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REPORT

Multiple mechanisms of transmission of the Caribbean coral disease white plague

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Abstract White plague is one of the most devastating coral diseases in the Caribbean, and yet important aspects of its epidemiology, including how the disease transmits, remain unknown. This study tested potential mechanisms and rates of transmission of white plague in a laboratory setting. Transmission mechanisms including the transport of water, contact with macroalgae, and predation via corallivorous worms and snails were tested on the host species *Orbicella annularis*. Two of the tested mechanisms were shown to transmit disease: water transport and the corallivorous snail *Coralliophila abbreviata*. Between these transmission mechanisms, transport of water between a diseased coral and a healthy coral resulted in disease incidence significantly more frequently in exposed healthy corals. Transmission via water transport also occurred more quickly and was associated with higher rates of tissue loss (up to 3.5 cm d^{-1}) than with the corallivorous snail treatment. In addition, water that was in contact with diseased corals but was filtered with a 0.22-μm filter prior to being introduced to apparently healthy corals also resulted in the transmission of disease signs, but at a much lower rate than when water was not filtered. This study has provided important information on the transmission potential of Caribbean white plague disease and highlights the need for a greater understanding of how these processes operate in the natural environment.

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Introduction

Throughout the Caribbean, coral diseases have played a major role in recent coral mortality and widespread declines among reefs (Richardson 1988; Weil 2004; Croquer et al. 2005). Coral diseases have the potential to threaten coral diversity and result in shifts to reef community structure and dynamics (Weil et al. 2003). Over thirty coral diseases have been reported globally, afflicting at least 150 species of coral (Richardson 1988; Green and Bruckner 2000; Jones et al. 2004). Understanding the relationship between coral and disease has proven difficult due to limited knowledge of the etiological agents and many of the fundamental processes associated with disease, including host resistance, pathogen virulence, and mechanisms of disease spread (Jones et al. 2004). However, as outbreaks of coral diseases continue to threaten reefs, there is a growing need for understanding of how disease initiates and propagates among corals.

The coral disease white plague (WP) was one of the first diseases identified (Dustan 1977), and since that time, it has been reported from all parts of the Caribbean, affecting nearly half of all Caribbean coral species (Sutherland et al. 2004). Outbreaks of this disease have resulted in localized but significant declines in coral cover (Nugues 2002; Croquer et al. 2005; Richardson and Voss 2005). Additionally, WP outbreaks following the 2005 mass coral bleaching event in the Caribbean resulted in losses of up to 40 % of coral cover on some reefs (Miller et al. 2009; Eakin et al. 2010). Several other WP outbreaks have been associated with environmental perturbation (Miller and

Williams 2007; Miller et al. 2009; Brandt et al. 2013), but the mechanisms that contribute to the spread of disease during these outbreaks often remain unknown. Due to the potential for WP to cause extensive loss of coral in short time frames, there is a definitive need to identify mechanisms of disease spread within coral communities.

Characteristic signs of WP include lesions that begin at the base or margin of a colony and enlarge rapidly (mm to cm d^{-1}), creating a smooth margin of tissue loss delineating the intersection between living tissue and denuded white skeleton (Fig. 1; Bythell et al. 2004). The name "white plague" encompasses three distinct disease "types" (WP-1, WP-2, and WP-3) with similar disease signs distinguished by their rates of tissue loss. WP-1 progresses slowly (1–10 mm d^{-1}) and is characterized by a smooth margin of tissue loss (Dustan 1977), WP-2 progresses rapidly (up to 2 cm d^{-1}) and occasionally shows a thin band of bleached tissue at the lesion edge (Richardson et al. 1998), and WP-3 progresses rapidly (>2 cm d^{-1}), but only exclusively affects large colonies (3–4 m in diameter) of *Colpophyllia natans* and *Orbicella* (formerly *Montastraea*) *annularis* (Richardson et al. 2001).

Efforts to identify an etiological agent of the WP types have been ongoing since the first identification. WP-1 was originally associated with bacteria, but Koch's postulates were not fulfilled (Dustan 1977), and WP-3 has not been associated with any microbial pathogen to our knowledge. Of the three types, WP-2 has been the most comprehensively characterized and has a known associated etiological agent. WP-2, originally documented in 1995, was first associated with a bacterium belonging to the group *Sphingomonas* (Richardson et al. 1998), later re-identified as a novel marine bacterium *Aurantimonas coralicida* (Denner et al. 2003). Nugues et al. (2004) showed that the macroalgae *Halimeda opuntia* has the potential to be a reservoir and can trigger infection by *A. coralicidia*. However, similar disease signs in different parts of the Caribbean have not been associated with *A. coralicida* (Pantos et al. 2003). For instance, Cook et al. (2013) detected *A. coralicida* in low abundances in samples from both WP affected corals and apparently healthy corals, suggesting it was not the etiological agent of the observed WP signs. However, Gray et al. (2013) identified PCR bias as a potential mechanism preventing the detection of *A. coralicida* in WP samples. Clearly, the debate over the etiology of WP-2 and other WP types continues, and most recently, viruses have been identified as playing a potential role in WP-1 in the US Virgin Islands (Soffer et al. 2013).

