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White Band Disease transmission in the threatened coral, *Acropora cervicornis*

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The global rise in coral diseases has severely impacted coral reef ecosystems, yet often little is known about these diseases, including how they are transmitted. White Band Disease (WBD), for example, has caused unparalleled declines in live *Acropora* cover, spreading rapidly throughout the Caribbean by unknown means. Here we test four putative modes of WBD transmission to the staghorn coral *Acropora cervicornis*: two animal vectors (*Coralliophila abbreviata* and *C. caribaea)* and waterborne transmission to intact and injured coral tissues. Using aquarium-based infection experiments, we determine that *C. abbreviata,* but not *C. caribaea*, acts as both a vector and reservoir for transmission of the WBD pathogen. We also demonstrate waterborne transmission to injured, but not intact staghorn coral tissues. The combination of transmission by both animal vectors and through the water column helps explain how WBD is spread locally and across the Caribbean.

Coral reefs have experienced unprecedented declines due to a global rise in disease epizootics over the last 30 years[1–3]. There are now more than 20 described coral diseases[4], many of which are linked to increasing ocean temperatures and anthropogenic development[5]. Despite the negative impacts of these diseases, we often lack key information about their etiology and ecology[6]. Few coral pathogens have been identified and little is known about how most coral diseases are transmitted, making efforts to manage outbreaks difficult.

White Band Disease (WBD) is a prime example of a devastating coral disease that is poorly understood[7–9]. WBD is a host-specific disease that infects both Caribbean *Acropora* species[7] – the staghorn coral *Acropora cervicornis* and the elkhorn coral *A. palmata* – and is identified by a progressing band of dying white tissue[10]. Since it was first reported in 1979[10], WBD has led to the destruction of up to 95% of Caribbean *Acropora* cover[7,11] and resulted in the listing of both species as threatened under the US Endangered Species Act[12] and as critically endangered on the IUCN Red List[13]. Transmission experiments using filtered homogenates to isolate bacteria combined with antibiotic treatments demonstrate that WBD is infectious and caused by bacteria[14]. While a specific WBD pathogen has not been isolated, *Vibrio* and *Rickettsiales* bacteria have both been associated with the disease[8,9,15].

WBD is highly transmissible experimentally, either via direct contact between diseased and healthy corals[16] or through application of gauze soaked in diseased tissue homogenate to a healthy coral[14]. Natural modes of transmission, however, are poorly understood. Two putative methods of WBD transmission are animal vectors and waterborne transmission. The corallivorous snail *Coralliophila abbreviata* is a vector for "rapid tissue loss" in *A. cervicornis*[17]. Other coral diseases can be transmitted through the water column[18] and transmission can be enhanced by prior injury[19,20].

Here we investigate the potential for WBD transmission via two putative snail vectors and the water column. First, we tested if *C. abbreviata* and *C. caribaea* (two common corallivorous snails found on *A. cervicornis*[21]) function as a vector and/or reservoir for WBD by exposing healthy *A. cervicornis* fragments to snails fed on healthy, diseased, or no corals (i.e. starved). Second, we tested the potential for waterborne transmission of WBD by adding diseased coral homogenates to closed-circuit aquaria containing intact and injured (i.e. lesioned) *A. cervicornis* fragments.

Results

When transmission was compared between *C. abbreviata* and *C. caribaea,* predation by *C. abbreviata* caused significantly higher rates of disease transmission (9 out of 19 fragments) than predation by *C. caribaea*, which failed to transmit WBD (Fisher's exact test, $p=0.0046$; Table 1). In contrast, prior feeding treatments (healthy, diseased, and starved) had no significant effect on WBD transmission rates by *C. abbreviata* (Fisher's exact test, $p=1$; Table 1).

Table 1 | White Band Disease transmission in corals exposed to the snails *C. abbreviata* and *C. caribaea* that were previously fed healthy or diseased corals, or were starved

Laboratory Feeding Treatment	n	WBD transmission in unbitten corals	WBD transmission in bitten corals	Total % WBD Transmission
C. abbreviata				
Healthy	6	-	3 / 6	**50.0%**
WBD	6	-	2 / 6	**33.3%**
Starved	9	0 / 2	4 / 7	**44.4%**
Total	**21**	**0 / 2**	**9 / 19**	**42.9%**
C. caribaea				
Healthy	6	0 / 5	0 / 1	**0.0%**
WBD	6	0 / 2	0 / 4	**0.0%**
Starved	3	0 / 1	0 / 2	**0.0%**
Total	**15**	**0 / 8**	**0 / 7**	**0.0%**

When WBD transmission was compared between *C. abbreviata* predation and lesion controls (mimicking predation), transmission was significantly higher in fragments fed on by *C. abbreviata* (21 out of 23) than lesion control fragments (1 out of 9) (Fisher's exact test, $p < 0.001$; Table 2). When fragments with WBD transmitted by *C. abbreviata* were grafted to healthy corals with lesions, 43 percent (4 out of 9) of the grafted fragments contracted WBD, demonstrating that WBD resulting from snail predation is transmissible by multiple means.

