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NOTE

Caribbean yellow-band syndrome on *Montastraea faveolata* is not transmitted mechanically under field conditions

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ABSTRACT: Caribbean yellow-band syndrome is a highly prevalent coral disease whose transmission mechanisms are unknown. Affected corals often show multifocal lesions of yellow-colored tissue. We tested the hypothesis that a mechanical vector was responsible for these multifocal lesions. Four presumably non-resistant colonies were experimentally manipulated. Tissue and microbial assemblages were taken from diseased tissue and injected into healthy-looking tissue. Seawater injections were used as controls. The manipulations created a small wound, as would be generated by a coral predator. After 1 mo, all lesions healed and showed no signs of disease. We therefore reject the hypothesis that a mechanical vector, acting in a similar way as our manipulations, was responsible for the multifocal lesions.

KEY WORDS: Coral · Disease · Caribbean yellow-band syndrome · *Montastraea* · Vector

INTRODUCTION

Prevalence of infectious coral diseases has increased worldwide since the first observations more than 30 yr ago (Ward & Lafferty 2004). This trend can be linked to changes in the environment that benefit potential pathogens and impair coral host immunity (Harvell et al. 2002, Muller et al. 2008). In spite of the increase in coral disease prevalence, few transmission models have been proposed. The alga *Halimeda opuntia* was suggested to be a reservoir and physical vector of *Aurantimonas coralicida*, the putative pathogen for white plague type II (Nugues et al. 2004), whereas the fire-worm *Hermodice carunculata* was shown to be a winter reservoir and vector of *Vibrio shiloi*, the pathogen for bacterial bleaching (Sussman et al. 2003, but see Leggat et al. 2007). Physical transmission has also been documented for the fungus *Aspergillus sydowii*, the causative agent of aspergillosis on sea fans (Jolles et al. 2002). In that case, floating fungus fomites, or direct contact of contiguous hosts, transmitted the disease. Williams & Miller (2005) also showed that a form of white disease on the coral *Acropora cervicornis* was transmissible by direct contact with the coral-eating snail *Coralliophila abbreviata*. Finally, black-band disease was transmissible in aquaria when a wound or a corallivorous fish was present (Aeby & Santavy 2006). Indirect evidence also suggests the role of fishes as possible vectors of 6 coral diseases from the Pacific Ocean (Raymundo et al. 2009).

Caribbean reefs contain 2 sets of coral species that comprise the main keystone reef-builders: *Acropora palmata* (and *A. cervicornis*) and species of the *Montastraea annularis* complex. These species have been heavily impacted by disease. White-band disease devastated acroporid populations around the Caribbean basin (Gladfelter 1982, Precht et al. 2002), while populations of the *M. anuularis* species complex are being severely affected by more than 8 different signs and syndromes described to date (Sutherland et al. 2004, Weil 2004). Among the various signs affecting

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Montastraea sp., the slow-progressing and lethal Caribbean yellow-band/blotch syndrome (CYB) is a great cause of concern because of its widespread mortality (Santavy et al. 1999, Jordán-Dahlgren et al. 2005, Bruckner & Bruckner 2006). Despite its widespread nature and high prevalence, the cause of CYB is still debated and its mode of transmission remains unknown. The disease seems to affect coral endosymbionts, causing a reduction in their mitotic rate, which leads to coral death (Cervino et al. 2001, 2004). In laboratory experiments, Cervino et al. (2008) found that a consortium of *Vibrio* sp. strains isolated from CYB-diseased tissue produced disease signs when inoculated into healthy coral fragments. However, *in situ* transmission has never been documented. Furthermore, large *M. faveolata* colonies commonly have multi-focal (focus is used here to describe a central point or locus of an infection) lesions, as the result of new lesions appearing on different colony locations. These new lesions expand and eventually may coalesce with other lesions (Bruckner & Bruckner 2006). However, it is unknown whether each lesion is a result of a new infection. We tested the hypothesis that CYB could be mechanically transmitted by simulating fish bites under field conditions.

MATERIALS AND METHODS

Study site and colony selection. The study was conducted on the back reef of Puerto Morelos, Yucatán, Mexico (20° 53.012′ N, 86° 50.913′ W), a well developed reef with abundant massive *Montastraea annularis* and *M. faveolata* (Jordán-Dahlgren & Rodríguez-Martínez 2003). *In situ* seawater temperature was measured with a submersible Stowaway Tidbit (TBI32, 0.3°C resolution) that was attached under a coral colony. The Tidbit was programmed to take measurements every hour.

