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Proc. R. Soc. B 2009 **276**, 2795-2803 first published online 6 May 2009
doi: 10.1098/rspb.2009.0481

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Dynamics of seasonal outbreaks of black band disease in an assemblage of *Montipora* species at Pelorus Island (Great Barrier Reef, Australia)

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Recurring summer outbreaks of black band disease (BBD) on an inshore reef in the central Great Barrier Reef (GBR) constitute the first recorded BBD epizootic in the region. In a 2.7 year study of 485 colonies of *Montipora* species, BBD affected up to 10 per cent of colonies in the assemblage. Mean maximum abundance of BBD reached 16 ± 6 colonies per 100 m^2 ($n = 3$ quadrats, each 100 m^2) in summer, and decreased to 0–1 colony per 100 m^2 in winter. On average, BBD lesions caused 40 per cent tissue loss and 5 per cent of infections led to whole colony mortality. BBD reappearance on previously infected colonies and continuous tissue loss after the BBD signs had disappeared suggest that the disease impacts are of longer duration than indicated by the presence of characteristic signs. Rates of new infections and linear progression of lesions were both positively correlated with seasonal fluctuations in sea water temperatures and light, suggesting that seasonal increases in these environmental parameters promote virulence of the disease. Overall, the impacts of BBD are greater than previously reported on the GBR and likely to escalate with ocean warming.

Keywords: coral reef; disease outbreak; seasonal dynamics; Great Barrier Reef; black band disease

1. INTRODUCTION

Infectious diseases in reef building corals have emerged at an accelerating rate over the last few decades (Richardson 1998; Harvell *et al.* 1999; Willis *et al.* 2004) and have contributed to a decline in hard coral cover, most notably in the wider Caribbean region (Green & Bruckner 2000; Gardner *et al.* 2003; Weil 2004). Although causative agents for the majority of coral diseases are difficult to identify and parameters contributing to increasing trends are often unclear (Harvell *et al.* 1999; Porter *et al.* 2001), global ocean warming (Harvell *et al.* 2002, 2007; Rosenberg & Ben-Haim 2002; Bruno *et al.* 2007) and human-induced marine eutrophication (Weil 2004; Jordan-Dahlgren *et al.* 2005; Kaczmarsky *et al.* 2005; Kline *et al.* 2006) have both been implicated as major drivers of increasing disease occurrence. However, there is an urgent need for longer term monitoring studies at small, detailed scales to more clearly elucidate links between environmental parameters and disease abundance.

Black band disease (BBD) is readily visible in the field and thus a good candidate for an intensive monitoring study. Macroscopic signs of the disease are a bacterial mat forming a black band that migrates across apparently healthy coral colonies, actively killing tissue and exposing skeleton (Richardson 2004). Progression rates of up to 2 cm d^{-1} have been recorded on Caribbean corals (Kuta & Richardson 1997), leading to death of entire coral colonies. BBD has been reported from reefs throughout the Caribbean, Red Sea and Indo-Pacific (reviewed in Sutherland *et al.* 2004), affecting at least 42 Caribbean and 57 Indo-Pacific coral species (Sutherland *et al.* 2004; Kaczmarsky 2006; Page & Willis 2006). In the Caribbean, while BBD prevalence (proportion of infected colonies in a population) is typically lower than 5 per cent, an exceptional event (50% prevalence) was reported for *Montastrea annularis* in the Florida Keys in 1993, and BBD has been a major contributor to the declines in coral cover in Caribbean populations (reviewed in Green & Bruckner 2000).

In the Great Barrier Reef (GBR) region, BBD was first reported from 19 reefs in 1993–1994 (Miller 1996). Low levels of BBD prevalence (0.0–0.7%) were reported throughout the GBR reef system in the summer of 2004 (Page & Willis 2006). Records of BBD abundance in yearly surveys on 48 reefs spanning the GBR between 1998 and 2004 also suggest that the disease typically remains at low background levels (Willis *et al.* 2004) and there have been no reports of destructive epizootics similar to those in the Caribbean. Although some investigations have been multi-year and multi-seasonal, most estimates of BBD prevalence have been based on 'snapshot' observations or infrequent surveys. However, population impacts of the disease are best evaluated from continuous monitoring of individually recognized colonies. Given potentially rapid rates of tissue loss caused by BBD, the disease is a potential threat to Indo-Pacific coral populations and warrants monitoring even in well-managed reef systems such as the GBR.

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Electronic supplementary material is available at http://dx.doi.org/10.1098/rspb.2009.0481 or via http://rspb.royalsocietypublishing.org.

Received 1 April 2009
Accepted 14 April 2009

Clumped BBD distribution patterns and apparent spread of disease to neighbouring colonies suggest that BBD is transmissible potentially through water movement and direct contact of colonies (Kuta & Richardson 1996; Bruckner & Bruckner 1997; Voss & Richardson 2006), although specific transmission modes and mechanisms of band formation are still poorly understood. A range of micro-organisms have been identified from the characteristic disease band, including cyanobacteria, sulphate-reducing *Desulfovibrio* bacterial species, sulphide-oxidizing *Beggiatoa* bacterial species, a marine fungus and other heterotrophic microbes (reviewed in Richardson 2004). These micro-organisms maintain a tightly organized synergistic community that causes host tissue necrosis (Carlton & Richardson 1995; Richardson 2004). Identification of the primary causative agent of BBD has been difficult, although complex microbial associations within the microbial mat suggest that the BBD is a polymicrobial disease. The effects of an anoxic micro-environment, in combination with sulphide production and cyanobacterial toxins, contribute to coral tissue degeneration (Richardson *et al.* 2007).

