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Ocean warming and acidification have complex interactive effects on the dynamics of a marine fungal disease

Running title: CCA fungal disease dynamics

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Abstract: Diseases threaten the structure and function of marine ecosystems and are contributing to the global decline of coral reefs. We currently lack an understanding of how climate change stressors, such as ocean acidification and warming, may simultaneously affect coral reef disease dynamics, particularly diseases threatening key reef-building organisms such as crustose coralline algae (CCA). Here we use coralline fungal disease (CFD), a previously described CCA disease from the Pacific, to examine these simultaneous effects using both field observations and experimental manipulations. We identify the associated fungus as belonging to the subphylum *Ustilaginomycetes* and show linear lesion expansion rates on individual hosts can reach 6.5mm per day. Further, we demonstrate for the first time that ocean warming events could increase the frequency of CFD outbreaks on coral reefs, but that ocean acidification-induced lowering of pH may ameliorate outbreaks by slowing lesion expansion rates on individual hosts. Lowered pH may still reduce overall host survivorship, however, by reducing calcification and facilitating fungal bioerosion. Such complex, interactive effects between simultaneous extrinsic environmental stressors on disease dynamics are important to consider if we are to accurately predict the response of coral reef communities to future climate change.

Keywords: coral reef; coralline fungal disease; ocean acidification; temperature; bioerosion; climate change

1. INTRODUCTION

Diseases alter ecosystems [1] and threaten marine community function and resilience [2]. On coral reefs, disease outbreaks are considered a key contributor to the recent global decline of reef health and resilience[3]. Both global impacts, such as sea-surface temperature anomalies, and local human impacts, such as pollution, drive disease dynamics and outbreaks in scleractinian corals on reefs [4, 5]. These stressors likely increase pathogen virulence and reduce host resistance, enhancing disease establishment and progression [2, 6]. Our understanding of diseases that threaten other key calcifying (reef-building) organisms, however, is rudimentary. Crustose coralline algae (CCA) serve essential functional roles in coral reef ecosystems, including facilitating reef accretion and consolidation [7], providing a settlement substrate for coral larvae [8], and forming a key successional state promoting reef recovery following acute disturbance [9]. While CCA can occupy up to 50 % of the living reef benthos [7, 10], relatively little is known about their biology and ecology [11], particularly their susceptibility to and subsequent impacts from disease [12].

Diseases can cause drastic reductions in CCA populations on coral reefs, with knock-on effects that promote regime shifts to fleshy macroalgal dominance and loss of functional resilience [13-15]. Several CCA diseases have been documented, although almost nothing is known about their etiology, spatiotemporal dynamics, and relationships with extrinsic environmental drivers [12]. This information is essential if we are to actively manage CCA disease occurrence and mitigate outbreaks at a local scale on coral reefs. While CCA are able to photoacclimate [16], they are still vulnerable to bleaching as a result of increased temperature, and their calcification, photophysiology, and survival are threatened by a lowering of pH and carbonate saturation state (Ω) as a result of ocean acidification (OA) [17-19]. However, the influence of these global-scale

stressors on local-scale CCA disease dynamics and occurrence on coral reefs is virtually unknown. Here, using a CCA disease previously described from the South Pacific, we shed light on this urgent research priority for the first time.

Coralline fungal disease (CFD) was first observed in 1997 on shallow (< 20 m) reef habitats in American Samoa. Based on gross morphology, the etiology of the disease was identified as an undescribed fungal pathogen [20]. Since 1997, CFD has been documented throughout other parts of the Pacific, with high prevalence at remote islands in the Central Pacific, in particular at Kingman Reef and Palmyra Atoll in the northern Line Islands [12]. While the distribution of CFD throughout the Pacific appears to be highly variable, evidence suggests that variations in sea-surface temperature (SST) may, in part, be driving spatial variation in disease occurrence, with higher mean SST at islands correlating with higher CFD occurrence [12]. However, the principal environmental drivers of temporal variation in CFD occurrence remain unknown.

In late 2009, a well-developed El Niño resulted in anomalous ocean warming across the equatorial Pacific Ocean. The sustained increase in temperature resulted in coral bleaching events and disease outbreaks at some of the Pacific's most remote coral reef systems, including Palmyra Atoll, where CFD is prevalent [21-23]. Using time-series field observations at Palmyra before, during, and after the 2009-2010 El Niño ocean warming event, followed by experimental manipulations, we show that CFD temporal dynamics are driven by variations in seawater temperature and that spatial variation in disease occurrence appears independent of host abundance. We show that CFD can exhibit very rapid progression across hosts, rivalling known rates for coral diseases, and that increased temperature accelerates CFD lesion progression. We show that these effects are counteracted by a reduction in pH; however, under simulated ocean acidification conditions, diseased hosts still experience greater rates of net dissolution than

healthy individuals. These complex interactions highlight the challenges associated with predicting disease outbreaks and their dynamics in a changing climate.

2. MATERIALS AND METHODS

(a) *Histopathology*

Fragments (n = 7, ~3 cm^2) of crustose coralline algae (CCA) displaying gross signs of coralline fungal disease (CFD) were collected at Palmyra Atoll (05º 52' N, 162º 06' W), at 10 m depth on the forereef (Fig. 1). Each sample was fixed in zinc-formaldehyde solution (Z-fix, Anatech) diluted 1:5 with ambient non-filtered seawater, decalcified using a formic acid/formaldehyde solution (Cal-Ex II, Fisher Scientific), embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin. Grocott's methenamine silver was used to confirm presence of fungal hyphae.

(b) *Culture conditions and phylogenetic determination of CFD fungus*

Culture conditions: To culture the fungus associated with CFD, Medium B, Wickerham's, Czapek-Dox agar, and cellulose agar [24] prepared with 25, 50, 75 or 100 % FSW as a base was used. Media were prepared with and without inclusion of the antibiotics ampicillin and spectinomycin at a concentration of 100 µg/ml. Media with antibiotics was used to suppress bacterial growth, and the same media were used without antibiotics to preclude any potential negative effects on fungal growth. Solid media included 0.7, 1.0, 1.2, or 1.5 % agar (Fisher Scientific). Cultures were incubated at 25, 27, or 30°C with aeration (solid cultures) or without aeration (liquid cultures) for 21 days.

DNA extraction and gene amplification: Frozen, ground CFD lesions were thawed on ice, 5 µl were spread on PALM PEN membrane slides (Carl Zeiss), and individual fungal filaments were isolated with a Zeiss PALM laser microdissection system. DNA was extracted from filaments as

in [25] with the following modifications: Two samples of 8-10 fungal filaments in 50 µl of sterile water were lysed with a mini-beadbeater (Biospec) and extracted with phenol-chloroform. DNA was precipitated, dried, and re-suspended in 20 µl of sterile 10 mM Tris buffer, pH 8.5. A 719 bp fragment of the 18S rRNA gene was PCR amplified with fungus-specific primers designed from mixed environmental samples: nu-SSU-0817-5' and nu-SSU-1536-3' [26] and Phusion High-fidelity Polymerase (New England Biolabs). The same primers were used for sequencing.

Phylogenetic analysis: The fungal 18S rRNA gene sequence was initially assessed using NCBI and the BLAST algorithm. To further assess its relatedness to other phylum *Basidiomycota* members, especially the subphylum *Ustilaginomycetes*, 653 bp 18S rRNA gene sequences were aligned in MEGA5 [27]. The fungal isolate's sequence was aligned with 22 other NCBI sequences, chosen based on a previously published analysis of the phylum *Basidiomycota* [28], using ClustalW. A phylogenetic tree was constructed using the neighbour-joining method with 1000 bootstrap replicates [29]. All positions containing gaps and missing data were eliminated, leaving a total of 647 positions. Evolutionary distances (number of base substitutions per site) were computed using the Maximum Composite Likelihood method. Rate variation among sites was modelled with a gamma distribution (shape parameter = 8). Evolutionary analyses were conducted in MEGA5.

(c) *CFD occurrence, host cover, and associated changes in seawater temperature*

To quantify CFD occurrence, 59 × 200 m^2 transects were surveyed in Jul-Aug and Oct-Nov 2008 (total of 11,800 m^2 of reef). Backreef (n = 24 transects, 1-5 m depth), reef terrace (n = 5, 4-5 m depth), and forereef (n = 30, 10 m depth) habitats were surveyed within 12 permanent sites (Fig.

1). Along each transect, percent CCA cover was estimated using the photoquadrat method. Each photoquadrat (n = 20 per 50 m) was 0.63 m^2. Percent cover was calculated *post-hoc* by identifying 100 points in a stratified random design for each photograph and averaging within each transect. Difficulties with delineating individual CCA crusts *in situ* meant a true CFD prevalence (proportion of individuals displaying signs of the disease) could not be calculated. Instead, the numbers of CFD cases were normalized to host cover (per m^2 of CCA) along each transect (see Supplemental Table 1 for mean raw number of CFD cases and percent cover of CCA within each transect over time). Forty of the 59 transects surveyed in 2008 became permanent as part of the Palmyra disease monitoring program and were re-surveyed for CFD occurrence and CCA cover in Oct-Nov 2009 and Mar 2010.

Throughout the study, *in situ* forereef temperature data were sampled at 30 min intervals at 10 m depth using Sea-Bird Electronics (SBE) 39 temperature sensors with a resolution and accuracy of 0.0001°C and ± 0.002°C, respectively. Satellite-derived weekly sea surface temperature (SST) and long-term monthly climatological SST for Palmyra were calculated following [30].

(d) *CFD vital rates during the El Niño*

On the central south forereef, 13 CFD cases were photographed weekly for four weeks in Oct-Nov 2009. Individual CFD cases were initially marked with a stainless steel pin as a reference point. Photographs were taken perpendicular to the substrate to minimize angle variations among images. CFD vital rates (lesion surface area and linear progression rate) were calculated *post-hoc* using ImageJ (http://rsbweb.nih.gov/ij). Active lesion surface area was the area displaying fungal cover (blue-black discolouration), not including dead CCA left in the lesion's path. Calculations

were averaged across three images of each lesion to account for slight variations in angle among photos.

(e) *Temperature and acidification experiments*

We used a factorial CO_2 bubbling and heating experiment (June 3rd to13th 2012) to examine independent and interactive effects of ocean acidification (OA) and warming on CFD disease dynamics (lesion surface area and linear progression rate) and CCA growth (net calcification). Samples were collected, as for histopathology, from independent diseased and healthy CCA crusts. Epiphytes were removed, and each CCA genus was confirmed using a dissecting microscope. Paired CCA fragments (~2 cm^2 *Neogoniolithon* sp.; diseased and healthy) were placed in 1L glass aquaria holding fresh seawater equilibrated to treatment conditions (~24 – 48 h before) [sensu 31]. Experimental OA conditions were created by bubbling pre-mixed air enriched with excess pCO_2 (AirGas Pro) to 1124 ± 88 µatm (mean ± SE hereafter) to reflect atmospheric CO_2 concentrations projected in 2100 [scenario IV; 32]. In control aquaria, present-day CO_2 conditions were created using a Pacbrake 12V HP625 Air Compressor delivering ambient air. Aquaria were immersed in flow-through water baths at 28.06 ± 0.01°C (seasonal average for Palmyra; ambient conditions) or 29.49 ± 0.02°C (mean SST during the El Niño warming event at Palmyra; warming conditions). Four independent water baths (two ambient and two warmed) held experimental aquaria that were randomly assigned to elevated or present-day CO_2 conditions to create every combination of warming and OA treatment level (n = 12 per level divided evenly among the two water baths).

Aquaria were covered to prevent evaporation and rainwater from affecting salinity and placed under a shade cloth to mimic the natural light environment at 10 m on the forereef. To control for

algal metabolism, two empty aquaria per water bath were subjected to the same four treatments described above. In each aquarium, temperature and light conditions were recorded every 15 min using Onset HOBO® Pendant UA-002-64 light and temperature loggers (Suppl. Fig. 1). Light intensities (lux) were converted to availability of photosynthetically active radiation (PAR) using the equation: 1 µmol quanta (400 – 700 nm) m/s = 51.2 lux [sensu 33]. These conversions were validated by midday PAR measurements with a Li-Cor LI 192 4π quantum sensor (492 ± 25 µmol photons m/s). Once daily, $pH_{seawater}$ (resolution ± 0.01), temperature (± 0.1 °C), and dissolved oxygen (± 0.2 mg/L, Suppl. Table 2) were monitored using a HACH HQ 40d handheld meter. These measurements were also taken at 0600h, 1200h, 1800h, and 2400h to quantify diurnal fluctuations in each aquarium (Suppl. Table 2). Twelve water samples for total alkalinity (A_T), total dissolved inorganic carbon (C_T), and salinity were collected at days 2, 6, and 10 of the experiment in 500 mL Corning brand Pyrex bottles and fixed with 200 µL saturated $HgCl_2$ solution (1 % headspace). Samples were collected (in duplicate) from experimental aquaria and control (empty) aquaria at each of the four treatment levels.

(f) ***CCA calcification and disease progression***

Net calcification rates of all fragments of *Neogoniolithon* sp. were quantified with the buoyant weight method [34] (to the nearest mg) using a weigh-below basket on a balance (Denver SI-403). Changes in buoyant weight over the 10-day experiment, normalized to initial fragment mass, approximated net calcification rate. For each diseased fragment, CFD disease vital rates (lesion surface area and linear progression rate) were calculated using ImageJ as described above.

(g) ***Experimental water chemistry analysis***

Carbonate chemistry and salinity were analysed in the Dickson Lab at Scripps Institution of Oceanography. C_T was determined using a Single Operator Multi-parameter Metabolic Analyzer (SOMMA) and a UIC Model 5011 CO_2 coulometer. A_T was determined by open-cell acid titration using a Metrohm Dosimat Model 665 and Metrohm potentiometric pH probe and meter. Salinity was determined using a Mettler Toledo Model DE45 density meter. Seawater dissolved inorganic carbon parameters (HCO_3^-, CO_3^{2-}, CO_2, pCO_2) and saturation state of carbonate minerals (Ω-calcite and Ω-Mg calcite) were calculated based on measured C_T and A_T using the computer program Seacarb [35] and stoichiometric dissociation constants [36] (Suppl. Table 3).

(h) ***Data analyses***

To test for differences in CCA cover and CFD occurrence across forereef sites in 2008, we ran a permutation-based analysis of variance and subsequent pairwise comparisons using PERMANOVA+ [37]. To test for any relation between CCA abundance and CFD occurrence in 2008, we used a permutational linear model using Distlm_*forward* [38]. Two-way nested analyses of variance (ANOVAs) tested whether OA (fixed) and warming (fixed) treatments independently or jointly affected net calcification; each fixed factor had two levels. Replicates from a water bath were nested within temperature treatments to test for location bias. Analyses were run independently for diseased and healthy samples. Normality and homoscedasticity were verified using the Shapiro-Wilk test. Growth responses were compared between diseased and healthy CCA within each treatment using t-tests. Proportional changes in lesion area were analysed using the Dunn's method for joint ranking, a non-parametric approach that compares means of treatments against a control (ambient SST and air); we confirmed that variances across treatments were equal with a Brown-Forsythe test. Unless otherwise stated, all analyses were completed using R 2.15.2 (R Development Core Team, http://www.r-project.org).

3. RESULTS

(a) *CFD gross morphology and histopathology; phylogeny of associated fungus*

CFD lesions were characterized by a diffuse area of mottled white discolouration separated from a pink CCA thallus by a blue-black band (approximately 1-3 cm wide) with irregular distinct undulating borders (Fig. 2a). Septated hyphae with branches originating near septal junctions were commonly observed (Fig. 2b). Many of the fungal filaments had highly branched structures resembling conidiophores, which in some cases appeared to have attached spherical conidia, the asexual spores of fungi. These were the dominant fungal structures in diseased CCA but were absent in healthy CCA. The CCA cuticle was overlaid and disrupted by mats of brown to colourless filamentous branching septate structures (Fig. 2c). These irregularly walled structures infiltrated into the CCA thallus at right angles to the cuticle to a depth of ~100 µm. These were morphologically compatible with fungi, and Grocott's methenamine silver confirmed a fungal infection (Fig. 2c).

Isolation by microdissection followed by genetic sequencing of the fungal hyphae revealed that 653 bp of the 18S rRNA gene sequence (Accession #KF255580) shared 97-98 % sequence identity with members of the phylum *Basidiomycota* and uncultured marine isolates. The sequence was most similar (98 % sequence identity) to members of the subphylum *Ustilaginomycetes*, in particular *Malassezia restricta* strain CBS-7877 and the marine isolates KM10-BASS and CK2-BASS (Suppl. Fig. 2). Repeated attempts to culture the fungus were unsuccessful.

(b) *Spatiotemporal patterns of CFD and associated changes in seawater temperature*

Atoll-wide mean (± 1SE) CFD occurrence on Palmyra's forereef habitat equalled 0.1 ± 0.06 cases/m^2 of CCA in 2008 (total reef area surveyed in this habitat equalled 6,000 m^2). Although cases of CFD were seen outside of our surveyed transects on the deeper (~10-15 m) reef terrace habitat, cases were rare in comparison; no CFD cases were documented within our surveyed transects on the shallow (<5 m) terrace or backreef habitats (total reef area surveyed in these two habitats equalled 5,800 m^2). Within the forereef habitat, CFD occurrence displayed spatial heterogeneity (*Pseudo-*$F_{5,29}$ = 13.456, $p < 0.0001$), with the central south forereef having the highest mean number of cases/m^2 of CCA (0.39 ± 0.10) in 2008 (Suppl. Table 1). Percent cover of CCA did not differ across forereef sites in 2008 (*Pseudo-*$F_{5,29}$ = 2.67, $p = 0.06$, mean cover 23.5 %), and there was no relationship between CFD occurrence and CCA cover (*Pseudo-*$F_{1,29}$ = 0.311, $p = 0.751$) (Suppl. Table 1).

Within the permanent backreef and reef terrace transects, mean CFD occurrence remained at 0 cases/m^2 of CCA in 2009 and 2010. However, within the permanent forereef transects, CFD mean occurrence increased approximately 14-fold from 0.10 cases/m^2 of CCA in 2008 to 1.37 cases/m^2 of CCA in late 2009 in association with the El Niño ocean warming event (Fig. 3, Suppl. Table 1). Seawater temperature had steadily increased over the latter part of 2009 and eventually peaked in November 2009, reaching 1.25°C and 1.51°C (satellite and forereef temperature observations, respectively) above the maximum long-term monthly climatological SST for Palmyra (Fig. 3). CFD occurrence increased in all permanent forereef transects during the El Niño event, with the central south forereef maintaining the highest levels (3.74 cases/m^2 of CCA, Suppl. Table 1). By March 2010, with a decrease in seawater temperatures, mean forereef CFD occurrence had returned to pre-El Niño levels (Fig. 3).

(c) *CFD vital rates during the El Niño event*

CFD lesions typically progressed in a radial manner across the surface of the CCA thallus, often crossing between individual CCA thalli but never spreading onto hard coral tissue (Suppl. Fig. 3). At time point zero, mean lesion surface area of the 13 CFD cases *in situ* was 108 mm^2 (± 25) (Suppl. Table 4). After one week, mean lesion surface area was 136 mm^2 (± 23), with a mean surface area progression rate of 3.5 mm^2/day (± 2.3) and a mean linear progression rate of 2.4 mm/day (± 0.5). Across the entire four-week time period, the maximum surface area progression rate and linear progression rate of any single CFD lesion was 12.9 mm^2/day and 6.5 mm/day, respectively (Suppl. Table 4).

(d) ***Experimental effects of warming and acidification on CCA calcification and disease progression rates***

Exposure to elevated temperature and atmospheric pCO_2, designed to simulate ocean acidification (OA), reduced CCA net calcification rates, but this effect was dependent on fungal infection (Table 1). All CCA samples gained $CaCO_3$ mass in the ambient air treatments, while all samples lost mass in the elevated pCO_2 (lower pH) treatments (Fig. 4A). However, when exposed to both elevated pCO_2 and temperature, diseased CCA lost nearly twice as much mass as when exposed to simulated OA alone (significant interaction term, Table 1). Mass loss was not intensified for healthy CCA (Fig. 4A); net calcification rates in healthy CCA were significantly depressed only by elevated pCO_2 and not by elevated temperature (Table 1). Accordingly, calcification rates for diseased and healthy samples were statistically similar in all treatments, except for the simultaneous acidified and warmed conditions, in which diseased CCA lost 40% more mass than healthy CCA (t-test, df = 15, p = 0.0343). Visible lateral progression of the CFD lesion occurred only in the elevated temperature treatment in ambient CO_2 conditions where lesion size and lethality increased by 60% over one week (Fig. 4B).

4. DISCUSSION

Using a previously described crustose coralline algae fungal disease (CFD) [20], we demonstrate that ocean warming and acidification can have complex interactive effects on marine disease dynamics. These relationships are to be expected, as they reflect intricate relationships among the putative pathogen, host, and environment [39].

(a) *Identification of CFD-associated fungus*

Fungal pathogens are prevalent throughout the marine environment [40, 41], are commonly associated with the coral holobiont [42], and are known to infect tropical sea-fans [43, 44] and marine algae [45]. An inability to culture fungal isolates and a reliance on morphology for identification, however, have caused fungal isolates to be misclassified and their distribution underestimated [46]. Using histopathology of CFD samples from Palmyra Atoll and genetic sequencing of the associated fungus, we confirm a fungal infection of the CCA. While species-level identification was not possible, our phylogenetic analysis strongly suggests that the CFD fungus belongs to the subphylum *Ustilaginomycetes*, which consists of a large number of plant parasites, including strains of smut fungi [47]. Our methods, which allowed isolation of the fungus without an axenic culture, could be used to compare the fungus present in the Palmyra CFD lesions with fungi associated with suspected CFD lesions found on other reefs. These genetic approaches allow us to better interpret spatiotemporal dynamics of this disease on coral reefs and postulate their underlying mechanisms.

(b) *Disease dynamics and sea surface temperature*

Many fungal pathogens in animals and plants respond positively to elevated temperatures [48-51], and ocean warming is predicted to favour pathogens for many marine diseases [2]. At our

study location, CFD displayed a dramatic (14-fold) increase in occurrence on the forereef during an El Niño in association with sustained seawater temperatures well above the long-term climatological mean. Furthermore, we found experimentally that elevated temperature increases lateral expansion rates of CFD lesions. The positive relationship between temperature and CFD occurrence was likely the result of elevated temperatures increasing the virulence of the pathogen, and hence speeding disease progression, and causing physiological stress to the CCA host that ultimately reduced resistance to infection [52]. The prevalence of many coral diseases, such as white syndromes [5], atramentous necrosis [53], and black band disease [54] are also positively related to temperature. Interestingly, Vargas-Ángel (2010) [55] documented higher overall CCA disease occurrence at islands experiencing higher mean annual sea-surface temperature in a Pacific-wide survey of U.S.-affiliated coral reefs, further highlighting the importance of temperature in governing CCA disease dynamics.

While temperature variation provides a strong explanation for temporal variation in overall CFD occurrence at our study location, it does not adequately explain the spatial variation we documented at any one point in time. If higher temperatures cause both CFD occurrence and rates of lesion progression to increase, why was the disease almost exclusively limited to the forereef, where temperatures are lower than the shallow reef terrace [21]? There are several possible explanations. Host density is often a crucial factor determining the spatiotemporal distribution patterns of disease [39], with the prevalence of many coral and CCA diseases positively related to host abundance [5, 56, 57]. For example, Vargas-Ángel (2010) [55] found a positive relationship between island mean CCA cover and overall CCA disease occurrence at an archipelago scale across coral reefs of the U.S. Pacific, including the Pacific Remote Island Areas, the geopolitical region within which Palmyra resides. However, within Palmyra's forereef

habitat, CFD occurrence appeared to be independent of host abundance. Moreover, CCA cover peaks on Palmyra's shallow (<5 m) western reef terrace [10], where CFD was virtually absent; previous surveys at Palmyra have documented CFD on the terrace habitat, but again at deeper (~15 m) depths (Vargas-Ángel, NOAA, *pers. comm.*) where host cover is lower [10].These findings suggest that host abundance alone does not explain the observed spatial variation in CFD occurrence at Palmyra. However, CCA species assemblages are known to vary spatially on reefs [58], and the peak of CFD occurrence on the forereef may simply reflect an increase in abundance of a preferred host species. While *in situ* species-specific assessments of CCA abundance would potentially resolve these issues, CCA taxonomy is difficult and requires microscopic examination, making it impossible in the field.

While potentially explaining between-habitat differences in disease occurrence, variation in host species abundance alone does not adequately explain the dramatic peak in CFD abundance on Palmyra's central south forereef. This CFD hotspot at Palmyra appears to be temporally stable, corroborating previous surveys conducted in 2006 [55]. The existence of this CFD hotspot at one site suggests that disease occurrence may be governed by external inputs of the pathogen, rather than by within-population transmission [44], or by an unmeasured extrinsic forcing. Palmyra's central south forereef is exposed to a particularly high level of lagoonal outflow during the change in tidal state (GJW *pers. obs.*), perhaps acting as a pathogen source and/or supplying more nutrient-rich waters that may enhance CFD establishment and progression, as has been shown for other fungal diseases on coral reefs [4]. Additionally, the south forereef of Palmyra has, on average, measurably higher seawater pH with less frequent or severe excursions than the north forereef or reef terrace [59]; our results indicate that the less acidic but warmer conditions characteristic of the southern forereef are most favourable for CFD occurrence. Regardless of the

mechanisms behind the fine-scale variations in CFD occurrence, it is clear that the disease is more abundant and virulent under elevated temperatures, suggesting that predicted increases in the frequency of temperature anomalies on coral reefs may result in more frequent CFD outbreaks.

(c) *Eco-physiological response of host to disease and climate change*

What will be the ecological consequences of increased CFD outbreaks on coral reefs? While elevated temperature increased overall CFD occurrence *in situ* and lateral rates of lesion expansion under experimental conditions, under the same experimental conditions elevated pCO_2 mediated these effects of temperature and slowed lesion expansion rates. While this suggests that future increases in the frequency of temperature anomalies will result in more frequent CFD outbreaks on coral reefs, the lowering of pH as a result of OA may actually slow overall spread of the disease across the reef landscape during such outbreaks. Importantly, however, while the lateral spread of CFD was not affected by reduced seawater pH and carbonate saturation state, all CCA thalli lost mass under OA conditions, suggesting net dissolution was occurring. For diseased thalli, these effects were exacerbated by warming.

