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Yuya Hibino<sup>a</sup>, Peter Todd<sup>b</sup>, Carey D. Ashworth<sup>a</sup>, Masami Obuchi<sup>c,d</sup> & James Davis Reimer<sup>a,c</sup>

<sup>a</sup> Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan

<sup>b</sup> Experimental Marine Ecology Laboratory, National University of Singapore, Republic of Singapore

<sup>c</sup> Molecular Invertebrate Systematics and Ecology Laboratory, Rising Star Program, Trans-disciplinary Organization for Subtropical Island Studies, University of the Ryukyus, Okinawa, Japan

<sup>d</sup> Biological Institute on Kuroshio, Otsuki, Kochi, Japan

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ORIGINAL ARTICLE

## Monitoring colony colour and zooxanthellae (*Symbiodinium* spp.) condition in the reef zoanthid *Palythoa tuberculosa* in Okinawa, Japan

YUYA HIBINO<sup>1</sup>, PETER TODD<sup>2</sup>, CAREY D. ASHWORTH<sup>1</sup>, MASAMI OBUCHI<sup>3,4</sup> & JAMES DAVIS REIMER<sup>1,3\*</sup>

<sup>1</sup>Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, Okinawa, Japan; <sup>2</sup>Experimental Marine Ecology Laboratory, National University of Singapore, Republic of Singapore; <sup>3</sup>Molecular Invertebrate Systematics and Ecology Laboratory, Rising Star Program, Trans-disciplinary Organization for Subtropical Island Studies, University of the Ryukyus, Okinawa, Japan; and <sup>4</sup>Biological Institute on Kuroshio, Otsuki, Kochi, Japan

### Abstract

Colony colour, as assessed using waterproof colour cards, is an accurate, economical and rapid way of field monitoring coral bleaching. This technique, however, has never been applied to zoanthids, nor have variations in colony colour been directly correlated with microscopic observation of *Symbiodinium* condition. In this study, we examined 12 colonies of the common reef zoanthid *Palythoa tuberculosa* at two sites (Odo and Miyagi) in Okinawa, Japan, for one year to observe the relationship between colour card scores, *Symbiodinium* morphological condition and sea surface temperature (SST); *Symbiodinium* types within the study specimens were also examined. Colonies became paler during periods of rapid SST increase and significant correlations between *P. tuberculosa* colony colour and internal zooxanthellar condition were found for 5 out of the 12 colonies monitored. Results were more pronounced at the Odo site (significant correlations for four out of six colonies) as compared to the Miyagi site (a significant correlation for one out of six colonies). Internal transcribed spacer ribosomal DNA (ITS-rDNA) sequencing results showed no changes in *Symbiodinium* types among colonies, or between summer and winter. Due to their wide distribution, abundance and ease of identification, zooxanthellate zoanthids are good candidates for monitoring bleaching events on coral reefs.

**Key words:** Bleaching, coral reef, ITS-rDNA, sea surface temperature, stress, zoanthid

### Introduction

In recent years, zooxanthellate corals have undergone dramatic reductions in numbers due to the phenomenon of bleaching, i.e. the breakdown in the symbiotic relationship between the host and their endosymbiotic, photosynthetic dinoflagellate symbiont *Symbiodinium* spp. (e.g. Glynn 1983, 1993; Hoegh-Guldberg 1999) and/or loss of photosynthetic pigments (Fitt & Warner 1995). Bleaching has various causes, including abnormally high (Mayor 1918; Coles & Jokiel 1977; Glynn 1996) or low ocean temperatures (Lirman et al. 2011), high levels of solar radiation (Dustan 1982; Lesser et al. 1997), increased terrestrial runoff (Goreau 1964; DeVantier et al. 1997), and changes in salinity (Goreau 1964; Reimer 1971) as well as other

environmental stressors (reviewed in Brown 1997). As stress increases, the condition of the symbiont degrades due to a breakdown of photosystem II (Warner et al. 1999) leading to *Symbiodinium* elimination and/or loss of photosynthetic pigment resulting in the host's colour becoming less intense or even white/transparent (hence 'bleaching').