Understanding potential links between environmental fluctuations and BBD abundance is an important component of epizootiological assessment and vital for identifying outbreak drivers. Seasonal patterns in BBD abundance have been documented in conjunction with a number of environmental parameters. For example, it has been suggested that sea water temperature is a major driver of seasonal variability of BBD since strong positive correlations between sea water temperature and BBD abundance have been demonstrated in field studies (e.g. Bruckner *et al.* 1997; Borger & Steiner 2005; Voss & Richardson 2006; Rodriguez & Croquer 2008). This is of particular concern given predicted increases in global ocean temperatures (e.g. Hansen *et al.* 2006). Light intensity is also an important seasonal variable that may contribute to seasonal patterns in the dynamics of BBD. Evidence that water depth and turbidity are negatively correlated with disease abundance (Kuta & Richardson 2002; Page & Willis 2006) also suggests that the availability of light may govern occurrence of the disease. High light has been demonstrated to elicit an immediate behavioural response in the microbial community, causing upward migration of *Beggiatoa* spp. within the cyanobacterium dominated BBD mat and shifting vertical gradients of oxygen and sulphide, which potentially contribute to pathogenesis (Carlton & Richardson 1995; Viehman & Richardson 2002). While these studies suggest that seasonal fluctuations in light intensity may affect the virulence of BBD, the role of annual photoperiod cycles in driving BBD outbreak dynamics has not been previously tested.

In the summer of 2006, large numbers of BBD infections were observed on laminar corals in the genus *Montipora* on an inshore reef within the Palm Island group in the central GBR region. Given that no BBD had been observed in field studies over the past 15 years at this site (B. L. Willis 2006, personal observation) or on adjacent reefs surrounding nearby islands in the Palm Island group in a recent survey (Page & Willis 2006), the sudden increase in BBD cases can be considered an epizootic (*sensu* Stedman 2000). Since first detecting BBD, this site has been systematically monitored for 2.7 years to document the dynamics of BBD in a *Montipora* assemblage. Our main objectives were: (i) to characterize seasonal and long-term trends in the incidence (rate of appearance of new disease cases per unit time) and abundance of BBD infections (appearance of the characteristic disease signs) in a host assemblage, (ii) to identify potential seasonal environmental factors driving BBD virulence, (iii) to assess the consequences of BBD outbreaks for the host assemblage, and (iv) to examine the frequency of direct and indirect transmissions of BBD, assuming that BBD is transmitted through physical contact between colonies and/or through the water column. Monitoring of BBD dynamics in conjunction with seasonally varying environmental parameters, i.e. annual sea water temperature and light cycles, will help to identify which environmental factors play important roles in governing progression and potential transmission of the disease. Results are pertinent to other Indo-Pacific reef populations and will aid the development of possible management strategies to mitigate impacts of coral disease outbreaks.

2. MATERIAL AND METHODS

(a) *Study site and field surveys*

In January 2006, laminar and encrusting colonies of *Montipora hispida*, *Montipora aequituberculata* and *Montipora mollis* were observed to have signs of BBD on reefs fringing the southeast corner of Pelorus Island (18°33′ S, 146°30′ E), in the central region of the GBR Marine Park (GBRMP; figure 1). The study site is located on the upper reef slope, where it is exposed to strong wave surges year round caused by predominantly southeasterly winds but minimal levels of terrestrial run-off or human impact. Three replicate 10×10 m permanent quadrats, haphazardly placed at 5–10 m intervals, were established at depths of 2.5–3.0 m. Percentage cover of the dominant scleractinian corals inside the quadrats was approximately 33 per cent for *Montipora* spp., 8 per cent for *Acropora* spp., and 4 per cent for *Porites* spp. (3 per cent for other species). Observations of BBD were collected for the assemblage of laminar and encrusting species of *Montipora* because identification to the species level requires microscopic observation of coenosteal features on skeletal samples (Veron 2000) and extractive sampling within the permanent quadrats was avoided. From comparisons of field characteristics between colonies inside the quadrats with those outside that had been sampled and checked microscopically, the majority of species within the quadrats were *M. hispida*, followed by *M. aequituberculata*, and *M. mollis* comprised a minor component of the assemblage. Each quadrat encompassed between 8 and 24 colonies with BBD lesions (the characteristic dark band and exposed skeleton). Locations of all BBD colonies inside the plots were mapped and marked with numbered tags attached to substratum near each colony to facilitate relocation in subsequent surveys. No coral species other than *Montipora* displayed BBD signs in the plots throughout the study.

