Table 2. Morphological conditions observed in symbiotic *Symbiodinium* (zooxanthellae, ZX) in *Zoanthus sansibaricus*.

Morphological Condition group	Morphotype	Reference	Description
NZ (normal ZX)	HZ (healthy ZX)	MISE and HIDAKA 2003	greenish-brown, chlorphyll-bearing, circular in shape, 6~10 μm diameter. See Figure 2b.
	DZ (dividing ZX)	KUROKI and VAN WOESIK 1999	HZ undergoing mitosis, useful in calculating mitotic index.
SZ (stressed ZX)	TZ (transparent ZX)	MISE and HIDAKA 2003	circular in shape, having a near or complete loss of chlorophyll and degradation of thylakoids and chloroplasts, 6~10 μm diameter. See Figure 2d.
	PZ (pale ZX)	MISE and HIDAKA 2003	intermediate stage between HZ and TZ. See Figure 2c.
	DDZ (dark degraded ZX)	KUROKI and VAN WOESIK 1999	smaller than HZ (approx. 3 μm diameter), irregularly-shaped. Figure see 2d.

Photographs of the same field of view (and the same *Symbiodinium* individuals) were taken at various intervals of 15 minutes (>length of time of *Symbiodinium* condition data collection as explained above). No changes in NZ% for these experiments were seen.

Environmental data

For samples collected from April 2001 to June 2002, tide pool (Yakushima and Amami sites) and ocean data temperature (all sites) were taken on site using a standard thermometer. Ocean temperature data at the surface (Yakushima and Amami) and at a depth of 3m (all sites) were recorded.

For samples collected from July 2002 to October 2003 environmental data were collected using a YSI 600 XLM Multi-parameter sonde and a YSI 650MDS handheld logging and display system (Yellow Springs, OH, USA). In addition to tide pool and ocean temperature as described above, salinity (ppt), pH, pHmV, dissolved oxygen saturation, dissolved oxygen content (mg/l), dissolved oxygen charge, conductivity (ms/cm), and special conductivity (ms/cm) were recorded. Annual average ocean temperatures for all four sites were calculated by using temperature data taken at a depth of 3m for the twelve-month period from July 2002 to June 2003.

We obtained daily water temperature data (April 2001 to October 2003) from the Kagoshima Prefectural Fisheries Experimental Center (KPFEC) for locations very close to our sampling sites (Tables 1 and 3, Fig. 1). Our obtained sampling site ocean temperature data (3m) were compared with KPFEC data and no major discrepancies were seen. Thus, KPFEC data were used in subsequent statistical analyses, as the data were available daily. Monthly rainfall and sunlight data were obtained from the Japan Meteorological Agency (JMA) homepage (http://www.data.kishou.go.jp/etrn/), again for locations very close to our sampling sites (see Tables 1 and 3). In addition, two-week average ocean temperature (hereafter referred to as 2-week temperature) was calculated from KPFEC temperature data. The 2-week temperature data reflected conditions in the environment for the two-week period before and up to NZ ratio data collection, and accordingly we theorized 2-week temperature would fit better than other temperature readings with our obtained NZ% data. No other environmental parameter was calculated as a two-week average as no daily data were available.

The total numbers of monthly NZ% data (=*n*) for each site were: Kokubu=27 (June 2001-August 2003), Sakurajima=28 (June 2001-September 2003), Yakushima=29 (May 2001-September 2003), and Amami=28 (May 2001-August 2003).

A summary of minimum and maximum observed temperatures for each sampling site is shown in Table 1. As would be expected, the northern sites of Kokubu and Sakurajima were coldest, followed by Yakushima, with southernmost Amami the hottest sampling site.

Transect Data

At both Yakushima and Amami one 50cm X 50cm transect was marked off in the

Table 3a. Correlation coefficient (*r* value) for all sites' data at "normal" temperatures (>18.0℃ - <28.5℃).

	NZ %	Samp t	℃ (3m)	℃ (2 wk)[1]	Sun[2]	Rain[2]	Sal	pH	pH mV	DO	DO cond	DO chrg	Cond	Sp cond
NZ %	1	0.056	0.055	0.12	0.138	-0.019	0.237	0.113	-0.114	0.238	0.246	0.277	0.316	0.241
Samp t		1	**0.473**	0.212	0.256	**0.438**	-0.369	-0.046	0.034	0.143	0.131	0.166	-0.291	-0.055
℃ (3m)			1	**0.758**	0.378	0.286	**-0.455**	-0.081	0.054	0.304	0.276	0.349	0.015	-0.003
℃ (2 wk)[1]				1	**0.508**	0.236	-0.304	-0.175	0.155	0.277	0.257	0.329	0.108	0.042
Sun[2]					1	-0.288	-0.227	-0.473	**0.458**	0.258	0.257	0.116	0.03	0.058
Rain[2]						1	**-0.514**	0.041	-0.045	0.141	0.139	0.326	**-0.512**	-0.395
Sal							1	0.288	-0.272	-0.092	-0.078	-0.103	**0.696**	**0.817**
pH								1	-1	-0.383	-0.383	-0.25	0.102	-0.304
pH mV									1	0.373	0.373	0.24	-0.102	-0.304
DO										1	**0.999**	**0.946**	0.029	0.095
DO cond											1	**0.943**	0.024	0.104
DO chrg												1	0.007	0.084
Cond													1	**0.542**
Sp cond														1

Values in bold are non-directionally statistically significant (p=<0.05).