Because some colonies may be resistant to the purported pathogen, we selected colonies which have had progressing multifocal yellow-band signs for several years (E. Jordán-Dahlgren pers. obs.). Knowing that the coral's immune system was unable to resist infections in the past, we assumed that those colonies would be susceptible to further infections (but see Reed et al. 2010). Four colonies of *Montastraea faveolata* were selected at 2 sites on the back reef of Puerto Morelos to be used for the inoculation experiments.

Yellow-band manipulations. To test whether CYB could be mechanically transmitted, tissue from a yellow-band lesion was extracted with a syringe and then injected into apparently healthy areas of the same colonies from which the extract was obtained. For each experimental series, a new syringe was used to inject tissue and microbial assemblages (~2 ml) following Cervino et al. (2004). Tissue was taken from the middle of a yellow-band lesion and injected into adjacent, apparently healthy, tissue. A similar procedure was followed using surrounding seawater (~2 ml) to control the experimental manipulations. Once the inoculum was injected, we purposefully destroyed 3 to 4 polyps with the tip of the syringe, creating lesions approximately 100 mm^2, to mimic the effect of a small bite with only slight damage to the skeleton (Rotjan & Lewis 2008). To guarantee no confusion in later recordings, nails were used as a benchmark to locate the inoculation foci (Fig. 1). Five injection series were done perpendicular to the coral edge under the benchmarks. The first injection was done closer to the colony edge (±10 cm), and subsequent injections were aligned perpendicular to the edge and spaced 5 cm from each other, so that the last injection was ±30 cm within the coral tissue (Fig. 1). If intra-colony variation existed,

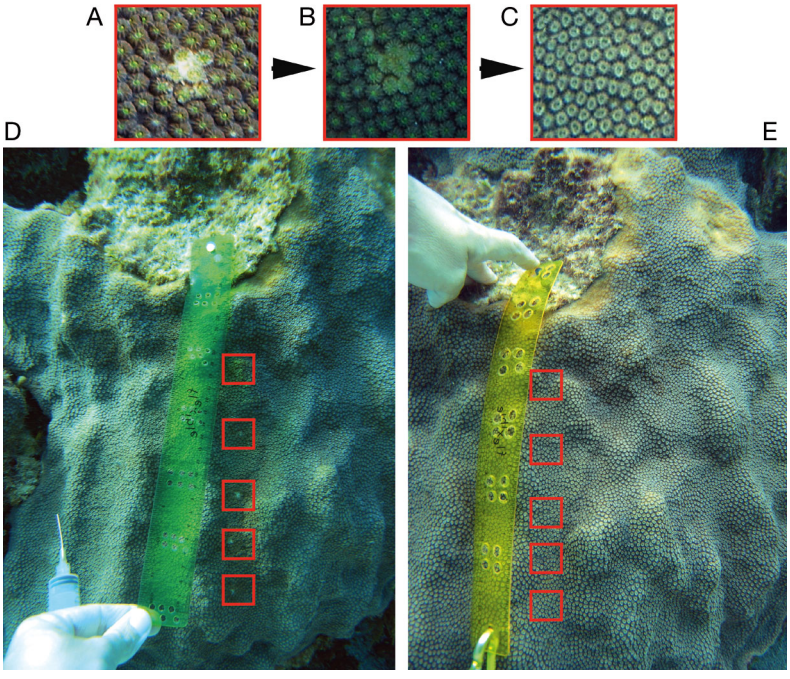

Fig. 1. *Montastraea faveolata.* Physically induced lesions on corals at Puerto Morelos, Mexico. (A) Recent lesion. (B) Lesion 1 mo later, showing weak yellowness. (C) Tissue after 5 mo; no lesion or signs are visible. (D) Every lesion of treatments and controls showed a yellow hue 1 d after manipulation (red squares). (E) After 5 mo, regeneration of the lesions was complete, and no signs of yellow-band disease were evident on any of the inflicted lesions (experimental or control)

e.g. different zooxanthellae clades (Rowan & Knowlton 1995) or allocation potential, then each injection, depending on its location within the colony, would be independent from other injections, but this was unknown. Six stainless-steel nails were placed on non-living areas on each colony, 3 adjacent to CYB signs (experimental inoculations); the same was done on locations adjacent to apparently healthy tissue within the same corals (control inoculations). On each coral colony, 6 series (3 experimental and 3 control) were made, resulting in 15 experimental lesions and 15 control lesions per colony, for a total of 60 experimental and 60 control lesions on the 4 studied coral colonies.

