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Pathogen Transmission Techniques and Genotypic Resistance to Disease in the Threatened Coral, Acropora cervicornis

Megan Bock
Nova Southeastern University, mb3154@nova.edu

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Thesis of
Megan Bock

Submitted in Partial Fulfillment of the Requirements for the Degree of

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M.S. Marine Environmental Sciences

Nova Southeastern University
Halmos College of Natural Sciences and Oceanography

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Approved:
Thesis Committee

Major Professor: Nicole Fogarty

Committee Member: Esther C. Peters

Committee Member: Erinn Muller

HALMOS COLLEGE OF NATURAL SCIENCES AND OCEANOGRAPHY

PATHOGEN TRANSMISSION TECHNIQUES AND GENOTYPIC RESISTANCE TO DISEASE IN THE THREATENED CORAL, *ACROPORA CERVICORNIS*

By
Megan Bock

Submitted to the Faculty of
Halmos College of Natural Sciences and Oceanography
in partial fulfillment of the requirements for
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Nicole D. Fogarty, Ph.D.

Committee Member: _____
Esther C. Peters, Ph.D.

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Abstract

Unprecedented population losses of the staghorn coral, *Acropora cervicornis,* since the 1970s have been attributed primarily to disease. Although a positive linear relationship between disease prevalence and increased water temperature has been described, the pathogen(s) causing disease and whether they are spread through the water or vectors is still poorly understood. Additionally, an increase in disease outbreaks and severity has provided an urgent need to identify natural genotypic resistance to disease in Caribbean acroporids. Studies to date have explored a variety of pathogen transmission methods, but prior to this study, there has been no examination of differences among common techniques. I investigated pathogen transmission and resistance to development of the disease known as rapid-tissue loss (RTL) in 11 different genotypes by comparing two common transmission methods (direct contact vs. waterborne). Additionally, I investigated changes in tissue condition over a 9-day acclimation period to determine the potential effect of acclimation on disease susceptibility. Overall, disease was significantly higher in the direct contact treatment, though resulting disease varied greatly by genotype, with only one genotype appearing resistant to developing disease. Acclimation time influenced tissue condition with a significant decline in condition occurring from day zero to day two, but significant improvements in surface body wall parameters were observed from days two to nine. These results highlight the differences between disease transmission methods and demonstrate the importance of selecting an appropriate transmission method and acclimation period for future studies.

Keywords: disease, transmission, acclimation, *Acropora*

CHAPTER 1: INTRODUCTION

1.1   Importance of Coral Reefs

Coral reefs are among the oldest reef systems on Earth, dating back more than 225 million years (Pandolfi 2011). Since this time, scleractinian corals have been responsible for creating vital oceanic habitat and structure. Today, coral reefs are considered one of the most biologically diverse ecosystems on the planet, providing habitat for more than a quarter of all marine organisms (Plaisance et al. 2011). Coral reefs also support hundreds of millions of people by providing shoreline protection and coastal buffering, a source of protein, ecotourism, a source of medicinal chemical compounds, and raw building materials (Moberg and Folke 1999, Spalding et al. 2001, Burke et al. 2011). While it is estimated that more than 850 million people across the world reside within 100 kilometers of coral reefs, even those far from reefs benefit from these numerous ecological services (Burke et al. 2011). In the Florida Keys alone, it is estimated that coral reefs and their associated tourism generate more than $1.2 billion in local sales annually (NOAA 2007). While this revenue only accounts for a small portion of Florida's economy, more remote locations can rely on reefs to support nearly their entire economy and livelihood. For example, in French Polynesia, exports for the aquaria trade comprise nearly 62% of the nation's gross domestic product (GDP; Burke et al. 2011). Reef-related tourism also accounts for more than 15% of the GDP in least 23 countries and territories (Burke et al. 2011). Globally, the total estimated value of these unique goods and services is more than $30 billion each year (Cesar et al. 2003).

Despite the many benefits to preserving functional reefs, coral reef health has been declining on a global scale for decades. More than 80% of coral cover has been lost in the Caribbean since the 1970s (Gardner et al. 2003), and by 2030 it has been estimated that more than 26% of the world's reefs will be lost (Wilkinson 2004). Multiple local anthropogenic pressures have been linked to reef degradation, including pollution, overfishing, sedimentation, and eutrophication (Jackson et al. 2001, Pandolfi et al. 2005, Williams et al. 2006), while large-scale stressors such as disease, ocean acidification, and increased sea-surface temperature have also had profound detrimental effects on reefs (Hoegh-Guldberg et al. 2007, Hoegh-Guldberg and Bruno 2010, Bruno and Valdivia

2016). Together, these pressures have compromised coral reefs throughout the world (Knowlton and Jackson 2008), putting the livelihood of millions of people at risk.

1.2   Acroporid Significance

Of the many scleractinian corals, the genus *Acropora* is the most speciose, containing more than 150 species (Wallace and Willis 1994). Although many species exist worldwide, only three acroporids are found in the Caribbean: *Acropora cervicornis*, *A. palmata* (Pandolfi 2002), and their hybrid, *A. prolifera* (Van Oppen et al. 2000, Vollmer and Palumbi 2002). Fossil records indicate the dominance of both parental species for millions of years, since the early Pleistocene (McNeill et al. 1997, Wallace 2012). However, *A. prolifera* abundance has only more recently been described (Fogarty 2012, Japaud et al. 2014, Aguilar-Perera and Hernández-Landa 2017). Historically, both *A. cervicornis* and *A. palmata* have been used to describe zonation in Caribbean reefs, primarily due to their distinct habitat ranges (Goreau 1959, Wallace and Dale 1978). For example, *A. cervicornis* can be found at intermediate depths along fore reefs, typically between 5 to 25 meters, while *A. palmata* is typically most abundant on reef crests and very shallow fore reefs, usually between depths of 0 to 5 meters (Adey and Bruke 1977, Hubbard 1988, Geister 1997). Habitat of the hybrid, *A. prolifera*, is still being studied, although it has been found to occupy both parental zones and can survive at extremely shallow depths and warm water temperatures (Fogarty 2012, Japaud et al. 2014, Aguilar-Perera and Hernández-Landa 2017).

While most reef builders in the Caribbean grow at a rate of only a few millimeters per year, acroporid corals grow faster, from 25 to 45 mm per year (Vaughan 1915, Huston 1985). However, attributes such as genotype, symbiont clade, and pre-existing coral size can influence the growth rate of acroporids, leading to rates beyond 45 mm per year (Lirman et al. 2014). These growth rates allow for the creation of expansive habitats, either in the form of *A. cervicornis* thickets, or large *A. palma*ta branches. These structures are utilized by many fishes and other invertebrates and are critical to the health of the Caribbean reef system. Acroporid corals have been considered ecologically irreplaceable due to this ability to create and maintain reef structure (Bruckner 2002).

However, since the late 1970s and early 1980s, the acroporid parental species have experienced unprecedented declines due to physical disturbance from storms, the loss of symbiotic algae (i.e., coral bleaching) from thermal stress, and disease. In 1980, Hurricane Allen devastated local acroporid populations in Discovery Bay, Jamaica (Woodley et al. 1981, Lang et al. 1990), leaving total coral cover at < 5% of benthic cover in some areas (Hughes 1994). Hurricanes David and Frederic, in 1979 in the U.S. Virgin Islands (USVI), reduced structural reef integrity and caused mortality of over 65% of experimental *A. palmata* fragments at Tague Bay eleven months after the storm (Rogers et al. 1982). Toward the end of the decade in 1989, Hurricane Hugo reduced *A. palmata* cover to 0.8% in Buck Island, USVI (Rogers 1993). Additional storms have caused significant fragmentation and dislodgment of both *Acropora* spp. including Hurricane Gerta (Highsmith et al. 1980), Gilbert (Kobluk and Lysenko 1992), and Andrew (Lirman and Fong 1997). Bleaching has also caused significant changes to reef structure. Between 1979 and 1998 there were six major mass bleaching events that affected reef communities throughout the world (Hoegh-Guldberg 1999), in addition to a more recent event in 2010 (Heron et al. 2016). The first report of bleaching in the Florida Keys occurred in the early 1980s (Wilkinson and Souter 2008). Following the mass bleaching event of 1998, *A. palmata* colonies in the Florida Keys experienced complete mortality at some study locations (Bruckner 2002), and in 2005 *A. palmata* colonies in St. Croix, USVI experienced 58% mortality in particular locations (Woody et al. 2008). However, most of the mortality associated with the Caribbean acroporids has been attributed to disease and not directly to bleaching (Aronson and Precht 2001, Lesser et al. 2007). In acroporid dominated reefs in the Florida Keys, Porter and Meier (1992) found a 44% decline in total coral cover at Looe Key and 33% decline at Carysfort Reef between 1984 and 1992, and even the complete mortality of *A. palmata* in some study sites due to disease. Disease outbreaks in Puerto Rico in the early 1980s also caused the complete mortality of *A. cervicornis* at some study sites (Bruckner 2002). Disease in Channel Cay, Belize also caused a rapid decline of *A. cervicornis* coral cover from 70% to nearly 0% between 1986 and 1993 (Aronson and Precht 1997). These sustained population losses consequently led to the classification of *A. cervicornis* and *A. palmata* as "threatened" under the United States' Endangered Species Act in 2006 (Hogarth 2006), and as

"critically endangered" under the International Union for the Conservation of Nature Red List in 2008 (Aronson et al. 2008, Carpenter et al. 2008, Kline and Vollmer 2011).

1.3   Coral Diseases

Following the first documentation of coral disease in 1973 (Antonius 1976), and subsequent publications in 1975 and 1976 (Garrett and Ducklow 1975, Antonius 1976), reports of coral diseases have rapidly increased (Richardson 2015). Today, reports of disease have spread to more than 65 countries (Garrett and Ducklow 1975, Woodley et al. 2008, Richardson 2015), and the number of described coral diseases ranges between 18 and 28 (Green and Bruckner 2000, Willis et al. 2004, Bourne et al. 2009), with many others recognized. Although major disease outbreaks have historically occurred in the Caribbean, recent outbreaks in the Indo-Pacific have become a significant concern and have demonstrated a global threat to reefs (Weil 2006, Aeby et al. 2011, Ushijima et al. 2012).

Four diseases have been reported globally (black-band disease, white plague-like disease, shut-down reaction, and skeletal anomalies); however, nine (white-band Type I, white-band Type II, white-plague Type I, white-plague Type II, white-plague Type III, white pox, aspergillosis, yellow-band, and dark spots) are found exclusively in the Caribbean (Sutherland et al. 2004). The frequent disease outbreak events, widespread associated morality, and high virulence of these diseases has led to the Caribbean's reputation as a "disease hot spot" (Weil 2006). However, despite the many decades of research, particularly in the Caribbean, these coral diseases are still poorly understood.

Disease can be defined as any impairment of normal function within the body, organs, or organ systems of an organism (Wobeser 1981). In order to classify a disease, at least two of the three following criteria must be met: (1) consistent anatomical alterations to the host, (2) an identifiable group of signs, and/or (3) recognized etiologic or causal agents (Peters 2015). Causal agents may be biotic (typically considered infectious diseases) or abiotic (non-infectious) in nature but are difficult to identify in marine organisms such as corals. For example, of the more than 18 different diseases described in corals, pathogens have been recognized for about half (Sutherland et al. 2004, Weil 2006, Harvell et al. 2007). Of these, unique bacterial pathogens for only five diseases

(white plague-II, white band-II, white pox, aspergillosis, and bacterial bleaching) have fulfilled Koch's postulates, the criteria established to determine the relationship between a microbe and disease (Weil 2006, Harvell et al. 2007).

Koch's postulates require that (1) the pathogen be found in every diseased individual, (2) the pathogen be isolated from a diseased individual and grown in pure culture, (3) the disease be induced in experimental organisms from culture, and (4) the same pathogen be re-isolated from the induced organism following development of disease (Sutherland et al. 2004). In a complex microbiome of bacteria, algae, viruses, protozoans, and fungi found in coral, collectively called the coral holobiont, it is difficult to isolate and grow a putative pathogen in pure culture, making the second, third, and fourth postulate difficult to fulfill for corals (Upton and Peters 1986, Bourne et al. 2009, Kline and Vollmer 2011, Weil and Rogers 2011). This is because many microbes are simply unculturable or require specific host cells to reproduce (Ritchie et al. 2001, Sutherland et al. 2004). As a result, very few coral diseases have undergone this type of research. Additionally, it has been discovered that some diseases which originally full-filled Koch's postulates are not always reproduceable, lacking the presence of the original identified pathogen (Polson et al. 2008). For these reason, many coral diseases are classified without fulfilling Koch's postulates and without identifiable causal agents.

In many cases, coral diseases are believed to be caused by a consortium of pathogens. Common techniques for identifying microbes and potential pathogens in corals include using genotype-based rRNA gene sequencing (16S and 18S), representational difference analyses (RDAs) (Ritchie et al. 2001), community DNA isolation sequencing from the surface mucopolysaccharide layer (Sutherland et al. 2004), and more recently through multi-locus sequence analysis (Ushijima et al. 2014). However, these techniques don't always reveal significant differences between communities of healthy and diseased corals, as was the case in Casas et al. (2004) when investigating a *Rickettsiales*-like bacterium associated with white-band disease (WBD) Type I. This suggests that multiple pathogens are acting on a diseased coral at any given time.

Because pathogens are difficult to identify, most coral disease efforts have focused on the accurate and consistent descriptions of disease lesions (Work and Aeby 2006, Woodley et al. 2008). Many guides and manuals have been produced to standardize disease identification; a huge challenge when managing observations from individuals around the world (Work and Aeby 2006, Galloway et al. 2007, Raymundo et al. 2008, Woodley et al. 2008, Rogers 2010). Still, many diseases are vaguely described, confused with pre-existing diseases, or classified as new when fitting disease characteristics already exist (Rogers 2010). Additionally, diseases such as white pox, white patch, white band, and rapid tissue necrosis, all with similar "white" characteristics, can easily be confused and misidentified. Predation marks, which often leave behind patches of white denuded skeleton, can also be interpreted as disease without careful examination.

While handbooks and guidelines help maintain consistency, they do little to improve the understanding of disease. Some researchers have begun exploring disease transmission in both field and laboratory experiments, and some have investigated potential disease resistance mechanisms in corals (E. Muller, unpub. data, Vollmer and Kline 2008, Aeby et al. 2010, Gignoux-Wolfsohn et al. 2012, Ushijima et al. 2012, Miller et al. 2014, Miller and Williams 2016, Randall et al. 2016, Hightshoe 2018). Though multiple methodologies for disease transmission have been applied, the most common in the literature are through direct contact and a form of water-borne transmission. In some cases, biological vectors have been explored in transmission studies, such as Gignoux-Wolfsohn et al. (2012), in which corallivorous snails *Coralliophila abbreviata* and *C. caribaea* were used in attempt to transmit white band disease in *A. cervicornis*. Despite these advances in coral disease research, pathogens, transmission, and virulence of most diseases are still unknown.

1.4   White-Band Disease and Rapid Tissue Loss

White-band disease (WBD) was one of the first described diseases affecting members of the genus *Acropora* (Antonius 1981, Gladfelter 1982, Aronson and Precht 2001), and has been responsible for widespread acroporid mortality since the late 1970s. Population losses of both *A. palmata* and *A. cervicornis* reached up to 95% in the 1980s as a result of WBD (Vollmer and Kline 2008), a decline that has not been seen in the

fossil record for hundreds of years (Aronson and Precht 2001). Acroporids affected by WBD exhibit clear tissue degradation that can be easily characterized by either a distinct line between coral skeleton and living tissue (Type I), or a temporary separation of bleached tissue (devoid of algal symbionts) between coral skeleton and normally pigmented tissue (Type II; Ritchie and Smith 1998). Tissue loss typically begins at the base or middle of a branch and spreads towards the tip, which can result in entire branch or colony mortality.

Like many other coral diseases, WBD prevalence increases with rising ocean temperature (Muller et al. 2008, Brandt and McManus 2009, Miller et al. 2009). As a result, a positive linear relationship between temperature and disease can be observed frequently during summer months (Muller et al. 2008). Large areas of adjacent colonies can be affected, but diseased colonies are frequently observed alongside unaffected colonies. These observations suggest multiple sources of the pathogen(s) causing WBD as well as possible pathogen resistance in certain colonies (Vollmer and Kline 2008).

Since the discovery of WBD in 1977, several possible etiologic agents for this disease have been proposed, including bacteria from the genera *Vibrio*, *Lactobacillus*, *Bacillus*, and the order *Rickettsiales*, but no single cause has been confirmed for WBD Type I (Peters et al. 1983, Ritchie and Smith 1998, Casas et al. 2004, Gil-Agudelo et al. 2006, Sweet et al. 2014). For this reason, it is possible that multiple bacterial pathogens may be responsible for WBD Type I (Gignoux-Wolfsohn and Vollmer 2015), or that WBD Type I may not be caused by a bacterial pathogen at all (Casas et al. 2004). WBD Type II, however, is believed to be associated with *Vibrio charcharia* (Ritchie and Smith 1998), although Koch's postulates have not been fulfilled for this pathogen.

Despite the lack of identification of a single pathogen for WBD Type I, many vectors and transmission routes have been described. These include direct contact with corals through predation of the corallivorous snail, *Coralliophila abbreviata* (Williams and Miller 2005, Gignoux-Wolfsohn et al. 2012), water-borne transmission through the application of diseased tissue into experimental tanks (Gignoux-Wolfsohn et al. 2012, E. Muller, unpub. data), and direct contact of a diseased coral to an apparently healthy coral fragment (Williams and Miller 2005, Vollmer and Kline 2008, Miller and Williams

2016). While other corallivores, such as damselfish and the fire-worm, *Hermodice carunculata*, may leave denuded skeletons that look like the described disease, these have not been confirmed as biological vectors of WBD.

Many other diseases and syndromes have been described with signs that are similar to WBD since the late 1970s (Williams et al. 2006). Rapid tissue loss (RTL) for example, is a common affliction that has been described more recently that can visually appear analogous to WBD (Williams and Miller 2005). However, RTL is characterized by acute tissue loss occurring within portions or entire branches of *A. cervicornis* or *A. palmata*. Affected corals exhibit rapid sloughing of tissue, at a rate of up to 4 cm per day, which leaves behind irregular areas of denuded skeleton (Miller et al. 2014). It is unclear whether previously published literature has correctly differentiated between WBD and RTL, or if these two afflictions are caused by different pathogens, further complicating identification and histological analysis of these coral diseases (Williams and Miller 2005). For these reasons, some authors have attempted to differentiate between RTL and WBD based on visual characteristics (Williams and Miller 2005, Miller et al. 2014), or may clump both together as "white syndromes" to be broader in their disease descriptions. This has led to some confusion within the literature when referring to acroporid diseases.

While some researchers are focusing on identifying a specific pathogen for diseases such as WBD and RTL, others have suggested that some diseases may be caused by opportunistic infections of pre-existing bacteria rather than distinct primary pathogens, or from a combination of the two (Lesser et al. 2007, Muller et al. 2008, Bourne et al. 2009, Muller and van Woesik 2012). These infections generally occur following a stressor that suppresses host immunity, which could include chemical pollutants, physical disturbance, or loss of their symbiotic algae ("bleaching") due to increased sea-surface temperature (SST).

1.5   *Climate Change and Coral Diseases*

Since the early 1900s, average global sea-surface temperature (SST) has increased at an average rate of 0.13°F each decade. A distinct increase in SST has occurred since around 1970, and in the last 30 years, SST has been consistently higher than any other

measurement since 1880 (Zhang et al. , EPA 2017). The emergence of new coral diseases and increase in total disease outbreaks since the 1970s have likely not occurred in coincidence (Harvell et al. 2002, Selig et al. 2006).

Rising ocean temperatures have repeatedly been linked to coral bleaching and disease prevalence, particularly in the late 20th and early 21st centuries (Selig et al. 2006, Lundgren and Hillis-Starr 2008, Muller et al. 2008, Randall 2014). Coral bleaching is described as a "thermally induced breakdown of host-zooxanthellae symbiosis" (Brown 1997, Porter et al. 2001, Douglas 2003), and results in a whitening appearance of corals due to the loss of these pigmented zooxanthellae. In many cases, disease outbreaks occur following bleaching events (Porter et al. 2001). For example, following abnormally high sustained water temperatures in 2005 and 2010, massive bleaching events and disease outbreaks occurred on a global scale, devastating coral populations. In areas of the U.S. Virgin Islands, up to 87% of *A. palmata* experienced partial or full mortality during the 2005 bleaching and disease event (Muller et al. 2008). Prior to this, the 1998 El Niño Southern Oscillation (ENSO) event caused mass bleaching and high mortality in the Florida Keys and entire Caribbean (Porter et al. 2001). Since the mid-1970s, ENSO events have become more frequent and have persisted longer than previously observed in the last 5,000 years (Trenberth and Hoar 1996). Increasing greenhouse-gas concentrations resulting in a warming atmosphere are predicted to cause more frequent ENSO events in the future (Timmermann et al. 1999, Donner et al. 2007), which will result in anomalously warm water temperatures for the Caribbean (Donner et al. 2007). As a result, bleaching is predicted to become a biannual or annual event in the Caribbean in 20 to 50 years (Donner et al. 2005, Donner et al. 2007, van Hooidonk et al. 2016). To date, severe bleaching events have already been observed in the years 1981–1982, 1997–1998, 2001–2002, 2005–2006, 2010, and 2014–2016 (van Oppen et al. 2017).

Mass bleaching events have also occurred in the Pacific. Bleaching events in 1998 and 2002 caused 42% bleaching and 54% bleaching, respectively, in nearly 650 monitored reefs in the Great Barrier Reef. Following these events, in the austral summers of 2001 and 2002, the first reports of a rapid-dark-spot-like disease occurred on Pacific corals in the Great Barrier Reef. Colonies of *Montipora aequituberculata* affected by the disease increased to 80% of the study population during this period, and mortality was

observed as 3–4 times above the average mortality (Jones et al. 2004). Bruno et al. (2007) also found a 20-fold increase in white syndrome in study locations in the Great Barrier Reef between 1998 and 2002. Numerous reports of coinciding disease, bleaching, and warm water temperatures have led to a strong association between water temperatures and disease. To date, at least four coral diseases have been strongly associated with warm water temperatures, including black-band disease, white plague, dark spot disease, and aspergillosis (Gil-Agudelo and Garzon-Ferreira 2001, Kuta and Richardson 2002, Patterson et al. 2002).

