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Disease dynamics and potential mitigation among restored and wild staghorn coral, *Acropora cervicornis*

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ABSTRACT

The threatened status (both ecologically and legally) of Caribbean staghorn coral, *Acropora cervicornis*, has prompted rapidly expanding efforts in culture and restocking, although tissue loss diseases continue to affect populations. In this study, disease surveillance and histopathological characterization were used to compare disease dynamics and conditions in both restored and extant wild populations. Disease had devastating effects on both wild and restored populations, but dynamics were highly variable and appeared to be site-specific with no significant differences in disease prevalence between wild versus restored sites. Disease affected up to 80% of colonies at one site following a tropical storm. A subset of 20 haphazardly selected colonies at each site observed over a single field season revealed widely varying disease incidence, although not in a consistent way between restored and wild sites, and a case fatality rate of 8%. Lastly, two field mitigation techniques, (1) excision of apparently healthy branch tips from a diseased colony, and (2) placement of a band of epoxy fully enclosing the diseased margin, gave equivocal results with no significant benefit detected for either treatment compared to controls. Tissue condition of associated samples was fair to very poor; unsuccessful mitigation treatment samples had severe degeneration of mesenterial filament cnidoglandular bands. Polyp mucocytes in all samples were infected with suspect rickettsia-like organisms; no bacterial aggregates were found. Overall results do not support differing disease quality, quantity, dynamics, or health management strategies between restored and wild colonies of *A. cervicornis* in the Florida Keys.

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INTRODUCTION

Disease, in conjunction with co-occurring stressors such as storms and warming temperatures, is the major driving factor placing the Caribbean staghorn coral, *Acropora cervicornis*, at risk of extinction (reviewed in Aronson & Precht 2001, IUCN 2012). Understanding the diagnostics and etiology of diseases affecting *A. cervicornis* populations remains problematic, and effective management strategies to combat this ongoing threat to species survival remain elusive. Despite more than a decade of focused research effort, there remains a dearth of strict diagnostic characterization for field cases of disease in *A. cervicornis.* Most authors simply apply the historical label of white-band disease (Gladfelter et al. 1977, Peters 1984) or the more general label rapid tissue loss to what is likely a range of disease conditions (Williams & Miller 2005, Raymundo et al. 2008). Corallivores, such as the snail *Coralliophila abbreviata*, the polychaete *Hermodice carunculata*, and damselfishes or butterflyfishes, further confound the ability to accurately assess disease by removing *A. cervicornis* tissue and leaving feeding scars that may be difficult to distinguish from disease (Bruckner 2001, Sutherland et al. 2004, Miller & Williams 2006). Further, Williams and Miller (2005) found that *C. abbreviata* that had been feeding at tissue loss margins on disease-affected colonies could transmit the condition to apparently healthy branches; thus predation may exacerbate disease spread through a population.

Acropora cervicornis' status under the USA Endangered Species Act carries a legal mandate to orchestrate its recovery (i.e., attain a sustainable status where ESA protections are no longer needed to prevent extinction). This mandate, combined with a growing consensus that decline has reached a point where natural resilience has likely been compromised, has led to increasing efforts to culture and restock populations of *A. cervicornis* (reviewed in Young et al.

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2012). This unprecedented movement toward proactive intervention and population engineering in a coral reef foundation species is occurring within a historical context of mixed success in previous case studies in the fields of fisheries and wildlife management (Hilborn & Eggers 2000, Carlsson et al. 2008, Champagnon et al. 2012). The primary concern for such an endeavor is the potential for unintended introductions of deleterious genetic or health consequences within the imperiled population or its ecosystem (Cunningham 1996, Baums 2008). For this reason, the genetic status of imperiled coral populations, including *A. cervicornis*, has received increasing attention in recent years and strides have been made in addressing the potential genetic risks of culturing and restoring *A. cervicornis* populations, such as outbreeding depression or genetic bottlenecks in cultured stocks (Baums et al. 2010, Hemond & Vollmer 2010).

Addressing potential health risks of transplanting *Acropora cervicornis,* on the other hand, is much more challenging. While explicit risk assessment and risk management frameworks have been proposed and applied in wildlife translocation projects, effective application requires at least qualitative knowledge of pathogens, vectors, and susceptibilities operating in the given species (e.g., Lenihan et al. 1999, Sainsbury & Vaughan-Higgins 2012). The lack of effective diagnostic tools and robust etiological characterization for coral disease in general, and in *A. cervicornis* in particular (Sutherland et al. 2004, Rogers 2010), impairs efficient health risk management. Until a better knowledge base is built for health management of coral populations, general risk-averse best practices are currently conducted in *A. cervicornis* culture and outplanting programs, including focus on field-based (rather than land-based) culture; avoiding outplanting of colonies with visual signs of ill health (discoloration or tissue loss); geographic restriction of source population, nursery site, and recipient populations; and focusing outplanting at sites where there is evidence of prior occupation, but where no live wild

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colonies currently exist (Johnson et al. 2011; L Gregg, Florida Wildlife Conservation Commission, pers. comm.).

The severe and ongoing impact of coral disease on coral populations begs the question of potential mitigation actions that could be applied in the context of local management (Bruckner 2002, Raymundo et al. 2008, Beeden et al. 2012). If effective, such targeted mitigation actions would seem particularly relevant and useful as part of an integrated health-risk management component in a population restocking program. Both nursery and field practitioners have anecdotally reported simple interventions, such as separating apparently healthy tissues from diseased colonies or applying a physical barrier (e.g., band of clay or epoxy) to the diseased tissue margin to control tissue loss (Raymundo et al. 2008, Johnson et al. 2011), but no controlled tests of such mitigation treatments have been published.

The objectives of the present study were to (1) characterize disease dynamics using targeted disease surveillance in outplanted/transplanted versus wild populations of *A. cervicornis* to provide a more robust scientific basis for judging the health risks associated with outplanting and, (2) perform controlled tests of two simple mitigation treatments *in situ* to determine if they significantly arrested tissue loss in affected colonies. For both objectives, and to improve our understanding of the tissue loss diseases in this species, the histopathology of selected fragments from unmanipulated and treated branches was evaluated using light microscopy.

MATERIALS AND METHODS

Study Sites

Disease prevalence surveys and mitigation treatments were conducted at restored and wild *A. cervicornis* populations in the upper Florida Keys National Marine Sanctuary. Restored

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populations were outplanted between 2007 and 2011 by the Coral Restoration Foundation (CRF) or the National Marine Fisheries Service-Southeast Fisheries Science Center and initially included a finite number (i.e., 18–50, some sites have proliferated considerably) of outplanted (i.e., from field nursery culture) or transplanted (i.e., from nearby wild populations) colonies. These restored sites were deliberately established in areas devoid of native wild colonies and are in shallow (3–8 m) fore-reef habitats, including Key Largo Dry Rocks, French, Molasses, Pickles, and Conch Shallow reefs (Table 1; Supplementary Fig S1). An additional restored site (Aquarius) was sampled in 2011 only and was located in the deeper fore-reef (14–16 m) of Conch Reef. Few wild *A. cervicornis* patches are extant in the upper Florida Keys; three were identified for the current study to provide comparison to the restored populations. These wild sites were all located in low-relief patch reefs with partially consolidated rubble bottom at about 5 m depth and included an unnamed patch reef off of Tavernier, FL (TavPatch sites A & B), and Little Conch reef. Periodic surveys were also conducted at the CRF field nursery (origin of most restored colonies).

Temperature data was collected at surveyed reefs during the survey seasons with HOBO pendant data loggers (UA-001-64; Onset Corporation). Loggers were not re-located after Tropical Storm Isaac in 2012 at TavPatch or Key Largo Dry Rocks, so no temperature data for those two sites in 2012 is presented.

Disease Characterization

The primary disease reported to affect *Acropora cervicornis* is termed white-band disease (WBD) (Gladfelter 1982, Peters et al. 1983, Peters 1984), and the tissue loss pattern resembling that seen in the 1970s is now known as type I (WBD-I), because a type II was recognized in the 1990s, WBD-II (Peters 1984, Ritchie & Smith 1998, Aronson & Precht 2001, Vollmer & Kline

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2008). The WBD-I disease was first reported from Tague Bay, St. Croix, US Virgin Islands, and Gladfelter (1982) characterized this disease in *A. palmata* as "a sharp line of advance where the distally located, brown zooxanthella-bearing coral tissue is cleanly and completely removed from the skeleton, leaving a sharp white zone about 1 cm wide that grades proximally into algal successional stages…." This is illustrated in Fig 1 A. Peters et al. (1983) found the same disease signs present on *A. cervicornis* colonies of the deeper forereef at Tague Bay. WBD-I has now been reported to occur throughout the Caribbean Sea (Aronson & Precht 2001, Raymundo et al. 2008), and is present in the Florida Keys (Fig 1 B). In WBD-II, a band of bleaching tissue 2–20 cm wide is present at the tissue loss margin, and its distribution had been limited to the Bahamas (Ritchie & Smith 1998), although more recently seen in Puerto Rico (Gil-Agudelo et al. 2006) and southeast Florida (EC Peters, unpubl).

The lesions resulting from tissue loss attributed to WBD on Caribbean acroporids have varied in their patterns (smooth or ragged tissue margins) and rate (less than 1 mm d^{-1} to more than 14 mm d^{-1}, Gladfelter 1982), and descriptions of the lesions have not always been clear (Rogers 2010). For example, "rapidly advancing white band of diseased tissue" (Vollmer & Kline 2008) is not appropriate because it is a band of white denuded skeleton, not white tissue, that appears progressively (does not itself advance) from the base or middle of a branch toward the branch tip as the necrotic tissue (confirmed by histological examination) peels off, sloughs, or lyses and disappears from the skeleton (Gladfelter 1982, Peters et al. 1983). However, recent observation of acute tissue loss in *A. cervicornis* in the Florida Keys indicates that disease rarely presents as a fairly uniform-in-width band of denuded skeleton beginning at the base of the colony or more rarely in the middle of a branch, as for WBD-I (Fig 1C). Rather, initial lesions often show irregular sloughing of tissue with rapid enlargement of lesions anywhere on the

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surface of a branch, yielding multifocal swaths of bright white denuded skeleton referred to as rapid tissue loss (RTL) (Fig 1D, Williams & Miller 2005). A similar but unnamed condition was described by Bak and Criens in the early 1980's (1981). In the current field surveys, bright white bare skeleton, either encircling a branch or in irregularly shaped patches, and adjacent to either a straight tissue margin (distinct, smooth to undulating) or a jagged margin (distinct, serpiginous) of sloughing tissue was classified as disease, WBD or RTL, respectively (Fig 1B-D). However, sometimes tissue loss on a colony can appear as a combination of lesion types (Fig 1E). Rarely, WBD-II was noted during this study (Fig 1F). Lesions where corallivorous snails (*Coralliophila abbreviata*) were present as well as lesions confined to branch tips, but not past a fork, were attributed to snail and fireworm (*Hermodice carunculata*) predation, respectively, rather than disease (Fig 1G-H). The key features of the types of tissue loss in *A.cervicornis* are compared in Table 2.