Tagged colonies were photographed at an angle approximately perpendicular to the colony surface to follow progression of the disease band and the fate of tagged individuals through time. A 10 cm scale was included in each photograph for calibration. During subsequent surveys, the number of BBD infected colonies and the presence or

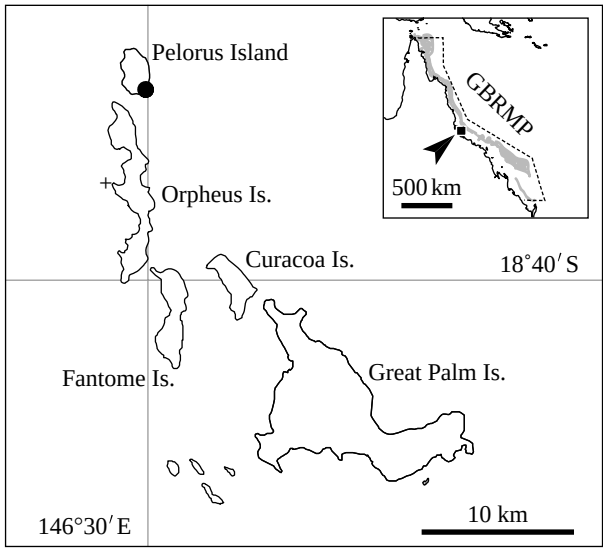

Figure 1. Location of study site (circle) at Pelorus Island in the central GBRMP (arrow), where BBD was monitored. Sea water temperature and surface light data were logged at Orpheus Island weather station (cross) throughout the study period.

absence of neighbouring colonies in direct contact with BBD infected colonies were recorded and all newly developed BBD cases were mapped and tagged in the same manner. Field data collections were conducted approximately monthly between January 2006 and August 2008, except for a two to three month interval during winter when abundance of BBD remained low. The total number of colonies of all *Montipora* species inside each quadrat was also recorded to examine prevalence of the disease in the assemblage.

(b) *Measurements of disease progression and coral tissue loss*

Disease progression and tissue loss due to BBD were measured from underwater photographs, taking advantage of the flat and easily discernable features of *Montipora* colonies, by superimposing pictures taken from two consecutive surveys (detailed in the electronic supplementary material 1). To ensure the measured tissue loss was caused only by BBD, tissue loss that had potentially occurred after disappearance of BBD signs was excluded, i.e. measurement of tissue loss caused by BBD was conservative. The original size of BBD infected colonies was measured as the area of apparently healthy tissue at the time when the infection was first recorded. Percentage tissue loss due to BBD was calculated as the area of tissue remaining when signs of BBD disappeared divided by the original tissue area.

(c) *Data analyses*

To assess the frequency of potential BBD transmission over time, the number of newly developed BBD cases per unit time (standardized as week^{-1}) and an index representing the extent of infectiousness (infectiousness index) were calculated for each quadrat between two consecutive surveys. The infectiousness index was defined as the number of newly contracted BBD cases between two surveys, divided by the number of BBD cases observed in the previous survey and the length of the intervening time interval (weeks). The index indicates the average number of potential disease transmissions per unit time for each infected colony in the susceptible assemblage, assuming that (i) abundance of BBD pathogens in the area within and surrounding the quadrat was represented by the abundance of BBD infected colonies within the quadrat, and (ii) waterborne transmission or direct contact of colonies were the sources of disease infection. When BBD was not present within the quadrat, the infectiousness index for the following survey was not calculated because the source of the infection could not be defined.

Sea water temperature data at a depth of 1 m and surface light levels (photon flux density of photosynthetically active radiation) were measured every 30 min at the Orpheus Island weather station (figure 1) throughout the study period (data available online from AIMS Weather Observing System <http://data.aims.gov.au/awsqaqc/do/advancedPlot.do>). Environmental data were used as indicators of seasonal fluctuations rather than as absolute measurements because there were minor hydrological differences between the weather station and study site. Patterns in the number of new BBD cases per unit time, the infectiousness index and linear progression rate of lesions were statistically compared against means for temperature and light data collected during the corresponding measuring period. The strengths of correlations and regressions between disease and environmental parameters were computed using a Pearson's Product Moment Correlation (r) and the General Linear Model, respectively. To meet the assumptions of normality and homogeneity of residuals required for the analyses, disease parameters were transformed as $X^{0.25}$. Relationships between living tissue area and linear progression rate of BBD or tissue loss per unit time were also examined with the same analyses. Regressions were carried out with the statistical analysis package, STATISTICA (StatSoft, Tulsa, OK).

A G-test was used to compare infection data between 2007 and 2008 to determine whether colonies previously infected with BBD had higher incidence of the disease than colonies that did not have BBD during at least the previous 12 months. Yates' correction for continuity was applied in the G-tests because the number of recurrences was limited.

3. RESULTS

(a) *Temporal patterns in BBD and environmental parameters*

Overall, a total of 485 colonies of laminar and encrusting *Montipora* species were monitored for 2.7 years, providing evidence of recurring BBD outbreaks annually in summers between 2006 and 2008. Outbreaks were positively correlated with sea water temperature and light fluctuations (figure 2*a*,*b*). Daily average sea water temperatures fluctuated between 20 and 30°C, but did not exceed 30°C throughout the study period, except for a short period in February 2006 when temperatures reached 30.4°C (figure 2*a*). Bleaching was not detected in *Montipora* species nor in any other coral during the 2.7 year period, providing corroborative evidence that temperatures did not exceed upper thermal thresholds for bleaching (Berkelmans & Willis 1999) at this site. Daily average light levels fluctuated extensively due to daily changing cloud cover, but overall, annual maximum levels occurred in November–December at approximately 710 μmol m^{-2} s^{-1} in both 2006 and 2007.

