NOTE

Appearance of an anomalous black band disease at upper mesophotic depths after coral bleaching

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ABSTRACT: In the summer of 2016, extensive coral bleaching occurred on the coral reefs of Okinawa, Japan, which was often lethal in shallow waters. In November 2016, after the coral bleaching event, an anomalous black band disease (ABBD) was noticed during regular monitoring of the Pachyseris-dominated upper mesophotic Ryugu Reef in Okinawa. We subsequently conducted 10 \times 1 m belt transects in deeper (30–35 m depth, n = 5) and shallower areas (27–29 m, n = 7) of the reef and obtained data on coral percentage coverage, Pachyseris percentage coverage, occurrence (numbers of lesions) of ABBD, and ABBD percentage coverage on *Pachyseris*. Both depths showed high live coral coverage (>90%), indicating little mortality from the summer 2016 bleaching event, and Pachyseris percentage coverage was significantly higher in the deeper area (mean \pm SD = 48.6 \pm 45.0%) compared to the shallower area (5.1 \pm 5.0%). Additionally, although numbers of ABBD occurrences (= lesions) were significantly higher in the deeper area (81.0 ± 52.8 as opposed to 8.3 ± 6.7 at shallower depths), total ABBD percentage coverage on Pachyseris was not significantly different between shallow and deep areas $(7.0 \pm 3.2\% \text{ versus } 4.7 \pm 3.6\%)$. ABBD was observed to be Pachyseris-specific at Ryugu Reef. These results indicate that similar to shallower reefs, mesophotic reefs can experience increased disease occurrences subsequent to non-lethal coral bleaching events.

KEY WORDS: ABBD · Coral · Mesophotic reef · Water temperature · Okinawa · Pachyseris

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INTRODUCTION

In the summer of 2016, extensive coral bleaching occurred on the coral reefs of Okinawa in southern Japan, with high rates of mortality in some regions and in 'shallow waters' (Kayanne et al. 2017, Ministry of the Environment 2017), although concrete numbers and depth information remain unpublished. In November 2016, during regular monitoring work of the upper (shallow) mesophotic Ryugu Reef, after summer coral bleaching had subsided, we noticed the appearance of purple-black bands on upper mesophotic *Pachyseris* colonies. This disease was confirmed via initial microscopic analyses to not be classic black band disease (BBD), but instead an anomalous black band disease (ABBD) due to the absence of large *Beggiatoa* sp. (Fig. 1; see also Rützler & Santavy 1983, Richardson 1992, 1998, Santavy & Peters 1997, Weil et al. 2006, Sussman et al. 2006). Although Ryugu has been monitored intensively since 2012 (Ohara et al. 2013), this was the first time any type of BBD was noticed at this site (absence of BBD from transect data reported by White et al. 2013, 2017).

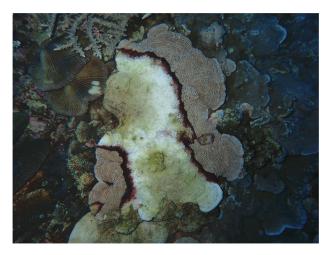


Fig. 1. Purple-black band of anomalous black band disease at 28 m depth on a *Pachyseris* colony at Ryugu Reef, Oki-nawa-jima, Japan, on 20 November 2016

Rützler & Santavy (1983) first discovered an 'anomalous BBD' on gorgonian colonies in Belize, and this BBD was initially considered as a 'suspected gorgonian black band disease'. This disease has been reported from coral reefs at 15-16 m depth in the Bahamas (Richardson 1992, 1998, Santavy & Peters 1997). For some time, the disease was believed to be a different disease, named red band disease (RBD). However, this disease is now believed to be the same as BBD, as Sussman et al. (2006) phylogenetically examined anomalous BBD in the Indo-Pacific from Palau, and isolated cyanobacteria from the BBD tissue, showing conspecificity with so-called 'normal BBD'. At the same time, the strains involved in BBD may vary regionally (Miller & Richardson 2011, Aeby et al. 2015). Anomalous BBD (formerly designated as RBD) resembles BBD in general appearance (Rützler & Santavy 1983, Richardson 1992, 1998, Sussman et al. 2006) and has been occasionally reported from somewhat deeper depths (e.g. 15-20 m; Richardson 1992, Weil et al. 2002, 2006). In total, anomalous BBD has been reported on 13 scleractinian coral species and 1 octocoral species in the Caribbean (Weil et al. 2002, 2006).

However, BBD has only been sporadically reported from Japan, including from the Ryukyu Islands (Yamashiro 2004, Irikawa 2006, Weil et al. 2012, Wada et al. 2017, 2018). In addition, there is a general lack of data and ecological studies on BBD in mesophotic reefs. Here, we report on the abundance and distribution of an ABBD in the upper mesophotic Ryugu Reef in 2016, examining if the ABBD occurrences differed by depth or by host coral, compared with previous BBD reports from southern Japan, and discuss possible causes of this occurrence.

MATERIALS AND METHODS

Coral bleaching occurred in summer 2016 at Ryugu Reef in Okinawa-jima Island (26° 30' 38" N, 127° 52' 53" E) at all depths (5–42 m; Stations 5 to 1, respectively, as defined in Fig. 1B in White et al. 2013), with water temperatures much higher at the site in summer 2016, even at depths of 35 m (= Stn 2), when compared to previous recent summers (2013, 2015) when no bleaching was observed (Fig. 2). ABBD was first noticed after bleaching had subsided on 1 November 2016, at Stns 2 and 3, but not at the more shallow Stns 4 and 5.

We subsequently investigated this ABBD occurrence via belt transects at Ryugu on 20 November 2016. Random belt transects $(10 \times 1 \text{ m})$ were placed at 5 locations near Stn 2 (30-35 m) and 7 locations near Stn 3 (27–29 m). As described by White et al. (2013, 2017), these 2 stations, despite being very similar in depth, have very different coral communities, with Stn 2 dominated by Pachyseris with some Acropora and Galaxea, and Stn 3 hosting a more diverse community including not only Pachyseris but also Acropora, Galaxea, Porites, Seriatopora, and Stylophora (White et al. 2017). Digital photographs were taken from directly above the substrate along each transect $(n = 21-31 \text{ images transect}^{-1})$, and from these images, we calculated coral percentage coverage, Pachyseris percentage coverage, occurrence (= numbers of lesions) of ABBD, and ABBD percentage coverage on *Pachyseris* for each transect using the image analysis software ImageJ (http://rsbweb.nih.gov/ij/).

We compared the average coral coverage, *Pachyseris* coverage, ABBD coverage, and number of ABBD lesions at Stns 2 and 3 for significant differences using the Mann-Whitney test. We also tested for correlation between *Pachyseris* coverage and number of ABBD lesions via Spearman's rho value. Statistical analyses were performed with RStudio v.1.1.456 (RStudio Team 2015).

We obtained temperature data from loggers placed at Stns 2 and 3 (HOBO U22 Temp Pro v2 logger) for the period from March 2013 to March 2014, and from March 2015 to March 2017. We used the data to calculate degree-heating weeks (DHW), following Liu et al. (2003) (Fig. 3). DHW values of >4.0°C-weeks usually indicate the start of bleaching, and DHW values of >8.0°C-weeks can result in widespread bleaching (Kayanne et al. 2017).

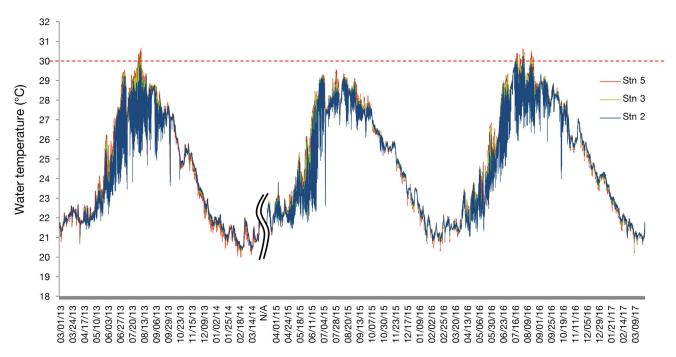


Fig. 2. Water temperatures at Stns 2, 3, and 5 (~35, 28 and 5 m depth, respectively) at Ryugu Reef, Okinawa, Japan. Temperature data were recorded from 1 March 2013 to 31 March 2014, and from 16 March 2015 to 31 March 2017. Red dashed line shows 30°C (average summer maximum sea surface temperature for Okinawa-jima)

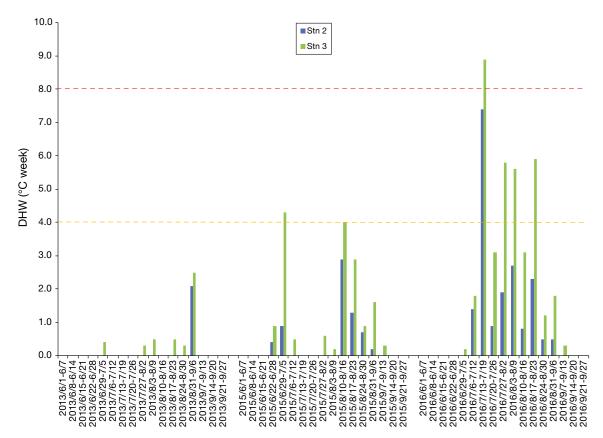


Fig. 3. Degree heating weeks (DHW) at Stns 2 and 3 on Ryugu Reef, Okinawa, Japan, in 2013, 2015, and 2016 from June to September. A DHW >4.0°C-week (yellow dashed line) generally induces bleaching, a DHW >8.0°C-week (red dashed line) induces widespread bleaching (Kayanne 2017, Kayanne et al. 2017). DHW was calculated using average water temperature in August 2013, 2015, and 2016 at Stns 2 and 3

RESULTS AND DISCUSSION

Both stations showed high coral coverage, with the average coverage being 93.9% at the shallower Stn 3, and 97.9% at the deeper Stn 2 (Table 1). In particular, Pachyseris coverage was significantly different between the 2 areas; mean \pm SD = 5.1 \pm 4.6% at Stn 3 and $48.6 \pm 40.2\%$ at Stn 2 (Mann-Whitney test, p < 0.027). Additionally, there was a significant difference in the number of occurrences of ABBD, with Stn 3 having 8.3 ± 6.2 occurrences (total occurrences = 56; Table 1) and Stn 2 having 81.0 ± 47.2 per 10 m transect (total occurrences = 405, Table 1) (Mann-Whitney test, p < 0.004). However, the ABBD coverage percentage on *Pachyseris* was not significantly different between the 2 stations (Stn 3, $4.7 \pm 3.3\%$; Stn 2, 7.0 \pm 2.9%). No other diseases were noted on corals from the transect images or during the surveys.

Although there was some bleaching observed at both Stns 2 and 3 during summer 2016 (T. Ohara pers. comm.), by November 2016 corals at Ryugu appeared to have recovered, as no bleaching from thermal stress was apparent. Moreover, based on high live coral cover (Table 1) and comparison with 2015 data (74% live cover at Stn 2, 62% at Stn 3; White et al. 2017), mortality from the summer 2016 event was apparently very low. However, coral disease occurs more often after sub-lethal coral bleaching events in the Caribbean (Cróquer & Weil 2009, Miller et al. 2009). In particular, coral reefs in deeper areas (>15 m) influenced by coral bleaching showed increases in yellow band disease (Cróquer & Weil 2009). Similarly, Bruno et al. (2007) demonstrated a positive relationship between coral bleaching in 2002 and increased disease incidence on the Great Barrier Reef (GBR). However, the GBR study was at 6-9 m

depth, and thus these previous reports are not from mesophotic reefs. Our observations show that upper mesophotic corals are not immune to post-bleaching disease, and we hypothesize that the *Pachyseris* colonies at Ryugu were weakened subsequent to the sublethal bleaching experienced in summer 2016 and became more susceptible to disease (ABBD).

During this survey, we did not record ABBD on any coral except *Pachyseris*, and the occurrences of ABBD had a proportionate and significant relationship to the amount of *Pachyseris* coverage present (p < 0.0001, R = 0.95622, Spearman's rho). Therefore, it is possible that the observed strain of ABBD is a coral disease specific to *Pachyseris*, at least at Ryugu Reef. Alternately, it may be that the *Pachyseris* colonies were simply more susceptible to ABBD in our transect areas, as differences in rates of BBD susceptibility have been reported in different corals from other regions (Willis et al. 2004, Page & Willis 2006, Aeby et al. 2015).

BBD has previously been reported as having a low abundance in southern Japan (Ishigaki Island in Yamashiro 2004, Irikawa 2006; summarized by Weil et al. 2012), and also with a mean prevalence of $3.3 \pm$ 3.2% at depths to 30 m from Maehama, Ginowan-Oyama, on Okinawa-jima Main Island in September 2010 (Weil et al. 2012). Interestingly, that study found BBD only on P. speciosa, suggesting the susceptibility of this genus to BBD. Another report from 2010 and 2011 reported up to 3.61% of encrusting Montipora spp. colonies with BBD to 8 m depth on a reef in Akajima, Kerema Islands, Okinawa (Wada et al. 2017). Additionally, a BBD prevalence of 0.1% was observed from the Miyako Islands in surveys conducted by Okinawa Prefecture between June 2011 and February 2012 (Wada et al. 2018). These previous

Table 1. Transects at Ryugu Reef, Okinawa-jima Island, Japan, in November 2016. Live coral and Pachyseris coverage,
anomalous black band disease (ABBD) percentage on <i>Pachyseris</i> , and number of occurrences (= lesions) of ABBD were noted.
Asterisks indicate significant differences between Stns 2 and 3 (Mann-Whitney test, $*p < 0.05$). na: not applicable

Transect -	Stn 2 (30–35 m)				Stn 3 (27–29 m)			
	Coral coverage (%) ——— ABBE			D——— Coral		verage (%)	ABBD	
	Total	Pachyseris	Coverage (%)	Lesions (n)	Total	Pachyseris	Coverage (%)	Lesions (n)
1	100.0	98.0	7.4	109	100.0	14.8	5.7	20
2	97.4	97.3	7.3	159	91.9	3.6	3.7	7
3	94.4	23.5	2.7	64	99.6	7.9	2.9	9
4	97.7	14.4	6.0	33	93.7	2.4	7.9	7
5	100.0	10.0	11.7	40	77.2	5.0	10.5	13
6	na	na	na	na	95.2	1.7	1.9	2
7	na	na	na	na	99.8	0.1	0.0	0
$Mean \pm SD$	97.9 ± 2.3	$48.6\pm45.0^*$	7.0 ± 3.2	$81.0 \pm 52.8*$	93.9 ± 8.1	$5.1 \pm 5.0*$	4.7 ± 3.6	$8.3 \pm 6.7*$
Totals				405				56

reports on BBD from southern Japan all reported a \geq 2-fold lower BBD prevalence than the ~7% we observed at Ryugu Reef.

Previous reports have suggested that the abundance and prevalence of coral diseases is increasing in southern Japan (Weil et al. 2012), and the recent coral bleaching events have negatively affected shallow coral reefs in the region (Kayanne et al. 2017, Ministry of the Environment 2017). Our observations at Ryugu Reef suggest that negative effects of bleaching extend beyond increased mortality to increased ABBD occurrence on upper mesophotic *Pachyseris* colonies. Whatever the causes of the ABBD observed at Ryugu Reef, continued monitoring, particularly after abnormally high thermal events, is needed.

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LITERATURE CITED

- Aeby GS, Work TM, Runyon CM, Shore-Maggio A and others (2015) First record of black band disease in the Hawaiian archipelago: response, outbreak status, virulence, and a method of treatment. PLOS ONE 10: e0120853
- Bruno JF, Selig ER, Casey KS, Page CA and others (2007) Thermal stress and coral cover as drivers of coral disease outbreaks. PLOS Biol 5:e124
- Cróquer A, Weil E (2009) Changes in Caribbean coral disease prevalence after the 2005 bleaching event. Dis Aquat Org 87:33–43
 - Irikawa A (2006) A report on the prevalence of 'growth anomalies', a disease of hermatypic corals, in waters around Okinawa Islands, Japan. Lagoon 7:5–11 (in Japanese)
- Kayanne H (2017) Validation of degree heating weeks as a coral bleaching index in the northwestern Pacific. Coral Reefs 36:63–70
- Kayanne H, Suzuki R, Liu G (2017) Bleaching in the Ryukyu Islands in 2016 and associated Degree Heating Week threshold. Galaxea J Coral Reef Stud 19:17–18
- Liu G, Strong AE, Skirving W (2003) Remote sensing of sea surface temperatures during 2002 Barrier Reef coral bleaching. EOS Trans Am Geophys Union 84:137–141
- Miller AW, Richardson LL (2011) A meta-analysis of 16S rRNA gene clone libraries from the polymicrobial black band disease of corals. FEMS Microbiol Ecol 75:231–241
- Miller J, Muller E, Rogers C, Waara R and others (2009) Coral disease following massive bleaching in 2005 cause 60% decline in coral cover on reefs in the US Virgin Islands. Coral Reefs 28:925–937

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- Ministry of the Environment (2017) Iriomote-Ishigaki National Park Survey: results of coral bleaching phenomenon of Sekisei lagoon. http://kyushu.env.go.jp/naha/ pre_2017/post_28.html (in Japanese)
- Ohara T, Fujii T, Kawamura I, Mizuyama M and others (2013) First record of a mesophotic *Pachyseris foliosa* reef from Japan. Mar Biodivers 43:71–72
 - Richardson LL (1992) Red band disease: a new cyanobacterial infestation of corals. In: Cahoon LB (ed) Proc 10th Am Acad Underw Sci, Sept 24–27, 1992, Wilmington, NC. American Academy of Underwater Sciences, Costa Mesa, CA, p 153–160
- Page C, Willis B (2006) Distribution, host range and largescale spatial variability in black band disease prevalence on the Great Barrier Reef, Australia. Dis Aquat Org 69: 41–51
- Richardson LL (1998) Coral diseases: What is really known? Trends Ecol Evol 13:438–443
 - RStudio Team (2015) RStudio: integrated development for R. RStudio, Boston, MA. www.rstudio.com/
- Rützler K, Santavy DL (1983) The black band disease of Atlantic reef corals. I. Description of the cyanophyte pathogen. Mar Ecol 4:301–319
- Santavy D, Peters EC (1997) Microbial pests: coral disease in the Western Atlantic. Proc 8th Int Coral Reef Symp, Panama 1:607–612
- Sussman M, Bourne DG, Willis BL (2006) A single cyanobacterial ribotype is associated with both red and black bands on diseased corals from Palau. Dis Aquat Org 69: 111–118
- Wada N, Mano N, Yanagisawa Y, Mori T (2017) Occurrence of coral diseases in Akajima, Okinawa, Japan in 2010 and 2011. Galaxea J Coral Reef Stud 19:35–44
 - Wada N, Ohdera A, Mano N (2018) Coral disease in Japan. In: Iguchi A, Hongo C (eds) Coral reef studies of Japan. Coral Reefs of the World, Vol. 13. Springer, Singapore, p 41–62
- Weil E, Urreiztieta I, Garzon-Ferreira J (2002) Geographic variability in the incidence of coral and octocoral diseases in the wider Caribbean. Proc 9th Int Coral Reef Symp, Bali 2:1231–1238
- Weil E, Smith G, Gil-Agudelo DL (2006) Status and progress in coral reef disease research. Dis Aquat Org 69:1–7
- Weil E, Irikawa A, Caserato B, Suzuki Y (2012) Extended geographic distribution of several Indo-Pacific coral reef diseases. Dis Aquat Org 98:163–170
- White KN, Ohara T, Fujii T, Kawamura I and others (2013) Typhoon damage on a shallow mesophotic reef in Okinawa, Japan. PeerJ 1:e151
- White KN, Weinstein DK, Ohara T, Denis V, Montenegro J, Reimer JD (2017) Shifting communities after typhoon damage on an upper mesophotic reef in Okinawa, Japan. PeerJ 5:e3573
 - Willis BL, Page CA, Dinsdale EA (2004) Coral disease on the Great Barrier Reef. In: Rosenberg E, Loya Y (eds) Coral health and disease. Springer, Berlin, Heidelberg, p 69–104
 - Yamashiro H (2004) Coral diseases. In: Coral reefs of Japan. Ministry of Environment and the Japanese Coral Reef Society, Tokyo, p 56–59

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