Surveillance

Disease surveillance was conducted from May to November periods in 2011 and 2012 to target the seasonal time-frame when acroporid disease was expected to be most active (Willis et al. 2004, Williams & Miller 2005, K. Nedimyer, pers. comm.). Surveys were conducted approximately biweekly in 2011 (total nine surveys) and monthly in 2012 (total seven surveys), each taking 2–3 days to complete. At the wild sites, a circular plot (8-m radius at Tav Patch A and B, 10-m radius at Little Conch) was marked with a center rebar stake and used to delineate the study population for which prevalence was determined (i.e., percent of colonies in the population that displayed signs of disease). Different plot sizes were used at the two wild sites to incorporate a minimum of 25 colonies. At restored sites, the sample population consisted of the outplanted and transplanted colonies. The number of colonies tallied for individual site

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prevalence ranged from 23 to 163 according to the number of colonies available and the extent of search during a given survey. During each survey, every colony was recorded as either affected or unaffected with acute tissue loss disease (bright white skeleton with either a straight or jagged tissue margin on basal portions of the colony or multifocal). Corallivores were sometimes present and lesions attributable to these predators (either denuded branch tips characteristic of fireworm feeding or usually basal lesions with snails present) were not counted as disease. Prevalence was calculated for each site for every survey and averaged for each site-by-year combination. A two-way, fixed-factor ANOVA, with factors being site-type (restored versus wild) and year (2011 or 2012) and sites as replicates, was conducted to determine if overall prevalence varied significantly between restored and wild sites or years. For reference, disease prevalence observations were also made during six surveys in 2011 and one in 2012 at the nearby field nursery (Coral Restoration Foundation) from which all the outplanted colonies in the study had originated.

To characterize disease incidence and mortality, 20 randomly selected colonies were tagged at each site in May 2012. At each survey, tagged colonies were photographed and a visual estimate of percent of dead colony surface, attributed as either predation, disease, or undefined, was recorded. After the fifth survey, disturbance from Tropical Storm Isaac damaged or removed several tagged colonies at most sites, thereby yielding observations of fewer than 20 colonies at the sixth survey. To determine disease incidence (rate of new disease cases) over a survey interval, each colony observed with active disease which had been observed as unaffected at the previous survey was counted as a new disease case. Incidence was expressed as a proportion of observed tagged colonies displaying new cases of disease since the previous survey

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and was standardized per week. A t-test was used to determine if the proportion of tagged colonies that remained unaffected during 2012 differed between restored and wild sites

We estimated partial mortality based on cumulative increase in rough visual estimates of percent dead on each colony that was observed with disease. We analyzed cumulative partial mortality for all cases which occurred through survey five, and then we included cases which were observed at survey six, to include the acute mortality following Tropical Storm Isaac. A z-test was used to compare the proportion of affected wild vs. affected restored colonies showing severe cumulative partial mortality (defined as greater than 80%). We also tallied the case fatality rate as the percent of cases (colonies which displayed disease signs during the course of the observation period) which displayed complete mortality.

Mitigation Treatments

Two disease mitigation treatments were implemented to test effectiveness in arresting tissue loss (Fig 2). The first treatment used a band of two-part marine epoxy (All-Fix Epoxy) applied around the branch to cover the disease margin of an affected colony, presumably functioning as a physical barrier over the tissue-loss margin. The second treatment involved a complete excision of live, apparently healthy, tips of branches distal to a disease margin using handheld wire cutters. The excised fragment was then reattached to the reef substrate with epoxy at a distance greater than 1 m from the parent colony. These treatments are referred to as epoxy band (EB) and excision (EX) (Fig 2B-C), respectively. Lastly, a control treatment consisted of a cable tie placed at or near a tissue loss margin on the same colony as a reference point to detect continued tissue loss (Fig 1C or 1D). To prevent potential contamination, nitrile gloves were used when manipulating colonies and were changed when moving between affected colonies. All

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equipment that came into contact with diseased colonies was rinsed in a 10% bleach solution following each dive.

The design and setup for this experiment (e.g., sample size, timing, and placement of replicates) were constrained by the availability of affected colonies with apparently active disease. Due to permitting constraints in 2011, no experimental mitigation treatments were performed on wild *A. cervicornis* colonies. In 2012, this stricture was lifted and treatments were conducted on both restored and wild colonies. Distribution of experimental replicates among sites and years is given in Table 1. Effort was taken to block treatments within the same colony if it contained three or four (to include a histology sample) affected branches. However, this was often not possible and so treatments were allocated sequentially to affected colonies as they were encountered.

Rates of tissue loss in the observed disease conditions were rapid so all experimental replicates were scored as either (1) continued or (2) arrested tissue loss at an interval of approximately one month after the treatment was implemented, and each treated colony was photographed to document disease progression. In some cases, corallivores were subsequently observed on a treated or control colony and these replicates were excluded from analysis. Proportion of replicates with continued tissue loss was compared among the three treatments using Chi-Squared tests (for each year separately and for the years pooled).

Histopathology

To better characterize the observed disease conditions, tissue samples were collected in 2011 from a subset of apparently healthy colonies (n=21, including n=1–4 from each site collected in June or late September 2011), diseased colonies observed in the vicinity of the

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surveys (n=12), and diseased samples collected from the colonies in the mitigation experiment (n=11), collected throughout the sampling season. In addition, two diseased samples were collected from wild site Little Conch in 2012 to compare with the apparently healthy samples collected at that site in 2011. Samples were removed by cutting a 5–10 cm portion of a branch (i.e., tissue and skeleton) using handheld wire cutters and placed in a labeled 50-ml plastic centrifuge tube. After surfacing, the sample was immediately immersed in a formaldehyde-based fixative solution (Z-Fix Concentrate, Anatech, Ltd., 1:4 dilution in seawater). Sample tubes were capped, kept at ambient temperature in the shade, and shipped to the Histology Laboratory at George Mason University for processing.

Each sample was photographed and the images compiled into trim sheets. Samples were trimmed into approximately 2-cm long fragments using a Dremel tool and diamond-coated tile-cutting blade. The location of each cut was marked on the sample image on the trim sheet and subsample numbers were assigned and marked on the trim sheet. Subsamples having a tissue loss margin were enrobed in 1.5% agarose to trap material that might be present on the denuded skeletal surface or in corallite or gastrovascular canal crevices. Subsamples were decalcified using 10% disodium ethylenediaminetetraacetic acid (EDTA) at pH 7, changing the solution every 24 to 48 hours. When completely decalcified, the subsamples were rinsed in running tap water for about 30 minutes, trimmed into 2–3 mm slices and placed in cassettes, processed through ethanols, cleared, and infiltrated with molten Paraplast Plus®, then embedded in Paraplast Xtra® (Peters et al. 2005). Sections (5-µm thickness) were mounted on microscope slides, stained with Harris's hematoxylin and eosin and Giemsa (Noguchi 1926) or other special staining procedures, and coverslipped with Permount™ mounting medium.

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The sections were examined with an Olympus BX43 compound microscope and photomicrographs obtained with an Olympus DP-72 camera. Semi-quantitative data (Jagoe 1996) were collected from each subsample based on relative condition (0=Excellent, 1=Very Good, 2=Good, 3=Fair, 4=Poor, 5=Very Poor) and severity or intensity of tissue changes from normal (0=Within Normal Limits, 1=Minimal, 2=Mild, 3=Moderate, 4=Marked, 5=Severe) (see Table S 1). Histoslides of *A. cervicornis* and *A. palmata* collected from the 1970s in the Florida Keys (the earliest tissue samples located, before tissue loss was reported from this region) were used to develop the "within normal limits" criteria for general coral tissue condition and zooxanthellae condition/abundance scores, six specific cell or tissue parameters of polyp health, bacterial aggregates (Peters et al. 1983), and suspect rickettsia-like organisms (RLOs) (Casas et al. 2004; CS Friedman, pers. comm.). Only presence was noted for hypertrophied calicodermis foci, necrotic cell spherules, suspect virus-like particles (VLPs) (PL Blackwelder, pers. comm.), apicomplexans (Upton & Peters 1986), and suspect ciliate predators. The developmental stage of gonads was noted, if present. Mean scores for each sample were obtained (one or multiple sections were made, especially if enrobed samples had been trimmed into four ~ 2–3 mm slices for embedding; some sections did not contain enough tissue for scoring) and checked for quality. Suspect RLO abundances were visibly higher in Giemsa-stained sections since it demonstrates *Rickettsia* well (Noguchi 1926), thus, estimates based on those sections were preferentially used. Descriptive statistics were calculated for the scored parameters in each group of samples (apparently healthy, disease characterization, and mitigation treatments). Frequency distributions of the scores were examined. Comparisons were made for the scored parameters between all apparently healthy and diseased samples, successful and unsuccessful mitigation treatments, and WBD- and RTL-affected samples using Student's t-tests and Mann-Whitney U-tests.

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RESULTS

Disease Dynamics

Disease prevalence was highly variable and largely site-specific with no consistent patterns between restored versus wild sites (Fig 3 A-D). In 2011, wild sites showed consistently low prevalence with means of 1.5 to 4.4% during the survey period and a peak of approximately 13% at TavPatch B in late June (Fig 3A). Meanwhile, four of six restored sites showed generally high disease prevalence (i.e., survey period means of 9–17% and max of 26–41%; Fig 3C) particularly from July through early October, while the remaining two restored sites showed consistently low prevalence throughout 2011 (i.e., Key Largo Dry Rocks and Conch Shallow had 2011 survey period means of 0.7 and 3.5% prevalence with one peak of 13%, lower or similar to the wild sites; Fig. 3C). Intermittent observations within the field nursery site throughout 2011 yielded consistently low prevalence of 0–1.7%. In contrast, during 2012, Key Largo Dry Rocks and Conch Shallow showed among the highest prevalence patterns with survey period means of 20% and peaks of 60–70% (Fig 3C). Little Conch (wild) consistently had the highest site prevalence throughout the 2012 survey period (20–57% range, mean 35%; Fig 3B).