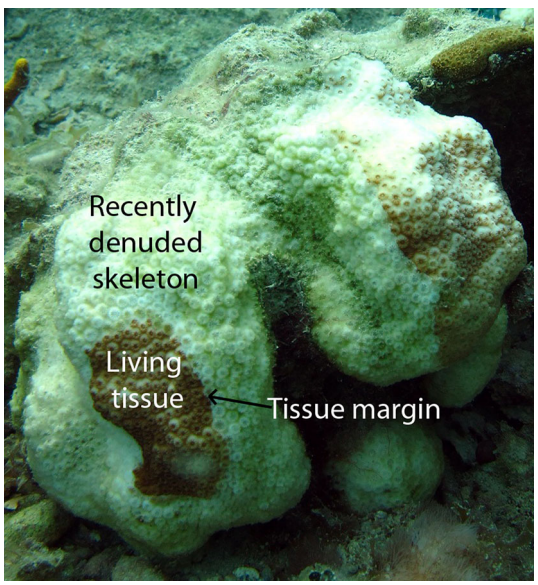

Fig. 1 White plague affecting a colony of *Orbicella annularis* in Brewers Bay, St. Thomas, USVI. Photograph taken September 23, 2010, 5 m depth

Studies of the etiological agent have suggested that WP is an infectious disease caused by a microbial pathogen versus an apoptotic state as has been found in a similar coral disease described from the Pacific (Ainsworth et al. 2007). Understanding how the infection transmits among corals is an important component of characterizing the disease and can also provide an ecological context for etiological agents that are identified. Our understanding of WP transmission in the marine environment has primarily been inferred from spatial patterns of disease. Clustered distributions of diseased individuals typically indicate an infectious nature of a disease (Diggle 1983), and several studies have associated WP with such a distribution (Borger 2003; Voss and Richardson 2006; Brandt and McManus 2009b). However, other studies have not found clustering (Nugues 2002; Borger 2005; Richardson and Voss 2005; Muller and van Woesik 2012), and these conflicting results may be due to inconsistent sampling regimes and scales. Due to the potential for high connectivity within marine environments (McCallum et al. 2003), a clustered distribution of coral disease may indicate transmission through some vector that is limited in its range, such as slow water flow, contact with algae, or via a mobile predator (Brandt and McManus 2009b). In laboratory experiments, Richardson et al. (1998) showed that the original WP-2 pathogen could spread through water borne transmission within a small tank. Research in the marine environment has demonstrated that WP-2 could be transferred between colonies via the calcareous green macroalga species *H. opuntia* (Nugues et al. 2004) and in the Virgin Islands WP-1 signs were transferrable via direct contact between two separate pieces of coral that were fixed together (Brandt et al. 2013). Clearly, more work is needed to identify mechanisms of WP transmission and their potential to aid WP propagation in the marine environment.

Multiple mechanisms of transmission have been identified in other disease systems of corals. In studies of black-band disease (BBD) of corals, an infection was able to be transmitted through direct contact (Rutzler et al. 1983) and through a corallivorous butterflyfish vector by oral and fecal transmission (Aeby and Santavy 2006). Other known vectors of disease transmission in corals include the polychaete *Hermodice carunculata* (Sussman et al. 2003), which is both a reservoir and vector for the bacterial bleaching pathogen *Vibrio shiloi* in the Mediterranean, and the mollusk *Coralliophila abbreviata*, which was identified as a potential vector for an unknown pathogen causing rapid tissue loss in acroporid corals in the Florida Keys (Williams and Miller 2005). Both *H. carunculata* and *C. abbreviata* serve as vectors of infectious agents that may cause disease by feeding on an infected colony and subsequently feeding on and infecting a healthy colony. Both *H. carunculata* and *C. abbreviata* are found, sometimes abundantly, feeding on corals affected by WP, but these potential vectors have not been tested as mechanisms of WP transmission.

In the US Virgin Islands (USVI), WP has been identified affecting corals in all monitored reef habitats for at least a decade (Smith et al. 2008). This disease had a devastating impact on many reef communities following the 2005 mass bleaching event (Miller et al. 2009) and continues to affect coral populations throughout the region (Smith et al. 2013). Recent targeted studies of WP in this region have shown that signs most closely match those described for WP-1 (Brandt et al. 2013). In addition to its association with mass bleaching, this disease was also associated with storm-related fragmentation and contact with sediment (Brandt et al. 2013). Its spatial distribution suggests infectious transmission (Brandt et al. 2013), and viruses may play an important role in the etiology of this disease (Soffer et al. 2013).

In this study, we examined multiple potential mechanisms of transmission of the Virgin Islands WP-1 in a controlled laboratory setting. Experiments consisted of using fragments of the susceptible reef-building coral *O. annularis* exposed to various vectors and media with the potential to transmit the unknown pathogen causing WP. We expected that multiple mechanisms would transmit the unknown pathogen causing disease, but that one would be the most effective. By identifying transmission mechanisms and targeting them for further study, we aimed to provide better understanding of the determinants behind the dynamics and ultimately the impact of this disease. This work may also lead to the identification of important aspects of the transmission mechanisms that could be targeted for intervention or in an effort to prevent future outbreaks and impacts.

Methods

Study region

All research took place in St. Thomas, USVI, at the Center for Marine and Environmental Studies (CMES) at the University of the Virgin Islands (UVI) from November 1 to December 22, 2012. Collection of experimental animals occurred at two locations on the south side of St. Thomas separated by a distance of 4.65 km, and both sites typically experience low disease prevalence (<1 %; Brandt pers. obs.) These sites (Perseverance Bay: 18°20′53.45″N 64°59′33.09″W and Saba Island: 18°18′19.70″N 64°59′55.27″W) are characterized by shallow reefs (<10 m) dominated by the coral species *O. annularis* and *O. faveolata*.

Specimen collection and acclimation

Experimental corals were exposed to treatments in three separate trials that took place in 2012 from 1 to 22 November, 12 November to 3 December, and 1 to 22 December. For each of the trials, fragments of *O. annularis* were collected from sites by divers on SCUBA. From each site, 2–3 coral fragments of <30 cm in maximum diameter were collected from 4 to 5 colonies that were separated by at least 5 m. The growth form of *O. annularis* is such that the colony grows in columns with living tops and dead sides. Fragments collected for the study consisted of the living tops of columns that were broken off at the dead area using a hammer and chisel. Large fragments were further fragmented such that a total of 25 pieces of apparently healthy *O. annularis* were used in each trial. Average fragment size had a length of 6.7 cm ± 0.2 SE, width of 5.4 cm ± 0.2, and area of 26.7 cm^2 ± 1.4.

Following collection, fragments were immediately transferred in seawater to the laboratory where they were placed in running seawater tables and allowed to acclimate for 7 d before being used in various treatments. The colony of origin was recorded and tracked for each coral fragment, and all fragments were photographed and monitored daily during a 7-d acclimation period. The sea water tables are part of a flow-through system located at CMES, which sits next to Brewer's Bay. The system consists of seawater that is pumped from Brewer's Bay into a head tank located physically higher than the elevation of the Marine Science building. Seawater flows by gravity to the culture tanks below via a 3 Sch. 40 PVC main supply line. Seawater temperatures in the water tables remained at ambient levels found on the reef during the same time period (Trial 1: 28.7–29.0 °C, Trial 2: 28.3–28.8 °C, Trial 3: 27.8–28.3 °C).

Two pieces of WP-diseased coral were collected separately and kept in a separate seawater table. Six additional fragments of apparently healthy *O. annularis* were directly exposed to these pieces, developed signs of WP, and were subsequently used in experiments as inoculant corals. Therefore, a total of eight diseased fragments were used in inoculant experiments in each trial. Each diseased fragment was assessed each day to determine whether the lesion was still progressing and active before being used in any experiments.

The mobile vectors (*H. carunculata* and *C. abbreviata*) were collected from intertidal areas around the dock at the Marine Science Building on Brewer's Bay. For each trial, three individuals of *H. carunculata* and three of *C. abbreviata* were used and allowed to acclimate in separate 2-L buckets with individual bubblers and daily water changes for 3 d. *H. carunculata* that were collected ranged in size from 7 to 9 cm long. The animals were not fed before being used in experiments, and during the experiment, all *H. carunculata* and *C. abbreviata* were documented feeding on experimental corals. Clumps of *Dictyota menstrualis* separated by 5 m were also collected from the dock area and dabbed dry before they were allowed to acclimate in separate 2-L buckets with bubblers and daily water changes for 3 d before being used in experiments.

Experimental design

After acclimation, coral fragments were removed from seawater tables and placed into numbered 2-L buckets with an individual bubbler and daily water changes. Coral fragments were then randomly assigned to be used as an inoculant, treatment, or control. Five sets of experiments explained below were performed, including (1) unfiltered water, (2) filtered water, (3) *Dictyota*, (4) *C. abbreviata*, and (5) *H. carunculata* (Fig. 2). Each experiment included a set of controls, and all buckets were washed with alcohol before each trial. In the unfiltered water experiment, treatment corals received 1/2 L of water from a bucket with a diseased inoculation coral during their daily water changes. Corresponding control corals received 1/2 L of water from a bucket with a healthy coral. The treatment and control corals were photographed and monitored each day for signs of disease. An identical design was used in the filtered water experiment, but water being transferred between buckets was first filtered using a 0.22-μm filter (Millipore Express, 47 mm, polyethersulfone, hydrophilic). For both the unfiltered and filtered water experiments, treatment and control corals were subjected to transferred water daily for the entirety of a 2-week study period.

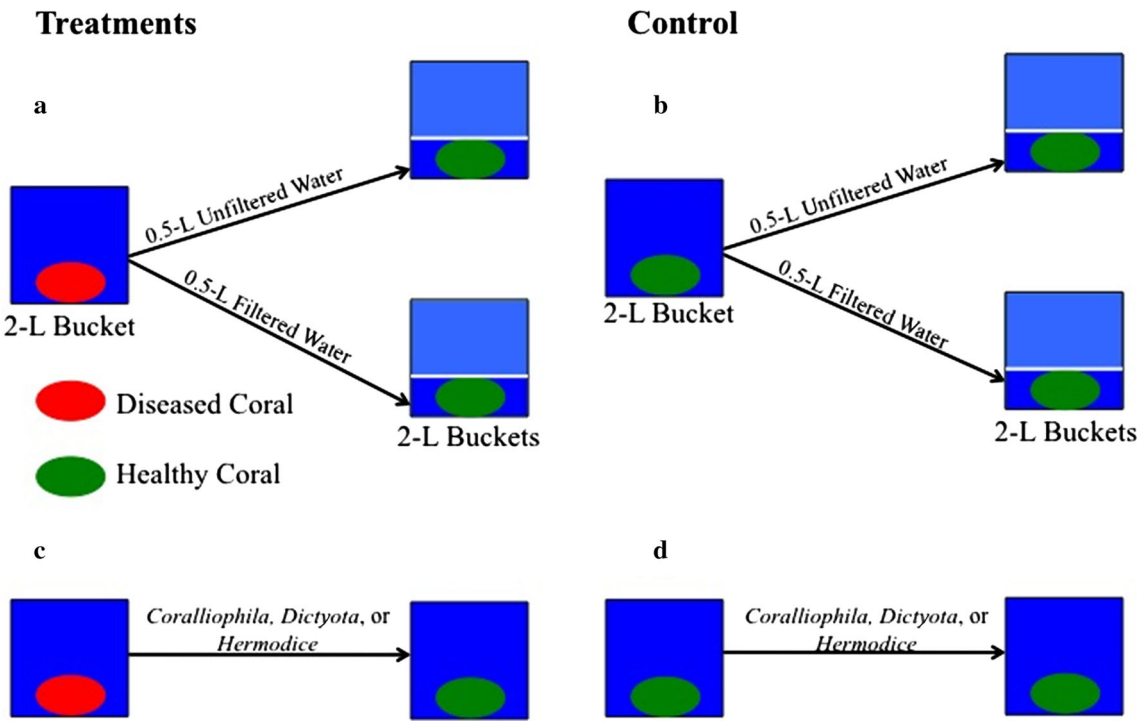

Fig. 2 Experimental design of transmission experiments. Replicates for the water transmission experiment included *a* treatment replicates where 0.5 L of filtered or unfiltered water was transferred from a 2-L bucket containing a diseased coral to a 2-L bucket containing a healthy coral, and *b* control replicates for the water transmission experiment consisted of the same design except water was transferred from a 2-L bucket containing a healthy coral. Replicates for the vector transmission experiments included *c* treatment replicates where *Coralliophila abbreviata* or *Hermodice carunculata* were allowed to feed on a diseased coral or *Dictyota* was placed in contact with a diseased coral and then transferred to a bucket with a healthy coral, and *d* control replicates where the same design was used, but the initial coral was a healthy coral. *Red ovals* represent diseased corals, and *green ovals* represent apparently healthy corals

Buckets with inoculation corals used as a source of water were refilled and allowed to sit for 24 h before being sampled again. Therefore, water that was used in the treatments had been in contact with either diseased or healthy corals for at least 24 h but no more than 36 h before being transferred to the treatment corals.

For *H. carunculata*, *C. abbreviata*, and *Dictyota* experiments, each suspected vector was placed in either a diseased or healthy coral bucket and allowed to feed for 2 d before being transferred into a treatment or control bucket. All buckets received individual bubblers and daily water changes. Each coral fragment was then photographed and monitored for signs of disease and mortality daily. Inoculation corals used for water experiments were not used in vector experiments.

Each trial lasted 2 weeks, and coral fragments were monitored until they died from disease or survived until the trial period was over. For each of the three trial periods, there were two controls and four treatments per trial for each of the filtered and unfiltered water experiments. This resulted in a total of $N = 12$ treatments and $N = 6$ controls for each experiment. For the *Dictyota*, *H. carunculata*, and *C. abbreviata* experiments, there was one control and three treatments per trial, resulting in a total of $N = 9$ treatments and $N = 3$ controls for each experiment. Low sample sizes were limited by logistical and permit constraints, and sequential trials were performed due to the scattered availability of diseased corals located in the natural environment.

Following previous descriptions (Remily and Richardson 2006), a WP lesion was defined as a smooth and undulating tissue margin adjacent to freshly denuded skeleton and exposed coral skeleton that continued expanding across the coral for at least 2 d. In contrast, feeding scars from the mobile vectors and other lesions due to fragmentation were distinguished from lesion signs based on their ragged edges the fact that the lesion did not enlarge following feeding or contact.

In order to calculate lesion enlargement rates, the area of recently denuded skeleton left by the lesion was traced using ImageJ software (NIH) for each daily photograph of fragments that developed lesions. Each lesion was measured anywhere from 3 to 6 times throughout the lesion's enlargement. The length of time for the disease signs to appear was recorded as the number of days between initial exposure to the treatment and start of tissue loss.

Statistical analyses

Rates of lesion appearance after exposure to inoculant corals were calculated as the number of days from the start of the trial until a WP lesion was observed. Where lesions appeared on multiple corals, a nonparametric Wilcoxon test was performed to test for a significant effect on the rate of lesion appearance between the two experiment types.

Tissue loss rate data were calculated per lesion as the area (cm^2) of tissue loss per day. These data were first square root transformed to approximate normality and then tested using a nested ANOVA with experiment type as the main factor and coral of origin as a nested factor. Once coral of origin was determined not to affect lesion progression rates, a repeated-measures ANOVA was used to test for an effect of time and experiment type on lesion progression rate.

To determine whether a relationship existed between the experiment type and the number of corals that became diseased, a contingency table analysis was used. A separate contingency table analysis tested for a relationship between the number of corals that became diseased and coral of origin.

Results

White plague disease signs were transmitted between diseased and healthy corals, but were never transmitted in any control treatments. Eleven of the 42 (26 %) treatment fragments became affected by WP during the study period (Fig. 3). The most effective mechanism of transmission was unfiltered water, which transmitted the disease 8/12 times, followed by filtered water (2/12) and *C. abbreviata* (1/6). Signs of disease transmission were not observed in either *H. carunculata* or *Dictyota* experiments; however, feeding was observed on all *H. carunculata* treatments. However, *Dictyota* caused partial bleaching in 2/3 controls and 5/6 experimental treatments. Fragments in the

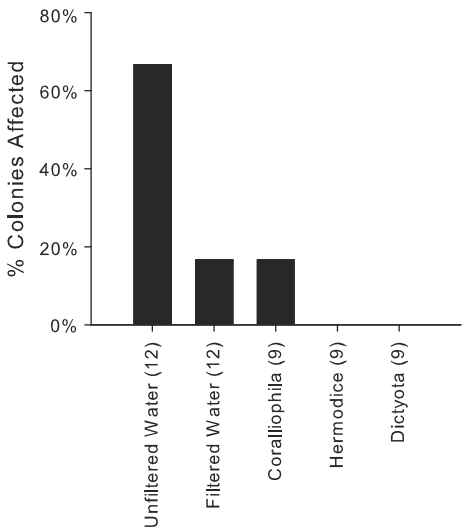

Fig. 3 Percent of experimental colonies that became affected by disease in each experiment. *N* are indicated in *parentheses*

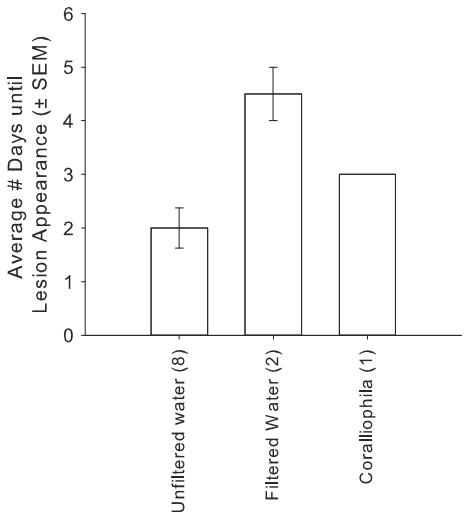

Fig. 4 Average rates of lesion appearance (±SEM) within treatments where disease transmission was identified. *N* are indicated in *parentheses*

unfiltered water experiment showed faster rates of lesion appearance than those in the filtered water experiments ($\chi^2 = 4.06$, $df = 1$, $p < 0.05$; Fig. 4). The average days until tissue loss began after exposure for unfiltered water, *C. abbreviata*, and filtered water were 2, 3, and 4.5 d, respectively (Fig. 4). Overall, new tissue losses were observed between 1 and 5 d after exposure to the different treatments.

Overall, average lesion enlargement rate on experimental corals was 0.8 $cm^2\ d^{-1} \pm 0.2$ (SEM), with a range of 0.007–3.6 $cm^2\ d^{-1}$. Most lesions resulted in total mortality, where total mortality was defined as the complete loss of tissue from the coral fragment. Average length of time until total mortality was 6.7 d (±1.5 SEM) after the first initiation of the lesion. Figure 5 shows an example of a lesion's initiation and tissue loss progression to completely denuded skeleton. The nested ANOVA revealed no significant effect of coral of origin ($F = 8.2637$, $df = 8$, $p > 0.05$). When tested without coral of origin as a factor, there was no significant difference in progression rates between experiments (Kruskal–Wallis test: $\chi^2 = 0.75$, $df = 2$, $p > 0.05$). However, throughout the lesion's enlargement, the new tissue loss rates at each time of measurement increased (repeated-measures ANOVA: $F = 9.23$, $df = 3.6$, $p < 0.05$; Fig. 6).

The results of the contingency table analyses indicated that the distribution of colonies that became diseased versus remaining healthy was significantly related to experimental mechanism, but this distribution was not significantly related to the colony of origin (Table 1). However, low sample sizes for both of these analyses indicate a cautious interpretation of the Chi-square statistic.

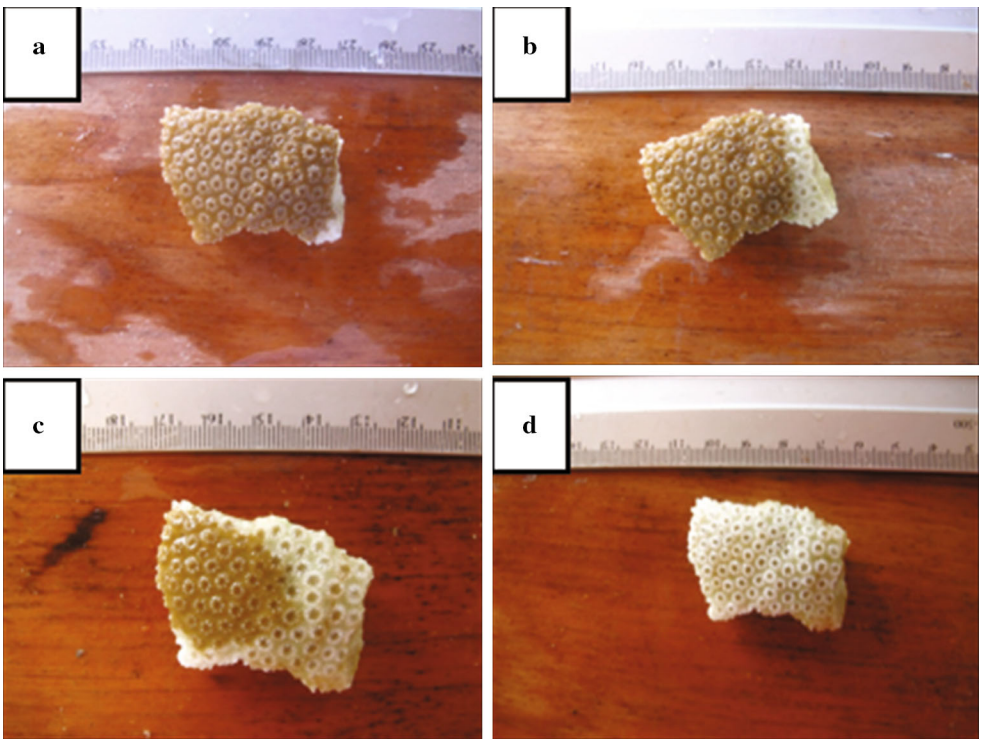

Fig. 5 Expansion of a white plague lesion in Trial 1 where the coral fragment was exposed to a *Coralliophila abbreviata* that had been allowed to feed on a separate diseased coral fragment. **a** Day 2 after exposure showing a feeding scar on the bottom right of the fragment, **b** day 3 showing the development of a smooth lesion line, **c** day 4 where the lesion has expanded across the colony fragment, and **d** day 5 where total mortality has occurred

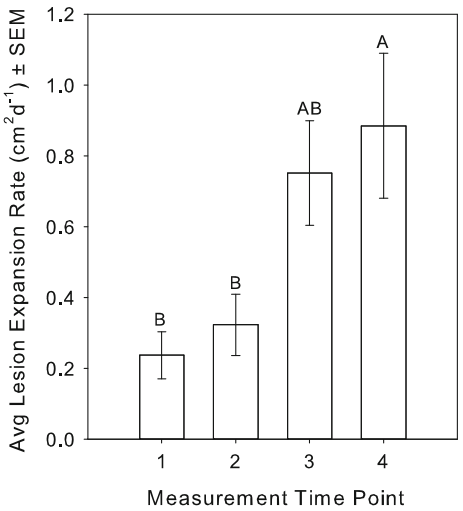

Fig. 6 Average rates of lesion expansion (±SEM) at successive time points across the lesion's enlargement. *Letters* indicate significant groups ($p < 0.05$) as determined by post hoc tests following a significant repeated-measures ANOVA. $N = 12$ at each measurement time point

Discussion

Disease transmission

The results of this study demonstrate that the causal agent of WP disease can be transmitted through multiple mechanisms, including water transport and snail predation. Transmission via water transport was the most effective mechanism, supporting the early observations by Richardson et al. (1998) that WP can be transmitted among colonies through water transport in laboratory conditions. While the experiments lasted 2 weeks, signs of disease generally occurred within the first 1–2 d for the unfiltered water treatment and 5–6 d for the filtered water and snail treatments, suggesting that transmission can occur rapidly. This is in contrast to field experiments in the same reef system where direct tissue-to-tissue transmission was not observed until after 3 weeks (Brandt et al. 2013). The discrepancy in timing was likely the result of laboratory conditions; laboratory coral fragments were kept in 2-L buckets with circulation limited to bubblers and daily water changes, while experimental corals in the field had natural circulation conditions and were constantly flushed. This may have allowed for potential pathogenic agents to be removed from field corals, while laboratory corals were in constant contact with materials that could have resulted in infection. Alternatively, laboratory conditions may have affected the resistance of experimental corals to disease and increased their susceptibility to infection. Follow-up experiments should investigate the timing of disease transmission in the field and in laboratory experiments that reflect more natural conditions.

In this study, water filtered to remove bacteria >0.22 µm was also able to induce disease signs, although at a highly reduced rate. Recent work has shown that viruses may play an important role in the etiology of WP in the Virgin Islands, and the transmission of disease using 0.2-µm filtered water supports a potentially viral infectious agent (Soffer et al. 2013). However, the low transmission rate of disease in the filtered water compared with unfiltered water treatments (2/12 vs. 8/12, respectively) suggests that the process of infection is more rapid when large microbial components such as bacteria are present. This could indicate a role of a filterable factor in the initiation of disease, similar to what has been demonstrated in WP signs from other regions (Barash et al. 2005). However, it is possible that these results are the result of contamination or incomplete efficiency, although all precautions were taken to reduce the risk of contamination and methods were applied equally to all treatments. The microbial communities found within a coral are diverse and include bacteria, viruses, and Archaea (Rosenberg et al. 2007). While less is known about the role of viruses and Archaea, bacteria have been noted to work as pathogen defense mechanisms and play a role in nutrient cycling (Lesser et al. 2007). Shifts in the overall microbial communities of corals can result in decreased resistance to disease (Ritchie 2006), and potentially increased virulence of pathogens (Vega-Thurber et al. 2012). Therefore, it would not be surprising if WP results from changes in multiple components of the microbial community and is not just the results of infection by one etiological agent.

The mobile vectors *C. abbreviata* and *H. carunculata* showed very low (1/6) and no transmission of the disease, respectively. Field studies have shown that feeding of *C. abbreviata* resulted in rapid tissue loss in *A. cervicornis* (Williams and Miller 2005) and have reported *H. carunculata* feeding on disease lesions (Miller and Williams 2007). Since these vectors have limited mobility and are not likely to travel between reefs, it is far more likely that water transmission may be the effective mechanism of transmission in the natural environment. However, as these predators feed on and negatively impact the coral's health, they may cause the coral to expend extra energy to heal feeding scars. This would stress the coral resulting in potential negative effects to the corals' immune system and possibly making it more susceptible to disease. Williams and Miller (2005) also hypothesized that once disease is introduced into a system, mobile predators that feed on one colony with disease can carry it to another, intensifying disease spread within the reef system. Although predators such as these may not be the primary mechanism of pathogen transmission within an ecosystem, they may have the capability of increasing the coral population's chances of infection or transfer the pathogen directly through feeding.