BBD abundance peaked at 16 ± 6, 15 ± 8 and 11 ± 3 infected colonies per 100 m^2 in the summers of 2006, 2007 and 2008, respectively (mean $\pm$ s.e., $n=3$ quadrats),

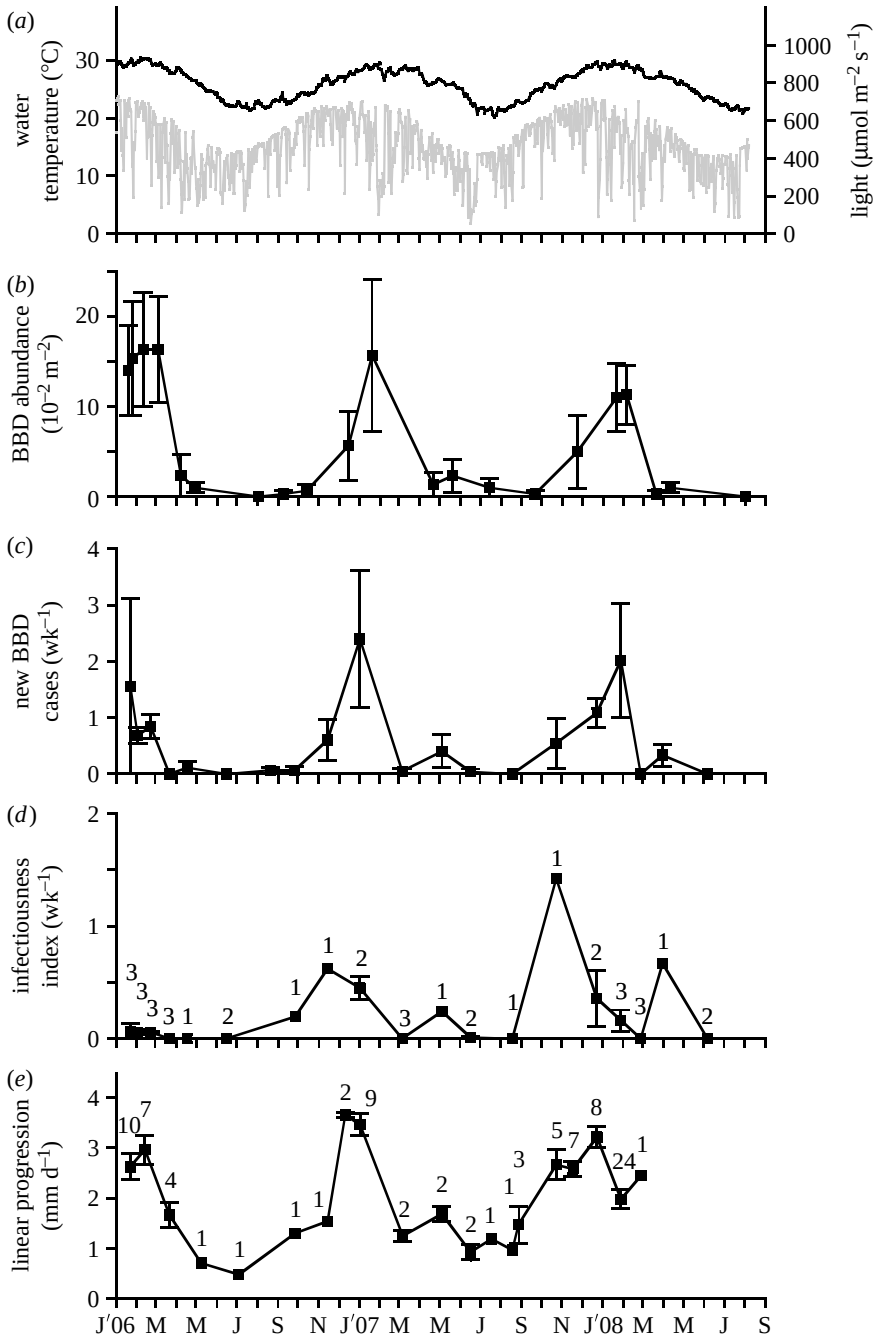

Figure 2. Temporal patterns in (*a*) daily average sea water temperature (black line) and surface light levels (grey line), (*b*) abundance of BBD per 100 m^2 $n=3$ quadrats, (*c*) number of new BBD infections per unit time (standardized as week^{-1}) $n=3$ quadrats, (*d*) infectiousness index of BBD (representing potential transmissibility), and (*e*) linear progression rate of BBD lesion. Data in (*b–e*) represent means$\pm$s.e. (*c–e*) are plotted in the middle of each measurement period as indicated on the horizontal axis. Numbers above plots are sample sizes for quadrats (*d*) and colonies (*e*).

during the period of maximum sea water temperature each year (January–February; figure 2*b*). The numbers of BBD infected colonies decreased when sea water temperature started to decline (figure 2*a*,*b*), and were stable at low levels between April and September. BBD was absent within the quadrats in July 2006, although BBD infections were observed locally outside the plots throughout the study. BBD was most prevalent in January 2007 when mean ($\pm$s.e.) prevalence reached 9.6$\pm$5.1% ($n=3$ quadrats each encompassing 157–168 colonies).