The temperature records indicate little temperature variation between sites during both years (Fig 3E-F), suggesting that site-specific disease increases or outbreaks were not triggered by temperature. Additionally, the accumulated thermal stress was greater in 2011 than in 2012, but the survey-period mean prevalence was higher in all three wild sites and four of six restored sites in 2012. However, the passage of Tropical Storm Isaac (26 Aug 2012) corresponded to a ubiquitous spike in prevalence and the survey period maximum prevalence observed across all sites (restored and wild). A two-way ANOVA using site means for each year showed a

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312 significant effect of year (p=0.032) but not of site-type (p=0.786) nor the interaction (p=0.237).
313 However, if the post-storm prevalence surveys are excluded in 2012, no factors are significant,
314 suggesting that higher overall disease prevalence in 2012 was attributable to the acute effect of
315 the storm.

316 Temporal patterns of disease incidence in 2012 are shown in Table 3 and further
317 emphasize the site-specific nature of disease dynamics in this population. Individual sites show
318 widely varying patterns of incidence, from persistent low incidence followed by a spike in the
319 fifth interval, following Tropical Storm Isaac (e.g., Pickles, TavPatch-A, TavPatch-B), to a
320 moderate level in the first three intervals followed by declining incidence (Molasses), to sites
321 with persistently high incidence from interval two (French, Little Conch), to sites with both an
322 early and a late peak (intervals two and six; Key Largo Dry Rocks)(Table 3).

323 Among the initial tagged population of 160 colonies in 2012, a total of 89 cases were
324 identified with a case fatality rate of 7.9%. The proportion of colonies that remained unaffected
325 throughout the study (non-cases, Table 3) was not significantly different between restored and
326 wild sites (t-test, p=0.686). Prior to the storm (up to survey 5; only n=53 cases occurred up to
327 this point), 52% of both restored and wild cases showed no detectable increment of partial
328 mortality (Fig. 4) and frequencies of cumulative partial mortality were very similar. When the
329 storm interval is included, disease-affected restored colonies had a significantly greater
330 likelihood of having severe (>80%) partial mortality than affected wild colonies (Fig. 4; z-test
331 p=0.005).

332 **Mitigation Testing**

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Approximately 60–70% of control replicates in each year showed continued tissue loss after one month (Fig. 5). In other words, around one-third of the replicates we thought to be in an active diseased state based on gross visual signs were, in fact, dormant during the following one-month period of observation. The proportion of experimental replicates displaying tissue loss about one month after the treatment application did not differ significantly among EB, EX, and Control treatments for either year analyzed separately (2011: χ^2=0.134, p=0.935; 2012: χ^2=1.502, p=0.472) nor for both years pooled (χ^2=0.953, p=0.621). However, the power of these tests is very low (0.059–0.173) so negative results should be treated with caution. Treatment success rate appears to be slightly greater in 2012, specifically suggesting more likely benefit from EX treatments than from EB treatments (Fig. 5).

Histopathological Observations

Summary statistics for the apparently healthy samples, diseased samples for characterization, and diseased mitigation samples are presented in Table 4. The apparently healthy samples were in very good to fair condition, had more zooxanthellae in gastrodermal cells, numerous mucocytes that were about the same height as the ciliated columnar cells of the epidermis (Fig 6A), and intact cnidoglandular bands of the mesenterial filaments (Fig. 6B). A third of the samples had minimal gaps in the calicodermis, mesoglea, and epidermis covering costal ridges. The calicodermis toward branch surfaces was squamous to culumnar, relatively thick, and contiguous over the mesoglea; calicoblasts often showed plasmallema extensions on their apical surfaces (toward the skeleton) and pale pink to clear cytoplasm (Fig. 6C). Deeper calicodermis was squamous and the cytoplasm contained fine eosinophilic granules. None of the samples contained bacterial aggregates, but almost all had mild to marked numbers of suspect

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RLOs in mucocytes on polyp oral discs and tentacles (Fig. 6D) and in cnidoglandular bands of the mesenterial filaments (Fig. 6E). Coccidia oocysts were seen in a couple of samples. Early oocytes were found in two samples, but no spermaries were observed.

Generally, characteristics of the diseased tissue samples collected from restored colonies at a range of sites and throughout the 2011 season included moderate to severe attenuation of the epithelia and mesoglea, numerous hypertrophied mucocytes or reduced number of mucocytes in the epidermis (Fig 6F), reduced numbers of zooxanthellae (but not entirely missing), and cells of the cnidoglandular bands showed varying degrees of atrophy, loss, necrosis or apoptosis, and dissociation (Fig. 6G). Moderate to severe costal tissue loss was noted, beginning in the apical polyp and increasing toward the tissue loss margin. The calicodermis varied in thickness and condition, but deeper and closer to the tissue loss margin was thinner, had fewer cells, and calicoblasts lysed or sloughed off the mesoglea (Fig 6H); sometimes foci of hypertrophied columnar calicoblasts with apical fine acidophilic granules were present at lysing tissue margins. None of these samples had bacterial aggregates, but all had suspect RLOs in mucocytes of the oral disc and tentacle epidermis, cnidoglandular bands, and infected mucocytes were also present in gastrodermis lining the gastrovascular canals and mesenteries (Fig. 6I-J). Suspect RLOs filling epidermal mucocytes were large and pleomorphic (Fig. 6H), whereas those in gastrodermal mucocytes were usually coccoid (Fig. 6I) and those in cnidoglandular band mucocytes could be either morphology; size of the RLOs also varied. Tissue loss margins displayed lysing coral cells with vacuolation and necrosis or apoptosis of cells remaining on the skeleton and sloughing of epithelial cells from mesoglea. Some agarose-enrobed samples had free-swimming ciliates containing zooxanthellae on the denuded skeleton in 24% of samples, but were very rarely in contact with tissue; ciliates without zooxanthellae were present in fewer numbers on 10% of

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samples, but farther away from tissue remnants. In addition, circumscribed masses of necrotic cell debris and zooxanthellae, in various states of further degradation and lysing, were present in 33% of the diseased samples. About 1–2 mm in diameter, they appeared to form as calicoblasts surrounding gastrovascular canals released from the skeleton and mesoglea surrounded gastrodermal cells or mesenterial filaments or epidermis fragments, trapping the degenerating epithelial cells within, but eventually becoming permeable to seawater and breaking apart. All of the diseased samples obtained from colonies used in the mitigation treatments had similar pathological changes (Table 4). Early to mid-stage developing oocytes were found in 10% and 5% of the samples, respectively, but no spermaries were observed.

Evaluation of the frequency distributions of the data to determine normality revealed that most parameters had a bimodal distribution, divided between the apparently healthy and diseased tissues (Table S 2), so the distributions were further examined within these categories. For example, Epidermal Mucocytes Condition had no overlap in scores, with apparently healthy samples showing mostly mild changes and diseased mostly severe changes. Parameters with minimal overlap included General Condition 100x, Zooxanthellae Condition 100x, Dissociation of Mesenterial Filaments, Costal Tissue Loss, and Calicodermis Condition. Parameters with broader frequency distributions of similar scores for both diseased and apparently healthy samples included Mesenterial Filament Mucocytes, Degeneration Cnidoglandular Bands, and Epidermal and Filament RLOs.

Comparison of the apparently healthy samples with all diseased samples (Fig. 7A) revealed that all parameter scores were significantly different, except for Epidermal and Filament RLOs ($p=0.165$ and 0.767, respectively, t-test, Table S 3). Epidermal RLOs were judged to be moderate to marked in severity; Filament RLOs were mostly judged to be minimal to marked in

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severity in both groups. For the samples in the mitigation treatments (Fig. 7B), histological parameters were significantly different in unsuccessful treatments only for Mesenterial Filament Mucocytes and Degeneration of Cnidoglandular Bands ($p=0.0097$ and 0.017, respectively, Mann-Whitney U-test, Table S 3). Number of mucocytes in the filaments was markedly fewer in samples from colonies where mitigation was not successful, in addition the filament epithelium had moderate to severe atrophy, loss of cnidocytes and acidophilic granular gland cells, and necrosis or apoptosis of remaining cells. Samples categorized as WBD or RTL in their patterns of tissue loss (Fig. 7C) only differed in Epidermal RLOs scores ($p=0.031$, Mann-Whitney U-test, Table S 3).

DISCUSSION

Surveillance of multiple wild and restored populations of staghorn coral in the Florida Keys during two years emphasize the ongoing toll that disease takes on this endangered species. Devastating disease outbreaks appear intermittently in both wild and restored patches that have appeared healthy for a number of years. For example, colonies at all three wild sites and restored colonies at Key Largo Dry Rocks appeared healthy with minimal partial mortality (mostly attributed to fireworm predation) throughout the 2011 surveillance, but two of these four sites (one wild, one restored) were devastated by disease in 2012. All apparently healthy and diseased samples collected in both years were infected with a suspect *Rickettsiales*-like bacterium (Casas et al. 2004) (Table 4, Fig. 7A). Although Casas et al. (2004) dismissed this microorganism as a potential pathogen of staghorn corals because it was present in apparently healthy and diseased samples, as well as other coral species, our histopathological examinations revealed that it infects

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polyp mucocytes and alters the coral's mucous secretions without causing gross disease signs, potentially increasing the susceptibility of the coral to other environmental stressors and tissue loss. There is no evidence that disease dynamics nor histological characterization are different between wild and restored colonies within the study population, which suggests that different disease risk management would not be warranted.

The high rates of disease prevalence documented in these populations are not unusual as overall average disease prevalences of more than 25% have been reported for individual site surveys in Panama, Belize, Cayman Islands, St. Thomas USVI, Antigua, and Curaçao for *A. cervicornis* (Vollmer & Kline 2008, Fogarty 2012) and for *Acropora* spp. (Ruiz-Moreno et al. 2012). Somewhat lower, but still substantial, average levels of *Acropora* spp. disease prevalence (8–12%) are reported in multi-year, Caribbean-wide, general coral condition surveys (Marks & Lang 2007, Ruiz-Moreno et al. 2012). In comparison, disease prevalence in acroporid corals across three sites in the Great Barrier Reef was also reported in the range of 9–13% (Willis et al. 2004), while more extensive surveys in three years across the entire Indo-Pacific region indicate an acroporid disease prevalence of around 4% (Ruiz-Moreno et al. 2012).

