AN ABSTRACT OF THE THESIS OF Roxanna Layne Miller for the Master of Science in Biology presented April 7, 2011.

Title: Partial Characterization of Growth Anomalies Affecting Massive *Porites* Species in Guam.

Approved: _____
Laurie J. Raymundo, Chairwoman, Thesis Committee

Growth anomalies (GAs) affect a range of coral species throughout the world, yet information regarding their etiology is incomplete. This study aimed to partially characterize how growth anomalies affected massive *Porites* spp. on Guam. Three types of GAs have been visually identified, and since etiologies are unknown, they are labeled Type I, Type II, and Type III. It was important to not only look at how GAs were affecting their host coral, but whether or not the three GA types were affecting the host coral differently, suggesting that these three types were distinct GAs. To determine GA affects on the host colony, physiological, microbiological, and ecological data were assessed by looking at GA growth over a 15 month period, GA transmission potential, zooxanthellae density, Chl a & c absorbance, tissue thickness, a suite of skeletal morphometric characteristics, and microbial abundance. GA growth was significantly different between GA Type II in Luminao and Ipan, and was also significantly different from both GA Types I and III. Type I GAs had little effect on its coral host. Type II, however, had effects on the coral host in mean corallite densities, mean maximum

corallite diameter, and mean corallite surface area. Type III had effects on the host in mean corallite diameter, mean corallite surface area, mean number of septae, and mean number of palli. There were also significant differences seen in microbial abundance between remote healthy colonies and those with GAs. These results suggest that not only do these GAs have detrimental effects on the host colonies normal function, but that the three morphologically different GAs affect their host species differentially and that these three GA types may be different types and not just three morphological types of one disease. A study with larger sample sizes needs to be conducted to elucidate these findings.

TO THE OFFICE OF GRADUATE STUDIES

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PARTIAL CHARACTERIZATION OF GROWTH ANOMALIES AFFECTING MASSIVE *PORITES* SPECIES IN GUAM

BY

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1: INTRODUCTION

1.1. Coral Disease

The degradation of coral reefs worldwide is a result of the increased frequency of both natural and anthropogenic disturbances (Harvell et al. 1999, 2002; Lesser 2007). These disturbances are hypothesized to add to the appearance of, and increase in, coral diseases which have become a pressing issue for the future of all coral reefs. Over the past 30 years there has been an approximate 30% decline in worldwide coral population; this coincides with the increase in frequency and magnitude of coral disease over the same time period (Hughes et al. 2003, Reshef et al. 2006). It has also been shown that within the past 20 years described coral diseases have increased in number, coral species affected, and geographic extent (Sutherland 2004, Weil 2004, Myers & Raymundo 2009). There are over 30 described coral diseases, several of which are known to affect coral species on a global scale (Lesser 2007, Sutherland 2004, Weil 2004, Willis et al. 2004). The full impacts of most of these diseases on coral populations are poorly understood. While coral diseases have been acquiring more attention within the past decade, comprehensive knowledge regarding these diseases is severely lacking and the description of disease signs is sometimes vague. Many diseases have not been formally characterized (Richardson 1998) and disease causation is challenging to prove (Work & Rameyer 2005). With this realization, there is need for more intensive and comprehensive analyses into coral disease etiology.

The Caribbean has long been thought as a coral disease "hot spot" while Indo-Pacific reefs have often been thought of as "healthier", with fewer reports of disease in

the past. While the Caribbean is still a "hot spot" for disease, the Indo-Pacific is not "healthier" and recent studies have shown that an increase of almost 50% of reported diseases since 1996 have come exclusively from the Indo-Pacific (Raymundo et al. 2003, Sutherland et al. 2004, Kaczmarsky 2006, McClanahan et al. 2009, Willis 2004, Gochfeld & Aeby 2008). Since coral diversity (and often abundance) is much higher in the Indo-Pacific, the potential is great for coral disease to cause detrimental impacts to biodiversity, leading to reef ecosystem shifts.

The Merriam-Webster Dictionary (1997) defines disease as "an abnormal bodily condition that impairs functioning and can usually be recognized by signs and symptoms". Disease, as defined by Wobeser (1997), is "any impairment that interferes with or modifies the performance of normal functions, including responses to environmental factors, such as nutrition, toxicants, and climate; infectious agents; inherent or congenital defects; or combinations of these factors". Both definitions refer to an alteration or modification of normal function. Disease in corals is believed to operate the same way though the clonal colonial nature of corals presents some interesting perspectives. Disease reduces the fitness and function of either the colony as a whole, or of individual polyps allowing later recovery or causing partial mortality (Antonius 1985, Sutherland et al. 2004, Harvell et al. 2007). Of the over 30 described diseases, a causative agent has been isolated and characterized for only nine (Sutherland 2004, Rosenberg 2007, Sussmen et al. 2008). These nine consist of the following: bacterial bleaching of *Oculina patagonica* by *Vibrio shiloi* (Kushmaro et al. 1997), bacterial bleaching of *Pocillopora damicornis* by *Vibrio corallilyticus* (Ben-Haim et

al. 2003), aspergillosis of *Gorgonia ventalina* caused by *Aspergillus sydowii* (Smith et al. 1996, 1998), white plague II of several Caribbean corals caused by *Aurantimonas coralicida* (Richardson et al. 1998, Denner et al. 2003), white pox (acroporid serratiosis) of *Acropora palmata* by *Serratia marcescens* (Patterson et al. 2002, Sutherland et al. 2010), white syndrome of *Pachyseris speciosa* in Palau by *Vibrio coralliilyticus* (Sussman et al. 2008), white plague of several Red Sea corals caused by *Thalassomonas loyana* (Thompson et al. 2006), skeletal eroding band of several Indo-Pacific corals by *Halofolliculina corallasia* (Antionius & Lipscomb 2000), and brown band of several coral within the genus *Acropora* by *Porpostoma guamensis* (Lobban et al. 2011). Black band disease has also been described and consists of a consortium of bacteria which varies depending on location (Sutherland et al. 2004). Although these diseases have been described and the field of coral disease research is expanding, most other diseases have no known causative agent. Several microorganisms which have been implicated in disease causation include marine bacteria (Ritchie & Smith 1995, Kushmaro et al. 1996, 1997, Ben-Haim & Rosenberg 2002, Ben-Haim et al. 2003, Denner et al. 2003, Cervino et al. 2004, Thompson et al. 2006, Sussman et al. 2008), cyanobacteria and associated members of microbial consortia (Myers et al. 2007, Voss et al. 2007), bacteria from terrestrial sources (Patterson et al. 2002), fungi (Smith et al. 1996), protozoans (Antonius & Lipscomb 2000, Page & Willis 2008, Bourne et al. 2008, Cróquer & Weil 2009), algae (Goldberg et al. 1984), and viruses (Davy et al. 2006).

Most infectious diseases are assumed to be caused by a microbial agent, but there is often little evidence to support this assumption and most described diseases

still await positive identification of such an agent (Sokolow 2009). Here, the difficulty lies in the reality that some diseases may exhibit similar signs and symptoms, when in fact they may be different diseases (white plague I, II, & III; white band I & II), or may not be infectious diseases at all ("rapid-wasting disease" was actually predation; Bruckner & Bruckner 1998). Also, some pathogens which have been previously identified from living tissue may lose their infectiousness or pathogenicity in laboratory challenges (Rosenberg et al. 2007). Moreover, the modes of transmission of coral diseases are not well understood – vector transmission of infectious agents is considered rare (though few data exist) and vertical transmission of disease from parent to larvae has not been evident in any case to date (Sokolow 2009). Transmission of an infectious agent, either by direct or water-borne contact, is still the method of infection proposed for many diseases (white plague type II, some growth anomalies, bacterial bleaching, black band, white syndrome, *Porites* ulcerative white spot), despite the paucity of information available.

The traditional microbiological method for proving disease causation has been the fulfillment of Koch's Postulates (Koch 1882). Koch's Postulates constitute four main experimental requirements: 1) the same pathogen must be present in every case of the disease, 2) the pathogen must be isolated from the diseased host and grown in pure culture, 3) the pathogen from the pure culture must cause the disease when it is inoculated into a healthy susceptible lab animal, and 4) the pathogen must be isolated from the inoculated animal and must be shown to be the original organism. Koch's Postulates make two major assumptions: 1) all diseases are caused by a culturable

infectious agent, and 2) there is only one pathogen for each disease (Sutherland et al. 2004, Lesser et al. 2007). However, limited evidence from coral disease research indicates that there may be more than one infectious agent, the agent may not be culturable, or the disease may have multifactorial etiologies (Sokolow 2009). In these cases, Koch's Postulates fail to provide evidence for causation and multiple approaches are necessary for definitive identification of a causative (i.e. infectious) agent, if there is one. This approach is particularly inappropriate for non-infectious diseases, diseases caused by multiple organisms, disease in which predisposing factors are important, diseases with a carrier state, and diseases caused by opportunistic agents that may not always cause disease when present (Wobeser 2007).

Lesser et al. (2007) suggests an alternative interpretation of the data, hypothesizing that most microbiological infections are a secondary phenomenon caused by opportunistic pathogens after physiological stress (i.e. elevated temperature). The role of environmental variables in coral disease dynamics is becoming apparent to researchers and is now coming to the forefront of coral pathology (Harvell et al. 2002, Rosenberg & Ben-Haim 2002, Cervino et al. 2004, Bruno et al. 2007, Harvell et al. 2007, Rosenberg et al. 2007, Muller 2008, McClanahan et al. 2009, Sokolow 2009). In light of predictions of future climate change and the current increase in sea surface temperature and ocean acidification, it is imperative to develop an understanding of effects these drivers may have on disease dynamics. With an increase in sea-surface temperatures estimated at 1.5 to 4.5 °C in the next century, it is predicted that this will not only have a direct effect on coral health, but also on the pathogens and disease

dynamics. Many diseases display seasonal variation in prevalence, often with higher prevalence in warmer months (Rosenberg et al. 2007). An increase in sea-surface temperature may increase host susceptibility due to temperature stress (Bruno et al. 2007). Pathogens respond to temperature based on species-specific optimal growth curves and can become more virulent at higher temperatures (Ben-Haim et al. 2003). There is also evidence suggesting that temperature-induced bleaching events can be followed by outbreaks of infectious diseases (Muller al. 2008). Recently, McClanahan et al. (2009) presented an apparent relationship between coral bleaching and growth anomalies in massive *Porites*. Given reported impacts to coral health and shifts in pathogen virulence with an increase in temperature, increases in frequency and duration of disease outbreaks in the face of global warming is likely (Hughes et al. 2003).

Host density and abundance are also thought to be influential on disease occurrence and distribution and positive relationships between host density and disease prevalence have been demonstrated in many host-pathogen systems (Anderson & May 1979, Rudolf & Antonovics 2005). Bruno et al. (2007) suggests that if coral cover is high, distance to nearest neighbor becomes reduced, as does distance between infected and healthy hosts, increasing the potential for horizontal disease transmission (i.e. colony to colony through direct contact or a vector). Aeby (2006) found that in the Northwest Hawaiian Islands (NWHI), both acroporid and poritid diseases were highest in areas where the abundance and diversity of these genera were highest. Poritids were the dominant coral and trematodiasis was the most common and widespread disease on *Porites* (Aeby 2006). In Guam (Myers & Raymundo 2009) and the Philippines

(Raymundo et al. 2009) *Porites* spp. abundance showed a significant and positive link with total disease prevalence. The reverse is also true (but less easy to prove); in reef systems where *Porites* is less common (Great Barrier Reef), trematodiasis (and other diseases affecting *Porites*) is less common (Willis et al. 2004). Lafferty & Holt (2003) suggest that as host populations decline, so will disease prevalence, assuming that diseases are density- and host-dependent.

However, this does not mean that host distribution is the only factor controlling disease occurrence. *Porites* spp. was abundant in the NWHI, but poritid diseases other than trematodiasis were relatively rare (Aeby 2006). Ward et al. (2006) found that in the Yucatán, *Porites* is the second most common genus (>20%), but is less affected by disease (<5% disease prevalence) than *Acropora* (>20% abundance with >25% disease prevalence). Other factors which can influence disease occurrence are:

1) host susceptibility: the more susceptible a host is to the pathogen or disease, the more likely it will become infected (Rosenberg & Ben-Haim 2002)
2) pathogen virulence and life history traits: some pathogens become more virulent and production of harmful enzymes increase with an increase in temperature, facilitating pathogen infection to the host (Ben-Haim et al. 2003)
3) temperature stress: climate warming can cause corals to bleach, making them more susceptible to disease, and increase pathogen virulence (Willis et al. 2004, Miller et al. 2006, Harvell et al. 2007, McClanahan et al. 2009)

4) anthropogenic stressors: human activity has greatly enhanced global transport of marine species and pathogens, and increased pollutant inputs to the marine system which can bring in anthropogenic pathogens and bacteria and alter host susceptibility to disease or pathogen virulence (Harvell et al. 1999, Aeby et al. 2011)
5) coral cover and community composition: some species or genera may be more susceptible to disease so those reefs with more susceptible species, or a large amount of one susceptible species, may see an increase in disease occurrence (Bruno 2007)
6) reef-fish diversity: reefs systems with higher fish diversity and protection status (MPA) were found to have less disease than those unprotected with less fish diversity (Raymundo et al. 2009).

Coral resistance to disease is also poorly understood. Corals possess innate immunity which is "a nonspecific ability to react to many potentially pathogenic organisms that is not altered with subsequent exposure" (Mullen et al. 2004). Their host defenses include mechanical or physiochemical barriers, secretion of chemicals or production of bioactive compounds (humoral defenses), and phagocytic cells that can engulf and destroy microorganisms on contact (cellular defenses) (Sutherland et al. 2004). There is evidence that hard corals can also release antimicrobials after exposure to stress (Geffen & Rosenberg 2005), possibly reducing the chance of opportunistic pathogenic infection. Lesser et al. (2007) proposes two methods of disease resistance: 1) coral acquisition of resistance through survival and reproduction of resistant

genotypes, or 2) coral resistance through immunological memory (which has been described for allorecognition; Sutherland et al. 2004). This alloimmune and adaptive-like immunological response has been demonstrated in the gorgonian *Swiftia exserta* (Salter-Cid & Bigger 1991) and the hard coral *Montipora verrucosa* (Hildemann et al. 1977) in which both corals demonstrated an immunorecognition system with a memory component of at least a short-term duration. The corals were able to recognize "self" and "non-self" after several repeated exposures, with "non-self" recognition time decreasing with subsequent exposures after short resting periods. The case for adaptation of corals for disease resistance comes from Reshef et al. (2006) who propose the coral probiotic hypothesis. After the discovery of bacterial bleaching of *Oculina patagonica* by *Vibrio shiloi* (Rosenberg and Ben-Haim 2002), Reshef et al. (2006) have noted that since 2004, *V. shiloi* can no longer be found on the corals, coral bleaching is rare, and that *V. shiloi* that previously infected corals are unable to infect existing corals. They proposed that *O. patagonica* has developed resistance to infection by *V. shiloi*. The coral probiotic hypothesis states "that the coral animal lives in a symbiotic relationship with a diverse metabolically active population of microorganisms. When environmental conditions change, the relative abundance of microbial species changes in a manner that allows the coral holobiont to adapt to the new condition" (Reshef et al. 2006). This suggests that while the coral animal may or may not be able to develop resistance, the coral's microbial population is able to adapt, change and subsequently allow the coral to resist infection over time. Given the near impossibility to treat and

cure coral diseases, promoting the study of coral adaptation of disease resistance may lead to one of the few disease management options available.

Coral disease, like any other disease, can have a wide range of impacts to a coral over varying time frames. The impacts a disease has on a coral are also crucial to understanding the impacts to the population. Diseases can generally be categorized into two types: acute and chronic. Acute diseases have a relatively rapid onset and can lead to partial and whole colony mortality while chronic diseases are those diseases or infections which have a relative slow onset and can have sub-lethal impacts to the coral (Raymundo et al. 2008, Page 2009). Page (2009) found that black band and skeletal eroding band contributed to significant mortality (acute impact) in corals on the Great Barrier Reef, whereas growth anomalies did not. Sub-lethal (chronic) impacts to corals, such as resource allocation for maintenance, growth, and reproduction, have not been studied as much (Cheney 1975, Bak 1983, Yamashiro et al. 2000). It is important to understand these sub-lethal impacts since they may be longer-lasting on the coral population, reducing reproductive output, and impacting population resilience.

1.2. Disease and Porites *spp.*

Throughout the world's tropical oceans, *Porites* spp. are abundant and, for many reefs, the dominant reef-building corals. In the eastern hemisphere, *Porites* spp. have the highest species richness in southeastern Asia, the western Pacific Ocean, southeastern Indian Ocean, and northern Australia (including the Great Barrier Reef) (Mohedano-Navarrete 2008). Within this area lies the Coral Triangle, containing over

75% of all know coral species. Even in Kenya, both branching and massive *Porites* spp. were among the dominant corals identified in Mombasa (Weil & Jordán-Dalgreen 2005), and in Hawaii, *Porites* spp. are among the four major genera (Hunter 1999, Aeby 2006). In Sulawesi, *Porites* spp.are among the two major coral genera in the surveyed areas with massive *Porites* spp. being the dominant growth form (Haapkylä et al. 2009).

Given that the *Porites* are dominant species on reefs, their potential for disease transmission is increased due to their abundance, and it is not surprising that the genus *Porites* is also a dominant host for many coral diseases in the Indo-Pacific (Aeby 2006, Raymundo et al. 2005, Kaczmarsky 2006, Harvell et al. 2007) and the Caribbean (Sutherland et al. 2004). Kaczmarsky (2006) found that disease primarily affected *Porites* spp. and affected few other taxa in the central Philippines. Table 1.1 lists the diseases identified to affect *Porities* spp.

Even though disease has been shown to affect *Porites* disproportionately compared to other genera, *Porites* are considered "hardy" species and are dominant reef builders (Raymundo 2005, Myers & Raymundo 2009). Clark & Edwards (1995) have found that massive *Porites* had the lowest mortality rates after transplantation, suggestive of their hardy nature. Sheppard (1999) also found *Porites* spp. survived the 1998 ENSO event better than all other genera that bleached in the Chagos Archipelago. Corals from the family Poritidae constituted an average of almost 50% of the live hard coral on Guam (Myers & Raymundo 2009). Kaczmarsky (2006) found that even though prevalence of *Porites* ulcerative white spot (PUWS) and "tumors" was high amoung *Porites* colonies in the Philippines, mortality rates were low, a large percentage of

colonies with PUWS recovered, and the rate of spread of "tumors" on individual colonies was slow. Given that *Porites* is such a robust genus, their apparent susceptibility to the majority of diseases affecting corals worldwide, and the fact that they are among the most common reef-builders in the Indo-Pacific suggests the need for more focused studies and long-term monitoring to determine what impacts diseases may have on the future community structure and diversity of these reefs.

While it is unknown exactly how disease may affect coral reproduction, it may have a detrimental effect on fecundity (Yamashiro et al. 2000, Work et al. 2008). *Porites* spp. in the Caribbean are both gonochoric and hermaphroditic brooders. In the wider Pacific, the trend is towards gonochoric spawners (Richmond & Hunter 1990). Szmant-Froelich (1984) found that the broadcast-spawning gonochoric Pacific *Porites* are large and long-lived. In *Montastrea annularis*, which oftentimes are massive and older, colonies needed to attain a certain minimum size before becoming fully reproductive; this applied to both young colonies and those that had been fragmented from old colonies (Szmant-Froehlich 1985). Fragmentation, therefore, can result in diminished colony size and cause a fragment to revert to a pre-productive state. Partial mortality can have the same effect. Given that *Porites* spp. are dominant reef-builders, disease causing partial mortality could impact reproductive output and larval production.

1.3. Skeletal Growth Anomalies

Skeletal growth anomalies (GA) are abnormalities of coral tissue and skeleton and appear as distinctly protuberant masses on corals; are found worldwide (Sutherland

et al. 2004); and affect at least 56 species of hard coral (Kaczmarsky 2006, Willis et al. 2004, Peters et al. 1986, Hunter 1999, Sutherland et al. 2004, Raymundo et al. 2005, Domart-Coulon et al. 2006, Gateño et al. 2003, Yamashiro et al. 2000, 2001, Cole & Seapy 1998, Breitbart et al. 2005, Loya et al. 1984). However, the etiologies of GAs are poorly known. The first report of GAs affecting scleractinian corals comes from Squires (1965) who described an anomaly on *Madrepora kauaiensis* as a neoplasm (i.e. tumor). Since then, a variety of classifications of skeletal growth anomalies have been made (Table 1.2). In an attempt to standardize nomenclature and protocols for describing GAs, Work et al. (2008) classified growth anomalies affecting acroporids according to their gross morphologies. It is important to note that description of growth anomalies has not been consistent.

GAs have been found on multiple species within the classes Hydrozoa and Anthozoa, in the suborder Anthomedusae and orders Alcyonacea and Scleractinia, respectively. Sutherland et al. (2004) lists 22 species (in 10 families) from the Caribbean and 24 species (in 9 families) from the Indo-Pacific which are susceptible to GAs. These conditions range from calicoblastic neoplasms on *Acropora palmata* (Peters et al. 1986, Table 1.2) to algal-induced "tumors" on *Gogonia ventalina* and *Pseudoplexaura* spp. (Morse et al. 1977, Goldberg and Makemson 1981). The calicoblastic neoplasms described by Peters et al. (1986) showed proliferation of calicoblastic tissue, the number of calicoblastic cells in the tumor area were significantly greater than in healthy areas, calicoblastic cells resembled those in apical polyps, skeletal composition was the same as in healthy areas, and neoplastic areas grew rapidly. On the basis of these

observations, the tumors observed on *A. palmata* were called neoplasms and were termed "calicoblastic epitheliomas" due to the nature and apparent origin of the tumor. Neoplasms are areas of uncontrolled cell proliferation and are the closest representation of a true tumor in corals. Peters et al. (1986) and Coles and Seapy (1998) describe neoplasms on *Acropora palmata*, and *Acropora valenciennesi* and *Acropora valida* respectively.

Other skeletal anomalies are characterized as nodules or galls, and are usually the result of the encapsulation of foreign organisms such as crabs or barnacles (Cheng & Wong 1974, Grygier & Cairns 1996, Work & Rameyer 2005, Williams et al. 2010). These are not considered disease, but rather just normal coral growth over or around an object resulting in an unusual morphology. In this study I use the term "growth anomaly" to avoid substituting causation or processes for morphology (Work & Aeby 2006) since it is unknown what causes all GAs, and I do not include here growth anomalies caused by macroscopic endolithic symbionts (i.e. nodules, galls, and algal-induced tumors).

Several aspects of skeletal growth anomalies have been partially characterized (Table 1.3). These studies indicate that growth anomalies can have detrimental effects to host colony function with the most noticeable character of GAs being the visible skeletal malformations. Most GAs appear as protuberances above normal tissue and illustrate rapid vertical growth instead of horizontal extension (as is seen in normal tissues; Bak 1983, Yamashiro et al. 2000). As a disease, GAs possess a unique characteristic in that they don't necessarily kill tissue like most other diseases. This

gives us a unique opportunity to study diseased tissue which is alive but chronically impacted.

Growth anomalies have been observed on massive *Porites* spp. in the Philippines (Kaczmarsky 2006, Raymundo et al. 2005), Hawaii (Hunter & Peters 1993; Hunter 1999), and Guam (Myers & Raymundo unpub. data, Taylor et al. in prep.). Most recently, Kaczmarsky and Richardson (2007) provided evidence for an infectious agent associated with the development of growth anomalies in massive *Porites* spp. in the Philippines through a transmission study. Conversely, Taylor et al. (in prep.) found that the spatial distribution of massive *Porites* spp. colonies with growth anomalies was random, suggesting the lack of involvement of an infectious agent in transmission of GAs between colonies. However, questions still remain regarding cause, rate of spread and recovery, and infectiousness.

McClanahan et al. (2009) suggest that bleaching may increase the likelihood of the development of GAs and Coles & Seapy (1998) suggest that a reduction in UV-absorbing compounds aids in the formation of GAs. UV radiation is another proposed cause of GA formation (Peters et al. 1986). While it is unclear how these processes may function to cause GA formation, it is not surprising that the sun and its byproducts may cause disease in invertebrates given the effects it can have on humans (skin cancer) which have an advanced immune system. Other putative causative agents include virus-like particles (VLPs) (Kaczmarsky 2009) and nutrient-rich conditions. Viruses are known to cause tumors in sea turtles (Lackovich et al. 1999) and given that VLPs have been found in tissue samples of GAs, it would not be surprising if viruses were a causative

agent of GAs. Also, GAs have been associated with areas of high human population and sewage input (Kaczmarsky 2006, Aeby et al. 2011). Given the lack of data suggesting causative agents for GAs and the increasing frequency of literature surrounding GAs, it is of interest to put make a greater effort in understanding how the above-mentioned processes contribute to GA formation, prevalence and severity.

1.4. Growth Anomalies on Guam

The first growth anomalies in Guam were on *Acropora formosa* (Cheney 1975) and provided some of the same data as recorded by Peters et al. (1986): growth anomalies lacked zooxanthellae; showed significant differences in structure of the skeleton as compared to normal areas; and had randomly scattered polyps and a porous coenosteum. Little progress was made concerning disease on Guam reefs until recently. Myers & Raymundo (2009) present the first information on current levels of disease for Guam which can be used to provide a reference for future comparative studies. GAs were found among the three most abundant coral families: Poritidae, Pocilloporidae, and Acroporidae.

On Guam, preliminary surveys showed that growth anomalies displayed at least three distinct forms on massive *Porites*; I refer to these as Type I, Type II, and Type III (Figure 1.1). All growth anomaly types display a surface texture visibly distinct from the surrounding healthy tissue and a discrete margin which distinguishes the two areas. Among the three types, Type I and II closely resemble GAs termed “hyperplasia” and “neoplasia” (Table 1.2), respectively, and are the best described in the literature. Type I

GAs showed a surface texture on the coral, distinct from surrounding healthy tissue which was most often a smoother surface. In some, but not all, GAs of Type I, a visible reduction in pigmentation was apparent.

In Type II GAs, the coral tissue was often non-pigmented or severely discolored in comparison with normal tissue, with few skeletal features visible and few or no corallites. The skeleton was often slightly raised relative to surrounding healthy tissue, often plaque-like, and skeletal densities in Type II GAs appeared to be lower compared to healthy areas. If accidentally touched, the skeleton of Type II GAs would compress and crumble. A discrete margin demarcating diseased from healthy areas was almost always present.

The third type of growth anomaly (Type III) was uncommon in Guam. Type III anomalies consisted of large irregular areas of skeleton which appear to be depressed relative to the surrounding healthy tissue. There was a discrete margin separating the diseased tissue from the healthy tissue and anomalous areas were optically very smooth and did not contain any surface texture characteristic of the healthy areas on the colony. Pigmentation often appeared decreased and the diseased area of the colony seemed to be sinking in the diseased areas.

While three GA types are grossly easy to distinguish in the extreme forms, it is unclear whether or not they are three discrete GA types or if they are three stages of one GA type. Sometimes, types I and II have appeared with characteristics of both types in one GA. It is also unclear whether or not these three types differ in their effect on the host colony. On Luminao Reef flat it has been observed that in a few instances, Type I

GAs have transformed into Type II GA's (pers. obs.), however these were not followed over time and was hard to confirm. Given these observations, the existence of three distinct GA types has not been quantified and it is a goal of this study to characterize each of these types.

1.5. The Purpose of This Study

The purposes of this study were to characterize and determine the impacts, if any, of the three types of skeletal growth anomalies (GA) affecting massive *Porites* spp. on Guam. Aspects of GAs have been characterized for several species of corals (Table 1.3) from the Pacific and Indian Oceans (Squires 1965; Cheney 1975; Loya et al. 1984; Peters et al. 1986; Hunter & Peters 1993; Coles & Seapy 1998; Yamashiro et al. 2000, 2001; Gateño et al. 2003; Breitbart et al. 2005; Domart-Coulon et al. 2006; Kaczmarsky & Richardson 2007; Work et al. 2008; McClanahan 2009). The few studies performed on massive *Porites* spp. have focused on morphological variation (Cheney 1975), GA prevalence (Kaczmarsky 2006), the influence of temperature on GA abundance (McClanahan et al. 2009), and transmissibility of GAs (Kaczmarsky & Richardson 2007). None of those studies distinguished the effect of GA on the colony by morphologic type. It is important to establish this basic information since good descriptions are lacking, especially since there is still the possibility that GAs may not badly impact a colony. Massive *Porites* are a dominant component to Guam's reef flats and in some areas can compose up to 39% of colonies on a reef, and up to 64% of colonies on a single transect (Raymundo unpub. data). GAs are found primarily on massive *Porites* and can affect up

to 5% of colonies on a reef compared to almost 30% of colonies affected by white syndrome (Raymundo unpub. data). Taylor et al. (in prep.) found a prevalence of 10% prevalence of GAs on massive *Porites* with GAs in a 50m X 50m area of Luminao reef (Guam) dominated by these massive colonies.

Previous studies have found growth rates for GA areas on *Acropora* range from 0.006 to 0.12 mm/day (0.219 to 4.38 cm/yr) (Cheney 1975, Peters et al. 1986, Coles & Seapy 1998). Coles & Seapy (1998) found this to be a significant increase in tumor diameter for the 5.5 month period between measurements. Given that acroporids possess high annual growth rates (up to 20 cm/year, Coral Reef Overview 2011), it is possible that the seemingly rapid growth rate (10 mm/yr, Coles & Seapy 1998) of GA affected tissues may have an overall effect on the growth of the entire colony. Cheney (1975) found that the rapid growth of "tumors" in *Acropora formosa* often accompanied a reduction of growth in the colony as a whole suggesting that the tumors were drawing nutrients from nearby normal tissue. Kaczmarsky (2006) found that mortality rates and rate of growth were slow for GAs affecting massive *Porites* in the Philippines and that the disease progression rate to full mortality was projected to be on the scale of years. However, given that most colonies in Kaczmarsky's (2006) study were large with ages assumed to be on the scale of decades or more, the possibility of mortality due to GAs in less than 10 years proposed a mortality rate which exceeded replacement rate. Therefore, the growth rate of GAs on massive *Porites,* in situ, needed to be determined to see if GA growth rates on Guam were negatively affecting the coral. I predict that GAs will have negative impacts on the host colony as measured by GA growth. Also,

given that Guam has three distinct GA forms, I plan to determine whether or not the three GA types affected their host colony differently. I predicted that the three GA types would not differ in their effect on the host colony.

GAs affecting various species have been demonstrated to have a variety of negative effects on the host species (Table 1.3). To address GA effects on massive *Porites* in Guam, I determined that lipid quantity, zooxanthellae densities, chl A and chl C absorbance, tissue thickness, and corallite morphometric characteristics were among the most important characters needed to assess potential detrimental effects to coral hosts due to the fact that these characters all speak to normal coral function and any differences could imply reduced health. Lipids in corals are used for respiration as well as for the building blocks of cell membranes (Benson & Muscatine 1974, Benson & Lee 1975, Stimson 1990) and any reduction in lipid content can impair cell structure and function. Zooxanthellae provide energy, remove metabolic wastes, recycle nutrients for the coral host (Wang & Douglas 1998), and enhance calcification (Muller-Parker & D'Elia 1997). When zooxanthellae densities are reduced, coral tissue biomass and reproductive abilities are negatively affected (Szmant & Gassman 1990). Individual polyps secret the skeleton underneath them establishing a close relationship with corallite structure. This would suggest that those corallites with different skeletal structure than normal are produced by polyps that may not be functioning within normal parameters. I predicted that there would be significant differences between healthy and GA areas for each of these characteristics. Further, the three distinct GA types were compared to see if they differed from each other in their effect on the host

colony. I predicted that the three GA types would not differ in their effects on the host colony.

Microbial agents are increasingly thought to be the cause of most coral diseases and, with the positive identification of six microbial causative agents, are the focus of much coral disease work (Harvell et al. 2007, Rosenberg et al. 2007). The surface mucopolysaccharide layer (SML) of corals contains a microbial consortium about which relatively little is yet known yet it is essential for the coral's health and part of the holobiont (Ritchie & Smith 2004). It is thought that disease may be caused by a shift in the composition of the microbial community of the SML to favor pathogenic microbes (Ritchie & Smith 2004, Ritchie 2006, Mao-Jones et al. 2010). Because only approximately 1% of marine bacteria are culturable on media (Schut et al. 1993), it is difficult to apply Koch's postulates as an approach to determine causation. Very little is known regarding surface microbial community composition of colonies affected by GAs. Breitbart et al. (2005) found that *Vibrio* spp. were preferentially associated with GA areas of *Porites compressa*. The significance of *Vibrio* spp. on those colonies is unknown, but given the information already known about *Vibrio* spp. and coral disease, this may be an avenue to pursue in future studies. However, Domart-Coulon (2006) found that bacterial aggregates were more abundant in the mucus of GA-affected polyps as opposed to the calicoblastic epithelium, which does not suggest a role of a bacterial infectious agent. Since no work has been done on the bacterial associates of GAs on massive *Porites* spp., I determined that microbial composition of the SML should be investigated to see if there was any difference, or shift, in the number or type of

microbial associates which could be implicated in the role of an infectious microbial agent associated with GAs on massive *Porites* on Guam. I predicted that GAs would possess a microbial community different from that of healthy areas on the same colony. Also, the three GA types will be compared to see how they differ from each other in their microbial consortium. I also predicted that the three GA types would not differ in the type or number of microbial associates.

Another factor which speaks to an infectious agent for GAs is transmissibility of GAs to healthy tissues. Gateño et al. (2003) found that two years of *in situ* isogenic and allogenic contacts between healthy and GA fragments of *Pavona clavus* did not reveal infection or transfer of the GA to healthy tissue; GAs appeared to be non-infectious. Conversely, Kaczmarsky and Richardson (2007) found a successful transmission of GAs between massive *Porites*. In both direct contact and water-borne experiments, corals became diseased with GAs. The authors concluded the possibility of an infectious agent associated with GAs for massive *Porites*. Taylor et al. (in prep.) found that GAs were randomly distributed throughout the *Porites* populations, which is consistent with a lack of involvement of an infectious agent in the disease process. Given the dearth of clear information on GA transmission in corals, it was determined that the infectious nature of GAs on massive *Porites* spp. should be investigated on Guam. Determination of infectiousness is considered a first in the in the investigation of a disease (Raymundo et al. 2008). I predicted that through direct contact, transmission of GAs to healthy colonies would not be a mode of infection for massive *Porites* spp. on Guam.