When waterborne transmission was tested, 6 out of 15 lesioned fragments inoculated with disease homogenate contracted WBD (Fisher's Exact test, $p=0.017$; Table 3), while none of the intact (not lesioned) fragments inoculated with disease homogenate showed signs of WBD. No WBD was transmitted to fragments inoculated with a healthy homogenate.

Discussion

We tested four possible modes of White Band Disease transmission in *A. cervicornis*: predation by *C. abbreviata*, predation by *C. caribaea*, waterborne transmission to intact coral fragments, and waterborne transmission to lesioned coral fragments. Transmission was highest in corals bitten by *C. abbreviata,* verifying that *C. abbreviata* acts as a vector for WBD. WBD was transmitted by *C. abbreviata* regardless of whether snails fed upon healthy corals, diseased corals, or were starved, indicating that *C. abbreviata* is able to retain the disease pathogen for at least two weeks and act as a reservoir for WBD. In contrast, *C. caribaea* did not transmit WBD to *A. cervicornis,* suggesting that not all corallivorous snails are capable of transmitting WBD. Waterborne WBD transmission only occurred when corals were injured (i.e. lesioned), indicating that tissue injury facilitates WBD transmission in corals.

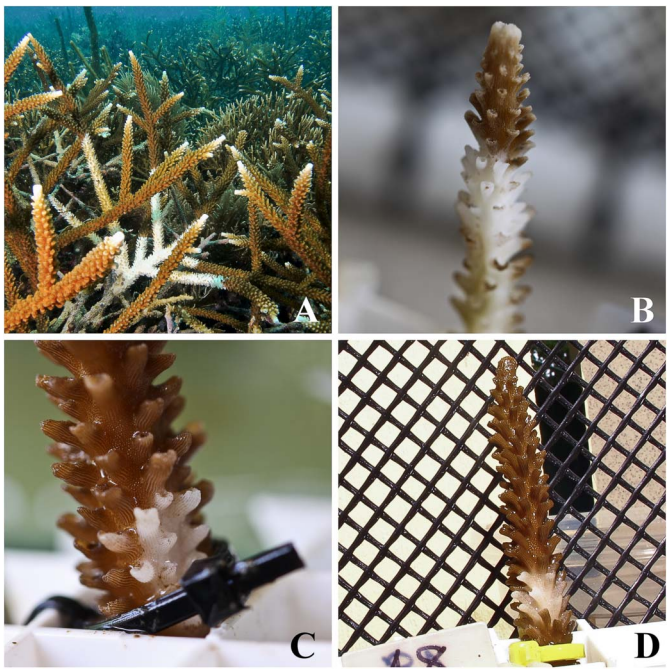

Figure 1 | White Band Disease and predation in *Acropora cervicornis*. WBD on *A. cervicornis* in the field (A) and experimentally transmitted in the laboratory (B), snail predation by *Coralliophila abbreviata* (C), and a lesion mimicking snail predation (D).

Previous experiments by Williams and Miller[17] show that *C. abbreviata* acts as a vector, but not a reservoir for "rapid tissue loss" in *A. cervicornis.* Their experiments differ from our results in that only snails that had fed on disease caused tissue loss. They did not test the possibility of the snails being a reservoir by starving their snails. Our results indicating that *C. abbreviata* acts as a reservoir for WBD is similar to the well-characterized *Vibrio-Oculina* disease system where the fireworm, *Hermodice carunculata,* acts as a vector and winter reservoir for the *Vibrio shiloi* bleaching pathogen on the Mediterranean coral *Oculina patagonica*[22]. WBD prevalence also varies seasonally, and is highest in summer months (Libro and Vollmer, unpublished data). Thus, *C. abbreviata* may act like *H. carunculata,* and house the WBD pathogen during the winter and other periods of low WBD prevalence. Another corallivorous snail, *Drupella cornus*, has been associated with multiple coral diseases in the Indo-Pacific and Red Sea[23,24], but no explicit tests of its potential as a vector or reservoir have been performed to our knowledge.