The existence of disease-resistant genets within *A. cervicornis* has been reported at a frequency of six percent in a studied population of 49 genets in Panama (Vollmer and Kline 2008). Four of the restored populations surveyed in this study are in fact genotypically depauperate, containing the same three genets, while the other two restored populations were genotypically more diverse (Table 1). Colonies at the three wild sites have not been genotyped, but multiple genets and high genetic diversity have been previously documented in wild populations of *A. cervicornis* in the Florida Keys (Baums et al. 2010; Hemond and Vollmer 2010). Thus, it is likely that multiple genets were present in each of these sites as well. The

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446 detection of potentially disease-resistant genets is extremely problematic. Among the three wild
447 sites, we might have surmised possible disease-resistant genets within these presumably
448 genotypically-diverse patches given low disease prevalence in 2011. However, all of the tagged
449 colonies at wild site Little Conch were observed with disease at some point during 2012 (Table
450 3). An important goal of Caribbean *Acropora* population enhancement strategies is the nursery
451 culture of stress-resistant genotypes or phenotypes in order to propagate hardier restored
452 populations (e.g., Bowden-Kerby & Carne 2012). The current results showing 1) extreme
453 variation in disease manifestation over sites and years, and 2) generally lower manifestation of
454 disease within the nursery environment than in nearby reef outplanted populations, (despite
455 similar RLO infection levels), reveal a challenge in accurately identifying these hardier
456 candidates.

457 Similarly, the site-specific nature of both disease prevalence and incidence patterns (i.e.,
458 patchy but not spatially autocorrelated) challenges the hope of identifying specific environmental
459 triggers for disease, at least on the site scale. While no severe warm thermal anomalies occurred
460 during the duration of this study, accumulated thermal stress was greater in 2011 than 2012—
461 corresponding to mild bleaching observed in some wild colonies during September–October
462 2011 (none in 2012)—but not greater disease impacts. Our temperature records do not indicate
463 substantial differences on the between-site scale that could account for spikes in disease among
464 our sites at different times (both within and between years, Fig 3E and F). Previous and repeated
465 reports of *A. cervicornis* disease in the Florida Keys have occurred in late spring to mid-summer
466 (April–July; Williams and Miller 2005; K. Nedimyer pers. comm.; M. Miller, pers. obs.), not
467 coinciding with the seasonal temperature peaks which occur in September–October. The only
468 coherent spike in disease prevalence and incidence that was discernible across all sites

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corresponded to the passage of Tropical Storm Isaac (Fig. 3), corroborating the hypothesis that storm disturbance may be an important coral disease trigger (Knowlton et al. 1981, Bruckner & Bruckner 1997, Miller & Williams 2006, Brandt et al. 2013).

The only significant difference we were able to discern between restored and wild colonies was in the degree of partial mortality during the storm interval, with restored colonies having greater partial mortality than wild colonies (Fig. 4). One limitation of the current study is in the spatial confounding of the restored and wild sites, with the former restricted to more exposed, mostly shallow fore-reef habitats and the latter in somewhat more sheltered patch reef habitats. It is likely that this habitat difference accounts for the apparent greater vulnerability of restored colonies to storm-associated disease mortality rather than any inherent characteristic of the colonies.

Our mitigation tests did not detect any significant benefit, in terms of preventing tissue loss over a four-week period, from either excision or epoxy-band treatment. However, high variability in response of both treatments, as well as the controls, yielded low power in the statistical tests. There was a greater suggestion of benefit from treatments conducted in 2012 (Fig. 5). One difference between years was the inclusion of wild colonies in 2012 (only restored colonies treated in 2011), but only a few replicates and only at Little Conch. Fishers' Exact Test for control vs. pooled treatments and considering only wild colonies was still not significant (but closer at p=0.06).

Several other observations may affect the interpretation of the somewhat inconclusive mitigation test. First, there was no hint of harm accruing to either treatment (Fig 5). However, we commonly observed in circumstances of high disease prevalence, a 'successful' (i.e., at one month assessment) excision or other areas on a successfully epoxy-banded colony might resume

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tissue loss at a later time, suggesting a re-activation of disease. On the other hand, if treatment replicates that were implemented at times and sites with high prevalence (i.e., >15%) are excluded, the remaining replicates indicate significantly lower frequency of tissue loss for treatments (especially excisions) vs. controls (X^2 test; p=0.014; see Table S 4). Our results and observations suggest that if mitigation interventions are attempted, branch-tip excisions are more likely to be successful. Histologically, tip tissue may be in better condition than that at the tissue-loss margin and resources are directed toward the tips rather than bases in this species (Highsmith 1982). Also, mitigation appears to be more successful in isolated cases rather than in areas with more disease. Unfortunately, conditions with <15% disease prevalence were surprisingly rare, occurring in only 31 of our 56 individual site surveys in 2012.

The histopathological examinations revealed several other reasons why mitigation treatment success can vary, despite the challenges in assigning a semi-quantitative score to observations because specific changes occurring in the coral tissues formed a continuum. The only significant differences in scores between the successful versus the unsuccessfully treated branches were the greater loss of mesenterial filament mucocytes and degeneration of the cnidoglandular bands of the filaments in samples from colonies that had unsuccessful treatments. The filament epithelium lines the free edges of mesenteries in the gastrovascular cavity below the actinopharynx in the polyp; the specialized acidophilic granular gland cells of this epithelium release enzymes to break down food particles (Raz-Bahat et al. In Prep). The number and size of gland cells and mucocytes in the cnidoglandular band increase, whereas ciliated cells decrease, aborally in normal *A.cervicornis* tissue. Cell loss, necrosis, and lysing indicate that the polyp is no longer able to process particulate food in the gastrovascular cavity. In addition, although zooxanthellae condition appears to remain unaffected until the host tissue is sloughing off the

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skeleton, their numbers are reduced as the host condition deteriorates. However, due to our inability to detect changes in coral pigmentation until zooxanthellae numbers are reduced by more than 50 percent (e.g., Jones 1997), the tissue grossly appears to be intact and "normal," when it may not be so microscopically. The ubiquitous presence of the suspect RLO infections suggests most, if not all, the *A.cervicornis* population's health is compromised. Thus, without microscopic examination, it is difficult, if not impossible, to identify the "best candidates" for mitigation treatment.

Exactly what the impact of the suspect RLOs is on the *A. cervicornis* colonies is conjecture at this point, but based on the behavior of similar obligate intracellular bacteria, their replication within host cells requires substantial energy (Fryer & Lannan 1994) resulting in tissue atrophy and necrosis (Friedman et al. 2000, Sun & Wu 2004). Nutritional stress may be a primary reason why the zooxanthellae are gradually lost and calicoblasts lyse (Weis 2008, Schoepf et al. 2013). The coral cannot maintain its tissues with the loss of these host and algal cells that are crucial to its survival. Infected mucocytes eventually die and are released from the epithelium and the coral may not be able to replace them. Loss of mucocytes means the loss of the coral's protection against sedimentation and microorganisms, as well as heterotrophic feeding (Brown & Bythell 2005, Ritchie 2006). Investigation of the pathogenesis of RLO infection is continuing, noting that other bacteria (*Vibrio harveyi*, *Serratia marcescens*, unspecified) have been implicated in the acute loss of tissue from Caribbean acroporids (Patterson et al. 2002, Gil-Agudelo et al. 2006, Kline & Vollmer 2011). Transcriptome analysis shows gene expression alterations in immunity, apoptosis, cell growth, and remodeling in WBD (Libro et al. 2013); and multiple pathogens may be involved or be different in specific cases requiring histopathological examinations (Work & Aeby 2011). Bacterial aggregates first

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538 proposed to be the pathogen (Peters et al. 1983) were not present in any of these samples and
539 ciliates do not seem to be a major factor in tissue loss in our study. Histologically, no differences
540 could be discerned between WBD- and RTL-affected colonies, suggesting that differences in the
541 patterns of tissue loss are due to the intensity and duration of suspect RLO infections or the
542 identity of other stressors that trigger the loss. Samples collected from the same colonies in this
543 study are also being processed for molecular characterization of the microbial communities
544 associated with them at the diseased margin and in apparently healthy tissue from diseased or
545 unaffected colonies to help explain the pathogenesis of tissue loss.
546 Overall, our results confirm the devastating toll that disease continues to have on both
547 wild and restored populations of Caribbean staghorn coral and suggests that wild and restored
548 populations display similar disease conditions, dynamics, and impacts. These results emphasize
549 the continuing need to understand and effectively address disease impacts in this species, as well
550 as discover methods and run experiments to try and determine a way to minimize tissue loss of
551 diseased colonies. Unfortunately, the straightforward mitigation treatments tested in this study
552 provided equivocal benefit. Given this situation, population restoration might be viewed as a
553 necessary but stop-gap recovery measure, particularly in light of the suspect RLO infections of
554 mucocytes in nursery and wild colonies. Additional assessments of factors affecting the staghorn
555 corals and their tissue loss diseases are needed, including pathogen interactions between the
556 stocks (Friedman & Finley 2003) and host genotype susceptibility (Vollmer & Kline 2008).
557
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LITERATURE CITED

Aronson RB, Precht WF (2001) White-band disease and the changing face of Caribbean coral reefs. Hydrobiologia 460:25-38

Bak RPM, Criens SR (1981) Survival after fragmentation of colonies of *Madracis mirabilis* , *Acropora palmata* and *A. cervicornis* (Scleractinia) and the subsequent impact of a coral disease. Proceedings of the 4th International Coral Reef Symposium 2:221–227

Baums I, Devlin-Durante M, Brown L, Pinzón J (2009) Nine novel, polymorphic microsatellite markers for the study of threatened Caribbean acroporid corals. Molecular Ecology Resources 9:1155-1158

Baums I, Johnson M, Devlin-Durante M, Miller M (2010) Host population genetic structure and zooxanthellae diversity of two reef-building coral species along the Florida Reef Tract and wider Caribbean. Coral Reefs 29:835-842

Baums IB (2008) A restoration genetics guide for coral reef conservation. Mol Ecol 17:2796-2811

Beeden R, Maynard J, Marshall P, Heron S, Willis B (2012) A framework for responding to coral disease outbreaks that facilitates adaptive management. Environ Manage 49:1-13

Bowden-Kerby A, Carne L (2012) Thermal tolerance as a factor in Caribbean *Acropora* restoration. Proceedings 12th International Coral Reef Symposium 20A:(Abstract)

Brandt ME, Smith TB, Correa AMS, Vega-Thurber R (2013) Disturbance induced coral fragmentation as a driver of a coral disease outbreak. PLoS ONE 8:e57164

Brown BE, Bythell JC (2005) Perspectives on mucus secretion in reef corals. Mar Ecol Prog Ser 296:291-309

Bruckner AW (2001) Coral health and mortality. Recognizing the signs of coral diseases and predators. In: Humann P, Deloach N (eds) Reef Coral Identification: Florida Caribbean Bahamas Including Marine Plants, 2nd edition

Bruckner AW (2002) Priorities for effective management of coral diseases. NOAA Technical Memorandum NMFS-OPR-22, Silver Spring, MD

