which this species of coral was documented.

Discussion

Looking strictly at the number of diseased corals, Narganá appears to have the lowest occurrence of diseases in all corals aside from lettuce coral. Without considering other data present, this may suggest that Narganá could possibly be considered the "healthiest" reef in terms of lowest prevalence of harmful diseases. However, looking at the diversity and distributions of coral species across sample sites as well as the total amounts of coral counted, Narganá had the lowest amount of live corals present. Digir had more than three times the amount of coral documented than at Narganá. With this information, it follows that Narganá had such a lower occurrence of disease in massive starlet or brain corals simply because there were fewer live corals to be infected. For this reason, the percentage of total corals with compromised health, Figure 4, is the most telling in the overall health and prevalence of disease. Though the coral affected was mainly lettuce coral, this graph shows the higher level of disease that is present at Narganá. This is also apparent in Figure 7. Narganá had an extremely high number of sick lettuce coral colonies compared to all the other sites. With such a high difference in percent sick, almost 30% compared to about 10% at the next highest site, it is safe to say that Narganá had the heaviest prevalence of coral disease. Looking at the presence of these diseases as the sole indicator, this would imply that the Narganá sample site was the unhealthiest site.

It is important to note that this reef was most heavily influenced by human activity. Just offshore from the main island, this site was subject to more boat traffic than other areas. Additionally, this site may be more heavily influenced by waste runoff and pollution. Extensive research has found that coral reefs are under immense stress and are highly impacted by human activities. In a study conducted in 2000, researchers found that 97% of all coral diseases are found in areas that are considered to be moderately to severely impacted by human activity. Some of these activities include coastal development, marine-based pollution, overexploitation and destructive fishing, and land-based pollution (Green & Bruckner, 2000). It has also been found that microbial contaminants associated with human waste can often be found in high concentrations within coral mucus. These contaminants pose harm to the corals as well as other organisms within reef environments (Lipp et. al., 2002). Harboring these potentially harmful substances within their tissues, the accumulation of such compounds within corals may act as another factor driving disease progression. Many of the problems listed, such as overfishing, pollution, both on land and in the water, and human waste runoff, are very prevalent within Guna Yala, and especially on the island of Narganá. Trash disposal is a major issue on the island, as much of the garbage is simply left on the shore, where it either amasses with other debris, or is taken by water currents elsewhere. Also, much of the human sewage leads directly into the water. Considering the results of prior research, this could explain why the Narganá reefs, which are in very close proximity to the inhabited island, had such a high incidence of coral disease.

Contemplating the presence of other diseases, Figure 6 shows that Digir had the highest number of diseased massive starlet corals. All three diseases documented were found in the highest abundance within the Digir sample area. Again, this may be slightly misleading, as the percentage of sick massive starlets was lowest at Digir, represented in Figure 5. Having more than double the number of live coral than the second most numerous site, the seemingly high number of diseased massive starlet corals actually ended up being the lowest overall percentage of disease in massive starlet corals.

In terms of percentage of diseased corals among all corals counted, the lowest total occurred at Ordud. This would suggest that this sample site was the "healthiest" of all sampled reefs, although this can only be said for certain when compared to Narganá. Prior research has concluded that measuring the prevalence of coral disease throughout a region can serve as a biological indicator of overall coral condition. This research found that healthy reefs will most often have about 6% of all corals afflicted with disease; this appears to be the base level for disease presence in many coral reefs. An area with disease occurrence significantly higher than 6% can be categorized as having an elevated disease level above the natural state. This suggests that the area is under high stress, possibly as a result of altered environmental quality (Santavy et al., 2005). Using this criteria, Ordud can be considered "healthy," with a disease prevalence of just 4.20%. This also gives further credence to designating the Narganá reefs as being "unhealthy," since the percentage of corals being affected by disease here was 29.21%. The other two sample sites cannot be listed as "unhealthy" since the percentage of sick coral colonies at these sites were not significantly higher than 6%.

Regarding human activity and influence, Digir should be expected to be considered the least influenced. Located inside of a marine protected area (MPA), it can be inferred that this site is not subject to the same level of anthropogenic disturbances as other sites. Such disturbances can include overfishing, physical damage from boats and divers, and even pollution and waste runoff. However, the border for this MPA ran alongside a fairly heavily populated island. Though the waters were protected on one side of the buoys, outside this area, all these practices were allowed. Having no true physical border the trash and waste dumped outside the MPA border was able to drift into the protected area, as was witnessed first-hand by researchers. For this reason, Digir may *not* have proven to be the site least affected by human activity.