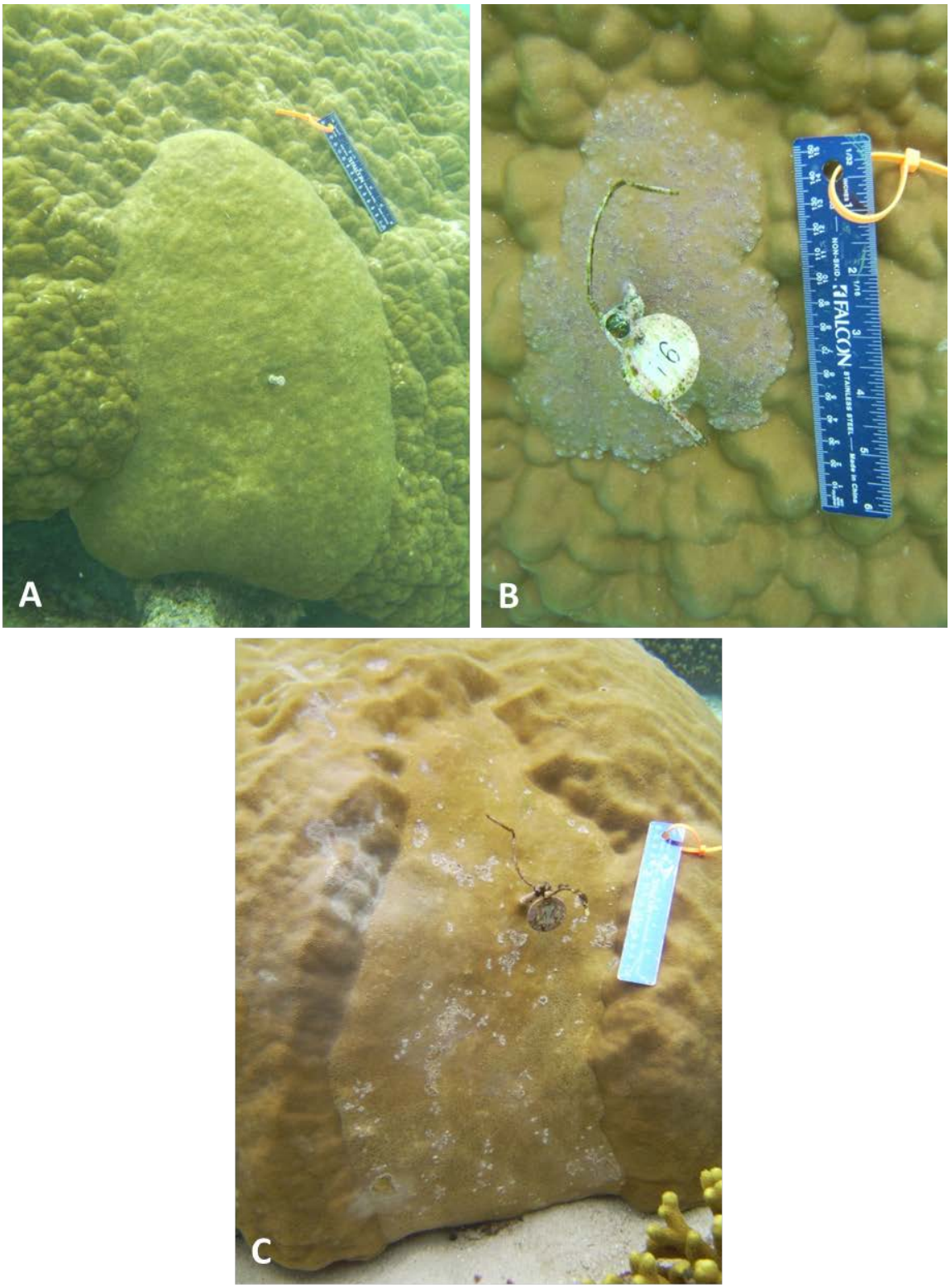

Figure 1.1. The gross appearance, in situ, of the three distinct GA types characterized on Guam: (A) Type I, (B) Type II, and (C) Type III.

Table 1.1. *Porites* spp. affected by disease (total number of reported species affected by each disease and total number of reported diseased affecting each species). Black letters indicate species from the Caribbean, red letters indicate species from the Indo-Pacific, and blue letters indicate species from the Red Sea. BBD = Black Band, WPL = White Plague, GA = Growth Anomalies, YBD = Yellow Band, PLS = Pink Line Syndrome, UWS = Ulcerative White Spot, SEB = Skeletal Eroding Band, Trem = Trematodiasis, WS = White Syndrome.

Species	BBD	WPL	GA	YBD	WBD	PLS	UWS	SEB	Trem	WS	No. of diseases	Source
Porites annae							X				1	Raymundo et al. 2005
Porites astreoides	X	X	X	X							4	Garzón-Ferreira et al. 2001
Porites attenuata							X				1	Raymundo et al. 2005
Porites australiensis					X						1	Antonius 1988
Porites compressa			X			X			X		3	Hunter 1999, Aeby 2006, Breitbart et al. 2005, Domart-Coulon et al. 2006
Porites cylindrica							X				1	Raymundo et al. 2005
Porites evermanni									X		1	Aeby 2006
Porites harrisoni				X							1	Riegl 2002
Porites horizontallata							X				1	Raymundo et al. 2005
Porites latistella							X				1	Raymundo et al. 2005
Porites lichen				X			X				2	Raymundo et al. 2005
Porites lobata			X		X				X		3	Antonius 1988, Hunter 1999, Aeby 2006
Porites lutea	X	X	X	X		X					5	Ravindran & Raghukumar 2002
Porites nigrescens							X				1	Raymundo et al. 2005
Porites nodifera				X							1	Riegl 2002
Porites porites		X		X							2	Garzón-Ferreira et al. 2001
Porites rus							X				1	Raymundo et al. 2005
Porites sillimaniana							X				1	Raymundo et al. 2005
Porites tuberculosa							X				1	Raymundo et al. 2005
massive *Porites* (Indo-Pacific and Caribbean)			X				X				2	Raymundo et al. 2003, Raymundo et al. 2005, Weil & Jordán-Dalgreen 2005, Kaczmarsky 2006, McClanahan et al. 2009
Porites spp. (Indo-Pacific, Caribbean, & Red Sea)	X		X				X	X		X	5	Raymundo et al. 2003, Willis et al. 2004, Myers & Raymundo 2009
Total no. of species	**2**	**3**	**5**	**6**	**2**	**2**	**10**	**>1**	**3**	**>1**		

Table 1.2. Different "types" of skeletal growth anomalies, their definitions (as stated by the authors), and their suggested causative agents.

Skeletal Anomaly Types	Definition	Suggested Causative Agent(s)	Source(s)
tumor	neoplasms; atypical growths	N/A	Cheney 1975
	raised roughly spherical masses projecting about 4.5 cm above the surface of the colony; hyperplasia	N/A	Willis et al. 2004
	spherical or irregulary shaped protuberances of the coral skeleton; distinct, gobular, bleached masses of coral skeleton	↓ in UVB absorption	Coles & Seapy 1998
	slightly hemispherical protuberances; bumps; abonormal skeletal growth	N/A	Yamashiro et al. 2000 & 2001
	conspicuous formations, easily observed over the colony surface as presenting unusual phenotypic expression	N/A	Gateño et al. 2003
	irregular patches of skeleton either raised or depressed relative to surrounding healthy tissue	N/A	Raymundo et al. 2005
	abnormal growths that lead to enlarged skeletal elements	possible *Vibrio* spp.	Breitbart 2005
	pale areas of tissue with an inconsistent morphology to very irregularly shaped depressed areas of white and pink tissue, sometimes causing tissue loss and mortality	N/A	Kaczmarsky 2006
	roughly spherical lesions covered with corallian epidermis and polyps of normal appearance; irregularly shaped flat or plaque-like lesions on *Gorgonia ventalina*	*Aspergillus sydowii*	Morse et al. 1977, 1981
neoplasia	unusually rapid growth and progressively disordered growth of skeletal structures	N/A	Squires 1965
	abnormal processess of calcification	N/A	Bak 1983
hyperplasia	circumscribed nodule-like areas of enlarged skeleton and tissue	chromophore infiltration	Domart-Coulon 2006
hypertrophy	nonneoplastic increase in cell size	N/A	Sutherland et al. 2004
skeletal biomineralization	aragonitic, hemispherical to conical outgrowths protruding from the walls of structural pores; pearl-like biomineralization	endolithic fungi	Le-Campion-Alsumard et al. 1995
nodule	conspicuous pink cysts on the coral colony	*Podocotyloides stenometra*	Aeby 1998
	abnormal growths , elevated, irregularly shaped, pink or yellowish protruding from the polyps	*Plagioporus* spp.	Cheng & Wong 1974
gall	parasite-induced tissue proliferation	*Petrarca madreporae*	Grygier & Cairns 1996
growth anomaly	a lesion with a focal or multi-focal distribution and predominantly nodular, exophytic, and umbonate; hyperplasia of basal body wall	fungi, algae, sponges, and Crustacea were seen in lesions, but no obvious causative agent overall	Williams et al. 2010

Table 1.2. (continued)

Skeletal Anomaly Types	Definition	Suggested Causative Agent(s)	Source(s)
	hyperplasia; patch of paling or fully bleached tissues that form tumor-like structures on the colonies surface or around depressions	microboring organism such as endolithic fungi	McClanahan et al. 2009
	an initial swelling, after which the surrounding unaffected coral appears to outgrow the affected area	pathogenic microorganism	Kaczmarsky & Richardson 2007
	hyperplasia; excessive or apparently uncontrolled growth of skeleton or soft tissues in relation to adjacent polyps	algae or metazoa (polychaete worms)	Work & Rameyer 2005
skeletal malformations	tumor; localized area of increased growth rate resulting in roughly circular protuberances extending up to 4.5 cm above the colony surface	a combination of environmental stress coupled with an injury inflicted on the coral may stimulate bacterial attack or the development of an aberrant polyp	Loya 1984
calicoblastic epithelioma	neoplasms; raised, whitened, irregularly shaped, protuberances; smooth white lumps that develop on all parts of the colony	UV radiation	Peters et al. 1986

* it is important to note that several studies use several of these names interchangably

Table 1.3. List of the findings of previous reports of growth anomalies and how they affect the host species.

Characteristic	Species	Finding	Source
corallite size	*Madrepora kauaiensis*	mean diameter up to 10X that of normal corallites	Squires 1965
	Porites lobata, *Porites* spp.	enlarged corallites as compared to normal areas	Hunter & Peters 1993, Kaczmarsky 2006
	massive *Porites*	larger calices in GAs	McClanahan et al. 2009
arrangement of septae	*Madrepora kauaiensis*	septae were inserted in a meaningless fashion, pattern lacked symmetry, portions of a 5th cycle had been inserted	Squires 1965
	massive *Porites*	higher than normal numbers of septae in GAs	McClanahan et al. 2009
coenosteum characteristics	massive *Porites*	less distance between calices in GAs	McClanahan et al. 2009
skeletal density	*Acropora formosa*, *Acropora palmata*, *Montipora informis*, *Pavona clavus*	less dense skeleton than normal areas	Cheney 1975, Peters et al. 1986, Yamashiro et al. 2000, Gateño et al. 2003
	Porites compressa, *Pavona clavus*	more porous skeleton than normal	Domart-Coulon et al. 2006, Gateño et al. 2003
	Acropora formosa	more porous coenosteum than normal areas	Cheney 1975
zooxanthellae density	*Acropora formosa*, *Acropora palmata*, *Montipora informis*, *Pavona clavus*, *Porites compressa*, *Acropora* spp., scleractinian corals	zooxanthellae were few or lacking compared to normal areas	Cheney 1975, Peters et al. 1986, Yamashiro et al. 2000, Gateño et al. 2003, Domart-Coulon et al. 2006, Work et al. 2008, Williams et al. 2010
	Platygyra pini & *sinensis*	zooxanthellae densities were similar between abnormal and normal areas	Loya et al. 1984
	Acropora cytherea & *Acropora abrotenoides*	cells uniformly devoid of zooxanthellae	Work & Rameyer 2005
growth rate	*Platygyra pini* & *sinensis*, *Pavona clavus*	higher growth rate in abnormal areas than in adjacent normal areas	Loya et al. 1984, Gateño et al. 2003
	Acropora valenciennesi	significant increase in tumor diameter	Coles & Seapy 1998
tissue thickness	*Acropora palmata*	less than in normal areas	Peters et al. 1986
polyp structures	*Acropora palmata*, *Acropora cytherea* & *Acropora abrotenoides*, *Acropora* spp., scleractinian corals	reduced and degenerating or absent in the "tumor"	Peters et al. 1986, Work & Rameyer 2005, Work et al. 2008, Williams et al. 2010
	Montipora informis	incomplete polyps in tumored areas	Yamashiro et al. 2000

Table 1.3. (continued)

mucous secretory cells	*Acropora palmata*	loss of mucous secretory cells in "tumor"	Peters et al. 1986
nematocycsts	*Acropora palmata*	loss of nematocycsts in "tumor"	Peters et al. 1986
UVB absorption	*Acropora valenciennesi*	lower than for extracts of normal tissue	Coles & Seapy 1998
polyp/corallite density	*Montipora informis, Porites compressa, Acropora* spp.	fewer number of polyps per surface area than normal areas	Yamashiro et al. 2000, Domart-Coulon et al. 2006, Work et al. 2008
fecundity	*Montipora informis*	reduced in tumored areas	Yamashiro et al. 2000
	Acropora spp.	fewer gonads in GAs	Work et al. 2008
dried tissue weight	*Montipora informis*	tissue from tumored areas weighed less than tissue from healthy areas	Yamashiro et al. 2000
lipid content	*Montipora informis*	decrease in the total lipid content as compared to healthy areas	Yamashiro et al. 2001
microbial community	*Porites compressa*	faster growth rate in in healthy areas of colonies with tumorous areas than remote healthy	Breitbart et al. 2005
GA transmission	*Porites lobata* & *lutea*	transmission of GAs through direct contact and waterborne transmission	Kaczmarsky 2007
	Pavona clavus	tumors do not appear to be transmitted between colonies, even after fusion of healthy and tumor fragmetns	Gateño et al. 2003

2: MATERALS AND METHODS

2.1. Study Areas

As part of the initiation of a coral health impacts monitoring program, coral disease surveys were conducted at 21 sites around Guam to determine baseline disease, what diseases were affecting Guam corals, and what coral spp. were hosts to these diseases (Raymundo, unpub. data; Myers & Raymundo 2009). To aid in the partial characterization of skeletal growth anomalies affecting massive *Porites* spp. in Guam, two sites were selected because they had large populations of massive *Porites* and surveys indicated GA prevalence was high: Luminao reef flat (13° 27' 54.17" N, 144° 38' 53.16" E) and Ipan reef flat (13° 21' 54.85" N, 144° 46' 19.49" E), in proximity to the Togcha River Channel (Figure 2.1.). Luminao reef flat is shallow (approx. 1-3 m depth) and dominated by *Porites* (71.6%) and *Acropora* (13.2%) species (Myers & Raymundo, unpubl. data). It lies on the north side of the Glass Breakwater in Southwest Guam. Baseline surveys conducted at Luminao reef flat revealed 5.1% growth anomaly prevalence on massive *Porites* spp. (Myers & Raymundo, unpubl. data). Ipan reef flat is also shallow (approx. 1-2 m depth) and dominated by massive *Porites* (35.3%) and *Pocillopora* (48.1%) species. (Myers & Raymundo, unpubl. data); the Togcha River channel bisects Ipan reef flat (approx. 7-8 m depth) and is also dominated by massive *Porites* along either side (pers. obs.). Ipan reef flat/Togcha Channel lies on the east side of Guam. Baseline surveys conducted at Ipan reef flat report 0.95% growth anomaly prevalence on massive *Porites* (Myers & Raymundo, unpubl. data).

2.2. Quantifying GA Growth on Individual Coral Colonies in situ

A total of 29 massive *Porites* colonies exhibiting growth anomalies (GA) were located and tagged within Luminao reef (18 colonies) and Ipan reef flat/Togcha Channel (11 colonies). Thirty-four growth anomalies were tagged on these 29 colonies, with some colonies having multiple GAs of differing types. A total of 15 Type I (8 Ipan; 7 Luminao), 15 Type II (8 Ipan; 7 Luminao), and 4 Type III (4 Luminao) growth anomalies were identified and tagged in September and October 2007. Nine remote healthy colonies (i.e. on the same reef flat but showing no visible signs of disease) were also tagged at each site. At the time of tagging, maximum diameter of each colony and the diameter perpendicular to the maximum were measured and recorded for each colony and GA. Growth anomaly size was measured to the nearest 0.1 cm monthly from September 2007 to November 2008, though poor weather conditions (i.e. high surf, dangerous current, etc.) curtailed censusing in certain months. Measurements were taken at the estimated maximum diameter and at the diameter perpendicular to the maximum. The initial and final measurements were converted into area using the equation for an ellipse ($A = \pi r_a r_b$). Overall growth over 15 months of each GA was calculated by taking the difference between the initial and final calculations.

2.3 Quantifying Evidence of Physiological Impacts to the Coral Host

In the field and in the lab, massive *Porites* spp. are difficult to impossible to identify to species. Corallite structure can be highly variable, and environmental variables can affect skeletal structures. Given the well-established difficulties in species

identification of massive *Porites* spp., I performed a tentative identification of all samples using Veron (2000) and then consulted Mr. Dick Randall, M.Sc. (University of Guam), to verify identification of the core samples. This was necessary to ensure that any variation seen was not due to between-species differences.

To assess the potential negative effects of growth anomalies on coral host function, an analysis of lipid quantity, zooxanthellae densities, chl A and chl C absorbance, corallite morphometrics, and tissue thickness were performed on remote healthy colonies and healthy and diseased areas of colonies with GAs. [These three areas (remote healthy, healthy and diseased) will be referred to as "health states" in the results section.] To examine all variables but tissue thickness, 29 mm cores were extracted from *Porites lutea* colonies on Ipan reef flat using a pneumatic drill (Figure 1). Cores were removed from 5 colonies of each of the following types: remote healthy, GA Type I, GA Type II, and GA Type III. From remote healthy colonies, two cores were removed. From GA-affected colonies, two sets of cores each were removed from GA and healthy areas (total 4 cores). This made a total of 80 cores removed. To assess tissue thickness, samples were chipped off, from the same areas which were used for all other analyses, using a hammer and chisel. All cores were taken from positions on the colony relative to GAs.

The first set of cores per colony were used for lipid analysis; in the field, each core was immediately placed into its own Whirl-Pak© filled with 10% formalin seawater. The set of chips used for tissue thickness were also immediately placed into a separate Whirl-Pak© filled with 10% formalin seawater. The second set of cores was used for

zooxanthellae densities, chl A and chl C absorbance, and morphometric analysis. Each second core was immediately wrapped in tinfoil and placed into a third Whirl-Pak© and transported on ice back to the UOGML laboratory. Upon arrival, the second set of cores was frozen at -80°C until a later date when zooxanthellae densities, chl A and chl C absorbance, and morphometric analysis would be performed. The first set of cores and chips were processed immediately for lipids and tissue thickness, respectively, as described below.

2.3.1. Lipid Analysis

The protocols of Harriott (1993) and Stimson (1987) were followed for lipid analysis. All cores were fixed in 10% formalin seawater for 24 hr. After fixation, cores were rinsed in fresh water three times to remove salts; cores were then immersed in fresh water overnight for additional soaking. The freshwater was then decanted and the cores were rinsed again four more times before air drying for approximately one hour. The cores were then placed in covered plastic cups containing a 2:1 chloroform:methanol solution to extract lipids. The samples soaked in the solvent for approximately 24 hr, upon which time the solvent was decanted out into clean plastic cups and the sample cup was refilled with new solvent. This process was carried out each 24 hr period until the solvents were clear for each sample -- in this case after three days. Combined extracts for each sample were then filtered through Whatman© Filter Papers (150mm) and the filter paper and sample were rinsed with chloroform:methanol solution to obtain any extract which remained. The extracts were then evaporated in aluminum dishes at 21.7°C and weighed to the nearest 0.0001 g.

The remaining tissue was decalcified in 5-10% HCl, rinsed with tap water twice and $MQ^{+}H_2O$ twice, and air dried at approximately 21°C for 24 hr. Tissue samples were then weighed to the nearest 0.001 g. They were then dried in an oven at 38°C for 2.5 weeks and were weighed to the nearest 0.001g. Samples were then returned to the oven, and reweighed every 24 hours. This was repeated until a constant dry weight was attained. A lipid index was calculated as:

$$\frac{weight\ of\ lipid\ extract}{weight\ of\ lipid\ extract + tissue\ weight}\ X\ 100\%$$

2.3.2. Zooxanthellae and Chlorophyll analysis

Zooxanthellae and chlorophyll samples were obtained from previously frozen samples by water-blasting the tissue off the skeleton using an artist's airbrush with cold, filtered seawater. Tissue was removed in the dark and "blastate" was collected in a Whirl-Pak©. The skeleton was placed in a 20% household bleach solution to remove remaining tissue and was set aside for later morphometric measurements. The blastate was homogenized by shaking vigorously for 5 s and 0.5 mL was aliquoted into a clean 1 mL centrifuge tube containing 0.5 mL of 10% formalin seawater. These samples were used for determining zooxanthellae density and were placed into the refrigerator at 0°C until counted.

Zooxanthellae were counted using a Reichert Bright-line hemacytometer (0.1 mm deep) and a compound microscope on 40X. Each sample was first vortexed on medium speed for 10 s. It was then loaded onto the hemacytometer and all cells in the eight corner 1 mm grids were counted using a push button counter (n = 8 counts per colony). Zooxanthellae present on the edge of each grid were only counted when 50%

or more of the cell was present in the box. The eight density counts were used to obtain a mean number of zooxanthellae per 0.1µL, which was then extrapolated up to the entire volume of the sample blastate.

The remaining blastate was homogenized again and 6 mL was transferred to a 15 mL centrifuge tube. The samples were then spun for 10 minutes at 1000 rpm at 4°C causing a pellet to form. Seawater was decanted without disturbing the pellet. These samples were then placed in the -80°C freezer until ready to be processed for chlorophyll. The remaining volume of the blastate was measured to the nearest 0.1 mL in a graduated cylinder and recorded to calculate the full volume of tissue removed.

Chlorophyll pellets were removed from the -80°C freezer and put on ice at room temperature to thaw. Three mL of ice-cold 90% acetone was added to each sample which was then sonicated 3 times using the VibraCell Sonicator, amplitude 60, pulser 2 seconds. The samples were then wrapped in tin foil and placed in the -20°C freezer on their sides to allow for a larger surface area of chlorophyll extraction. The samples were then shaken at the following time periods after placement in the freezer: 12, 16, 19, and 21.5 hours. Between each time interval samples were placed back into the -20° C freezer to continue chlorophyll extraction. After 24 hours, samples were spun for 10 minutes at 1000 rpm at 4°C. The chlorophyll extract in 90% acetone was then transferred into a new, sterile 15 mL tube. Three or 4 more mL of 90% acetone was added to each sample and the volume was measured to the nearest 0.1 mL. To measure chlorophyll absorbance, a Spectronic 20D spectrophotometer was used. A cuvette was rinsed with 1 mL of 90% acetone, then 1 mL of sample. To calculate the

amount of chlorophyll using the equations listed below (Parsons et al. 1984), the absorbencies were measured at the following wavelengths: 630, 647, and 664 nm. Using the equation for dinoflagellates extracted in acetone (see equation below; Jeffrey & Humphrey 1975), the concentrations of chl A and chl C were calculated as:

$$\text{Chlorophyll a} = 11.85E_{664} - 1.54E_{647} - 0.08E_{630}$$

$$\text{Chlorophyll c} = 24.52E_{630} - 1.67E_{664} - 7.60E_{647}$$

where E stands for the absorbance of the sample at different wavelengths.

2.3.3. Morphometric Analysis

Once the skeletons were stripped of tissue, they were rinsed in tap water and dried for use in skeletal morphometric analyses. A power analysis for the 2-way ANOVA (Zar 1999) was performed for each morphometric to determine adequate sample size. For each sample the following measurements and observations were recorded: corallite density (#/cm^2) (n = 15 per core sample), maximum corallite diameter (CD) (mm), distance from wall to closest neighboring calice (CSM) (mm), distance from wall to furthest neighboring calice (CSX) (mm), the corallite surface area (CSA) (mm^2), the # of septa per corallite, and the # of palli per corallite (Figure 2.3.). Sample size (n) is 20 corallites per core sample (unless otherwise stated) with two core samples per colony (1 healthy and 1 diseased). From these measurements and observations, comparisons between GAs and healthy tissue could be assessed.

2.3.4. Tissue Thickness

All chips were fixed in 10% formalin seawater for 24 hr. After fixation, chips were rinsed in fresh water three times to remove excess formalin. The freshwater was

then decanted and replaced with a 5-10% HCl solution to decalcify the skeleton. Once the skeleton had adequately decalcified, the HCl solution was decanted and replaced with $MQ^{+}H_2O$. A straight line was then cut through the thickest part of the sample using a scalpel. Using calipers, tissue thickness was measured to the nearest 0.01 mm in five different areas of the sample (n = 5 per sample) (D. Gochfeld, pers. comm.).

2.4. Microbial Community Characterization

To determine if GAs show any difference in the surface microbial community from the rest of the colony, the surface mucopolysaccharide layer (SML) of healthy and diseased areas was collected from 15 *Porites lutea* (5 from each of the three GA types) and from 5 remote healthy colonies on the Ipan reef flat. The SML was collected from healthy and diseased areas using a sterile 5 ml needleless syringe. Using the tip of the syringe, colonies were agitated at their surface to produce excess mucus, which was then sucked up into the syringe. Once collected, samples were immediately transferred to sterile 15 ml centrifuge tubes. Five seawater samples were collected by opening a sterile 15 ml centrifuge tube at 0.5m depth at haphazardly selected locations around Ipan reef flat. All samples were placed in an ice-water bath while in transport to the UOGML. Samples were collected between 1000 to 1300 h, which coincided with low tide.

Mucus and seawater samples were immediately placed in a refrigerator at ~ 12°C. Serial dilutions were made from the original samples (1:1 concentration) at the following concentrations: 1:10, 1:100, and 1:1000. This was done by aliquoting 900 µl

of sterile seawater into a 1 ml microcentrifuge tube, and then adding 100µl of sample to obtain the 1:10 sample. Before adding sample to the microcentrifuge tube, samples were vortexed for 10 sec to evenly distribute the microbial community in the sample. From the 1:10 sample, 100 µl was put into another microcentrifuge tube with 900 µl of sterile seawater, making the 1:100 sample. 100 µl from this sample was then placed into another microcentrifuge tube with 900 µl of sterile seawater, making the 1:100 sample. This made for 160 samples plated out on Marine Agar (MA) and Thiosulfate Citrate Bile Salts Sucrose (TCBS) Agar. Marine Agar was chosen for its ability to grow a broad diversity of heterotrophic marine bacteria; TCBS Agar was chosen for its selective isolation properties on *Vibrio* spp. (several of which are verified causative agents of specific coral diseases). Each sample was divided into four 100µl subsamples and plated out onto MA (n = 2 plates per colony per dilution) and TCBS (n = 2 plates per colony per dilution). The plates were incubated at 25.5°C for 24 hours. Colony forming units (CFUs) were counted using a Bio-Technologies Colony Counter and a hand counter. The plates were then resealed with parafilm, incubated for an additional 24 hrs and recounted at 48 hrs to allow slower-growing colonies to develop.

For those plates which had too many bacterial colonies to be able to count, the "value" of TNTC (too numerous to count) was recorded. For statistical analyses, TNTC plates were given a numerical value which was above the maximum number of bacteria counted on any plate. This number was 350 and was used in statistical analyses.

Since it is not understood how different bacterial communities may be implicated in GA formation, if at all, colony counts were counted at 24 and 48 hours and

in a dilution series in order to incorporate as many different types of bacteria as possible. Some bacterial species have a longer incubation time than others while others grow depending on microbial population density. While bacterial species were not identified for this study, information regarding population densities, incubation time, and density-dependence is nonetheless valuable when analyzing bacterial population differences and their potential influence on coral health.

For storage for future work in bacterial identification, individual bacterial CFUs displaying unique colony morphologies were counted and described and then subcultured to purification. Those colonies cultured on TCBS agar were transferred to marine agar and subcultured to purification. Once pure colony strains were obtained, individual CFUs were picked using sterile toothpicks and placed into individual wells in a 96-well culture plate, each well containing 160 µl of Marine Broth. Bacteria were incubated at ~25.5 °C for 24 hours. After 24 hours 40 µl of sterile glycerol was added to each well. The plate was then sealed with sealing film, labeled, and stored in a -80°C freezer.

2.5. Testing for Growth Anomaly Transmissibility

To test for infectiousness of growth anomalies, a transmission experiment was performed in the lab. Fifteen small ($\leq$ 20 cm in diameter), clinically healthy individual colonies of massive *Porites* spp. were collected from Tanguisson reef flat on the western shore of Guam (Figure 2.1.; 13° 32' 48.76" N, 144° 48' 35.49" E). Five healthy control chips were also collected from the Tanguisson reef flat (maximum diameter 3.9 – 5.6

cm). Five chips of Type I GAs and five of Type II GAs were collected from colonies on the Ipan reef flat (maximum diameter 3.9 – 5.7 cm and 4.2 – 7.8 cm, respectively) (Figure 2.1.). Type III was not tested as they are rare and an appropriate sample size was not possible. All colonies and chips were immediately transported to the lab in buckets containing aerated fresh seawater. Once at the lab, the corals were placed in continuous flow saltwater tables and provided with aeration. The chips were epoxied onto small tiles and placed into another continuous flow saltwater table with aeration. The colonies and chips were checked daily over the next two weeks of acclimatization.

Fifteen 10 L aquaria were provided with a continuous flow of saltwater and aeration. These tanks were placed in two larger holding tanks filled with circulating seawater to modulate temperature fluctuation (mean temp. 29.68 $\pm$ 0.004 °C for the duration). Tanks were covered with shadecloth and translucent roofs, which protected against rainfall and excess sunlight (Figure 2.2.). After acclimatization, healthy corals were placed into each 10L tank and allowed to acclimatize for several days. A single healthy (control), GA Type I, or GA Type II chip was then randomly selected to be put in direct contact with the healthy colonies. Each tank was censused weekly for 10 weeks. During each census period the health status of both the healthy coral points of contact were carefully examined and the experimental chip was recorded and were cleaned of fouling algae and placed back into the original touching position. Pictures for documentation were also taken on days during weeks 3, 7, 8, and 10. The transmission experiment lasted from April 4 – June 12, 2008.

2.6. Data Analyses

During analysis, it was recognized that one colony which was visually assessed as having a GA of type II did not have a GA but rather a hyper-pigmentation and swelling of tissue. This assessment was confirmed by Dr. Bernardo Vargas-Angel (pers. comm.). This colony was taken out of the analysis, reducing the number of colonies within GA Type II to an *n* of 4. As a result, one colony/sample was randomly taken out of each GA, healthy, and seawater sample group to attain even sample sizes. Therefore, each GA, healthy, and seawater sample type has and *n* of 4 colonies/samples. Sample sizes per colony are indicated in Nested 1-way PERMANOVA tables and are the sample size for all analyses of that character.

Data were analyzed using the programs IBM SPSS Statistics v.19 and Primer 6 & PERMANOVA +. All data were analyzed in SPSS for normality and homoscedasticity. Those data which did not need to be transformed, or could be transformed, to meet the assumptions of normality and homoscedasticity for 1-way and 2-way ANOVAs were analyzed using SPSS. Data still not meeting these assumptions and which required nested 1-way and nested 2-way analyses were analyzed using Primer 6 & PERMANOVA +. Data analyzed using PERMANOVA were also analyzed for normality and, when possible, transformed to meet the assumption of normality. This was due to the fact that in univariate PERMANOVA analysis, those data which conform to the traditional assumptions of ANOVA will have permutational P-values which converge on the traditional P-value (Anderson et al. 2008).

Transformations for SPSS and PERMANOVA analyses are outlined in Table 2.1. All PERMANOVA data were analyzed using 9999 permutations and Monte Carlo tests when needed. The Euclidean distance resemblance measure and Type III sums of squares were used to analyze all data in PERMANOVA. When performing a strict 1-way PERMANOVA, the permutation method of "unrestricted permutation of raw data" was used as it provides an exact test for the one-way case (Anderson et al. 2008). All other data (nested 1-way, 2-way, and nested 2-way) were analyzed using the "permutation of residuals under a reduced model" permutation method because it yields the best power and is theoretically the closest to the exact test (Anderson et al. 2008). Pair-wise comparison tests both between and within colonies were performed for those data which produced a p-value ≤ 0.05. Note that the data were first tested using nested PERMANOVAs. If the main terms or interaction terms were not significant in the nested analysis, a 1- or 2-way PERMANOVA was performed on the main terms.

When analyzing total GA growth between Ipan and Luminao, a correlation test was run between GA size and colony size, using initial and final calculations by GA type, to test for a correlation of GA size with colony size. All correlations were run in Microsoft Excel. All R^2 values were analyzed for significance using the Calculators for Statistical Table Entries. A significant correlation was found between all final calculations of GA I (R^2 = -0.258, p = 0.026) and GA II (R^2 = 0.2639, p = 0.0251). To eliminate this correlation, the residual differences of each value were taken from their respective line of best fit, and a correlation was run on the residual differences. The correlation of the residuals was not significant therefore the residual differences were analyzed in SPSS.

Since some colonies experienced partial mortality or shrinkage of the GA area, a constant (3016) was added to each number to make them positive and non-zero. One-Way ANOVAs were performed to compare GAs within each site and 2-way ANOVAs were performed to compare GAs between sites.