Bruckner AW, Bruckner RJ (1997) Outbreak of coral disease in Puerto Rico. Coral Reefs 16:260-260

Carlsson J, Carnegie RB, Cordes JF, Hare MP, Leggett AT, Reece KS (2008) Evaluating recruitment contribution of a selectively bred aquaculture line of the oyster, *Crassostrea virginica* used in restoration efforts. J Shellfish Res 27:1117-1124

Casas V, Kline DI, Wegley L, Yu YN, Breitbart M, Rohwer F (2004) Widespread association of a Rickettsiales-like bacterium with reef-building corals. Environ Microbiol 6:1137-1148

Champagnon J, Elmberg J, Guillemain M, Gauthier-Clerc M, Lebreton J-D (2012) Conspecifics can be aliens too: A review of effects of restocking practices in vertebrates. J Nat Conserv 20:231-241

Cunningham AA (1996) Disease risks of wildlife translocations. Conserv Biol 10:349-353

Fogarty ND (2012) Caribbean acroporid coral hybrids are viable across life history stages. Mar Ecol Prog Ser 446:145-159

Friedman CS, Andree KB, Beauchamp KA, Moore JD, Robbins TT, Shields JD, Hedrick RP (2000) 'Candidatus Xenohaliotis californiensis', a newly described pathogen of abalone, *Haliotis* spp., along the west coast of North America. International Journal of Systematic Bacteriololgy 50:847:855

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.328v1 | CC-BY 4.0 Open Access | received: 31 Mar 2014, published: 31 Mar 2014

611 Friedman CS, Finley CA (2003) Anthropogenic introduction of the etiological agent of withering
612 syndrome into northern California abalone populations via conservation efforts. Can J
613 Fish Aquat Sci 60:1424-1431
614 Fryer JL, Lannan CN (1994) Rickettsial and chlamydial infections of freshwater and marine
615 fishes, bivalves, and crustaceans. Zool Stud 33:95-107
616 Galloway SB, Woodley C, McLaughlin S, Work T, Bochsler V, Meteyer C, Sileo L, Peters E,
617 Kramarsky-Winters E, Morado J (2007) Coral disease and health workshop: coral
618 histopathology II. NOAA Technical Memorandum NOS NCCOS 56 and CRCP 4, Silver
619 Spring MD
620 Gil-Agudelo DL, Smith GW, Weil E (2006) The white band disease type II pathogen in Puerto
621 Rico. Revista Biologia Tropical 54:59-67
622 Gladfelter WB (1982) White-Band Disease in *Acropora palmata* : Implications for the structure
623 and growth of shallow reefs. Bull Mar Sci 32:639-643
624 Gladfelter WB, Gladfelter EH, Monahan RK, Ogden JC, Dill RF (1977) Coral destruction.
625 Environmental Studies of Buck Island Reef National Monument, US National Park
626 Service Report,144 pp
627 Hemond EM, Vollmer SV (2010) Genetic diversity and connectivity in the threatened staghorn
628 coral (*Acropora cervicornis*) in Florida. PLoS ONE 5:e8652
629 Highsmith RC (1982) Reproduction by fragmentation in corals. Mar Ecol Prog Ser 7:207-226
630 Hilborn R, Eggers D (2000) A review of the hatchery programs for pink salmon in Prince
631 William Sound and Kodiak Island, Alaska. Transactions of the Americal Fisheries
632 Society 129:333-350
633 IUCN (2012) IUCN Red List of Threatened Species Version 2012.2.
634 Jagoe CH (1996) Responses at the tissue level: Quantitative methods in histopathology applied
635 to ecotoxicology. In: Newman MC, Jagoe CH (eds) Ecotoxicology: A Hierarchical
636 Treatment. Lewis Publishers, Boca Raton, FL
637 Johnson ME, Lustic C, Bartels E, Baums IB, Gilliam DS, Larson L, Lirman D, Miller MW,
638 Nedimyer K, S.. S (2011) Caribbean Acropora Restoration Guide: Best Practices for
639 Propagation and Population Enhancement The Nature Conservancy, Arlington, VA.
640 Jones RJ (1997) Changs in zooxanthellar densities and chlorophyll concentrations in corals
641 during and after a bleaching event. Mar Ecol Prog Ser 158:51-59
642 Kline DI, Vollmer SV (2011) White Band Disease (type I) of endangered Caribbean acroporid
643 corals is caused by pathogenic bacteria. Scientific Reports 1:Article 7
644 Knowlton N, Lang JC, Rooney MC, Clifford P (1981) When hurricanes kill corals: Evidence for
645 delayed mortality in hurricane-damaged Jamaican staghorn corals. Nature 294:251-252
646 Lenihan H, Micheli F, Shelton S, Peterson C (1999) The influence of multiple environmental
647 stressors on susceptibility to parasites: An experimental determination with oysters.
648 Limnol Oceanogr 44:910-924
649 Libro S, Kaluziak ST, Vollmer SV (2013) RNA-seq profiles of immune related genes in the
650 staghorn coral *Acropora cervicornis* infected with White Band Disease. PLoS ONE
651 8:e81821
652 Marks KW, Lang JC (2007) AGRRA Summary Products, version (10/2007). Available online
653 <http:wwwagrraorg/Release_2007-10/>, Book Available online
654 <http:www.agrra.org/Release_2007-10/>
655

PeerJ PrePrints | http://dx.doi.org/10.7287/peerj.preprints.328v1 | CC-BY 4.0 Open Access | received: 31 Mar 2014, published: 31 Mar 2014

Miller MW, Williams DE (2006) Coral disease outbreak at Navassa, a remote Caribbean island. Coral Reefs 26:97-101

Noguchi H (1926) Cultivation of *rickettsia*-like microorganisms from the Rocky Mountain spotted fever tick, *Dermacentor andersoni*. Jouranal Experimental Medicine 43:515-532

Patterson KL, Porter JW, Ritchie KE, Polson SW, Mueller E, Peters EC, Santavy DL, Smiths GW (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 EC (1984) A survey of cellular reactions to environmental stress and disease in Caribbean scleractinian corals. Helgo Meeresunters 37:113-137

Peters EC, Oprandy JJ, Yevich PP (1983) Possible causal agent of "white band disease" in Caribbean acroporid corals. J Invertebr Pathol 41:394-396

Peters EC, Price KL, Borsay Horowitz DJ (2005) Histological preparation of invertebrates for evaluating contaminant effects. In: Ostrander GK (ed) Techniques in Aquatic Toxicology Vol 2. Taylor & Francis, Boca Raton, FL

Raymundo L, Couch C, Harvell CD (eds) (2008) Coral Disease Handbook: guidelines for assessment, monitoring & management. Coral Reef Targeted Research & Capacity Building for Management, St. Lucia, Queensland, Australia www.gefcoral.org

Ritchie KB (2006) Regulation of microbial populations by coral surface mucus and mucus-associated bacteria. Mar Ecol Prog Ser 322:1-14

Ritchie KB, Smith GW (1998) Type II white-band disease. Rev Biol Trop 46:199-203

Rogers C (2010) Words matter: Recommendations for clarifying coral disease nomenclature and terminology. Dis Aquat Org 91:167

Ruiz-Moreno D, Willis BL, Page AC, Weil E, Cróquer A, Vargas-Angel B, Jordan-Garza AG, Jordán-Dahlgren E, Raymundo L, Harvell CD (2012) Global coral disease prevalence associated with sea temperature anomalies and local factors. Dis Aquat Org 100:249-261

Sainsbury AW, Vaughan-Higgins RJ (2012) Analyzing disease risks associated with translocations. Conserv Biol 26:442-452

Schoepf V, Grottoli AG, Warner ME, Cai W-J, Melman TF, Hoadley KD, Pettay DT, Li Q, Xu H, Wang Y, Matsui Y, Baumann JH (2013) Coral energy reserves and calcification in a high-CO2 world at two temperatures. PLoS ONE 8:e75049.

Sun J, Wu X (2004) Histology, ultrastructure, and morphogenesis of a *Rickettsia*-like organism causing disease in the oyster *Crassostrea ariakensis* Gould. J Invertebr Pathol 86:77-86

Sutherland KP, Porter JW, Torres C (2004) Disease and immunity in Caribbean and Indo-Pacific zooxanthellate corals. Mar Ecol Prog Ser 266:273-302

Upton SJ, Peters EC (1986) A new and unusual species of Coccidium (Apicomplexa: Agamococcidiorida from Caribbean scleractinian corals. J Invertebr Pathol 47:184-193

Vollmer SV, Kline DI (2008) Natural disease resistance in threatened staghorn corals. PLoS ONE 3:e3718. doi:3710.1371/journal.pone.0003718

Weis VM (2008) Cellular mechanisms of Cnidarian bleaching: stress causes the collapse of symbiosis. J Exp Biol 211:3059-3066

Williams DE, Miller MW (2005) Coral disease outbreak: pattern, prevalence, and transmission in Acropora cervicornis. Mar Ecol Prog Ser 301:119-128

Willis BL, Page CA, Dinsdale EA (2004) Coral disease on the Great Barrier Reef. In: Rosenberg E, Loya Y (eds) Coral Health and Disease. Springer-Verlag, Berlin

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Work TM, Aeby GS (2006) Systematically describing gross lesions in corals. Dis Aquat Org 70:155

Work TM, Aeby GS (2011) Pathology of tissue loss (white syndrome) in *Acropora* spp. corals from the Central Pacific. J Invertebr Pathol 197:127-131

Young CN, Schopmeyer SA, Lirman D (2012) A review of reef restoration and coral propagation using the threatened genus *Acropora* in the Caribbean and Western Atlantic. Bull Mar Sci 88:1075-1098

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Table 1: Characteristics of study sites/populations in the upper Florida Keys. Number of genets indicates number of *Acropora cervicornis* multi-locus genotypes (based on seven microsatellite markers (Baums et al. 2009, Baums et al. 2010)) within the surveyed populations at each site. Distribution of experimental replicates for the mitigation treatments among sites and years is summarized in the last two columns. UNK= Unknown, C=Control, EB = Epoxy Band, EX = Excision.