Occurrence of new BBD infections per unit time increased from October to January, reaching a mean peak of 2.0$\pm$1.0 and 2.4$\pm$1.2 colonies per week ($n=3$ quadrats) in 2007 and 2008, respectively, and was lowest ($<0.4\pm0.3$ colonies per week) between March and August each year (figure 2*c*). Increases in the number of new cases per unit time between October and January reflected an accelerating increase in the cumulative number of colonies infected, although this is not apparent from figure 2*b* due to the concurrent disappearance of BBD signs on a number of colonies. The number of new infections per unit time was significantly and positively associated with both sea water temperature ($r=0.4697$, d.f.$=1$, $F=18.967$, $p<0.001$, $n=60$ measurements) and light ($r=0.5252$, d.f.$=1$, $F=25.909$, $p<0.001$, $n=60$ measurements; electronic supplementary materials 2 and 3). Variability among quadrats was also significant in the tests for both temperature (d.f.$=2$, $F=5.501$,

Table 1. Comparison of BBD incidence between colonies with and without a history of BBD infection in the previous 12 months. (Data were collected during BBD outbreaks in an assemblage of *Montipora* species throughout 2007 and 2008. Incidence of BBD infections was tested between the non-infected assemblage and previously infected assemblage using a *G*-test with Yates' continuity correction.)

	2007		2008	
	not infected previously	infected previously	not infected previously	infected previously
population size (colonies)	559	16	685	50
BBD infections (colonies)	54	6	43	11
incidence	9.7%	37.5%	6.3%	22.0%
G_{Yates}	7.666		17.129	
d.f.	1		1	
p-value	<0.01		<0.001	

$p=0.007$) and light (d.f.$=2$, $F=6.010$, $p=0.004$), reflecting consistently lower numbers of new BBD cases in one quadrat. However, patterns of increasing or decreasing new BBD cases with temperature and light were identical in all three quadrats.

The infectiousness index peaked at 0.62 in September 2006 and at 1.45 in October 2007 (figure 2*d*), prior to January peaks each year in both BBD abundance and new BBD cases per unit time. There was a smaller peak in the infectiousness index of 0.67 in April 2008, which corresponded to a rise in light levels from an anomalous dip in March (figure 2*a*). Overall, however, the index started to decrease in December–January, when the light levels were declining from their annual maxima, yet sea water temperatures were still rising (figure 2*a*,*d*). The index was not significantly associated with temperature ($r=0.2072$, d.f.$=1$, $F=2.908$, $p=0.097$, $n=38$ measurements) but it was positively associated with light levels ($r=0.5550$, d.f.$=1$, $F=17.578$, $p<0.001$, $n=38$ measurements). Variability among quadrats was not significant in the regression models for either temperature (d.f.$=2$, $F=2.079$, $p=0.141$) or light (d.f.$=2$, $F=2.278$, $p=0.118$).

Mean ($\pm$s.e.) linear progression rate of BBD was greatest between December and February each year and reached a maximum of 3.7 ± 0.1 mm d^{-1} in 2007 (maximum linear progression rates ranged from 3.0 ± 0.3 mm d^{-1} in 2006 to 3.2 ± 0.2 mm d^{-1} in 2008; figure 2*e*). Mean rates of linear progression were lowest between autumn and spring (<1.7 mm d^{-1}). Linear progression rates of the disease band were significantly and positively correlated with both temperature ($r=0.4034$, d.f.$=1$, $F=8.331$, $p=0.007$, $n=92$ measurements) and light ($r=0.6383$, d.f.$=1$, $F=24.086$, $p<0.001$, $n=92$ measurements). While sample size was small during winter due to the low abundance of BBD infections, colony effect was not significant in the regression models for either temperature (d.f.$=60$, $F=1.290$, $p=0.226$) or light (d.f.$=60$, $F=1.287$, $p=0.227$).

(b) *Reappearance of BBD and probability of direct transmission*

During BBD outbreaks in 2007 and 2008, recurrent BBD infections were observed on colonies that were deemed to be in remission because BBD signs had disappeared. In both 2007 and 2008, *G*-tests indicated that the incidence of BBD on previously infected colonies was significantly higher than in the proportion of the assemblage that had no signs of BBD in the previous 12 months (table 1). A total of 31 per cent of recurrent BBD lesions appeared at the same site on colonies as the previous lesion ($n=13$ infections).