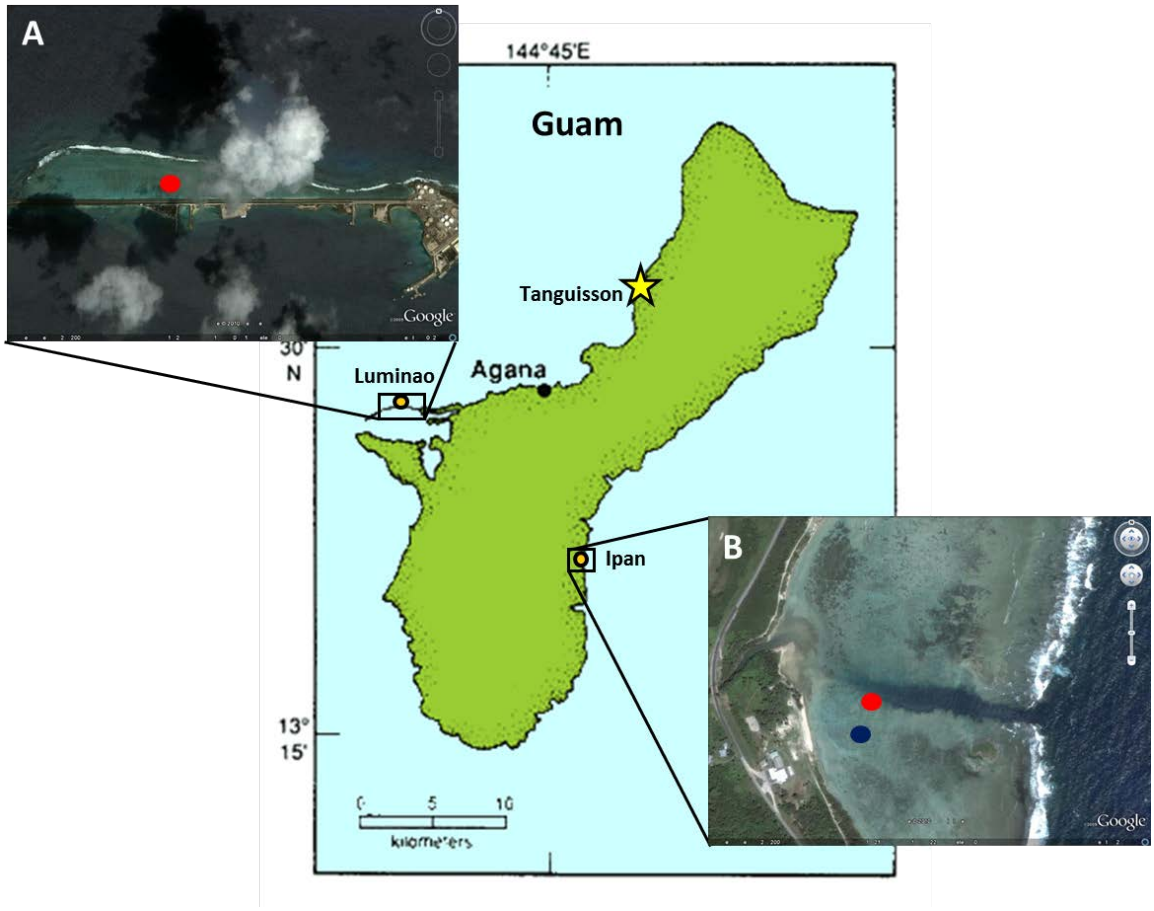

Figure 2.1. Map of the study sites, (A) Luminao Reef and (B) Ipan Reef Flat/Togcha Channel. Each insert shows a close-up of the study site. Red dots (●) denote approximate monitoring sites. Blue dot (●) denotes the approximate core sampling site. Star (★) denotes donor site for healthy colonies for the lab transmission experiment.

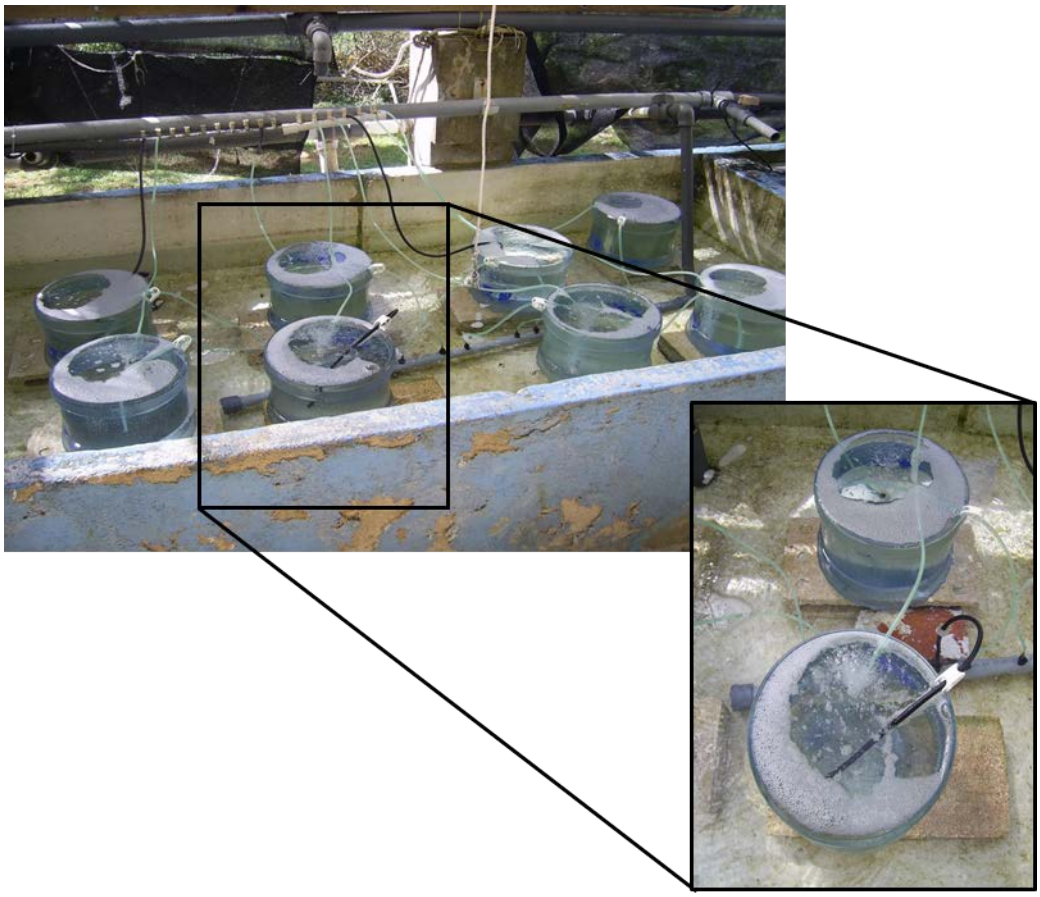

Figure 2.2. Transmission experimental setup. Insert shows the setup of each individual tank which has its own air and water supply.

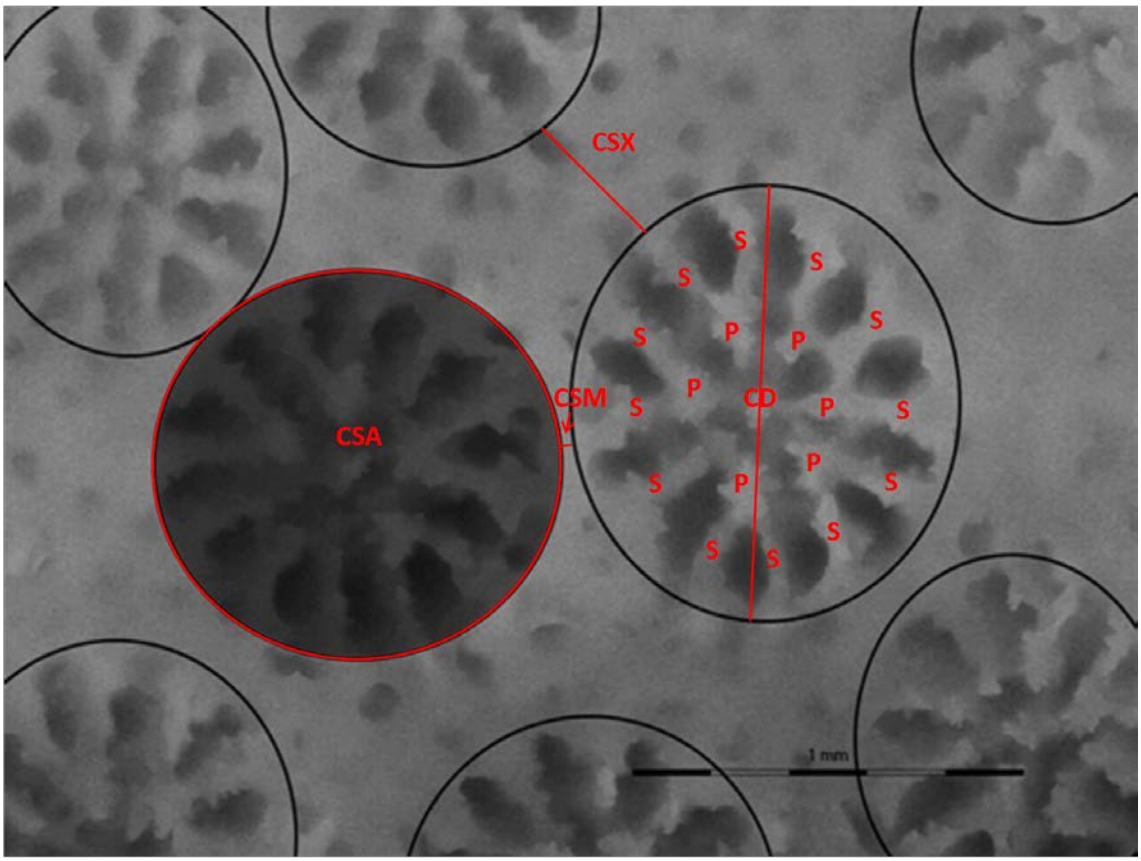

Figure 2.3. Skeletal morphometric measurements: CSA = corallite surface area (shaded area), CSX = distance from wall to furthest neighboring calice, CSM = distance from wall to closest neighboring calice, CD = maximum corallite diameter, S = septa (# of septa were counted), P = palli (# of palli were counted). Corallite density was also noted.

Table 2.1. Data transformations for all statistical analyses.

PERMANOVA		1-Way	1-Way Nested	2-Way	2-Way Nested
Analyses		Trans.	Trans.	Trans.	Trans.
Total GA Growth (Res.) (+3016)		----------	----------	no trans.	----------
tissue thickness (cm)		no trans./ square root	no trans.	square root	square root
zooxanthellae density (#/mm^2)		4th root	4th root	square root	square root
Morphometric Analysis					
number of septae		no trans.	no trans.	no trans.	no trans.
corallite diameter		4th root	4th root	log 10	log 10
number of palli		no trans.	no trans.	no trans.	no trans.
corallite surface area		square root/4th root	square root	log 10	log 10
CSM		no trans.	no trans.	no trans.	no trans.
CSX		square root	square root	sqare root	square root
corallite density		no trans./square root	no trans.	sqare root	square root
Microbial Analysis					
24 Hours	MA 1:1	4th root	4th root	4th root	4th root
	MA 1:10	no trans./square root	no trans.	square root	square root
	MA 1:100	no trans./square root	no trans.	no trans.	no trans.
	MA 1:1000	no trans.	no trans.	no trans.	no trans.
	TCBS 1:1	log (x+1)	log (x+1)	log (x+1)	log (x+1)
	TCBS 1:10	no trans.	no trans.	log (x+1)	log (x+1)
	TCBS 1:100	no trans.	no trans.	no trans.	no trans.
	TCBS 1:1000	no trans.	no trans.	no trans.	no trans.
48 Hours	MA 1:1	no trans./4th root	no trans.	square root	square root
	MA 1:10	no trans./4th root	no trans.	4th root	4th root
	MA 1:100	4th root (x+1)	4th root (x+1)	log (x+1)	log (x+1)
	MA 1:1000	no trans.	no trans.	no trans.	no trans.
	TCBS 1:1	square root/log (x+1)	square root	log (x+1)	log (x+1)
	TCBS 1:10	square root (x+1)/square root	square root (x+1)	log (x+1)	log (x+1)
	TCBS 1:100	no trans.	no trans.	no trans.	no trans.
	TCBS 1:1000	no trans.	no trans.	no trans.	no trans.

SPSS	1-Way	2-Way
Analyses	Trans.	Trans.
Chlorophyll A	no trans.	no trans.
Chlorophyll C	no trans.	no trans.

3: RESULTS

3.1. GA Type Gross Morphology

Type I GAs were grossly characterized in the field as being skeletal protuberances beyond that of healthy areas with discrete margins demarcating diseased areas from healthy areas (Figure 1.1 A). Diseased areas possessed normal or nearly normal pigmentation. Often times the diseased surface areas were optically smoother than surrounding healthy areas, but they could also possess small "crinkled" patches of skeleton within the GA. Rarely, "crinkled" patches showed signs of fish predation with the top layer of tissue and skeleton scraped off. Corallites were mostly uniform in size with occasional enlarged corallites.

Type II GAs (Figure 1.1 B) were the most distinct of the GA types. Coral tissue in these GAs was often non-pigmented or had an abnormal pigmentation of a pink/purple hue. GA tissue was only slightly raised above adjacent healthy tissue with a discrete margin demarcating diseased areas from healthy in almost all instances. The shape of these GAs were much more abnormal and often had a patchy arrangement of tissue within the GA area. Skeletal density appeared to be reduced in GA areas, and when accidentally touched, the skeleton would compress and crumble. Corallites were often larger in diseased areas than in healthy areas, with noticeable disorganization and alteration of structural elements.

Type III GAs (Figure 1.1 C) were uncommon and consisted of irregular patches of skeleton which were depressed relative to the surrounding healthy tissue. There was a discrete margin separating the diseased tissue from the healthy tissue. Anomalous

areas were optically very smooth and did not contain any surface texture characteristics of the healthy areas on the colony characteristic of the species. Pigmentation was often decreased while corallites were often arranged regularly and of the same size as healthy areas.

3.2. GA Prevalence and Massive Porites *Abundance*

GAs were found on massive *Porites* at five out of 21 sites surveyed on Guam: Ipan, Luminao, Anae Island, Haputo, and Fouha (Myers and Raymundo, unpub. data). When comparing GA prevalence with massive *Porites* abundance at all sites, there was no significant correlation between the two variables (Figure 3.1 A). However, when the five sites containing GAs on massive *Porites* were analyzed separately, there was a negative, though not significant, correlation between GA prevalence and massive *Porites* abundance (Figure 3.1 B). This suggests that as massive *Porites* abundance increases, GA prevalence on massive *Porites* declines.

3.3. GA Growth in situ

There were significant differences in residuals of GA growth between GA types both within sites and between sites (Table 3.2 2-way; Figure 3.2.). Type II GAs grew significantly less than GAs I and III when sites were combined and Type II GAs in Luminao grew significantly less than Type II GAs in Ipan (Pair-wise Tests: I t = 3.2256, p = 0.0024; III t = 3.6969, t = 0.0033; L,I t = 3.2639, p = 0.0075). Total GA growth over 15 months ranged from a high (growth) of 4175.004 cm^2 (Type II Luminao) to a low

(shrinkage) of -132.779 cm^2 (Type I Luminao), with a mean total growth of 182.91 cm^2 over the study period. Individual growth of the maximum diameter ranged from a high (growth) of 23.2 cm to a low (shrinkage) of -57.3 cm (see Appendix A Figure A.1a-i). While these numbers may seem large given that massive corals grow between 5 and 25 mm per year (Coral Reef Overview), over 83% of GAs grew less than 10 cm along either axis over the time period. This suggests that most GAs grow slowly. Three colonies monitored for GA growth had multiples GAs per colony – one colony with two type I GAs, one colony with one of each type I and II, and one colony with two type I and two type II. Individual GAs comprised from less than 0.02% of the colony surface to up to 24% of the colony at the beginning of the monitoring (Figure 3.3), and almost 80% of GAs were 0.1 m^2 (Figure 3.4). Colony size ranged from 0.128 to 45.689 m^2, with over 82% of colonies being less than 10 m^2 (Figure 3.5). At the end of the monitoring, GA size did not change in percent of colony affected, and the percent of the colony affected at the beginning was significantly correlated with percent of the colony affected at the end ($R^2 = 0.6455$, $p < 0.0001$). All GA-impacted areas experienced a variety of health states throughout the experiment (Table 3.1), with one GA experiencing full disappearance (i.e. tissue reverted back to normal appearance).

3.4. Physiological Impacts to the Host

3.4.1. Total Lipids

After the first extraction, lipid extracts, when evaporated, were found to contain large and visible salt crystals, the amount of which varied widely between samples. Due

to the amount of large, heavy salt crystals, and the relatively lighter lipid extract, lipid content of healthy and GA tissue could not be properly estimated. A second attempt at extraction was made. I concluded that the duration of the freshwater rinse (2 h) was not long enough for all salt from the coral to be completely rinsed from the samples. During the second failed attempt, the duration of freshwater rinse was increased to 24 h. This duration of time in freshwater rinse still did not fully remove all salts from the samples either. It was not possible to run a third extraction, so this portion of the experiment was aborted and samples were unable to be analyzed due to excess salt.

3.4.2. Chl A & C Concentrations and Zooxanthellae Density

While differences in chlorophyll concentrations were observable, no overall pattern in mean chlorophyll a and c concentrations was seen between remote healthy areas, healthy areas of GA colonies or diseased areas of GA colonies for any GA Type (Table 3.3 & Figure 3.6). Although not significant, GA II did have a greater concentration of chlorophyll a in the diseased area compared to healthy areas. There was also a great deal of variation seen in all samples including the remote healthy colonies. Given that there was only one chlorophyll sample per colony, statistical power was extremely low to account for the inherent between-colony variation, which made it difficult to determine if there was an impact of GAs on chlorophyll concentrations.

Zooxanthellae densities were significantly different between and within colonies. Colony variation between health states was small, however colonies did vary significantly within health states of each GA type (Table 3.5a, Di X Colony(Type)) and remote healthy colonies showed similar high variation (Figure 3.7). One colony within

GA III did, however, have a significantly greater zooxanthellae density in healthy areas than in diseased areas ($t = 3.4054$, $p = 0.0037$), and there was greater variation between diseased areas than healthy areas of colonies within GA III. GAs I and II also varied significantly between colonies within health states, however GA I had a larger amount of variation between colonies (Figure 3.7). My results show inherent high variation between colonies regardless of health state and suggest that for the sampled population, GAs do not significantly impact zooxanthellae densities or chlorophyll a and c concentrations.

3.4.3. Morphometric Differences Between Healthy and Diseased Skeletal Structures

Corallite Density

Corallite density varied within remote healthy colonies, though generally averaged 60-75 corallites per cm^2 (Figure 3.8). Corallite density was significantly higher in healthy areas than in diseased areas of individual colonies within GA Types II and III (Figure 3.8). Colonies also varied significantly within health states in all GA Types (Table 3.5a Di X Colony(Type)). Of 12 colony pairs, four had significantly fewer corallites per area in diseased areas than in healthy areas and one colony had significantly more per area in diseased. Approximately 42% of colonies were significantly different between healthy and diseased areas. Type III GAs showed the most consistent pattern: 75% of colonies have significantly more corallites in healthy areas than in diseased areas.

Corallite Size

Corallites were significantly larger in diseased areas than healthy areas for most colonies (Figure 3.9) and also when healthy and diseased areas were combined for all

types (corallite diameter: Figure 3.10; Table 3.5, Pair-wise Tests: t = 7.154, p = 0.0001). Colonies also varied within health states in all GA Types (Table 3.5, Di X Colony(Type)). Approximately 83% of colonies had significant differences between healthy and diseased areas. Of 12 colony pairs, eight had significantly higher mean CDs in diseased areas than in healthy areas. These results were consistent across GA type which suggests that, regardless of type, GAs affect the host colony by causing it to produce larger corallites.

Corallite surface area (CSA) varied in accordance with corallite diameter for colonies having GA II and III, with occasional colonies showing very large deviation from the normal range or 0.75-1.75 mm^2 (Figure 3.11). All samples deviating sharply from the mean were those taken from GAs. Colonies also varied within healthy and diseased areas in all GA Types (Table 3.5, Di X Colony(Type)). Of 12 colony pairs, nine differed in mean CSA between healthy and diseased areas, with seven showing significantly greater mean CSAs in diseased areas and two with smaller corallites within GAs. Results point to the overall enlargement of corallites in diseased GA areas.

Distance Between Corallites

Three out of four remote healthy colonies were similar in the arrangement of corallites, though one showed much more variability, however they did not differ significantly from each other (Figure 3.12). Mean distance from a corallite wall to its closest neighboring calice (CSM) and furthest neighboring calice (CSX) were highly variable between all tested with no discernable pattern (Figures 3.12 & 3.13). Colonies varied both between and within healthy and diseased areas of GA Types (CSM & CSX

Table 3.5, Di X Colony(Type)). Of 12 colony pairs, six had significantly different mean CSMs between healthy and diseased areas, though the relationship did not show a consistent pattern (Figure 3.12). Four colonies (2 each of GA I and II) had corallites that were more widely spaced within diseased areas than in healthy and two colonies (1 each of GA II and III) showed the opposite trend.

When looking at CSX colonies, of 12 colony pairs, two (1 each of GA I & III) had significantly greater mean CSXs in healthy areas than in diseased areas (Figure 3.13). There was much less mean maximum distances between neighbors (CSX). GA I and II corallites tended to be more widely spaced in healthy areas of colonies than in diseased while there was no clear pattern in colonies with Type III GAs. These results are consistent with the combination of corallite density and size interacting to affect their spacing. These characters appear to affect the host colony differentially, according to GA Type.

Number of Septae and Palli

Remote healthy colonies did not vary in the number of septae and palli they contained and neither did healthy areas of colonies with GAs. However, diseased areas of two colonies had more septae per corallite than healthy areas (Figure 3.14). Of 14 colony pairs, four had significantly different mean number of septae between healthy and diseased areas and displayed more variability in diseased then in healthy corallites (Table 3.5, Di X Colony(Type)). This suggests that the number of septae within healthy areas is quite stable but that diseased corallites show more variation in this trait.

While the number of septae within healthy corallites of *Porites lutea* is quite consistent (12 septae), the number of palli within a corallite of this species is inherently variable (5-8 palli; Randall pers. comm., Veron 2000). Remote healthy colonies showed little variation in the number of palli within a corallite. Most healthy areas of colonies had a number of palli that fell within the normal range of five to eight palli, however significant differences were clear. Of the 14 colony pairs, five had significantly different mean number of palli between healthy and diseased areas (Table 3.5, Di X Colony(Type); Figure 3.15). There was much more variation in diseased areas of colonies than in healthy areas, with all healthy areas having between 4-6 palli per corallite and diseased areas ranging between 2-8 palli per corallite. These results suggest that corallite structure is more dysfunctional in diseased areas and more structured in healthy areas.

3.4.4. Tissue Thickness

Tissue thickness varied significantly between colonies within both healthy and diseased areas of GA types (Table 3.5a, Di X Colony(Type); Figure 3.16). Four of 12 colonies (GA I & III) had significantly thicker tissue in healthy areas than in diseased areas. Two colonies (GA I & II) had thicker tissue in diseased areas than in healthy areas. It is also interesting to note that in GA II, most colonies had greater tissue thickness in diseased areas though differences were not significant in three out four colonies.

3.5. Differences in Microbial Community

3.5.1. Non-specific Culturable Bacteria on Marine Agar

There was very little variation in bacterial populations grown on MA between health states within colonies at any dilution for either time period. Likewise, the bacterial communities in seawater samples did not vary for all dilution sets and all time periods (See Appendix Table A.5). There were, however, significant differences between colonies within health states and between remote healthy colonies.

At 24 hours, remote healthy colonies (RH) had significantly greater mean CFU counts than seawater (SW) samples and healthy areas of GA II and III on the full strength and 1:10 dilution plates (Table 3.6; Figure 3.18 & 3.20). After 48 hours at the 1:10 dilution, all healthy areas of GAs and RH were significantly greater than SW. Healthy areas of GA types had mean CFU counts resembling those of SW more than those of RH.

After 24 hours at the 1:100 dilution, diseased areas of GA I had significantly more CFUs than healthy areas (Table A.2, Di X GA Pair-wise Tests: $t = 3.0349$, $p = 0.0077$) (Figure 3.22) and healthy areas of GA III had significantly more CFUs than those of GA I (Pair-wise Tests: $t = 2.2361$, $p = 0.0401$). After 48 hours, there was no longer any difference between health states for GA types, or between healthy areas of GA types. This suggests that all colonies harbored a larger array of bacterial types which were better suited for growth under less dense conditions and needed a longer incubation time to utilize resources.

At the 1:1000 concentration, it was hard to examine differences between tested factors, as many samples had no bacterial growth at all (Figure 3.23). After 24 hours,

mean CFUs were too low to detect any differences between colonies or GA types at any level. There was little change after 48 hours of growth. Given that this concentration yielded so few CFU counts, I assumed that all unique isolates were isolated in previous concentrations, rendering this dilution unnecessary in future efforts.

3.5.2. Vibrio-*specific TCBS Agar*

There were significant differences between colonies within health states and between remote healthy colonies which is consistent with current theory that bacterial communities in coral SML can be highly diverse and variable. As with MA, there was very little significant variation between health states within colonies at any dilution set for either time period. Seawater, again, appeared relatively stable with little variation seen for all dilutions at all time periods (See Appendix Table A.6).

Within the full strength sample, the number of mean CFUs within remote healthy colonies increased dramatically from 24 to 48 hours, while all other counts from that dilution set remained nearly the same (Figure 3.24). When comparing the amount of *Vibrio* spp. within a given sample, RH colonies had the highest amount, which is consistent with mean CFU counts from MA, and diseased samples had very few *Vibrio* spp. When combined within type, RH had significantly greater mean CFUs than healthy areas of GA II (Figure 3.25). At the 1:10 concentration, there was no significant variation, although RH colonies still had the greatest mean CFU counts (Figure 3.26).

At the 1:100 (Figure 3.27) and 1:1000 (Figure 3.28) concentrations there were no significant differences between any samples at either time period. At these concentrations, like for MA, it was hard to determine significant differences given the

amount of samples which had no bacterial growth at all. This suggests that both the 1:100 and 1:1000 concentrations are too much of a dilution and should not be used in subsequent studies of GAs.

3.6. Transmission Experiment

After 68 days of direct contact, no healthy colonies developed GAs. One healthy colony and four GA chips experienced full mortality by the end of the experiment (Table 3.10). Eighty percent of GA Type II chips experienced partial mortality and 100% of GA Type I chips experienced compromised health states (bleaching, tissue loss, algal overgrowth, etc.). Of the healthy control chips, however, 25% experienced partial mortality, and none experienced full mortality. All colonies and chips experienced either bleaching or slight tissue loss at the contact point between colonies. This was not counted as a compromised health state if the reaction did not spread further than the contact area.

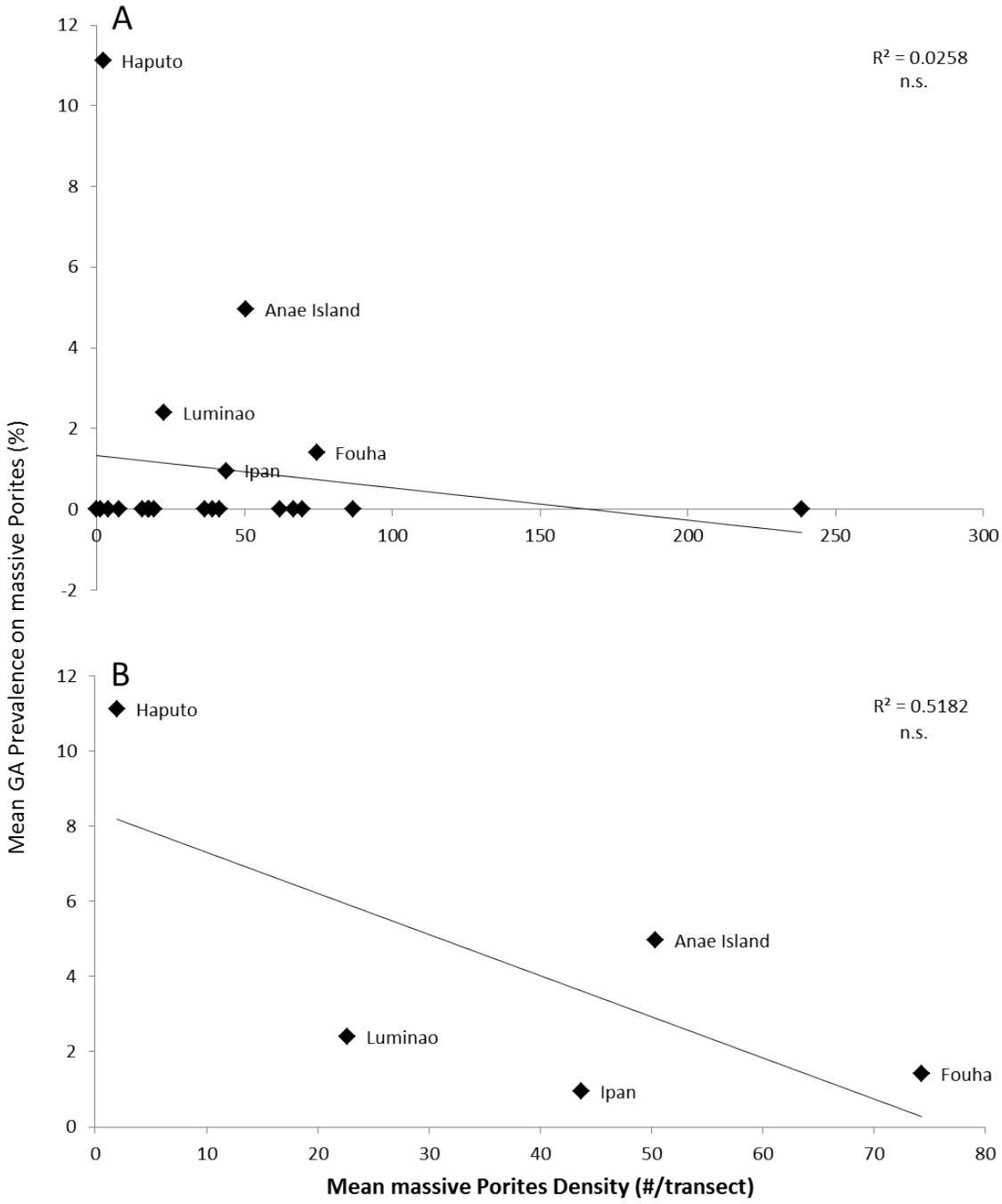

Figure 3.1. Mean GA prevalence vs. mean massive *Porites* density (A) for all 21 sites (n = 2-3 transects per site) and (B) for the 5 sites with GAs present on massive *Porites* (n = 3 transects per site) (n.s. = not significant).

Table 3.1. Overall growth/shrinkage and compromised health states of tissue within GA-affected areas over the 15 month observation period.

Overall Change in GA	GA Type Type I (n = 15)	 Type II (n = 15)	 Type III (n = 4)
Healed	---	1	---
Full Mortality	---	---	---
Overall Growth	9	12	3
Overall Stasis	2	1	---
Overall Shrinkage	4	1	1
Compromised Health States			
Partial Mortality	6	8	2
Predation	---	1	---

Table 3.2. Univariate 2-way PERMANOVA results for Total GA Growth. Significant p-values are in bold print.

Characteristic	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
GA Total Growth	Site	1	4.03E+06	8.1974	**0.0072**	9837
(ALL GA Types)	GA Type	2	4.22E+06	8.5999	**0.0009**	9944
	Site X GA	1	3.00E+06	6.1014	**0.0168**	9856

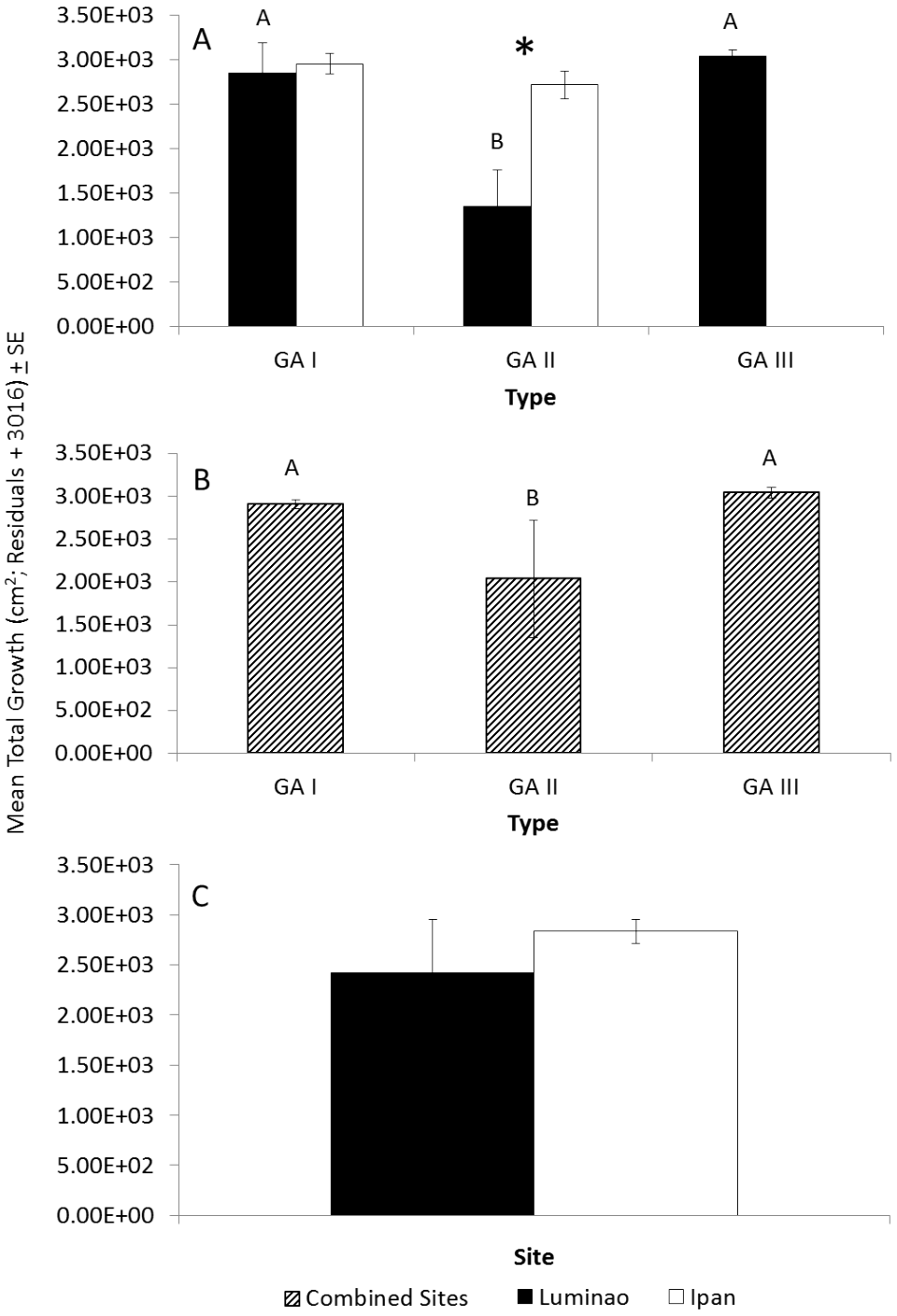

Figure 3.2. Mean total GA growth residuals in (A) GA Types by Sites (B) GA Types combined between Sites, and (C) GA Types combined within Sites. Letters in (A) indicate significant differences between GA Types in Luminao ($p < 0.05$). Letters in (B) indicate significant differences between GA Types when combined between sites ($p < 0.05$). Asterisks indicate significant differences between sites ($p < 0.05$).