	Colony Origin	**Site Type**	**Number (#) of Genets**	**Coordinates**	**Depth (m)**	**# 2011 replicates (C/EB/EX)**	**# 2012 replicates (C/EB/EX)**
Molasses	Nursery	Restored	3	25° 00.599'N 80° 22.373'W	8-10	2/8/6	1/1/0
Aquarius	Transplant & Nursery	Restored	11	24o57.2'N 80o27.15'W	14	9/6/3	NA
Conch Shallow	Transplant	Restored	14	24° 57.083' N 80° 27.594' W	6	1/1/1	4/4/5
French	Nursery	Restored	3	25° 07.314' N 80° 17.851'W	10	5/3/4	6/5/1
KL Dry Rocks	Nursery	Restored	3	25° 07.451'N 80° 17.839'W	6	NA	3/3/4
Pickles	Nursery	Restored	3	24° 59.300'N 80° 24.737'W	8-10	0/1/1	NA
Tav Patch A	Wild	Wild	UNK*	24° 59.228'N 80° 27.173'W	6	NA	NA
Tav Patch B	Wild	Wild	UNK	24° 59.242'N 80° 27.159'W	6	NA	NA
Little Conch	Wild	Wild	UNK	24° 56.780'N 80° 28.21o'W	6	NA	10/10/2
CRF Nursery		Nursery	>20	24° 59'N 80° 26'W	11	NA	NA

*Previous haphazard genotype sampling at this site yielded 6 unique genets in 20 sampled colonies (Miller & Baums, unpubl)

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723 Table 2: Comparison of field manifestations of lesions seen in *A. cervicornis* and morphologic diagnoses. See Work and Aeby (2006)
724 and Galloway et al. (2007) for definitions of terms.

Field Name	Tissue Loss Type	Location of Lesion on Colony*	Lesion Margin Appearance	Lesion Shape And Size	Lesion Number and Color	Lesion Progression	Morphologic Diagnosis
White-band disease type I (WBD-I)[1]	Acute to subacute	Base or middle of branch, encircling branch	Distinct areas of tissue loss, smooth to serpiginous margin, tissue tan to brown (due to symbiotic algae pigment-ation)	Band of intact bare skeleton, well-different-iated from more distal skeleton	Focal to multifocal to diffuse, white (denuded skeleton), normally pigmented tissue margin	White band typically 2–10 cm wide; rate of tissue loss usually a few mm per day but can vary or stop; at branch bifurcation tissue loss continues on both branches at about the same rate; freshly denuded skeleton grades into green to brown algal growth on the skeleton, first visible after 5–7 days and becoming increasingly dense with time	Severe, basal to mid-branch band, diffuse, acute tissue loss, polyp, coenenchyme
White-band disease type II (WBD-II)[2]	Acute to subacute	Tip or base of branch, encircling branch	Distinct areas of tissue loss, smooth margin, 2–20 cm wide band of bleaching tissue (loss of brown algal pigmentation) between tissue	Band of intact bare skeleton, well-differentiated from more distal skeleton, developing green to brown algal	Focal to multifocal, white (denuded skeleton), bleaching tissue margin	White band typically 2–10 cm wide; rate of tissue loss usually a few mm per day; bleaching margin tissue disappears, normally pigmented tissue starts bleaching; however, bleaching margin tissue may also disappear and then the normally pigmented tissue	Severe, basal, band, diffuse, acute tissue loss, bleaching margin, polyp, coenenchyme

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			loss margin and normally pigmented tissue	growth		disappears, as in WBD-I; freshly denuded skeleton grades into green to brown algal growth on the skeleton, first visible after 5–7 days and becoming increasingly dense with time	
Rapid Tissue Loss (RTL)[3]	Acute	Basal, medial, or colony-wide, partially to completely encircling branch	Distinct areas of tissue loss, undulating to serpiginous margin, tan to brown tissue, sloughing	Irregularly shaped areas of intact bare skeleton	Focal to multifocal and coalescing to diffuse, white (denuded skeleton)	Intact bare skeleton appears quickly along branches, new lesions may coalesce; rate of tissue loss usually cm per day; denuded skeleton develops green to brown algal growth that becomes uniformly visible after 5–7 days covering entire denuded area	Severe, basal to complete, band or irregular, diffuse, acute to subacute tissue loss, polyp, coenenchyme
Fireworm (*H. carunculata*) predation feeding scars[1]	Acute	Apical 1 to 5 cm of branch, but not extending beyond a branch bifurcation	Distinct areas of tissue loss encircling apex of branch, smooth to serpiginous margins, tissue tan to brown	Intact bare skeleton, tip of branch, developing green to brown algal growth	Focal to diffuse, white (denuded skeleton)	None, denuded skeleton develops uniform green to brown algal growth	Severe, focal, branch tip, acute tissue loss, polyp, coenenchyme
Snail (*C. abberviata*) predation feeding scars[1]	Acute	Colony base, skeletal-tissue margin	Distinct areas of tissue loss, smooth to serpiginous rounded or	Intact bare skeleton, usually adjacent to one or more	Focal or multifocal, white (denuded skeleton)	None, denuded skeleton gradually colonized by green to brown algal growth	Severe, diffuse, basal, acute tissue loss, polyp, coenenchyme

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		inward and vertically	scalloped margins, tissue tan to brown	*Coralliophila abbreviata*			

*First lesion on all of these may be a single small focus of acute tissue loss, either at the base or in the middle of a branch, lesion enlargement pattern then varies.

[1]Illustrated in Williams et al. (2006) but only for *A.palmata*

[2]Described in Ritchie and Smith (1998)

[3]Described in Williams and Miller (2005); described but not named in Bak and Criens (1981)

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Table 3: Survey intervals (dates and duration in weeks), incidence, and proportion of colonies that remained unaffected by disease for the population of tagged colonies (n=20) at each site throughout the 2012 sampling period. Incidence is expressed as the proportion of new cases observed during each survey interval (i.e., diseased tagged colonies observed without disease in the previous survey) standardized per week. Shading is scaled with incidence value. Tropical Storm Isaac passed during Interval V.

	Interval Dates (#weeks)	*I* *5/15-6/2* *(2.71)*	*II* *6/2-6/30* *(4.00)*	*III* *6/30-7.23* *(3.29)*	*IV* *7/23-8/15* *(3.29)*	*V* *8/15-9/10* *(3.71)*	**Unaffected**
Restored	*Conch Shallow*	0.018	0.000	0.000	0.064	0.126	0.400
	Pickles	0.000	0.025	0.000	0.000	0.094	0.400
	Molasses	0.037	0.025	0.000	0.016	0.000	0.800
	French	0.000	0.050	0.046	0.076	0.075	0.250
	KL Dry Rocks	0.018	0.088	0.000	0.015	0.184	0.150
Wild	*Little Conch*	0.037	0.063	0.046	0.076	0.099	0.000
	Tav Patch A	0.018	0.013	0.000	0.000	0.038	0.800
	Tav Patch B	0.018	0.000	0.016	0.015	0.058	0.700

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Table 4: Summary statistics for histopathological observations on all apparently healthy (n = 21), diseased (n = 11), and mitigation treatment samples (n = 11).

Parameter	Apparently Healthy			Characterization Diseased			Mitigation Treatments		
Assigned Scores	**Mean**	**St.Dev.**	**Range**	**Mean**	**St.Dev.**	**Range**	**Mean**	**St.Dev.**	**Range**
General Condition (100x)	1.6	0.7	1–3	4.5	0.5	3–5	4.4	0.7	3–5
Zooxanthellae (100x)	1.2	0.5	0–2	3.6	0.4	3–4	3.4	0.3	3–4
Epidermal Mucocytes Condition	1.7	0.5	1–2	4.3	0.5	3–5	4.3	0.6	3–5
Mesenterial Filament Mucocytes	2.7	1.1	1–5	4.4	0.7	3–5	4.2	0.9	2–5
Degeneration Cnidoglandular Bands	1.5	1.3	0–5	4.3	1.0	2–5	3.8	1.3	2–5
Dissociation Mesenterial Filaments	0.5	0.9	0–3	2.8	1.5	0–5	1.9	1.2	0.2–3.7
Costal Tissue Loss	0.3	0.5	0–1	3.5	1.3	1–5	3.2	1.4	0.9–4.8
Calicodermis Condition	1.4	0.6	1–3	4.0	0.7	2–5	3.8	0.9	2.1–4.9
Bacterial Aggregates	0.0	0.0	0–0	0.0	0.0	0–0	0.0	0.0	0–0
Epidermal RLOs	3.2	0.6	2–4	3.6	0.5	3–4	3.4	0.5	2.5–4
Filament RLOs	2.8	0.5	2–4	2.8	1.2	1–5	2.9	0.9	2–5
Percent Affected (Presence/Absence)									
Coccidia	10			14			10		
Calicodermis Repair	0			43			33		
Necrotic Cell Spherules	0			33			33		
Zooxanthellate Ciliates	0			24			24		
Non-zooxanthellate Ciliates	0			10			14		
Oocytes	10			10			5		
Spermaries	0			0			0		

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Figure Legends:

Figure 1: Illustration of disease and predation conditions categorized in this study. A) Loss of necrotic tissue from skeleton of A. palmata during WBD outbreak, Tague Bay, St. Croix, 1980 B) Typical disease-affected colony with multifocal lesions of denuded skeleton, C) WBD-I, D) initial stages of RTL, (E) Colony manifesting signs of both WBD-I (base) and RTL (tips), F) WBD-II signs, G) fireworm predation with two older preyed tips (partially colonized by algal turfs) visible, and H) snail predation scar on basal portion of branch (removed snails indicated by arrow) .

Figure 2: Illustration of the treatments used in the mitigation trials. A) Excision (EX) of healthy looking tips snipped from a nearby disease colony and re-attached to the reef, B) Epoxy band (EB) surrounding the diseased tissue margin. One month later (C) this 'successful' EB replicate shows no additional tissue loss and initial regrowth over the epoxy. Control treatments are illustrated in Fig 1C and 1D.

Figure 3: Disease prevalence in *Acropora cervicornis* colonies in Wild (A and B) and Restored (C and D) populations over two survey periods (May–Nov 2011 and May–Nov 2012). Dotted lines indicate close passage of Tropical Storm Isaac in Aug 2012. Panels E and F show the temperature records from the same sites and time periods.

Figure 4: Frequencies of severity of cumulative partial mortality in tagged diseased colonies during the 2012 survey period before (A and B, Surveys 1-5, n=32 and 21, respectively) and after (C and D, Surveys 1-6, n=51 and 27 respectively) passage of Tropical Storm Isaac at Restored and Wild sites. More cases occurred after the post-storm disease spike. The bin labeled zero includes colonies that accumulated less partial mortality than could be resolved in coarse visual estimates.

Figure 5: Results of experimental mitigation trials showing response in each year for Epoxy-Band (EB), Excision (EX) and Control (cable tie placed around disease margin on a branch) treatments as the percent of replicates showing continued tissue loss after one month. Number of

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replicates implemented given above each bar. Chi-Squared Goodness of Fit tests indicate no significant difference in the proportions of the three treatments showing continued tissue loss when all replicates across years are pooled.