[1]-Calculated from Kagoshima Prefecture Fisheries Experimental Center (KPFEC) data. For locations see Table 1.

[2]-Sunlight and rainfall data from Japan Meteorological Agency (JMA): Kokubu data from Makinohara site (31°40' N, 130°51' E), Sakurajima data from Kagoshima (31°33' N, 130° 33' E), Yakushima data from Onoaida (30°14' N, 130°33' E), and Amami data from Naze (28°23'N, 129°30'E).

Abbreviations: NZ %=normal zooxanthellae ratio %, Samp t=sampling time of day, ℃ (3m)=ocean temperature on day of sampling at 3m depth, ℃(2wk)=average ocean temperature for 2 weeks' time previous to sampling, Sun=monthly sunlight, Rain=monthly rainfall, DO=dissolved oxygen saturation, DO cond=dissolved oxygen conductivity, DO chrg=dissolved oxygen charge, Cond=conductivity, Sp cond=special conductivity. *n*=23 for all coefficients.

Table 3b. Correlation coefficient (*r* value) for data at non-"normal" (<18.0 ℃ or >28.5 ℃) ocean temperatures.

	NZ%	Sampling time	Temp (tidepool)	Temp (surface)	Temp (3m)[1]	Temp (2 wk avg)[1]	Monthly sunlight[2]	Monthly rainfall[2]
NZ%	1	-0.105	NA	NA	0.389	**0.745**	**-0.769**	0.055
Sampling time	-0.179	1	NA	NA	-0.133	-0.134	-0.082	-0.307
Temp (tidepool)	-0.415	-0.064	1	NA	NA	NA	NA	NA
Temp (surface)	-0.889	-0.204	0.189	1	NA	NA	NA	NA
Temp (3m)[1]	-0.229	0.496	-0.492	0.202	1	0.775	--0.707	0.277
Temp (2 wk avg)[1]	-0.716	-0.492	0.421	**0.838**	-0.151	1	**-0.671**	0.407
Monthly sunlight[2]	-0.363	-0.565	0.258	0.511	-0.142	0.862	1	0.075
Monthly rainfall[2]	0.010	-0.064	0.665	0.021	-0.696	0.138	-0.094	1

Right triangular matrix: Northern (Kokubu, Sakurajima) data for "cold" temperatures <18.0℃. *n*=16
Left triangular matrix: Southern (Yakushima, Amami) data for "hot" temperatures >28.5℃. *n*=6

n=monthly NZ% and other data from one site.
Values in bold are non-directionally statistically significant (p=<0.05).
[1]-Calculated from Kagoshima Prefecture Fisheries Experimental Center (KPFEC) data. For locations see Table 1.
[2]-Sunlight and rainfall data from Japan Meteorological Agency (JMA). For locations see Table 2a.
Abbreviations: NA=not available.

inter-tidal zone, and photographs taken monthly between November 2001 and September 2003. Host *Z. sansibaricus* cover was calculated from images. For some months data were not recorded due to inclimate conditions (*i.e.* large waves during sampling trips).

Data Analyses

Collected NZ% data and transect data were compared and analyzed with all chronologically corresponding data sets (including 2-week temperature data). All statistical analyses were performed using StatView 4.0J (Japanese version). Data were examined for correlation with NZ% and for significant difference at different ocean temperatures. Regression analyses between NZ% and ocean temperature data were also performed.

Results

Correlation of environmental data

Table 3 shows correlation coefficient values between our collected data. Based on Fig. 3a-d, in which NZ% appears to follow seasonal trends (see below), with NZ% decreasing during cold weather (*i.e.* winter months) at northern Kokubu and Sakurajima, and during hot weather (*i.e.* summer) at Yakushima and Amami, with no apparent NZ% decreases at mild temperatures, we theorized that correlations would be different during different seasons. Accordingly, using 2-week temperature data, we divided data for correlation analyses into three groups: a. "cold" <18.0℃, roughly the temperature at which Kokubu and Sakurajima NZ% appeared to decrease in winter, b. "normal", 18.0℃-28.5℃, and c. "hot", >28.5℃, which is approximately the expected annual maximum temperature at Yakushima and Amami (Table 1). No large (*i.e.* $r > 0.4$) correlation coefficient was observed at "normal temperatures" (Table 3a, $n = 23$) between NZ% and other collected data. Additionally, no statistical significance was observed between NZ% and any environmental parameter at "normal temperatures" (Table 3a, n =23). However, NZ% had a large positive correlation with 2-week temperature (r =0.745, n =16, p =<0.001) during "cold" temperatures at northern sites (Table 3b), and a large negative correlation at "hot" southern samplings with surface temperature (r =-0.889, n =6, p =0.018) and 2-week temperature (r =-0.716, n =6, p =0.110). The first two of these r values were statistically significant (p =<0.05, Table 3b). No other r value correlated with NZ% was statistically significant. NZ% showed a lower (r =0.389, n =16) correlation with 3m temperature at "cold" northern sampling times that was not statisctically significant (p =0.136) and therefore for examining NZ% over time we used 2-week temperature data.

We could not compare "cold" northern data correlation with salinity (ppt), pH, pH mV, dissolved oxygen saturation, dissolved oxygen content (mg/l), dissolved oxygen charge, conductivity (ms/cm), or special conductivity (ms/cm), as the total number of "cold" month data (4) between July 2002 to September 2003 (when we recorded these data) was less than the minimum number required (6) to examine correlation (see LOWRY 2006). We could examine correlation of these factors with our "hot" southern data however (n =8). No significant correlation (p=<0.05) was found between NZ% and all non-temperature environmental data except for a strong negative correlation with sunlight during "cold", northern samplings (r =-0.769, n =16, p =<0.001). No correlation greater than r =0.2 was seen between NZ% and sampling time of day. Interestingly, the correlation between tide pool temperature at Yakushima and Amami and NZ% was not very strong (r =-0.415, n =6, p =0.413).

However, some correlations are apparent in our data. For "cold" northern data, NZ% and sunlight had a strong negative correlation of r=-0.769 (n=16). Sunlight showed a large negative correlation with all temperature data during such conditions (Table 3b).

With "hot" southern data correlations between NZ% and pH (r =0.469), pHmV

(r = - 0.494), dissolved oxygen content (r = - 0.656) and dissolved oxygen charge (r = -0.466) (all n =8) were evident. pH and pHmV were shown to be very highly correlated with 2-week temperature (r =-0.916, r =0.915 respectively, n =8), as well as with surface temperature (r =-0.880, r =0.947, n =8), and thus can be consistently linked with ocean temperature.

Monthly NZ%

No clear trends for within site variation between colonies were found at all four sampling locations.

In general, large NZ% decreases were seen at Kokubu and Sakurajima for both winter (December-April) 2001-2 and winter 2002-3. At Yakushima and Amami NZ% decrease most in summer (July-September) 2001, with smaller decreases in summer 2002 and 2003. At Yakushima an NZ% decrease was also seen in winter 2001-2.

To test the significance of such apparent trends, NZ% data were divided into the three groups used for correlation coefficient analyses (cold, normal, and hot). For all sites' NZ% data combined, NZ% at "cold" temperatures of <18.0℃ (avg. 62.8±6.6%, n =15, p =<0.0001) and at "hot" temperatures of >28.5℃ (avg. 69.0±10.0%, n =15, p =0.0454) were found to be significantly lower than "normal" temperatures (avg. 73.3±7.3%, n =82) (Table 4) (all Fisher's Post-Hoc PLSD Test).

Data were grouped together into northern (Kokubu and Sakurajima) and southern (Yakushima and Amami) groups based on similarity of NZ% changes over time. In "northern" Kokubu and Sakurajima data, NZ% were significantly lower at "cold" temperatures (avg. 62.8±6.6%, n =15, p =0.0003) than at "normal" temperatures (avg. 71.4±7.7%, n =36). Unexpectedly, "hot" NZ% data were significantly higher (avg. 79.2±3.6%, n=4, p=0.0446) than "normal" temperatures (all Fisher's Post-Hoc PLSD test).

Southern Yakushima and Amami NZ% decreased significantly at "hot" temperatures (avg. 65.3±8.9%, n =11, p =0.0002) when compared to "normal" temperatures. No "cold" 2-week average temperatures were recorded at Yakushima or Amami during the course of this study.

Additionally, Sakurajima NZ% data showed differences compared with all other sites' data (Fig. 3b), as reflected by unstable and more variable NZ% values. Sakurajima NZ% data were significantly different from both Yakushima (p=0.0141) and Amami (p=0.0386) NZ% data (both n=27, Fisher's PLSD test), and less similar to Kokubu NZ% data (p=0.1326, n=27) than all other sites' NZ% data (Kokubu-Amami p=0.5770, n=27, Kokubu-Yakushima p=0.3479, n=27, and Yakushima-Amami p =0.7032, n =28, all Fisher's PLSD test).

Regression Analyses

For the three temperature groups ("cold", "normal" and "hot") a very clear relationship between NZ% and 2-week temperature was seen (Fig. 4). "Cold" temperatures and NZ% had a statistically significant r value (p=<0.05), while at normal

Table 4. Significance of ocean temperature on NZ%.

All sites

Temperature class	Average NZ% ± S.D.	*n*	*p* (compared with 18.0℃-28.5℃ data)
"cold" (<18.0℃)	62.76 ± 6.63	15	***
"normal" (18.0-28.5℃)	73.31 ± 7.28	82	not determinable
"hot" (>28.5℃)	68.98 ± 10.0	15	*

Northern sites (Kokubu and Sakurajima)

Temperature class	Average NZ% ± S.D.	*n*	*p* (compared with 18.0℃-28.5℃ data)
"cold" (<18.0℃)	62.76 ± 6.63	15	***
"normal" (18.0-28.5℃)	71.38 ± 7.66	36	not determinable
"hot" (>28.5℃)	79.21 ± 3.62	4	*

Southern sites (Yakushima and Amami)

Temperature class	Average NZ% ± S.D.	*n*	*p* (compared with 18.0℃-28.5℃ data)
"cold" (<18.0℃)	not determinable	0	not determinable
"normal" (18.0-28.5℃)	74.82 ± 6.66	46	not determinable
"hot" (>28.5℃)	65.26 ± 8.93	11	*

*p=<0.05, ***p=<0.001 (Fisher's post-hoc PLSD test)

temperatures and at "hot" temperatures correlation coefficients were not statistically significant. In general, NZ% increased with temperature in the "cold" group, slightly increased as "normal" temperature increased, and decreased as "hot" temperatures increased (Fig. 4). Note that based on Table 4 results, "hot" data from the two northern sites of Sakurajima and Kokubu were not included in Figure 4. Transect data.

Yakushima and Amami

Zoanthus colony cover decreased in summer months and increased in winter months in both Yakushima and Amami populations, showing an apparent seasonal influence (Fig. 5a-b). To confirm this observation, *Zoanthus* % cover data were divided into two groups (winter=December-May, summer=June-November). On Yakushima, while % *Zoanthus* cover was lower (11.2 ± 3.7%, n =6) in summer than in winter (12.1 ± 1.6%, n=11), the difference was not significant (p=0.4822, Fisher's Post-hoc PLSD test). On Amami, however, there was a significant (p=0.0003) difference between summer (25.6 ± 3.9%, n=8) and winter (35.4 ± 5.5%, n=13) *Zoanthus* colony cover (Fish-

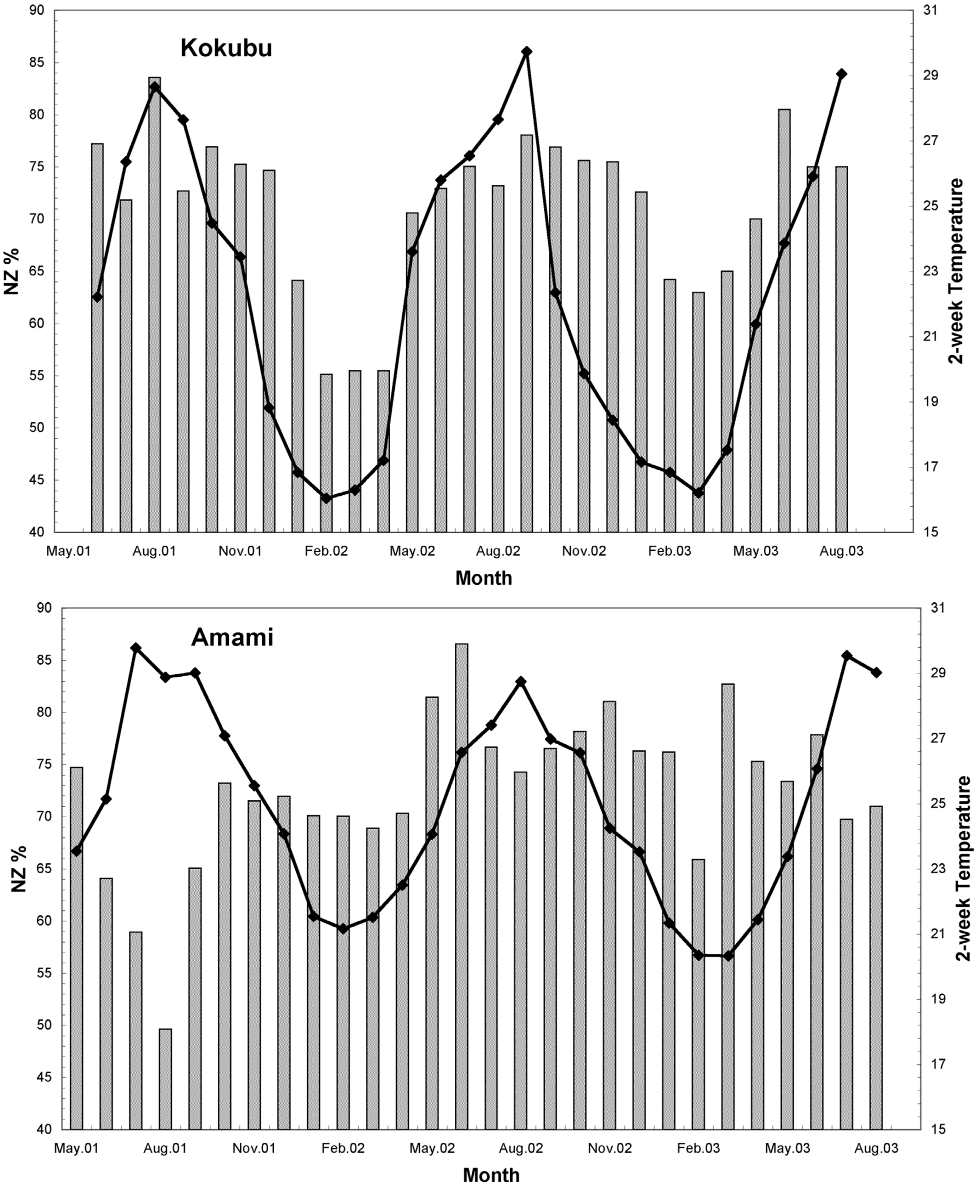

Fig. 3. Percentage of healthy morphological condition *Symbiodinium* (NZ%) (black bars) in *Zoanthus* and 2-week average ocean temperatures (℃) (line), May 2001-September 2003, by sampling location. a) Kokubu (n=27), b) Sakurajima (n=28), c) Yakushima (n=29), and d) Amami (n=28). Each monthly NZ% data point for each location is the average of five sampled *Zoanthus* polyps (sampled from marked colonies) (5 field of view counts/polyp).

er's Post-Hoc PLSD test). However, the correlation between *Zoanthus* colony cover and NZ% at all temperatures at both southern sites combined was negligible (r =-0.003, n =38). Correlation between colony cover and NZ% was high at temperatures above 28.5℃ (r =-0.792), but due to the small sample size (n =4) care should be taken in interpreting this result (see Lowry 2006).

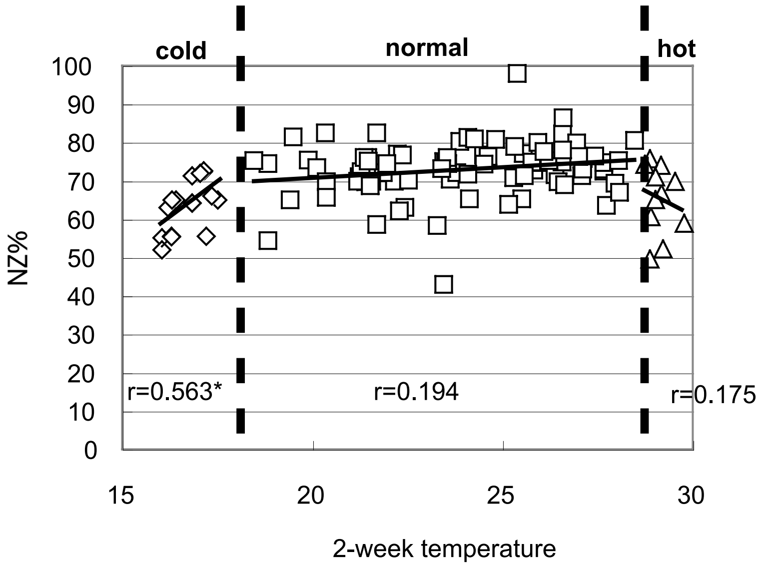

Fig. 4. Regression analyses between NZ% and 2-week average ocean temperature (℃) for data collected from all sampling locations. Data were divided into “cold” (diamonds, <18.0℃, *n*=15), “normal” (squares, 18.0~28.5℃, *n*=82), and “hot” (triangles, 28.5℃, *n*=11) analyses.*=statistically significant (p=<0.05). Please note that based on Table 4 results, "hot" NZ% data from the two northern sites of Sakurajima and Kokubu were not included here.

Discussion

How accurate is the NZ% method ?

From our results it is clear that our NZ% data do reflect seasonal environmental stress on symbiotic *Symbiodinium* in *Zoanthus*, albeit with a varying degree of accuracy. We used a benchmark of 65% for NZ% to indicate holobiont “stressed” conditions, which is approximately equal to our observed NZ% at both “cold” and “hot” temperatures, and over 5% lower than NZ% at normal temperatures for both northern and southern sites. Observed NZ% at three of four sampling sites (excepting Sakurajima) showed decreases to less than 65% only in summer (June-September) or winter (December-March) months. The reasons for Sakurajima’s variability under seemingly normal temperature conditions may be explained by its close proximity to the active volcano Mt. Sakurajima. Ash fall from eruptions irregularly but not infrequently falls on the Sakurajima sampling site, and this has been shown negatively impact on *Zoanthus* and other macrobenthos (ONO *et al*. 2003). Additionally, unlike the other three sampling locations, Sakurajima experiences regular spring (April-May) growth of *Sargassum* sp. seaweed, and this has been shown to decrease the number of *Symbiodinium* in *Zoanthus* spp. (ONO *et al*. 2003). Another factor possibly contributing to non-predictable Sakurajima NZ% data is the high variability of visibility at this site (discussed further below).

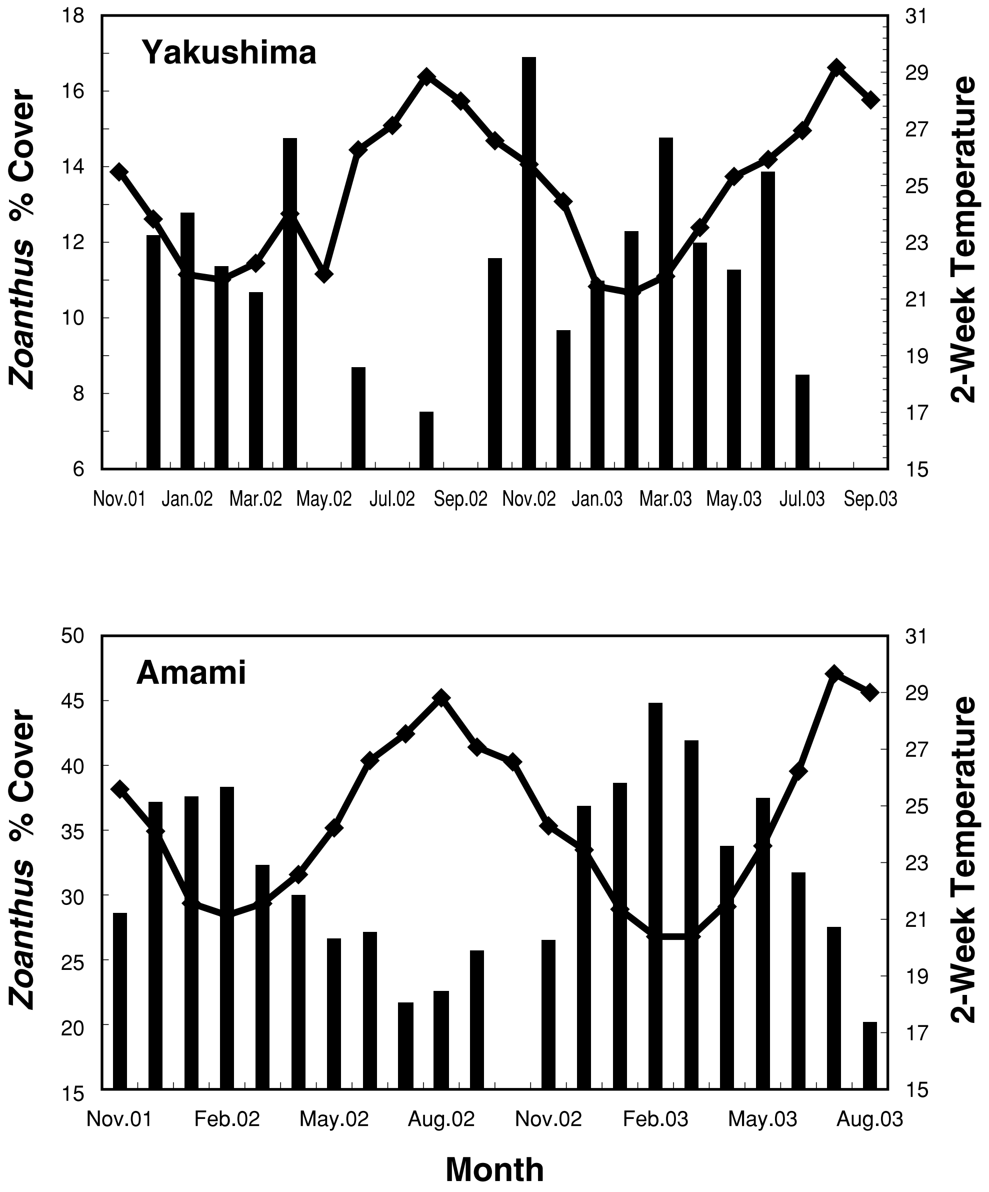

Fig. 5. *Zoanthus* colony % transect cover and 2-week average ocean temperature, November 2001-August 2003, by sampling location; a) Yakushima, b) Amami. Black bars represent *Zoanthus* % cover and 2-week average temperatures (℃) are signified by black line. Occasionally % cover data were not collected due to high waves.

Similarly, differences between observed NZ% and *Zoanthus* cover patterns are somewhat difficult to explain. While NZ% and *Zoanthus* cover were correlated for "hot" temperatures at Yakushima and Amami, our sample size is too small to safely draw conclusions. Unfortunately we do not have 2001 summer *Zoanthus* cover data, but we expect that if such data were available the correlation between *Zoanthus* cover and NZ% data would be reinforced. Additionally, it appears that *Zoanthus* cover decreases in summer are an expected or "annual" occurrence as *Zoanthus* cover decreased in summer 2002 despite no decreases in NZ%. This phenomenon may be partially due to a combination of high light levels and temperatures during daytime in summer (as opposed to winter) during extreme daytime low tides. *Zoanthus* at Sakurajima is found below the extreme low tide line, and in another study no decreases in cover in summer were observed (ONO *et al*. 2003). Another factor such as *Zoanthus* directing energy into the production of gametes for summer mass spawning (ONO *et al*. 2005) may also be partially responsible for colony cover decreases. More research is needed to properly explain the relation between our observed differences in seasonal patterns of NZ% and *Zoanthus* cover.

Winters and "Cold" Temperatures

At the two northern sites of Kokubu and Sakurajima NZ% decreased significantly in winter when ocean temperatures dropped below 18.0℃. However, the annual expected minimum sea temperature (in late February) is well below 18.0℃ (15.83℃, Table 1). During the monthly sampling temperatures below 18.0℃ were never observed at Yakushima and Amami. Temperatures below 15.83℃ were seen at Kokubu and Sakurajima on one day (non-sampling) in 2002 and for three non-sampling days in 2003, reaching a minimum of 15.53℃ on 13-14 Feb. 2003. Despite winter temperatures being not much colder than in an "average" year (especially when compared to extremely high summer temperatures observed - see later in the Discussion) during our study period at Kokubu and Sakurajima, we observed significant NZ% decreases during winter, suggesting that a winter decrease in NZ% is an annual event for *Zoanthus* at these two northern locations. Similarly, HOEGH-GULDBERG and FINE (2004) have suggested "bleaching during winter months may be a natural phenomenon for high-latitude coral reefs". Further evidence that *Zoanthus* at Sakurajima and Kokubu are living at the low end of their thermal tolerance can be seen in distribution data. *Zoanthus* are found along the Pacific coast of Japan as far north as mid-Honshu, as well as on the Pacific coasts of Shikoku and Kyushu (UCHIDA and SOYAMA 2001). At Kushimoto, Wakayama, near the northern limit of *Zoanthus* distribution (see Fig. 1), average ocean temperatures for March reach 16.59℃, compared to a monthly average of 17.36℃ at Sakurajima and Kokubu in February (data from Japan Oceanographic Data Center - JDOC). Further north, at Izu Peninsula, Shizuoka (February average 15.07℃) and Boso Peninsula, Chiba (February average 15.94℃) *Zoanthus* is not found (all data JDOC).

Winters at Yakushima (expected annual minimum 19.88℃, Table 1) and Amami (expected annual minimum 20.74℃, Table 1) are approximately 4-5℃ warmer than winters at Kokubu and Sakurajima. It comes as no surprise that winters at Yakushima and Amami appeared to be much less detrimental to NZ% and *Symbiodinium* than at the northern sampling sites, despite both 2001-2 and 2002-3 winters being colder than average winters at both southern sampling sites. Only in the winter of 2001-2 at Yakushima did NZ% stay below 65% for more than single month, despite winter 2002-3 having lower temperatures at both sites. This unusual result could be a "carryover" result due to the poor condition (and very low NZ%) of *Symbiodinium* in *Zoanthus* during summer 2001, when temperatures were much higher than usual, and coral bleaching was observed in southern Japan (STRONG *et al.* 2002). Alternatively, short exposures to very low air temperatures (approx. 12℃) and strong winds during extreme low tides have been shown to induce bleaching one to two weeks later in Acroporoid corals (HOEGH-GULDBERG and FINE 2004). During extreme winter low tides at night on 11-12 Feb. 2002 air temperatures at Yakushima reached as low as 7.5℃ to 7.8℃ (2.0-2.3℃ colder than the expected coldest winter temperature, JMA), with strong winds of 32.4km/h. Our unexpectedly low NZ% data from February 2002 (collected 28 Feb.) may reflect these cold conditions.

Further evidence of the lack of strength of negative winter effects at the southern sampling sites is seen in the growth and expansion of *Zoanthus sansibaricus* colonies during winter months. From these results it may be inferred that expected winter temperatures at Yakushima and Amami do not negatively impact the *Zoanthus*-*Symbiodinium* holobiont.

Summer and "Hot" Temperatures

Summer ocean temperatures at all four sampling sites exceeded expected annual maximum temperatures for all three summers (2001-3) that the study was conducted. Contrary to what was expected, however, at Kokubu and Sakurajima NZ% did not decrease and instead significantly increased during "hot" conditions. ONO *et al.* (2003) similarly saw no decrease in *Zoanthus* cover at Sakurajima during summer 2001. When compared to Yakushima and Amami, where NZ% decreases were seen in "hot" temperatures, there are significant differences in the northern sampling sites' environments: Both Kokubu and Sakurajima *Zoanthus* are sub-tidal (unlike inter-tidal *Zoanthus* sampled at Yakushima and Amami), and visibility is significantly worse in Kagoshima Bay (where Kokubu and Sakurajima are located) than on the open Pacific Ocean at Yakushima and Amami (SO and JDR, personal observation). Studies have shown that light (both irradiance and UV) has a compounding detrimental effect in conjunction with temperature on *Symbiodinium* condition (JONES *et al.* 1998; Salih *et al.*, 1998) and that both irradiance and UV can be inversely correlated to *Symbiodinium* density (STIMSON 1997). The combination of cloudy water, no direct exposure to sunlight, and slightly lower "hot" temperatures (Kokubu and Sakurajima maximum observed temperatures 0.2℃ lower than at Yakushima and 0.7℃ lower than at Amami) appears to be enough to prevent any serious decrease in NZ% at the northern sampling sites.

On the other hand, NZ% significantly dropped during "hot" weather at the tidal southern sampling of Yakushima and Amami. The largest drops were seen in the summer of 2001, when widespread coral bleaching was observed throughout the Ryukyu Islands coinciding with high ocean surface temperatures due to the El Nino-Southern Oscillation (ENSO) phenomenon (STRONG *et al*. 2002). According to our KPFEC data, ocean temperatures were higher than expected (annual expected maximum) at Yakushima for 64 days in 2001, including 36 days continuously (July 14-August 18). At Amami, extreme conditions were even more prolonged in 2001, with 90 days over the annual expected maximum, including 52 days continuously (June 28-August 18), reaching of 31.33℃ in mid-August.

In addition to the severe NZ% decreases seen at both Yakushima and Amami in summer 2001, several bleached *Zoanthus* colonies were observed at both southern sites outside of our sampling areas. *Symbiodinium* samples taken from bleached colonies at Yakushima showed NZ% (25.3 ± 19.7%, n=10) to be highly variable but much lower than *Symbiodinium* NZ% in non-bleached *Zoanthus* adjacent to bleached colonies (63.0 ± 11.0%, n=10). The large majority of observed stressed *Symbiodinium* were clear, having lost their chlorophyll (=morphotype TZ, Fig. 2). It should be remembered that the correlation between tide pool temperature at Yakushima and Amami and NZ% was not very strong, suggesting that short-term inter-tidal exposure to extreme temperatures (ranging from 12.97-37.80℃ at Amami) does not impact NZ% in *Zoanthus* as much as long-term ocean temperature.

Other Environmental Factors

Sunlight was negatively correlated with "cold" NZ% northern data, suggesting that clear skies in winter are linked invariably to colder ocean temperatures, which are in turn correlated with a decrease in NZ%. Similarly, "hot" southern data correlations between NZ% and pH and pHmV can be consistently linked with ocean temperature (both 2-week temperature and surface temperature). However, no definite explanation exists for the correlation between dissolved oxygen data and NZ%. For NZ% at normal temperatures the correlation is positive and much smaller (r =0.238, n =23). Similar to our observed NZ%-DO correlation at "hot" temperatures, FAGOONEE *et al.* (1999) noted a large negative correlation between *Symbiodinium* concentration and dissolved oxygen concentration and could offer no likely explanation for the phenomenon. Further research into this matter is warranted.

Possible Mechanisms Behind NZ% Decreases

JONES *et al*. (1998) have proposed that initial heat damage in dinoflagellate symbionts is in carboxylation in the Calvin cycle, similar to the damage seen in plants from heat. This damage then leads to PSII damage and photoinhibition caused by high light (irradiance/UV) levels. As outlined in SALIH *et al*. (1998) and TCHERNOV *et al*. (2004), stressed *Symbiodinium* undergo thylakoid breakdown. Thylakoid membrane breakdown from abnormal heat and light levels results in the production of oxygen radicals, leading to lipid peroxation and the shrinkage and eventual rupture of chloroplasts. This corresponds closely to our observed clear "stressed" (TZ) *Symbiodinium*. This theory could help explain why bleaching and strong NZ% decreases at sub-tidal northern sites (with lower irradiance and UV levels) were not seen in summer 2001 but were seen inter-tidal southern sites.

Studies have shown that many different strains, or clades, of *Symbiodinium* spp. exist in symbioses with marine invertebrates (KINZIE and CHEE 1979), and these clades may have slightly different physiological limits (WARNER *et al*. 1999, TCHERNOV *et al*. 2004). Many clades may be present in the natural environment in each area (LAJEUNESSE 2002), and it is possible that many clades may exist within a single host species (POCHON *et al*. 2001) or even an individual host (LOH *et al*. 1998). It is believed that latitudinal flexibility in a host species is a trait used to adapt to different environments (LAJEUNESSE and TRENCH 2000). Genetic data (REIMER *et al*. 2006b) show that *Zoanthus* colonies at Kokubu, Sakurajima, and Yakushima often host different species-/clades (C1/C3-related and C15-related, see LAJEUNESSE 2005) of *Symbiodinium* than Amami *Zoanthus* (C1/C3-related and A1). However, unlike many other algae, it appears that regardless of clade, *Symbiodinium* are not very flexible with regards to their thermal tolerance thresholds (TCHERNOV *et al*. 2004), and small changes in ocean temperatures of +<2.0℃ can lead to bleaching and/or drops in *Symbiodinium* density (PODESTA and GLYNN 2001). Despite possessing *Symbiodinium* of different clades, *Zoanthus* at Yakushima and Amami displayed almost identical seasonal NZ% patterns, suggesting that *Symbiodinium* in *Zoanthus* are affected by even small ocean temperature changes observed here despite genotypic differences.

Conclusions

For *Symbiodinium* in *Zoanthus* under normal ocean temperature conditions (between 18.0℃ and 28.5℃), NZ% of greater than 70% are to be expected. NZ% data showed slight variation from month to month at each site, but were correlated to 2-week ocean temperature, reflecting the utility of this method in long-term investigations into holobiont condition. Different seasonal patterns of NZ% data were seen at the four investigated sampling sites, with northern sites (Kokubu, Sakurajima) having "cold" temperature (<18.0℃) winter NZ% decreases below 70%, and southern sites (Yakushima, Amami) suffering NZ% decreases under "hot" summer conditions(>28.5℃). No other investigated environmental data set had a large and consistent correlation with observed NZ% data patterns. We investigated all environmental parameters we felt could possibly cause NZ% decreases, but other parameters such as bacterial infection and disease do remain possibilities, although no evidence was seen during the course of this study. Winter NZ% decreases at Kokubu and Sakurajima appear to an annual event, as winters were not colder than expected at these sites during our study.

On the other hand, summer NZ% decreases only occurred at ocean temperatures above expected annual maximum temperatures, which were observed for all three summers during the study, suggesting this is not an annual or "expected" phenomenon, and that much like coral bleaching, this may be a relatively recent development related to increasing ocean temperatures. It appears that the *Zoanthus-Symbiodinium* holobiont is living at both the high (at Yakushima and Amami during ENSO events) and low ends (at Kokubu and Sakurajima in winter annually) of its thermal tolerance.

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