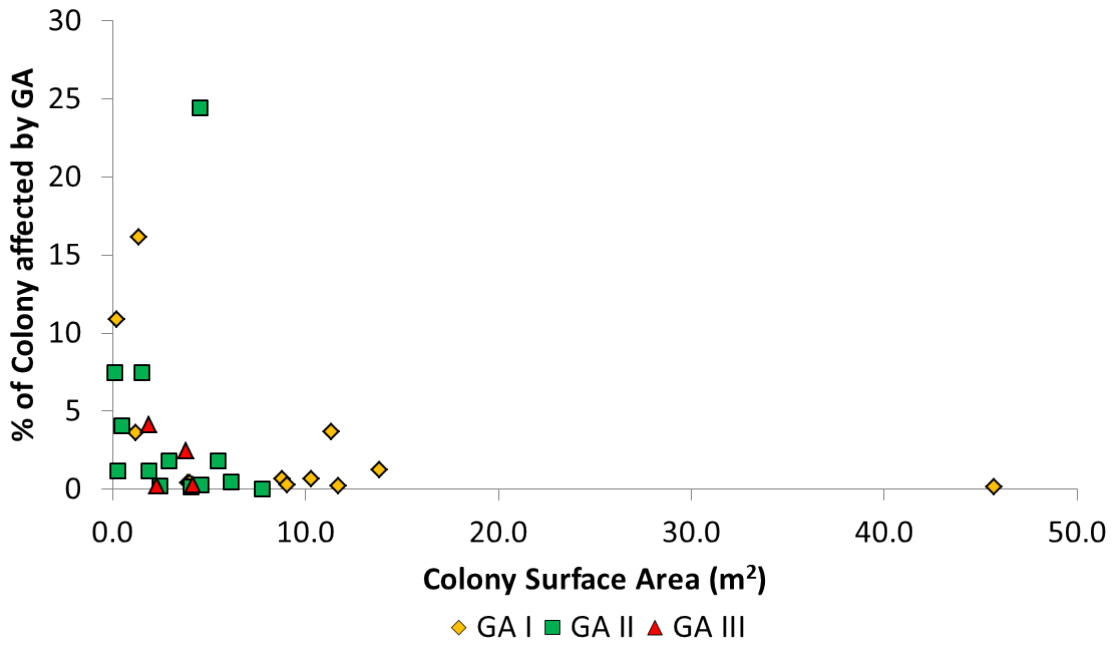

Figure 3.3. Percent (%) of colony affected by a GA vs. the size of the colony itself (surface area in m^2). Each GA type is represented by a different symbol/color: GA I = ◆ , GA II = ■, and GA III = ▲.

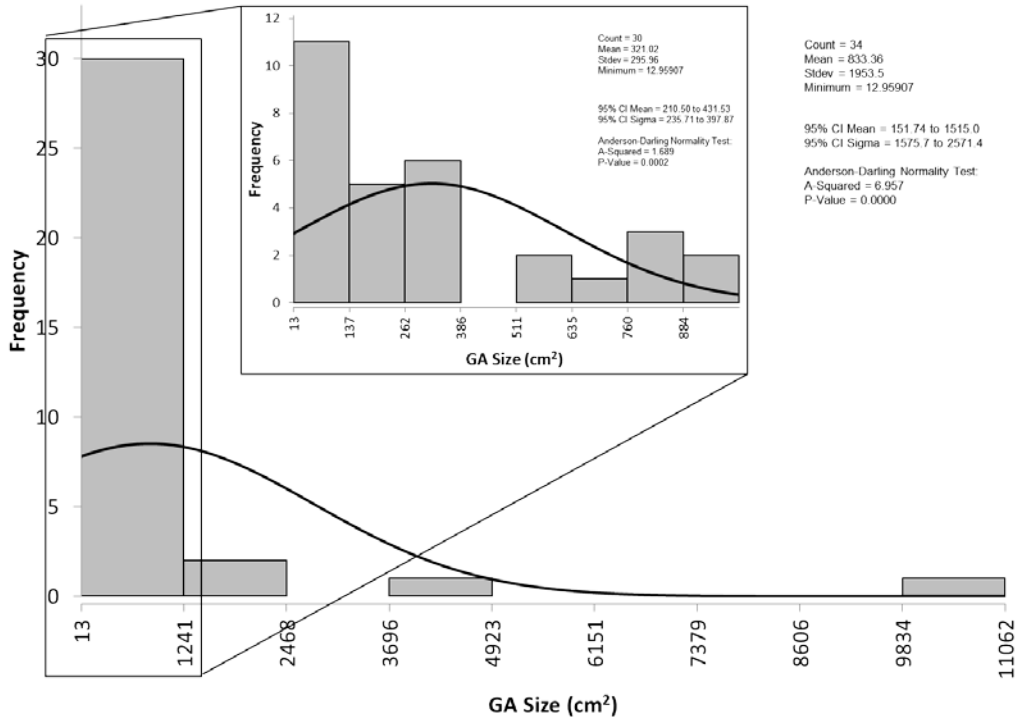

Figure 3.4. Size frequency distribution of those GAs monitored for growth. Inset shows the size frequency distribution of the first bin between 13 and 1241 cm^2.

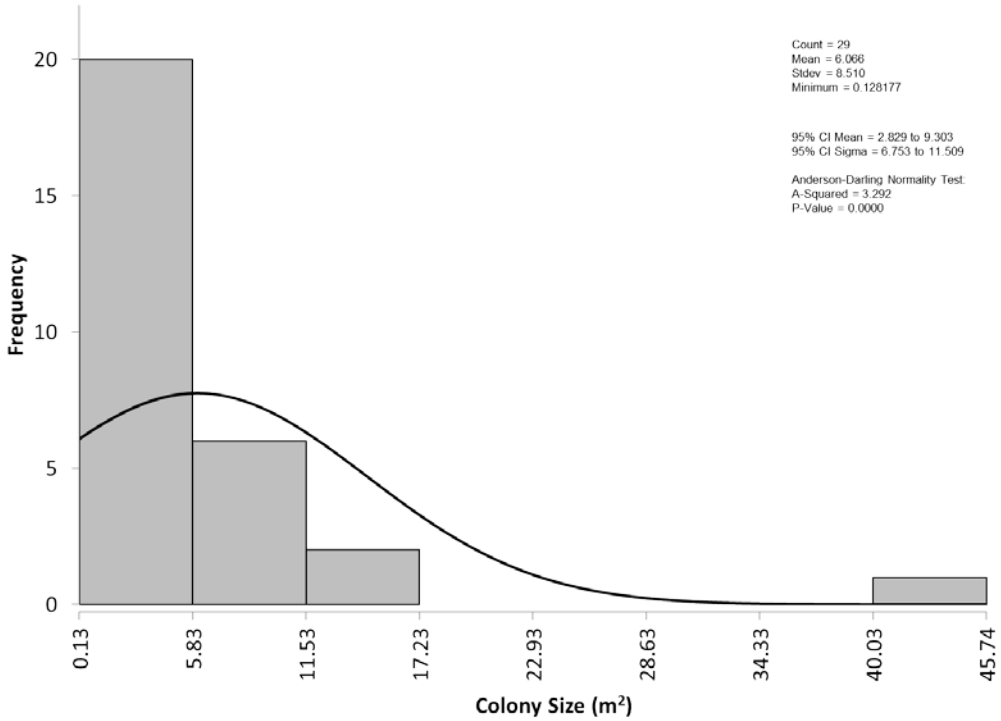

Figure 3.5. Size frequency distribution of those colonies which had GAs monitored for growth.

Table 3.3. Univariate ANOVA results for Chlorophyll A and C concentrations. 1-way designs for all healthy samples and 2-way designs for comparisons between health states for each characteristic are presented.

1-Way

Characteristic	Source of Variation	df	MS	F	P
Chlorophyll A	GA Type	3	7.439	0.772	0.532
Chlorophyll C	GA Type	3	14.704	1.793	0.202

2-Way

Characteristic	Source of Variation	df	MS	F	P
Chlorophyll A	Disease State	1	18.47	1.747	0.203
	GA Type	2	11.762	1.113	0.35
	Di. X GA	2	5.197	0.492	0.62
Chlorophyll C	Disease State	1	2.051	0.258	0.617
	GA Type	2	9.081	1.144	0.341
	Di. X GA	2	13.816	1.740	0.204

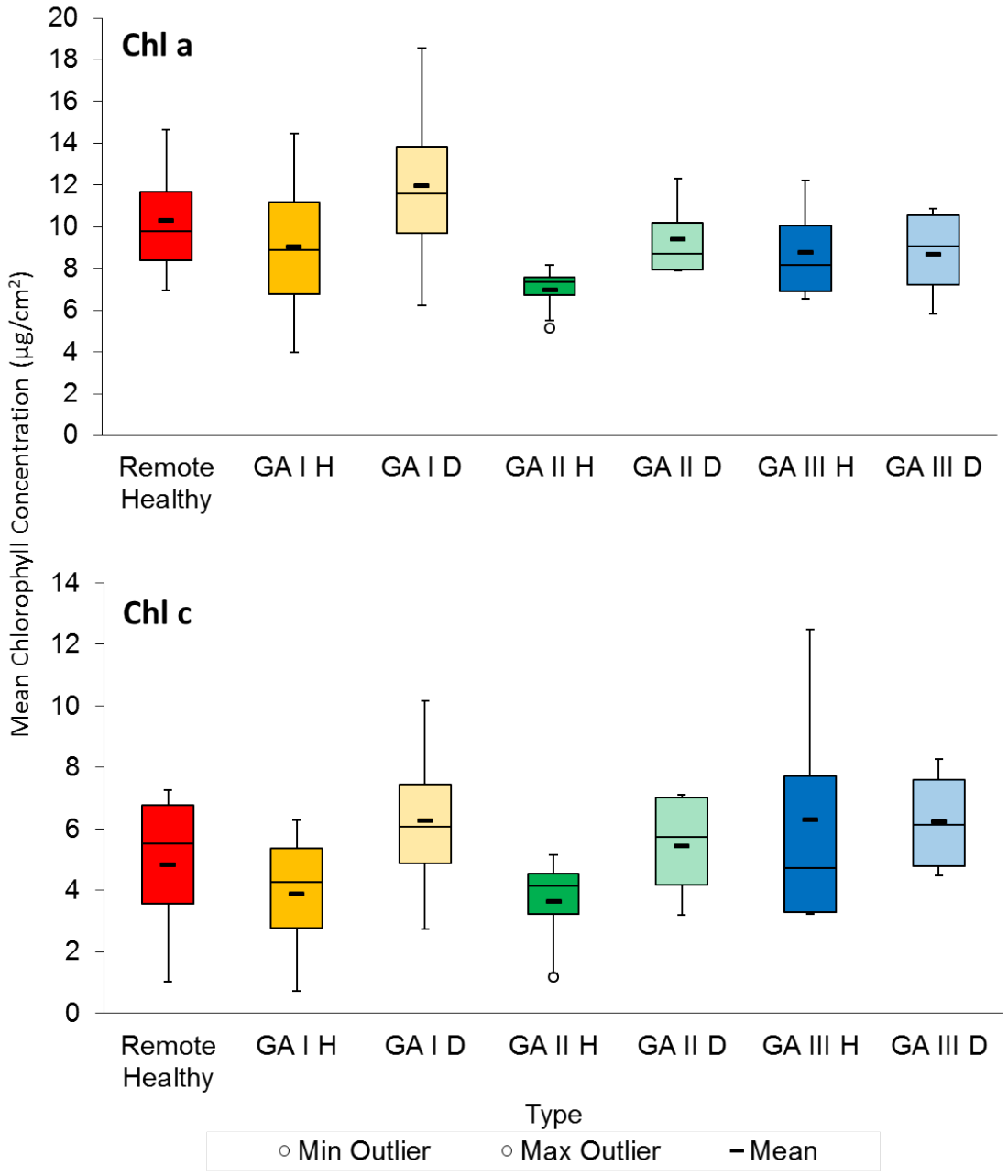

Figure 3.6. Box plots of mean Chl A & C concentrations of *Porites lutea* in Remote Healthy and healthy (H) and diseased (D) areas of GA Types. Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

Table 3.4. Univariate PERMANOVA results for physiological parameters. Nested 1-way designs for all healthy samples of each characteristic are presented. Significant p-values are in bold print and MC indicates the p-value was derived from Monte Carlo sampling.

Characteristic	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
Zooxanthellae Density	GA Type	3	63.929	0.96531	0.4478	9936
	Colony(Type)	12	66.226	8.355	**0.0001**	9933
Corallite Density	GA Type	3	677	0.42077	0.7505	9937
	Colony(Type)	12	1608.9	7.6748	**0.0001**	9930
Corallite Diameter	GA Type	3	1.58E-02	0.17983	0.9239	9938
	Colony(Type)	12	8.81E-02	9.6145	**0.0001**	9914
Corallite Surface Area	GA Type	3	1.11E-02	0.13357	0.9445	9943
	Colony(Type)	12	8.32E-02	20.281	**0.0001**	9924
CSM	GA Type	3	2.55E-02	0.38862	0.7348	9929
	Colony(Type)	12	6.56E-02	15.021	**0.0001**	9922
CSX	GA Type	3	2.32E-02	0.26708	0.8366	9944
	Colony(Type)	12	8.67E-02	5.1299	**0.0001**	9930
Number of Septae	GA Type	3	1.15E-02	4.80E-02	0.9839	MC
	Colony(Type)	12	0.23854	1.1996	0.2894	9919
Number of Palli	GA Type	3	2.1083	1.7539	0.2032	470
	Colony(Type)	12	1.2021	2.2174	**0.0121**	9939
Tissue Thickness	GA Type	3	2.11E-02	1.0112	0.4136	9730
	Colony(Type)	12	2.09E-02	8.1882	**0.0001**	9940

Table 3.5. Univariate PERMANOVA results for physiological parameters. Nested 2-way designs for each characteristic are presented. Significant p-values are in bold print.

Characteristic	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
Zooxanthellae Density	Disease State	1	5173	4.04E-02	0.8381	9829
	GA Type	2	5.49E+05	0.82109	0.52	4734
	Colony(Type)	9	6.69E+05	14.634	**0.0001**	9936
	Di. X GA	2	2.53E+05	1.9727	0.188	9951
	Di. X Colony(Type)	9	1.28E+05	2.8028	**0.0047**	9929
Corallite Density	Disease State	1	14.804	2.9844	0.1183	9838
	GA Type	2	18.701	0.94348	0.4228	4737
	Colony(Type)	9	19.821	29.101	**0.0001**	9943
	Di. X GA	2	10.073	2.0307	0.1924	9959
	Di. X Colony(Type)	9	4.9604	7.2826	**0.0001**	9941
Corallite Diameter	Disease State	1	0.2465	5.9689	**0.04**	9837
	GA Type	2	1.25E-02	0.18269	0.8261	4730
	Colony(Type)	9	6.81E-02	22.919	**0.0001**	9925
	Di. X GA	2	3.10E-02	0.75036	0.5035	9951
	Di. X Colony(Type)	9	4.13E-02	13.889	**0.0001**	9935
Corallite Surface Area	Disease State	1	0.72207	3.798	0.0824	9854
	GA Type	2	3.46E-02	0.11182	0.8862	4764
	Colony(Type)	9	0.30946	29.553	**0.0001**	9942
	Di. X GA	2	0.10812	0.56871	0.5845	9964
	Di. X Colony(Type)	9	0.19012	18.156	**0.0001**	9939
CSM	Disease State	1	3.60E-03	9.35E-02	0.7633	9865
	GA Type	2	1.15E-02	9.76E-02	0.9168	4715
	Colony(Type)	9	0.11754	19.255	**0.0001**	9934
	Di. X GA	2	8.53E-02	2.2141	0.1604	9947
	Di. X Colony(Type)	9	3.85E-02	6.3131	**0.0001**	9940
CSX	Disease State	1	5.52E-02	0.71824	0.4181	9825
	GA Type	2	1.60E-02	5.71E-02	0.9448	4725
	Colony(Type)	9	0.27956	14.101	**0.0001**	9936
	Di. X GA	2	2.83E-02	0.36786	0.6864	9956
	Di. X Colony(Type)	9	7.69E-02	3.8768	**0.0001**	9955
Number of Septae	Disease State	1	73.633	1.9765	0.2106	9826
	GA Type	2	25.39	0.65399	0.6909	2338
	Colony(Type)	9	38.829	11.228	**0.0001**	9925
	Di. X GA	2	25.265	0.67817	0.5711	9949
	Di. X Colony(Type)	9	37.254	10.773	**0.0001**	9948
Number of Palli	Disease State	1	2.1333	0.15182	0.7079	9853
	GA Type	2	5.5271	0.27827	0.749	1728
	Colony(Type)	9	19.862	11.246	**0.0001**	9930
	Di. X GA	2	17.827	1.2687	0.3318	9961
	Di. X Colony(Type)	9	14.051	7.9556	**0.0001**	9943
Tissue Thickness	Disease State	1	1.70E-03	8.35E-02	0.7759	9822
	GA Type	2	1.07E-02	1.2144	0.3508	4713
	Colony(Type)	9	8.79E-03	5.8381	**0.0001**	9943
	Di. X GA	2	1.67E-02	0.8177	0.4583	9964
	Di. X Colony(Type)	9	2.04E-02	13.543	**0.0001**	9938

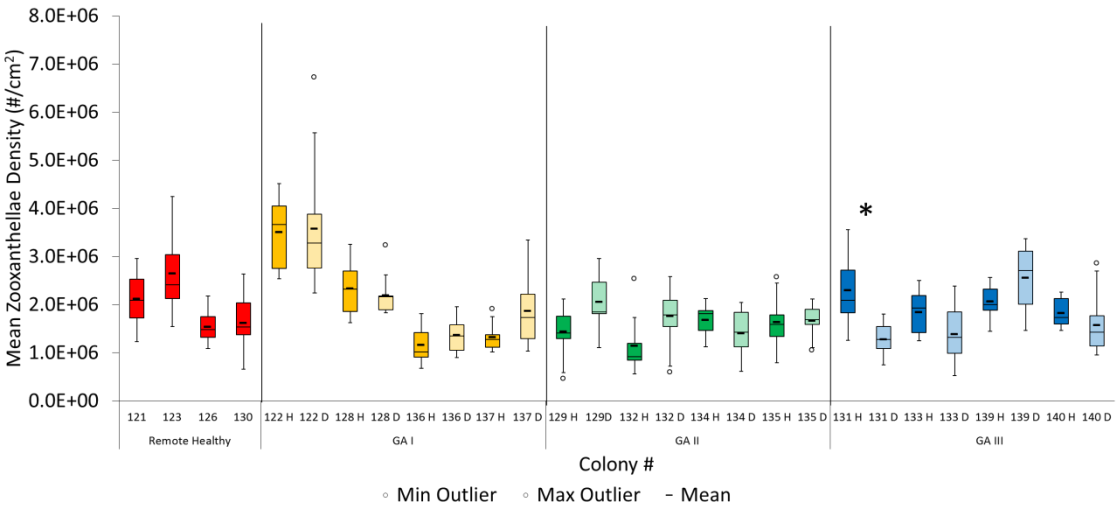

Figure 3.7. Box plots of mean zooxanthellae densities of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate a significant difference between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

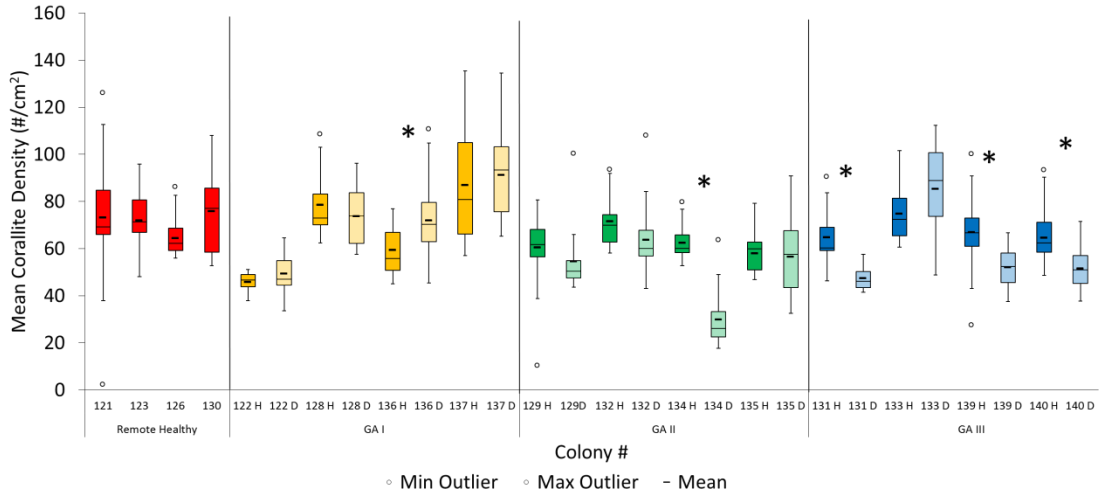

Table 3.8. Box plots of mean corallite densities of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

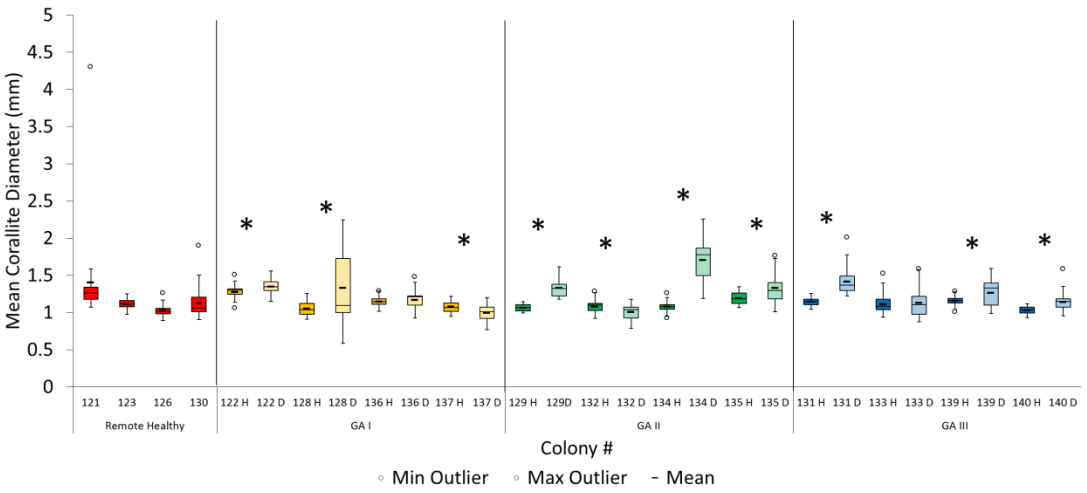

Figure 3.9. Box plots of mean maximum corallite diameter of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

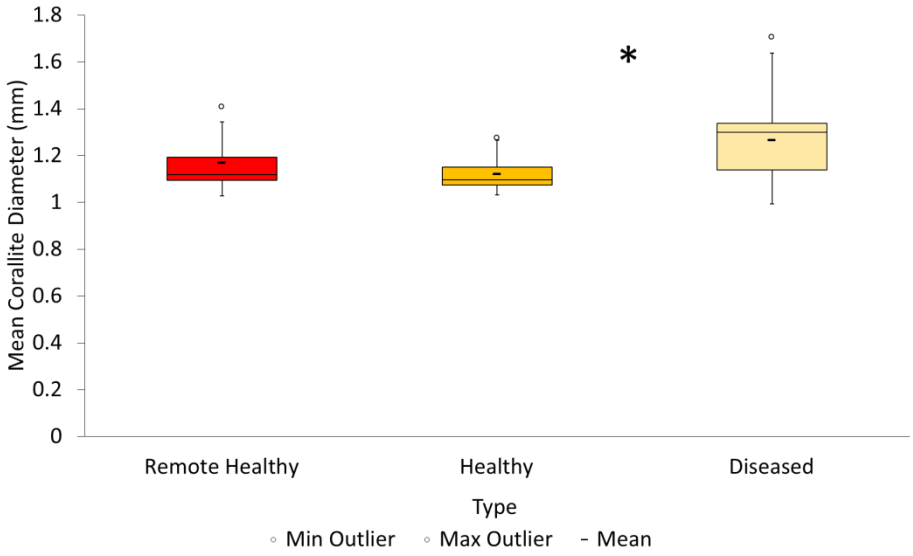

Figure 3.10. Box plots of mean maximum corallite diameter of *Porites lutea* within Remote Healthy, Combined Healthy and Combined Diseased samples. Asterisks (*) indicate differences between healthy and diseased samples ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

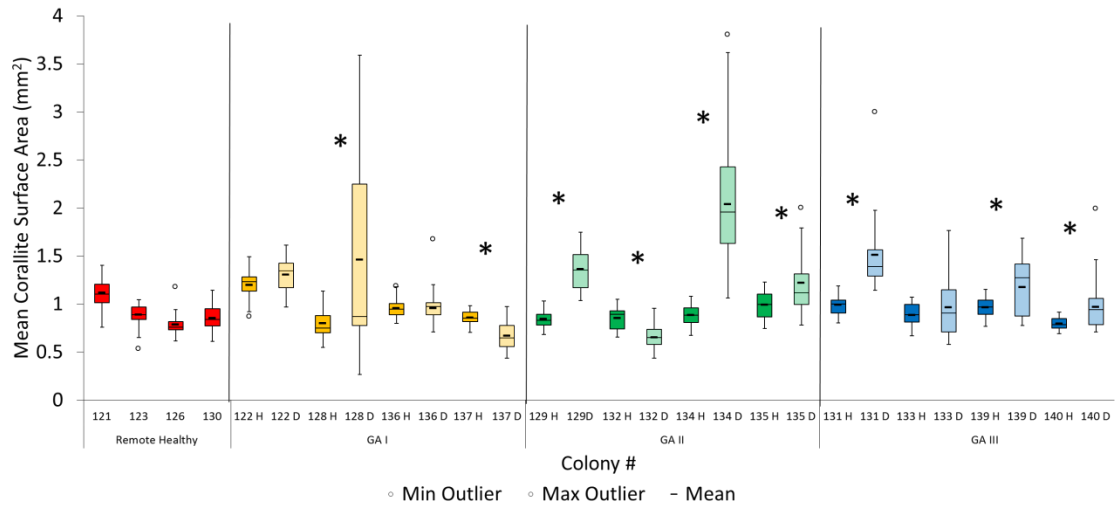

Figure 3.11. Box plots of mean corallite surface area of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

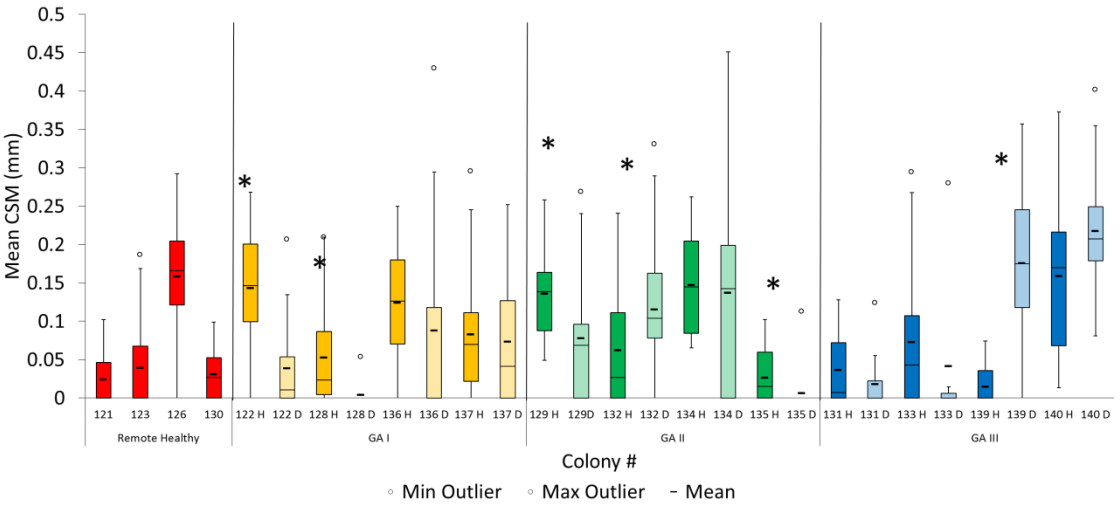

Figure 3.12. Box plots of mean distance from wall to closest neighboring calice (CSM) of *Porites lutea* between Remote Healthy colonies and between healthy (H) and diseased areas (D) of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

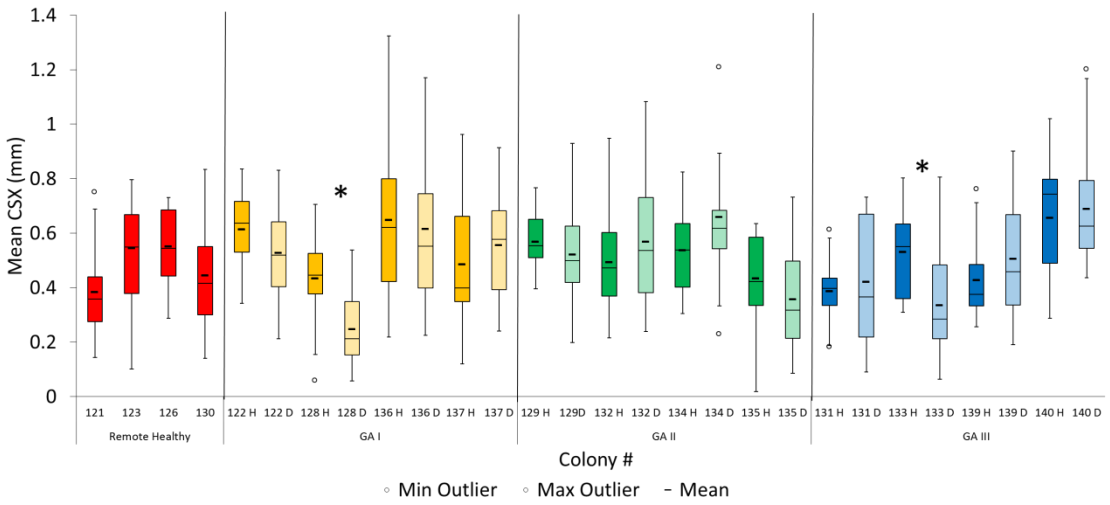

Figure 3.13. Box plots of mean distance from wall to furthest neighboring calice (CSX) of *Porites lutea* between Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

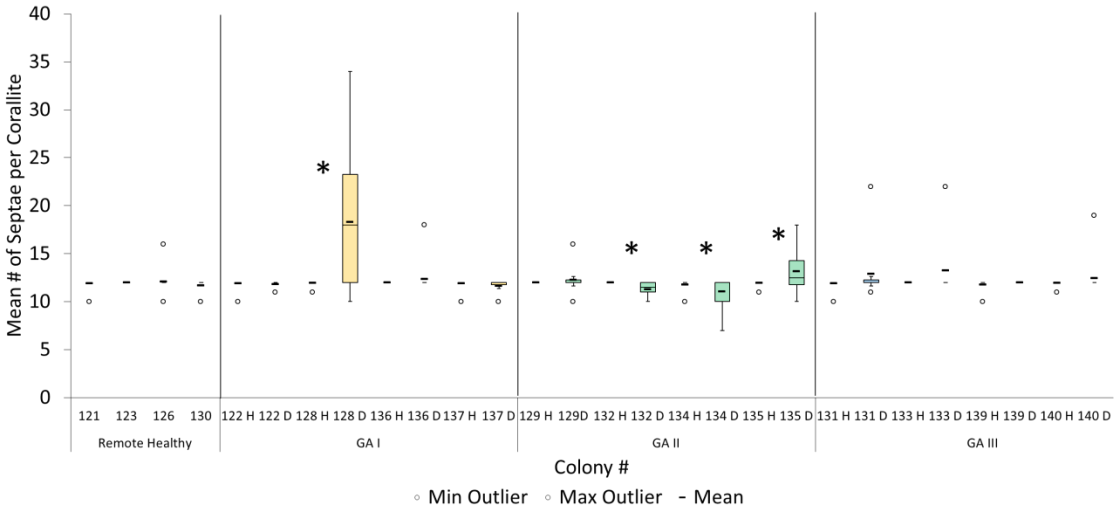

Figure 3.14. Box plots of mean number of septae of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

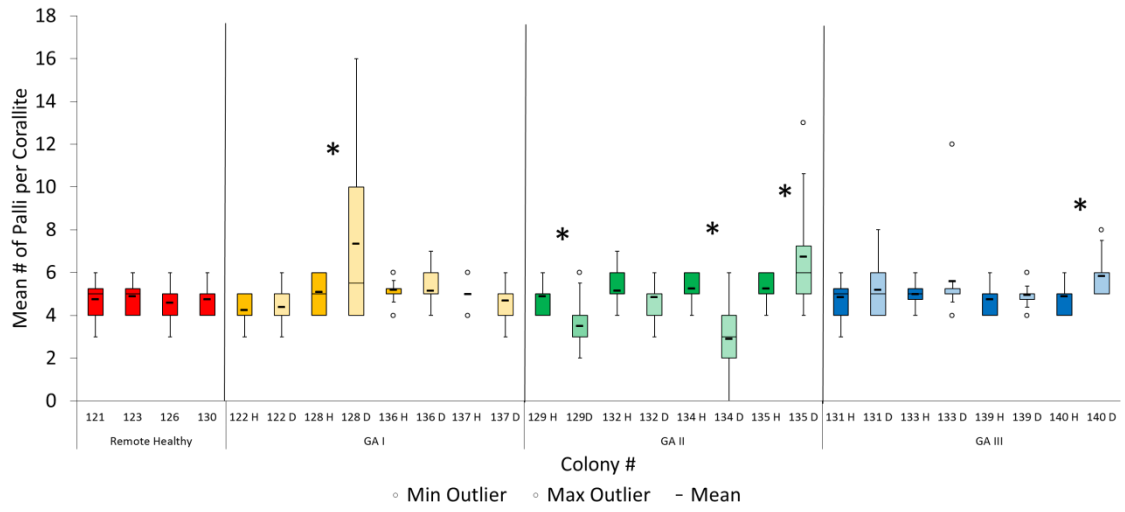

Figure 3.15. Box plots of mean number of palli of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

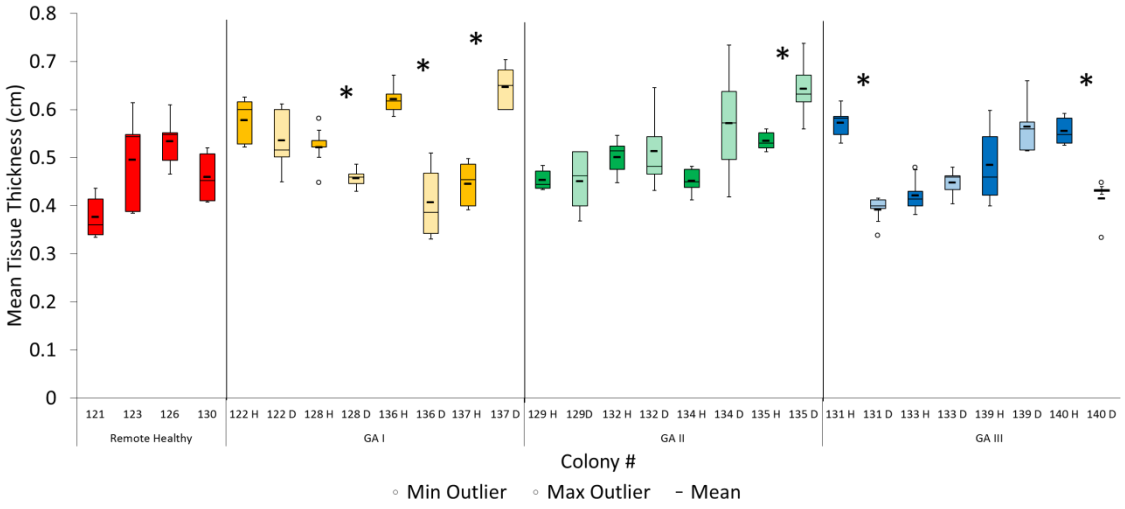

Figure 3.16. Box plots of mean tissue thickness of *Porites lutea* within Remote Healthy colonies and between healthy (H) and diseased (D) areas of colonies within GA Types. Asterisks (*) indicate significant differences between healthy and diseased states of individual colonies ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

Table 3.6. Univariate PERMANOVA results for microbial communities. Nested 1-way designs for all healthy samples at each time period, agar type, and dilution are presented. Significant p-values are in bold print and MC indicates the p-value was derived from Monte Carlo sampling. Sample size is indicated under each dilution.