Figure 6: Histology observations. A) Coenenchyme epidermis from apparently healthy *Acropora cervicornis* branch tip, columnar mucocytes of surface body wall (large arrow), suspect RLOs in gastrodermal mucocytes of basal body wall (small arrows), Giemsa. B) Mesenteries showing sections through cnidoglandular bands (large arrow), H&E. C) Apparently healthy staghorn sample, epithelia lining gastrovascular canals with columnar calicoblasts having extensions of plasmallema (large arrows), H&E. D) Section through tentacles (= T) and oral disc from apparently healthy colony sample, mucocytes infected with suspect RLOs stain dark purple (large arrow pointing to oral disc), Giemsa. E) Cnidoglandular bands from apparently healthy colony sample, suspect RLOs in mucocytes (large arrows) and mucocytes in the epithelium (small arrows). F) Coenenchyme epidermis from *A. cervicornis* showing signs of RTL, note atrophy of epithelium and loss of mucocytes (large arrow), suspect RLOs in gastrodermal mucocytes of basal body wall (small arrows), Giemsa. G) Sections through mesenteries from RTL-affected sample with degeneration (necrosis, lysing) and dissociation of cells of the cnidoglandular bands, note pink-staining acidophilic granular gland cells are rounding up and atrophied, ciliated cells and mucocytes are reduced in number compared to Fig. 6B, H&E. H) RTL-affected sample epithelia lining gastrovascular canals, severe atrophy of the calicodermis, loss of calicoblasts from mesoglea (large arrows); adjacent gastrodermis is swollen, fragmented, and vacuolated compared to cuboidal cells in upper left corner of image, H&E. I) Suspect RLOs infecting gastrodermal cells (large arrows) lining the mesenteries (= MES) of an apparently healthy sample, Giemsa. J) High magnification of infected epidermal mucocytes from apparently healthy sample, showing pleomorphic suspect RLOs (large arrow) and mucocytes (small arrows, = MUC), Giemsa.

Figure 7: Histology parameter scores comparisons. A) Apparently healthy samples vs. diseased samples. B) Successful vs unsuccessful mitigation treatment samples. C) Microscopic characteristics of WBD vs. RTL samples.

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Figure 1:

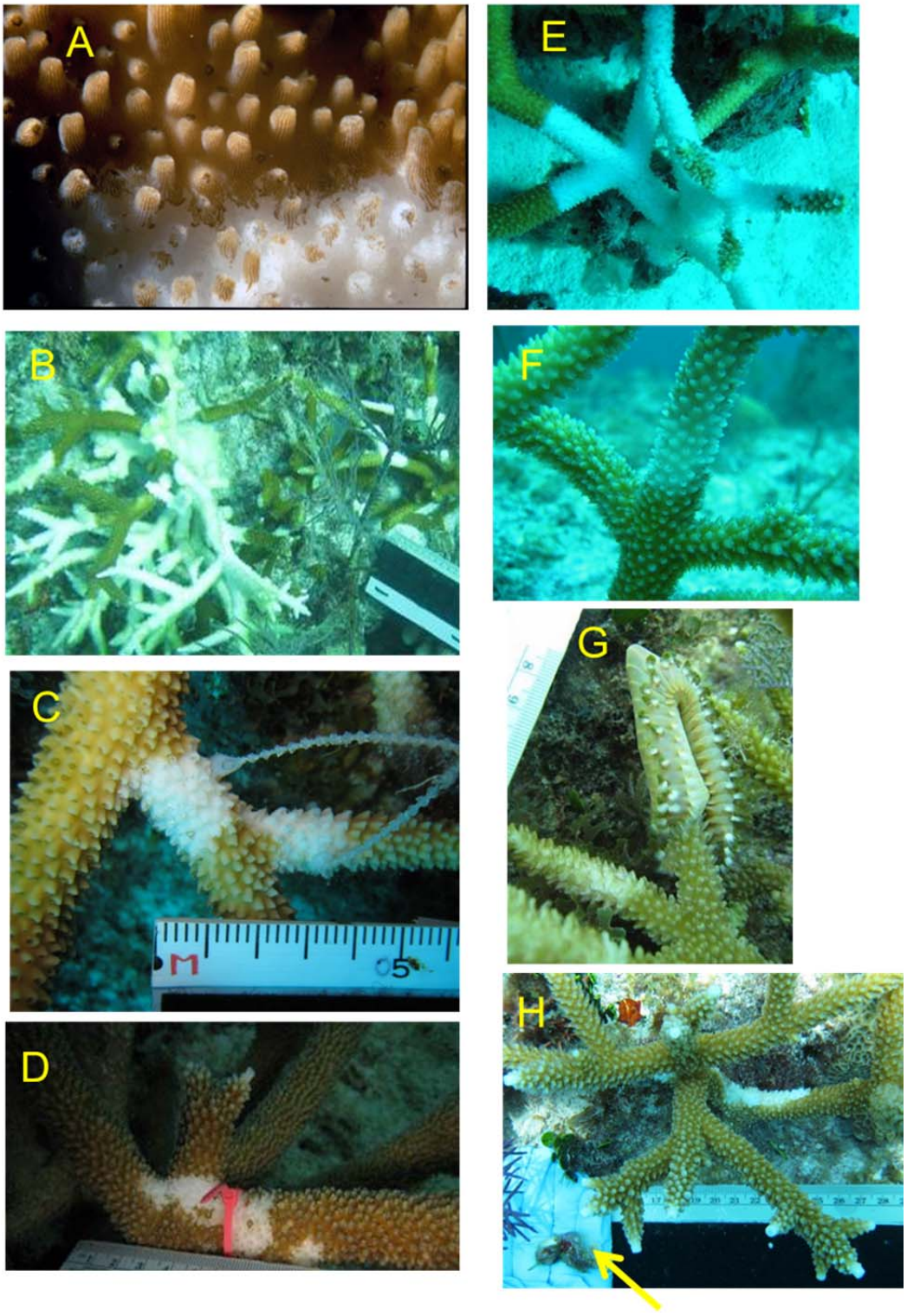

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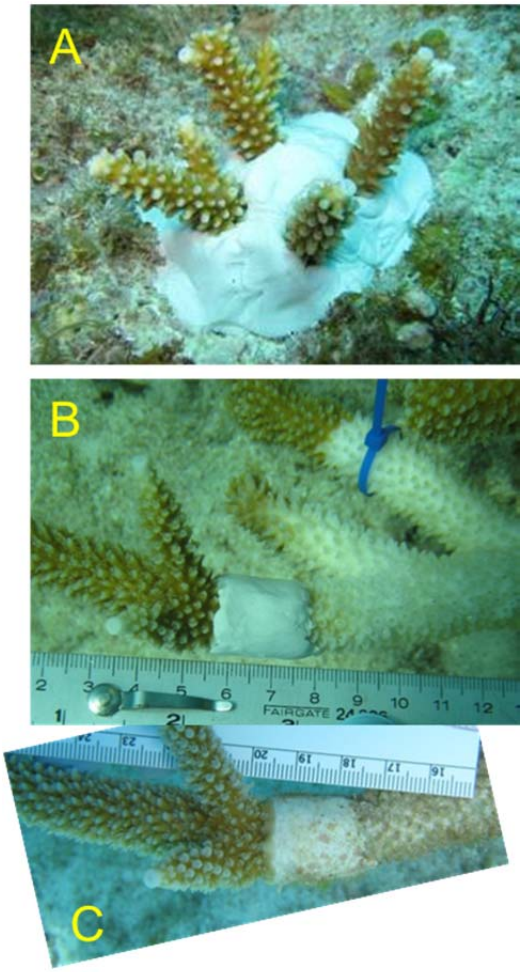
Figure 2:



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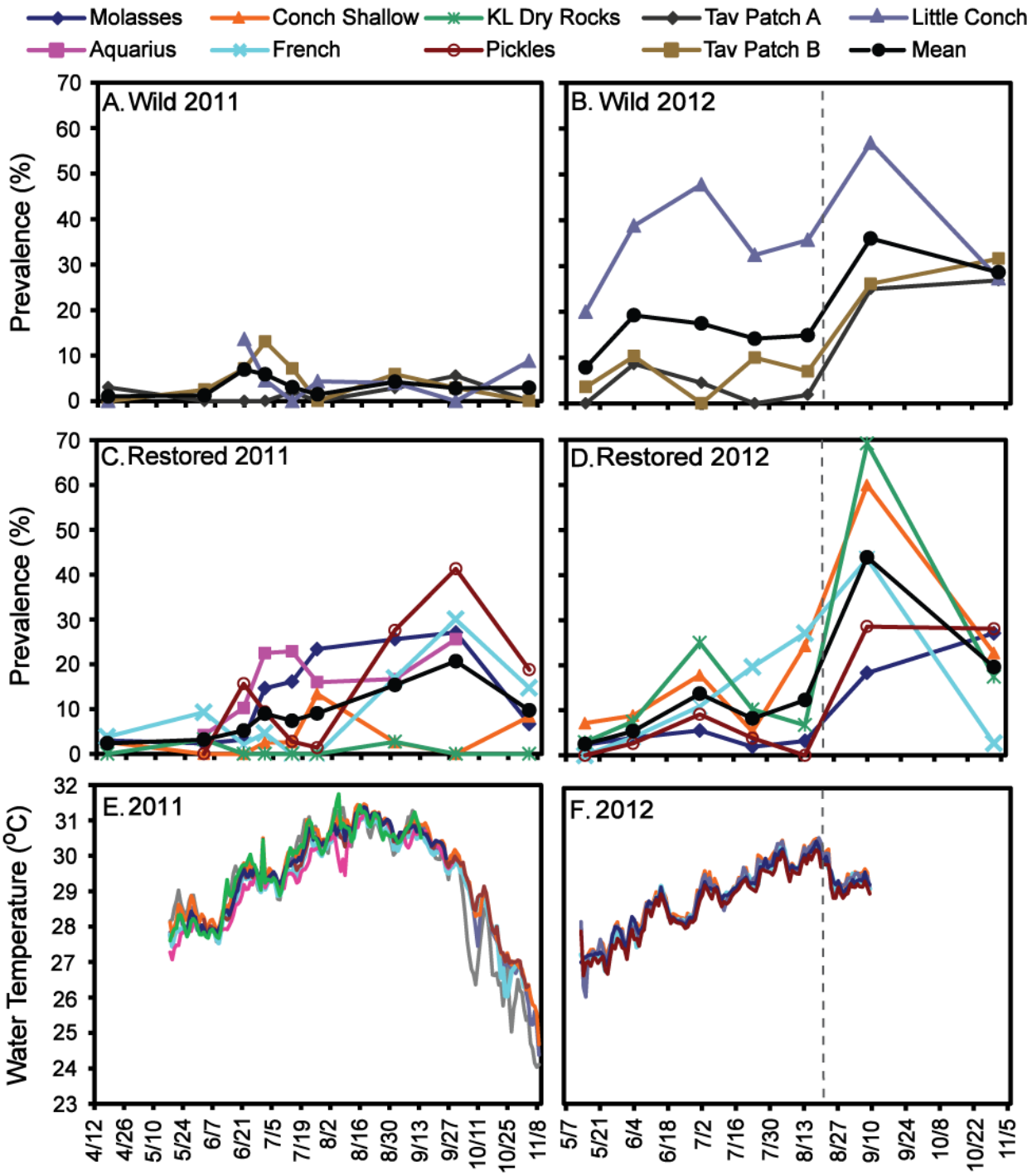