It may be important to note that larger fish were noted within the area and in higher numbers. Also, species such as snappers, which were not seen elsewhere, were found in notable numbers in this site. Finding larger fish here would be a reasonable observation, considering that MPAs have proven to increase both carnivorous and herbivorous fish biomass (Mora, 2008), considering size and populations. However, higher fish populations may be the reason that there were a high number of diseases here, especially black band disease. Research has proven that areas with higher grazing behavior by fish such as various species of damselfish (genus *Stegastes*) lead to higher occurrences of coral diseases, specifically black band disease. Higher rates of disease may occur due to damselfish tendency to exclude macroalgae to maintain turf algae on coral heads to provide a stable food source. The damselfishes' grazing behavior allows for pathogens to establish themselves within the coral tissue. This includes types of cyanobacteria that are linked with the development of black band disease. For this reason, the continued farming behavior that these fish demonstrate only increases the prevalence of coral diseases (Casey et al., 2014). If there were in fact larger populations of these fishes, it would provide an explanation for why there was a high occurrence of coral diseases, even though it was a low percentage of the total corals found within the Digir sample area. Since the populations of these fish were not documented, a study investigating fish sizes and populations would have to be conducted to further substantiate this statement.

Although it is difficult to measure and evaluate, the lowest level of human disturbance may have been within Ordud. The sample area here was a patch reef, meaning that it lay in open water and did not border an island, as was the case with every other sample site. Also, Ordud was the sample site farthest away from any nearby inhabited islands. For this reason, it can be assumed that the reef was not subject to the same levels of waste runoff and other possible detrimental human activities. In addition to the research discussed earlier, a positive relation has been found between proximity to sewage runoff and the amount of infected corals. This research found that reefs nearby a sewage discharge were more prone to having corals contract black band disease and white plague. Ultimately, these reefs affected by the sewage runoff also experienced higher rates of mortality (Kaczmarsky et al., 2005). If Ordud is the site with the lowest human disturbance, this would have important implications. The findings would show that Narganá, with the highest human influence, had the highest percentage of sick corals, and Ordud, with potentially the lowest human disturbance, had the fewest diseased corals. The resulting assumption would be that coral disease presence is directly influenced by human activity. Considering these findings, it would be clear to reason that as proximity to human inhabited areas and human disturbance increases, so does the prevalence of coral disease.

Before these conclusions could be stated concretely, similar research would have to be conducted to attempt to repeat these findings. It must be stated that some of these results might not be truly representative. The decision to ignore certain coral species, such as golf ball and great star, could have greatly altered the final results for this project. These species were observed fairly often at certain sites. As stated earlier, since they were not originally recorded in the first transects, doing so later on in the research would have given skewed data. Repeating this experiment to include such species would yield more conclusive and definitive results. It may also have an effect on the calculated diversity indexes, possibly leading to significant differences between sample sites. Along these lines, there were some problems with disease identification encountered through the duration of this project. Ailments were identified to the best of the researcher's ability, though diseases encountered below water often look different from pictures in field guides. Certain key characteristics were looked for and noted, but the possibility remains that some health issues were misidentified. Further, other variables must be monitored and sampled to clearly state whether a reef can be classified as healthy or unhealthy. This project simply defined "unhealthy" as having a high amount of diseased corals. In truth, many other factors contribute to the overall health of a reef. For this reason, other research measuring and monitoring these aspects must be considered in conjunction with the findings from this project to make definitive statements on the health of a reef. Finally, the measure of human disturbance was very subjective. An effective way to measure human disturbance and activity must be determined and practiced to make further conclusions and test if disease presence truly is dependent on human influence.

Conclusions

This project was successful in documenting the prevalence of different coral diseases in specific hard coral species at four different reefs near Narganá in Guna Yala, Panamá. The findings of this project supported the hypothesis that diversity would not significantly differ among sample sites. A significant difference in percent of corals with compromised health was found between certain sites, Narganá and Ordud, as well as between Narganá and Digir. The difference in disease prevalence across sample sites also suggests that there is a relation between human activity and disease occurrence. The reefs most heavily suffering from coral diseases were in close proximity to a highly populated island. The sample site with the lowest incidence of coral disease was farthest away from any nearby inhabited islands and the only noted human use here was for recreational snorkeling purposes. These findings support the second hypothesis that reefs that experience heavier human disturbance and activity would have higher severity of coral diseases. However, more replicates would prove useful in validating these conclusions and a means for measuring human influence must be created to verify this relation.

A future project that might give more insight to coral disease progression and the effect of human activity may be to monitor the spread of disease across a coral colony and its transmittance to others. Locating diseased corals in the sampled reefs and monitoring the change in size of the diseased region over a set period of time would yield results on the spread of disease. It may be possible to determine if the human induced environmental stresses that lead to increased disease occurrence also lead to increased disease growth on individual corals. If this were true, the experiment would find that the disease would advance across a single colony at a higher rate in Narganá then it would at Ordud. Other research that may be useful for investigating the interactions between organisms within coral reef ecosystems may be to explore the effect of removing grazing fishes from the environment. In a controlled system, such as a tank, tests could be conducted to find if taking these fish out of the environment can help prevent the spread of coral disease. Since these fish are believed to contribute to coral diseases due to their tendency to “farm” algae, removing these fish and allowing for natural algal growth may prevent the infectious cyanobacteria from establishing within coral and causing disease. This may not help with the treatment of coral diseases, but could potentially prevent further spread.

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