Increased ocean temperatures can induce coral stress and disease for a variety of reasons. As with many terrestrial organisms, the growth rate of marine bacteria and fungi increase with higher temperature (Harvell et al. 2002). This has been documented in pathogens of black-band disease (*Phormidium corallyticum*) and in *Vibrio*, a bacterial pathogen that induces bleaching in the coral *Oculina patagonica* (Kushmaro et al. 1997, Toren et al. 1998, Porter et al. 2001). Additionally, habitat and/or range expansion of potential pathogens can occur as temperatures increase (Harvell et al. 2002). Meanwhile, host immunity can decrease as a physiological stress response to rising temperatures (Bruno 2015), making these stressed individuals more susceptible to infection (Scott 1988). This combined host susceptibility and pathogen virulence caused by increased SST put coral reefs at risk for widespread mortality (Harvell et al. 1999, Harvell et al. 2002, Bruno et al. 2007).

However, SST is not solely responsible for increased disease prevalence. Humans have facilitated disease in marine systems through direct transport of pathogens and through habitat degradation for decades (Harvell et al. 1999). Pollutants, increased nutrient input, and increased sediment on coral reefs are believed to affect the holobiont communities within corals (Kuta and Richardson 2002). Kuta and Richardson (2002) described an increase in black-band disease at sites with higher concentrations of ortho-phosphate and nitrite in the Florida Keys. Additionally, Porter et al. (2001) suggested that proximity to densely populated areas may increase the chance of infection. Anthropogenic stressors can disrupt the balance of bacterial and viral communities in the coral, making them more susceptible to pathogens or opportunistic infections (Rosenberg and Ben-Haim 2002, Bruno et al. 2003, Kuntz et al. 2005). Together, increased SST and

anthropogenic degradation of water quality are likely to cause an increase in disease severity in future years (Bruno et al. 2003, Bruno et al. 2007).

The Intergovernmental Panel on Climate Change (IPCC) estimates that the average SST will continue to rise between 1.8–4.0 °C by the end of the 21st century (Hoegh-Guldberg et al. 2007). As temperatures rise, more frequent mass bleaching events and subsequent disease outbreaks can be expected, putting the health of global reefs at risk (Hoegh-Guldberg 1999, Porter et al. 2001, Knutson et al. 2010). To survive in the future climate, Donner et al. (2005) suggested that corals would need to increase their thermal tolerance by 0.2–1.0 °C per decade. This may only be achievable by temperature- and/or disease-resistant genotypes that are able to survive and reproduce.

1.6   Coral Restoration Efforts

Decades of reef degradation and coral mortality from bleaching and disease have led to an increased need for restoration. To date, more than 117 coral species have been grown for restoration purposes in coral nurseries throughout the world (Rinkevich 2014). As restoration techniques continue to improve, an increase in the number of species grown, colonies outplanted, and survival of these colonies can be expected (Rinkevich 2014). Restoration strategies have already expanded in the form of coral transplantations, production of artificial reefs, and more recently, "coral gardening," through *in situ* and *ex situ* coral nurseries (Rinkevich 2005). These nurseries can contain thousands of fragments growing simultaneously and are considered more effective than coral transplantation due to the ability to preserve donor colonies and increase genetic diversity of outplanted corals (Rinkevich 2006, Shafir et al. 2006, Rinkevich 2014).

The concept of coral nurseries (Rinkevich 1995, Shafir et al. 2006) consists of two major objectives: (1) culturing small fragments of wild coral in either *in situ* or *ex situ* nurseries and (2) planting the grown corals on degraded reef sites (Shafir et al. 2006). These are typically established through the collection of very small fragments from local donor, or wild, colonies (Rinkevich 2005, Young et al. 2012), which are grown on substrate-based table nurseries, hanging line nurseries, or floating "tree" nurseries (Rinkevich 2006, van Oppen et al. 2015). As with the coral species of interest, the environment and geographic location determine the appropriate nursery type. Coral

fragments are typically grown and maintained in nurseries for 1–2 years before reaching optimal size for outplantation to a local degraded reef (Rinkevich 2014). There are a variety of effective outplanting methods, such as attaching colonies to the substrate with underwater epoxy, fixing corals onto reef rubble using cable ties, or mounting colonies onto fixed structures to be placed onto the reef (Jaap 2000, Rinkevich 2014). This process has successfully added thousands of coral colonies to degraded reefs throughout the world.

While the species in culture vary by nursery and location, a majority of nurseries focus on corals with high growth rates and/or those that are under environmental pressures or endangerment (Rinkevich 2006). In the Caribbean, *A. cervicornis* and *A. palmata* are common nursery species for all of these reasons (Young et al. 2012). More than 60 restoration projects involving acroporid corals exist in the Caribbean alone, including locations in Florida, Mexico, Belize, Honduras, Puerto Rico, Jamaica, Antigua, Barbados, the Dominican Republic, and the Bahamas (Young et al. 2012). Nearly 40 of these utilize a nursery concept, many of which have evolved from substrate-supported to mid-water floating nurseries (Young et al. 2012). However, creating and maintaining coral nurseries can be costly. While the cost to produce one coral from start to finish (outplant) is estimated at only 50 cents to $1 (Shafir et al. 2006), the amount of coral required to make substantial habitat improvement is vast. It has been estimated that outplanting alone can cost approximately $10,000 per hectare (Spurgeon 2001, Edwards 2010). Additionally, the cost of frequent boat trips and SCUBA staff for regular nursery maintenance can make these efforts even more expensive. For these reasons, current restoration research and advancements focus on developing efficient practices to reduce costs and improving coral survival.

However, long-term success and survival of nursery-raised corals will remain low if ocean conditions fail to improve (Baums 2008, van Oppen et al. 2015). Controlling and improving factors, such as CO_2 emissions and SST rise require a massive global effort, and even immediate and extreme policy changes would take years to affect environmental conditions. Alternatively, culturing corals that are well adapted to survive in current and future conditions can be done relatively quickly, and may be necessary to

preserve today's coral reefs, as corals may not be able to rapidly adapt to these changing conditions (van Oppen et al. 2015).

It has been proposed that selectively breeding corals with the ability to withstand bleaching events or disease outbreaks can increase the probability of their survival as water quality continues to deteriorate (van Oppen et al. 2015). Recent studies have demonstrated that increasing thermal tolerance to bleaching events may be done by manipulating the coral symbiont community. Evidence of specific algal symbiont communities, or clades, with a higher tolerance to warm water temperatures have been found in corals that have previously been exposed to warm water anomalies (Baker et al. 2004, Maynard et al. 2008, Stat and Gates 2011, Guest et al. 2012, Cunning et al. 2015). Introducing more resistant clades to corals in the laboratory is one example of coral modification that can be used to influence resiliency (Stat and Gates 2011, van Oppen et al. 2015). Additionally, exposing certain species of corals to heat in the laboratory may also enhance their thermal tolerance (Middlebrook et al. 2008, Fitt et al. 2009).

The need to quantify disease resistance in corals was also established following the report of natural disease resistance *A. cervicornis* by Vollmer and Kline (2008) in Panama (Hunt and Sharp 2014, Miller and Williams 2016). Multiple studies have since focused on identification of resistant genotypes, many of which have done so using nursery-raised corals in the state of Florida (E. Muller, unpub. data, M. Miller, unpub. data, N. Fogarty, unpub. data, Hightshoe 2018). While focusing restoration efforts on disease-resistant genotypes may increase the survival success of corals during similar disease outbreaks (Vollmer and Kline 2008, Drury et al. 2017), this may not be the case if a new disease caused by a different pathogen or a natural catastrophic event occurs. Instead, these data can help nursery managers plan to repopulation reefs with coral genotypes of diverse traits, thus increasing their probability of reproduction and survival.

1.7   Study Standardization – Acclimation Periods

As resistance to disease in local populations is identified, scientific methods must be standardized to draw comparable conclusions between regions (Miller and Williams 2016). Disease resistance has already been tested using a variety of pathogen-introduction methods, including direct contact and water-borne transmission (Williams and Miller

2005, Vollmer and Kline 2008, Aeby et al. 2010, Kline and Vollmer 2011, Gignoux-Wolfsohn et al. 2012, Hightshoe 2018, E. Muller, unpub. data, N. Fogarty, unpub. data), and conclusions about population resistance have been made using both. However, pathogen virulence may differ based on these methods, which could result in the underestimation of disease resistance and/or susceptibility.

Additionally, in many coral disease transmission studies, acclimation periods differ and there is little discussion of this important step in the extant literature. While Miller and Williams (2016) suggest an acclimation period of at least 2 weeks *in situ* to allow clipped fragment margins to heal, acclimation time has varied greatly among previous studies. For example, Gignoux-Wolfsohn et al. (2012) acclimated *A. cervicornis* fragments in aquaria tanks for 72 hours prior to disease exposure via homogenate. Many other transmission studies fail to mention acclimation period all together and it is possible that some have omitted acclimation from their design completely. This lack of consensus and emphasis on the importance of acclimating manipulated organisms is concerning, particularly in disease studies, as it can make the initial cause of disease indistinguishable from stress from collection and transportation.

1.8   Study Objectives

To predict future population success of *A. cervicornis* in a changing environment, I addressed the following research questions in this study: (1) Do different genotypes of *A. cervicornis* in a local nursery-raised population respond differently to disease-causing pathogens? If so, what genotypes appear to be more susceptible and/or more resistant to tissue-loss disease? (2) Does pathogen transmission method (grafting vs. homogenate) influence the probability of tissue-loss disease? (3) How does acclimation period influence the condition of coral tissue prior to pathogen-transmission application?

I used *A. cervicornis* fragments from the Coral Restoration Foundation (CRF) nursery to provide data that can be useful to nursery management and future restoration efforts locally. My results help identify disease susceptibility and possible resistance in specific genotypes raised at CRF, which can be used to strategically plan growth efforts and outplanting of cultured fragments. This investigation into disease transmission method and acclimation length also fills gaps in current research that will help produce

more standardized and comparable data in the future. This information is crucial for maintaining reef integrity and preventing the extinction of *A. cervicornis* in the years to come.

CHAPTER 2: PATHOGEN TRANSMISSION EXPERIMENT

2.0   Abstract

Unprecedented population losses of the staghorn coral, *Acropora cervicornis,* since the 1970s have been attributed primarily to disease. Although a positive linear relationship between disease prevalence and increased water temperature has been documented, the pathogen(s) causing disease and whether they are spread through the water, or vectors are involved in transmitting them, is still poorly understood. Additionally, an increase in disease outbreaks and severity has provided an urgent need to identify natural genotypic resistance to disease in Caribbean acroporids. Studies to date have explored a variety of pathogen transmission methods, but prior to this study, there has been no examination of differences among common techniques. I investigated pathogen transmission and resistance to development of the disease known as rapid-tissue loss (RTL) in 11 different genotypes by comparing two common transmission methods (direct contact vs. waterborne). Overall, disease was significantly higher in the direct contact treatment; however, tissue-loss rates were not significantly different between treatments. The number of diseased fragments varied greatly by genotype, with only one genotype appearing resistant to developing disease, showing no signs of disease throughout the study. These results highlight the differences between pathogen transmission methods and demonstrate the importance of selecting an appropriate method for future studies.

2.1   Introduction

Although coral cover has declined worldwide, Caribbean acroporids have experienced the highest mortality. Population losses in the Caribbean have reached up to 98 percent at different sites (Aronson et al. 2008), and as a result, both *Acropora cervicornis* and *A. palmata* have been listed as "threatened" under the United States' Endangered Species Act (Hogarth 2006) and as "critically endangered" under the International Union for the Conservation of Nature Red List in 2008 (Aronson et al. 2008, Carpenter et al. 2008, Kline and Vollmer 2011). Many factors have been attributed to mortality, including physical disturbance from storms, overfishing, excess nutrient

input, the loss of symbiotic algae (i.e., coral bleaching) from thermal stress, and most notably, disease (Aronson and Precht 2001).

Disease can be defined as any impairment of normal function within the body, organs, or systems of an organism (Wobeser 1981) that must meet at least two of the following criteria: (1) consistent anatomical alterations to the host, (2) an identifiable group of signs, and/or (3) recognized etiologic or causal agents (Peters 2015). Causal agents may be biotic (typically considered infectious diseases) or abiotic (non-infectious) in nature, but are difficult to identify in marine organisms, such as corals. For example, of the over 18 different diseases described in corals, biotic pathogens have been recognized in about half (Sutherland et al. 2004, Weil 2006, Harvell et al. 2007). Of these, unique bacterial pathogens for only five diseases (white plague-II, white band-II, white pox, aspergillosis, and bacterial bleaching) have fulfilled Koch's postulates, the criteria established to determine the causal biotic pathogen of a disease (Weil 2006, Harvell et al. 2007).

White-band disease (WBD) was one of the first described diseases affecting Caribbean members of the genus *Acropora* (Aronson and Precht 2001). Acroporids affected by WBD exhibit clear tissue degradation, which can be characterized by either a distinct line between coral skeleton and living tissue (Type I), or a temporary separation of bleached tissue (devoid of algal symbionts) between coral skeleton and normally pigmented tissue (Type II) (Ritchie and Smith 1998). Tissue loss rate from WBD in *A. palmata* have ranged from 0.2 to 2 cm per day, with an average rate of 0.5 cm per day (Antonius 1981, Gladfelter 1982). Since the discovery of WBD Type I in 1977, several possible etiologic agents for this disease have been proposed, but no single cause has been confirmed (Peters et al. 1983, Ritchie and Smith 1998, Casas et al. 2004, Gil-Agudelo et al. 2006, Sweet et al. 2014). Many other diseases and syndromes have been described with signs similar to WBD since the late 1970s, such as rapid tissue loss (RTL), a common affliction that can visually appear analogous to WBD (Williams and Miller 2005, Weil 2006, Williams et al. 2006). However, this disease is characterized by tissue loss occurring within portions or entire branches of *A. cervicornis* or *A. palmata* without a clear progression pattern. Affected corals exhibit rapid sloughing of tissue, up to 4 cm per day, which leaves behind irregular areas of denuded skeleton (Williams and

Miller 2005). It is unclear whether previously published literature has correctly differentiated between WBD and RTL, or if these two afflictions are caused by different pathogens, further complicating identification and histological analysis of these coral diseases (Miller et al. 2014).

The pathogen(s), vectors, and transmission of pathogen(s) for WBD and RTL are poorly understood (Richardson and Aronson 2000, Lesser et al. 2007, Merselis et al. 2018). However, a positive correlation has been established between WBD prevalence and rising ocean temperatures (Muller et al. 2008, Brandt and McManus 2009, Miller et al. 2009). The Intergovernmental Panel on Climate Change (IPCC) estimates that the average sea-surface temperature (SST) will continue to rise between 1.8–4.0 °C by the end of the 21st century (Hoegh-Guldberg et al. 2007). As temperatures rise, more frequent disease outbreaks are expected, putting the health of global reefs at further risk (Hoegh-Guldberg 1999, Porter et al. 2001, Knutson et al. 2010). Survival of populations may only be achievable by thermally tolerant (Donner et al. 2005) and/or disease-resistant genotypes (Miller and Williams 2016).

The need to quantify disease resistance in corals was established following the report of natural disease resistance in *A. cervicornis* by Vollmer and Kline (2008) in Panama (Hunt and Sharp 2014, Miller and Williams 2016). Multiple studies have since focused on identification of resistant genotypes, many using nursery-raised corals in the state of Florida (Hightshoe 2018, E. Muller, unpub. data, M. Miller, unpub. data). Data on genotypic resistance may help nursery managers reduce costs and improve restoration efficiency by understanding which genotypes are disease resistant and likely to survive in future disease outbreaks (Vollmer and Kline 2008, Drury et al. 2017). Disease resistance has been tested using a variety of pathogen-introduction methods, most commonly tested using direct contact and exposure to homogenized diseased tissue (Williams and Miller 2005, Vollmer and Kline 2008, Aeby et al. 2010, Kline and Vollmer 2011, Gignoux-Wolfsohn et al. 2012, E. Muller, unpub. data). Pathogen virulence may differ based on these methods; therefore, there is a clear need to standardize pathogen-transmission methods to produce comparable genotype screenings or at least identify if virulence differs between methods for proper interpretation.

To compare pathogen-transmission methods and predict future population success of *A. cervicornis* in a changing environment, I addressed the following research questions in this study: (1) Do different genotypes of *A. cervicornis* in a Florida nursery population respond differently to pathogen(s) associated with diseased corals? If so, what genotypes appear to be more susceptible and/or more resistant to developing disease? (2) Does pathogen-transmission method (grafting vs. homogenate) influence the probability of disease?

2.2   *Materials and Methods*

In October 2016, apparently healthy and diseased *Acropora cervicornis* fragments were collected from the Coral Restoration Foundation nursery (N 24° 58.933', W 80° 26.180') in Tavernier, Florida Keys. Temperature in the nursery was estimated using data from nearby long-term monitoring sites established by the Florida Fish and Wildlife Coral Reef Evaluation and Monitoring Project (CREMP) as approximately 26.5 °C on collection date (10/24/16). Based on preliminary research, the following genotypes were selected for the pathogen-transmission study as "disease-susceptible" genotypes: U72, U30, U22, U21, U17, U68 (M. Hightshoe, unpub. data), K2, M5, U25, and U41 (M. Miller, unpub. data). U77 was the only collected genotype with previous evidence of relative disease resistance (M. Miller, unpub. data). To eliminate the possibility of divers acting as vectors of disease, all apparently healthy and diseased coral fragment collections were conducted on separate dives, using designated clippers and gloves, as well as separate coolers for transportation. Twelve (~8 cm) fragments were collected from each of the 11 different genotypes (n = 132). Additionally, three apparently healthy fragments from 11 additional randomly selected genotypes (n = 33) were collected from the nursery to serve as control fragments for the graft treatment. Based on the availability of diseased corals in the nursery, 72 diseased fragments showing signs of RTL were collected from additional randomly selected genotypes following the collection of all apparently healthy fragments. All collected samples were separated by health into two 45-L Igloo© coolers and genotypes were wrapped together using seawater-soaked bubble wrap for transportation to the Nova Southeastern University (NSU) Oceanographic Center's SEACOR experimental aquaria system. Coolers were filled to ¼ of their volume with ambient seawater, and wrapped fragments were arranged in a single floating layer.

A 50-percent water change was conducted after approximately 1.5 hours of transportation (½ of distance) to remove excess mucus from the water and provide oxygen exchange.

At NSU, experimental fragments were separated into a total of 12, 113-L tanks, such that each tank contained one fragment from each genotype. Three tanks were designated as either control tanks or experimental tanks for each exposure method (homogenate and control) (Fig. 1). Fragments were suspended in the tanks using monofilament and crimps attached to an egg crate screen, and organization of fragments was randomized within each tank (Fig. 2). Each tank contained a powerhead for water circulation, an air stone, and a heater. Immediately before introduction into the tanks, clippings (~2 cm) were taken from all fragments for later histological analysis. Clippings from each fragment in the two exposure method treatment tanks (6 tanks, n = 66 clippings) were saved for later histological analysis, while only a subset of fragments from one control tank per exposure method (grafting or homogenate) were saved for analysis (2 tanks, n = 22 clippings). However, all fragments were clipped to standardize potential stress experienced from clipping. Diseased fragments were kept in separate holding tanks during this time and were monitored for continuing tissue loss by placing a cable-tie on the tissue-loss margin of each fragment. An additional 1 cm per day increase in denuded skeleton was required for the fragment to be used in this study. Because of rapid tissue loss from diseased fragments in the holding tanks, the acclimation time of experimental fragments was limited to 40 hours.
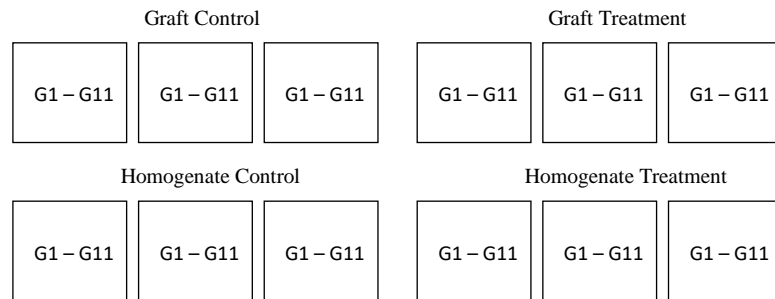

Figure 1. Experimental design of transmission study. Tanks were designated as graft or homogenate exposure controls and graft or homogenate exposure treatments, each containing replicates of 11 different genotypes (G1-11) hanging in random order within their tank.

Figure 2. Experimental hanging design. Fragments were hung into tanks from egg crate using 12-inch long monofilament and crimps. Each fragment was spaced approximately 2.5 inches apart and the location of each fragment was randomized in each tank. The egg crate rested on the top of the tank and corals were suspended in the middle of water column.

Following acclimation, grafting and homogenate pathogen(s) transmission methods described in previous studies were compared (Vollmer and Kline 2008, Kline and Vollmer 2011, Gignoux-Wolfsohn et al. 2012, E. Muller, unpub. data). For the grafting treatment, randomly selected active-diseased fragments were cable-tied directly onto experimental fragments. Apparently healthy fragments were randomly cable-tied to experimental fragments in control tanks. To directly compare the transmission of pathogen(s) through contact (grafting) vs. the water column (homogenate), the homogenate was prepared using the same number of fragments used in the grafting treatment (11 per tank). Beaded cable-ties were also applied to all homogenate treatment and their control fragments to account for abrasion experienced in the grafting treatment. Each homogenate slurry was prepared by removing all present tissue from 11 diseased fragments using an airbrush and 0.2 µm-filtered seawater, which was collected in a re-sealable plastic bag. This process was replicated for each homogenate exposure tank and the total volume of the slurry was approximately 250 mL per tissue collection. This slurry was added directly to the water column of each homogenate treatment tank at the start of the experiment, and their control tanks received an equal volume of 0.2 µm-filtered seawater.