It is unclear why *C. abbreviata,* but not *C. caribaea*, can transmit WBD given that both are corallivores of *A. cervicornis*. Failure of *C. caribaea* to transmit WBD could be due to a combination of factors. *Coralliophila caribaea* consumed less coral tissue than *C. abbreviata* (unpublished data) and thus, may not have acquired enough pathogen for transmission or produced a large enough lesion on healthy corals to allow the pathogen to infect the coral. An alternate and potentially more interesting possibility is that *C. caribaea* is inhospitable to the WBD pathogen.

In terms of waterborne transmission, our data indicate that WBD is transmissible through the water column only if the coral has been injured (i.e. lesioned). The potential for injury and thus infection seems high given the many ways that tissue injury can occur in nature, including competition, mechanical damage, or corallivory by snails, damselfish, butterflyfish, fireworms and other taxa.

Table 2 | White Band Disease transmission in corals fed on by *C. abbreviata* versus experimental lesion controls

Treatment	n	# WBD	% Transmission
C. abbreviata	23	21	91.3%
Lesion	9	1	11.1%

Table 3 | White Band Disease transmission in corals (with and without lesions) inoculated with healthy or diseased coral homogenates

Treatment	n	# WBD	% Transmission
Healthy Homogenate			
Lesion	15	0	0.0%
No Lesion	15	0	0.0%
Diseased Homogenate			
Lesion	15	6	40.0%
No Lesion	15	0	0.0%

Evidence for waterborne WBD transmission helps explain how WBD spread across the Caribbean since direct contact between healthy and diseased corals occurs between neighbors and *C. abbreviata* generally do not travel long-distances[17].

Understanding the nature of WBD transmission provides insights into how the disease might be managed and controlled. While controlling waterborne WBD transmission will prove difficult, management of *C. abbreviata* populations may be a practical method of reducing WBD incidence, especially in heavily impacted *Acropora* populations. More research is needed to assess the viability of *C. abbreviata* population control as a management strategy, to identify the WBD pathogen, and to understand pathogen dynamics within *C. abbreviata*.

Methods

Healthy and diseased (active WBD) *A. cervicornis* fragments (6 cm in length), and the snails *C. abbreviata* and *C. caribaea* were collected from Cayo Corales (9° 15′ 16″ N, 82° 7′ 40″ W) in Bocas del Toro, Panama in February 2012. Organisms were transported to the Smithsonian Tropical Research Institute in separate buckets. Coral fragments were cable-tied to plastic louver and acclimated in flow-through aquaria for three days prior to each experiment.

In order to test whether *C. abbreviata* and *C. caribaea* are capable of acting as a vector and reservoir for WBD, both species of snails were starved for three days and then randomly fed healthy *A. cervicornis* tissue (Healthy), diseased *A. cervicornis* tissue (WBD), or nothing (Starved) for three days. Snails were then placed in individual cages each containing one healthy coral fragment. Four cages were randomly placed into nine 500 L flow-through aquaria. Predation scars and WBD progression were measured (length x width) twice daily for five days. Once fragments exhibited signs of predation, the snails were removed from the cages. This allowed us to isolate predation (i.e. feeding) from disease progression.

A subsequent experiment was conducted to confirm that the WBD transmitted by *C. abbreviata* was not a response of the coral to mechanical damage. To do this, the first experiment was repeated with three cages containing one coral fragment and one *C. abbreviata*, and one cage containing a coral fragment that had been experimentally lesioned by removing ~7.5 mm^2 of coral tissue with an airbrush and 0.2 μm filtered seawater (Figure 1d). WBD resulting from either snail predation or lesion controls was monitored as above. To confirm that WBD caused by *C. abbreviata* was due to a transmissible pathogen, fragments that had been fed on by *C. abbreviata* and contracted WBD were grafted to experimentally lesioned healthy corals and monitored.

To test waterborne WBD transmission, twelve replicate fragments of five genotypes were collected and placed in six closed-circuit 500 L aquaria containing a water circulation pump. Each aquarium contained two fragments of each of the five genotypes; one fragment per genotype was left intact and the other fragment was lesioned. Six fragments of healthy and six fragments of diseased *A. cervicornis* were individually vortexed in 50 mL plastic conical tubes filled with 15 mL 0.2 μm filtered seawater and 3 mm glass beads, and combined to make our separate diseased and healthy inoculates. Aquaria were then randomly inoculated with either a diseased or healthy homogenate.

Transmission data comparing presence and absence of WBD in each experiment were analyzed using Fisher's exact tests[25].

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Author contributions

SAGW, CJM and SVV designed the experiments. SAGW and CJM conducted the fieldwork, experiments, and analyzed the data. SAGW, CJM, and SVV co-wrote, reviewed, and edited the manuscript.

Additional information

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