Time Period	Agar & Dilution	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
24 Hours	MA 1:1	GA Type	4	0.69686	3.2799	**0.0386**	9961
	(n = 2 per colony)	Colony(Type)	15	0.21246	16.121	**0.0001**	9938
	MA 1:10	GA Type	4	181.37	4.3227	**0.0158**	2407
	(n = 2 per colony)	Colony(Type)	15	41.958	2.5391	**0.0304**	9939
	MA 1:100	GA Type	4	2.15	2.7158	0.0684	MC
	(n = 2 per colony)	Colony(Type)	15	0.79167	0.67376	0.8013	9905
	MA 1:1000	GA Type	4	3.75E-02	0.75	0.5619	MC
	(n = 2 per colony)	Colony(Type)	15	5.00E-02	1	0.419	1046
	TCBS 1:1	GA Type	4	3.3248	2.6288	0.0781	9954
	(n = 2 per colony)	Colony(Type)	15	1.2648	2.4428	**0.0266**	9926
	TCBS 1:10	GA Type	4	0.4	0.32432	0.8555	MC
	(n = 2 per colony)	Colony(Type)	15	1.2333	1.1746	0.3114	9874
	TCBS 1:100	GA Type	4	0.1875	1.3235	0.3168	MC
	(n = 2 per colony)	Colony(Type)	15	0.14167	0.80952	0.6855	7824
	TCBS 1:1000	GA Type	4	2.50E-02	1	0.4317	MC
	(n = 2 per colony)	Colony(Type)	15	2.50E-02	1	0.482	MC
48 Hours	MA 1:1	GA Type	4	8068.7	2.1247	0.1092	9591
	(n = 2 per colony)	Colony(Type)	15	3797.6	13.436	**0.0001**	9924
	MA 1:10	GA Type	4	165.81	3.591	**0.0272**	2512
	(n = 2 per colony)	Colony(Type)	15	46.175	1.8378	0.0959	9938
	MA 1:100	GA Type	4	1.56E-02	0.15795	0.9598	9954
	(n = 2 per colony)	Colony(Type)	15	9.85E-02	2.8746	**0.0131**	9929
	MA 1:1000	GA Type	4	3.8375	0.58071	0.6802	MC
	(n = 2 per colony)	Colony(Type)	15	6.6083	3.4329	**0.0119**	9933
	TCBS 1:1	GA Type	4	26.709	3.3744	**0.0341**	9953
	(n = 2 per colony)	Colony(Type)	15	7.9152	3.0356	**0.0064**	9924
	TCBS 1:10	GA Type	4	1.0071	2.1466	0.1064	8415
	(n = 2 per colony)	Colony(Type)	15	0.46916	2.7755	**0.0206**	9920
	TCBS 1:100	GA Type	4	4.2125	1.7737	0.1815	MC
	(n = 2 per colony)	Colony(Type)	15	2.375	4.1304	**0.0003**	9892
	TCBS 1:1000	GA Type	4	0.3375	1.35	0.2976	MC
	(n = 2 per colony)	Colony(Type)	15	0.25	0.83333	0.7101	8807

Table 3.7. Univariate PERMANOVA results for microbial communities. Nested 2-way designs for each time period, agar type, and dilution are presented. Significant p-values are in bold print and MC indicates the p-value was derived from Monte Carlo sampling.

Time Period	Agar & Dilution	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
24 Hours	MA 1:1	Disease State	1	5.35E-02	0.30198	0.5897	9827
		GA Type	2	0.50719	0.99571	0.4363	4745
		Colony(Type)	9	0.50937	24.275	**0.0001**	9952
		Di. X GA	2	1.19E-02	6.70E-02	0.9401	9961
		Di. X Colony(Type)	9	0.17712	8.4411	**0.0001**	9948
	MA 1:10	Disease State	1	0.11274	7.79E-02	0.7822	9834
		GA Type	2	4.7546	1.1614	0.3756	4720
		Colony(Type)	9	4.0939	7.2545	**0.0001**	9948
		Di. X GA	2	0.48213	0.33327	0.7223	9950
		Di. X Colony(Type)	9	1.4467	2.5636	**0.0292**	9944
	MA 1:100	Disease State	1	1.3333	1.4769	0.2548	9718
		GA Type	2	0.14583	0.21429	0.8066	MC
		Colony(Type)	9	0.68056	1.4848	0.2052	9932
		Di. X GA	2	2.5208	2.7923	0.1213	9795
		Di. X Colony(Type)	9	0.90278	1.9698	0.0878	9936
	MA 1:1000	Disease State	1	10267	1.0057	0.3959	9300
		GA Type	2	10150	0.99431	0.4105	MC
		Colony(Type)	9	10208	2.45E+05	**0.0001**	9932
		Di. X GA	2	10179	0.99714	0.4676	9862
		Di. X Colony(Type)	9	10208	2.45E+05	**0.0001**	9934
	TCBS 1:1	Disease State	1	1.324	0.96955	0.354	9823
		GA Type	2	1.5051	0.57054	0.5579	4751
		Colony(Type)	9	2.638	7.2722	**0.0003**	9942
		Di. X GA	2	0.13647	9.99E-02	0.9092	9955
		Di. X Colony(Type)	9	1.3656	3.7646	**0.0029**	9946
	TCBS 1:10	Disease State	1	0.2835	0.81145	0.3908	9825
		GA Type	2	0.76984	2.4339	0.1593	250
		Colony(Type)	9	0.3163	0.99702	0.4618	9929
		Di. X GA	2	0.1373	0.39299	0.686	9960
		Di. X Colony(Type)	9	0.34937	1.1012	0.3963	9935
	TCBS 1:100	Disease State	1	0.1875	1	0.3367	4446
		GA Type	2	2.08E-02	0.15789	0.8557	MC
		Colony(Type)	9	0.13194	0.7037	0.7084	9842
		Di. X GA	2	0.1875	1	0.4034	3314
		Di. X Colony(Type)	9	0.1875	1	0.4544	9890
	TCBS 1:1000	Disease State	1	54.187	1	0.3783	3864
		GA Type	2	54.188	1	0.4102	MC
		Colony(Type)	9	54.187	1	0.4979	4250
		Di. X GA	2	54.188	1	0.4699	1423
		Di. X Colony(Type)	9	54.188	1	0.5102	5187

Table 3.7. (continued)

48 Hours	MA 1:1	Disease State	1	1.6131	0.44076	0.5165	9830
		GA Type	2	20.618	1.2189	0.3502	4719
		Colony(Type)	9	16.915	25.101	**0.0001**	9945
		Di. X GA	2	0.22447	6.13E-02	0.9383	9952
		Di. X Colony(Type)	9	3.6598	5.4309	**0.0003**	9954
	MA 1:10	Disease State	1	7.72E-02	1.02	0.3335	9828
		GA Type	2	0.86512	4.5306	**0.0341**	4751
		Colony(Type)	9	0.19095	3.562	**0.0061**	9961
		Di. X GA	2	0.11782	1.5562	0.2638	9949
		Di. X Colony(Type)	9	7.57E-02	1.4123	0.2403	9948
	MA 1:100	Disease State	1	0.28545	0.43247	0.5255	9844
		GA Type	2	0.30316	0.37204	0.6944	1718
		Colony(Type)	9	0.81486	2.2025	0.0648	9951
		Di. X GA	2	0.23437	0.35508	0.6982	9960
		Di. X Colony(Type)	9	0.66004	1.784	0.128	9941
	MA 1:1000	Disease State	1	10473	1.0474	0.3833	9621
		GA Type	2	10139	0.98551	0.4133	MC
		Colony(Type)	9	10288	5676.2	**0.0001**	9933
		Di. X GA	2	10169	1.017	0.4608	9943
		Di. X Colony(Type)	9	9998.7	5516.5	**0.0001**	9944
	TCBS 1:1	Disease State	1	0.39669	0.30992	0.5699	9851
		GA Type	2	1.9317	0.63773	0.5509	4607
		Colony(Type)	9	3.0291	7.1091	**0.0001**	9955
		Di. X GA	2	0.10014	7.82E-02	0.9261	9958
		Di. X Colony(Type)	9	1.28	3.0041	**0.0095**	9947
	TCBS 1:10	Disease State	1	0.4543	1.1489	0.3149	9836
		GA Type	2	0.76528	2.0857	0.1818	465
		Colony(Type)	9	0.36692	1.0618	0.4141	9926
		Di. X GA	2	0.23429	0.5925	0.5777	9955
		Di. X Colony(Type)	9	0.39542	1.1443	0.3647	9936
	TCBS 1:100	Disease State	1	0.1875	1	0.3524	4436
		GA Type	2	2.08E-02	0.15789	0.8569	MC
		Colony(Type)	9	0.13194	0.7037	0.7059	9864
		Di. X GA	2	0.1875	1	0.3948	3339
		Di. X Colony(Type)	9	0.1875	1	0.4584	9887
	TCBS 1:1000	Disease State	1	588	0.98817	0.3932	9385
		GA Type	2	591.52	0.99408	0.4033	MC
		Colony(Type)	9	595.04	1	0.4906	9908
		Di. X GA	2	598.56	1.0059	0.4596	8627
		Di. X Colony(Type)	9	595.04	1	0.5022	9920

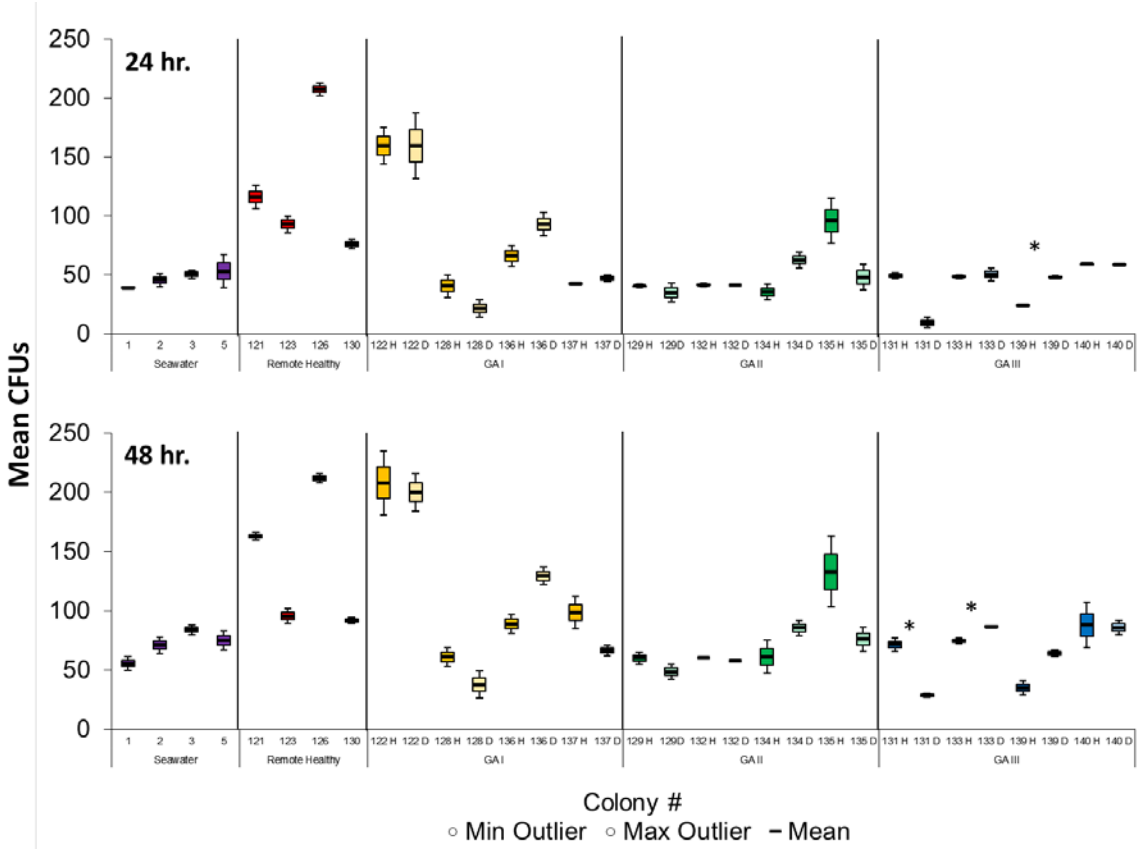

Figure 3.17. Box plots of mean CFUs on Marine Agar at full strength between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Asterisks (*) indicate a significant difference between healthy and diseased states ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

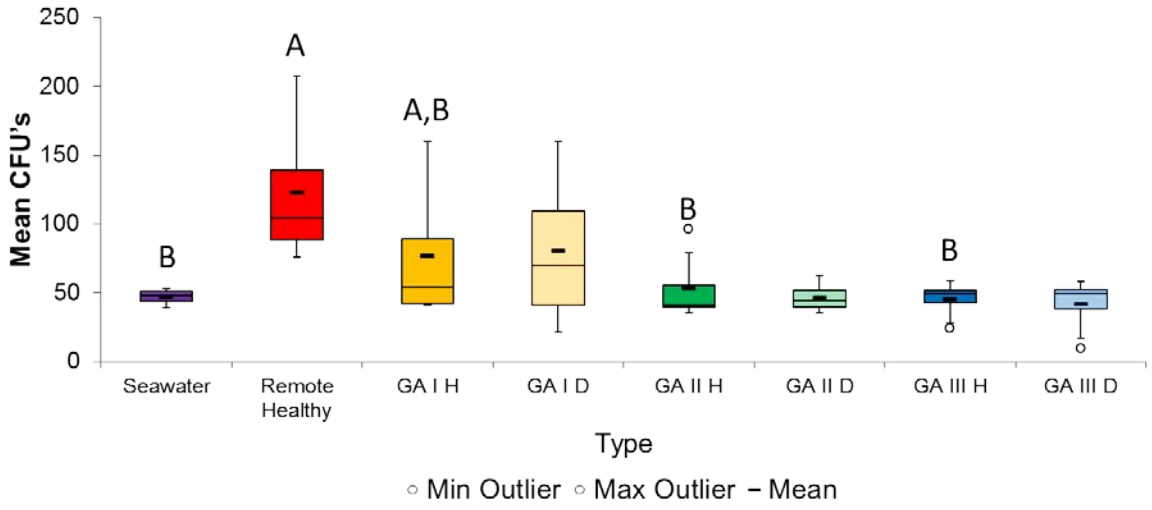

Figure 3.18. Box plots of mean CFUs on Marine Agar at full strength at 24 hours in Seawater, Remote Healthy, and healthy (H) and diseased (D) areas of GA Types. Capital letters indicate significant differences between Seawater, Remote Healthy, and healthy areas of GA Types ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

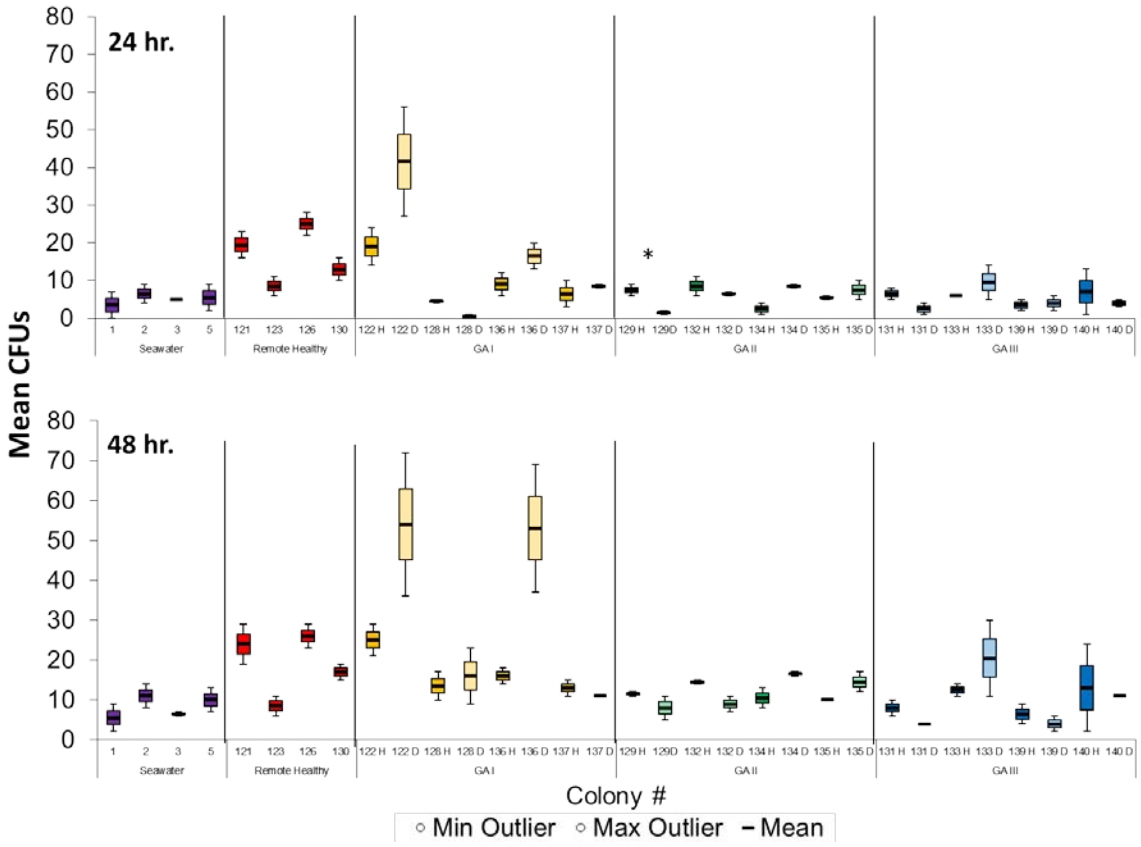

Figure 3.19. Box plots of mean CFUs on Marine Agar at the 1:10 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Asterisks (*) indicate a significant difference between healthy and diseased states ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

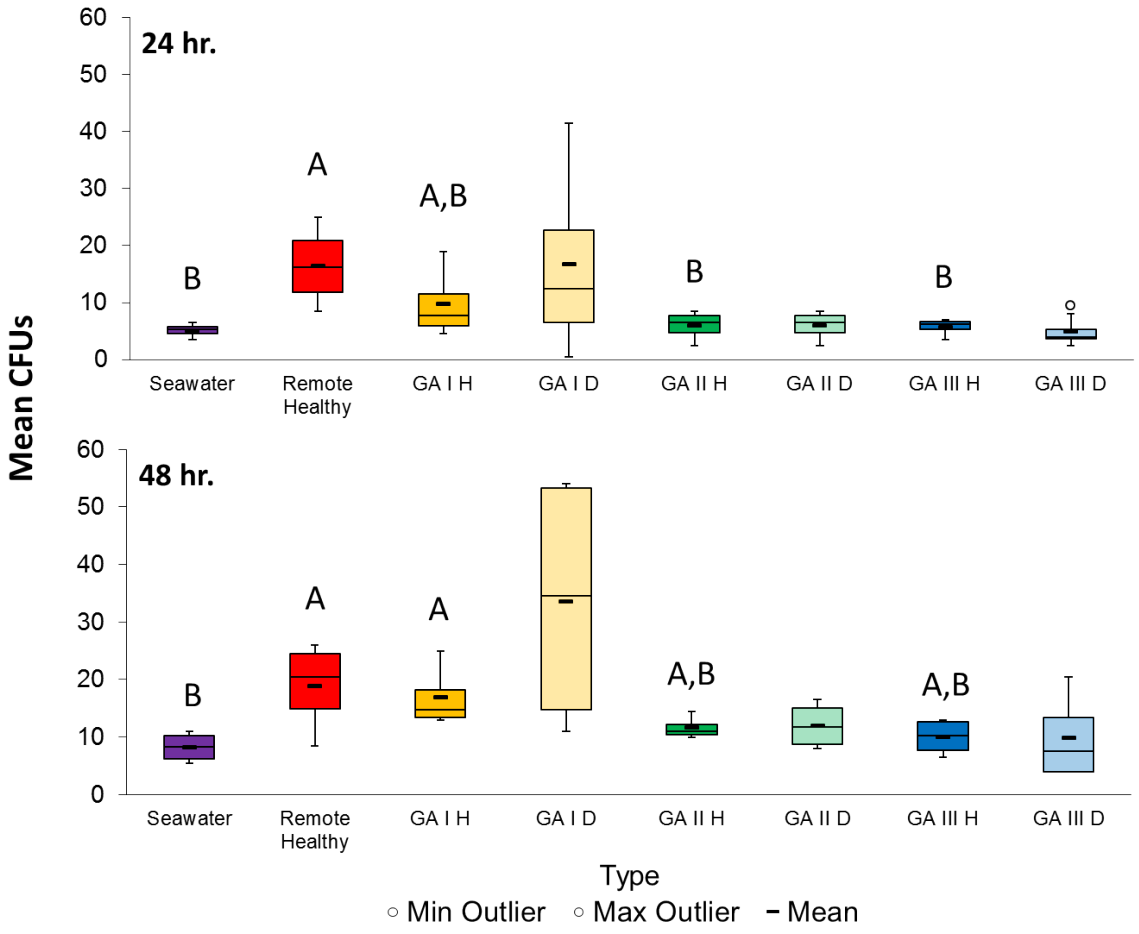

Figure 3.20. Box plots of mean CFUs on Marine Agar at the 1:10 dilution in Seawater, Remote Healthy, and healthy (H) and diseased (D) areas of GA Types at 24 & 48 hours. Capital letters indicate significant differences between Seawater, Remote Healthy, and healthy areas of GA Types ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

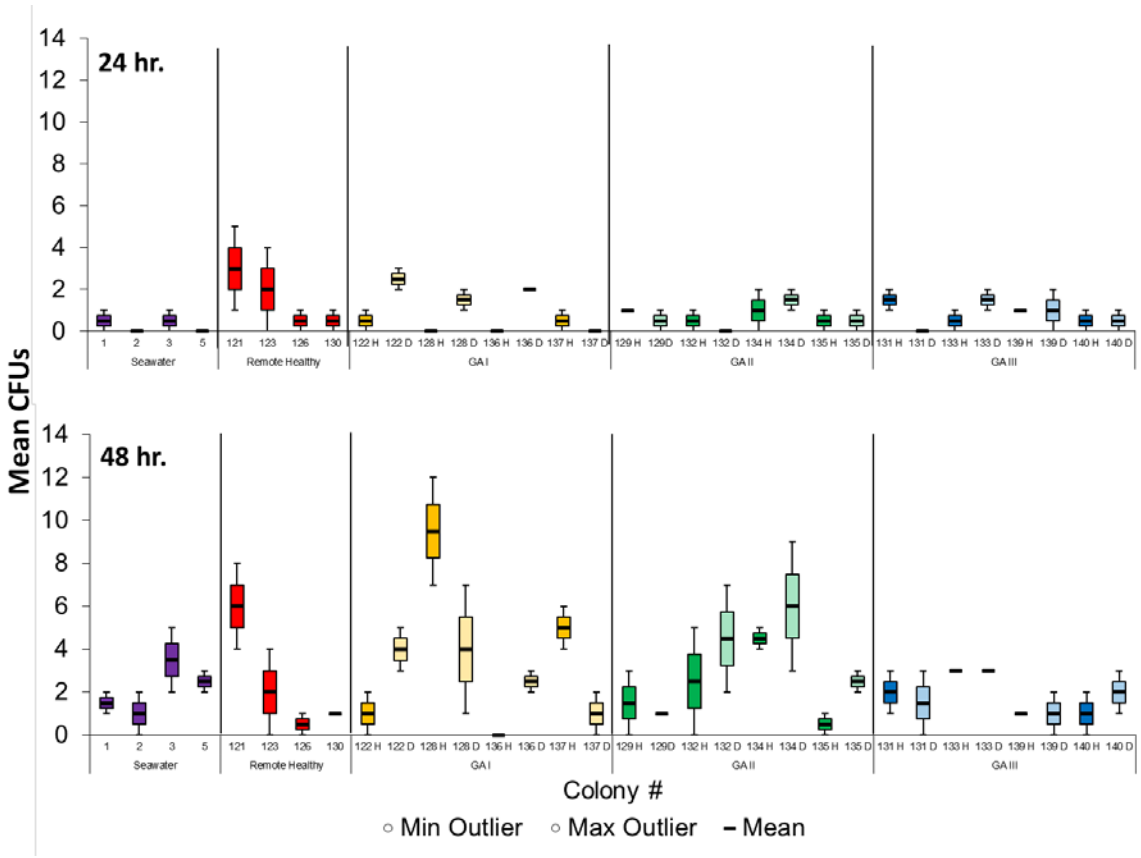

Figure 3.21. Box plots of mean CFUs on Marine Agar at the 1:100 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

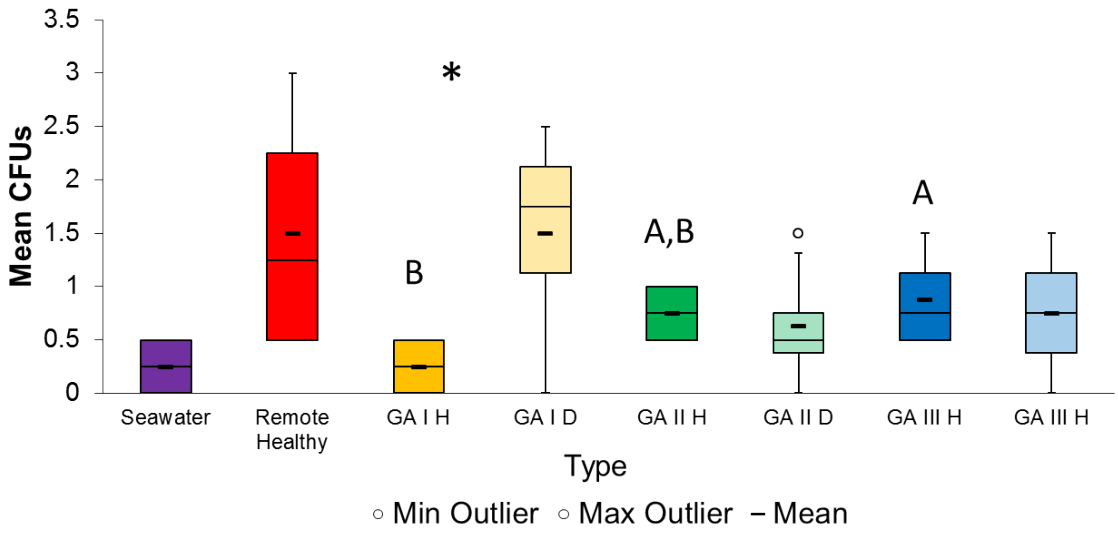

Figure 3.22. Box plots of mean CFUs on Marine Agar at the 1:100 dilution at 24 hours in Seawater, Remote Healthy, and healthy (H) and diseased (D) areas of GA Types. Capital letters indicate significant differences between healthy areas of GA Types, and asterisks (*) indicate significant differences between healthy and diseased areas of GA Types ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

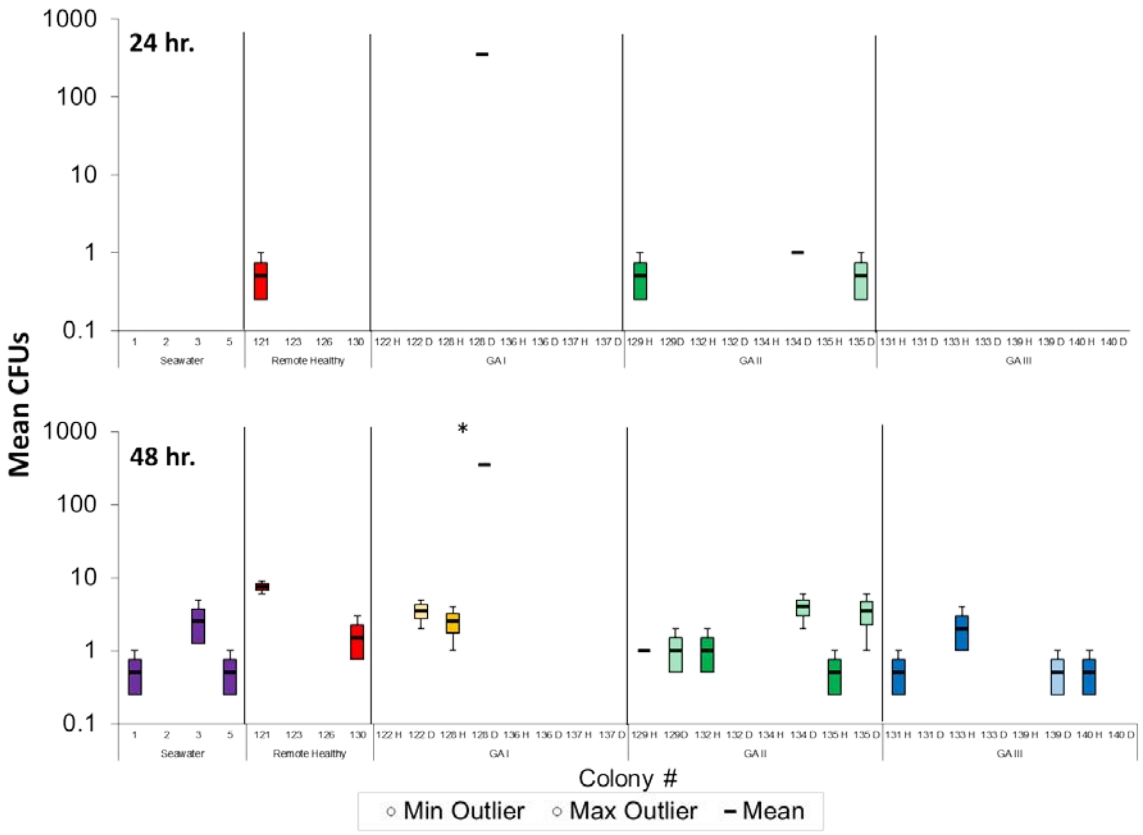

Figure 3.23. Box plots of mean CFUs on Marine Agar at the 1:1000 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Asterisks (*) indicate a significant difference between healthy and diseased states ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