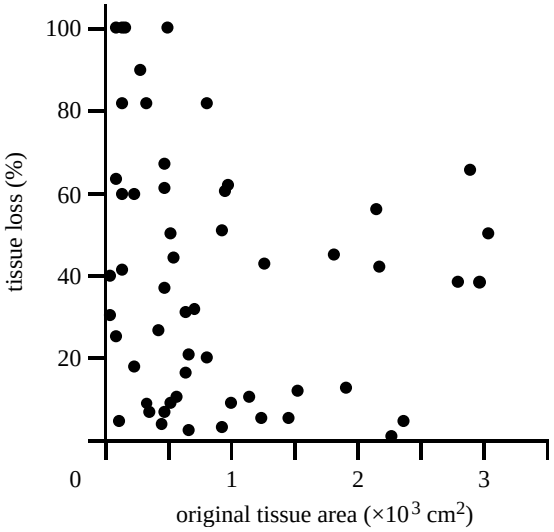

Figure 3. Relationship between original tissue area of *Montipora* colonies and percentage tissue loss by BBD ($n=57$ colonies).

Most BBD lesions appeared for the first time on physically isolated colonies. Throughout the study period, only 3 per cent of BBD infections ($n=178$ infections) appeared to be transmitted from a neighbouring colony through direct contact.

(c) *BBD impacts on infected coral assemblage*

The average area of tissue loss caused by a single BBD infection was 304 cm^2 ($n=57$ infections), although 75 per cent of disease cases caused less than 300 cm^2 of tissue loss (electronic supplementary material 4). While the average percentage tissue loss was 40 per cent ($n=57$ colonies), 37 per cent of BBD infected colonies lost more than 50 per cent of their original tissue area. There was a negative relationship between original tissue area and percentage tissue loss, with smaller colonies suffering a larger percentage tissue loss, including death of entire colonies (figure 3), although the largest area of tissue loss caused by one BBD lesion was more than 1800 cm^2, which accounted for 65 per cent of the original live tissue area of one large (2900 cm^2) colony. However, no significant correlation was detected between linear

rate of BBD progression (between January 2008 and February 2008) and remaining tissue area on the corresponding colony ($r=-0.023$, d.f.$=1$, $F=0.011$, $p=0.918$, $n=23$ colonies, tissue areas ranged from 48 to 2447 cm^2). Similarly, no correlation was detected between tissue area loss per unit time during the same period and original colony size ($r=0.033$, d.f.$=1$, $F=0.023$, $p=0.881$, $n=23$ colonies, colony size ranged from 48 to 7419 cm^2). Case fatality (number of infections that led to death, divided by the total number of infections) during the outbreak each year was approximately 5 per cent ($n=23$, 57 and 51 colonies in the 2006, 2007 and 2008 outbreaks, respectively) and only three BBD cases in 2007 remained until the next summer outbreak, thus the majority of infected colonies (90–95%) survived BBD infection. Most of the surviving colonies remained apparently healthy after signs of BBD disappeared. However, 7.6 per cent of surviving colonies that had ceased to have any visible signs of disease subsequently died ($n=105$ colonies). On average, such colonies had remaining tissue areas of 59 cm^2 ($n=8$ colonies).

4. DISCUSSION

Annual summer outbreaks of BBD were observed between 2006 and 2008 in an assemblage of *Montipora* species on reefs surrounding Pelorus Island in the central, inshore GBR region. The average prevalence of BBD peaked at 9.6 per cent, which is the highest prevalence of BBD recorded on the GBR to date (cf. Dinsdale 2002; Page & Willis 2006) and constitutes the first report of a BBD epizootic (*sensu* Stedman 2000) in the region. Peak prevalence during the outbreak was greater than the previous highest record of BBD prevalence on an Indo-Pacific reef (7.8% for a Philippine population of *M. aequituberculata*; Kaczmarsky 2006), but comparable to BBD prevalences on some of the most severely affected sites in the Caribbean (Edmunds 1991; Bruckner *et al.* 1997; Voss & Richardson 2006). Maximum rates of BBD progression (3.7 ± 0.1 mm d^{-1}) in our study are also comparable to rates on massive corals in the Caribbean (mean$=3$ mm d^{-1}; maximum$=2$ cm d^{-1}; Kuta & Richardson 1997). This study confirms that coral diseases such as BBD, which have caused significant mortality in Caribbean coral assemblages (Green & Bruckner 2000), can also reach epizootic proportions on Indo-Pacific reefs.

(a) *Effects of seasonal fluctuations in sea water temperature and light on BBD dynamics*