Tanks were maintained at a constant ambient collection temperature of approximately 27°C throughout the experiment, and temperature, dissolved oxygen, and

pH were monitored daily. Fifty percent water changes were conducted every two days to maintain water quality within tanks. All corals were monitored for signs of disease and photographed daily for nine days. General health and percentage of tissue versus denuded skeleton were recorded. Photographs were taken beside a Coral Health Chart established by The University of Queensland's CoralWatch, and a ruler for scale. Post-exposure treatment histology samples were taken when fragments were removed from the experiment, which occurred when tissue loss had affected 50 percent of the fragment or at the end of the 9-day period. Overall disease prevalence at the end of the 9-day experimental period was calculated in each treatment group and their control group, as the proportion of individuals with tissue-loss disease. Mean proportion of disease present among treatments and controls were compared using a one-way ANOVA and between the pairs of homogenate-exposed and grafting-exposed treatments and their controls using separate two-tailed two-sample *t*-tests. To directly compare the number of diseased fragments in exposed and controls by treatment method per genotype, a Bayesian relative risk assessment transformed to the log scale was used. Survival (or probability of becoming diseased) in each treatment method was compared using a right-censored Mantel-Haenszel survival analysis. Rate of tissue loss was also calculated for each fragment using ImageJ software, and was compared among genotypes using a one-way ANOVA, and between treatment methods using a two-tailed two-sample *t*-test.

2.2.1   Histological Analysis

Tissue samples that were taken prior to and after grafting or homogenate exposure were used to identify changes occurring on the cellular/tissue level in response to the pathogen transmission methods (Work and Meteyer 2014). All clippings (~2 cm) were taken using handheld wire cutters and were placed in labeled 50-mL plastic falcon tubes with Z-Fix Concentrate (Anatech, Ltd.) in a 1:4 dilution in seawater. Samples were stored indoors in the NSU Histology Laboratory for 2–4 months prior to processing. To decalcify, each sample was removed from fixative, photographed, and trimmed if necessary. Diseased samples were trimmed to include their tissue loss margin. Samples were decalcified using a 5% decalcifying solution (1.5 g ethylenediaminetetraacetic acid, 150 mL hydrochloric acid, and 2.85 L seawater), which was changed every 24 hours for 3–4 days until samples were completely decalcified. Once decalcified, samples were cut

longitudinally using a clean razor blade and were placed in cassettes with 70% ethanol. Cassettes were processed through a graded series of ethanol concentrations, cleared with xylene, and infiltrated with molten paraffin wax. Samples were embedded into blocks using paraffin wax and were sectioned at a 4-µm thickness. Sections were mounted onto glass microscope slides and were stained with Harris's hematoxylin and eosin before being coverslipped using Cytoseal 60 ™ mounting medium.

Samples were examined using an Olympus BX 43 light microscope and computer imaging. Samples were scored using a semi-quantitative (Jagoe 1996) approach in collaboration with Dr. Esther Peters and Morgan Hightshoe (pers. comm.) using rubrics previously developed in Miller et al. (2014) (Table A1). Histoslide of *A. cervicornis* and *A. palmata* from the Florida Keys in the 1970s, collected before tissue loss was reported, were used to develop a baseline for excellent condition (Miller et al. 2014). The following parameters were ranked by severity of the change compared to the 1970s samples or relative condition, respectively (0 = No Change, Excellent; 1 = Minimal, Very Good; 2 = Good, Mild; 3 = Moderate, Fair; 4 = Marked, Poor; 5 = Severe, Very Poor): epidermal mucocytes, costal tissue loss, zooxanthellae in the surface body wall, cnidoglandular band epithelium mucocyctes, degeneration of cnidoglandular bands, dissociation of cells on mesenterial filaments, gastrodermal architecture in the basal body wall (BBW), and calicodermis condition. Parameter scores were summed to produce an overall condition score for each sample.

To confirm that visually healthy samples exposed to diseased fragments by grafting or homogenate were healthy on the microscopic scale, tissue scores for post-exposure treatment apparently healthy (n = 14), and post-exposure treatment visually diseased samples (n = 6), were compared to apparently healthy post-exposure control fragments (n = 8) collected at the end of the experiment. To identify the condition of samples prior to disease exposure, pre-exposure treatment samples that later became diseased (pre-exposure treatment later visually diseased samples; n = 14) were also compared to pre-exposure treatment samples that later appeared healthy (pre-exposure treatment later apparently healthy samples; n = 14), and pre-exposure control samples that later appeared healthy (unexposed controls later apparently healthy samples; n = 8) at the end of the experiment. Overall condition of pre-exposure treatment apparently healthy

(n = 14) and post-exposure treatment apparently healthy samples (n = 14) were also compared, in addition to pre-exposure treatment later visually diseased (n = 14) and post-exposure treatment visually diseased (n = 6) to determine if the condition of the same samples changed over time. Descriptive statistics were calculated for each parameter in each sample group (Table 2, Table 3). Differences between each parameter score for each group were determined using non-parametric Kruskal-Wallis tests, whereas overall specific condition scores were compared using a one-way ANOVA or two-sample *t*-test.

2.2.2 Pathogen Containment and Disinfection

Because the pathogen(s) causing WBD and RTL are not well understood, precautions were taken to eliminate the potential of spreading them. During collections, divers handled apparently healthy and diseased fragments on separate dives. Additionally, diseased fragments were collected last, and handling involved the use of gloves, designated clippers, and separate containers/coolers for transportation. All clippers, containers, and coolers that were used for diseased sample collection were washed with a 10 percent bleach solution during clean-up. Precautions were also taken when changing water on tanks containing diseased samples. Water from diseased fragment holding and treatment tanks was siphoned into a collection container, and then pumped into a designated wastewater tank provided by NSU. All power heads, air stones, and heaters that were used in experimental tanks were also washed with a 10% bleach solution during clean-up. Coral fragments exposed to diseased fragments in treatments were bleached, and skeletons were donated for educational purposes. All apparently healthy fragments were maintained in captivity for future research.

2.3   Results

2.3.1 RTL Pathogen Transmission

Throughout the acclimation and experimental period, tanks were maintained at an average temperature of 27.19 ± 0.08 °C, 99.32 ± 0.18 % dissolved oxygen (mean ± S.E.), and pH between 8.1 and 8.2. During the 40-hour acclimation period, 8 fragments from the following genotypes exhibited signs of disease: U21 (n = 1), U22 (n = 4), U30 (n = 2), and U17 (n = 1; Table 1).

Table 1. Total number of diseased fragments during acclimation and during the experiment by genotype. Each of the treatments and controls were summed to produce the total number of diseased fragments during the experiment (maximum n = 6).

Genotype	Total number diseased during acclimation	Total number diseased controls during experiment	Total number diseased in treatment during experiment
K2	0	3	1
M5	0	0	1
U17	1	0	2
U21	1	0	1
U22	4	1	3
U25	0	1	3
U30	2	2	2
U41	0	1	1
U68	0	1	1
U72	0	0	0
U77	0	0	2

Fragments that became diseased during acclimation were removed from tanks and from all later analyses, because this disease occurred prior to the introduction of pathogen transmission methods. Total number of fragments in each tank and number of replicates were adjusted during statistical analyses to account for the removal of these fragments.

Overall, the grafting treatment resulted in a higher mean proportion of diseased individuals when compared to the homogenate treatment (two-sample t-test, $t(3.82) = 3.10$, p = 0.038, Table 1, Fig. 3), and the probability of remaining apparently healthy was significantly lower in the grafting treatment than the homogenate (right-censored Mantel-Haenszel survival analysis, $\chi^2 (1) = 7.9$, p = 0.005, Fig. 4). However, disease prevalence was not significantly different when compared among all treatments and controls (one-way ANOVA, $F(3,8) = 2.89$, p = 0.101), or between each treatment and their corresponding controls (two-sample t-test, $t(3.95) = -0.085$, p = 0.936 homogenate, $t(3.49) = -1.80$, p = 0.155 graft).

Table 2. Number of diseased fragments after the 9-day experimental period by tank. Proportion reflects the total number of fragments within the tank after pre-experimentation (acclimation) diseased corals were removed.

Tank/Treatment	Number of Diseased Fragments	Total Fragments in Tank	Proportion Diseased	Genotypes Diseased
Homogenate 1	1	11	0.09	U77
Homogenate 2	0	9	0	
Homogenate 3	2	9	0.22	U30, U25
Control 1 Homogenate	2	10	0.20	U22, K2
Control 2 Homogenate	0	10	0	
Control 3 Homogenate	1	11	0.09	K2
Graft 1	6	11	0.55	U22, U30, U21, U17, K2,U68
Graft 2	3	11	0.27	U22, U25, U41
Graft 3	5	11	0.45	U22, U77, U25, U17, M5
Control 1 Graft	4	10	0.40	U68, U25, U41, K2
Control 2 Graft	1	11	0.09	U30
Control 3 Graft	1	10	0.10	U30

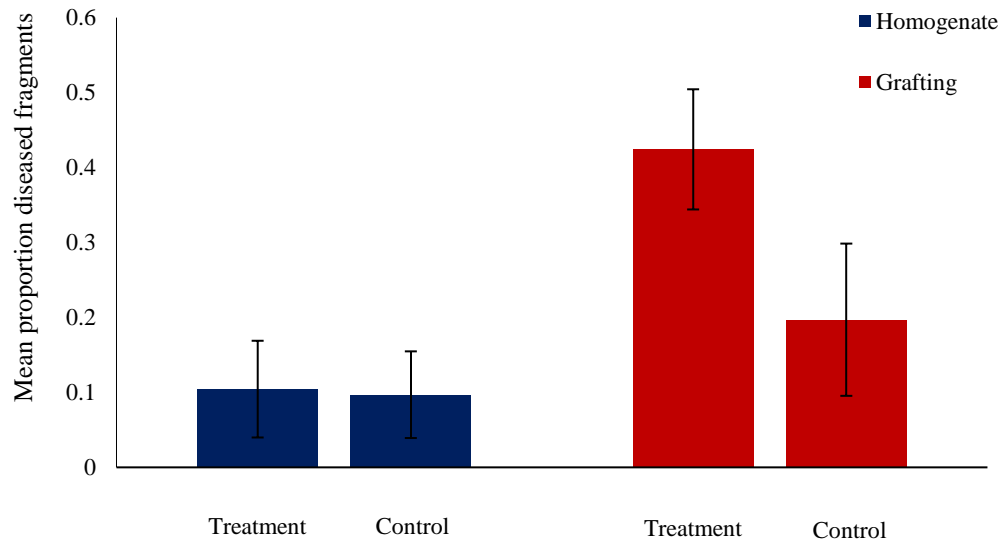
Figure 3. Mean proportion of total fragments that showed signs of disease per treatment over the 9-day experimental period. Bars represent ± 1 S.E. Mean proportion of diseased fragments in the grafting treatment was significantly greater than the homogenate treatment (two-sample *t*-test, $t(3.82) = 3.10$, p = 0.038) but not between each treatment and their corresponding controls (p>0.05).
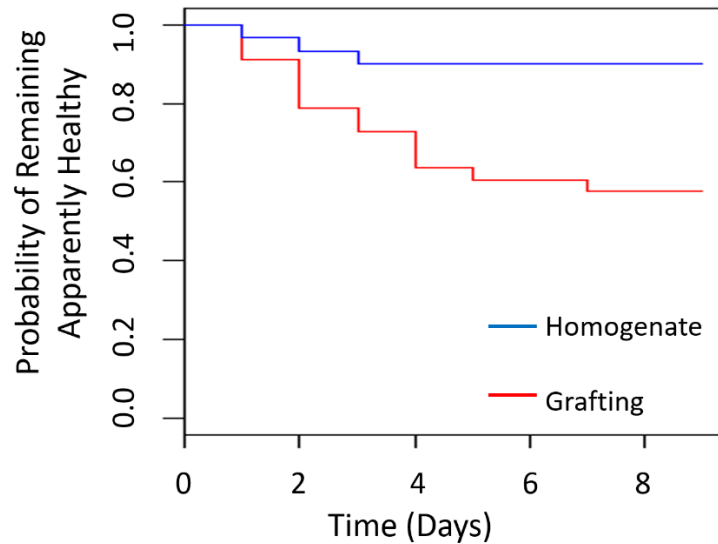
Figure 4. Mantel-Haenszel survival analysis. Treatment significantly affected the probability of remaining apparently healthy over time. The probability of becoming diseased was significantly higher in the grafting treatment when compared to the homogenate (χ^2 (1) = 7.9, p = 0.005).

Number of diseased individuals after exposure varied greatly by genotype and by treatment (Fig. 5, Fig. 6). Only three genotypes (n = 3 total fragments) exhibited signs of disease in the homogenate treatment, whereas 10 genotypes (n = 14 total fragments) exhibited signs of disease in the grafting treatment. In the homogenate treatment, genotype K2 appeared to be slightly less susceptible to disease, although no genotypes were significantly resistant or susceptible because all values were not greater or less than 1 in the Bayesian relative risk assessment (Fig. 7). Similarly, in the grafting treatment, some genotypes, such as U17, M5, and U77, appeared slightly more disease susceptible, but none were significantly different from their controls (Fig. 8). When comparing the homogenate treatment to the grafting treatment using the same approach, all genotypes except for U72, U77, U25, and U30 appeared slightly more susceptible to disease in the grafting treatment. However, no significant differences between treatments were found when comparing the number of diseased fragments with their controls (Fig. 9).

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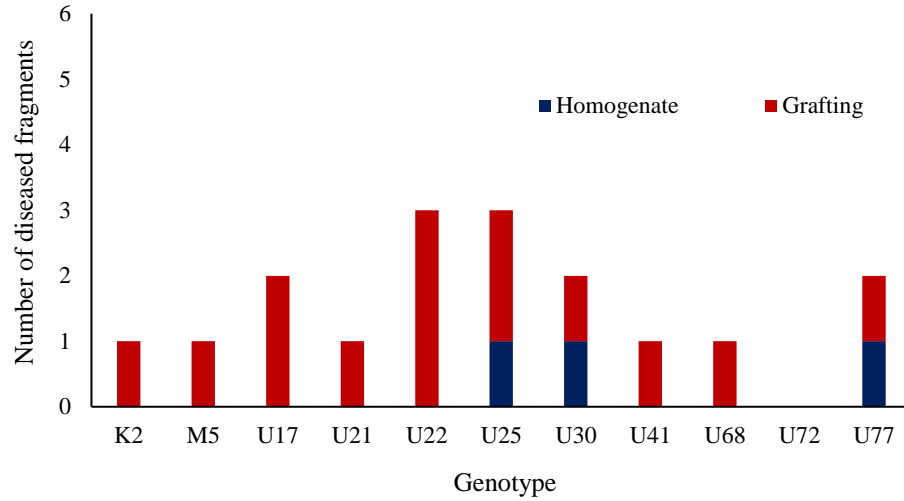
Figure 5. Number of experimental fragments that showed signs of disease over the 9-day experimental period by genotype in the homogenate and grafting exposure treatments.
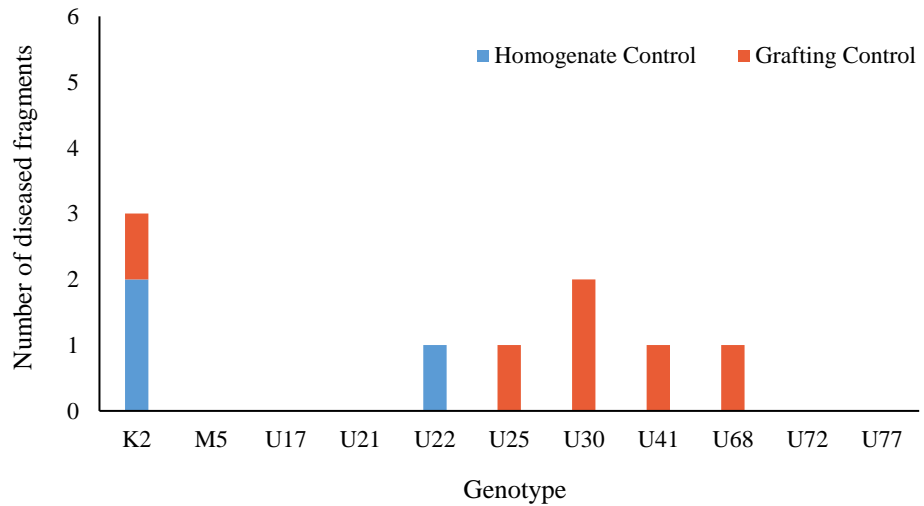
Figure 6. Number of control fragments that showed signs of disease over the 9-day experimental period by genotype in the homogenate control and grafting control exposure treaments.
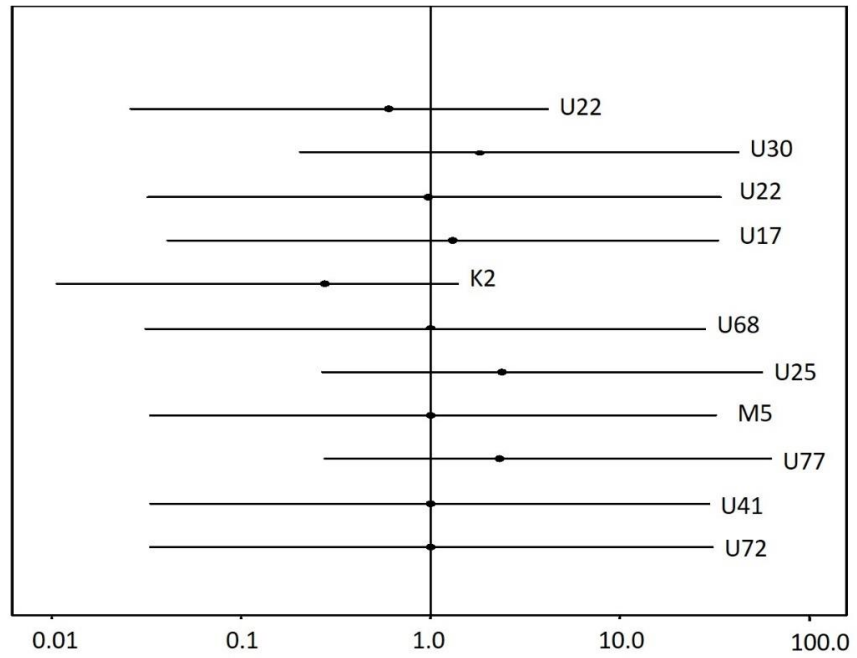
Figure 7. Bayesian relative risk comparison between disease incidences in each genotype for the homogenate treatment and control. All values were transformed to a logarithmic scale.
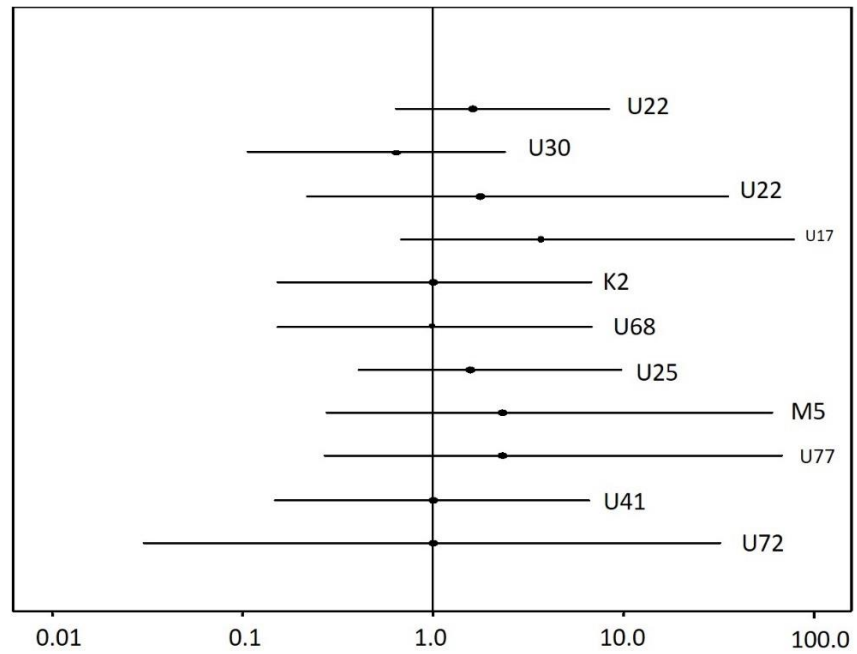
Figure 8. Bayesian relative risk comparison between disease incidences in each genotype for the grafting treatment and control. All values were transformed to a logarithmic scale.
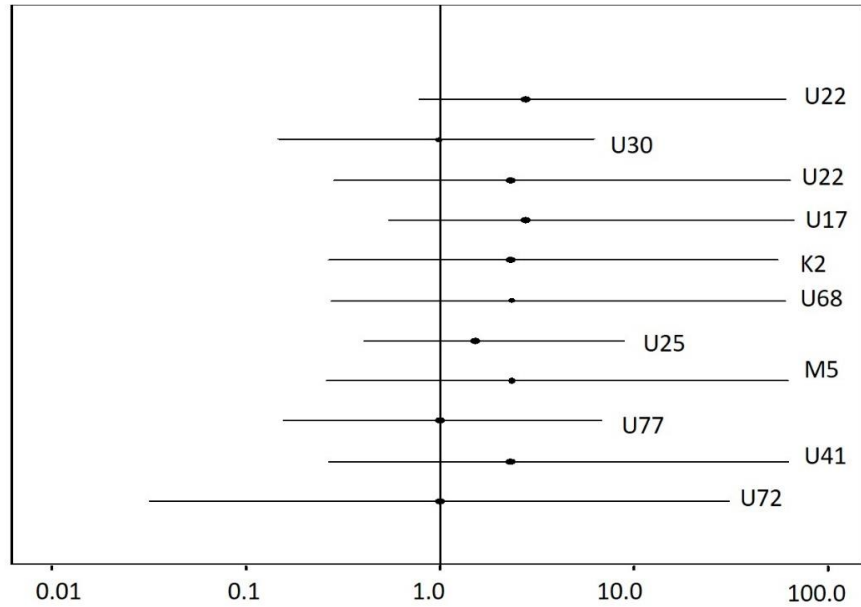
Figure 9. Bayesian relative risk comparison between disease incidences in each genotype for the homogenate treatment and the grafting treatment. All values were transformed to a logarithmic scale.