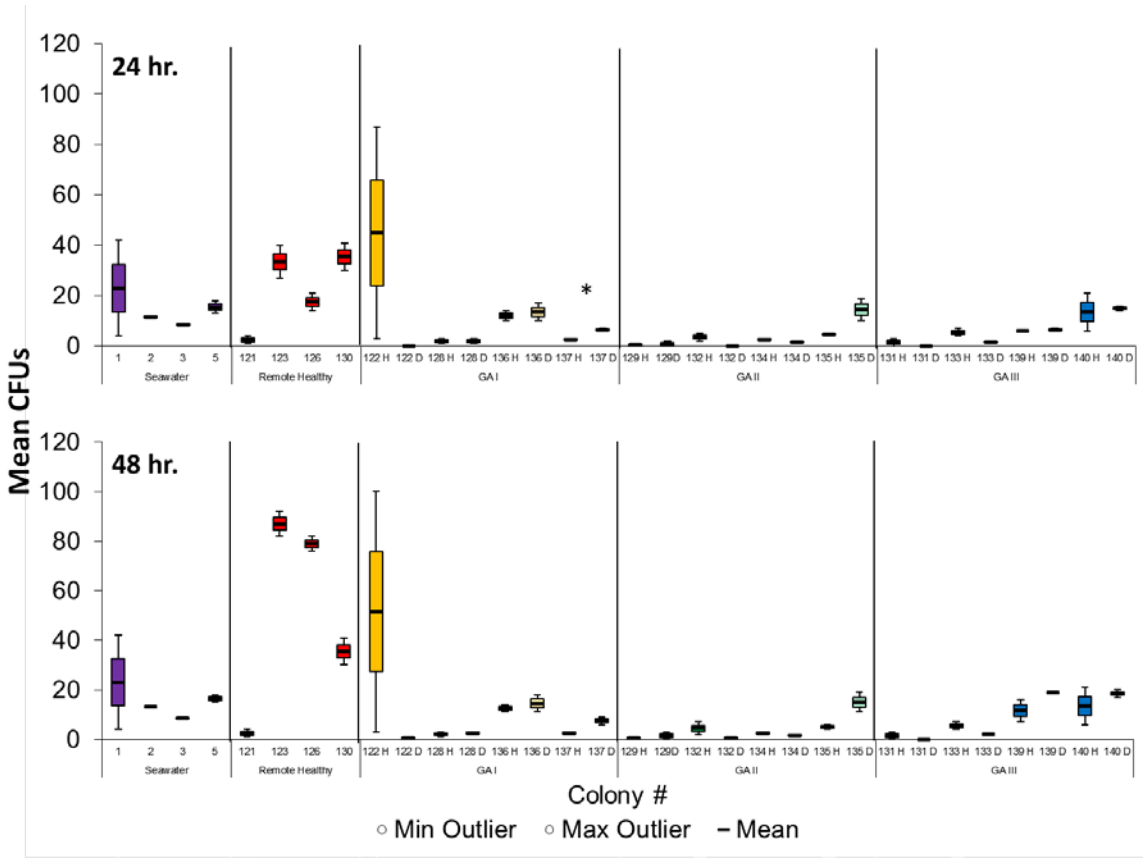

Figure 3.24. Box plots of mean CFUs on TCBS Agar at full strength between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Asterisks (*) indicate a significant difference between healthy and diseased states ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

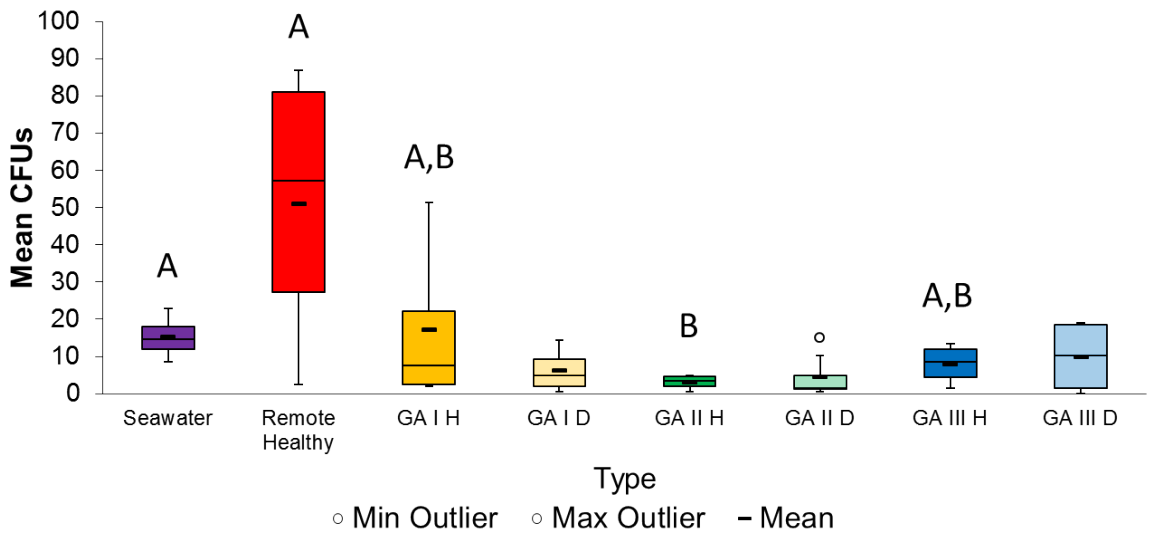

Figure 3.25. Box plots of mean CFUs on TCBS Agar at full strength at 48 hours in Seawater, Remote Healthy, and healthy (H) and diseased (D) areas of GA Types. Capital letters indicate significant differences between healthy areas of GA Types ($p < 0.05$). Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

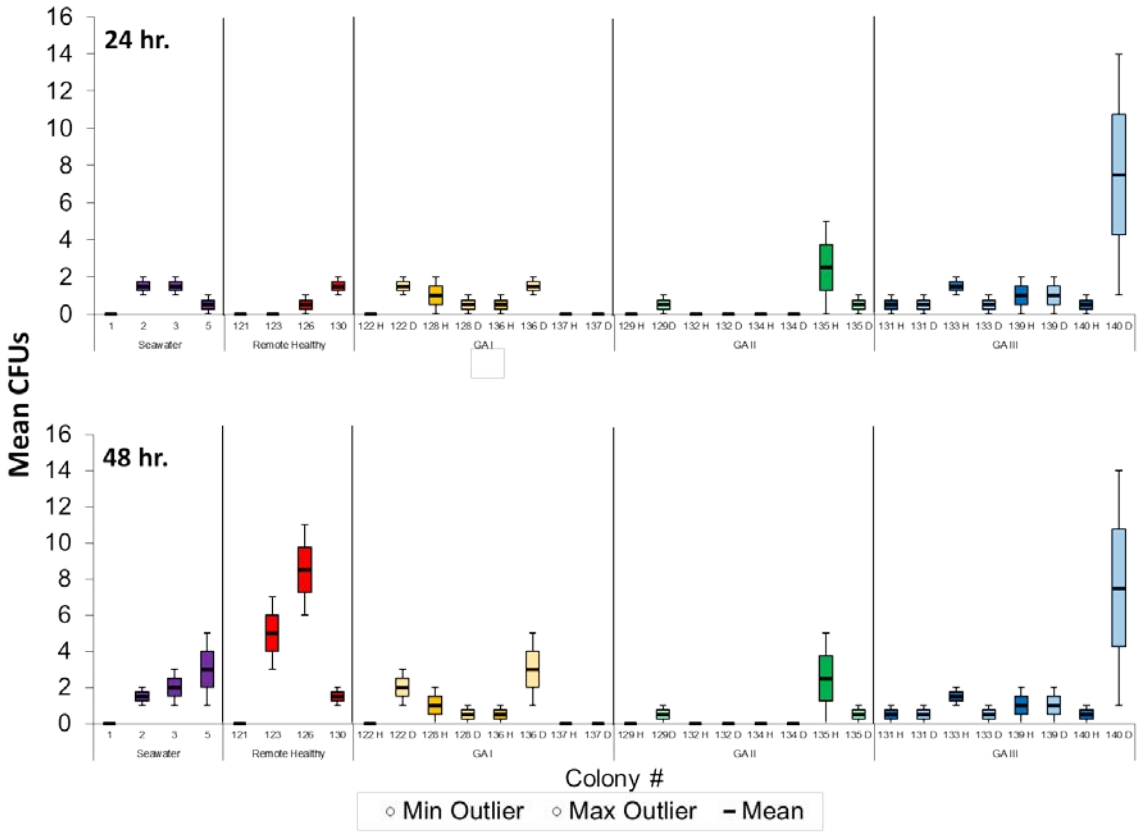

Figure 3.26. Box plots of mean CFUs on TCBS Agar at the 1:10 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

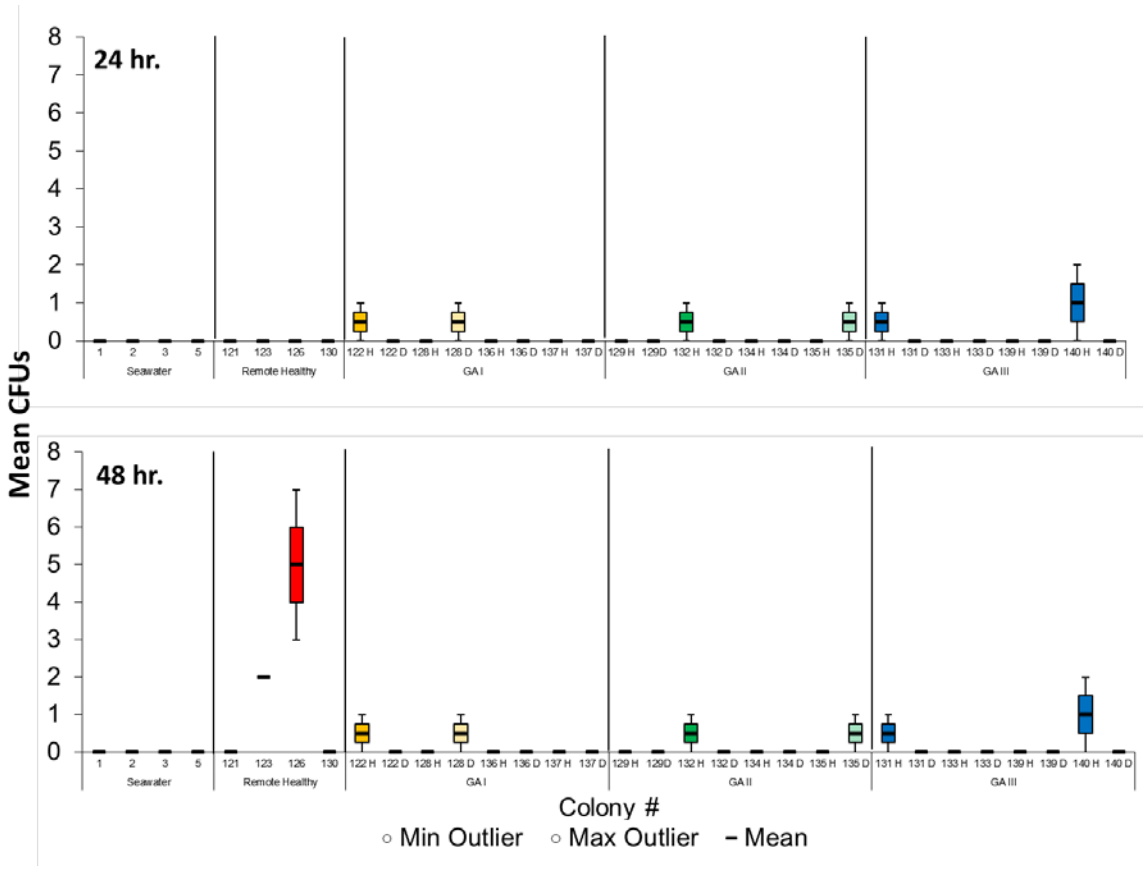

Figure 3.27. Box plots of mean CFUs on TCBS Agar at the 1:100 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

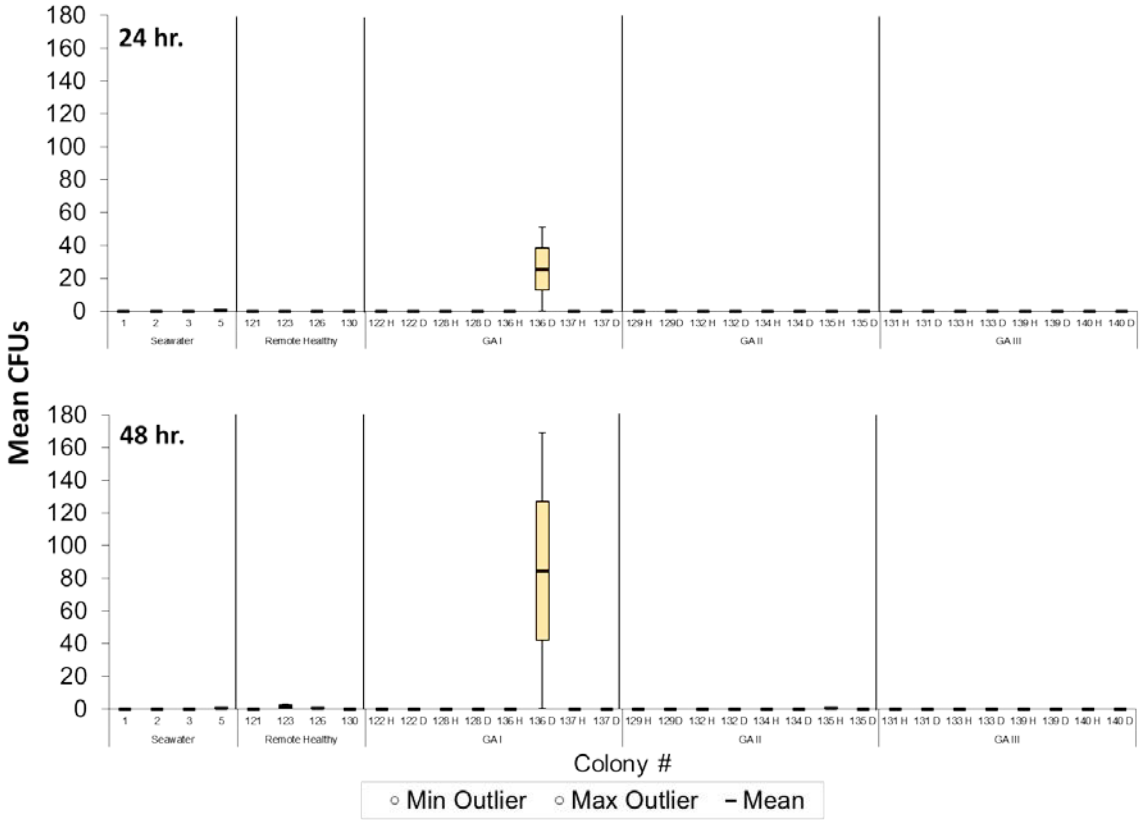

Figure 3.28. Box plots of mean CFUs on TCBS Agar at the 1:1000 dilution between seawater samples, remote healthy colonies, and healthy (H) and diseased (D) areas of colonies within GA Types at 24 and 48 hours. Plots display the median (thin horizontal line), mean (thick horizontal bar), the lowest datum still within 1.5 IQR of the lower quartile, the highest datum still within 1.5 IQR of the upper quartile, and minimum and maximum outliers (if present).

Table 3.8. Results of transmission experiment after 60 days. Numbers marked with an asterisk (*) also had algal overgrowth (quantified in last column).

	100% Mortality	**Partial Mortality**	**Bleaching**	**small dead/bleached patches where touching**	*** Algal overgrowth**
Healthy Colony	1 *	3 *	1	10	4
Control Chip	---	1	---	4	---
GA Type I Chip	2 *	1 *	2	---	3
GA Type II Chip	2 (*1)	2 *	---	1	3

4: DISCUSSION

The lack of understanding of coral disease dynamics and etiology prevents us from being able to predict and plan for future changed in reef-diversity and community-structure. Given the gaps in information regarding coral diseases, a first step is defining and describing diseases on ecosystem, species specific, and colony scales. Since coral diseases affect multiple species both in different ways but can also have similar impacts across species, it is important to characterize their effects on individual species before making broad statements of their effects on scleractinians as a whole. In this study, I examined several characteristics of growth anomalies on massive *Porites* spp. in Guam. These results will be a valuable contribution to the understanding of the overall effects GAs have on all corals.

Growth anomalies on massive *Porites* spp. on Guam are comprised of three visually distinct morphological types—Types I, II, and III. In this study I looked at how, overall, GAs were affecting their host coral and if individual GA types were affecting their host coral differentially. Due to small sample sizes and high inherent inter-colony variation, it was difficult to determine definitively if the three GA types did significantly affect their host coral differently. However, patterns did emerge when looking at individual colonies and even when colonies were combined within types, strongly suggesting differences did exist. Type I GAs, overall, had the least effect on their host coral. Type II GAs had the most consistent results between health states within colonies and seemed to affect the host most dramatically. Type III GAs had slightly more

significant effects on their coral hosts than Type I but fewer effects than Type II. However, these observed differences were not statistically significant.

The evidence from this study shows that impacts are variable between colonies and between GA types. Some authors have pooled morphologically different GA types across different species to assess differences between GAs and normal tissue for a specific genus (Work et al. 2008). In my study, the goal was to see if the GA types affected their hosts differently within a single species, and my results suggest that while individual GA types may not be affecting their host colony significantly differently, the amount of variability within one species suggests that GAs should be analyzed at a species level to differentiate effects.

Of the GAs monitored for growth, all GAs grew over the study period and the majority grew less than 10 cm over the 15-month period. This suggests GAs grow slowly and may have persistent effects on their host colony. There were also significant differences between GA types and type II grew less than types I and III over the study period. Three colonies which were monitored for GA growth *in situ* had multiple GAs on one colony. Type III GAs were never encountered on the same colony as another GA type within this study. I did not find evidence that the three types transition from one type to another. However, in the field I have observed type I and II GA tissue connected on a massive *Porites* colony (pers. obs.; Figure 4.1). This suggests successive developmental process from one GA type to another. While other studies have identified morphologically different GA types (Gateño et al. 2003, Work et al. 2008, Kaczmarsky et al. 2009), and Kaczmarsky (2009) has noted the appearance of more than

one type of GA on a colony, no study has described connections between GA types. It would be valuable to investigate the developmental process of GAs to see if they develop separately or successively.

For those corals monitored for growth, effect of sites is interesting given that Type I GAs did not differ, while GA II growth did. The two sites, Luminao and Ipan, are on opposite sides of the island. Luminao is on the western leeward side of the island, just outside of the harbor, and can be from 0.5 to 3 m deep in some places. The fringing reef flat in Ipan is on the eastern windward side of the island, has a river channel through it, but otherwise is quite shallow (0.5 – 2 m deep). The Togcha River receives sewage effluent from the Baza Gardens sewage treatment plant. It is unknown what the nutrient enrichment is at Ipan, and the chronically enriched water in Ipan has unknown effects on coral health or on the growth of Type II GAs. Aeby et al. (2011) and Kaczmarsky (2006) have both found GAs to be more prevalent around areas of high human population and higher nutrient loads, respectively. Coral reefs adjacent to areas of high human population commonly possess higher nutrient loads, which can result in reduction of coral reproduction, coral mortality (Koop et al. 2001), and exacerbation of disease severity (Bruno et al. 2003). Given that Raymundo et al. (2011) found sewage N sources in Guam coastal waters, I speculate that poorer water quality in Ipan may have a deleterious impact on coral health leading to the formation of GAs.

Certain skeletal morphometric characteristics were significantly different between health states of colonies within GA types. Corallite size was different between healthy and diseased areas of GAs II and III (Figure A.3, A.4, A.5) and mean numbers of

septae (Figure A.8) and palli (Figure A.9) were different between healthy and diseased areas of GA III. Previous studies of growth anomalies have found that there are fewer corallites per surface area, larger corallites, and more and abnormally arranged septae within corallites of diseased areas (Squires 1965, Hunter & Peters 1993, Yamashiro et al. 2000, Domart-Coulon et al. 2006, Kaczmarsky 2006, Work et al. 2008, McClanahan et al. 2009). The findings in my study are consistent with the findings in these studies. Given that individual polyps secrete the skeleton underneath them, polyp structure and function are in close relation to corallite structure. This implies that corallites with different skeletal structure than normal are produced by polyps that may not be functioning within normal parameters. Cheney (1975) found that specimens of *Madrepora kauaiensis* had larger corallites and an abnormal arrangement of septae. While he was not able to perform histological analysis, he posited that the arrangement of skeletal features within GAs suggests that mesentery formation was disordered, leading to a disordered septal arrangement. Subsequent studies have shown disordered mesenteries or missing mesenterial filaments (which are used for defense) within GAs (Peters et al. 1986, Yamashiro et al. 2000, Work & Rameyer 2005, Work et al. 2008, Williams et al. 2010). Since gonads develop along mesenteries, disordered mesenteries or missing filaments is expected to have an adverse effect on the number, presence, or function of gonads or defensive capacity of the polyp. Yamashiro et al. (2000) and Domart-Coulon et al. (2006) have found that gonads were only partially developed in GA tissues and subsequently lowered fecundity within the GA. Given that differences in corallite structure are most often present in types II and III GAs, this suggests that these

GAs may have a negative effect on host fecundity, especially if these GA areas are large. If the differences within GAs extend to areas larger than that delineated by the GA, this has the possibility to reduce fecundity of the whole colony.

My study demonstrated that massive *Porites* display high inherent variability in a number of characters related to their structure and function. Given that distance between corallites and numbers of septae and palli are analyzed when trying to distinguish species, variability in these characteristics may confound taxonomic work since the characters used for species ID are affected by GAs. These results may be explained by phenotypic plasticity seen in other poritids (Brakel 1977, Forsman et al. 2009) and other species of corals (Klaus et al. 2007). Variability in skeletal morphology, even within a species, often makes it difficult to identify species calling into question the use of traditional morpho-taxonomic descriptions. Forsman et al. (2009) discuss the plasticity of morphology in *Porites* corals and suggests that the species *Porites lutea* can be separated into three genetically divergent groups. It is known that coral skeletal morphology can respond to a variety of environmental parameters (Veron 2000, Klaus et al. 2007, Todd 2008) which can make proper identification of species challenging. Forsman et al. (2009) also found that mounding and branching morphospecies of *Porites* corals were genetically indistinguishable, and that corallite-level characters, such as number and size of palli and free or fused triplets, were highly variable. However, specimens that appeared to match the morphological species description of *Porites lutea* were sorted into several clades that were deeply genetically divergent. Given that my samples were morphologically identified as *Porites lutea* and large variability was

seen between samples, there is a possibility of these samples being different morphospecies which may warrant genetic analyses of Guam poritids. This also brings into question what characters are good to use, both when taxonomically identifying species and when studying disease impacts to coral. The results of my study suggest that corallite size, corallite density, and the number of septae are good morphological characters to analyze in description of GA impacts to their host.

Zooxanthellae provide energy, remove metabolic wastes, recycle nutrients for the coral host (Wang & Douglas 1998), and enhance coral calcification (Muller-Parker & D'Elia 1997). When zooxanthellae densities are reduced due to bleaching, coral tissue biomass and reproductive abilities are negatively affected (Szmant & Gassman 1990) and if colonies do not regain normal zooxanthellae densities within a certain time, the colonies can die. This process of bleaching is known to occur due to a variety of stressors, such as increased temperature, high solar irradiance, presence of disease, and presence of bacterial pathogens and pollutants (Brown 1997, Brown et al. 2000, Bruno et al. 2007, Douglas 2003, Glynn 1996, Jones 2004, Vidal-Dupiol et al. 2009). The presence of skeletal growth anomalies has also been shown reduce zooxanthellae to few or none within affected tissues (Cheney 1975, Peters et al. 1986, Yamashiro et al. 2000, Gateño et al. 2003, Domart-Coulon et al. 2006, Work et al. 2008, Work & Rameyer 2005, McClanahan et al. 2009, Williams et al. 2010). The GA-affected tissues in these studies were described as being white or having little coloration (pale). In the present study, mean zooxanthellae densities were not significantly different between healthy and diseased areas of any GA type (Figure 3.4). Type II GAs, however, did have reduced

zooxanthellae densities in both diseased and healthy areas in comparison with Type I GAs. While Type II GAs tended to have a visual reduction and/or difference in pigmentation compared to healthy areas and to GAs I and III, some pigmentation was always present, in contrast to the plaque-type GA seen elsewhere.

Since zooxanthellae enhance coral calcification (Muller-Parker & D'Elia 1997), it is interesting that GA II had fewer zooxanthellae in both healthy and diseased areas than GA I. Type II GAs have been observed in the field to have a reduction in skeletal density as GA areas are easily crushed when small amounts of pressure are applied. I did not observe this on either GA I or III. Several studies (Cheney 1975, Peters et al. 1986, Yamashiro eta l. 2000, Gateño et al. 2003, Domart-Coulon et al. 2006) have found a reduction in skeletal density within GA areas. My results suggest that GA type II negatively impacts the coral via a reduction in zooxanthellae densities with a possible effect on calcification. However, low skeletal density can be the result of several different processes (i.e. less $CaCO_3$ laid, very rapid secretion, etc.) and it would be useful to investigate this relationship further. The other GA types did not appear to have this effect. GAs I and III were only slightly paler than healthy areas. Loya et al. (1984) found that zooxanthellae densities in GA areas of *Platygyra* colonies were similar to densities within healthy areas, consistent with my findings for all types. Also, Peters et al. (1986), while finding no zooxanthellae in their samples of *Acropora palmata* from Key Largo, FL, found other "tumors" from *Acropora cervicornis* from Jamaica revealed abundant zooxanthellae in their tissue. And while Williams et al. (2010) found a depletion of zooxanthellae in GAs, this was only true for 31% of the samples and it was not

determined if this was different from normal tissues. In my study I found fewer zooxanthellae in ~41% of my samples (Figure 3.4. one significant difference, five overall differences in mean densities); however when all samples were combined, there was no significant effect of GAs on zooxanthellae densities. These contrasting results suggest that not all GA morphotypes display a reduction in zooxanthellae density.

Tissue thickness, varied between health states of all GAs though the differences were not significant (Figure A.10). Previous studies have found that tissue thickness (Peters et al. 1986, Coles & Seapy 1998) and dried tissue weight (Yamashiro et al. 2000) is reduced in GA areas. My study suggests a reduction of tissue thickness in GA areas in types I and III GAs which a larger sample size may have been able to demonstrate. Reduced tissue thickness can negatively affect polyp structures; Peters et al. (1986) found that nematocysts and mucous secretory cells were few or absent from the gastrodermis of GA-affected polyps. This suggests that GAs affect the coral's ability to defend itself and produce a mucus sheet which acts as a defense against a wide range of environmental stresses (Brown & Blythell 2005), potentially weakening its ability to resist infection and increasing its likelihood of at least partial mortality. This has been seen in an observed increase in fish predation bites focused within GAs resulting in partial mortaliy (pers. obs.). In contrast, greater tissue thicknesses of type II GAs than healthy areas of the same colony present new insight into GA type dynamics.

Of GAs monitored for growth, 16 experienced some partial mortality and one experienced predation. Of those GAs with partial mortality, eight (5 type I, 2 type II, 1 type III) showed partial mortality solely within the GA. Partial mortality seen within the

GA may or may not have been caused by the GA and along with tissue loss, filamentous algal invasion of central areas has been found within GA areas (Cheney 1975, Peters et al. 1986, Domart-Coulon 2006,). It is still unclear, however, if this loss is due to the presence of the GA or from other factors such as fish predation (Cheney 1975, Bak 1983, Peters et al. 1986, Coles & Seapy 1998, Domart-Coulon 2006, Kaczmarsky 2006, Work et al. 2008). Takabayashi et al. (2008) found that in some colonies the GA occupied >90% of the entire colony which then experienced full mortality within a year of monitoring. GAs in my study started to experience partial mortality after the 5^{th} month of monitoring, however none experienced full mortality by the end of the 15 month monitoring period and none occupied over 25% of the colony. This suggests that if partial mortality is due to GA presence it can develop slowly and affect the colony over a long period of time.

Reduction in colony size due to partial mortality or fragmentation of tissue also has negative implications to the reproductive ability of corals. Szmant-Froehlich (1985) found that colonies of *Montastrea annularis* smaller than 83 cm^2 (surface area) were not fully reproductive. These occurred whether the colonies were young or were generated from fragments of older colonies. Kojis & Quinn (1984) also found that colony size primarily determines whether a colony will reproduce (i.e. a minimum size is necessary for genetic production). Therefore, regardless of why areas of GAs experience tissue loss, tissue loss can reduce colony size below that which can support reproduction. This has a two-fold negative impact in that tissue loss automatically reduces reproductive output and then fragmentation of tissues into smaller areas again reduces reproductive

output. The effects of tissue fragmentation on fecundity within GAs should be an area to look at when monitoring GA growth.

Bacterial communities in the coral surface mucopolysaccharide layer (SML) are known to be an important component of coral health (Ritchie & Smith 2004, Mao-Jones 2010). When corals are stressed, these communities can change in number and species composition (Ritchie 2006, Gil-Agudelo et al. 2006, Gil-Agudelo et al. 2007). Since it is known that coral SML has a greater concentration of microbes than the surrounding seawater (Ducklow & Mitchell 1979, Ritchie & Smith 1995, Rohwer et al. 2001, Ritchie & Smith 2004, Gil-Agudelo et al. 2006), it is interesting to find in my study that the number of microbes on healthy areas of GA-affected colonies were generally significantly less than remote healthy colonies and not significantly different from seawater samples. Also, healthy and diseased areas were generally not significantly different within GA types. This suggests one possible effect of GAs that extends beyond the immediate area of the GAs. Also, perhaps microbes associated with diseased coral mucus are in greater abundance, but are not culturable. Breitbart et al. (2005) found microbial growth rates of tissue extracts from both healthy and diseased areas were faster than remote healthy colonies. This study, along with Breitbart's, suggests a colony-wide effect of GAs on the SML. Gil-Agudelo et al. (2006) also found more differences between remote healthy colonies and diseased areas of colonies, than between diseased areas and healthy areas of the same colony of *Gorgonia ventalina*. The authors found that the communities living on the healthy and diseased areas of diseased colonies were very similar. This compliments the results that I found. It is also interesting that combined remote

healthy areas had greater mean CFUs of bacteria than almost all other samples. Given the amount of literature which supports an increase in microbiological activity (i.e. numbers of bacterial CFUs) when corals undergo stress, it could be assumed that I would find greater mean CFUs in GA samples. Remote healthy colonies also had the highest *Vibrio* spp. counts out of all samples. This seems quite odd, given that *Vibrio* spp. are more often associated with stressed and diseased corals. However, *Vibrio* spp. are also the most common marine genus of microbes and many are undoubtedly useful components of coral health. Domart-Coulon (2006) found bacterial aggregates only in the upper polyp areas of GA tissue, and not in the calicoblastic epithelium, as is the case in normal *Porites* tissue. This does not support the role of a bacterial infectious agent in GA formation. If a bacterial infectious agent is to be implicated in GA formation, genetic identification of the bacterial communities within each SML is needed to determine if any isolates could be implicated in disease causation.

Another reason why there may be such marked differences between bacterial samples was to the amount of time between sampling and plating. Breitbart et al. (2005) suggests the amount of time between collecting the mucus and starting the experiments can significantly alter the results. In my experiment, there was a lag time of up to 7 hours between when mucus samples were collected, and when they were plated out onto agar media, due to the large amount of samples processed. Breitbart et al. (2005) notes that even a lag of 2 h between collection and experiment start can lead to dramatic differences in microbial production rates which will be reflected in CFU counts. The lag can also potentially affect microbial growth and survival.

An important consideration for my results is the experimental design. The high amount of variability within certain characters suggests a need to increase the sample size. It was hard to determine true significant differences between diseased and healthy areas due to small sample size; however, the results were intriguing and suggested patterns to the effects GAs have on the host colony and also differences between how GA types affect the colony. In order to tease out significant differences there is much which needs more detailed work.

GA causation is another avenue needing more assessment. Those GAs described in the literature have many suggested causative agents including UV radiation (Peters et al. 1986), UVB absorption (Coles & Seapy 1998), a pathogenic microorganism (Breitbart 2005, Kaczmarsky & Richardson 2007), combinations of environmental stress and injury (Loya 1984), and nutrient enrichment (Kaczmarsky 2006, Aeby et al. 2011). It is also unclear whether or not the different morphological types have different etiologies. Work et al. (2008) found that five out of the seven morphologically distinct GAs had a consistent pattern of microscopic morphology, which may suggest a common causative agent.

Given that the two studies which have looked at GA transmission on massive *Porites* show conflicting results, the infectiousness (or lack thereof) is still not established and the presence of a pathogenic microorganism as a causative agent is not supported. However, given the short transmission time in my study (~10 weeks) these results do not eliminate the possibility of transmission and also imply that GAs develop slowly. Kaczmarsky (2006) found that the rate of spread for “tumors” was slow, which

supports the finding of my study in GA growth. If this is the case, it will take longer transmission, or a vector or mechanical injury may be required for transmission, so an extended transmission experiment is suggested.

The presence of viral constituents which can cause GA formation is also a new avenue being pursued in GA pathogenesis. Previous work done by Davy & Patten (2007) has assessed the presence of viral-like particles (VLPs) in the corals SML. They found that most of the VLPs were specific to the SML micro-niche and that there was not a lot of VLP transfer between the water column and the SML. Kaczmarsky (2009) found an abundance of VLPs in GAs from massive *Porites* spp. from the Philippines. More studies (Couch pers. comm.) will be looking at the presence of VLPs within coral tissue and looking at which tissue layers or particles they affect. Along with viral pathogenesis, genetic mutation and gene expression in GAs is another area which will be studied in GA initiation/formation (Couch pers. comm.).

Future Directions

GAs appear to be a chronic, rather acute stress to corals, but not one which leads to high mortality. My results suggest diverse effects of the three GA types on the host colony, making it important to describe gross morphology of lesions in addition to their pathology and etiology. Type I GAs had the least overall effect on their host colonies. There were few differences between healthy and diseased areas within GA I among the ten physiological parameters explored. There was moderate between-colony variation for healthy areas among almost all characters (except for mean # of septa and palli). Work and Aeby (2006), Work et al. (2008), and Williams et al. (2010) stress the

importance of systematically describing lesions and naming diseases to eliminate ambiguity in disease descriptions. They provide a list of terms to help in lesion description and morphologic diagnosis that are useful when coming across a novel affliction. They divide *Acropora* spp. GA types into morphology-based sub-types, as I have done in this study. If I had initially aggregated the GAs, the increased variation would have prevented me from seeing the observed patterns in diseased versus healthy areas. To further develop this idea of differing effects of different GA sub-types on their host corals, a larger sample size is required to see significant results. Perhaps since GA Type III are less common, they should be left out of subsequent studies with larger sample sizes.