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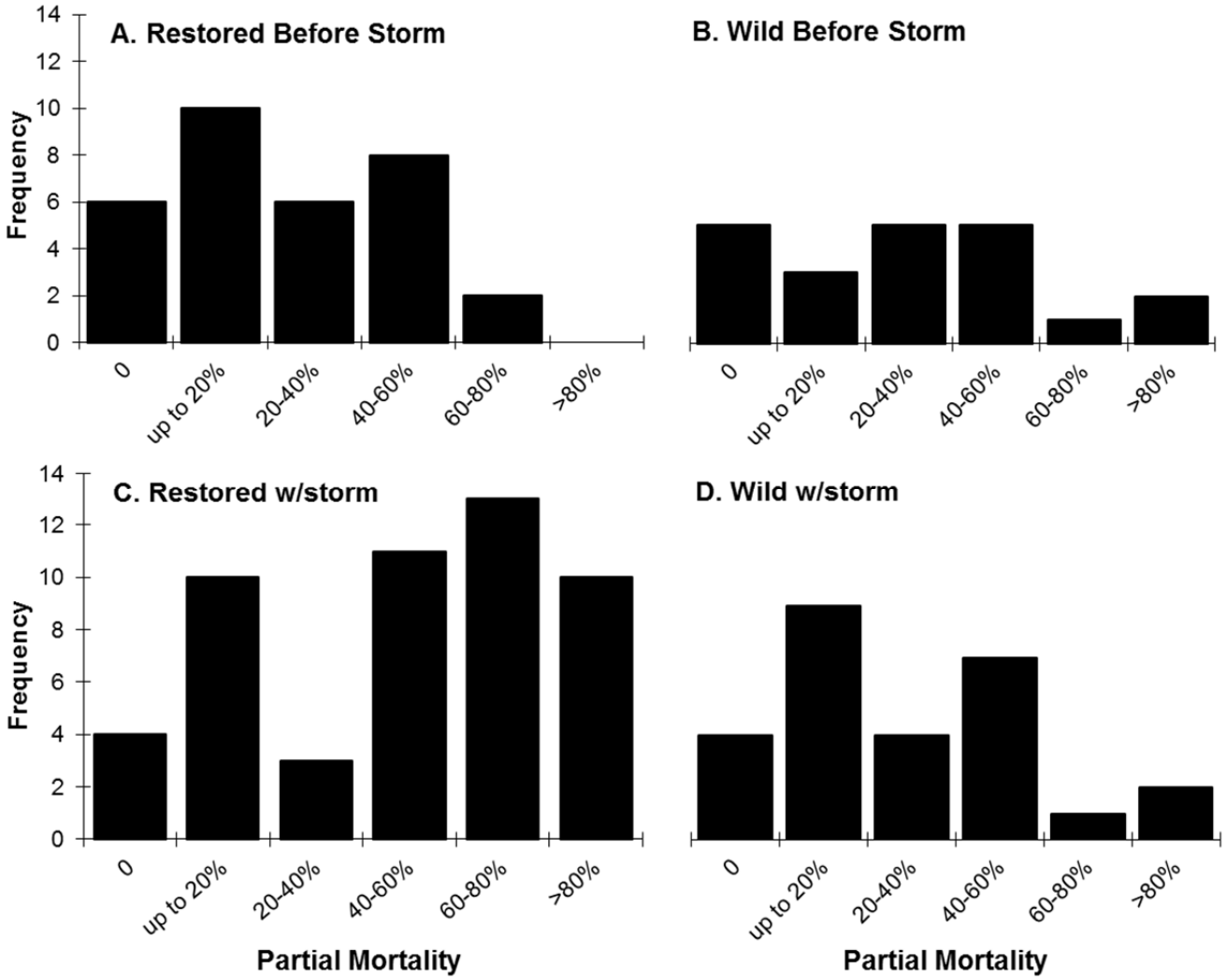

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Figure 5:

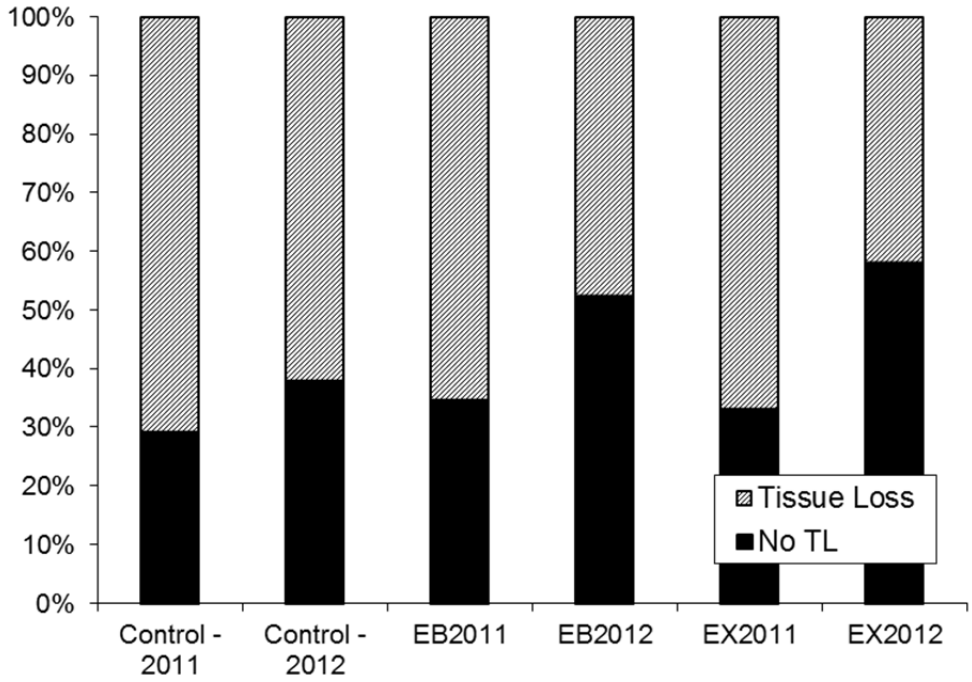

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Figure 6:

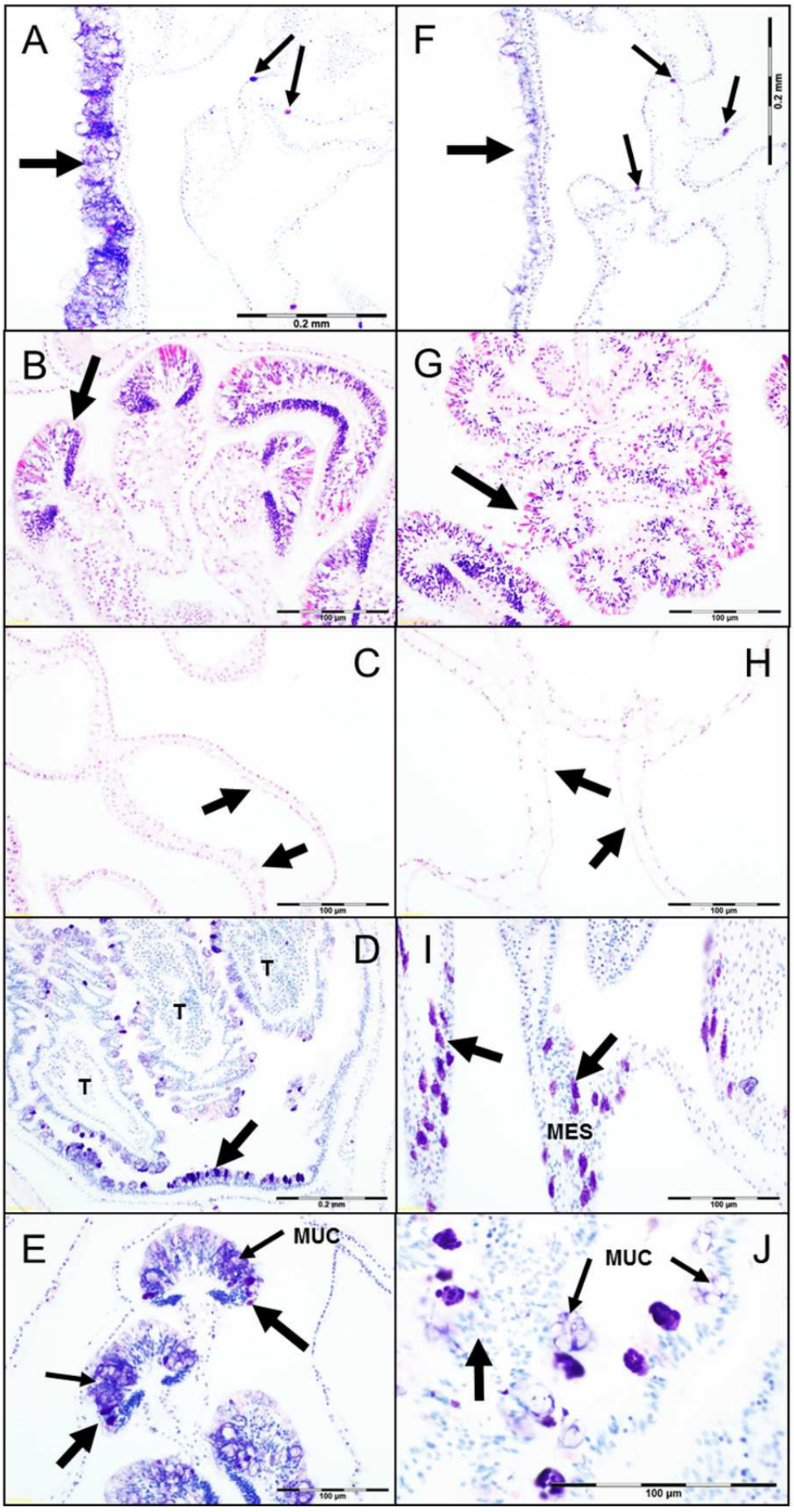

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Figure 7: