Vol. 594: 95–106, 2018
https://doi.org/10.3354/meps12525

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published April 26

Selective feeding by corallivorous fishes neither promotes nor reduces progression rates of black band disease

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ABSTRACT: Black band disease (BBD) is a virulent coral disease, and although its microbiology has been studied extensively, the aetiology of BBD remains poorly understood. Here we used aquaria and field experiments to determine if feeding on BBD lesions by corallivorous fishes influences disease progression rates. Although selective predation on lesions was observed in both controlled laboratory experiments and field-based observations, we found no evidence that fish feeding either reduced or enhanced progression rates of BBD. Variability in disease progression rates in the field was explained by variation among coral colonies (24.46%) and among sample days (37.77%) rather than by predation treatment (<0.1%). Also, disease progression rate was significantly correlated with the width of the disease band. This suggests that properties of the disease band, potentially the complexity of the microbial community forming the band, influence rates of tissue loss. Results highlight that natural variation in host resistance and dynamics of the disease band play a greater role in BBD progression rate than selective feeding by corallivorous fish.

KEY WORDS: Coral disease · *Chaetodon plebeius* · Disease progression rate · Selective feeding

INTRODUCTION

Diseases in marine ecosystems are major drivers of population dynamics and can severely reduce biodiversity and influence community composition (Harvell et al. 2002, Plowright et al. 2008, Maynard et al. 2015). In the past few decades, outbreaks of marine diseases have affected a wide range of species, and explanations for the increased prevalence of diseases in marine organisms are often related to climate-induced ocean warming (Burge et al. 2014, Randall et al. 2014, Eisenlord et al. 2016). On coral reefs, increased water temperature has been linked to both increased prevalence (i.e. the number of disease cases at a particular time) and progression rates (i.e. speed at which disease causes tissue loss) of several diseases affecting reef-building corals (Sato et al. 2009, Zvuloni et al. 2009, Vargas-Ángel 2010, Williams et al. 2014). Numerous other abiotic and biotic factors can also influence prevalence and progression rates, including nutrient enrichment (Kuntz et al. 2005, Kline et al. 2006, Voss & Richardson 2006, Kaczmarsky & Richardson 2011), light intensity (Boyett et al. 2007, Sato et al. 2011), injury and breakage (Miller & Williams 2007, Nicolet et al. 2013, Lamb et al. 2014), sedimentation (Frias-Lopez et al. 2002, Haapkylä et al. 2011), proximity of algae (Haas et al. 2011, Barott & Rohwer 2012, Casey et al. 2014) and coral cover (Bruno et al. 2007, Hoff 2007, Williams et al. 2010, Aeby et al. 2011). Whereas studies over several decades (i.e. since the first disease–temperature study conducted by Antonius (1981) on black band disease) reveal consistent patterns in the role of environmental factors in disease onset and progression, studies on other biological factors, such as the

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role of corallivores in disease progression, have so far shown ambiguous results.

Coral-feeding fishes and invertebrates have the potential to influence coral disease dynamics through the continual removal of coral tissue, causing the coral to redirect energy towards tissue regeneration (Gochfeld 2004), thereby potentially lowering its resistance to infections. Indeed, predation by corallivores can hinder the recovery of corals after stressful events like bleaching (Rotjan et al. 2006). Alternatively, invertebrates could, in some cases, be beneficial and defend corals against pathogens. For example, feeding by *Cymo* crabs on white syndrome lesions debrides coral lesions and, therefore, slows rates of disease progression (Pollock et al. 2013). Similarly, corallivorous fishes have been observed feeding selectively on diseased sections of prey corals (Cole et al. 2009, Chong-Seng et al. 2011). Such behaviour has been postulated to slow the progression of disease by removing infectious microorganisms (Cole et al. 2009), but it may also spread diseases to adjacent healthy colonies, either via feeding, waterborne contamination (i.e. release of infected tissue into the water column) and/or feeding-related injuries that provide an entry site for pathogens (Raymundo et al. 2009). Such selective feeding has been observed on 2 coral diseases thus far, black band disease (BBD) and brown band disease (Cole et al. 2009, Chong-Seng et al. 2011, Nicolet et al. 2013). However, the role of corallivores in suppressing disease progression remains equivocal (Aeby & Santavy 2006, Cole et al. 2009, Chong-Seng et al. 2011).

BBD is a virulent coral disease found on the Australian Great Barrier Reef and has been reported to infect corals worldwide (Garrett & Ducklow 1975, Antonius 1985, Korrubel & Riegl 1998). BBD is characterized by a dark polymicrobial mat that progresses across the host coral colony, killing coral tissue and exposing white skeleton (Richardson 2004). The pathogenicity of BBD derives from the anoxic and sulphide-rich microenvironment created by the synergistic effects of a consortium of cyanobacteria, sulphur cycle-related bacteria and other heterotrophic microorganisms present in the disease mat (Sato et al. 2013, 2016). Although the ecology and microbiology of BBD have been studied extensively (Antonius 1985, Frias-Lopez et al. 2002, Voss & Richardson 2006, Sato et al. 2009, 2010, 2013), its aetiology and dynamics remain largely unknown. In a study at Lizard Island, Chong-Seng et al. (2011) reported that 8 species of corallivorous fishes (6 of which were chaetodontids) and 4 species of non-corallivorous fishes selectively fed on BBD lesions *in situ*, and speculated that reef fishes could be disease vectors transmitting pathogens to neighbouring corals (see also Aeby & Santavy 2006). Alternatively, Cole et al. (2009) suggested that this intense selective feeding could slow the progression of BBD and, at very high levels of predation pressure, even stop progression of the disease. However, neither of these studies quantified the impact of selective feeding on progression rates of BBD (i.e. linear progression of the disease band along infected coral branches).

To determine the effect of predation on disease progression in experimental studies, natural variation among colonies must be accounted for. Recent studies have found that intraspecific variation can explain up to 70% of the variation in disease dynamics *in situ* (Rodriguez & Croquer 2008, Nicolet et al. 2013). Such differences among colonies of the same species are likely to be influenced by the characteristics of the pathogen (e.g. some diseases increase virulence over time as microbial communities accumulate; Glas et al. 2012), but might also be influenced by the health state and natural resistance of the coral host prior to infection. However, variation in resistance among corals is seldom accounted for in coral disease ecology, often being treated as random variation that obscures treatment effects. Understanding and quantifying this natural variation in disease susceptibility among coral colonies is critical for understanding the effects of coral disease on coral population dynamics.

The aim of this study was to determine the effects of selective predation by coral reef fishes on progression rates of BBD (i.e. the rate of disease-related coral tissue loss), using a combination of laboratory and field experiments. In the field, the impact of natural predation on BBD progression rate was evaluated by comparing progression rates of BBD on coral branches that were caged (i.e. protected from coral-feeding fishes) versus exposed to naturally-occurring fish assemblages. In aquaria, we experimentally tested whether high levels of predation pressure by the blueblotch butterflyfish *Chaetodon plebeius* (Chaetodontidae) enhance or inhibit disease progression.

MATERIALS AND METHODS

Study site

This study was conducted between March (end of austral summer) and June (beginning of austral winter) 2013 at Lizard Island (14° 40′ 08″ S, 145° 27′ 34″ E), a mid-shelf island in the northern Great Barrier Reef,

Australia. Lizard Island has well-developed fringing reefs along the exposed (south and east) fore reef, an extensive semi-enclosed lagoon with largely continuous reef structures and a mosaic of patch reefs on the sheltered (north and west) sides of the island. To locate a study site with a high abundance of BBD, we completed extensive surveys across 12 reefs, including exposed reef fronts, lagoon and back reef habitats, in March 2013 using timed-swims (3 replicates of 10 min, ~100 m^2, per reef). Disease prevalence, especially of BBD and growth anomalies, was highest on staghorn colonies of *Acropora muricata* at Trawler Reef, a shallow reef in the northern part of the lagoon, which was subsequently selected for the field experiment.

Field experiment: BBD progression and predation by resident fish communities

To test whether natural predation by local fish assemblages influences progression rates of BBD, we conducted a controlled caging experiment in the field. A total of 15 colonies of *A. muricata* showing well-developed BBD infections were tagged, using cable ties secured on the exposed skeleton below the disease band. All colonies were located at the same site, at depths between 2 and 4 m depending on the tide, and separated by a maximum of 40 m from one another. Disease progression along infected branches (referred to as progression hereafter) was monitored every 2 d for 10 d by taking pictures of diseased branches including a ruler for scale. Since virulence of BBD pathogens and migration patterns of cyanobacteria that dominate the BBD microbial consortium vary with light intensity (Sato et al. 2011), all photographs were taken during high light intensity hours (between 11:00 and 15:00 h) to avoid additional variation in lesion progression rate and width. Initially, 1 lesion colony^{-1} was monitored, but when lesions progressed past bifurcation points on branches, newly diseased branches were considered separately from the original branch to avoid overestimating progression rates. As a consequence, the number of diseased branches colony^{-1} varied from 1 to 6, producing an unbalanced dataset in which the total number of branches increased over time.

At the same time as progression rates were measured, rates of natural predation on disease bands by fishes were determined using digital underwater video cameras (GoPro Hero 2) placed 30 to 60 cm away from BBD-infected colonies, and focussed on coloured bands bordering disease lesions adjacent to seemingly healthy coral tissue. All 15 colonies were filmed before caging during 5 replicate 30 min long video recordings. After 10 d, branches of 5 colonies were caged using 0.5 × 0.5 cm wire mesh to prevent predation on infected coral tissues without reducing water flow, 5 colonies were left uncaged, and branches on the remaining 5 colonies were only partially caged to control for the presence of caging material around the corals without preventing natural predation (branches enclosed by a frame but with wire mesh on only 2 sides of the cube; see Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m594p095_supp.pdf). After caging, disease progression was monitored every 2 d for another 15 d using the same methodology. All pictures were taken using an underwater camera (Panasonic DMC-TZ15), and BBD progression rate (standardized to cm d^{-1}) was measured on each diseased branch using the image analysis program ImageJ (1.44o, Java 1.6.0_29, public domain). Each infected branch was photographed once every second day, and care was taken to ensure that images taken on successive days were taken from the same angle and distance from the band to minimise this potential source of variation in the data. Progression rate was estimated by measuring the linear distance the disease band moved along coral branches over time (over the total 25 d of the experiment), from the tag (cable tie on exposed skeleton) to the margin of healthy tissue. Band width was measured in a similar fashion, from the interface between the skeleton and the disease lesion to the margin of the healthy tissue.

Laboratory experiment: BBD progression and predation by *Chaetodon plebeius*

To closely monitor corallivore impact on disease progression, aquarium trials were conducted at Lizard Island Research Station using the blueblotch butterflyfish *C. plebeius* (Chaetodontidae), the corallivore that interacted most frequently with BBD lesions in the field. Adult and subadult individuals of *C. plebeius* (total length 5 to 8 cm) were collected from multiple reefs (at least 1 km away from Trawler Reef). Fish were caught using a 5 × 1.5 m barrier net and hand nets. To minimise the potential effects of fish size and potential previous exposure to BBD, fishes of different sizes and from different reefs were mixed together in the aquarium experiment. Healthy nubbins of *A. muricata* were collected from different reefs within the lagoon at least 500 m away from the Trawler site, and the absence of BBD

lesions was confirmed under a dissecting microscope (Olympus SZX7). Fish and coral nubbins were allowed to acclimate to laboratory conditions for 48 h prior to the experiment. *C. plebeius* were fed healthy coral branches (renewed every 3 d) and coral nubbins were fed every day at dusk with brine shrimp (*Artemia* sp.) hatched in 0.5 µm filtered and UV sterilized seawater. After the acclimation period, heavily diseased nubbins of *A. muricata* were collected from the reef and placed directly in experimental aquaria (each 120 × 40 × 50 cm), supplied with flow-through seawater filtered to 0.5 µm and UV sterilized.

To determine the impact of predation levels on BBD progression rates under controlled aquarium conditions, healthy coral nubbins, diseased coral nubbins and fish were randomly allocated to 1 of 3 experimental (high, medium and no predation) and 2 control treatments (Fig. 1). The high predation treatment comprised a healthy nubbin, a diseased nubbin and 4 *C. plebeius* (2 replicate tanks). The medium predation treatment comprised an infected nubbin with 2 *C. plebeius* in 1 half of the tank and 2 *C. plebeius* and a healthy nubbin separated by a divider in the other half of the tank (2 replicate tanks); this maintained the total number of fish tank^{-1} at 4 to control for nutrient input due to fish presence, as nutrient enrichment can increase BBD virulence (cf. Kuta & Richardson 2002, Richardson & Ragoonath 2008). Fish feeding behaviour on experimental diseased nubbins was determined every second day using underwater videos (GoPro Hero 2). The no-predation treatment comprised a diseased nubbin on one side of the tank separated by a divider from 4 fish and a healthy nubbin on the other side of the tank (1 replicate tank with fish). Plexiglas dividers were perforated to enable water to flow normally between the 2 compartments, but to prevent the fish from moving freely. All fish in experimental treatments were fed, as outlined above, with non-experimental healthy nubbins that were renewed every 3 d to maintain fish health (4 non-experimental nubbins per *C. plebeius* pair, renewed every 3 d over the duration of the trial, in addition to the 1 experimental healthy nubbin tank^{-1}). Control treatments comprised: (1) 1 healthy and 1 infected nubbin in a tank without direct contact (control for BBD progression in the absence of fish), and (2) 1 healthy nubbin in a tank to test for water-borne transmission of potential pathogens from the aquarium system (water control) (Fig. 1).

Due to space limitations in the aquarium facility, replicate trials (n = 7) were run successively, rather than concurrently. During each trial, the 5 treatments (high, medium and no predation treatments, and BBD progression and water controls), including duplicates of the high and medium predation treatments, were randomly allocated to 1 of 7 experimen-

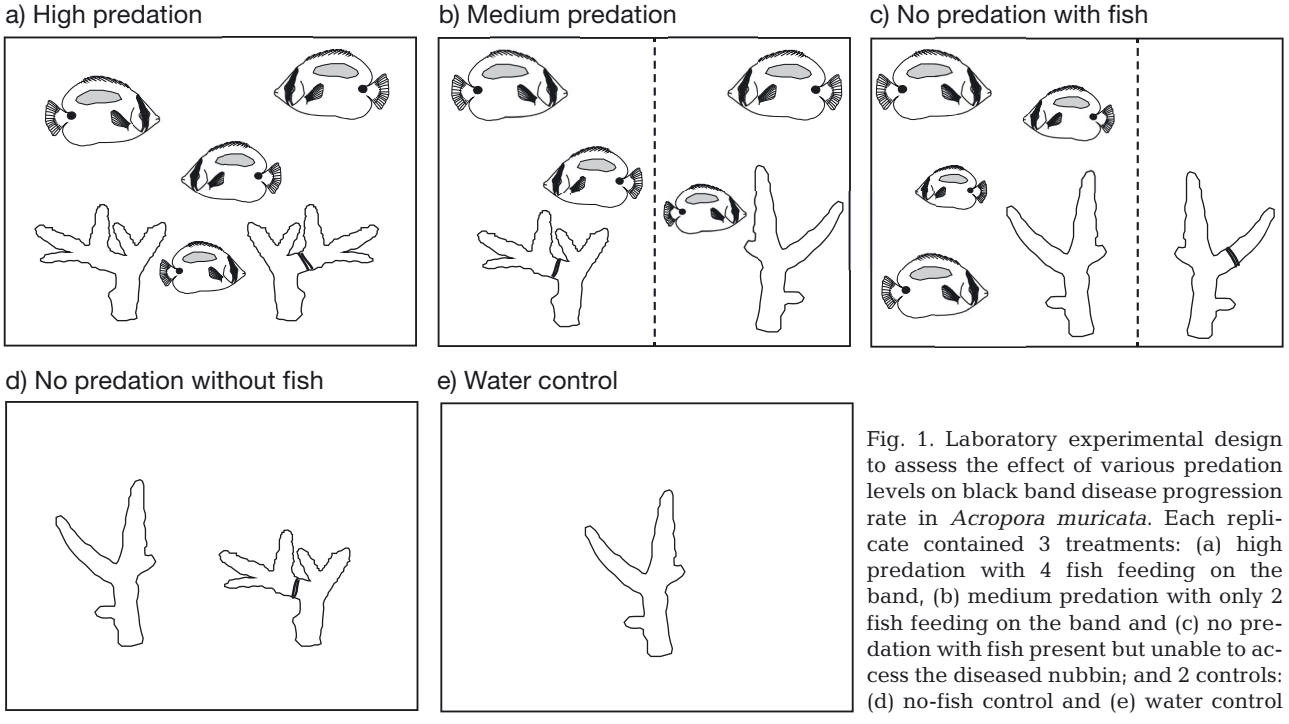

Fig. 1. Laboratory experimental design to assess the effect of various predation levels on black band disease progression rate in *Acropora muricata*. Each replicate contained 3 treatments: (a) high predation with 4 fish feeding on the band, (b) medium predation with only 2 fish feeding on the band and (c) no predation with fish present but unable to access the diseased nubbin; and 2 controls: (d) no-fish control and (e) water control

tal aquaria. Each replicate trial was run for 6 d to allow sufficient time to detect an effect of selective feeding on disease progression. During this period, large proportions of coral tissue on infected nubbins were killed by the disease. Progression rate was quantified in the same manner as in the field, using an underwater camera (Panasonic DMC-TZ15) and with a ruler as scale. Pictures were taken every day to better estimate progression rates. In total, the experiment ran for 42 d (6 d × 7 trial replicates), and between each replicate trial, all diseased corals, healthy nubbins and fish were changed to avoid pseudo-replication. New fish, freshly caught from the reef, replaced 'used' fish (after a 48 h acclimation period) whenever possible. A total of 80 *C. plebeius* were used to run the 7 replicate trials.

Video analysis and statistical analysis

Analyses of *in situ* video recordings enabled identification of all species of fish that took bites from coral tissues within the field of view, as well as quantification of bite rates and qualitative observations of feeding behaviour. Similarly, fish feeding behaviour and bite rates during the laboratory study were determined from video analysis. In both cases, the number of bites taken by each fish was recorded in 2 categories: bites on the disease band (comprised of a cyanobacterial mat, potentially with other pathogens and necrotic tissue) and bites on apparently healthy coral tissue. All bites that were visually estimated to be between 1 and 5 mm above the disease band were considered diseased tissue, as this region typically comprises pathogens, mucus and necrotic coral tissue (i.e. non-healthy tissue). The observer only counted bites when the mouth of the fish and the food source were clearly visible; hence it is likely that the recorded counts slightly underestimated actual bite rates. Predation data on both diseased and healthy corals were standardized to bites min^{-1} for both field and aquarium experiments.

Progression rate data from the 15 colonies of *A. muricata* monitored in the field were graphically and statistically analysed to tease apart major influences on BBD progression rates, including inter-colony variation, seasonal variation and predation effects. The relationships between progression rate and both experimental stage (before and after caging) and band width (cm) were investigated using a linear mixed-effects model using the function 'lme' in the 'nlme' package in RStudio (R Version 3.0.2). Experimental stage (before and after caging) was treated as a fixed effect, and band width as a continuous covariate, while colony was included as a random effect. The width of the disease band at each time point was measured and included in this analysis because it was hypothesised to be positively correlated with disease progression rate. Variation in residuals was heterogeneous (residuals increasing with fitted values) and, hence, an additional argument 'weights' was included in the mixed-effects model using band as the variance covariate. To account for repeated measurements over time (days of experiment, 1 to 25 as a factorial variable), which violates the independence assumption of the linear mixed-effects model, a 'correlation' argument was also added to model the auto-correlation between residuals of different time points. After various trials of auto-correlation models for residuals, the ARMA structure ($p = 2$, $q = 0$) was selected using Akaike's information criterion (AIC; see the Supplement for all R codes). The effect of treatment (cage, cage control and control) on disease progression rate was tested in a separate analysis, which only included the dataset after caging. In this model, treatment was considered a fixed effect and band width treated as a continuous covariate, and a generalised least squares analysis was run including a correlation argument for repeated measurements ('gls' using the 'nlme' package).

In addition, given the combination of fixed, random and repeated factors included in the analysis, a variance components analysis was used to assess how much of the observed variance in progression rates was explained by each factor (i.e. experimental stage, caging treatment, band width, colony and time [days of experimental exposure: 1 to 25]). Variance was partitioned by running a linear mixed-effects model, with progression rate as the response variable, and time, band width and colony as random effects. Time and experimental stage were combined because stage (before/after caging) also captures variation through time. The standard deviations of each random effect (time, treatment, band width and colony) were extracted from the model summary, squared to calculate variances, then expressed as a percentage of the total variance.

In the aquarium experiment, lesion progression rates were highly variable and non-normally distributed. A non-parametric Kruskal-Wallis test comparing mean progression rates among predation levels in the different treatments revealed that mean predation rates did not differ significantly between the medium and high predation treatments (Kruskal-Wallis test, $\chi^2 = 0.05$, df = 1, p = 0.83). Thus, progression rates for the 2 treatments were pooled and tested

against predation rate (i.e. predation present vs. absent) in the final model. The linear mixed-effects model was used to investigate the effect of predation on progression rate, where predation (as a continuous variable, bites min^{-1}) was included as a fixed factor, band width as a continuous covariate, and experimental replicate was included as a random factor. Again, the arguments 'weights' and 'correlation' were added to the model to deal with heterogeneity and repeated measures over time. Finally, a linear mixed-effects model was used to test the effect of time (1 to 42 d of experimental exposure; treated as a factorial variable) and band width on BBD progression rate in the absence of predation (experimental replicate treated as a random factor). All statistical analyses were performed with RStudio (Version 3.0.2 – ©2013 RStudio). Linear mixed-effects models and generalised least squares models were computed using the nlme package and the multiple comparison test (kruskalmc) using the 'pgirmess' package. Kruskal-Wallis, Kolmogorov-Smirnov and Pearson's correlation tests were calculated using the 'stats' package.

RESULTS

Field experiment

In the field study, 10 fish species were observed feeding on BBD lesions during 37.5 h of video recording. *Chaetodon plebeius* was responsible for more than half (52.7%) of the total bites taken on BBD lesions, followed by *C. lunulatus* (22.3%), *C. rainfordi* (9.2%) and *C. aureofasciatus* (5.6%). The other 6 fish species were *C. baronessa*, *C. trifascialis*, *Labrichthys unilineatus* (juvenile), *Oxymonacanthus longirostris*, *Pomacentrus amboinensis* and *P. grammorhynchus*.

Among-colony variation in disease progression

Mean progression rates of BBD were found to vary significantly among coral colonies in the field (Kruskal-Wallis test $\chi^2 = 121.75$, df = 14, $p < 0.001$; Fig. 2a). Mean ± SE progression rate was 0.79 ± 0.05 cm d^{-1}, but ranged from 0.005 to 5.2 cm d^{-1}

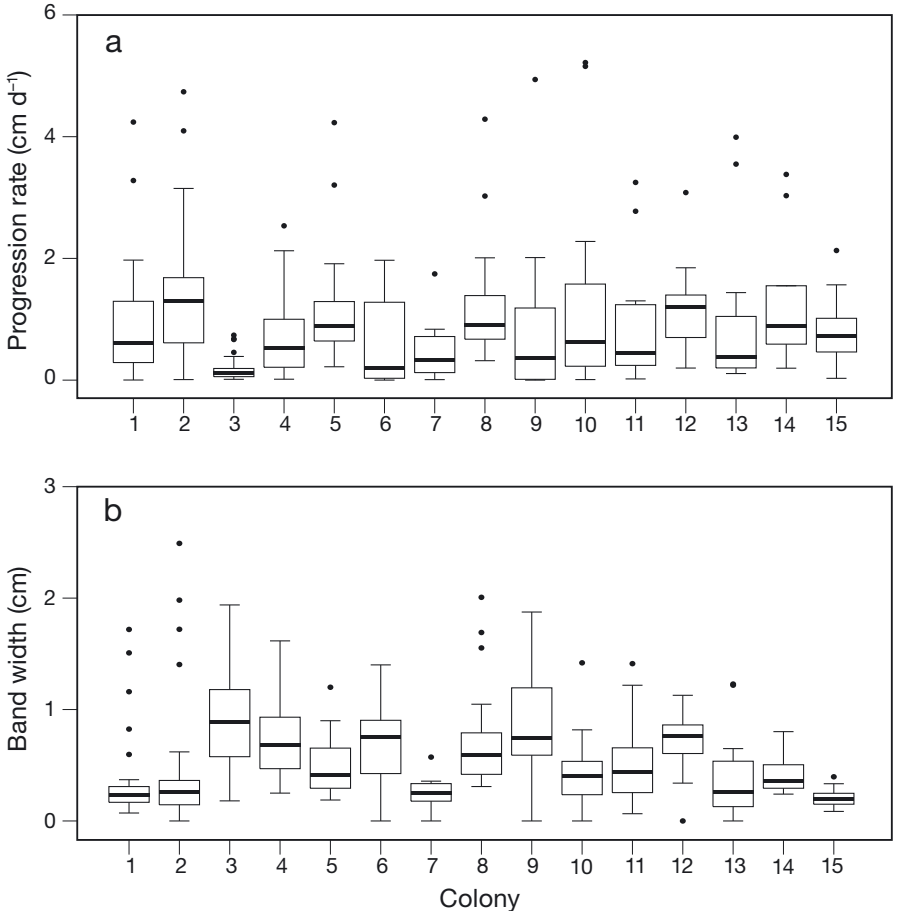

Fig. 2. Natural variation in black band disease (a) progression rate (cm d^{-1}) and (b) band width (cm) across 15 colonies of *Acropora muricata* before the onset of the caging experiment. Thick lines inside boxes: median (or second quartile); lower and upper lines of boxes: quartiles 1 and 3 (box = inter-quartile range); whiskers: 1.5 times the inter-quartile range; individual points on the graph: data outside the range of the whiskers

among colonies. Intra-colony variation was also observed, with SEs of colony means varying from 0.037 to 0.49. Band width differed significantly among colonies (Kruskal-Wallis test $\chi^2 = 146.14$, df = 14, $p < 0.001$), varying from 0 (e.g. at the end of the experiment when very little live tissue remained on branches) to 4.9 cm, with a mean of 0.5 ± 0.02 cm across all colonies (Fig. 2b). However, only 3 (from n = 606) data points had band widths >3 cm; these were considered outliers and were removed from the dataset for subsequent analyses. When considering all of the data (before and after caging), there was a significant positive relationship between band width and disease progression rate (linear mixed-effects model, band width: denDF = 589, $F = 13.98$, $p < 0.01$; slope = 0.33, Fig. S2 in the Supplement). Moreover, correlation coefficients were positive for most of the colonies (13 out of 15) when considered individually, supporting the interpretation that band width and progression rate are positively correlated in natural conditions. Progression rate varied significantly through time as well. Mean BBD progression rate was significantly faster in the summer months before caging (0.92 ± 0.05 cm d^{-1}) compared to in cooler months after the onset of the caging experiment (0.61 ± 0.04 cm d^{-1}; linear mixed-effects model, experimental stage: denDF = 589, $F = 12.63$, $p < 0.001$, Fig. 3).

Effect of predation on disease progression

Overall, mean ± SE predation rate before caging was 0.46 ± 0.14 bites min^{-1}, but rates ranged widely from 0 to 6.6 bites min^{-1}. Considering only the data after caging, progression rate was independent of predation level (generalised least squares, treatment: numDF = 2, $F = 2$, $p = 0.11$, Fig. S3 in the Supplement) but positively correlated with band width (generalised least squares, band width: numDF = 1, $F = 12$, $p < 0.001$). Variance components analyses revealed that variability in BBD progression among treatments (cage, partial cage control and uncaged control) was negligible (<0.1%). Instead, variability in disease progression was due to 3 other factors: inter-colony variation (~24%), temporal variation (i.e. days of experimental exposure; ~38%) and band width variation over time for each colony (~38%). The high variability over time (~38%) is likely due to fluctuating or seasonally varying environmental factors, especially during the experimental months of March and April, when seawater temperatures cool down relatively rapidly.

Laboratory experiment: effect of predation on disease progression

In the aquarium study, mean ± SE predation rate was 1.76 ± 0.19 bites min^{-1} $nubbin^{-1}$, an average 3.5-fold greater than the field value, and ranged from 0 to 10.3 bites min^{-1}. Overall, *C. plebeius* took more bites from the disease band (14 323 bites) than from healthy tissue (13 417 bites), despite the lesion representing <10% of the available substratum. Mean disease progression rate in aquaria was 0.36 ± 0.03 cm d^{-1} but ranged from 0.002 to 1.32 cm d^{-1} among nubbins. Due to high variation in fish predation rates within treatments, and the lack of a clear difference in predation between medium and high predation treatments, data were analysed using predation as a continuous variable. In this model, disease progres-

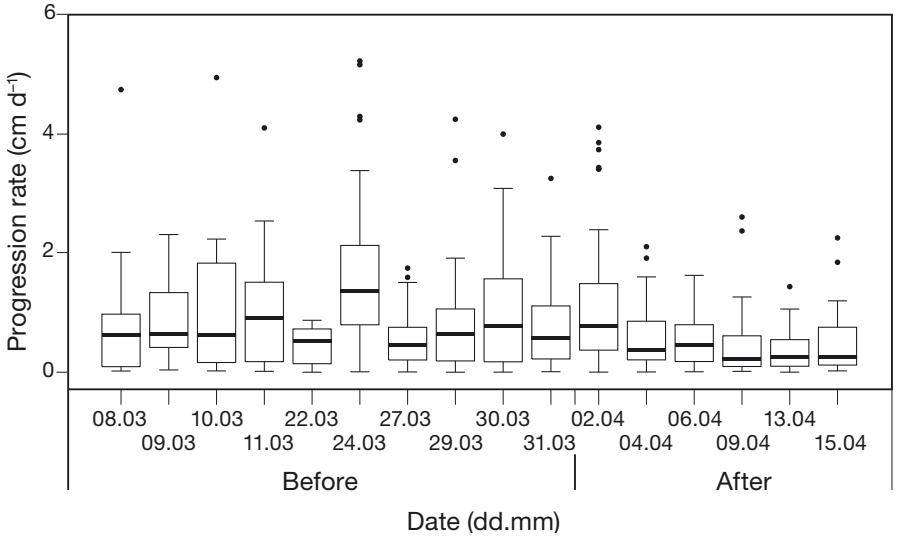

Fig. 3. Progression rate (cm d^{-1}) of black band disease of *Acropora muricata* over time in the field, including data both before and after the onset of the caging experiment. Dots: extreme data points with values far above the mean of the day. The same 15 colonies were observed before and after the onset of the caging experiment. Due to the uneven number of branches per colony, and the longer observation period before caging, 354 data points were used for the 'before' analysis and 255 for the 'after'. Box plot parameters as in Fig. 2

sion rate was significantly positively correlated with predation rate (bites min^{-1}) but not band width (cm) (Fig. 4, linear mixed-effects model using only progression data under predation; predation: denDF = 150, $F = 8.16$, p = 0.005; band width: denDF = 150, $F = 0.04$, p = 0.8). In the absence of predation (i.e. no predation treatment), lesion progression rates reached a higher maximum (3.02 cm d^{-1}) and mean (0.56 cm d^{-1}) than under predation. However, no significant difference was found in the frequency distribution of the disease progression data with or without predation (Kolmogorov-Smirnov, $D = 0.25$, p = 0.69), meaning that the difference in progression rate with and without predation was not significant. Furthermore, disease progression rate in the absence of predation varied significantly over time (linear mixed-effects model, time: denDF = 44, $F = 2.09$, p = 0.008), increasing during the cooler months.

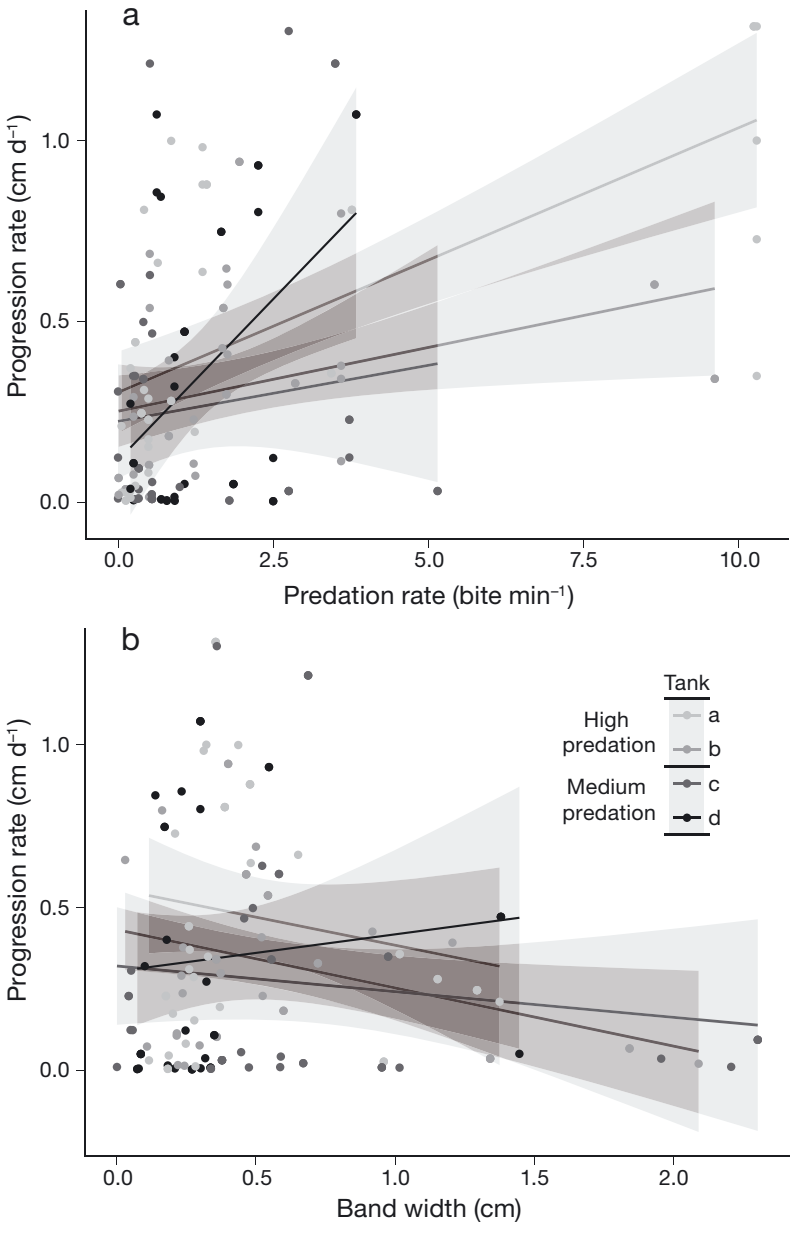

Fig. 4. Correlation between black band disease progression rate (cm d^{-1}) in *Acropora muricata* and either (a) *Chaetodon plebeius* predation rate (bites min^{-1}) or (b) band width (cm) in the aquarium experiment. Each aquarium is represented by a specific shade of grey to help visualise the correlations accounting for variation per aquarium. Aquaria a and b were high predation treatments with 4 fish feeding on the diseased coral nubbin; aquaria c and d had 2 fish feeding on the nubbin (medium predation treatments). Dots: data points; lines: regression (for each tank, a, b, c or d). Shaded area: 95% confidence region

DISCUSSION

We found no evidence that selective predation by corallivorous fishes led to declines in the progression rates of BBD. These results do not support inferences from previous studies (Cole et al. 2009) that predation by corallivorous fishes might suppress coral disease progression. Although mean progression rates were highest in the complete absence of predators, they did not differ significantly from progression rates in predation treatments. Similarly, in field experiments, predation treatments had no effect on progression rate, explaining <0.1% of the overall variance in BBD progression rates. Variation in disease progression in the field was mostly explained by inherent variation among coral colonies (24%) and among sampling days (38%).

Chaetodon plebeius was the predominant fish species to feed on *Acropora* colonies infected with BBD in the field during this study. Other species that fed on BBD included *C. lunulatus*, *C. rainfordi*, *C. aureofasciatus*, *C. baronessa*, *C. trifascialis*, *Labrichthys unilineatus* (juvenile), *Oxymonacanthus longirostris, Pomacentrus amboinensis* and *P. grammorhynchus.* In a previous study on Lizard Island reefs, Chong-Seng et al. (2011) reported that 12 fish species from 3 different families (Labroidae, Pomacentridae and Chaetodontidae) targeted BBD, with the primary coralli-

vores being *Neoglyphidodon melas* and *C. baronessa*. Inconsistencies between the 2 studies may simply reflect differences in study sites; the Trawler Reef site in our study is a shallow back reef that typically harbours a different fish community with a lower diversity than the crest site where the 2011 study was located (Berumen et al. 2005). These contrasting results are unlikely to indicate seasonal variation in fish feeding activity, as Chong-Seng et al. (2011) also conducted their experiment during the austral summer (2008/2009). Despite differences in the species of corallivore studied, Chong-Seng et al. (2011) reported similar rates of predation on BBD by corallivores in the field (0.31 bites min^{-1}) to those found here (0.46 bites min^{-1}).

Fish predation on disease lesions is highly selective, often accounting for half of the bites taken on diseased colonies, despite lesions typically representing <10% of the available coral surface area (Chong-Seng et al. 2011, this study), but the reason why fish actively target BBD lesions is unknown. Such selective predation may be related to increases in the density of microbial communities, increased mucus production or because pathogens have inactivated coral nematocysts making the tissue more palatable to fish predators. As very few studies of reef fish corallivory report bite rates per colony where the health state of coral tissues is specified (but see laboratory and field experiments of Dirnwoeber & Herler 2013 and Gochfeld 2010, respectively), it is difficult to distinguish whether disease lesions attract predators to the infected colony (increasing overall predation on diseased colonies), or whether these lesions simply focus predation on diseased tissue (releasing healthy tissue from predation). These 2 alternatives could have very different impacts on overall coral health, and on the likelihood that infected colonies will survive. In the laboratory experiment, we found that exposure to high numbers of corallivores increased BBD progression rate (Fig. 4), suggesting that attraction of predators to infected colonies might reduce coral health and indirectly promote disease progression. However, increased progression of BBD was not detected in colonies exposed to natural levels of predation in the field.

In field studies of BBD progression, the proportion of variance explained by colony (~24%), time (~38%) and band width (~38%) was far greater than the variance explained by caging treatment and, by extension, predation (<0.01%). Thus, inter-colony variation, environmental variation over the study period and changes in the width of the disease band had stronger impacts on disease progression rate than fish feeding behaviour. Collectively, these findings suggest that any effects of natural levels of predation on disease lesions in the field are largely overshadowed by other biotic and abiotic factors. However, high variability among and between colonies, in addition to small sample sizes (n = 5 colonies $treatment^{-1}$), could have reduced statistical power and minimised the likelihood of detecting a predation effect. Finding over 15 coral colonies of the same species infected with the same disease at the same time and on the same reef proved to be impossible at the study site, even after monitoring the reef over a 2 yr period, thereby constraining the sample size. Nonetheless, regardless of the sample size, the impacts of natural sources of variation among colonies and over time are likely to remain greater than any effect of fish feeding behaviour. This low impact of fish predation on rates of BBD progression might be specific to branching corals, as mounding or encrusting species attract a distinct fish community. Further studies are needed to clarify the impact of corallivory on disease dynamics using other coral and fish species.

In the aquarium experiment, bite rates did not differ significantly between treatments with high and medium densities of fish. This could be because fishes excluded each other from the food source in the high density treatment, resulting in only 2 fishes feeding on the band at any one time — a hypothesis supported by analysis of the video footage. Direct competitive exclusion has been studied extensively in many taxa, usually at the species level, where 2 species limited by the same resource cannot coexist (Armstrong & McGehee 1980). At the within-species scale, aggressive or competitive behaviour between individuals targeting the same resource is also common across taxa (e.g. crayfish: Bovbjerg 1970; chipmunks: Brown 1971; birds: Murray 1971; marsupials: Dickman 1986). Diets of chaetodontids, especially hard coral feeders, typically overlap by 30 to 50% but sometimes by up to 70% (e.g. Pratchett 2005). When dietary overlap is not minimised by spatial or temporal partitioning, intense competition can occur, with frequent aggressive interactions between conspecifics and congenerics (Berumen & Pratchett 2006, Blowes et al. 2013). This aggressive behaviour could result in only a few individuals feeding at once and thus prevent excessive predation rates on disease lesions and corals in general.

Variation among coral colonies in both BBD progression rate and disease lesion size was likely due to a combination of intrinsic factors (e.g. genotypic differences in disease susceptibility or differences in colony condition; Pisapia et al. 2014), and extrinsic

factors (e.g. differences in the specific micro-habitat and recent disturbance history for colonies). This inter-colony variation remained after the onset of the caging experiment, with variation among colonies over time accounting for ~25% of the total variance. These results are consistent with other studies, which have also found that colony typically accounts for the greatest amount of variability in rates of disease progression. For example, Rodriguez & Croquer (2008) reported that variability within and among colonies explained 52 and 48% of the total variance, respectively. Similarly, variability among colonies over time explained 73% of the total variance in rates of brown band disease (Nicolet et al. 2013). As all colonies of *Acropora muricata* used in the field experiment co-occurred within 40 m of each other, they experienced similar ranges in environmental factors like wave action, coral cover, water temperature, salinity, water quality and light intensity. Only microhabitat variation, such as the presence of territorial damselfish that influence disease dynamics by harbouring potential BBD pathogens (Casey et al. 2014), could be an alternative explanation for among-colony variation observed in this study. Benthic primary producers around colonies can also alter microbial processes by modifying biochemical cycling in their surrounding environment (Haas et al. 2011). The close proximity of algae releasing dissolved organic carbon into their surroundings could promote bacterial growth and increase the virulence or likelihood of infection in neighbouring coral colonies (Kuntz et al. 2005, Kline et al. 2006, Haas et al. 2011). Understanding how these fine-scale processes influence coral health, and how much this explains among-colony variation, requires further study.

Disease progression rate was temporally variable in both the field and aquaria, which may be attributable to changing environmental factors. Progression significantly decreased over time, regardless of caging treatment or colony-level variation, and this response might have been influenced by the decrease in light intensity and water temperature between the months of March and June. BBD prevalence and rate of related tissue loss have previously been linked to seasonal fluctuations in environmental variables such as water temperature and light intensity (Boyett et al. 2007, Sato et al. 2011), sedimentation (Bruckner et al. 1997) and nutrient enrichment (Kuta & Richardson 2002, Voss & Richardson 2006). A manipulative experiment testing both light and temperature reported that BBD progression rate was greatest (0.52 cm d^{-1}) at high temperatures (30.5°C) and high light intensities (Sato et al. 2011). The natural decrease in both water temperature and light exposure between the austral summer and winter could have influenced the progression rate of BBD through time.

Positive correlations between the width of the disease band and BBD progression rate, both in the field and in aquaria (in the absence of predation), highlight that characteristics of the pathogenic consortium are also likely to contribute to variation in BBD progression rates. A wider band, potentially comprising more pathogens, is likely to break down coral tissue more rapidly. The correlation was consistent for all 15 colonies in the field, although the strength of the correlation varied. To our knowledge, this is the first time that a correlation between disease band width and disease progression rate has been found. It is possible that the 2 factors, progression and lesion size, are not directly correlated but instead are the product of another aspect of black band dynamics. For example, Glas et al. (2012) found that biogeochemical microgradients within the complex microbial community of the band, particularly through the creation of an anoxic and sulphide-rich environment, are responsible for the disease virulence. Because the microbial community within the band changes over time, mean progression rate and potentially the width of the band increase as the community becomes more vertically stratified. However, although microbial community complexity and stratification are responsible for pathogen virulence, coral tissue loss is a mere by-product of this process. Consequently, reducing the disease band width, for example via fish predation, would not impact progression rate of the disease. Further research is needed to tease apart factors underlying virulence of the BBD microbial community and rate of coral tissue loss, and how both can be moderated.

In conclusion, this study shows that corallivorous fish have little to no potential to either constrain or enhance the progression of BBD on the staghorn coral *A. muricata*. Instead, variation in progression rate was better explained by variations among coral colonies (e.g. differences in disease susceptibility or health), among sampling days (i.e. variation through time) and in the width of the disease band. The precedence of inter-colony variability in explaining progression rate variability highlights that some colonies are naturally more resistant to black band disease than others. Such genotypic variation is commonly acknowledged in studies of coral immunity (Palmer & Traylor-Knowles 2012, Pinzón et al. 2014, Toledo-Hernandez & Ruiz-Diaz 2014) but is often disregarded in coral disease ecology research. Overall, the potential of biotic (e.g. coral immunity and/or

health state) and abiotic factors (e.g. environmental factors) to influence coral disease progression rates is greater than the impact of selective fish feeding on the infected tissue. Further studies will focus on elucidating the impact of predation on disease transmission, and assess the influence of environmental factors on transmission, to better predict and manage the impact of BBD on coral reefs.

Acknowledgements. We are grateful for field and logistic support provided by Anne Hoggett and Lyle Vail and the rest of the Lizard Island Research Station staff. This study has benefited from discussion with N. Gardiner, K. Chong-Seng and Y. Sato and field support from K. Quigley, A. Vail and T. Rueger. This work was supported by The Australian Natural History Museum (Lizard Island Research Station Internship), James Cook University and the ARC CoE for Coral Reef Studies. The research was covered by James Cook University Fisheries Permit 103256, Marine Parks Permit G11/32003.1 and Ethics approval A1717.

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Editorial responsibility: Peter Edmunds, Northridge, California, USA

Submitted: December 8, 2018; Accepted: February 9, 2018
Proofs received from author(s): April 19, 2018