Rate of tissue loss was not significantly different between treatments (two-sample *t*-test, $t(2.72) = -0.78$, p = 0.493), suggesting that there is no difference in tissue-loss rate between treatment methods once a fragment is exposed to a diseased fragment, or diseased homogenate (Fig. 10). Mean tissue loss rate was 1.82 ± 0.35 cm day^{-1} in the grafting treatment, 2.53 ± 0.84 cm day^{-1} in the homogenate treatment, 2.48 ± 1.14 cm day^{-1} in the control grafting treatment, and 2.00 ± 0.40 cm day^{-1} in the control homogenate treatment (mean $\pm$ S.E). To calculate tissue-loss rate by genotype, all diseased samples were included in the mean calculation, regardless of treatment. This was necessary to increase sample size and produce a mean tissue-loss rate for each genotype. Rate of tissue loss was highest in genotypes U30 and U22; however, there was no significant difference among any genotypes (non-parametric Kruskal-Wallis test, χ^2 (9) = 13.14, p = 0.156, Fig. 11, Fig. 12).
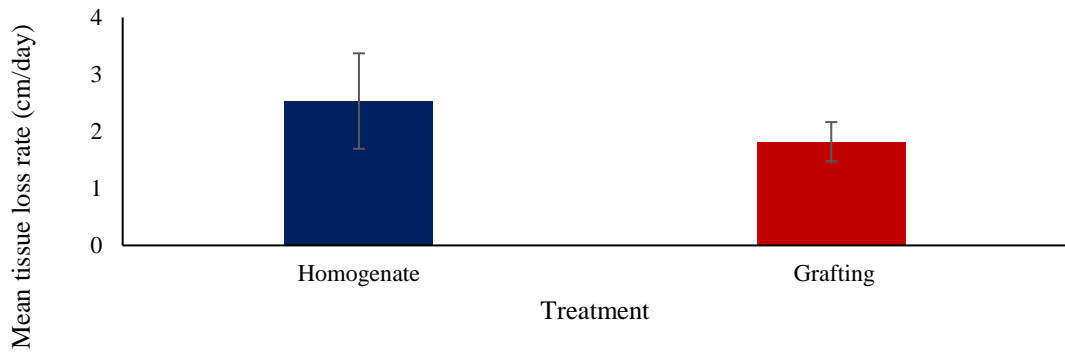
Figure 10. Mean rate of tissue loss between homogenate and grafting exposure treatment. Bars represent ± 1 S.E. No significant difference between tissue-loss rate was found (two sample *t*-test, $t(2.72) = -0.788$, p = 0.493).
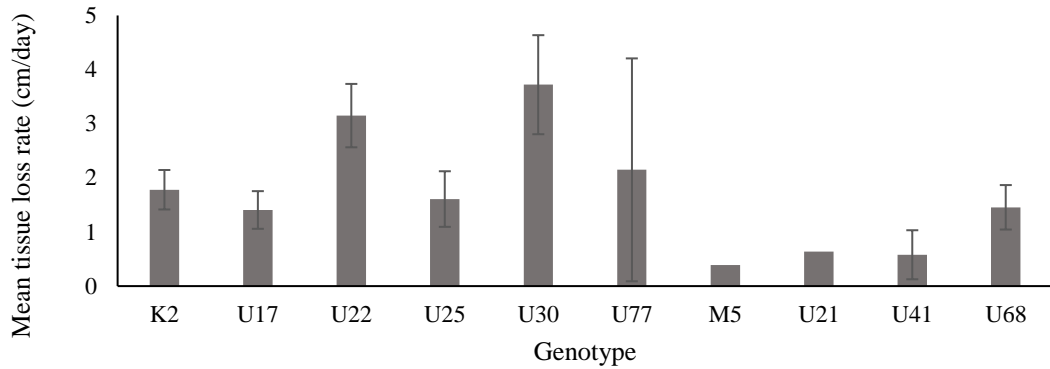
Figure 11. Mean rate of tissue loss by genotype. All fragments with disease were included to obtain a larger sample size and therefore included all treatment and control diseased individuals. Bars represent ± 1 S.E. No significant differences were found between genotypes (non-parametric Kruskal-Wallis test, $\chi^2 (9) = 13.14$, p = 0.156).
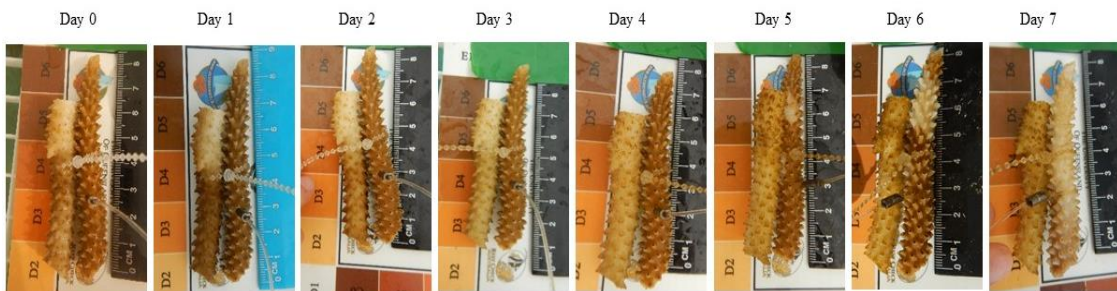
Figure 12. Photo series of U17 fragment exposed to the grafting treatment. Signs of disease were first observed on day 5 and continued to progress until the fragment was void of tissue on day 7. This time series illustrates the rapid progression observed in many experimental fragments.

2.3.2 Histopathology Results

Overall condition score of post-treatment visually diseased samples was significantly worse than post-treatment apparently healthy and control-post samples (one-way ANOVA, $F(2,25) = 3.74$, $p = 0.037$). The following parameter scores were significantly different among groups: dissociation of cells on mesenterial filaments (Kruskal-Wallis test, $\chi^2 (2) = 8.30$, $p = 0.015$), gastrodermal architecture (Kruskal-Wallis test, $\chi^2 (2) = 12.05$, $p = 0.002$), and calicodermis condition (Kruskal-Wallis test, $\chi^2 (2) = 8.69$, $p = 0.012$, Fig 13). In all three parameters, post-exposure treatment visually diseased samples were in significantly worse condition than both post-exposure treatment apparently healthy samples, and post-exposure control apparently healthy samples. There were no significant differences between post-exposure treatment apparently healthy samples and post-exposure control apparently healthy samples in any parameter (Table 3).

Figure 13. Mean parameter score for post-exposure treatment and control samples. Bars represent ± 1 S.E. Significant differences were found between sample groups in the following parameters: dissociation of cells on mesenterial filaments (p= 0.015), gastrodermal architecture (p= 0.002), and calicodermis condition (p= 0.012, Kruskal-Wallis tests).

Table 3. Summary of histopathology results for post-exposure samples. Severity/intensity or condition scores ranged from 0 = No Change, Excellent; 1 = Minimal, Very Good; 2 = Mild, Good; 3 = Moderate, Fair; 4 = Marked, Poor; and 5 = Severe, Very Poor for each parameter.

Parameter	Post- exposure control apparently healthy				Post-exposure treatment apparently healthy				Post-exposure treatment visually diseased			
	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max
Epidermal Mucocytes	2.3	0.2	2	3	2.4	0.1	2	3	2.8	0.4	2	4
Costal Tissue Loss	1.9	0.2	1	3	2.0	0.2	1	3	2.2	0.4	1	3
Zooxanthellae in SBW (40X)	2.9	0.3	2	4	3.1	0.2	2	4	2.8	0.2	2	3
Cnidoglandular Band Epithelium Mucocytes	1.8	0.3	1	3	1.5	0.2	1	3	1.5	0.2	1	2
Degeneration of Cnidoglandular Bands	3.0	0.3	2	4	2.9	0.2	2	4	2.8	0.4	2	4
Dissociation of Cells on Mesenterial Filaments	2.8	0.2	2	3	2.9	0.2	2	4	3.8	0.3	3	5
Gastrodermal Architecture: BBW	1.9	0.3	1	3	2.1	0.2	1	4	3.7	0.2	3	4
Calicodermis Condition	2.5	0.3	2	4	2.4	0.2	2	4	3.7	0.3	2	4
Overall Specific Condition Score	18.9	1.1	16	25	19.1	0.7	16	25	22.5	1.3	18	26

Similarly, pre-exposure treatment later visually diseased samples were in significantly worse overall condition than both pre-exposure treatment later apparently healthy samples and control later apparently healthy samples (one-way ANOVA, $F(2,31)$ = 11.29, p = 0.0002). The same parameters were also significantly different between these sample groups: dissociation of cells on mesenterial filaments (Kruskal-Wallis test, $\chi^2 (2) = 10.31$, p = 0.005), gastrodermal architecture (Kruskal-Wallis test, $\chi^2 (2) = 10.64$, p = 0.004), and calicodermis condition (Kruskal-Wallis test, $\chi^2 (2) = 11.93$, p = 0.002), in addition to costal tissue loss (Kruskal-Wallis test, $\chi^2 (2) = 7.09$, p = 0.028, Fig 14, Table 4).
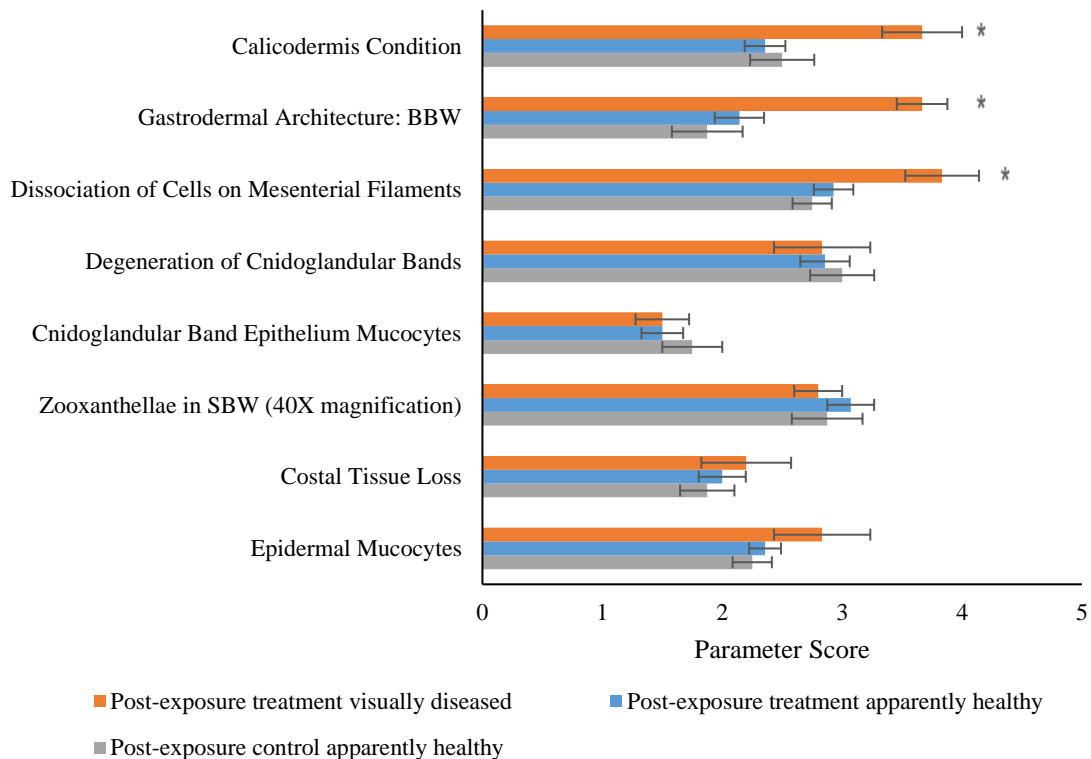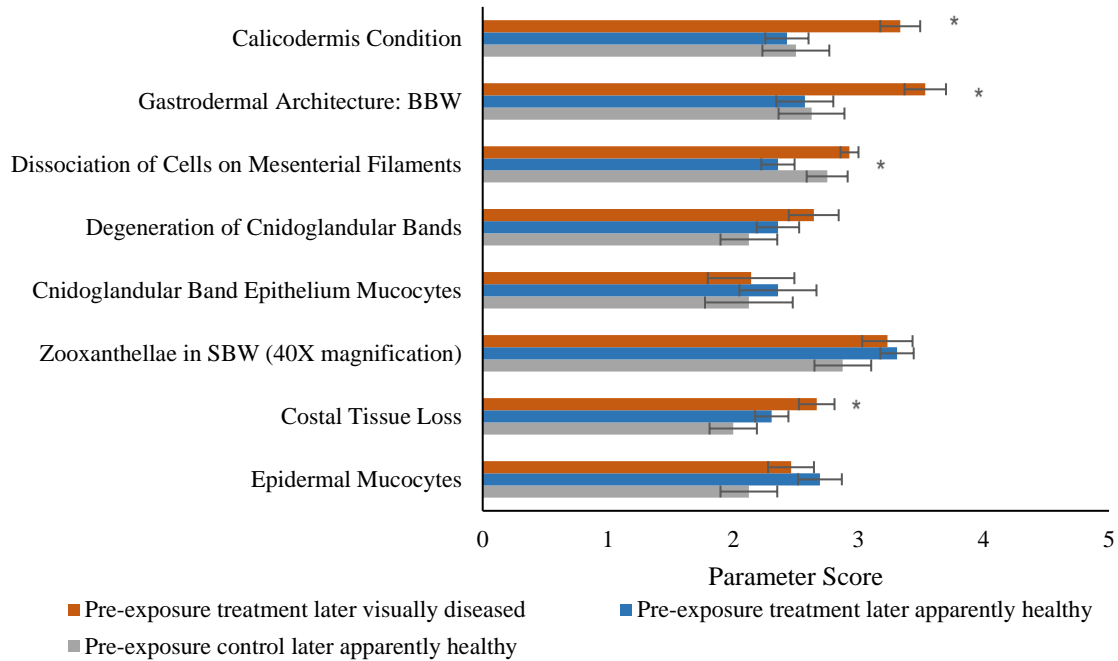
Figure 14. Mean parameter score for pre-exposure treatment and control samples. Bars represent ± 1 S.E. Significant differences were found between sample groups in the following parameters: costal tissue loss (p = 0.028), dissociation of cells on mesenterial filaments (p = 0.005), gastrodermal architecture (p = 0.004), and calicodermis condition (p = 0.002, Kruskal-Wallis tests).

Table 4. Summary of histopathology results for pre-exposure samples. Severity/Intensity or Condition scores ranged from 0 = No Change, Excellent; 1 = Minimal, Very Good; 2 = Mild, Good; 3 = Moderate, Fair; 4 = Marked, Poor; and 5 = Severe, Very Poor for each parameter.

Parameter	Pre-exposure control later apparently healthy				Pre-exposure treatment later apparently healthy				Pre-exposure treatment later visually diseased			
	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max
Epidermal Mucocytes	2.1	0.2	1	3	2.7	0.2	1	3	2.5	0.2	2	4
Costal Tissue Loss	2.0	0.2	1	3	2.3	0.1	2	3	2.7	0.1	2	3
Zooxanthellae in SBW (40X)	2.9	0.2	2	4	3.3	0.1	3	4	3.2	0.2	2	4
Cnidoglandular Band Epithelium Mucocytes	2.1	0.4	1	4	2.4	0.3	1	4	2.1	0.3	1	4
Degeneration of Cnidoglandular Bands	2.1	0.2	1	3	2.4	0.2	1	3	2.6	0.2	2	4
Dissociation of Cells on Mesenterial Filaments	2.8	0.2	2	3	2.4	0.1	2	3	2.9	0.1	2	3
Gastrodermal Architecture: BBW	2.6	0.3	2	4	2.6	0.2	2	4	3.5	0.2	2	4
Calicodermis Condition	2.5	0.3	2	4	2.4	0.2	2	4	3.3	0.2	2	4
Overall Specific Condition Score	19.1	0.5	18	22	20.0	0.5	17	24	22.8	0.6	18	27

No significant difference was found between the overall condition score of pre-exposure treatment later apparently healthy and post-exposure treatment apparently healthy samples (two-sample *t*-test, $t(23.629) = -1.12$, p = 0.270). In fact, many of their parameters shared similar condition scores. In the epidermis, mucocytes were in good to fair condition with pale-staining mucus, and ciliated columnar cells were visible (Fig. 15A). Costal tissue loss was typically mild, with about 25% of the costae exposed. Zooxanthellae in the surface body wall were in fair condition, with generally one or two zooxanthellae present in gastrodermal cells, which were slightly atrophied (Fig. 15A). Basal body wall gastrodermis architecture ranged from good to fair, with evidence of lipid droplet formation present in most of the gastrodermis. Between 25% and 50% of the gastrodermis was swelling due to ruptures or necrotic tissue in the gastrodermis and a release of zooxanthellae was often visible (Fig. 15C). Calicodermis condition seemed to follow gastrodermis condition, with few ruptures and atrophy in about 50% of the calicoblasts in a given sample (Fig. 15C). Within cnidoglandular bands, mucocytes were typically around 50% of the area or less, with mild loss of cells. In mesenterial filaments, loss of cells resulted in about 50% of filaments present in slides that were intact (Fig. 15E). Post-exposure control samples appeared similar to their corresponding post-exposure treatment apparently healthy samples.

Similarly, post-exposure treatment visually diseased samples and pre-exposure treatment later visually diseased samples had similar observed condition and no significant difference between overall condition scores (two-sample *t*-test, $t(7.69) = -0.24$ p = 0.812). Epidermal mucocytes were typically irregularly sized and shaped, and sometimes stained dark in color (Fig. 15B). Costal tissue loss was mild to fair, with typically 25% to 50% of the costae exposed in slides. Zooxanthellae in the surface body wall were typically one to two layers thick, and in some cases, were released into the gastrovascular canal due to ruptured gastrodermis (Fig. 15B). Gastrodermal architecture was fair to poor, often with 75% of the BBW gastrodermis showing signs of swelling and a release of zooxanthellae into the gastrovascular canals due to ruptures in the gastrodermis (Fig 15D). Calicodermis condition was similarly in poor condition, with separation of the calicodermis from mesoglea in some instances, and atrophy of

calicoblasts (Fig. 15D). Condition of the cnidoglandular bands was generally good with less than 50% of the band composed of mucocytes. Fewer mesenterial filaments were intact than in apparently healthy samples, with 50% or more not intact (Fig. 15F). A list of all histology samples analyzed can be seen in Table A2.
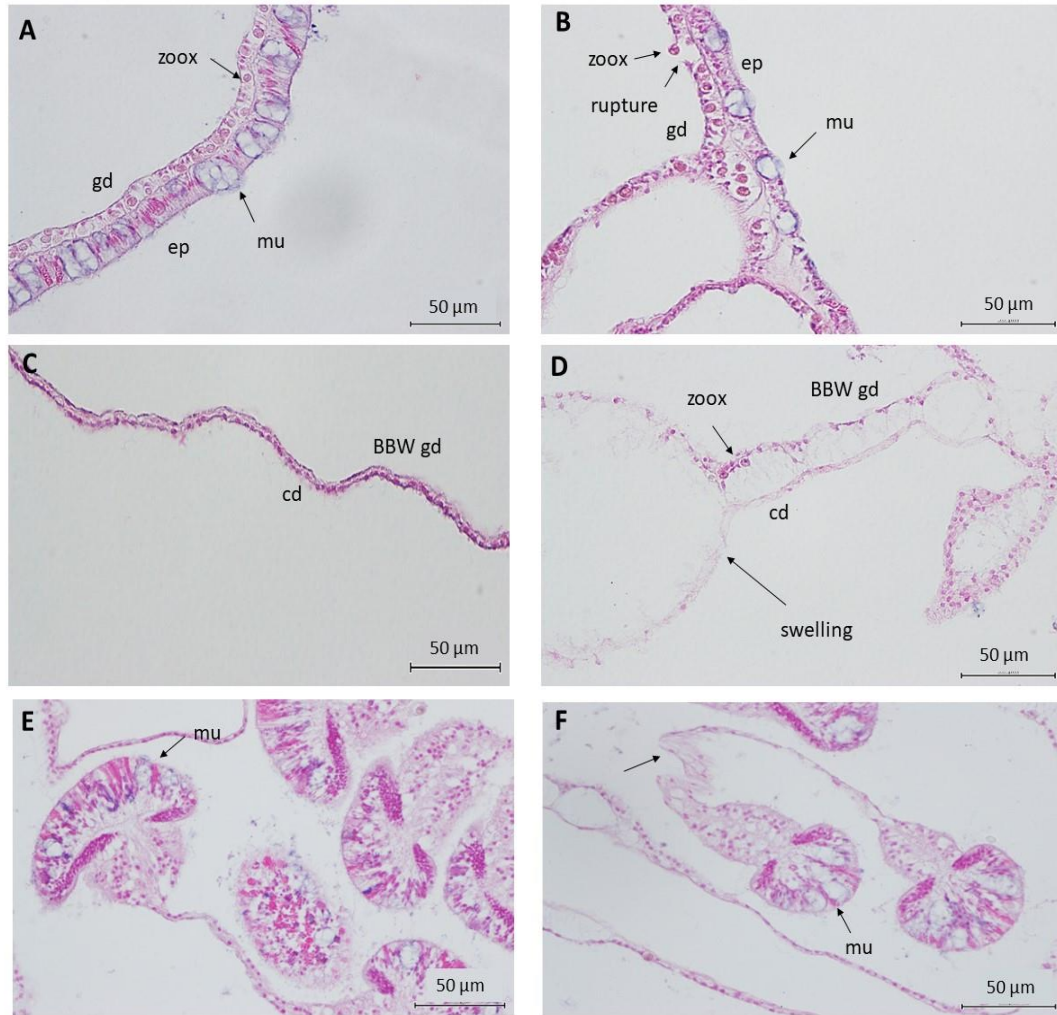
Figure 15. Representative histological sections of *Acropora cervicornis* surface body and basal body walls at 40X magnification from experimental groups. (A) Apparently healthy fragment with visible ciliated columnar cells and mucocytes and a thick layer of zooxanthellae. (B) Diseased fragment with irregularly shaped and stained mucocytes, and a single layer of zooxanthellae in the SBW. (C) Healthy fragment with no hypertrophy in the BBW gastrodermis and clear calicodermis. (D) Diseased fragment with swelling of the BBW gastrodermis and calicodermis. (E) Healthy fragment cnidoglandular band with about 50% epithelium containing mucocytes and intact filament. (F) Diseased fragment with about 50% mucocytes in cnidoglandular band epithelium but degradation to mesenterial filament. Scale bars = 50 µm. ep = epidermis, gd = gastrodermis, mu = mucocyte, zoox = zooxanthellae, cd= calicodermis, BBW gd = basal body wall gastrodermis.