During the past three decades, bleaching events have increased on a global scale, and this has been linked to climate change caused by anthropogenic factors (Hoegh-Guldberg 1999). While the mechanism(s) of bleaching is generally understood, and publicly available tools can help predict broad-scale bleaching events (e.g. NOAA Coral Reef Watch 2000), the ability to identify bleaching at smaller scales often requires direct local observation (Siebeck et al. 2006). To facilitate such work, Siebeck

\*Correspondence: James Davis Reimer, Department of Chemistry, Biology and Marine Science, Faculty of Science, University of the Ryukyus, 1 Senbaru, Nishihara, Okinawa 903-0213, Japan. E-mail: [jreimer@sci.u-ryukyu.ac.jp](mailto:jreimer@sci.u-ryukyu.ac.jp)

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et al. (2006) developed waterproof colour cards ('CoralWatch Coral Health Chart') for observer-based field monitoring of bleaching that does not require in-depth training or specialist knowledge. Analysis of in-situ pictures of corals next to the card resulted in good correlations between chlorophyll content and symbiont density (Siebeck et al. 2006), providing evidence that the technique, in addition to being rapid and economical, is an accurate way of monitoring individual coral colony condition.

Previously, symbiont density and chlorophyll *a* (chl *a*) concentration have been used as indicators of a zooxanthellate coral's health, but both measurements possess drawbacks. Kuroki & Van Woesik (1999) found that symbiont density alone was not a good indicator of coral health because degraded symbionts were not always expelled, but instead remained in the host. Furthermore, chl *a* concentrations of *Symbiodinium* have been known to increase in some corals after bleaching events (Jones 1997). An alternative approach is determining *Symbiodinium* cell morphological condition, which is straightforward and requires no special equipment other than a light microscope. Even though symbiont degradation is easy to see, and density and chl *a* have been demonstrated to have some disadvantages, very few studies have utilized the examination of *Symbiodinium* cell morphological condition in anthozoans. However, variations in colony colour have not yet been directly correlated with microscopic observation of *Symbiodinium* cells, which degrade under stress. The condition of *Symbiodinium* cells can be quantified based on their morphology (shape, colour) and size (Titlyanov et al. 1996; Kuroki & Van Woesik 1999; Mise & Hidaka 2003; Reimer et al. 2007).

To date, the colour card technique has not been tested on other zooxanthellate anthozoans, for example zoanthids. Zoanthids, an order within the subclass Hexacorallia, are colonial cnidarians and common components of subtropical and tropical coral reef ecosystems (Ruppert et al. 2003; Irei et al. 2011). The order contains at least 17 genera of which at least seven include species with endosymbiotic *Symbiodinium*. *Palythoa* (Sphenopidae) and *Zoanthus* (Zoanthidae) are found in tropical and subtropical reef environments throughout the world and seasonal changes in morphological condition of *Symbiodinium* (Reimer et al. 2007) and thermal bleaching (Kemp et al. 2006) have previously been recorded in these two genera. Due to their non-endangered status and ease of identification and sampling, they could be used for monitoring bleaching events on coral reefs. Furthermore, non-scleractinian zooxanthellate anthozoans will also be impacted by rising sea temperatures, and it is important to more fully understand their responses to thermal stress. *Palythoa* spp. have been noted to bleach early (Williams & Bunkely-

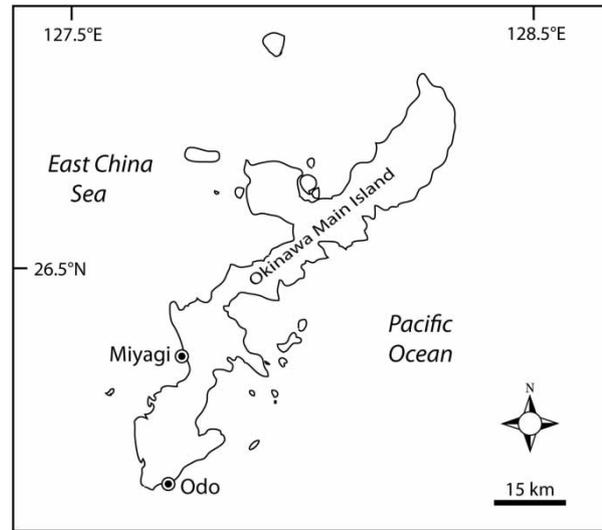


Figure 1. Map of Okinawa Island, Japan showing the sampling sites where *Palythoa tuberculosa* and its *Symbiodinium* were investigated for this study.