Our results indicate that seasonal fluctuations in both sea water temperature and light drive the occurrence of BBD infections and virulence of BBD lesions. The observed temperature-driven increases in BBD are potentially explained by host- and/or pathogen-responses to seasonal thermal fluctuations. High (but not anomalous) summer sea water temperatures cause stress to coral hosts (Fitt *et al.* 2001) and increase their susceptibility to disease infections such as fungal pathogens (Alker *et al.* 2001). Cyanobacterium species dominating the biomass of BBD bacterial mats (i.e. *Geitlerinema* species, formerly referred to as *Phormidium corallyticum*; Myers *et al.* 2007) have an optimal photosynthetic production rate at or above 30°C (Richardson & Kuta 2003). Other cyanobacterial species associated with BBD mats, such as strains closely related to an *Oscillatoria* species, have been shown to occupy the same ecological niche within BBD mats as *Geitlerinema* species (Myers & Richardson 2009) and have been detected worldwide (reviewed in Myers *et al.* 2007). Molecular analysis of cyanobacterial 16S rRNA gene sequences associated with BBD on *Montipora* species at the study site demonstrated 99 per cent sequence similarity to that of the ubiquitous BBD *Oscillatoria* strain (Y. Sato 2008, unpublished data). Although an optimal temperature for the ubiquitous BBD *Oscillatoria* has not been reported thus far, the summer outbreaks of BBD reported here indicate that higher temperatures may also be favourable for this strain. Increased cyanobacterial biomass under higher temperatures may be important in BBD pathogenesis by increasing local cyanotoxin production (Richardson *et al.* 2007) and/or generating dynamic vertical micro-gradients of oxygen and sulphide, which have been implicated in coral tissue degeneration (Carlton & Richardson 1995; Richardson *et al.* 1997). Enhancement of BBD progression rates under higher temperatures on GBR corals has also been demonstrated experimentally (Boyett *et al.* 2007), which further supports the positive association between temperature and BBD virulence found in the present study. Importantly, positive correlations between BBD activity and sea water temperature suggest that warmer ocean conditions will lead to longer BBD outbreak events and more rapid tissue loss, thus more intense degradation of Indo-Pacific coral populations.

Our results also support light as an environmental driver of both linear progression and incidence of BBD in the *Montipora* host assemblage. Previous microbial studies have shown that BBD-associated cyanobacterial species are adapted to low light levels and known to have a 'self-shading' behaviour under high light conditions (Kuta & Richardson 2002; Richardson & Kuta 2003). Clumping behaviour of cyanobacteria has been suggested to contribute to the pathogenesis of BBD by providing anoxic conditions that favour other pathogenic community members, such as sulphate-reducing *Desulfovibrio* species and sulphide-oxidizing *Beggiatoa* species (Kuta & Richardson 2002; Richardson & Kuta 2003; Myers *et al.* 2007). In particular, sulphide produced by *Desulfovibrio* species causes coral tissue lysis (Richardson *et al.* 1997) as potentially does the cyanotoxin and microcystin (Richardson *et al.* 2007). Thus, cyanobacterial clumping in response to high light may accelerate rates of disease progression. Evidence that coral-associated microbial communities vary with depth (Klaus *et al.* 2007) further corroborates conclusions that solar irradiance is a key factor structuring coral microbial communities, and thus seasonally changing light levels may affect the virulence of BBD microbial communities. An experimental study also reported no difference in the probability of BBD transmission under different temperature regimes (Aeby & Santavy 2006). Therefore, seasonally increasing light levels may be more important in the frequency of new BBD infections than seasonally rising sea water temperatures.

Identifying an independent effect of a specific environmental factor is often difficult in field studies because environmental variables are typically correlated with each other. However, in the current study, seasonal patterns in light preceded seasonal patterns in sea water temperatures

by approximately two months. The significant association of our infectiousness index with light but not with sea water temperature suggests that light plays an important role in driving new infections. Boyett *et al.* (2007) also proposed that strong light enhances BBD progression rates under elevated temperatures. Enriquez *et al.* (2005) describe the physical mechanism by which high solar radiation synergistically exacerbates oxidative stress in heat-stressed corals, and a number of studies have experimentally demonstrated that solar radiation increases damage to both coral tissues and symbiotic algae experiencing thermal stress (e.g. Brown 1997; Lesser & Farrell 2004). Such synergistic effects of temperature and light may also contribute to the observed seasonal patterns of BBD virulence. There is need for a manipulative experiment, with temperature and light as independent variables, and a larger scale field study, considering the small (300 m^2) spatial scale of the current study, to unequivocally separate temperature and light effects on BBD transmission and progression rates.

(b) *Potential source of BBD infection*

Apparent direct transmission of BBD between physically connected colonies was recorded but was not the major mode of spread of the disease. Similar observations have been recorded in past studies (e.g. Kuta & Richardson 1996; Sutherland *et al.* 2004), although specific transmission mechanisms are still unknown. One potential transmission mechanism is transport of the BBD bacterial community by water movement since a developed BBD bacterial mat is easily sloughed off into the water column (Richardson 2004). Bruckner *et al.* (1997) recorded spread of BBD infections over 3 km in a down-current direction, suggesting mechanical transport of BBD pathogens by water movement. The study site at southeast Pelorus Island is constantly exposed to strong wave surges, therefore discharge and local transport of BBD bacterial mats by water movement is possible.

Recurrence of BBD on previously infected colonies is common (Kuta & Richardson 1996; Bruckner & Bruckner 1997; Voss & Richardson 2006; Rodriguez & Croquer 2008), suggesting that colonies which survive BBD may act as reservoirs for pathogens. While results suggest that 31 per cent of recurrent lesions in our study may have been caused by residual pathogens, we cannot distinguish whether recurrent BBD lesions observed at different sites than previous infections were caused by pathogens from the water column or by pathogens remaining on the colony that were motile and present at visually undetectable levels. It is also possible that members of BBD-associated microbial communities present in either healthy coral tissues (Frias-Lopez *et al.* 2002; Klaus *et al.* 2007), dead coral skeleton (Frias-Lopez *et al.* 2002) or sediment on live coral (Richardson 1997) caused the disease in response to environmental or biological triggers (Rohwer *et al.* 2002). Additionally, both vectors (see Aeby & Santavy 2006) and reservoirs for BBD pathogens other than infected coral colonies (Richardson 1997) may play important roles in BBD transmission.