2.4   Discussion

The present study was the first to directly compare and quantify differences between direct contact (grafting) and waterborne (homogenate) pathogen(s) transmission methods. These results indicate that pathogen transmissibility may vary based on the method used, which should be considered in future transmission studies, and support the potential for higher natural pathogen(s) transmission through direct contact rather than through the water column. Gignoux-Wolfsohn et al. (2012) were the first to demonstrate that water-borne transmission was only possible when tissue was first abraded. Although most coral tissue is likely abraded in some form in the wild due to predation or fragmentation, the concentration of free-floating diseased tissue in the open ocean is likely much smaller than what is used to expose corals in experimental tanks in the form of a homogenate. Additionally, water movement is constant in the open ocean, and may control or limit the length of exposure to diseased tissue depending on the velocity and direction of water motion. On the contrary, water flow could potentially increase the amount of free-floating diseased tissue in localized areas where necrotic tissue is present on diseased coral colonies. Direction of water flow in relation to diseased colonies, the number of diseased colonies in an area, and abrasion of apparently healthy colonies would likely affect the probability of pathogen transmission through the water-column. Direct contact to biotic pathogen(s) through exposure to diseased fragments that are actively losing tissue or to biological vectors, although also sporadic in nature, may be more of a concern as they are not influenced as greatly by water movement. Therefore, once vectors are present, or diseased colonies are in contact with healthy colonies, transmission could potentially continue until these sources are removed. However, because the pathogen(s) for this tissue-loss disease are still unknown, it is difficult to predict exactly how this disease will spread and therefore how it could be managed. While management for direct contact transmission is possible, if additional abiotic causal agents are contributing to disease, this disease may be difficult to control.

Scientists have explored disease management approaches that target direct-contact transmission, such as covering tissue-loss margin areas with epoxy or a chlorine-epoxy mixture, shading corals during bleaching events, and antibiotic treatments (Raymundo et al. 2008, Muller and Van Woesik 2009, Miller et al. 2014, Aeby et al. 2015). Current

management practices in the nursery setting include clipping out disease fragments to prevent enlargement of lesions within colonies or spread of biotic pathogens among coral nursery structures (Coral Restoration Foundation, pers. comm.). However, it may not be feasible to manage the more frequent and severe disease outbreaks anticipated with a changing climate through manual labor. Instead, by identifying corals that are genetically more resistant to disease and incorporating this information into restoration efforts, the likelihood that these corals survive may increase. For example, outplanting colonies of a mixture of susceptible and resistant colonies, or separating susceptible genotypes in outplanting arrays, may prevent the spread of infectious pathogens. As new diseases emerge, or pathogens change, even those genotypes that appear susceptible to current diseases may fare well. Of course, disease resistance must not be the only characteristic considered when attempting to improve restoration efforts. Other characteristics, such as tolerance to temperature anomalies, growth rate, and fragmentation, must be considered to increase the chances of sexual reproduction of these colonies in the future (Hunt and Sharp 2014).

The results of the present study also demonstrated the high variability in response to pathogen exposure among the tested *A. cervicornis* genotypes. Evidence of disease prior to the start of the experiment in multiple fragments from genotypes U22 and U30 seemed to coincide with relatively high disease susceptibility later in this experiment. Genotype U22 had the highest number of diseased fragments in the grafting treatment (n = 3), and one fragment was diseased in its post-exposure control tanks as well. A similar trend was seen in genotype U25, where a relatively high number of fragments became diseased in the grafting and homogenate treatments (n = 3 total), as well as one control fragment. Genotype U30 also had a relatively high number of fragments diseased in their treatments (n = 2), controls (n = 2), and prior to the start of the experiment (n = 2). Other researchers also found evidence of disease susceptibility in these genotypes in previous studies (M. Hightshoe, unpub. data, M. Miller, unpub. data). However, U77, which was believed to be a relatively resistant genotype based on these past studies, appeared to be relatively disease susceptible in the present study. The only genotype that showed no signs of disease throughout the entirety of this study, and thus appeared to be relatively disease resistant, was U72. This was previously believed to be a relatively susceptible

genotype (M. Miller, unpub. data). Differences in relative susceptibility found in this study compared to others could be due to a variety of factors. Seasonality, environmental conditions, experimental design, pathogen exposure methods, or variability within individual fragments from each colony and genotype could have played a role in relative disease susceptibility. However, the high level of disease appearing in the post-exposure control tanks adversely affected the present study's results on relative resistance. No genotype was found to be significantly more or less resistant to disease than their control, as indicated by the Bayesian relative risk assessments. While sample size likely influenced these results as well, reducing the amount of disease in control fragments would likely produce clearer results on relative susceptibility or resistance to disease. This could be accomplished by conducting the experiment during a different time of year when background disease prevalence is not high, or by improving the condition of all fragments prior to the start of the experiment. This may be done by acclimating fragments for a longer period prior to pathogen exposure, which may reduce stress in the fragments or allow for the removal of fragments in poor condition prior to the start of the experiment.

Histological analysis revealed that many pre-exposure treatment and control samples were in very poor condition prior to the introduction of diseased tissue in this experiment. While the cause of this condition is unclear, it is possible that fragments had encountered pathogens in the field prior to collection, experienced stress during the collection and transportation processes, or that their condition was the result of exposure other unknown abiotic factors while in the nursery that eventually resulted in tissue loss. Although these variables may imply possible limitations in earlier findings, they still allow for the identification of relative susceptibility in genotypes, regardless of when fragments became diseased. However, these findings certainly support the need for further histological analysis in disease resistance studies. All fragments collected for this experiment appeared visually healthy, although histological analysis showed that some pre-exposure treatment samples were diseased on the microscopic level. Histological analysis also determined that post-exposure treatment visually healthy samples were indeed healthy both on the macro- and microscopic levels.

Histological parameters that were in significantly worse condition in post-exposure treatment visually diseased samples included dissociation of cells on mesenterial filaments, gastrodermal architecture, and calicodermis condition. Loss of cells on mesenterial filaments and damaged filaments may influence the ability of coral polyps to capture and digest prey, while deterioration in gastrodermal architecture in the BBW prevents corals from processing particulate food and storing lipids (Miller et al. 2014). Additionally, the loss of integrity in the calicodermis indicates potential problems with skeleton accretion, and therefore growth. These same parameters were significantly worse in pre-exposure treatment later visually diseased samples than in pre-exposure treatment later apparently healthy samples, suggesting that poor condition of these parameters may influence disease susceptibility.

Although no evidence of bacteria or rickettsia-like organisms (RLOs) were observed in our histology samples, we did observe ciliates in three post-exposure treatment visually diseased samples. While it is unclear if these ciliates were involved with the cause of tissue loss, or were opportunistically present on the diseased tissue, further investigation into the roles of ciliates in diseased corals should be conducted. Additional staining techniques may also help improve the detection of bacteria and RLOs in histoslides, such as Giesma staining used in Miller et al. (2014). These techniques may help identify potential pathogens or organisms involved in visually diseased fragments.

Overall, results from this study support variability in disease susceptibility among genotypes and demonstrate the potential for identifying disease-resistant genotypes in local populations. This information is crucial to coral nursery managers who wish to effectively plan restoration efforts. Repopulating reefs with corals of high genetic diversity and the ability to withstand a variety of stressors will hopefully improve the future success of *A. cervicornis* populations. Additionally, these results demonstrate the importance of gaining a better understanding of biotic pathogen transmission mechanisms and the condition of coral fragments used in experiments, as well as the need for standardizing these methods for future susceptibility studies. As researchers continue to identify disease resistance in populations, it is important to consider that results may differ based on exposure methods. Methods should be carefully considered and selected based on research questions and resource availability. The high prevalence of disease and

rates of tissue loss observed in this study, in addition to the high host susceptibility amongst genotypes, demonstrate the clear need to increase our understanding of disease dynamics in *A. cervicornis* and limit the impact of more severe disease outbreaks in the future.

CHAPTER 3: ACCLIMATION EXPERIMENT

3.0   Abstract

The acclimation period for experimental organisms being exposed to stressors is often neglected in study designs. Many published coral disease pathogen transmission studies lack the mention of acclimation periods altogether. To determine if tissue condition changes during acclimation, potentially influencing results from these studies, I took clippings for histological analysis during a 9-day period from four different genotypes of the Caribbean staghorn coral, *Acropora cervicornis*. I found that there was a significant decline in overall condition of samples between days zero and two, and significant improvement in surface body wall parameters (epidermal mucocytes and zooxanthellae in the surface body wall) from day two to nine. These findings support a delay in response to new environmental conditions and suggest the need to acclimate experimental fragments of *A. cervicornis* for periods of at least nine days, if possible. While repeated clipping may have caused deterioration to internal basal body walls, and prevented significant changes in them over time, a similar acclimation period without clipping would likely result in an adequate acclimation period that allows for tissue repair and adaptation to new experimental environmental conditions.

3.1   Introduction

Following the first report of natural disease resistance in a Panama population of *A. cervicornis* (Vollmer and Kline 2008), multiple studies have begun to focus on genotypic resistance to disease in coral populations using a variety of *in situ* and *ex situ* pathogen exposure methods (Hunt and Sharp 2014, Miller and Williams 2016, Hightshoe 2018, E. Muller, unpub. data). However, in many coral pathogen transmission studies, acclimation periods differ and there is little discussion of this important step in the extant literature. Although Miller and Williams (2016) suggested an acclimation period of at least 2 weeks *in situ* to allow clipped fragment margins to heal, acclimation time has varied greatly among previous studies. For example, Gignoux-Wolfsohn et al. (2012) acclimated *A. cervicornis* fragments in aquaria tanks for 72 hours prior to pathogen exposure by homogenate and the biological vector, *C. abbreviata*, and E. Muller (unpub. data) acclimated *A. cervicornis* fragments for 72 hours prior to pathogen exposure in

aquaria by homogenate. Many other studies fail to mention acclimation period all together, and it is possible that some studies have omitted acclimation from their design completely. This lack of consensus and emphasis on the importance of acclimating manipulated organisms is concerning, particularly in coral disease studies, as it can make it difficult to differentiate between unidentified biotic (pathogens) and abiotic (stressful conditions) causal agents that may lead to tissue-loss disease. To better understand how acclimation time may affect these results, in addition to many other types of studies, I scored changes in tissue condition observed by light microscopy in histological sections of *A. cervicornis* fragments during a 9-day acclimation period.

3.2 Materials and Methods

In June 2017, three fragments (~10 cm) from four genotypes of *A. cervicornis* (n = 12) were collected from the Coral Restoration Foundation nursery (N 24° 58.933', W 80° 26.180') in Tavernier, Florida Keys, based on availability (genotypes: U25, U21, U30, and U77). Temperature in the nursery was estimated using data from nearby long-term monitoring sites established by the Florida Fish and Wildlife Coral Reef Evaluation and Monitoring Project (CREMP) as approximately 28.9 °C around collection day (6/27/18). All fragments were collected using handheld wire cutters, wrapped in seawater-soaked bubble wrap, and placed in coolers for transportation to the Nova Southeastern University (NSU) Oceanographic Center's outdoor SEACOR experimental aquaria system. Total time from collection to arrival at NSU was approximately 4 hours. At NSU, experimental fragments were randomly arranged and suspended into a 113-L tank using monofilament and crimps, attached to an over-hung egg-crate screen. Immediately upon introduction into the tanks, clippings (~2 cm) for histology were taken on all fragments. These clippings were considered "day 0" samples and were taken to determine tissue condition immediately following transportation.

Additional histology samples were taken every 2–3 days on two out of three fragments from each genotype (day 2, 4, 6, and 9), while the remaining fragment from each genotype was sampled only on days 0, 2, and 9 to limit handling and potential damage/repair in tissue from frequent sampling (n = 52 histology samples total). Water changes (50%) were conducted every two days, to maintain water quality, and remove

any mucous and tissue/skeletal debris that resulted from clipping. Tanks were maintained at a constant ambient collection temperature of approximately 28°C throughout the 9-day experiment. Temperature, dissolved oxygen, and pH were monitored daily.

All histology clippings (~2 cm) were taken using handheld wire cutters and were placed in labeled 50-mL plastic centrifuge tubes with Z-Fix Concentrate (Anatech, Ltd., 1:4 dilution in seawater). Samples were stored indoors in the NSU Histology Laboratory for 2–7 days prior to processing. For decalcification, each sample was removed from fixative, photographed, and trimmed if necessary. Samples were decalcified using a 5% decalcifying solution (1.5 g ethylenediaminetetraacetic acid, 150 mL hydrochloric acid, and 2.85 L seawater), which was changed every 24 hours for 3–4 days until samples were completely decalcified. Once decalcified, samples were cut longitudinally using a clean razor blade and were placed in cassettes in 70% ethanol. Cassettes were processed through a graded series of ethanol concentrations, cleared with xylene, and infiltrated with molten paraffin wax. Samples were embedded into blocks using paraffin wax and were sectioned at a 4-μm thickness. Sections were mounted onto glass microscope slides and were stained with Harris's hematoxylin and eosin before applying a coverslip using Cytoseal 60 ™ mounting medium.

Samples were examined using an Olympus BX 43 light microscope and computer imaging to identify changes occurring on the cellular/tissue level (Work and Meteyer 2014). Each sample was scored using a semi-quantitative (Jagoe 1996) rubric modified from Miller et al. (2014) (Table A1, modified to exclude degeneration of cnidoglandular bands, cnidoglandular band epithelium, and dissociation of cells on mesenterial filaments). The following parameters were ranked by severity and relative condition (0 = No Change, Excellent, 1 = Minimal, Very Good, 2 = Mild, Good, 3 = Moderate, Fair, 4 = Marked, Poor, 5 = Severe, Very Poor): epidermal mucocyte condition, costal tissue loss, zooxanthellae in the surface body wall (SBW), gastrodermal architecture in the basal body wall (BBW), and calicodermis condition. Histoslides of *A. palmata* from the Florida Keys in the 1970s, collected before tissue loss was reported, were used to develop a baseline for excellent condition (Miller et al. 2014). To avoid reporting damage from the physical clipping, fragments were scored above the clipping margin, if evident. Descriptive statistics were calculated for each scored parameter in each group of samples

(day 0, day 2, day 4, day 6, and day 9; Table 4) and were compared among days for each parameter using non-parametric Kruskal-Wallis tests. Parameter scores were also summed to produce an overall condition score for each sample at each period, which was compared among genotypes and among days using a two-way ANOVA and individual non-parametric Kruskal-Wallis tests when necessary. Change in overall fragment condition was also observed in each fragment within each genotype and was compared using a repeated measures ANOVA to follow the same fragment over time.

3.3   Results

Water quality was maintained at an average 27.8 ± 1.17 °C, 95.07 ± 1.69 % dissolved oxygen (mean ± S.E.), and pH between 8.1 and 8.2 throughout the study. No significant differences in overall condition scores were found among genotypes or days (Kruskal-Wallis test, $\chi^2 (19) = 20.5$, $p = 0.361$); however, mean condition scores of the epidermal mucocyte parameter was significantly different among genotypes or days (Kruskal-Wallis test, $\chi^2 (19) = 36.1$, $p = 0.010$), although these differences were not detectable in a post-hoc Steel test. Because tissue conditions were similar among genotypes, genotypes were grouped together to determine change in condition over time.

When comparing changes in tissue condition over time among all samples, significant improvements in condition of the epidermal mucocytes and zooxanthellae in the SBW were found between day 2 and 9 (Kruskal-Wallis test, $\chi^2 (4) = 18.0$, $p = 0.001$, Kruskal-Wallis test, $\chi^2 (4) = 16.6$, $p = 0.002$, respectively, Fig. 16A, Fig. 16C). Zooxanthellae condition in the SBW was also significantly worse at day 2 than day 0 as identified in a post-hoc Steel test (Fig. 16C). Overall condition score was only significantly different between day 0 and 2 (Kruskal-Wallis test, $\chi^2 (4) = 10.3$, $p = 0.034$), indicating that condition worsened in samples at day 2 (Fig. 16F, Table 5). Day 0 and 2 samples were typically observed with irregularly shaped and stained mucocytes, and a thin layer of zooxanthellae in the SBW. The SBW gastrodermis often contained ruptures and release of zooxanthellae (Fig. 17).

Table 5. Summary of histopathology results of fragment acclimation study. Parameter scores ranged from (0 = No Change, Excellent, 1 = Minimal, Very Good, 2 = Mild, Good, 3 = Moderate, Fair, 4 = Marked, Poor, 5 = Severe, Very Poor).

Parameter	Day 0				Day 2				Day 4				Day 6				Day 9			
	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max	Mean	S.E.	Min	Max
Epidermal Mucocytes	2.7	0.2	2	4	3.5	0.2	3	4	3.3	0.3	2	4	2.5	0.3	2	4	2.4	0.1	2	3
Costal Tissue Loss	1.9	0.1	1	3	2.0	0.0	2	2	2.1	0.1	2	3	2.0	0.0	2	2	1.7	0.1	1	2
Zooxanthellae in SBW (40X)	2.9	0.2	1	4	3.8	0.1	3	4	3.3	0.3	2	4	3.5	0.2	3	4	2.8	0.2	2	4
Gastrodermal Architecture: BBW	2.3	0.1	2	3	2.8	0.2	2	4	2.8	0.3	2	4	2.9	0.3	2	4	3.3	0.3	1	4
Calicodermis Condition	2.3	0.1	2	3	2.5	0.2	2	3	2.5	0.2	2	3	2.5	0.2	2	3	3.0	0.2	2	4
Overall Specific Condition Score	12.1	0.4	9	14	14.6	0.5	12	17	13.9	0.9	10	17	13.4	0.6	11	15	13.2	0.6	10	17

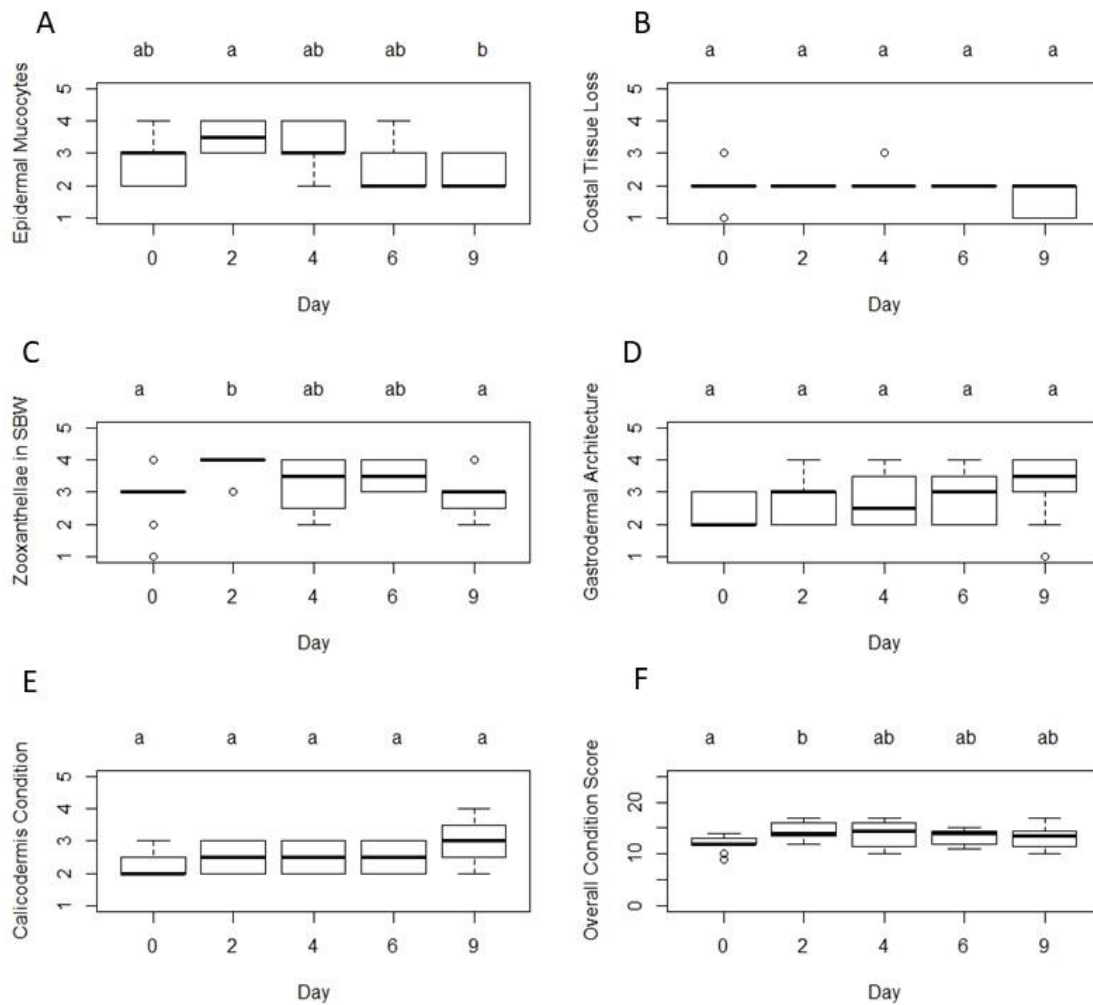**Figure 16.** Mean parameter and overall condition score by day. Significant differences were found in the following: (A) epidermal mucocytes between day 2 and 9 (Kruskal-Wallis test, χ^2 (4) = 18.0, p = 0.001), (C) zooxanthellae in the SBW between day 0 and 2 and 2 and 9 (Kruskal-Wallis test, χ^2 (4) = 16.6, p = 0.002) ,and (F) overall condition score between day 0 and 2 (Kruskal-Wallis test, χ^2 (4) = 10.3, p = 0.034).
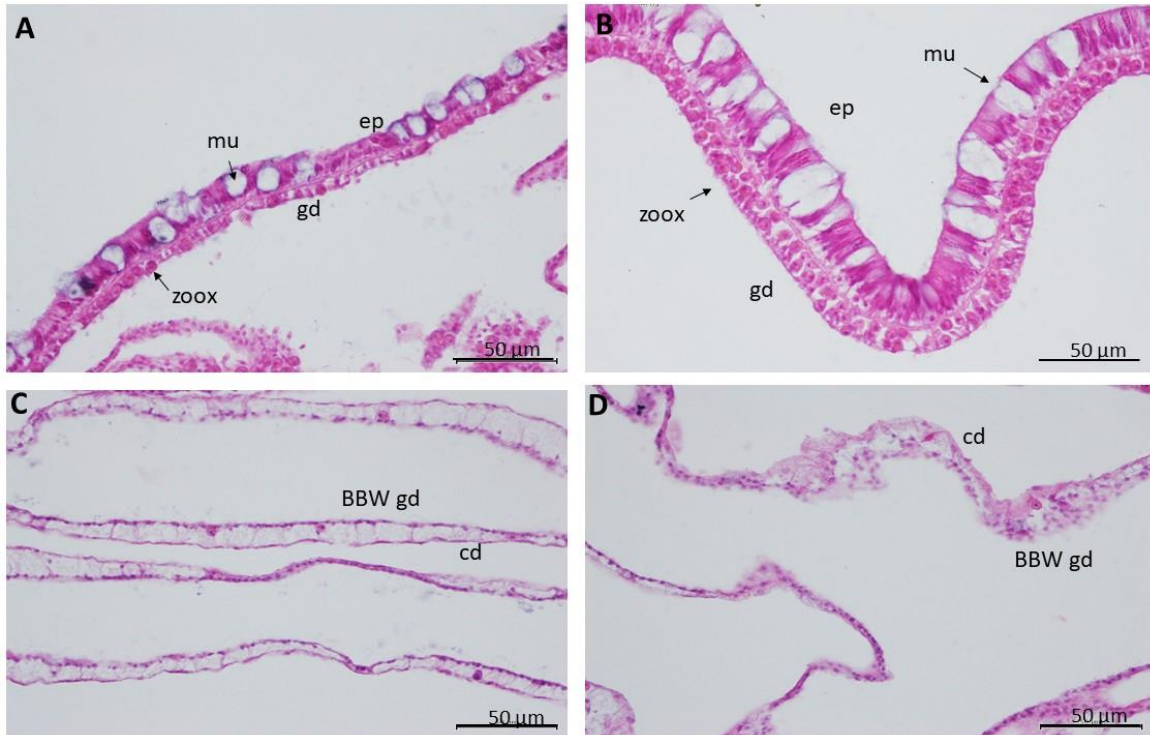
Figure 17. Histological sections of *Acropora cervicornis* surface body and basal body wall at 40X magnification over different time periods. (A) Day 2 sample from genotype U21 showing signs of irregularly shaped and stained mucocytes (parameter score of 3), and a single layer of zooxanthellae in the SBW (parameter score of 4). (B) Day 9 sample of genotype U21 with visible columnar cells and mucocytes (parameter score of 2) and a thick layer of zooxanthellae (parameter score of 2). (C) Day 2 sample of genotype U30 with visible signs of lipid droplet formations in the BBW gastrodermis, few zooxanthellae, (parameter score of 3) and squamous calicoblasts in the calicodermis (parameter score of 3). (D) Day 9 sample of genotype U30 day with few zooxanthellae, small ruptures in the BBW gastrodermis, and partial swelling, (parameter score of 4) and clear calicoblasts (parameter score of 3). Scale bars = 50 µm. ep = epidermis, gd = gastrodermis, mu = mucocyte, zoox = zooxanthellae, cd = calicodermis, BBW gd = basal body wall gastrodermis.