Two areas that had been analyzed in this study should be reassessed – lipid content, Chl A & C absorbance – and skeletal density should be added to the list of analyses. Lipid content was not analyzed due to the contamination of samples in two separate trials. Lipids in corals are used for respiration as well as for the building blocks of cell membranes (Benson & Muscatine 1974, Benson & Lee 1975, Stimson 1990). Yamashiro et al. (2001) found a depletion of storage lipid in GA tissue as compared to normal tissue. The authors suggested the depletion may be explained by an increase in energy demand in the coral tissue for tumor synthesis. Since this implies that polyps in GAs may have a reduced capacity to respire and form cell membranes, it is important to quantify lipid content within GA and healthy areas for massive *Porites* spp. on Guam. Chlorophyll a & c absorbance were hard to quantify and compare due to a low sample size, so this analysis should be performed again with a larger sample size. Skeletal

density, was not quantitatively assessed by this study, but field observations showed it to be reduced in GAs of type II. This suggests a quantitative analysis of skeletal density within GAs on massive *Porites* spp. should be conducted to validate these observations.

While this information is valuable, there is still more which needs to be understood. One intriguing area is the effect of poor water quality (i.e. an increase in nutrients). In this study, colonies at the Ipan site were subjected to chronic exposure of sewage from a housing development up river. During times of intense rainfall, the Togcha River channel becomes overwhelmed with runoff from terrestrial anthropogenic sources, providing sewage and other harmful substances to come in contact with the coral reef substrate. It is not yet understood how an increase in nutrients or terrestrial runoff can affect (i.e. stress to host, delivery of pathogen, genetic trigger, etc.) GA prevalence or progression, however some suggest that GA prevalence is correlated with proximity to anthropogenic sources of pollution and human population size (Kaczmarsky 2006, Aeby et al. 2006, Work et al. 2008, Aeby et al. 2011).

In regards to the bacterial community associated with GAs, a culture-independent approach may be much more lucrative in establishing differences between healthy and diseased areas and possibly even between types. Culture-independent techniques are able to provide much more information than culture-based methods since it is well known that the majority of microbes cannon be cultured (Fuhrman & Campbell 1998). Rohwer et al (2001) found no common 16S rDNA sequences between cultured isolates and 16S rDNA sequences obtained without culturing. These methods

produced dramatically different SML bacterial profiles for the same coral, speaking to the importance of combining techniques.

Histological examination of GAs on massive *Porites* spp. also needs to be performed to understand possible effects GAs have on colony reproduction. Yamashiro et al. (2000) found that *Montipora informis* colonies possessing "tumors" had a significant reduction in fecundity. Domart-Coulon et al. (2006) found only partially developed gonads in anomalous tissue and a significant reduction in gonad diameter compared to healthy tissue and there were fewer gonads in GAs from *Acropora* spp. from the Indo-Pacific (Work et al. 2008). Given that coral recruitment on Guam is low (Minton & Lundgren 2006) and that *Porites* spp. are dominant reef-builders here (Myers & Raymundo 2009), the possibility of GAs negatively affecting reproduction of massive *Porites* spp. has implications for the future of Guam's reefs. This information, combined with my study results, is critical to the understanding of disease dynamics of a not fully understood diseased in a relatively understudied area of the world.

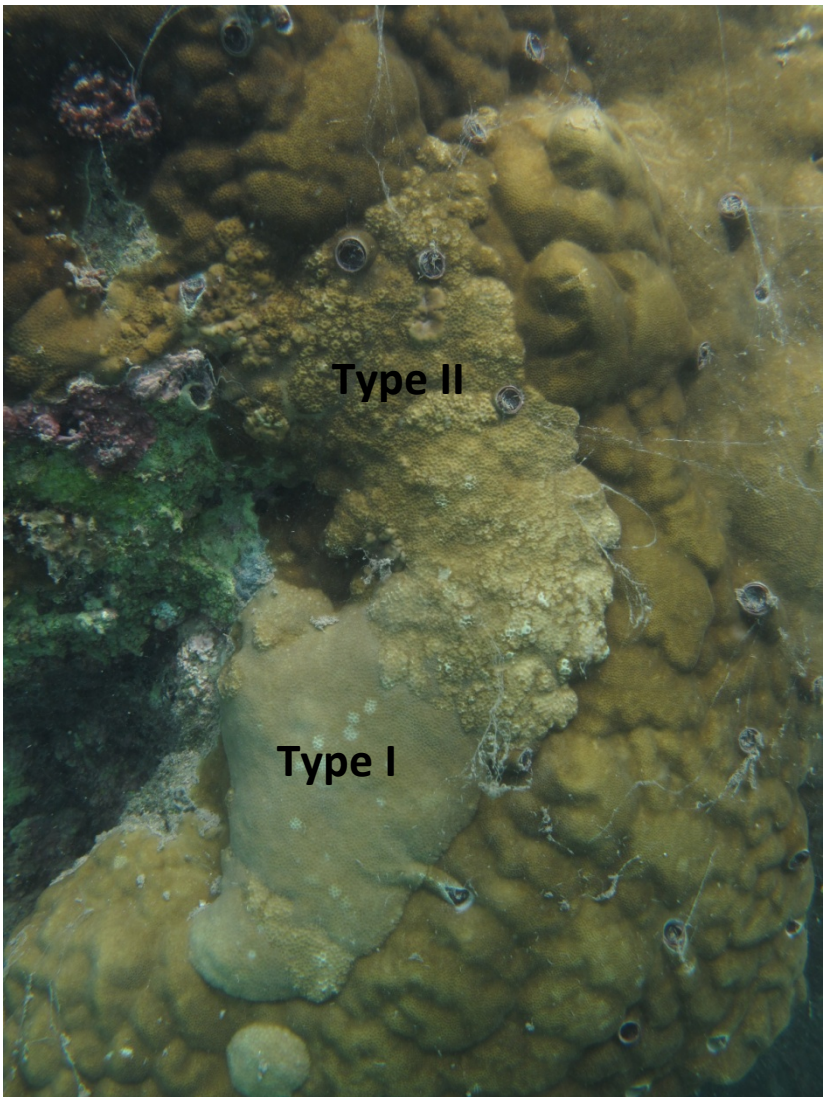

Figure 4.1. Photograph showing the presence of Type I and Type II GAs with tissue connection.

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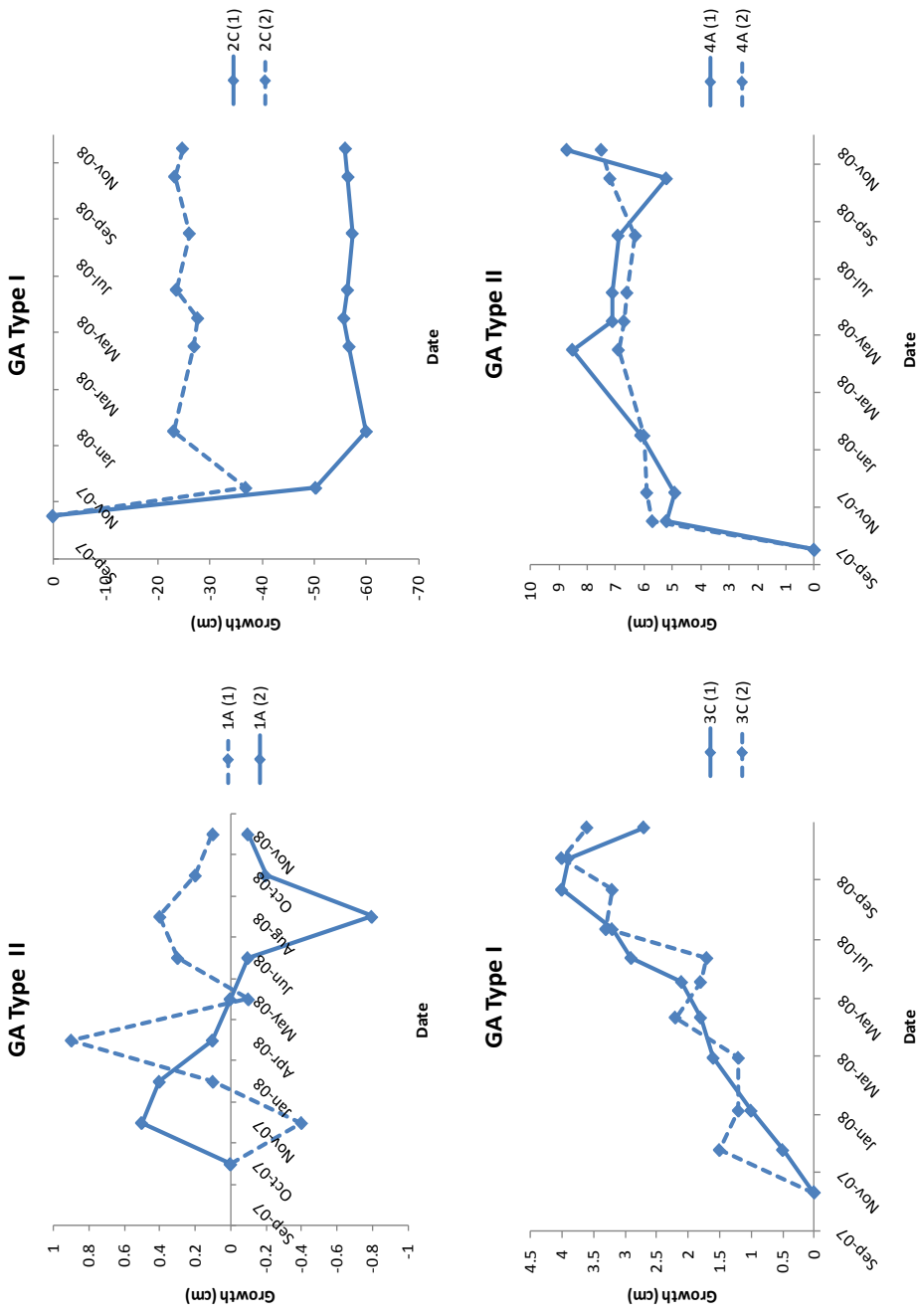

Figure A.1a. Growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

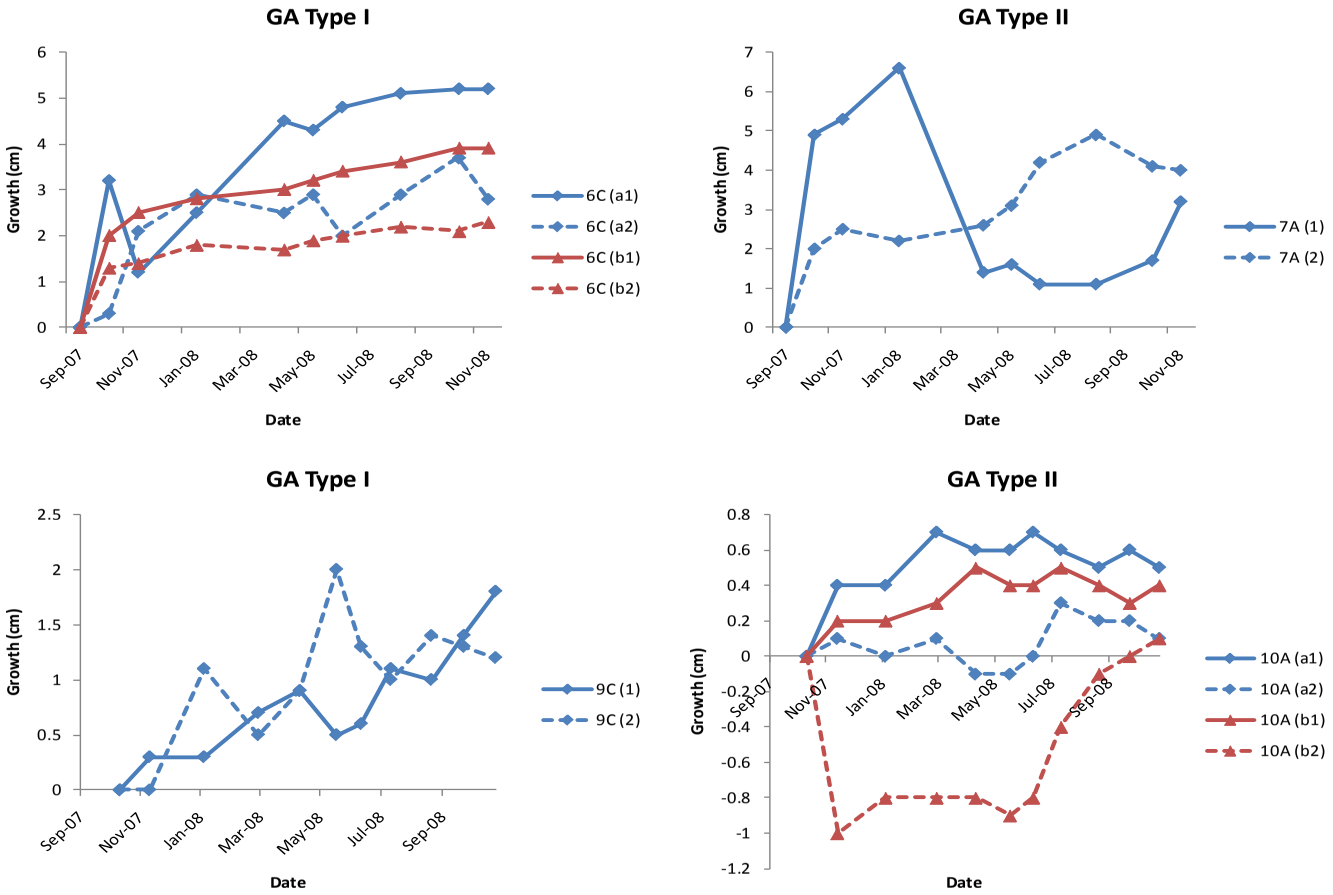

Figure A.1.b. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum). Lowercase letters in front of the numbers in parentheses (i.e. a1, b1) indicate specific measurements for each GA on the colony if there was more than 1 per colony.

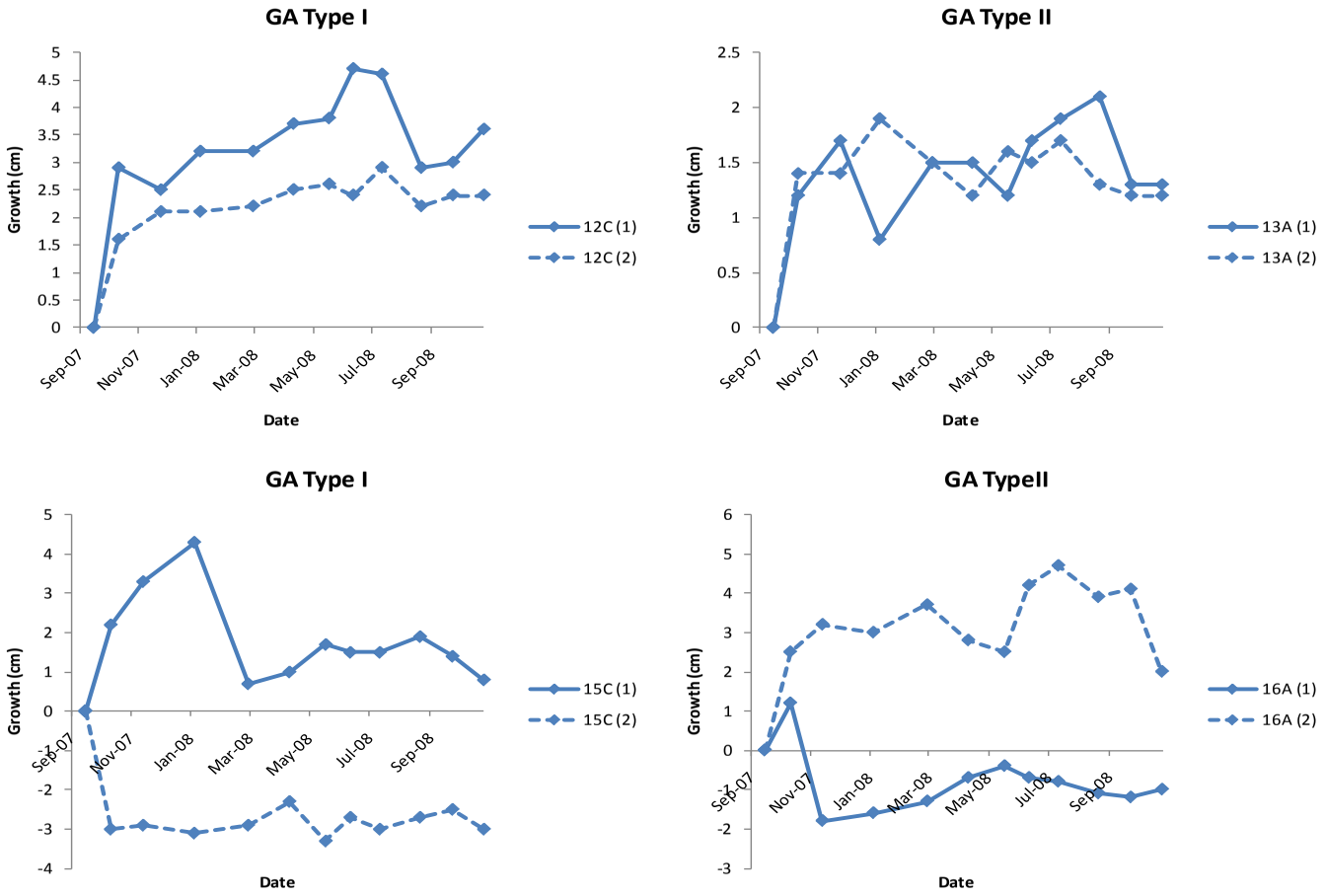

Figure A.1c. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

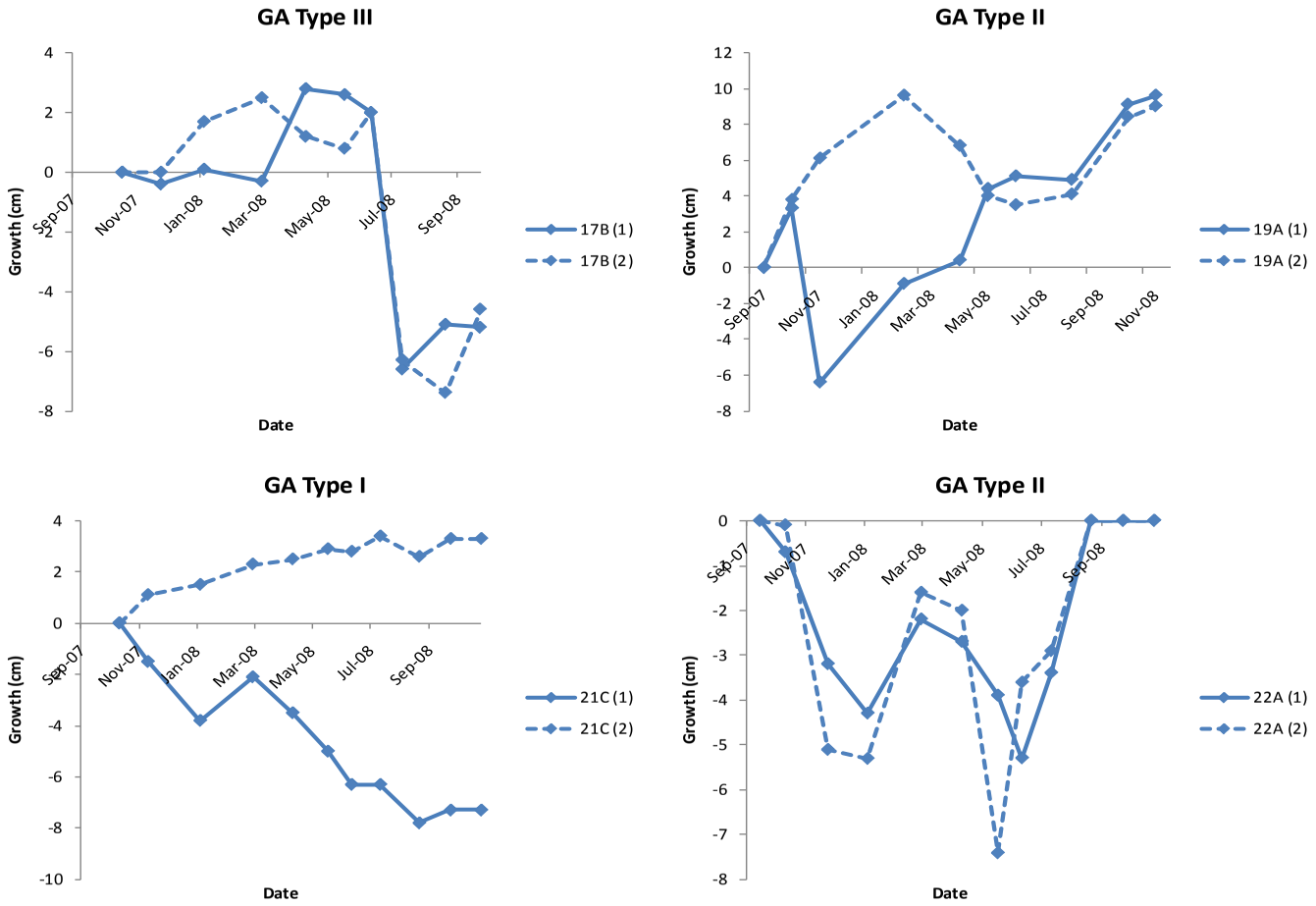

Figure A.1d. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

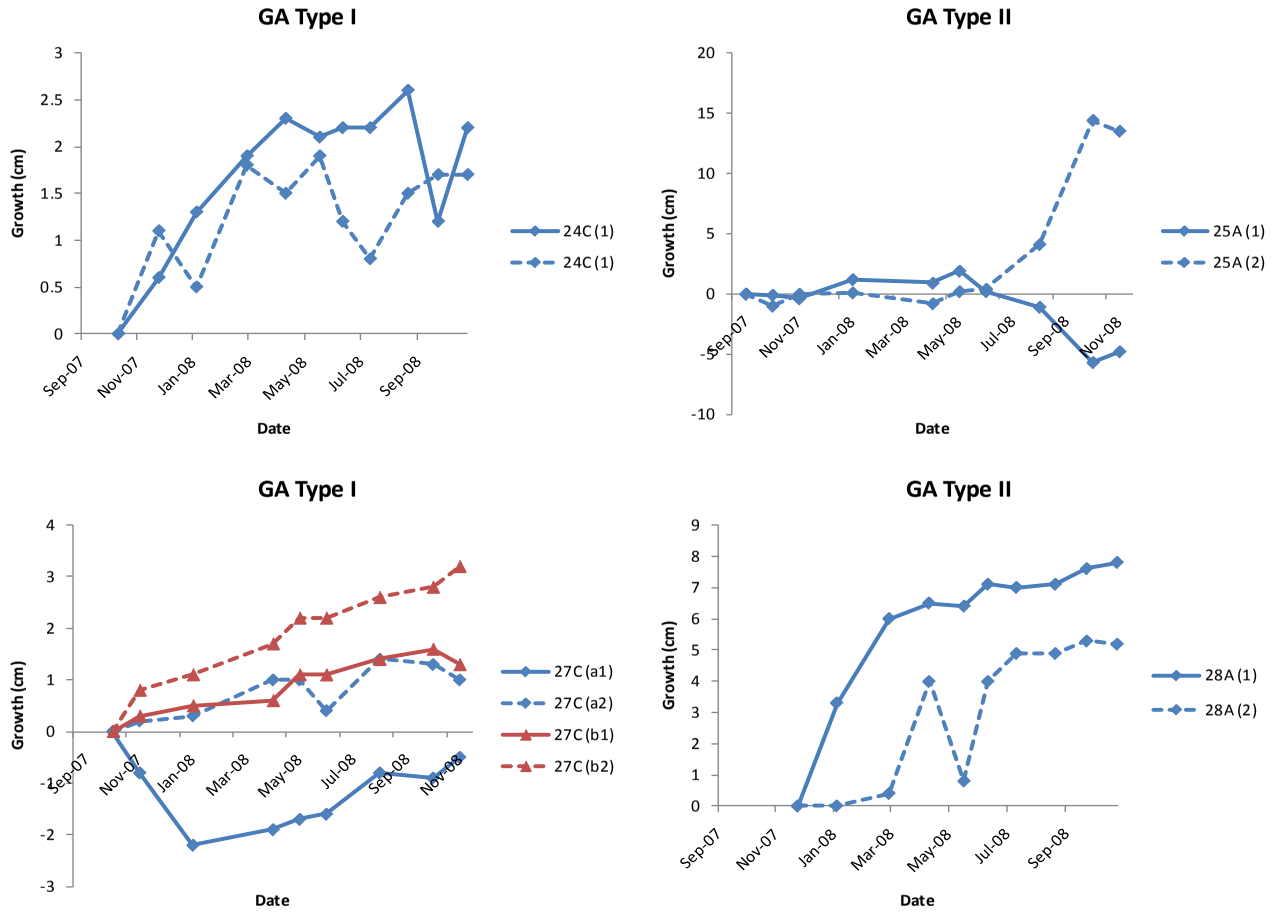

Figure A.1e. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum). Lowercase letters in front of the numbers in parentheses (i.e. a1, b1) indicate specific measurements for each GA on the colony if there was more than 1 per colony.

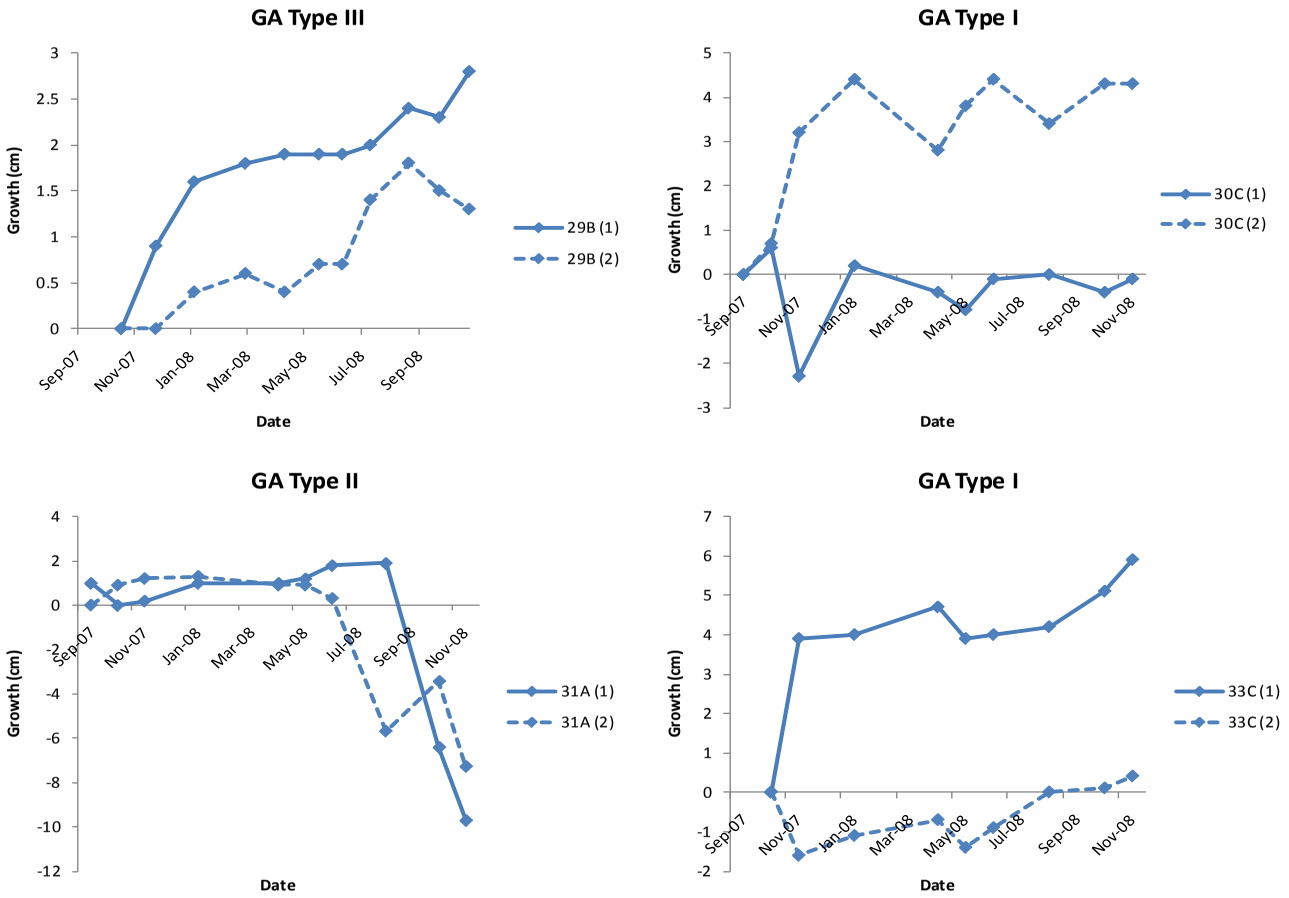

Figure A.1f. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

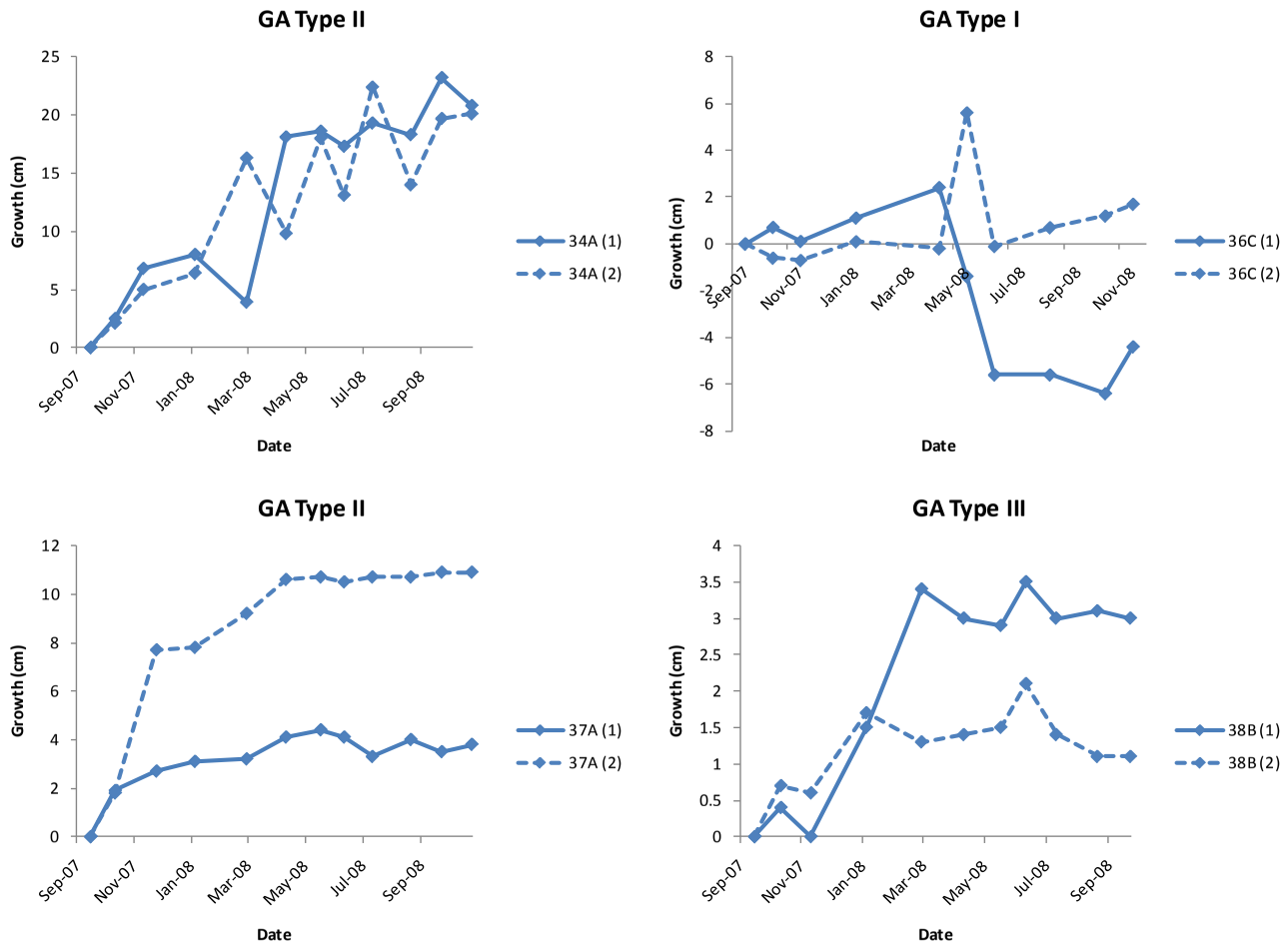

Figure A.1g. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

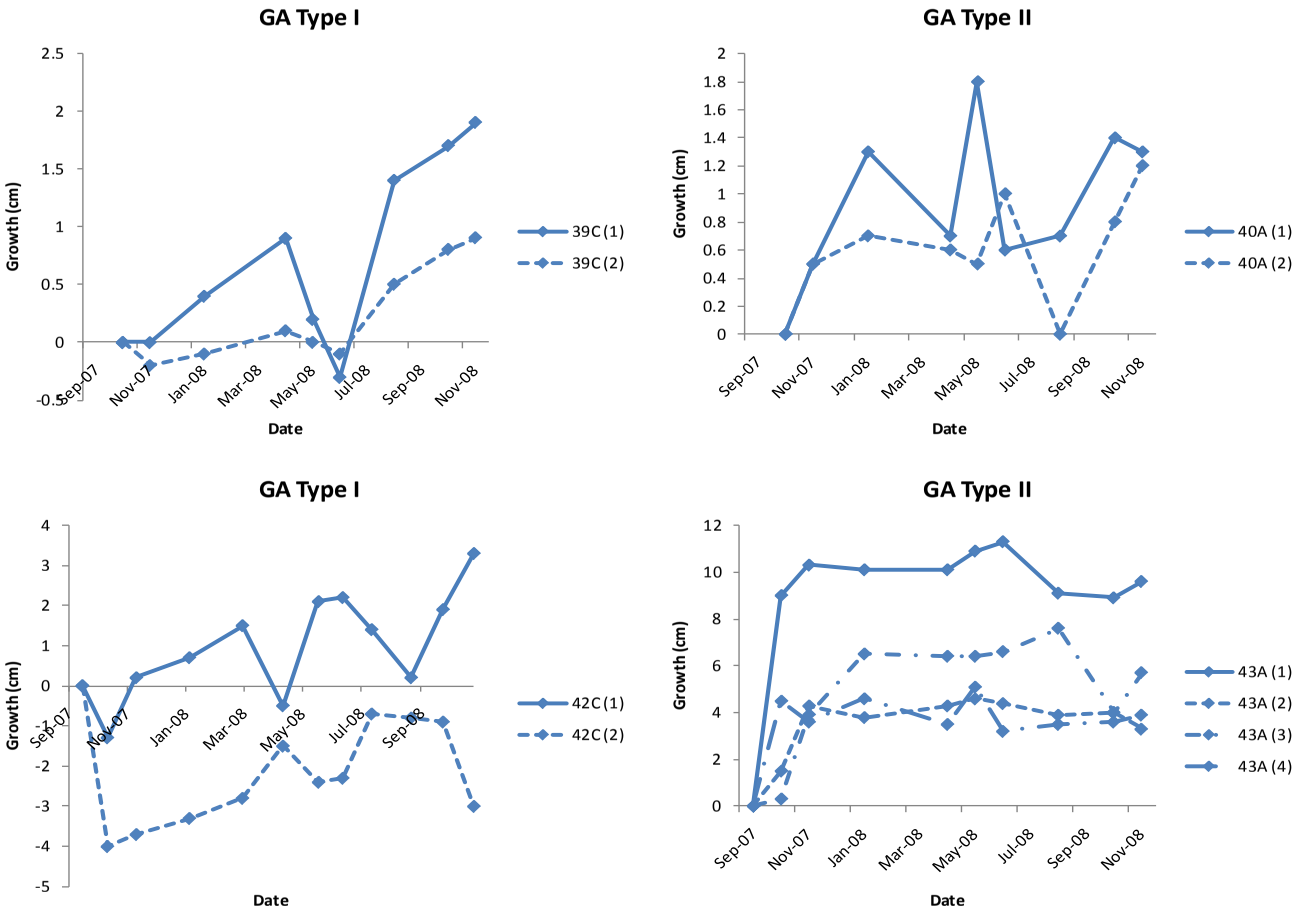

Figure A.1h. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

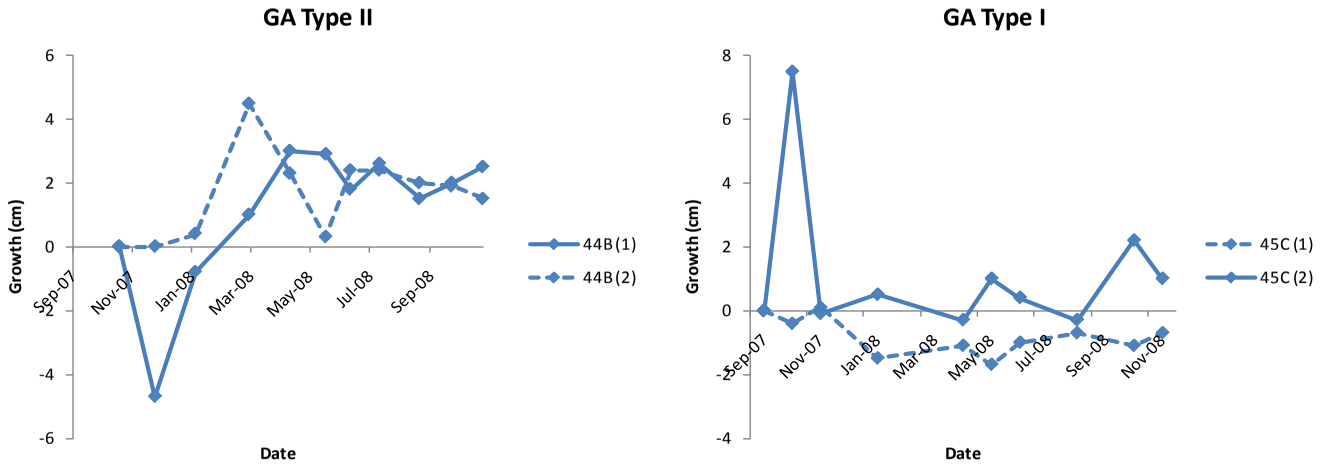

Figure A.1i. Continuation of growth (cm) of each GA from original size (a value of "0" indicates original size). Number and letter combinations (i.e. 1A, 2C, etc.) indicate colony number, and numbers in parentheses indicate axis of measurement (1 = maximum diameter, 2 = perpendicular to maximum).

Table A.1. Univariate PERMANOVA results for microbial communities. If the nested term Colony(Type) was not significant in the Nested 1-way analysis, colonies were pooled within GA Type and a 1-way PERMANOVA was performed on the main term Type. Significant p-values are in bold print and MC indicates the p-value was derived from Monte Carlo sampling.

Time Period	Agar & Dilution	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
24 Hours	MA 1:100	GA Type	4	2.15	2.1272	0.1003	MC
	MA 1:1000	GA Type	4	3.75E-02	0.75	0.5685	MC
	TCBS 1:10	GA Type	4	0.4	0.35443	0.8388	MC
	TCBS 1:100	GA Type	4	0.1875	0.4874	0.3483	MC
	TCBS 1:1000	GA Type	4	2.50E-02	1	0.4101	MC
48 Hours	TCBS 1:1000	GA Type	4	0.3375	1.2115	0.3211	MC

Table A.2. Univariate PERMANOVA results for microbial communities. If the nested term Colony(Type) or Di. X Colony(Type) was not significant in the Nested 2-way analysis, colonies were pooled within GA Type and a 2-way PERMANOVA was performed on the main terms. Significant p-values are in bold print and MC indicates the p-value was derived from Monte Carlo sampling.

Time Period	Agar & Dilution	Source of Variation	df	MS	Pseudo-F	P	Unique Perms
24 Hours	MA 1:100	Disease State	1	1.3333	2.2178	0.1496	9641
		GA Type	2	0.14583	0.24257	0.7791	9952
		Di. X GA	2	2.5208	4.1931	**0.0211**	9960
	TCBS 1:10	Disease State	1	0.2853	0.87518	0.3617	9813
		GA Type	2	0.76984	2.3766	0.0989	9959
		Di. X GA	2	0.1373	0.42385	0.6666	9954
	TCBS 1:100	Disease State	1	0.1875	1.0678	0.3374	5023
		GA Type	2	2.08E-02	0.11864	0.903	7970
		Di. X GA	2	0.1875	1.0678	0.3672	9877
	TCBS 1:1000	Disease State	1	54.188	1	0.4355	2569
		GA Type	2	54.188	1	0.4702	1368
		Di. X GA	2	54.188	1	0.4722	9475
48 Hours	MA 1:100	Disease State	1	0.28545	0.54117	0.4673	9852
		GA Type	2	0.30316	0.57475	0.561	9947
		Di. X GA	2	0.23437	0.44433	0.6377	9961
	TCBS 1:10	Disease State	1	0.4543	1.2591	0.2637	9826
		GA Type	2	0.76528	2.1209	0.133	9956
		Di. X GA	2	0.23429	0.64933	0.5293	9953
	TCBS 1:100	Disease State	1	0.1875	1.0678	0.3328	4991
		GA Type	2	2.08E-02	0.11864	0.9034	7954
		Di. X GA	2	0.1875	1.0678	0.3677	9846
	TCBS 1:1000	Disease State	1	588	0.98817	0.4485	9864
		GA Type	2	591.52	0.99408	0.4766	9860
		Di. X GA	2	598.56	1.0059	0.4661	9923

Table A.3. Overall results of physiological parameters measured (n.s. = not significant).

Characteristic	GA I	GA II	GA III	Combined	Remote Healthy
mean zooxanthellae density	significant differences between healthy areas of colonies and between diseased areas of colonies	significant difference only between healthy areas of colonies 132 & 134 and between diseased areas of 129 & 134	1 colony H>D; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	n.s.	significant differences between colonies
mean Chl a	n.s.	n.s.	n.s.	n.s.	n.s.
mean Chl c	n.s.	n.s.	n.s.	n.s.	n.s.
mean corallite density	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	2 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	3 colonies H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	significant differences between 126 & 130
mean corallite diameter	2 colonies D>H; 1 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	3 colonies D>H; 1 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	3 colonies D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	D>H	significant differences between colonies
mean CSM	2 colonies H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	2 colonies H>D; 1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	significant differences between colonies
mean CSX	1 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	significant differences between healthy areas of colonies 129 & 135; significant differences between diseased areas of colonies	1 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	significant differences between colonies
mean corallite surface area	1 colony H>D; 1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	3 colonies D>H; 1 colony H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	3 colonies D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	significant differences between colonies
mean # of septa	1 colony D>H; no significant differences between healthy areas of coloines; significant differences between diseased areas of colonies	2 colonies H>D; 1 colony D>H; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	1 colony D>H; no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	n.s.
mean # of palli	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	2 colonies H>D; 1 colony D>H; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	1 colony D>H; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies 139 & 140	n.s.	n.s.
mean tissue thickness	2 colonies H>D; 1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	2 colonies H>D; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	significant differences between colonies

Table A.4. Overall results of physiological parameters measured per colony (n.s. = not significant).

Characteristic	GA I				GA II				GA III				Combined	Remote Healthy
	122	128	136	137	129	132	134	135	131	133	139	140		
mean zooxanthellae density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**H>D**	n.s.	n.s.	n.s.	n.s.	significant differences between colonies
mean Chl a	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	n.s.	n.s.
mean Chl c	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	n.s.	n.s.
mean corallite density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**H>D**	n.s.	**H>D**	n.s.	**H>D**	**H>D**	n.s.	significant differences between 126 & 130
mean corallite diameter	**D>H**	**D>H**	n.s.	**H>D**	**D>H**	**H>D**	**D>H**	**D>H**	**D>H**	n.s.	**D>H**	**D>H**	D>H	significant differences between colonies
mean CSM	**H>D**	**H>D**	n.s.	n.s.	**H>D**	**D>H**	n.s.	**H>D**	n.s.	n.s.	**D>H**	n.s.	n.s.	significant differences between colonies
mean CSX	n.s.	**H>D**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**H>D**	n.s.	n.s.	n.s.	significant differences between colonies
mean corallite surface area	n.s.	**D>H**	n.s.	**H>D**	**D>H**	**H>D**	**D>H**	**D>H**	**D>H**	n.s.	**D>H**	**D>H**	n.s.	significant differences between colonies
mean # of septa	n.s.	**D>H**	n.s.	n.s.	n.s.	**H>D**	**H>D**	**D>H**	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
mean # of palli	n.s.	**D>H**	n.s.	n.s.	**H>D**	n.s.	**H>D**	**D>H**	n.s.	n.s.	n.s.	**H>D**	n.s.	n.s.
mean tissue thickness	n.s.	**H>D**	**H>D**	**D>H**	n.s.	n.s.	n.s.	**D>H**	**H>D**	n.s.	n.s.	**H>D**	n.s.	significant differences between colonies

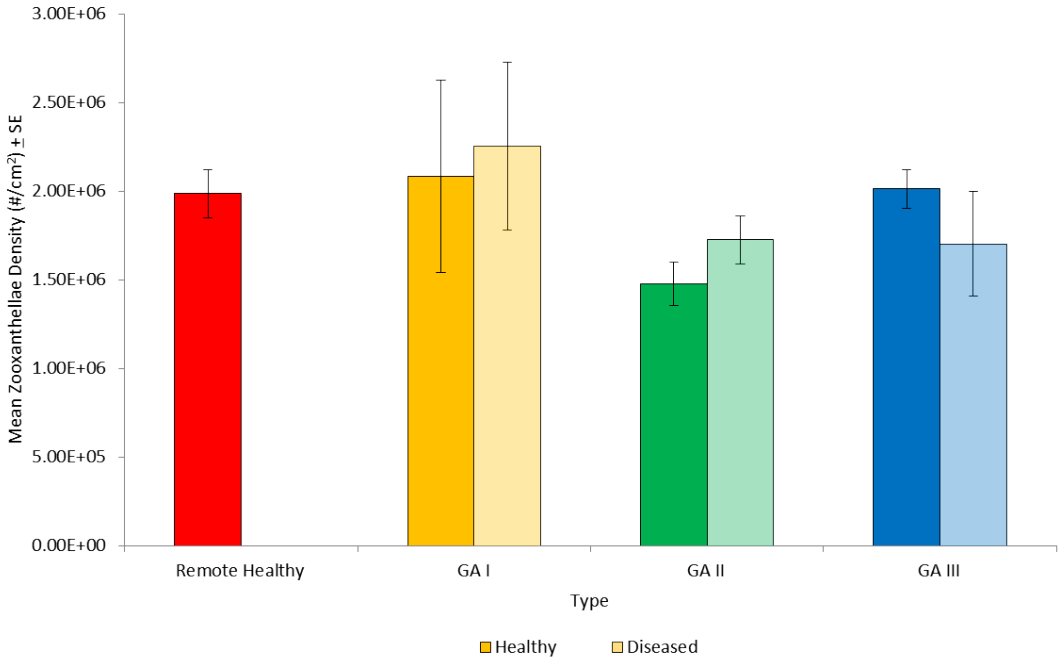

Figure A.2. Mean zooxanthellae densities of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

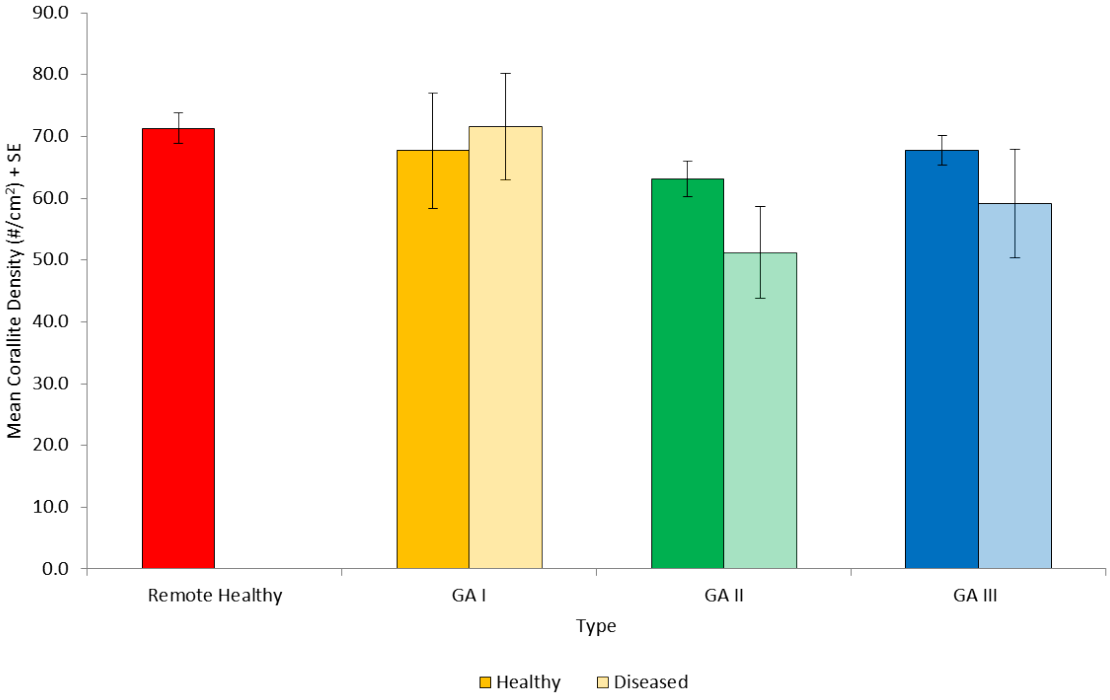

Figure A.3. Mean corallite densities of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

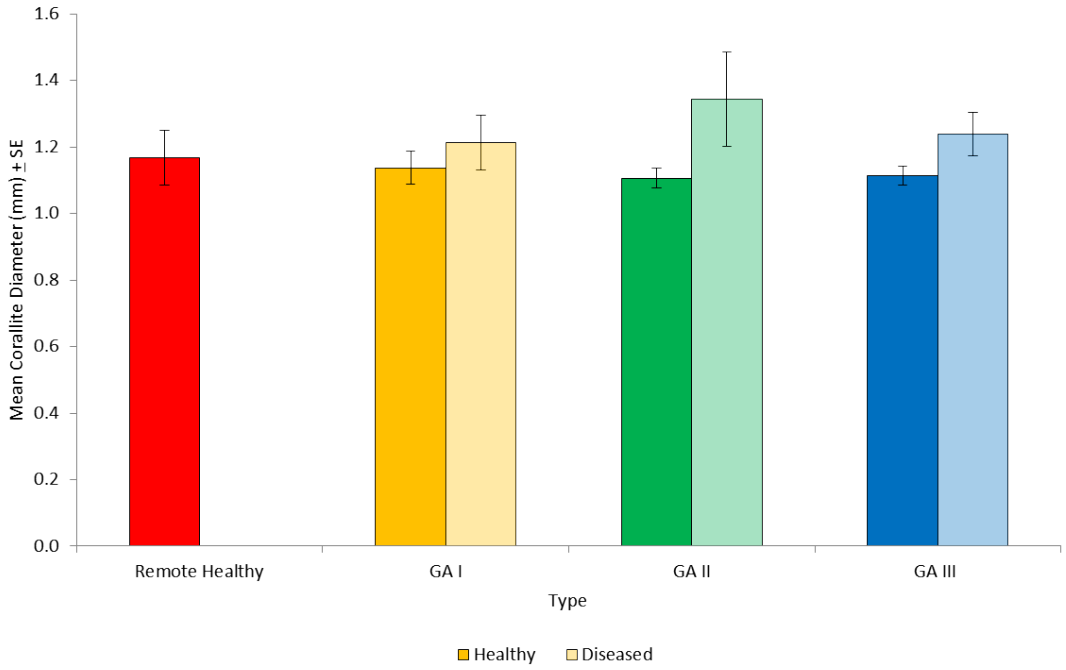

Figure A.4. Mean corallite diameter of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

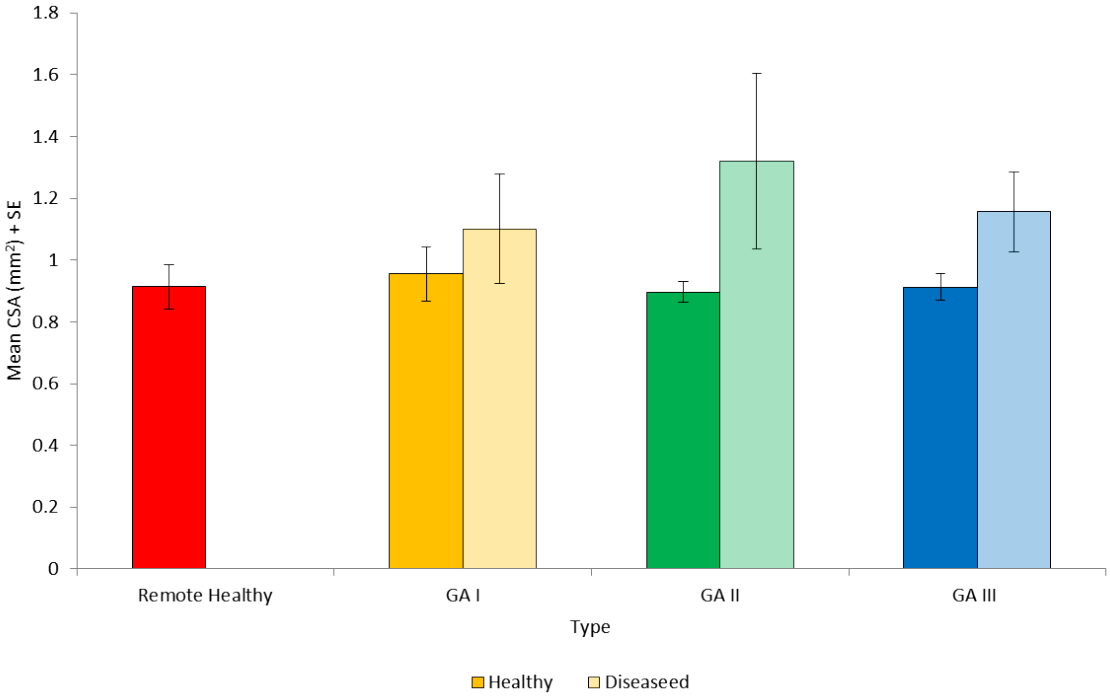

Figure A.5. Mean corallite surface area (CSA) of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

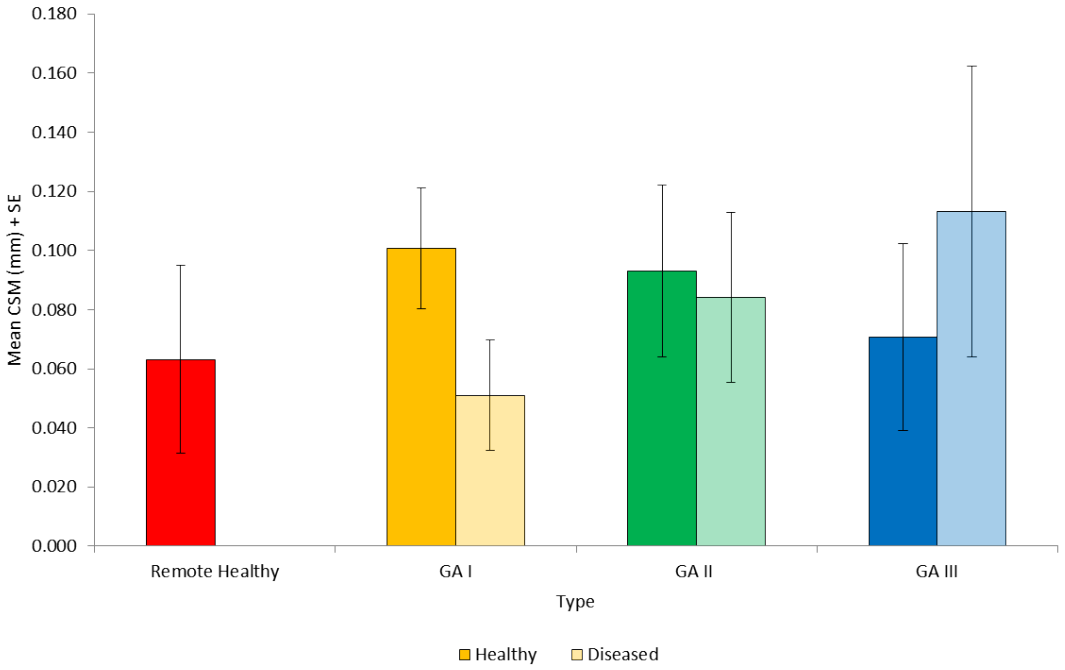

Figure A.6. Mean distance from wall to closest neighboring calice (CSM) of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

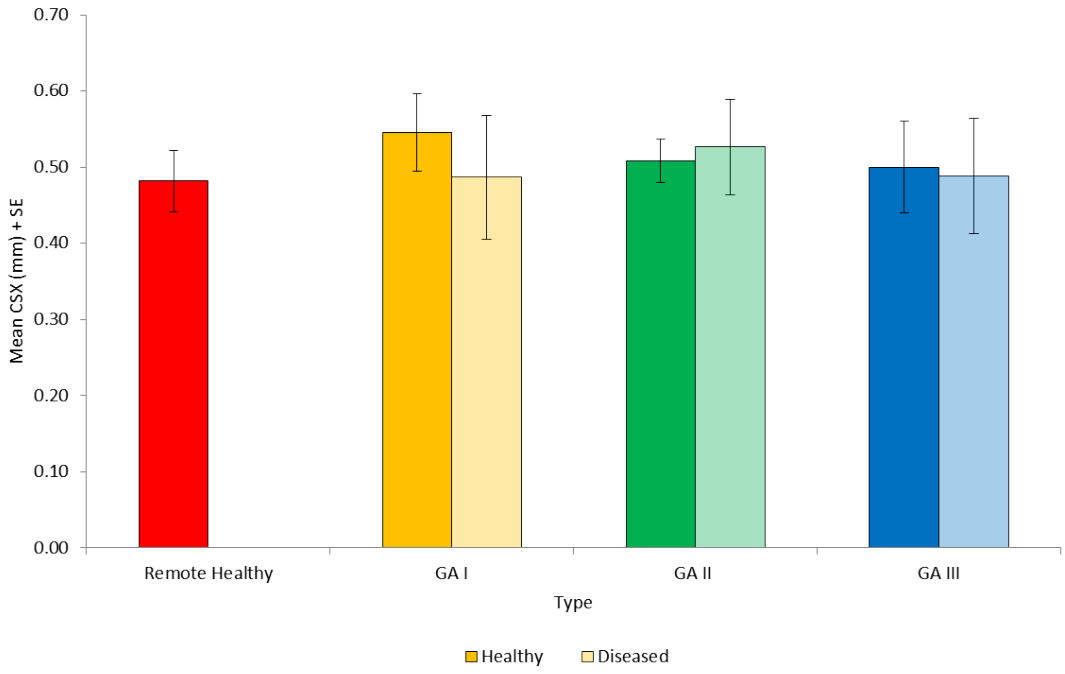

Figure A.7. Mean distance from wall to furthest neighboring calice (CSX) of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

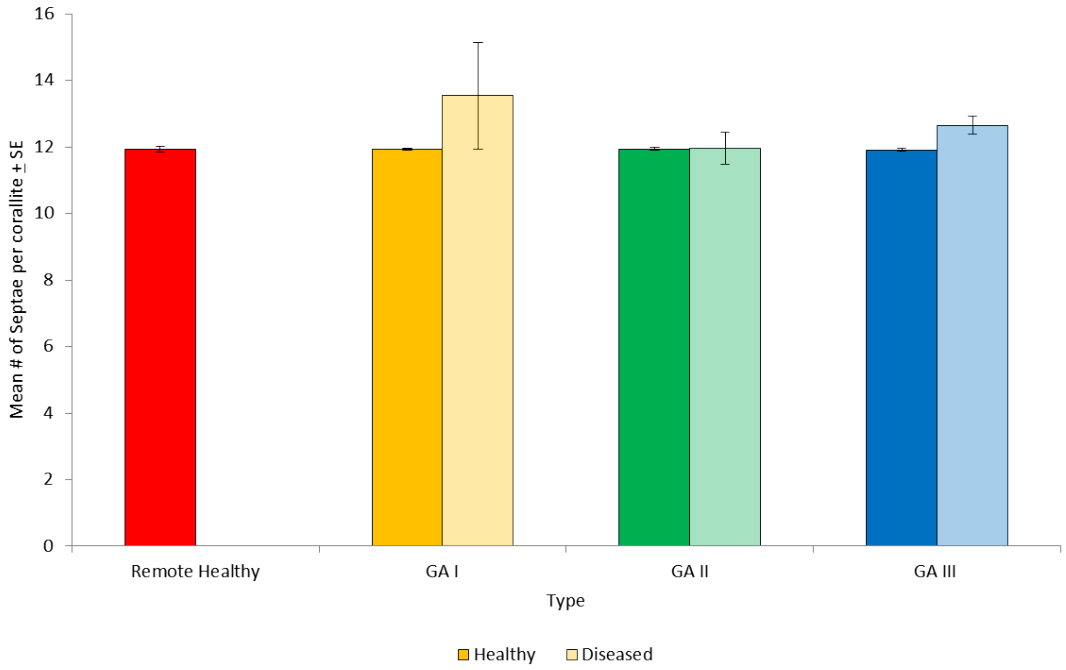

Figure A.8. Mean number of septae per corallite of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

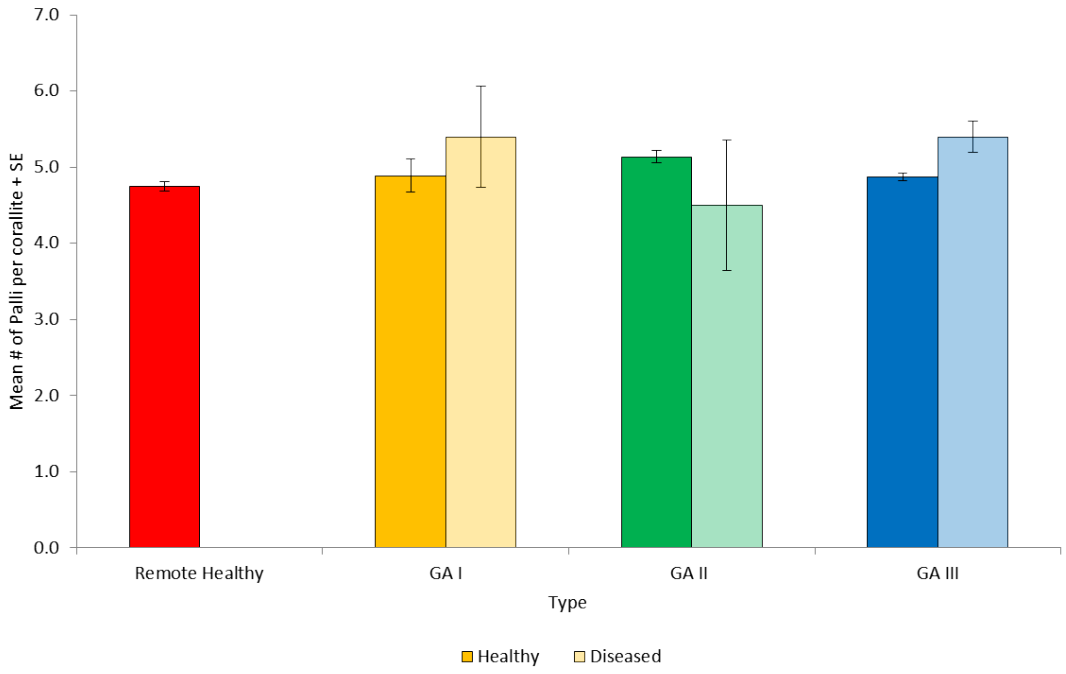

Figure A.9. Mean number of palli per corallite of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

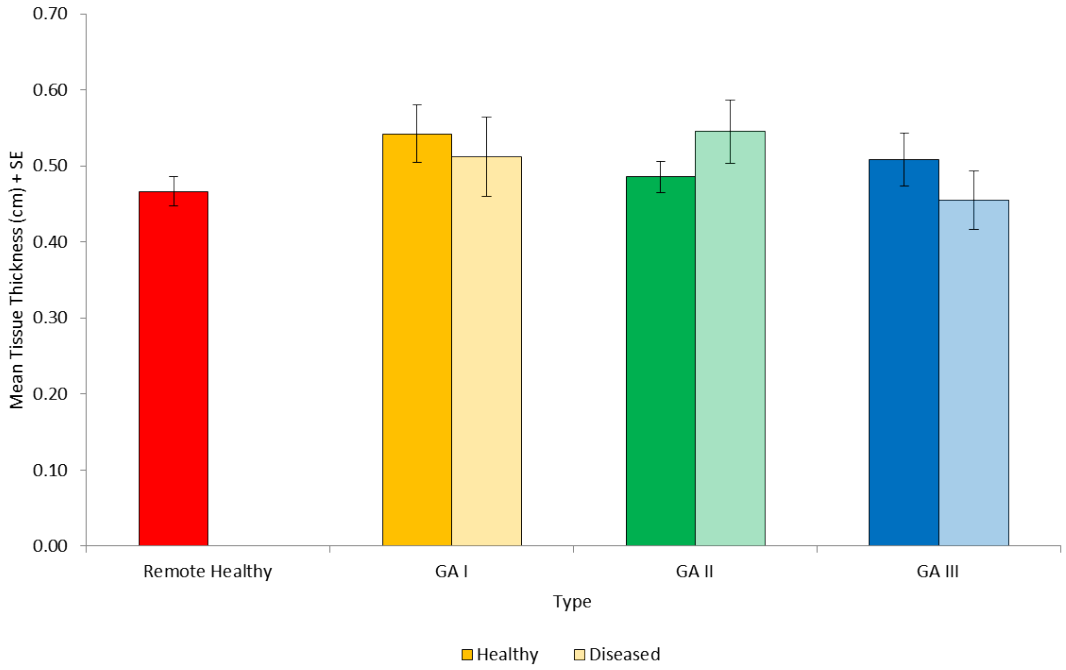

Figure A.10. Mean tissue thickness of *Porites lutea* within Remote Healthy and healthy and diseased areas of GA Types.

Table A.5. Overall results of microbial analysis on MA (n.s. = not significant).

Agar/Time/Dilution	GA I	GA II	GA III	Combined	Remote Healthy	Seawater
MA 24 1:1	significant differences between healthy areas of colonies and between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	n.s.	RH>SW,GAIIH,GAIIIH; no significant differences between colonies	no significant differences between samples
1:10	no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	RH>GAIIH; 1 colony H>D; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	RH>GAIIIH; no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	RH>SW,GAIIH,GAIIIH; no significant differences between colonies	SW<RH; no significant differences between samples
1:100	D>H; GAIH<GAIIIH; no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	GAIIIH>GAIH; no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	significant differences between colonies	no significant differences between samples
1:1000	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	significant differences between colonies	no significant differences between samples
MA 48 1:1	significant differences between healthy areas of colonies and between diseased areas of colonies	no significant differences between healthy areas ofcolonies; significant differences between diseased areas of colonies 132 & 134	1 colony D>H; 1 colony H>D; significant differences between healthy areas of colonies 133 & 139; significant differences between diseased areas of colonies	n.s.	significant differences between colonies	no significant differences between samples
1:10	GAIH>SW; no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	significant differences between colonies 123 & 126	SW<RH,GAIH; no significant differences between samples
1:100	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples
1:1000	1 colony D>H; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies 122 & 128	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	significant differences between colonies	no significant differences between samples

Table A.6. Overall results of microbial analysis on TCBS (n.s. = not significant).

Agar/Time/Dilution	GA I	GA II	GA III	Combined	Remote Healthy	Seawater
TCBS 24 1:1	1 colony D>H; significant differences between healthy areas of colonies and between diseased areas of colonies	no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	n.s.	significant differences between colonies	no significant differences between samples
1:10	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples
1:100	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples
1:1000	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples
TCBS 48 1:1	significant differences between healthy areas of colonies 136 & 137; significant differences between diseased areas of colonies	GAIIH<SW,RH; no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	no significant differences between healthy areas of colonies; significant differences between diseased areas of colonies	n.s.	RH>GAIIH; significant differences between colonies	SW>GAIIH; significant differences between samples
1:10	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	significant differences between colonies 121 & 126	no significant differences between samples
1:100	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples
1:1000	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	no significant differences between healthy areas of colonies or between diseased areas of colonies	n.s.	no significant differences between colonies	no significant differences between samples