Williams 1990) but generally experience low mortality in bleaching events (Jiménez 2001). Hence, in the present study, we monitored several colonies of the common reef zoanthid *Palythoa tuberculosa* (Esper, 1791) at two locations in Okinawa, Japan, over one year to answer the following questions. First, did *Palythoa* colony colour and the condition of their *Symbiodinium* cells change over the year of study? Second, could the colour of *Palythoa* colonies be directly correlated with *Symbiodinium* cell morphological condition? Finally, given that some variation in *Symbiodinium* type has been previously observed in *P. tuberculosa* (Burnett 2002; Reimer & Todd 2009), were there any shifts in dominant *Symbiodinium* type during the monitoring period?

## Materials and methods

### *Study design, image capture, colour scoring and temperature data acquisition*

Two locations were selected on Okinawa-jima Island, Japan, where *Palythoa tuberculosa* colonies are common: Odo Beach (26°05'N, 127°42'E) near Itoman City and Miyagi Beach (also called Sunabe; 26°19'N, 127°44'E) near Chatan Village (Figure 1). At each location, six *P. tuberculosa* colonies (designated colonies Odo1 to Odo6 and Miyagi1 to Miyagi6, respectively) were selected haphazardly, with the caveat that each colony was >50 cm<sup>2</sup> in surface area. The distance between any pair of colonies ranged from 5 to 15 m. At both locations, colony 1 was closest to shore, and colony 6 was furthest from shore (mean depth and SE at Odo = 1.13 ± 0.21 m, range 0.70–2.10 m; mean depth and SE at Miyagi = 1.77 ± 0.010 m, range 1.60–2.10 m). The location of each colony

was marked with a yellow plastic tag held in place with a concrete nail driven into the nearby substrate. Monitoring began at Odo on 16 January 2009 and at Miyagi on 23 January 2009.

Marked *P. tuberculosa* colonies were examined once a month during high tide using SCUBA. Digital images of each colony were taken with a Canon G10 camera in an underwater housing on default settings. The laminated colour card used by Siebeck et al. (2006) was included in each shot. Back in the laboratory, the gradation of the intensity of colour was then scored from 1 (low) to 6 (high) based on the colour card; here called 'colour card score'. As *P. tuberculosa* colonies often have subtle differences in shading, we scored by 0.5 increments (e.g. 1.0, 1.5, etc., to 6.0). All observations of colour were assessed by a single observer.

Daily sea surface temperature (SST) data for both Odo and Miyagi for the period of 1 January to 21 December 2009 were acquired from the Ministry of the Environment (MOE) of Japan (<http://www.jma.go.jp/jma/index.html>), which utilizes satellite, buoy and ship data in their calculations.

#### *Symbiodinium cell condition*

At each sampling time, a small portion (1–2 cm<sup>3</sup>; 3–5 polyps) of tissue from each marked *P. tuberculosa* colony was excised. These specimens were kept alive in seawater in sealable plastic bags and brought back to the laboratory within 1 h for further examination.

Preparation of the *Symbiodinium* and classification of cells into different morphological conditions followed a modified Reimer et al. (2007) technique. A subsample of each specimen was placed in a Petri dish, cut into fine pieces and the resulting slurry mixed with 0.1 ml of ambient temperature filtered (0.7 µm) seawater. The slurry was then extracted into a 1 ml syringe and shaken thoroughly. Contents of the syringe were placed on a slide and *Symbiodinium* cells were observed at 400 × magnification with a light microscope. Digital images of >100 *Symbiodinium* cells per sample were acquired. *Symbiodinium* cells were classified into healthy zooxanthellae (HZ), partially healthy zooxanthellae (PZ), transparent zooxanthellae (TZ), small dark degraded zooxanthellae (DDZ), and dividing zooxanthellae (DZ) according to Reimer et al. (2007). All assessment of *Symbiodinium* cells was conducted by a single observer. HZ and DZ were pooled and divided against the combined counts for PZ, TZ and DDZ, changed to percentage, and termed 'normal zooxanthellae percentage' (NZ%). NZ% was calculated for each colony at both sites for each month.

#### *Statistical analyses*

Based on the monthly monitoring data, Spearman rank correlations between colony colour and *Symbiodinium* cell condition (NZ%) were calculated for each colony. In addition, to determine whether it is possible to predict NZ% from the colour score, the overall relationship between mean colony colour and mean NZ% was assessed with regression analysis. Finally, the mean colony colour and mean NZ% for each site was calculated.

#### *Symbiodinium typing*

Approximately half (0.5–1.0 cm<sup>3</sup>) of the monthly specimens of each colony were preserved in 99.5% ethanol. DNA was extracted from the February 2009 (the coldest month for ocean temperature in Okinawa; *n* = 11 colonies) and August 2009 (the hottest month; *n* = 10 colonies) specimens using a spin column Dneasy Animal Extraction kit following the manufacturer's protocol (Qiagen, Tokyo). A ~300-base pair region of the ITS2 rDNA was amplified and direct sequenced using the following primers: forward primer 'ITSintfor2' (5'-GAATTG CAGAACTCCGTG-3'), reverse primer 'primer ITS-reverse' (5'-GGGATCCATATGCTTAAGTT CAGCGGGT-3') (Manning & Gates 2008). PCR using HotStarTaq ReadyMix (QIAGEN, Tokyo, Japan) was performed under the following conditions: an initial denaturation for 5 min at 95°C and 35 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 30 s, followed by a final extension at 72°C for 10 min. All positive PCR reactions were cleaned using a SAP (shrimp alkaline phosphatase) protocol: 0.30 µl of SAP, 0.15 µl ExoI, and 2.55 µl of water were added to each sample and run under the following conditions: 20 min at 37°C and 30 min at 83°C. Samples were sent to FASMAG (Yokohama, Japan) for direct sequencing.

New *Symbiodinium* ITS-rDNA sequences acquired in this study were deposited in GenBank (accession numbers KC335554–KC335574). Sequences were compared for similarity with previous sequences by the National Center for Biotechnology Information nucleotide BLAST (<http://www.ncbi.nlm.nih.gov/BLAST/>), and by manual visual comparison using the software Mega Version 5.05 (Tamura et al. 2011). Sequences were DNA barcoded to the nearest type/subclade of *Symbiodinium*.

#### **Results**

At Odo Beach, colonies were examined monthly (= 11 times) between 16 January and 11 December 2009. Due to very strong currents and high waves,

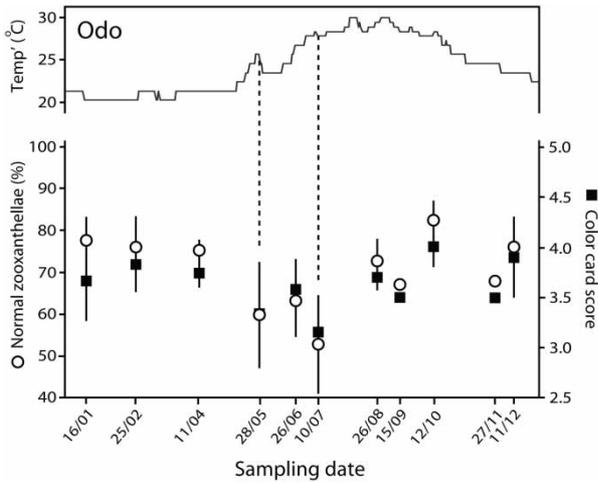


Figure 2. Odo, Okinawa. Upper plot: mean sea surface temperature (SST). Lower plot: mean ( $\pm$ SE) NZ% and mean ( $\pm$ SE) colour card score for colonies sampled each month. The vertical dotted lines have been inserted manually to highlight possible relationships between the two lowest colour card scores and rapid increases in SST.

no data were obtained in March 2009. Occasionally, data were unobtainable for some colonies in other months due to inclement sea conditions (therefore,  $n=6-10$  for each colony). Results from the 6 colonies showed that mean monthly colour scores varied between 3.2 and 4.0 during the year with the lowest scores (highest bleaching levels) in May and July. The lowest scores aligned with contemporaneous sharp increases in temperature (Figure 2). NZ% scores also had similar patterns, with the lowest results again in May and July. Despite reduced numbers of surveys (compared to Miyagi Beach), four out of six colonies showed significant correlations between NZ% and colour card score, while the remaining two colonies also had relatively high  $R^2$  values (Table I). For the research period, the mean ( $\pm$ SE) NZ% (mean of each month's mean) at Odo Beach was  $70.1 \pm 7.1\%$ , and the mean ( $\pm$ SE)

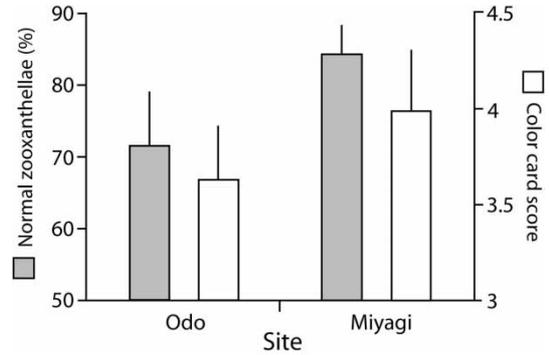


Figure 3. Mean ( $\pm$ SE) NZ% and colour card scores for each site.

colour score was  $3.63 \pm 0.27$  (Figure 3). NZ% scores for individual colonies ranged from 53.0% to 93.1% and colour scores from 2.0 to 5.0.

At Miyagi Beach, colonies were examined at 12 time points between 23 January and 12 December 2009. Results from the six colonies showed that average colour scores were generally higher, but varied less (3.7–4.3) than at Odo. Low mean colour card scores occurred during rapid temperature rises in May and July (Figure 4). A low colour score was also observed in winter (December). The lowest NZ% scores were in summer (August) and winter (January, December). Only one out of six colonies exhibited a significant correlation between NZ% and colour card score (Table I). For the research period, the mean ( $\pm$ SE) NZ% (mean of each month's mean) at Miyagi Beach was  $84.2 \pm 4.2\%$  and the mean ( $\pm$ SE) colour score was  $4.06 \pm 0.29$  (Figure 3). NZ% scores for individual colonies ranged from 11.2% to 97.8% and colour scores from 2.5 to 5.0.

Regression analysis indicated that the overall relationship between mean colony colour and mean NZ% was positive ( $R^2 = 0.479$ ) and significant ( $p = 0.013$ ) (Figure 5).

During the period between January and December 2009, no substantial bleaching of anthozoans, in-

Table I. Summary data for all colonies, including the correlations between colour card scores and NZ%. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ .

Colony	# sampling occasions	Mean ( $\pm$ SE) NZ%	Mean ( $\pm$ SE) colour card score	$R^2$ for NZ% vs. colour card score
Odo1	10	62.0 (7.24)	3.6 (0.13)	0.90***
Odo2 <sup>a</sup>	6	51.5 (11.64)	2.9 (0.29)	0.73
Odo3	9	75.9 (3.63)	3.4 (0.17)	0.92***
Odo4	8	88.1 (2.05)	4.4 (0.18)	0.87**
Odo5	9	66.5 (4.47)	3.6 (0.17)	0.57
Odo6	9	73.0 (5.97)	3.7 (0.20)	0.81**
Miyagi1	12	88.3 (2.48)	3.8 (0.12)	0.63*
Miyagi2	12	87.0 (2.57)	3.8 (0.12)	0.33
Miyagi3	12	80.5 (3.15)	3.8 (0.09)	0.56
Miyagi4	12	91.1 (1.99)	4.9 (0.06)	-0.23
Miyagi5	12	78.7 (3.03)	3.4 (0.12)	0.43
Miyagi6	12	79.9 (3.87)	4.8 (0.17)	0.46

<sup>a</sup> Disappeared between the July and August sampling.

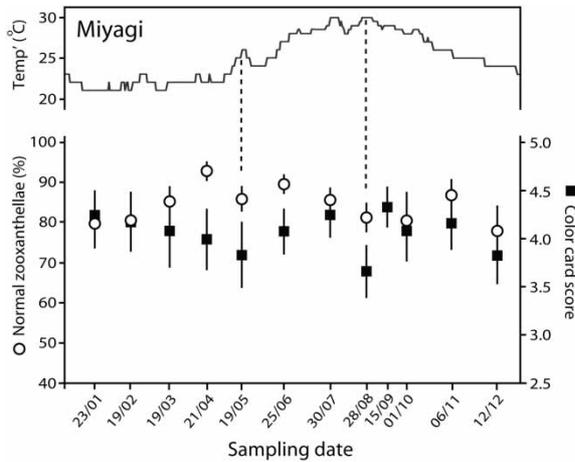


Figure 4. Miyagi, Okinawa. Upper plot: mean sea surface temperature (SST). Lower plot: mean ( $\pm$ SE) NZ% and mean ( $\pm$ SE) colour card score for colonies sampled each month. The vertical dotted lines have been inserted manually to highlight possible relationships between the two lowest colour card scores and rapid increases in SST.

cluding *Palythoa tuberculosa*, was noted at either study site other than for colony Odo2 (colour card score of 2.0) before it disappeared in July/August. No other colony had a colour card score below 2.5 over the course of the study.

MOE daily SST data were similar at both locations, although temperatures before May were often slightly higher ( $1.0^{\circ}\text{C}$ ) at Miyagi Beach. Temperatures rose quite rapidly in early May, from  $22.0^{\circ}\text{C}$  at both sites on 8 May to  $26.0^{\circ}\text{C}$  at both sites on 24 May. Temperatures then dropped slightly to  $24.0^{\circ}\text{C}$  until 11 June, and then rose again during June until a peak of  $30^{\circ}\text{C}$  at both sites in August. SSTs then gradually dropped from late August/early September onwards, reaching  $23.0^{\circ}\text{C}$  by 21 December (Figures 2 and 4).

The temporal changes in *P. tuberculosa* colour appeared to be broadly related to SST. The two lowest readings at Odo corresponded to rapid

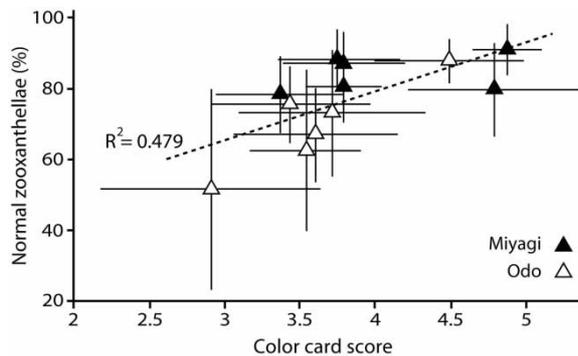


Figure 5. The relationship between NZ% and colour card score. Each data point represents the mean ( $\pm$ SE) of the NZ% and colour card scores collected through the year for each marked *Palythoa tuberculosa* colony (hence the large error bars).

increases in SST, i.e. immediately prior to the 28 May 2009 sampling there was a  $4.0^{\circ}\text{C}$  rise over 15 days and just before the 10 July 2009 sampling there was a  $4.5^{\circ}\text{C}$  rise over 14 days (dotted lines in Figure 2). The lowest colour card score for Miyagi (on 28 August 2009) was after a  $2.0^{\circ}\text{C}$  rise over 11 days, while one of the other two lowest scores (on 19 May 2009) directly followed a  $3.0^{\circ}\text{C}$  rise over 10 days (dotted lines in Figure 4).

### Sequence analyses

The same dominant symbiont subclade for colonies sampled in February and August was consistently observed in all samples examined. For all but one colony, the symbionts' ITS2 sequence identity matched 100% with numerous *Symbiodinium* sequences isolated from many hosts, including soft corals (Aratake et al. 2012) and zoanthids in Japan, such as *Palythoa tuberculosa* (Reimer et al. 2006) (e.g. GenBank Accession Numbers AB665719, DQ480631, respectively). This symbiont type belongs to the 'host generalist' subclade C1/C3 (LaJeunesse 2005; Reimer et al. 2006). The lone exception was a single base pair difference observed for Odo colony 6 from both sampled months, and these sequences were also identical to numerous *Symbiodinium* sequences isolated from many hosts, including *P. tuberculosa* from Japan (Reimer et al. 2006) (e.g. GenBank Accession Numbers DQ889744, DQ480613, etc.). As with the other sequences above, this symbiont type belongs to the 'host generalist' subclade C1/C3 group (Reimer et al. 2006).

### Discussion

Bleaching (expulsion of *Symbiodinium* and/or loss of photosynthetic pigments) is a phenomenon recorded in all groups of tropical zooxanthellate anthozoans, but programmes to monitor such events generally focus on scleractinian corals due to their importance as reef builders. There is, however, a lack of monitoring of other zooxanthellate anthozoans, despite the fact that many groups such as octocorals (Jeng et al. 2011), anemones (Chen & Dai 2004) and zoanthids (Irei et al. 2011) may be the dominant benthos. Here we observed temporal changes in colony colour in 12 *Palythoa tuberculosa* colonies at 2 sites in Okinawa. At Odo in particular, drops in mean colour card scores appeared to be related to increases in SST. Significant correlations between *P. tuberculosa* colony colour and internal zooxanthellar condition (NZ%) were also found for four out of six colonies at Odo. The associations between colony colour and temperature, and colour card score and

NZ%, were less pronounced at Miyagi. For both sites, the pattern of relatively subtle changes through the year represents 'seasonal thermal bleaching' (*sensu* Fitt et al. 2000) as opposed to catastrophic *Symbiodinium* loss due to extreme heating events. No major bleaching of any anthozoans was noted at either site during this study, and SST temperatures over the research period were not abnormally high or low. *Symbiodinium* ITS-rDNA sequencing results showed no changes in types among colonies at either site, or between summer and winter.

The temporal shifts in *P. tuberculosa* colour appeared to be broadly related to SST. Short-term changes in SST and solar radiation have been shown previously to affect the status of the algal–coral symbiosis (e.g. Brown et al. 1999) and a similar response from zoanthids is not unexpected. The low colour card scores in winter for Odo (27 November 2009) may be due to freshwater input from a creek flowing over the reef crest, perhaps as a result of heavy rainfall during 22–25 October (Typhoon 20; total 235 mm of rain during this period, Japan Meteorological Agency, from Itoman site < 10 km from Odo). There are also some small creeks along the Miyagi coastline, which may have contributed to the winter low colour card score (12 December 2009) at this site. Previous research in Okinawa has demonstrated that lowered salinity can cause mortality in corals (Sakai & Nishihira 1991) and it is reasonable to suggest that zoanthids are stressed by freshwater input. Overall, the responses in relation to temperature at Miyagi were less pronounced and the correlations between NZ% and colour card score less strong. The zoanthid colonies at Miyagi were deeper (mean = 1.77 m) compared to Odo (mean = 1.13 m) and this, to some degree, may have buffered this site from factors inducing bleaching. For instance, greater depth coupled with more turbidity at Miyagi (Ministry of the Environment and Japanese Coral Reef Society 2004) would have reduced the level of solar irradiance (which can contribute towards bleaching) reaching the zoanthids.

In a follow up to the original 2006 study, Siebeck et al. (2008) monitored various coral colonies of several species utilizing colour cards and concluded that the technique was a useful tool for 'rapid, wide-area assessment of changing coral condition'. It is surprising, however, that despite many papers citing Siebeck et al. (2006, 2008), no other research group has yet specifically and critically assessed this technique for any other coral reef organism – especially as the authors themselves strongly suggested in both studies that this is a necessary step. Our results provide the first evidence that these colour cards can be used for monitoring zooxanthellate zoanthids. Short-term single survey or single colony monitoring

using this technique, however, is not recommended, as cases of NZ% declining without corresponding colour changes were occasionally observed (Figures 2 and 4). Siebeck et al. (2006) also suggested that drops of less than 2 colour points may not be indicative of a degradation in *Symbiodinium*. Our results suggest that, at least for *P. tuberculosa*, a finer scale of sensitivity may be achieved and is appropriate. Furthermore, without acquiring long-term (e.g. several months) data, baseline levels of NZ% or colour cannot be determined for individual colonies, which may have inherent colour differences.

The colonies at Odo had more significant NZ% vs. colour card score correlations than those at Miyagi (4 of 6 colonies compared to 1 of 6 at Miyagi; Table I). *Palythoa tuberculosa* colonies at the Miyagi site were more stable, and did not experience bleaching or exhibit as much variation as Odo colonies. It seems that, under these conditions, the colour card technique may not perform so well. In contrast, despite a lower number of samplings per colony ( $n=6-10$ ), at Odo there were strong correlations between NZ% and colour card scores. Thus, even over shorter time spans (e.g. <10 months), utilizing colour cards to monitor individual colonies can give significant results at locations where small to moderate levels of bleaching occur.

Regardless of which environmental factors are responsible for the changes in colour card score and NZ% observed in this study, the results demonstrate that not only are there likely to be different patterns of bleaching among species (e.g. Montano et al. 2010), but also varying responses for the same species at different sites (e.g. Guest et al. 2012), and variation within individual sites even when hosts have identical symbiont types.

Previous investigations of *P. caribaeorum* in subtropical Florida reported higher rates of symbiont release in winter (Kemp et al. 2006), while Siebeck et al. (2008) noted lower colour scores in spring and higher scores in autumn in corals on the Great Barrier Reef. Reimer et al. (2007) observed decreases in *Symbiodinium* NZ% in *Zoanthus sansibaricus* in both summer and winter in Kagoshima, north of Okinawa. As stated in Siebeck et al. (2008), it is clear that colour card data are needed for many different zooxanthellate species, and for varying environmental conditions, to develop a better understanding of how the technique can be refined and interpreted.

All colonies in this study had the same generalist C1/C3 subclade of *Symbiodinium*, suggesting that, even though some minor bleaching (e.g. colony Odo2 in February and July 2009) was observed, *P. tuberculosa* does not shuffle or change its symbiont types. *Palythoa tuberculosa* colonies from the Indian

Ocean (Burnett 2002) and Singapore (Reimer & Todd 2009) have been shown to be in symbioses with both *Symbiodinium* clades C and D, as have colonies of the sister species *P. caribaeorum* in Florida (Kemp et al. 2006), but clade D was not found in this study and has not been observed in the north-western Pacific in *Palythoa*. Our results are almost identical to previous research examining *Symbiodinium* in *P. tuberculosa* in Japan, which found 'generalist' C1/C3 in all colonies examined (Reimer et al. 2006). *Palythoa* spp. are considered some of the most sensitive anthozoans to bleaching (e.g. Williams & Bunkley-Williams 1990), but mortality due to these events is relatively rare (Jiménez 2001). This may be because *Palythoa* spp. are heterotrophic (Reimer 1971) and hence do not rely solely on their symbionts. A stable symbiosis with generalist C1/C3 *Symbiodinium* is apparently a successful strategy that facilitates *P. tuberculosa*'s ability to thrive in a variety of environments (Reimer et al. 2006).

Our results highlight that bleaching is not just a coral phenomenon, and that zooxanthellate zoanthids (and other taxa that host symbiotic algae such as anemones, soft corals and giant clams) should be considered for inclusion in monitoring programmes. *Palythoa* spp. are common throughout subtropical and tropical waters in both the Indo-Pacific and Atlantic. Two of the most common species, *P. tuberculosa* and its Atlantic sister species *P. caribaeorum*, are often abundant in shallow waters, readily viewable by snorkelling. *Palythoa* spp., unlike hard corals, are not CITES-listed, not endangered and are easy to sample. Hence, they are good candidate organisms for monitoring bleaching events on coral reefs.

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