The gastrodermal epithelium in day 0 and 2 samples appeared visibly thicker than day 9 samples, with evidence of past lipid formation. Although few ruptures were present, which resulted in some areas of swelling tissue, both the gastrodermis and calicodermis were generally in good to fair condition. Day 9 samples generally seemed to have overall better SBW integrity, with more regularly shaped and stained mucocytes. Columnar epidermal cells were more visible, and the gastrodermal layer in the SBW contained a thicker layer of symbionts and was in better structural condition. The gastrodermal tissue layer in the BBW typically had few signs of lipid droplet formation, which may have been due to clipping location in proximity to the apical polyp. However, the gastrodermis usually contained ruptures, which resulted in swelling, and often

necrotic cells. Calicoblasts were also less apparent and sometimes not visible in the calicodermis due to swelling and lysing (Fig. 18).
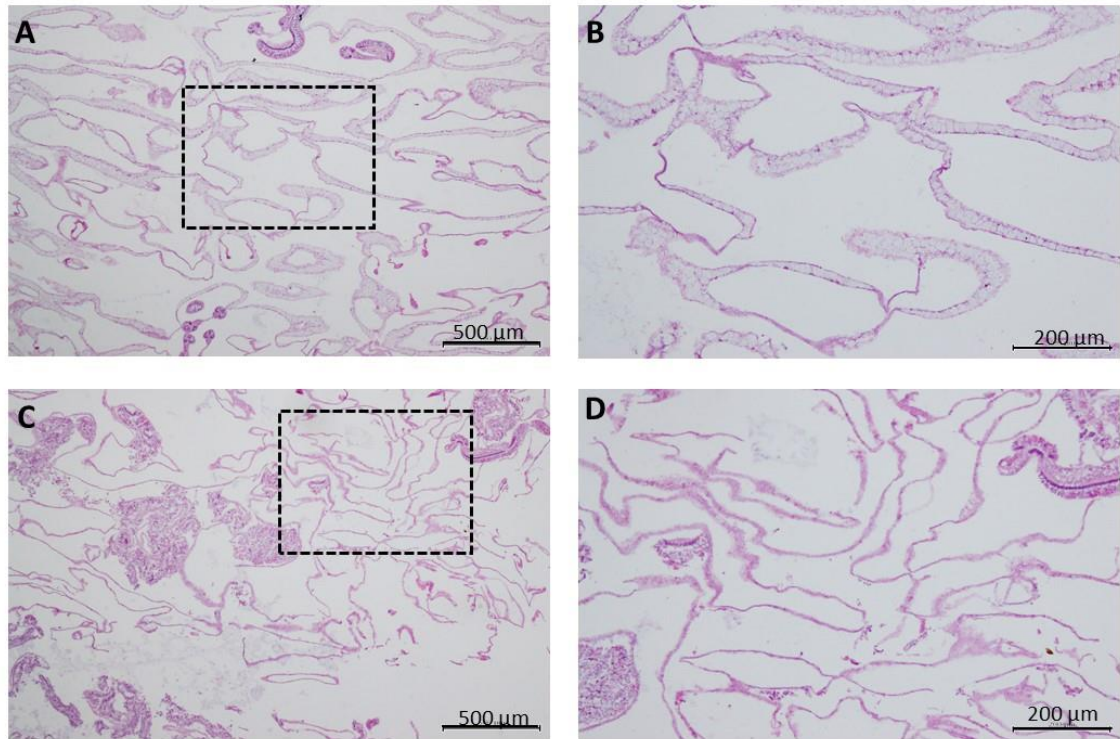
Figure 18. Histological sections of *Acropora cervicornis* basal body wall at 4X and 10X magnification illustrating condition of the basal body wall gastrodermis over time. All images were taken from the same fragment. Dashed boxes on images (A) and (C) represent the area magnified in (B) and (D), respectively. (A) Day 0 sample of genotype U25 at 4X. A thick BBW due to lipid droplet formation is visible throughout entire BBW gastrodermis, with well-defined tissue layers. (B) Day 0 sample of genotype U25 at 10X. (C) Day 9 sample of genotype U25 at 4X. A fragmented gastrodermis with many ruptures and necrotic or lysing cells in both the gastrodermis and calicodermis is visible. (D) Day 9 sample of genotype U25 at 10X. Scale bars = 500 or 200 μm, respectively.

When examining overall condition score in all four genotypes, each fragment's condition varied over time, with generally a worsening of condition between days 2–6 and either a slight improvement or worsening by day 9 when compared to day 0 samples (Fig. 19). No significant differences in overall condition score were found in any genotype when fragment was considered in separate repeated measures ANOVAs ($p > 0.05$). Additionally, when comparing the overall condition score of fragments that were not clipped on days 4 and 6 to fragments clipped on those days, there was no significant difference between condition at the end of the experiment (Kruskal-Wallis test, $\chi^2 (2) = 2.1$, $p = 0.342$).
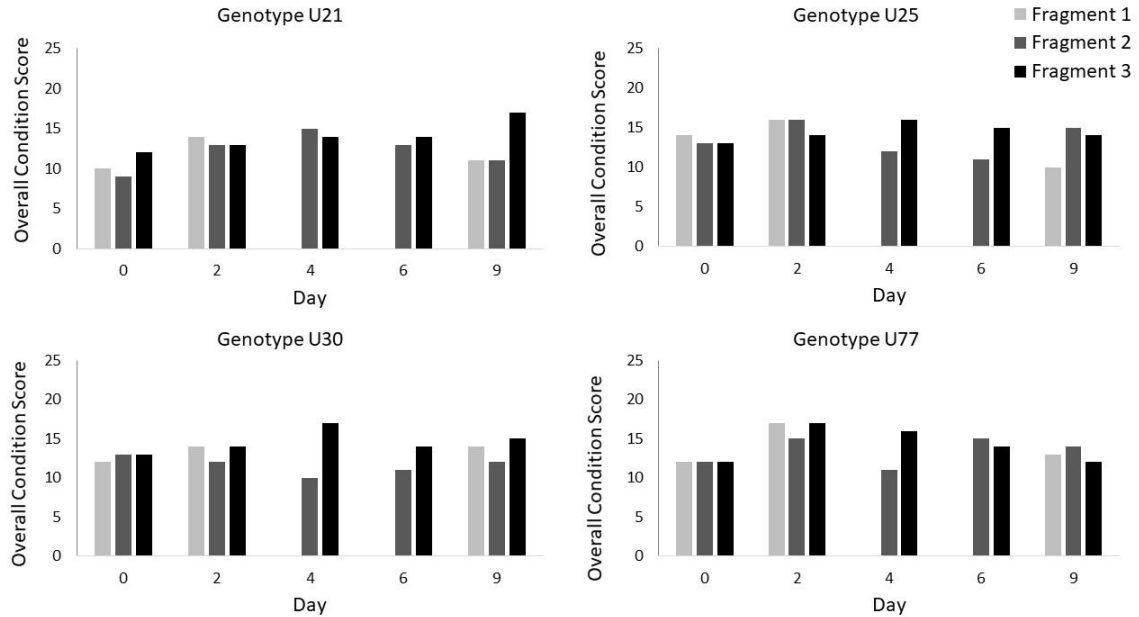
Figure 19. Change in overall condition score (sum of all parameters) over time in each fragment. Increasing values represent worsening condition score. Fragment one was not clipped on days 4 and day 6, represented by the missing bar. No significant differences in overall condition score were found in any genotype when fragment was considered in separate repeated measures ANOVAs ($p > 0.05$).

3.4 Discussion

Throughout the 9-day acclimation period, I observed a significant decline in condition between days 0 and 2 both in overall condition and zooxanthellae in the SBW. This may have been caused by a delayed acclimation effect, in which corals were stressed by the change in environmental condition and showed microscopic changes in their tissues during a multi-day period after being introduced to their new environment. Laboratory conditions often differ from natural conditions experienced *in situ*. In this study specifically, tank temperature was maintained at a lower mean temperature than anticipated, resulting in water that was approximately 1 °C cooler than the temperature in the field during collection. It is possible that this change of temperature, and additional factors such as light levels and food availability may have altered the fragments conditions during this two-day period. These changing tissue conditions illustrate the importance of an acclimation period greater than 2 days. Significant findings in the zooxanthellae parameter indicated that zooxanthellae presence may be driving the overall decline in condition. While humans can visually detect a decrease in zooxanthellae

concentrations through a "paling" appearance in coral color, this is not detectable until zooxanthellae have already decreased 50% (Jones 1997). Lack of a thick layer of zooxanthellae may prevent corals from meeting energy requirements (Sumich 1996), which in turn could result in poor maintenance of their tissues.

Only between days 2 and 9 did I observe significant improvements in surface body wall (SBW) parameters, such as the epidermal mucocytes and zooxanthellae. These improvements to the SBW may be crucial for maintaining coral health. Mucocytes are one of few defense mechanisms that exist within corals. Through the production and secretion of a polysaccharide-protein-lipid complex, also known as mucus, these cells are responsible for protection against unwanted particles, microorganisms, and potential pathogens, and prevent these from entering coral tissues (Brown and Bythell 2005, Ritchie 2006). A loss of mucocytes may severely compromise the coral's immunity against diseases and may also play a role in feeding, desiccation resistance, and calcification mechanisms (Brown and Bythell 2005). Zooxanthellae within the SBW are also critical components of the coral holobiont, and produce an estimated 90% of energy for the coral host (Sumich 1996). They also contribute to the composition of mucus produced by the coral, as 20 – 45% of the photosynthate they produce daily is released as mucus (Crossland et al. 1980, Davies 1984, Brown and Bythell 2005). Nutritional stress may cause zooxanthellae to be lost (Weis 2008, Miller et al. 2014), and without these cells, corals cannot meet the energy demand required to maintain its tissues and survive (Miller et al. 2014).

I found no improvement in overall condition score after day 2 when genotypes were grouped together or when examining genotypes separately. Although not statistically significant, I observed gastrodermis deterioration in day 9 samples, in the form of ruptures, swelling, and necrosis, which may have influenced the overall condition scores. It is possible that these observations were caused by repetitive clipping of the fragments, which accelerated BBW deterioration; often the first sign of stress in *A. cervicornis* (E. Peters, pers. comm.). When comparing the overall condition score of fragments that were not clipped on days 4 and 6 to fragments clipped on those days, there was no significant difference between conditions; however, all fragments may have been affected by clippings on days 0, 2, and 9. Additionally, the sum of the significant

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improvement of the SBW and anecdotal evidence of BBW deterioration may have resulted in an insignificant finding in the overall condition score.

Results from this acclimation study suggest that samples may be the most disease susceptible at the 40-hour, or 2-day mark, due to poor tissue condition. Past pathogen transmission studies, such as Bock et al. (in prep.), E. Muller (unpub. data), or Gignoux-Wolfsohn et al. (2012), which acclimated coral fragments for 40 and 72 hours respectively, may have also experienced a decline in tissue condition at the start of their experiments. However, exact collection methods and transportation of corals in these studies may have differed, and without histological analysis, tissue condition in these experiments cannot be confirmed on the microcropic level. This host condition may influence the susceptibility to disease infection; therefore, it is important to consider using histological techniques in disease transmission experiments to interpret results correctly.

No *in situ* samples immediately after collection were taken for histological analysis in this study. However, identifying the state of tissue condition *in situ* and observing how this changes from collection to arrival at experimental facilities would help determine how fragments are affected during different transportation periods. Transportation distances, conditions that fragments are kept in during transportation, and health of fragments *in situ* may all affect tissue condition and should be examined. Identifying optimal transportation times that limit exposure to stressors damaging tissue may also be useful in planning future experimental methods.

Although some improvements in tissue condition were observed in this study, these results can only describe improvements up to 9 days after collection in *A. cervicornis*, which was transported for a 4-hour period. Because no significant improvements were observed prior to day 9, a minimum of 9 acclimation days should be considered for *A. cervicornis* when transporting approximately 4-hours, and when study design allows, before exposing fragments to additional stressors. Because signs of stress were still apparent on day 9, such as necrosis in the gastrodermis, this supports the need for an acclimation period of greater than 9 days, if possible. However, if collection and transportation times are minimal, the necessary acclimation period may be greatly

reduced, and an acclimation of less than 9 days may be sufficient. Additional studies should be conducted to determine any additional changes in tissue condition beyond this 9-day period, if this period varies among species, and how tissue conditions change following various collection and transportation methods.

CHAPTER 4: SUMMARY AND CONCLUSIONS

Quantifying pathogen transmission and disease development between the two common existing pathogen transmission methods (direct contact vs. water-borne) provides valuable knowledge for future disease studies and for nursery managers. In my study, I found higher disease transmission through the direct contact, or grafting method. These results may have been influenced by an overall longer constant exposure of experimental fragments to diseased fragments, which was not experienced in the homogenate treatment. While only three total incidences of disease occurred in the homogenate treatments, all three occurred within the first three days of the experiment. Therefore, after day four, when two 50% water changes were completed, no further disease occurred in the tanks. While continuously adding diseased homogenate after water changes would have allowed for consistent concentration within the tanks, this was not possible in my study due to the distance between NSU experimental facilities and the CRF nursery, where the diseased fragments were collected. Additionally, water changes may represent natural flushes of water that occur in the open ocean. Avoiding water changes or continuously adding diseased tissue homogenate may be unrepresentative of natural conditions; however, additional experiments should be conducted using various homogenate concentrations to determine its feasibility in future studies.

Low success of homogenate method pathogen transmission may have also been caused by a relatively low concentration of diseased homogenate in the tanks. While I did not measure the surface area of tissue used to produce each homogenate slurry, each slurry produced from 11 diseased fragments was approximately 250 mL. In a study design with smaller tanks, and therefore less water quantity, it may be possible to transmit pathogen(s) causing disease more efficiently using the homogenate treatment, without collecting as many fragments (E. Muller, pers. comm.). Additionally, mixing a stock solution of homogenate and administering this to tanks may decrease the necessary number of fragments, as well as decrease variability within homogenate treatment tanks. Investigating the effectiveness of pathogen transmission using smaller tanks (less water volume) is necessary to support water-borne transmission, and may have implications for the aquaculture industry, although it is unclear how these concentrations would relate to the open ocean and exposure to particulate diseased tissue experienced in certain regions.

There was no advantage to using the homogenate method in this study based on my experimental design, as the same number of diseased fragments could be collected and easily cable-tied onto experimental fragments with more success in tissue loss development. Additionally, I found no significant difference in tissue-loss rate between the two methods once a fragment was affected by disease. These results, however, are limited by a sample size of only three diseased fragments in the homogenate treatment. While mean tissue-loss rate may change with a higher sample size, these results do not suggest a trade-off between pathogen transmission methods in terms of tissue-loss. Higher success in developing disease by using the grafting treatment support the potential for higher natural pathogen transmission through direct contact rather than through the water column and should be considered when developing management techniques. While current disease management techniques include coating the diseased areas with epoxy or a chlorine-epoxy mixture, shading corals during bleaching events, and antibiotic treatments (Raymundo et al. 2008, Muller and Van Woesik 2009, Miller et al. 2014, Aeby et al. 2015), additional future practices may include controlling direct-contact vectors, such as biological corallivorous snails or removing actively-diseased tissue. However, it may not be feasible to manage the more frequent and severe disease outbreaks anticipated with a changing climate.

Instead, identifying genotypes of coral that are genetically more resistant to disease and focusing restoration efforts on these genotypes may increase the chance of future survival. In this study, I identified clear differences in genotypic response to disease. Genotype U22 had the highest number of diseased fragments in the grafting treatment, and some fragments became diseased in its control treatments. A similar trend was seen in genotype U25, where a relatively high number of fragments became diseased in the grafting and homogenate treatment, as well as the controls. Genotypes U30 and K2 also had a somewhat high number of fragments become diseased in their exposure treatments, in addition to their controls and/or prior to the start of the experiment. Other researchers also found evidence of disease susceptibility in these genotypes (M. Hightshoe, unpub. data, M. Miller, unpub. data). However, U77, which was believed to be a relatively resistant genotype based on these past studies, appeared to be relatively disease susceptible in my study. The only genotype that showed no signs of disease

throughout the entirety of my study, and thus appeared to be relatively disease resistant, was U72. This was previously believed to be a relatively susceptible genotype (M. Miller, unpub. data). Differences in relative susceptibility found in my study compared to others could be due to a variety of factors. Study times, environmental conditions, experimental design, pathogen transmission methods, or variability within individual fragments from each colony and genotype could have played a role in relative disease susceptibility. Increasing replicates of fragments may help to eliminate some of these inconsistencies. However, it is possible that other factors not investigated in this study were driving differences in disease response other than genotype. The microbial community found on each fragment and symbionts within the coral tissue may have played a role in relative disease susceptibility (Bourne et al. 2009), in addition to individual fragment health. Although outside the scope of this study, these may be driving susceptibility or resistance and should be investigated in future studies.

The high level of disease in the control treatments also greatly impacted my results on relative resistance. No genotype was found to be significantly more or less resistant to disease than their control, as indicated by the Bayesian relative risk assessments. Additionally, each pathogen transmission treatment was not significantly different from their corresponding control due to high level of disease in controls. While histological analysis helped to identify the poor condition of many fragments at the start of the pathogen transmission experiment, I was not able to process enough samples to understand how each genotype may have differed histologically if they later remained healthy or became diseased. However, in my later acclimation experiment, I determined that genotype did not significantly affect overall condition score, suggesting that there may not be observable trends between genotypes using histology. Reducing the amount of disease in control fragments would likely produce more clear results on relative susceptibility or resistance to disease and may be done by experimenting during a different time of year when background disease prevalence is lower or by improving the condition of these fragments prior to the start of the experiment.

Results from the acclimation study suggest that increasing the acclimation period may help to improve the condition of fragments prior to disease application. To preserve rapidly disappearing diseased tissue on actively diseased fragments during the pathogen

transmission experiment, I was only able to acclimate fragments for 40 hours. During the acclimation study, I observed a significant decline in overall tissue condition from day 0 to day 2. Therefore, it is possible that fragments in the pathogen transmission study were even more susceptible to disease due to stress than when they were originally transported to the experimental facilities. Later improvements from this stress in the acclimation study were not seen until day 9 in epidermal mucocytes and zooxanthellae in the surface body wall. This suggests that by acclimating fragments for nine or more days, health of fragments may improve. Miller and Williams (2016) have suggested an acclimation period of at least 2 weeks *in situ* to allow clipped fragment margins to heal, which my results support. In the case of the pathogen transmission experiment, an acclimation period of this time likely would have revealed which fragments were diseased on the microscopic level over time. Fragments that exhibited signs of disease during this longer acclimation period could easily be removed from the experiment prior to pathogen transmission application. This would help obtain a better idea of disease resistance, if this reduced the amount of disease in control fragments. Adopting standard acclimation periods for each species of coral may help produce more comparable results between studies and improve our understanding of response to biotic and abiotic stressors.

Although not investigated in my study, it is crucial to determine the extent to which tissue may change from collection in the field to introduction into the laboratory. In my studies, fragments experienced about four hours of transportation time from the time they were collected in the upper Florida Keys, driven to Ft. Lauderdale, and placed in tanks at NSU. Histology samples were only taken once back at the laboratory. It is unknown how quickly tissue degeneration may occur and is important to consider this when planning the transportation of corals. However, based on the rapid tissue degradation and removal of tissue visually observed in the diseased fragments, quick degeneration may be possible on the microscopic scale. Some limitations in my histological analyses occurred because of this. Even when visually observing fragment health daily, in some cases fragments had lost all tissue before clippings could be taken for histological analysis. Additionally, many of the diseased tissue fragments that were collected disintegrated during the decalcification process due to the extremely poor and delicate condition of the tissue. In the future, fragments should be monitored for disease

greater than once daily, and agarose enrobing techniques should be used to maintain tissue structure for processing (E. Peters, pers. comm.).