Synergistic effects of ocean warming and acidification that together cause greater reduction in calcification of CCA than either stressor alone have been reported elsewhere [17-19, 60], but synergistic global climate change effects were only observed in this study when the CCA were also infected with the CFD fungus. Microboring organisms, or euendoliths, like fungi or cyanobacteria, burrow and erode carbonate at rates that can exceed biogenic $CaCO_3$ precipitation, leading to net dissolution of reef-building organisms [61, 62]. OA is expected to reduce resistance to eudondolith penetration in both hermatypic corals and CCA by weakening

structural integrity of the $CaCO_3$ crystals [63], reducing skeletal density [64], and facilitating chemical dissolution [65, 66]. Further, colony formation is stimulated by natural reductions in pH, so OA has the potential to radically elevate abundance of marine fungi [67]. Not only can acidification weaken host resistance to bioerosion, but reduced saturation states and enhanced disease infestation of the CCA thallus could further accelerate corrosion. Thus synergistic interaction of pathogen infection, warming, and OA may exacerbate reef degradation under projected global climate change scenarios.

(d) *Conclusions*

Our study represents the first attempt to understand the interactive effects of two major global stressors, ocean warming and acidification, on disease dynamics on coral reefs. Using a fungal disease affecting crustose coralline algae (CFD), we show that while outbreaks of CFD should become more common on coral reefs as temperature anomalies become more frequent, ocean acidification may ameliorate lesion progression rates but still decrease overall survivorship of diseased hosts. The ecological consequences of such interactions are difficult to predict; however, it is clear that CFD possesses a tremendous capacity for lateral spread across the reef landscape during ocean warming events. Our results highlight the intricate nature of disease-host-environment interactions and the importance of adopting a multi-factor approach to modelling disease dynamics on coral reefs in order to accurately predict dynamics in a changing climate.

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Table 1. Two-way ANOVA results for calcification rates of diseased and healthy CCA crusts (n = 8 per treatment) immersed in the ocean acidification ('CO_2 enrichment') and ambient conditions across duplicate flow-through seawater tables ('Table') nested within a warming El Niño ('Temperature') or a seasonal average scenario.

CCA state	**Source**	**DF**	***F***	**p**
Healthy, no lesions	CO_2 enrichment	1	64.327	< 0.0001
	Temperature	1	0.018	0.896
	CO_2 x Temp.	1	0.050	0.824
	Table [Temp.]	2	1.667	0.208
Diseased, lesions present	CO_2 enrichment	1	163.153	< 0.0001
	Temperature	1	3.008	0.095
	CO_2 x Temp.	1	4.593	0.042
	Table [Temp.]	2	0.396	0.677

Figure legends

Figure 1. Location of Palmyra Atoll and the permanent monitoring sites established in 2008 (1-6 = shallow terrace; 7-12 = forereef).

Figure 2. Field signs of coralline fungal disease (CFD) (A). Fungal infection appears as a blue-black lesion (arrows). Days-old exposed substrate becomes colonised by microalgae and turf algae (1) and appears bleached white when freshly exposed (2), while the CCA tissue remains pink and healthy on the leading edge of the lesion (3). Bar = 1 cm. (B) Appearance of isolated fungal hyphae associated with CFD (1000x magnification using light microscopy). Bar = 15 µm. (C) Section of a coralline alga infected with CFD and positively confirmed as a fungal infection using Grocott's methenamine silver. Note the fungal hyphae invading the algal thallus and conceptacles (arrows). Cu = cuticle. Bar = 30 µm.

Figure 3. Representative *in situ* temperatures at Palmyra Atoll at 10 m on the forereef and sea-surface temperatures (SST) from satellite-derived sources during 2008, 2009, and 2010 and the associated change in coralline fungal disease (CFD) occurrence (forereef-wide mean number of cases/m^2 of CCA are shown by black arrows).

Figure 4. (A) Mean (± SE) calcification rate for diseased and healthy CCA in experimental aquaria (n = 12). Change in weight shown as mg $CaCO_3$/week for each thallus. Asterisk indicates when response within treatment differs significantly between diseased and healthy specimens

(Table 2). (B) Mean (± SE) lesion lateral expansion rate for diseased CCA in experiments. Using the ambient air x 28°C treatment as a control value, the asterisk indicates a significant effect of elevated temperature (Dunn's, $Z = 3.156$, $p = 0.0048$).