Table 1 Observed and expected (in parentheses) frequencies of colonies that became diseased or remained healthy (not diseased) with respect to treatment types with the results of the tests of independence

	Diseased	Not diseased	Statistical results
Treatment			$\chi^2 = 15.283$, $df = 4$, $p = 0.0042$
Dictyota	0 (1.57)	9 (4.42)	(Significant)
Filtered water	2 (3.14)	16 (8.86)	
Fireworm	0 (1.57)	9 (4.42)	
Snail	1 (1.57)	8 (4.42)	
Unfiltered water	8 (3.14)	8 (8.86)	
Coral of origin			$\chi^2 = 3.613$, $df = 6$, $p = 0.7289$
2	1 (1.78)	5 (4.21)	(Not significant)
3	3 (2.37)	5 (5.62)	
20	3 (1.48)	2 (3.51)	
1	1 (1.48)	4 (3.51)	
27	1 (0.89)	2 (2.11)	
7	1 (1.46)	4 (3.51)	
12	1 (1.46)	4 (3.51)	

Although no *Dictyota* treatments were found to transmit disease, the presence of *Dictyota* caused mild paling to complete bleaching of several experimental corals, indicating a negative interaction between this macroalga and the corals in this study. Macroalgae can negatively affect coral health (Nugues et al. 2004), and *Dictyota* negatively affects coral by causing hypoxia (Barott et al. 2012) or emitting dissolved compounds (Smith et al. 2006). This macroalgal species group has also been associated with bacterial shifts in coral tissue (Morrow et al. 2012). This may provide insight into the correlation Brandt et al. (2012) reported between increased *Dictyota* cover and the expansion of WP disease when there were no other physical or biological factors influencing the outbreak of disease. The bleaching of coral tissue and shifts in the bacterial communities due to the interaction with the macroalgae may increase the risk of infection. Brandt and McManus (2009a) found that the incidence of WP increased in *Orbicella* after a bleaching event, and similar patterns have been identified in different coral disease systems (Harvell et al. 2002; Weil et al. 2006; Muller et al. 2008). Although most disease lesions in this study appeared within the first 4 d, in the case of *Dictyota*, more time may have been needed to see the full effects of this macroalga on disease incidence.

Enlargement rates

Interestingly, in this study the enlargement rates of disease on individual corals increased through time. Although these results derive from laboratory experiments, it is possible that this trend also exists in the field and that measuring the rate of WP lesion progression at only one or two points in time (compared to the entirety of the lesion's life) may cause incorrect classification of WP types.

The change in enlargement rates on individual corals through time could be explained by progressive changes in the microbial community, which is known to change within the coral holobiont in response to diseases and other stressors (Ritchie 2006). In some instances, bacteria within the holobiont coordinate their behavior by regulating gene expression and creating a community wide response. This phenomenon is known as quorum sensing (QS), which can take place when density dependent cell-to-cell communication occurs among bacteria within the coral (Fuqua et al. 1994). QS has also been noted to regulate bacteria's response to virulence (Bandara et al. 2012). For bacterial pathogens, QS molecules are used in the establishment of an infection and help regulate a switch from a non-pathogenic to a pathogenic state. These regulated systems have the potential to suppress the coral's immune response to pathogens until an adequate number of bacteria have accumulated to have a successful infection (Bassler 1999). Recent studies have found a QS signal in isolates from coral diseases such as BBD (Goldberg et al. 2011). In this study, QS could be causing the large range of progression rates by delaying responses or by increasing the virulence of a pathogen. However, if QS is disrupted, it can block production of antibiotics or even reduce the virulence of a pathogen, which may also explain the wide ranges of progression rates recorded in the field (Teplitski and Ritchie 2009).

In summary, WP is one of the most important sources of coral mortality on Caribbean reefs, yet ambiguity remains regarding its emergence and distribution in the natural environment. In this study, we have provided insight into the potential pathogen transmission mechanisms and dynamics of this disease, although our studies were limited to the laboratory environment. Future research should investigate transmission dynamics in wild populations,

especially in light of the severity and increasing number of reports of WP outbreaks. Overall, more research is needed into the epidemiological properties of this important disease in conjunction with studies of its etiological agents, which will enable the coral disease community to predict future disease outbreaks.

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