Despite these restraints, I was able to identify differences in diseased fragments and healthy fragments using histological techniques. This was crucial for determining poor initial condition at the start of the experiment, which was unknown prior to histological analysis. This also helped to confirm which tissue layers were most affected by disease, and which may be good indicators of susceptibility. Based on my results, the inner-most coral polyp parameters, including the mesenterial filaments, gastrodermis, and calicodermis, were most affected by disease and were also in worse condition in pre-exposure treatment samples that later showed signs of disease. This supports the observation that *A. cervicornis* appears to die from the inside-out (E. Peters, pers. comm.). These parameters influence the corals' ability to capture food, digest food, and store lipids (Miller et al. 2014). Having little energy due to the lack of these functioning tissue types may also explain the poor condition of the calicodermis I observed, which is the skeleton-producing epithelium of the coral. Without sufficient energy, it is unlikely that the coral would be able to deposit skeleton. Similarly, it is possible that this lack of energy would limit the coral's immune response and decrease its ability to defend against diseases (Sheldon and Verhulst 1996, Sandland and Minchella 2003, Sadd and Schmid-Hempel 2009).

Overall, these data enhance our understanding of pathogen transmission and response in a local *A. cervicornis* nursery population. While disease management techniques may be useful for controlling small areas of disease, they may not be a feasible option for preventing the extinction of threatened species, such as *A. cervicornis,* in future environmental conditions. Properly identifying genotypes that are more disease resistant will help to direct conservation efforts and inform nursery managers on which genotypes to use in restoration efforts, which will likely affect future population success of this species within southern Florida. This can only be done using carefully considered pathogen transmission methods and acclimation periods accounted for in study design. The results from my studies help to highlight the importance of these details for producing more accurate conclusions in the future. Specifically, I demonstrated that: (1) an acclimation period of at least nine days is recommended when transferring *A.*

cervicornis approximately four hours or more, (2) histological analysis should be used to determine the condition of fragments at the start of transmission experiments, and (3) disease susceptibility varies by genotype and pathogen transmission method used. Incorporating these findings to design future studies may help with identifying corals capable of maintaining reef integrity and preventing the extinction of *A. cervicornis* in the years to come.

LITERATURE CITED

Adey, W. H., and R. Bruke. 1977. Holocene bioherms of the Lesser Antilles, geological control of development. Studies in Geology **4**:67-81.

Aeby, G., M. Ross, G. Williams, T. Lewis, and T. Work. 2010. Disease dynamics of Montipora white syndrome within Kaneohe Bay, Oahu, Hawaii: distribution, seasonality, virulence, and transmissibility. Diseases of aquatic organisms **91**:1-8.

Aeby, G. S., G. J. Williams, E. C. Franklin, J. Kenyon, E. F. Cox, S. Coles, and T. M. Work. 2011. Patterns of coral disease across the Hawaiian Archipelago: relating disease to environment. PLoS ONE **6**:e20370.

Aeby, G. S., T. M. Work, C. M. Runyon, A. Shore-Maggio, B. Ushijima, P. Videau, S. Beurmann, and S. M. Callahan. 2015. First record of black band disease in the Hawaiian Archipelago: response, outbreak status, virulence, and a method of treatment. PLoS ONE **10**:e0120853.

Aguilar-Perera, A., and R. Hernández-Landa. 2017. Occurrence of large thickets of Acropora prolifera (Scleractinia: Acroporidae) in the southern Gulf of Mexico. Marine Biodiversity:1-3.

Antonius, A. 1976. New observations on coral destruction in reefs.*in* 10th Meeting of the Association of Island Marine Laboratories of the Caribbean, Mayaguez, PR.

Antonius, A. 1981. The "band" disease in coral reefs. Pages 7-14 *in* Proceedings of the 4th International Coral Reef Symposium, Manila.

Aronson, R. B., A. Bruckner, J. Moore, B. Precht, and E. Weil. 2008. Acropora cervicornis. The IUCN Red List of Threatened Species 2008: e.T133381A3716457.

Aronson, R. B., and W. F. Precht. 1997. Stasis, biological disturbance, and community structure of a Holocene coral reef. Paleobiology **23**:326-346.

Aronson, R. B., and W. F. Precht. 2001. White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia **460**:25-38.

Baker, A. C., C. J. Starger, T. R. McClanahan, and P. W. Glynn. 2004. Coral reefs: corals' adaptive response to climate change. Nature **430**:741-741.

Baums, I. B. 2008. A restoration genetics guide for coral reef conservation. Molecular Ecology **17**:2796-2811.

Bourne, D. G., M. Garren, T. M. Work, E. Rosenberg, G. W. Smith, and C. D. Harvell. 2009. Microbial disease and the coral holobiont. Trends in Microbiology **17**:554-562.

Brandt, M. E., and J. W. McManus. 2009. Disease incidence is related to bleaching extent in reef-building corals. Ecology **90**:2859–2867.

Brown, B. E. 1997. Coral bleaching: causes and consequences. Coral reefs **16**:129-138.

Brown, B. E., and J. C. Bythell. 2005. Perspectives on mucus secretion in reef corals. Marine Ecology Progress Series **296**:291-309.

Bruckner, A. W. 2002. Proceedings of the Caribbean Acropora workshop: potential application of the U.S. Endangered Species Act as a conservation strategy. Page 199. NOAA Technical Memorandum, NMFS-OPR-24, Silver Spring, MD.

Bruno, J. F. 2015. Marine biology: The coral disease triangle. Nature Climate Change **5**:302-303.

Bruno, J. F., L. E. Petes, C. Drew Harvell, and A. Hettinger. 2003. Nutrient enrichment can increase the severity of coral diseases. Ecology Letters **6**:1056-1061.

Bruno, J. F., E. R. Selig, K. S. Casey, C. A. Page, B. L. Willis, C. D. Harvell, H. Sweatman, and A. M. Melendy. 2007. Thermal stress and coral cover as drivers of coral disease outbreaks. PLOS Biology **5**:e124.

Bruno, J. F., and A. Valdivia. 2016. Coral reef degradation is not correlated with local human population density. Scientific Reports **6**.

Burke, L., K. Reytar, M. Spalding, and A. Perry. 2011. Reefs at risk revisited.

Carpenter, K. E., M. Abrar, G. Aeby, R. B. Aronson, S. Banks, A. Bruckner, A. Chiriboga, J. Cortés, J. C. Delbeek, L. DeVantier, G. J. Edgar, A. J. Edwards, D. Fenner, H. M. Guzmán, B. W. Hoeksema, G. Hodgson, O. Johan, W. Y. Licuanan, S. R. Livingstone, E. R. Lovell, J. A. Moore, D. O. Obura, D. Ochavillo, B. A. Polidoro, W. F. Precht, M. C. Quibilan, C. Reboton, Z. T. Richards, A. D. Rogers, J. Sanciangco, A. Sheppard, C. Sheppard, J. Smith, S. Stuart, E. Turak, J. E. N. Veron, C. Wallace, E. Weil, and E. Wood. 2008. One-third of reef-building corals face elevated extinction risk from climate change and local impacts. Science **321**:560-563.

Casas, V., D. I. Kline, L. Wegley, Y. Yu, M. Breitbart, and F. Rohwer. 2004. Widespread association of a Rickettsiales-like bacterium with reef-building corals. Environmental microbiology **6**:1137-1148.

Cesar, H., L. Burke, and L. Pet-Soede. 2003. The economics of worldwide coral reef degradation. Cesar environmental economics consulting (CEEC).

Crossland, C. J., D. J. Barnes, and M. A. Borowitzka. 1980. Diurnal lipid and mucus production in the staghorn coral Acropora acuminata. Marine Biology **60**:81-90.

Cunning, R., R. N. Silverstein, and A. C. Baker. 2015. Investigating the causes and consequences of symbiont shuffling in a multi-partner reef coral symbiosis under

environmental change. Proceedings of the Royal Society B: Biological Sciences **282**.

Davies, P. S. 1984. The role of zooxanthellae in the nutritional energy requirements of Pocillopora eydouxi. Coral reefs **2**:181-186.

Donner, S. D., T. R. Knutson, and M. Oppenheimer. 2007. Model-based assessment of the role of human-induced climate change in the 2005 Caribbean coral bleaching event. Proceedings of the National Academy of Sciences **104**:5483-5488.

Donner, S. D., W. J. Skirving, C. M. Little, M. Oppenheimer, and O. V. E. Hoegh-Guldberg. 2005. Global assessment of coral bleaching and required rates of adaptation under climate change. Global Change Biology **11**:2251-2265.

Douglas, A. E. 2003. Coral bleaching—how and why? Marine Pollution Bulletin **46**:385-392.

Drury, C., D. Manzello, and D. Lirman. 2017. Genotype and local environment dynamically influence growth, disturbance response and survivorship in the threatened coral, Acropora cervicornis. PLoS ONE **12**:e0174000.

Edwards, A. 2010. Reef Rehabilitation Manual.

EPA, U. S. 2017. Climate change indicators: sea surface temperature.

Fitt, W. K., R. D. Gates, O. Hoegh-Guldberg, J. C. Bythell, A. Jatkar, A. G. Grottoli, M. Gomez, P. Fisher, T. C. Lajuenesse, O. Pantos, R. Iglesias-Prieto, D. J. Franklin, L. J. Rodrigues, J. M. Torregiani, R. van Woesik, and M. P. Lesser. 2009. Response of two species of Indo-Pacific corals, Porites cylindrica and Stylophora pistillata, to short-term thermal stress: The host does matter in determining the tolerance of corals to bleaching. Journal of Experimental Marine Biology and Ecology **373**:102-110.

Fogarty, N. D. 2012. Caribbean acroporid coral hybrids are viable across life history stages. Marine Ecology Progress Series **446**:145-159.

Galloway, S., C. Woodley, S. McLaughlin, T. Work, V. Bochsler, C. Meteyer, L. Sileo, E. Peters, E. Kramarsky-Winters, and J. Morado. 2007. Coral disease and health workshop: coral histopathology II.

Gardner, T. A., I. M. Côté, J. A. Gill, A. Grant, and A. R. Watkinson. 2003. Long-term region-wide declines in Caribbean corals. Science **301**:958-960.

Garrett, P., and H. Ducklow. 1975. Coral diseases in Bermuda. Nature **253**:349-350.

Geister, J. 1997. The influence of wave exposure on the ecological zonation of caribbean coral reefs. Rosenstiel School of Marine and Atmospheric Science, Miami.

Gignoux-Wolfsohn, S. A., C. J. Marks, and S. V. Vollmer. 2012. White band disease transmission in the threatened coral, Acropora cervicornis. Scientific Reports **2**:804.

Gignoux-Wolfsohn, S. A., and S. V. Vollmer. 2015. Identification of candidate coral pathogens on white band disease-infected Staghorn Coral. PLoS ONE **10**:e0134416.

Gil-Agudelo, D., G. Smith, and E. Weil. 2006. The white band disease type II pathogen in Puerto Rico.

Gil-Agudelo, D. L., and J. Garzon-Ferreira. 2001. Spatial and seasonal variation of Dark Spots Disease in coral communities of the Santa Marta area (Colombian Caribbean). Bulletin of Marine Science **69**:619-629.

Gladfelter, W. B. 1982. White-band disease in Acropora palmata: implications for the structure and growth of shallow reefs. Bulletin of Marine Science **32**:639-643.

Goreau, T. F. 1959. The ecology of Jamaican coral reefs: species composition and zonation. Ecology **40**:67-90.

Green, E. P., and A. W. Bruckner. 2000. The significance of coral disease epizootiology for coral reef conservation. Biological Conservation **96**:347-361.

Guest, J. R., A. H. Baird, J. A. Maynard, E. Muttaqin, A. J. Edwards, S. J. Campbell, K. Yewdall, Y. A. Affendi, and L. M. Chou. 2012. Contrasting patterns of coral bleaching susceptibility in 2010 suggest an adaptive response to thermal stress. PLoS ONE **7**:e33353.

Harvell, C. D., K. Kim, J. M. Burkholder, R. R. Colwell, P. R. Epstein, D. J. Grimes, E. E. Hofmann, E. K. Lipp, A. D. M. E. Osterhaus, R. M. Overstreet, J. W. Porter, G. W. Smith, and G. R. Vasta. 1999. Emerging marine diseases--climate links and anthropogenic factors. Science **285**:1505-1510.

Harvell, C. D., C. E. Mitchell, J. R. Ward, S. Altizer, A. P. Dobson, R. S. Ostfeld, and M. D. Samuel. 2002. Climate warming and disease risks for terrestrial and marine biota. Science **296**:2158-2162.

Harvell, D., E. Jordán-Dahlgren, S. Merkel, E. Rosenberg, L. Raymundo, G. Smith, E. Weil, and B. Willis. 2007. Coral disease, environmental drivers, and the balance between coral and microbial associates. Oceanography **20**:172-195.

Heron, S. F., J. A. Maynard, R. van Hooidonk, and C. M. Eakin. 2016. Warming trends and bleaching stress of the world's coral reefs 1985–2012. Scientific Reports **6**:38402.

Highsmith, R. C., A. C. Riggs, and C. M. D'Antonio. 1980. Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. Oecologia **46**:322-329.

Hightshoe, M. V. 2018. Identifying disease-resistant and thermal-tolerant genotypes in the threatened staghorn coral, Acropora cervicornis. Nova Southeastern University, Halmos College of Natural Sciences and Oceanography.

Hoegh-Guldberg, O. 1999. Climate change, coral bleaching and the future of the world's coral reefs. Marine and Freshwater Research **50**:839-866.

Hoegh-Guldberg, O., and J. F. Bruno. 2010. The impact of climate change on the world's marine ecosystems. Science **328**:1523-1528.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, and M. E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science **318**:1737-1742.

Hogarth, W. 2006. Endangered and threatened species: final listing determinations for elkhorn coral and staghorn coral. Federal Register **71**:26852-26861.

Hubbard, D. K. 1988. Controls of modern and fossil reef development: common ground for biological and geological research.*in* Proceedings: Sixth International Coral Reef Symposium, Townsville.

Hughes, T. P. 1994. Catastrophes, phase shifts, and large-scale degradation of a Caribbean coral reef. Science-AAAS-Weekly Paper Edition **265**:1547-1551.

Hunt, J., and W. Sharp. 2014. Developing a comprehensive strategy for coral restoration in Florida

Huston, M. 1985. Variation in coral growth rates with depth at Discovery Bay, Jamaica. Coral reefs **4**:19-25.

Jaap, W. C. 2000. Coral reef restoration. Ecological Engineering **15**:345-364.

Jackson, J. B. C., M. X. Kirby, W. H. Berger, K. A. Bjorndal, L. W. Botsford, B. J. Bourque, R. H. Bradbury, R. Cooke, J. Erlandson, J. A. Estes, T. P. Hughes, S. Kidwell, C. B. Lange, H. S. Lenihan, J. M. Pandolfi, C. H. Peterson, R. S. Steneck, M. J. Tegner, and R. R. Warner. 2001. Historical overfishing and the recent collapse of coastal ecosystems. Science **293**:629-637.

Jagoe, C. H. 1996. Responses at the tissue level: quantitative methods in histopathology applied to ecotoxicology. Ecotoxicology: A Hierarchical Treatment. Lewis Publishers, Boca Raton, Florida:163-196.

Japaud, A., C. Fauvelot, and C. Bouchon. 2014. Unexpected high densities of the hybrid coral Acropora prolifera (Lamarck 1816) in Guadeloupe Island, Lesser Antilles. Coral reefs **33**:593-593.

Jones, R. J. 1997. Changes in zooxanthellar densities and chlorophyll concentrations in corals during and after a bleaching event. Marine Ecology Progress Series **158**:51-59.

Jones, R. J., J. Bowyer, O. Hoegh-Guldberg, and L. L. Blackall. 2004. Dynamics of a temperature-related coral disease outbreak. Marine Ecology Progress Series **281**:63-77.

Kline, D. I., and S. V. Vollmer. 2011. White Band Disease (type I) of endangered Caribbean acroporid corals is caused by pathogenic bacteria. Scientific Reports **1**:7.

Knowlton, N., and J. B. C. Jackson. 2008. Shifting baselines, local impacts, and global change on coral reefs. PLOS Biology **6**:e54.

Knutson, T. R., J. L. McBride, J. Chan, K. Emanuel, G. Holland, C. Landsea, I. Held, J. P. Kossin, A. K. Srivastava, and M. Sugi. 2010. Tropical cyclones and climate change. Nature Geoscience **3**:157-163.

Kobluk, D. R., and M. A. Lysenko. 1992. Storm features on a southern Caribbean fringing coral reef. PALAIOS **7**:213-221.

Kuntz, N. M., D. I. Kline, S. A. Sandin, and F. Rohwer. 2005. Pathologies and mortality rates caused by organic carbon and nutrient stressors in three Caribbean coral species. Marine Ecology Progress Series **294**:173-180.

Kushmaro, A., E. Rosenberg, M. Fine, and Y. Loya. 1997. Bleaching of the coral Oculina patagonica by Vibrio AK-1. Marine Ecology Progress Series **147**:159-165.

Kuta, K. G., and L. Richardson. 2002. Ecological aspects of black band disease of corals: Relationships between disease incidence and environmental factors.

Lang, J., N. Knowlton, and B. D. Keller. 1990. Case study of natural population collapse: post-hurricane prediction on Jamaican staghorn corals.

Lesser, M. P., J. C. Bythell, R. D. Gates, R. W. Johnstone, and O. Hoegh-Guldberg. 2007. Are infectious diseases really killing corals? Alternative interpretations of the experimental and ecological data. Journal of Experimental Marine Biology and Ecology **346**:36-44.

Lirman, D., and P. Fong. 1997. Patterns of damage to the branching coral Acropora palmata following hurricane Andrew: damage and survivorship of hurricane-generated asexual recruits. Journal of Coastal Research **13**:67-72.

Lirman, D., S. Schopmeyer, V. Galvan, C. Drury, A. C. Baker, and I. B. Baums. 2014. Growth dynamics of the threatened Caribbean staghorn coral Acropora cervicornis: influence of host genotype, symbiont identity, colony size, and environmental setting. PLoS ONE **9**:e107253.

Lundgren, I., and Z. Hillis-Starr. 2008. Variation in Acropora palmata bleaching across benthic zones at Buck Island Reef National Monument (St. Croix, USVI) during the 2005 thermal stress event. Bulletin of Marine Science **83**:441-451.

Maynard, J., K. Anthony, P. Marshall, and I. Masiri. 2008. Major bleaching events can lead to increased thermal tolerance in corals. Marine Biology **155**:173-182.

McNeill, D. F., A. F. Budd, and P. F. Borne. 1997. Earlier (late Pliocene) first appearance of the Caribbean reef-building coral Acropora palmata: Stratigraphic and evolutionary implications. Geology **25**:891-894.

Merselis, D. G., D. Lirman, and M. Rodriguez-Lanetty. 2018. Symbiotic immuno-suppression: is disease susceptibility the price of bleaching resistance? PeerJ **6**:e4494.

Middlebrook, R., O. Hoegh-Guldberg, and W. Leggat. 2008. The effect of thermal history on the susceptibility of reef-building corals to thermal stress. Journal of Experimental Biology **211**:1050-1056.

Miller, J., E. Muller, C. Rogers, R. Waara, A. Atkinson, K. R. T. Whelan, M. Patterson, and B. Witcher. 2009. Coral disease following massive bleaching in 2005 causes 60% decline in coral cover on reefs in the US Virgin Islands. Coral reefs **28**:925-937.

Miller, M. W., K. E. Lohr, C. M. Cameron, D. E. Williams, and E. C. Peters. 2014. Disease dynamics and potential mitigation among restored and wild staghorn coral, Acropora cervicornis. PeerJ **2**:e541.

Miller, M. W., and D. E. Williams. 2016. A standard field protocol for testing relative disease resistance in Acropora palmata and Acropora cervicornis. PeerJ Preprints **4**:e2668v2661.

Moberg, F., and C. Folke. 1999. Ecological goods and services of coral reef ecosystems. Ecological economics **29**:215-233.

Muller, E., and R. Van Woesik. 2009. Shading reduces coral-disease progression.

Muller, E. M., C. S. Rogers, A. S. Spitzack, and R. van Woesik. 2008. Bleaching increases likelihood of disease on Acropora palmata (Lamarck) in Hawksnest Bay, St John, US Virgin Islands. Coral reefs **27**:191-195.

Muller, E. M., and R. van Woesik. 2012. Caribbean coral diseases: primary transmission or secondary infection? Global Change Biology **18**:3529-3535.

NOAA. 2007. Florida Keys National Marine Sanctuary Revised Management Plan. Page 5.

Pandolfi, J. 2002. Coral community dynamics at multiple scales. Coral Reefs **21**:13-23.

Pandolfi, J. M. 2011. The paleoecology of coral reefs. Pages 13-24 Coral Reefs: An Ecosystem in Transition. Springer.

Pandolfi, J. M., J. B. C. Jackson, N. Baron, R. H. Bradbury, H. M. Guzman, T. P. Hughes, C. V. Kappel, F. Micheli, J. C. Ogden, H. P. Possingham, and E. Sala. 2005. Are U.S. coral reefs on the slippery slope to slime? Science **307**:1725-1726.

Patterson, K. L., J. W. Porter, K. B. Ritchie, S. W. Polson, E. Mueller, E. C. Peters, D. L. Santavy, and G. W. Smith. 2002. The etiology of white pox, a lethal disease of the Caribbean elkhorn coral, Acropora palmata. Proceedings of the National Academy of Sciences, USA **99**:8725-8730.

Peters, E. C. 2015. Diseases of coral reef organisms. Pages 147-178 *in* C. Birkeland, editor. Coral Reefs in the Anthropocene. Springer Dordrecht, The Netherlands.

Peters, E. C., J. J. Oprandy, and P. P. Yevich. 1983. Possible causal agent of "white band disease" in caribbean acroporid corals. Journal of Invertebrate Pathology **41**:394-396.