The experiment began in September 2007 when seawater temperatures were at the annual maximum and pathogens were more likely to proliferate (Harvell et al. 2002). After the first series of inoculations, the colonies were examined every 2 d for 2 wk, then every month until the last survey in January 2008.

RESULTS AND DISCUSSION

The tissue surrounding the artificial lesions, both for CYB inoculations and controls, showed a yellow hue following the first manipulations. We discerned 3 color stages, in comparison to surrounding tissue: a strong yellowness, a weak yellowness and no signs at all (Fig. 2). The time to complete tissue regeneration was noted. No significant differences in the proportion of lesions with strong and weak yellowing between colonies, 2 wk after the start of the study, were noticed for controls ($\chi^2 = 0.63$, df = 3, p = 0.89) or for CYB inoculum ($\chi^2 = 0.53$, df = 3, p = 0.91). Data from all 4 colonies were pooled, and the median percentage of tissue with signs of CYB and the average absolute deviation of the total number of lesions were calculated for the first 2 wk of observations and then every month until the end of the experiments (Fig. 2). The yellow hue decreased in intensity with time, until no signs were visible at all.

In situ mean (± SD) seawater temperature recorded for September 2007 was 29.9 ± 0.4°C (n = 720), which was within the 11 yr average for this month (29.3 ± 0.5°C; Rodríguez-Martínez et al. 2010). In laboratory conditions, inoculations have successfully transmitted signs of CYB on *Montastraea* sp. fragments at temperatures ranging from 28 to 31°C (Cervino et al. 2004, Weil et al. 2008). Therefore, we expected September water temperatures to be adequate for transmitting new infections at the study sites. Immediately after the manipulations, the tissue surrounding the lesions took on a yellowish hue for all treatment levels. Within 1 mo, the coral colonies had regenerated tissue and polyps on the experimental and control lesions, and a less intense yellow hue was still visible on the majority of the inflicted lesions. After 5 mo, regeneration was complete and there were no signs of yellow tissue on any of the injection foci (Fig. 2). No successful transmission of CYB signs and no difference in tissue regeneration were observed, between or within, the 4 coral colonies and treatment levels (experimental, controls) at the end of the 5 mo observational study. It seems evident that the change in color on the produced lesions

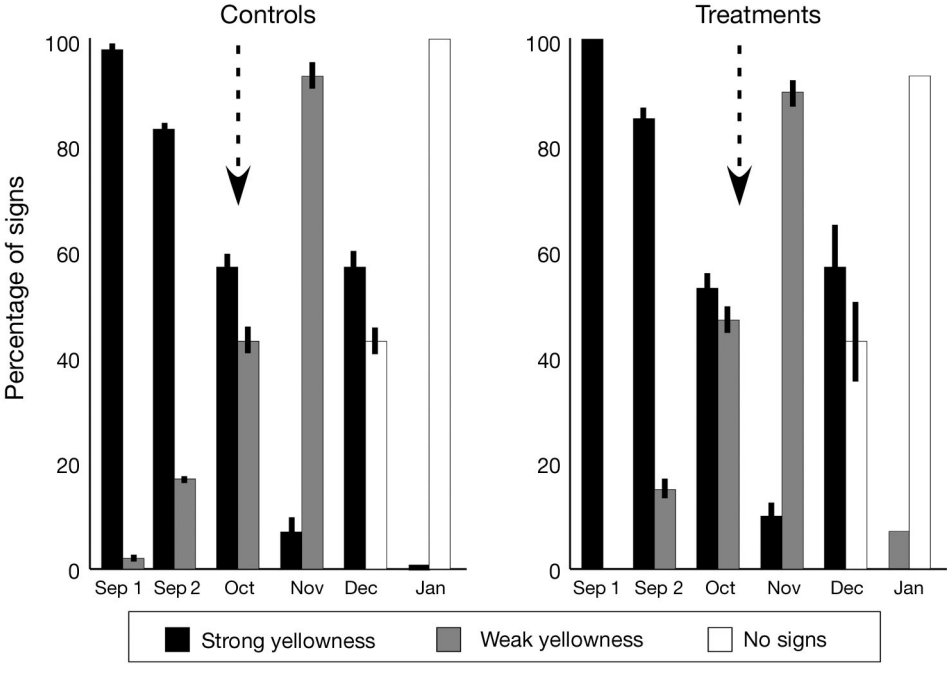

Fig. 2. *Montastraea faveolata.* Percent of experimentally induced lesions in corals with yellow-band syndrome and controls. Data expressed as median (bars) and median absolute deviation (solid lines) of the total number of lesions. Dotted arrows indicate almost complete tissue regeneration on the artificial lesion area. September 1 and 2 correspond to weekly observations during the first 2 consecutive weeks. Afterwards, a single observation per month was made (n = 4 coral colonies, n = 60 treatment lesions, n = 60 control lesions)