(c) *Impact of BBD on coral assemblages*

Our study suggests that small colonies are most likely to suffer whole colony mortality, as indicated by a negative correlation between original tissue area of colonies and percentage tissue loss caused by BBD infections. Two factors contributed to this pattern: (i) linear progression rate of the band was not dependent on host tissue area, and (ii) most BBD infections started as small lesions and disappeared within a season, thus smaller colonies were more likely to lose larger proportions of live tissue area. However, an extensive (more than 1800 cm^2) tissue loss caused by one BBD lesion was recorded on one large (2900 cm^2) colony, indicating that a BBD lesion can potentially kill a substantial proportion of host tissue if environmental conditions (e.g. sea water temperature, light) are favourable. Moreover, the impact of BBD on host population dynamics is potentially larger than the apparent loss of tissue area reported here because substantial tissue loss may result in cessation of reproductive activity regardless of colony age (Szmant-Froelich 1985). It is also important to note that the current study underestimated tissue loss by excluding mortality on tagged colonies when BBD signs disappeared between visits.

It is notable that, after the disappearance of visible BBD lesions on some small colonies, whole colony mortality nevertheless occurred. These observations suggest that coral health may be impaired even after BBD signs disappear and/or that continued tissue loss may be caused by BBD pathogens remaining at visually undetectable levels, potentially within the skeleton (Ainsworth *et al.* 2007). Photoinhibition of symbiotic algae has been demonstrated in apparently healthy tissue areas of coral hosts near BBD lesions (Roff *et al.* 2008), suggesting that BBD affects the host before the band migrates over nearby tissues. Considering the small size of most colonies that suffered whole colony mortality after a BBD infection in our study, this potential distant impact of BBD before the band disappeared may have been lethal to tissues remaining on small colonies. Patterns of susceptibility to BBD infection for specific *Montipora* species are needed in future epizootiological studies to further validate colony size and mortality patterns found in the present study.

The approximate 3.5-fold higher incidence of BBD we observed on previously infected colonies, in comparison to colonies that had no previous history of BBD signs, accords with high probabilities of BBD recurrence reported for a Venezuelan population of *Diploria strigosa* (Rodriguez & Croquer 2008) and highlights the vulnerability of large colonies to recurrent infections. Although the source of pathogens in recurrent cases is unclear, the following hypotheses may contribute. BBD may compromise a coral's immune responses, which may include amoebocytes (Hildemann *et al.* 1977), melanin deposition (Palmer *et al.* 2008) and antibacterial chemicals (Koh 1997; Gochfeld *et al.* 2006; Ritchie 2006), increasing its susceptibility to recurring summer infections. Secondly, although undetectable in the field, pathogens may remain on or within apparently healthy colonies and act as winter reservoirs, contributing to reappearance of BBD signs in the following summer. It is, thus, possible that the history of past disease infections is a colony-specific factor governing susceptibility to BBD. It has also been suggested that susceptibility to coral disease increases with decreasing colony size (Kramarsky-Winter 2004; Sutherland *et al.* 2004; Kaczmarsky *et al.* 2005).

Therefore, while larger colonies are more likely to survive a BBD infection despite a potentially greater loss of tissue area, the surviving colonies have high probability of recurrence of BBD, leading to further tissue loss in subsequent infections. Small colonies, on the other hand, have higher percentage tissue loss overall and may suffer further tissue loss after disappearance of BBD signs, potentially causing whole colony mortality. The case fatality was calculated at 5 per cent; however, this study suggests that the long-term consequences of BBD on host coral population dynamics can be greater due to 'post BBD infection' effects, particularly disease recurrence on large colonies and continuous tissue loss on small colonies.

(d) *Conclusions*

Our study documents the first BBD epizootic on the GBR and highlights size-related patterns in mortality caused by BBD infections that have significant long-term implications for Indo-Pacific populations of *Montipora* species. Sea water temperatures and light levels were identified as environmental drivers governing the abundance and virulence of BBD, with light having a potentially greater role in infectiousness. The long-term nature of our study revealed the seasonally fluctuating nature of BBD dynamics, with infections increasing exponentially in summer and declining to low levels in winter. Therefore, frequent reef monitoring should be encouraged to detect potential disease outbreaks that otherwise terminate, resulting in loss of important information relating to disease impacts. It is likely that warmer sea water temperatures predicted in association with global warming will exacerbate the impacts of BBD on Indo-Pacific reefs by increasing rates of tissue loss and the duration of outbreak events.

This study was funded by the ARC Centre of Excellence for Coral Reef Studies and the GEF Disease Working Group in the Coral Reef Targeted Research and Capacity Building for Management Program, and was supported logistically by AIMS@JCU. Authors thank staff of James Cook University's Orpheus Island Research Station for their logistic support, Dr Craig Syms for his support in statistics, and numerous volunteers for their field assistance.

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