Plaisance, L., M. J. Caley, R. E. Brainard, and N. Knowlton. 2011. The diversity of coral reefs: what are we missing? PLoS ONE **6**:e25026.

Polson, S. W., J. L. Higgins, and C. M. Woodley. 2008. PCR-based assay for detection of four coral pathogens. Pages 247-251 *in* 11th International Coral Reef Symposium, Ft. Lauderdale, FL.

Porter, J. W., P. Dustan, W. C. Jaap, K. L. Patterson, V. Kosmynin, O. W. Meier, M. E. Patterson, and M. Parsons. 2001. Patterns of spread of coral disease in the Florida Keys. Hydrobiologia **460**:1-24.

Porter, J. W., and O. W. Meier. 1992. Quantification of loss and change in floridian reef coral populations. American Zoologist **32**:625-640.

Randall, C. J., A.G. Jordan-Garza, E.M. Muller, R. Van Woesik. 2014. Relationships between the history of thermal stress and the relative risk of diseases of Caribbean corals. Ecology **95**:1981–1994.

Randall, C. J., A. G. Jordán-Garza, E. M. Muller, and R. van Woesik. 2016. Does dark-spot syndrome experimentally transmit among Caribbean corals? PLoS ONE **11**:e0147493.

Raymundo, L. J., C. S. Couch, C. D. Harvell, J. Raymundo, A. W. Bruckner, T. M. Work, E. Weil, C. M. Woodley, E. Jordan-dahlgren, and B. L. Willis. 2008. Coral disease handbook guidelines for assessment, monitoring & management.

Richardson, L. L. 2015. Arnfried Antonius, coral diseases, and the AMLC. Revista de Biología Tropical **60**.

Richardson, L. L., and R. B. Aronson. 2000. Infectious diseases of reef corals. Proceedings 9th International Coral Reef Symposium, Bali, Indonesia **2**:23-27.

Rinkevich, B. 1995. Restoration strategies for coral reefs damaged by recreational activities: the use of sexual and asexual recruits. Restoration Ecology **3**:241-251.

Rinkevich, B. 2005. Conservation of coral reefs through active restoration measures: recent approaches and last decade progress.

Rinkevich, B. 2006. The coral gardening concept and the use of underwater nurseries.

Rinkevich, B. 2014. Rebuilding coral reefs: does active reef restoration lead to sustainable reefs? Current Opinion in Environmental Sustainability **7**:28-36.

Ritchie, K. B. 2006. Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Marine Ecology Progress Series **322**:1-14.

Ritchie, K. B., S. W. Polson, and G. W. Smith. 2001. Microbial disease causation in marine invertebrates: problems, practices, and future prospects. Pages 131-139 *in* J. W. Porter, editor. The Ecology and Etiology of Newly Emerging Marine Diseases. Springer Netherlands.

Ritchie, K. B., and G. W. Smith. 1998. Type II white-band disease. Revista de Biología Tropical **46**:199-203.

Rogers, C. S. 1993. A matter of scale: damage from Hurricane Hugo (1989) to U.S. Virgin Islands reefs at the colony, community and whole reef level. Pages 127-133 Proceedings of the 7th International Coral Reef Symposium. University of Guam Press, Guam, Micronesia.

Rogers, C. S. 2010. Words matter: recommendations for clarifying coral disease nomenclature and terminology. Diseases of aquatic organisms **91**:167-175.

Rogers, C. S., T. H. Suchanek, and F. A. Pecora. 1982. Effects of hurricanes David and Frederic (1979) on shallow Acropora palmata reef communities: St. Croix, U.S. Virgin Islands. Bulletin of Marine Science **32**:532-548.

Rosenberg, E., and Y. Ben-Haim. 2002. Microbial diseases of corals and global warming. Environmental microbiology **4**:318-326.

Sadd, B. M., and P. Schmid-Hempel. 2009. PERSPECTIVE: Principles of ecological immunology. Evolutionary Applications **2**:113-121.

Sandland, G. J., and D. J. Minchella. 2003. Costs of immune defense: an enigma wrapped in an environmental cloak? Trends in Parasitology **19**:571-574.

Scott, M. E. 1988. The impact of infection and disease on animal populations: implications for conservation biology. Conservation Biology **2**:40-56.

Selig, E., D. Harvell, J. Bruno, B. Willis, C. Page, K. Casey, and H. Sweatman. 2006. Analyzing the relationships between ocean temperature anomalies and coral disease outbreaks at broad spatial scales. Coral reefs and climate change:111-128.

Shafir, S., J. Rijn, and B. Rinkevich. 2006. A mid-water coral nursery.

Sheldon, B. C., and S. Verhulst. 1996. Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends in Ecology & Evolution **11**:317-321.

Spalding, M., C. Ravilious, and E. P. Green. 2001. World atlas of coral reefs. Univ of California Press.

Spurgeon, J. 2001. Improving the economic effectiveness of coral reef restoration.

Stat, M., and R. Gates. 2011. Clade D Symbiodinium in scleractinian corals: a "nugget" of hope, a selfish opportunist, an ominous sign, or all of the above?

Sumich, J. L. 1996. An Introduction to the Biology of Marine Life. Jones and Bartlett Publishers.

Sutherland, K. P., J. W. Porter, and C. Torres. 2004. Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Marine Ecology Progress Series **266**:273-302.

Sweet, M. J., A. Croquer, and J. C. Bythell. 2014. Experimental antibiotic treatment identifies potential pathogens of white band disease in the endangered Caribbean coral Acropora cervicornis. Proceedings of The Royal Society B **281**:20140094.

Timmermann, A., J. Oberhuber, A. Bacher, M. Esch, M. Latif, and E. Roeckner. 1999. Increased El Nino frequency in a climate model forced by future greenhouse warming. Nature **398**:694-697.

Toren, A., L. Landau, A. Kushmaro, Y. Loya, and E. Rosenberg. 1998. Effect of temperature on adhesion of Vibrio strain AK-1 to Oculina patagonica and on coral bleaching. Applied and Environmental Microbiology **64**:1379-1384.

Trenberth, K. E., and T. J. Hoar. 1996. The 1990–1995 El Niño-Southern Oscillation Event: longest on record. Geophysical Research Letters **23**:57-60.

Upton, S. J., and E. C. Peters. 1986. A new and unusual species of coccidium (Apicomplexa: Agamococcidiorida) from Caribbean scleractinian corals. Journal of Invertebrate Pathology **47**:184-193.

Ushijima, B., A. Smith, G. S. Aeby, and S. M. Callahan. 2012. Vibrio owensii induces the tissue loss disease Montipora white syndrome in the Hawaiian reef coral Montipora capitata. PLoS ONE **7**:e46717.

Ushijima, B., P. Videau, A. H. Burger, A. Shore-Maggio, C. M. Runyon, M. Sudek, G. S. Aeby, and S. M. Callahan. 2014. Vibrio coralliilyticus strain OCN008 is an etiological agent of acute montipora white syndrome. Applied and Environmental Microbiology **80**:2102-2109.

van Hooidonk, R., J. Maynard, J. Tamelander, J. Gove, G. Ahmadia, L. Raymundo, G. Williams, S. F. Heron, and S. Planes. 2016. Local-scale projections of coral reef futures and implications of the Paris Agreement. Scientific Reports **6**:39666.

Van Oppen, M., B. Willis, H. Van Vugt, and D. Miller. 2000. Examination of species boundaries in the Acropora cervicornis group (Scleractinia, Cnidaria) using nuclear DNA sequence analyses. Molecular Ecology **9**:1363-1373.

van Oppen, M. J. H., R. D. Gates, L. L. Blackall, N. Cantin, L. J. Chakravarti, W. Y. Chan, C. Cormick, A. Crean, K. Damjanovic, H. Epstein, P. L. Harrison, T. A. Jones, M. Miller, R. J. Pears, L. M. Peplow, D. A. Raftos, B. Schaffelke, K. Stewart, G. Torda, D. Wachenfeld, A. R. Weeks, and H. M. Putnam. 2017. Shifting paradigms in restoration of the world's coral reefs. Global Change Biology **23**:3437-3448.

van Oppen, M. J. H., J. K. Oliver, H. M. Putnam, and R. D. Gates. 2015. Building coral reef resilience through assisted evolution. Proceedings of the National Academy of Sciences **112**:2307-2313.

Vaughan, T. W. 1915. The geologic significance of the growth-rate of the Floridian and Bahaman shoal-water corals. Journal of the Washington Academy of Sciences **5**:591-600.

Vollmer, S. V., and D. I. Kline. 2008. Natural disease resistance in threatened staghorn corals. PLoS ONE **3**:e3718.

Vollmer, S. V., and S. R. Palumbi. 2002. Hybridization and the evolution of reef coral diversity. Science **296**:2023-2025.

Wallace, C., and M. B. Dale. 1978. An information analysis approach to zonation patterns of the coral genus Acropora on outer reef buttresses. Atoll Research Bulletin **220**:95-110.

Wallace, C. C. 2012. Acroporidae of the Caribbean. Geologica Belgica **15**:388-393.

Wallace, C. C., and B. L. Willis. 1994. Systematics of the coral genus Acropora: implications of new biological findings for species concepts. Annual Review of Ecology and Systematics **25**:237-262.

Weil, E., and C. S. Rogers. 2011. Coral reef diseases in the Atlantic-Caribbean. Pages 465-491 Coral reefs: an ecosystem in transition. Springer.

Weil, E., Smith, Garriet, Gil-Agudelo, Diego, L. 2006. Status and progress in coral reef disease research. Diseases of aquatic organisms **69**:1-7.

Weis, V. M. 2008. Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. Journal of Experimental Biology **211**:3059.

Wilkinson, C., and D. Souter. 2008. Status of Caribbean coral reefs after bleaching and hurricanes in 2005. Townsville.

Wilkinson, C. C. 2004. Status of coral reefs of the world: 2004. Australian Institute of Marine Science (AIMS).

Williams, D. E., and M. W. Miller. 2005. Coral disease outbreak: pattern, prevalence and transmission in Acropora cervicornis. Marine Ecology Progress Series **301**:119-128.

Williams, D. E., M. W. Miller, and K. L. Kramer. 2006. Demographic monitoring protocols for threatened Caribbean Acropora spp. corals.

Willis, B. L., C. A. Page, and E. A. Dinsdale. 2004. Coral disease on the great barrier reef. Pages 69-104 Coral health and disease. Springer.

Wobeser, G. A. 1981. Diseases of Wild Waterfowl. Plenum Press, New York, NY.

Woodley, C., A. Bruckner, A. McLenon, J. Higgins, S. Galloway, and J. Nicholson. 2008. Field manual for investigating coral disease outbreaks.

Woodley, J., E. Chornesky, P. Cliffo, J. Jackson, L. Kaufman, N. Knowlton, J. La, M. Pearson, J. Porter, and M. Rooney. 1981. Hurricane Allen's impact on a Jamaican coral reef. Science **214**:13.

Woody, K., A. Atkinson, R. Clark, C. Jeffrey, I. Lundgren, J. Miller, M. Monaco, E. Muller, M. Patterson, C. Rogers, T. Smith, T. Spitzack, R. Waara, K. Whelan, B. Witcher, and A. Wright. 2008. Coral bleaching in the U.S. Virgin Islands in 2005 and 2006.

Work, T., and C. Meteyer. 2014. To understand coral disease, look at coral cells. EcoHealth **11**:610-618.

Work, T. M., and G. S. Aeby. 2006. Systematically describing gross lesions in corals. Diseases of aquatic organisms **70**:155-160.

Young, C. N., S. A. Schopmeyer, and D. Lirman. 2012. A review of reef restoration and coral propagation using the threatened genus Acropora in the Caribbean and western Atlantic. Bulletin of Marine Science **88**:1075-1098.

Zhang, H.-M., B. Huang, J. Lawrimore, M. Menne, and T. M. Smith. NOAA Global Surface Temperature Dataset (NOAAGlobalTemp), Version 4.0 NOAA National Centers for Environmental Information.

Appendix

Table A1. Histology scoring rubric developed by Dr. Esther Peters, Megan Bock, and Morgan Hightshoe. Adopted and modified from Miller et al. 2014. Characteristics noted in cells and tissues using light microscopic examination of *A. cervicornis*.

Parameter Viewed at 100x or 250+x, Description of "Normal"	Numerical Score — Intensity or Severity Score				
0 (No Change)	1	2	3	4	5
High Magnification (40-60x)	Minimal	Mild	Moderate	Marked	Severe
Epidermal Mucocytes 0 = In 1970s sample, thin columnar cells, uniform distribution and not taller than ciliated supporting cells, pale mucus	Slightly hypertrophied, numerous, pale-staining frothy mucus. Ciliated supporting cells still very abundant.	Many cells hypertrophied, abundant release of pale-staining mucus. Increase of mucus may reduce detection of columnar cells.	Uneven appearance of mucocytes, some hypertrophied but some reduced in size and secretion, darker staining mucus	Some epidermal foci lack mucocytes entirely, atrophy of epidermis and mucocytes evident, darker staining and stringy mucus, necrosis mild to minimal	Loss of many mucocytes, epidermis is atrophied to at least half of normal thickness or more, if mucus present it stains dark, thick, necrosis moderate to severe
Costal Tissue Loss 0 = Tissue covering costae intact, epidermis similar in thickness to epidermis of surface body wall with gastrodermis as it covers the costae, although this may vary with location and be thinner; calicodermis thick, pale to clear cytoplasm, or thinner with cytoplasmic extensions apically	Atrophy of epidermis, mesoglea, and calicodermis, but still intact over costae. Minimal costae exposed.	Up to one-quarter of costae on corallite surfaces exposed due to loss of epithelia and mesoglea	Up to one-half of costae exposed	About three quarters of costae exposed	Most costae exposed or gaps in surface body wall present, tissues atrophied
Zooxanthellae in SBW (40-60X) 0 = Gastrodermal cells packed with well-stained algal symbionts in surface body wall, tentacles; scattered algal symbionts deeper in gastrovascular canals and absorptive cells next to mesenterial filaments	Similar to 1970s samples, thick layer of well-stained algal symbionts in gastrodermis of surface body wall, tentacles, and scattered cells in gastrovascular canals and absorptive cells next to	Thick layer of well-stained algal symbionts, but not quite as abundant as in 1970s samples. Mild atrophy of zooxanthellae and gastrodermis	Algal symbionts fewer in gastrodermis which is mildly atrophied, most zooxanthellae still stain appropriately. About ½ of the zooxanthellae appear atrophied.	Single row of algal symbionts in surface body wall gastrodermis and markedly fewer in tentacle gastrodermis, some are misshapen, shrunken, or have lost acidophilic staining as proteins are no longer present or nucleus/cytoplasm has lysed,	No zooxanthellae present in cuboidal gastrodermal cells of colony (bleached)

Parameter Viewed at 100x or 250+x, Description of "Normal"	Numerical Score Intensity or Severity Score				
0 (No Change)	1	2	3	4	5
	mesenterial filaments			accumulation body (vacuole) enlarged compared to algal cell or missing	
Cnidoglandular Band Epithelium Mucocytes 0 = Oral portion lacks mucocytes, increasing in number aborally, may be abundant with pale mucus; difficult to assess significance of appearance	Less than half the area of cnidoglandular band is mucocytes, but could be more depending on location along the filament, size of mucocytes variable (seen in one or a few cnidoglandular bands)	About half the area is mucocytes, some hypertrophied (seen secretions in ¼ of cnidoglandular bands)	About half the area is mucocytes, all hypertrophied (seen in ½ of cnidoglandular bands)	About three quarters of the area is mucocytes, mucus production reduced, some vacuolation and necrosis present (seen in ¾ of cnidoglandular bands)	Loss of mucocytes, vacuolation and necrosis of most cells present (seen in majority of cnidoglandular bands)
Degeneration of Cnidoglandular Bands 0 = Ciliated columnar cells, nematocytes, acidophilic granular gland cells, and mucocytes abundant (but varying with location), tall, thin columnar, contiguous, terminal bar well formed	Mild reduction in cell height in one or a few areas	Cell height more reduced, mild loss of mucocytes or secretions in ¼ of cnidoglandular bands	Atrophy, loss of cells in ½ of cnidoglandular bands	Moderate atrophy of epithelium, some granular gland cells stain dark pink and are rounded, not columnar, terminal bar not contiguous, some pycnotic nuclei present, loss of cells by detachment and sloughing in ¾ of cnidoglandular bands	Severe atrophy of epithelium, detachment from mesoglea and loss of cells, necrosis or apoptosis of remaining cells, no terminal bar present, loss of cilia in majority of cnidoglandular bands
Dissociation of Cells on Mesenterial Filaments 0 = All cells intact and within normal limits, contiguous, thin columnar morphology, terminal bar present, cilia visible along apical surface	Minimal loss of cilia, but will not be present where mucocytes are predominant <u>in one or few areas</u>	Minimal to mild loss of cells, terminal bar has minute gaps indicating loss of ciliated cells in ¼ of mesenterial filaments	Atrophy of cells, vacuolation, reduced cilia, but filament still intact in ½ of mesenterial filaments	Rounding up and loss of granular gland cells, some pycnotic nuclei present, cell loss evident, terminal bar gaps, terminal web (junctions) between cells lost, starting to spread apart along cnidoglandular band in ¾ of mesenterial filaments	Marked to severe separation of cells, most necrotic with pycnotic nuclei, vacuolated, lysing and loss of mucocytes, nematocysts, granular gland cells and ciliated columnar cells in majority of mesenterial filaments

Parameter Viewed at 100x or 250+x, Description of "Normal"	Numerical Score / Intensity or Severity Score				
0 (No Change)	1	2	3	4	5
Gastrodermal Architecture (BBW) 0= Gastrodermis in BBW is uniform, no apparent swelling, scattered zooxathellae present but not as abundant as SBW (similar to 1976 controls). Thickness of gastrodermis variable based on lipid droplet formation. Swelling indicative of potential intrusion, lysing, necrosis not seen.	None to a few areas of swelling and cell lysing in gastrodermis, scattered zooxanthellae but less than controls	¼ of gastrodermis is swollen, cell lysing present, less zooxanthellae and some released into gastrovascular canals	½ of gastrodermis is swollen, few areas of necrotic tissue, zooxanthellae abundance reduced by ½ or ½ released into gastrovascular canals	¾ of gastrodermis is swelling, necrotic tissue, zooxanthellae abundance reduced by ¾ or ¾ released into gastrovascular canals	Entire BBW gastrodermis is necrotic, extreme swelling is visible, few to no zooxathellae present or majority of zooxanthellae released into gastrovascular canals
Calicodermis Condition 0 = Calicoblasts numerous both peripherally and internally, squamous but thick cytoplasm	Calicoblasts slightly reduced in height focally (more likely interior of colony, basal body wall) more squamous	About half of calicoblasts atrophied, loss of proteins in cytoplasm. Calicoblasts reduced in number	Most calicoblasts atrophied, fewer in number, spread out thinly on mesoglea, still cuboidal to columnar and active under surface body wall and in apical polyps	Most calicoblasts markedly atrophied, fewer in number, some separating from mesoglea	Basal and surface body wall calicoblasts severely atrophied or vacuolated, detaching and sloughing, or missing entirely from mesoglea

Table A2. List of histology samples compared for pathogen transmission experiment. Treatment IDs represent grafting or homogenate and grafting or homogenate controls (G, H, CG, CH, respectively). Number refers to replicate/tank number. When possible, the same fragment was compared between pre-exposure and post-exposure treatments. Due to rapid tissue loss, samples were limited in the post-visually diseased group.

Genotype	Treatment	Type	Note	Genotype	Treatment	Type	Note
K2	G2	Pre	later apparently healthy	K2	G2	Post	apparently healthy
K2	H1	Pre	later apparently healthy	K2	H1	Post	apparently healthy
M5	C1G	Pre	later apparently healthy	M5	C1G	Post	apparently healthy
M5	G1	Pre	later apparently healthy	M5	G1	Post	apparently healthy
U17	C1G	Pre	later apparently healthy	U17	C1G	Post	apparently healthy
U17	G2	Pre	later apparently healthy	U17	G2	Post	apparently healthy
U21	C1H	Pre	later apparently healthy	U21	C1H	Post	apparently healthy
U21	H1	Pre	later apparently healthy	U21	H1	Post	apparently healthy
U22	C1G	Pre	later apparently healthy	U22	C1G	Post	apparently healthy
U22	H1	Pre	later apparently healthy	U22	H1	Post	apparently healthy
U25	G1	Pre	later apparently healthy	U25	G1	Post	apparently healthy
U25	H1	Pre	later apparently healthy	U25	H1	Post	apparently healthy
U30	G2	Pre	later apparently healthy	U30	G2	Post	apparently healthy
U30	H1	Pre	later apparently healthy	U30	H1	Post	apparently healthy
U41	C1H	Pre	later apparently healthy	U41	C1H	Post	apparently healthy
U41	G1	Pre	later apparently healthy	U41	G1	Post	apparently healthy
U68	C1H	Pre	later apparently healthy	U68	C1H	Post	apparently healthy
U68*	H1	Pre	later apparently healthy	U68	H1	Post	apparently healthy
U72	C1G	Pre	later apparently healthy	U72	C1G	Post	apparently healthy
U72	G1	Pre	later apparently healthy	U72	G1	Post	apparently healthy
U77	C1H	Pre	later apparently healthy	U77	C1H	Post	apparently healthy
U77	G1	Pre	later apparently healthy	U77	G1	Post	apparently healthy
M5*	G3	Pre	later diseased	M5	G3	Post	visually diseased
U17	G1	Pre	later diseased				
U17	G3	Pre	later diseased				
U21	G1	Pre	later diseased	U21**	G1	Post	visually diseased
U22	G1	Pre	later diseased				
U22	G2	Pre	later diseased				
U22 **	G3	Pre	later diseased	U22	G3	Post	visually diseased
U25	G2	Pre	later diseased				
U25	G3	Pre	later diseased	U25	G3	Post	visually diseased
U30	G1	Pre	later diseased	U30**	G1	Post	visually diseased
U30	H3	Pre	later diseased				
U41	G2	Pre	later diseased	U41	G2	Post	visually diseased
U68	G1	Pre	later diseased	U68**	G1	Post	visually diseased
U77	G3	Pre	later diseased	U77	G3	Post	visually diseased
U77	H1	Pre	later diseased	U77	H1	Post	visually diseased
* = Two or more parameters unable to be scored. Overall condition score omitted from analysis							
** = Not enough tissue on slide to read. Excluded from statistics							