reflected the healing process, rather than successful transmissions of CYB. Controls and experimental lesions recovered at a similar rate, and the time taken for the damaged polyps to regenerate was consistent with the period observed by Meesters et al. (1992) on healthy colonies of *Montastraea* sp. The experimental coral colonies in this study were chosen because they already had multifocal CYB signs, and thus it is known that they were unable to fight infection on several foci in the past. However, none of our manipulations resulted in new CYB lesions, and furthermore the colonies appeared to retain normal regenerative capacities on their apparently healthy tissue, independently of treatment (controls or CYB inoculations). We reject the hypothesis that a mechanical vector, acting similarly as our manipulations, would cause the multifocal lesions within a coral colony, under natural conditions.

Our findings also suggest a local effect of the disease, given the regeneration time observed. The diseased tissue of the colonies dies at relatively slow rates (A. G. Jordán-Garza pers. obs. *in situ*, also see rates in Cervino et al. 2001), but at the same time, the apparently healthy tissue of the experimental colonies showed a normal capacity to regenerate the small wounds (Meesters et al. 1992). In contrast, Weil et al. (2009) suggested that *Montastraea* sp. yellow-band diseased coral colonies reacted in a systemic manner, as these authors found an apparent, but not statistically significant, reduction in the reproductive effort of the healthy-looking tissues of diseased colonies. Diseased colonies may be allocating resources to preserve tissue integrity and prevent disease advancement (Mydlarz et al. 2009), resulting in an impairment in reproductive output. Alternatively, because reproduction in corals is linked to tissue surface area, it is also possible that partial mortality resulting from the disease progression indirectly affects their reproductive output (Soong & Lang 1992). In accordance with trade-off theory, the energy used toward reproduction may impair the immune system (Sheldon & Verhulst 1996). Our study did not control for the reproductive state of the experimental coral colonies, but our results suggest they maintained the ability to resist new infections, probably by the release of antibacterial materials after an induced wound, as shown by Geffen & Rosenberg (2005).

It is important to examine the inoculation method used here, because it could be related to the lack of transmission. We produced a small wound with a single inoculation of microbial assemblages; this could be similar to a predator with high motility or an extensive territory, that would bite the colony a few times while swimming by, e.g. the spot-biting behavior of parrotfishes (Rotjan & Lewis 2008). If the predators were territorial and with limited movement around a single coral colony, e.g. butterflyfishes (Reese 1989), then the probability that they repeatedly bite the same area within the colony would be higher; this could increase the pathogen dosage and may facilitate infection, if mechanical transmission is feasible at all. On the other hand, CYB on *Montastraea faveolata* may be transmitted by a biological vector, although so far only mechanical transmission has been successful under laboratory conditions, using inoculations directly extracted from CYB (Cervino et al. 2008). Experimental field manipulations attempting to transmit CYB signs have been unsuccessful (present study; E. Weil pers. comm.).

Presently, it remains unknown how CYB, and the multiple infections associated with this coral disease, are transmitted. Corals are modular organisms, it may be that intra-colony variation exists on the defense against diseases, as has been shown for plants (Pavia et al. 2002), and that environmental drivers could exacerbate or impair those defenses (Harvell 1986). Lesser et al. (2007) suggested that coral diseases are infections that appear after a stress has hampered the coral immune system. If this is so, compromised coral immunity may vary within a coral colony, and the multiple lesions of yellow-band might be dependent on this variability. However, it is not yet known if the purported causative agents of CYB are primary or opportunistic pathogens. Given the continuous emergence of diseases in corals (as in many other organisms), we can state that the rapidly degrading coral reef environment may be compromising all 3 major components of the holobiont immune system: the animal, the zooxanthellae, and the coral's microbial community. In such a situation, multiple mechanisms may exist for an infection to occur, and more experimental approaches are needed to understand how this disease spreads in nature.

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