

EVIDENCE OF SEASONAL AND GEOGRAPHICAL VARIATIONS IN *ZOANTHUS PACIFICUS* AND ITS ENDOSYMBIOTIC ZOOXANTHELLAE DUE TO ENVIRONMENTAL FACTORS

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Abstract

Despite much progress, many questions regarding coral bleaching in cnidarians are still unanswered. One specific phenomenon that has received little attention thus far is the variability and change in zooxanthellae body types due to environmental changes. Our previous studies have shown that zooxanthellae are strongly affected by abnormal light and especially, abnormally high seawater temperatures. However, all previous research has been conducted in the lab, with samples from only one site. Confirmation of these hypotheses in the field from multiple sites would go a long way in helping solidify our previous theories, and shed light on other aspects of the cnidarian-zooxanthellae symbiosis.

To accomplish this samples of the encrusting anemone *Zoanthus pacificus* were collected each month from four field sites (Kokubu-Wakamiko (K), Sakurajima-Hakamagoshi (S), Yakushima-Kurio (Y), and Amami-Oshima-Kasari (A)). Immediately after sampling, endosymbiotic zooxanthellae were isolated from each sample and examined under a microscope using a hemocytometer, and classified by body type. % cover transect data was also collected. Initial (15 month results) show that the sites can be divided into Northern (K and S) and Southern (Y and A) Groups. The Northern Group shows little decrease in NZ during summer, but a significant drop in NZ during the winter months while the Southern Group displays an NZ decrease in hot summer months, and either no drop (A) or a slight drop (Y) in NZ during winter. (A) shows no drop in NZ ratios during winter, and (Y) shows a decrease in NZ (although not as dramatic as K or S). These results indicate that seawater minimum and maximum temperatures approach 17 and 30 °C, respectively, based on temperature readings taken from the field sites. This is supported by the transect data, which shows a marked decrease in % *Zoanthus* cover over winter at Y but not at A. Additionally, *Zoanthus* appears to be able to withstand short-term (up to 6 hours) intertidal temperatures ranging from 13 °C to 37 °C.

Key words: Zoanthid, Zooxanthellae, Body type, Temperature, *Zoanthus*, Bleaching, Seasonal variation

Introduction

Zoanthus and other cnidarians such as coral rely heavily upon endosymbiotic zooxanthellae as a source of energy. Even though the problem of coral bleaching (the expulsion and/or degradation of endosymbiotic zooxanthellae) has become a worldwide issue (GLYNN & D CROZ, 1990; HOEGH-GULDBERG & SMITH, 1989), and many studies have been conducted on coral and zooxanthellae, there are still many unanswered questions. Previous studies in the lab on *Zoanthus* (REIMER et al. in review 2002) have shown that at 30 °C and 33 °C *Zoanthus* ' normal zooxanthellae (NZ)

become less frequent than at normal temperatures (23 °C), and the degraded zooxanthellae (DZ) form DDZ (dark degraded zooxanthellae) becomes more frequent. (For detailed explanations of zooxanthellae body types please see Figure 1, and TITLYANOV et al., 1996, and KUROKI & van WOESIK, 1999) There is also direct lab evidence of digestion of zooxanthellae by *Zoanthus* (REIMER et al. in review 2002). However, whether zooxanthellae digestion and coral bleaching occurs in *Zoanthus* under natural conditions in the field or not is unknown. As well, is there any variation in the upper critical temperature observed in the lab of 30 °C for the onset of bleaching and increase in DDZ with *Zoanthus* over a wide geographic range?

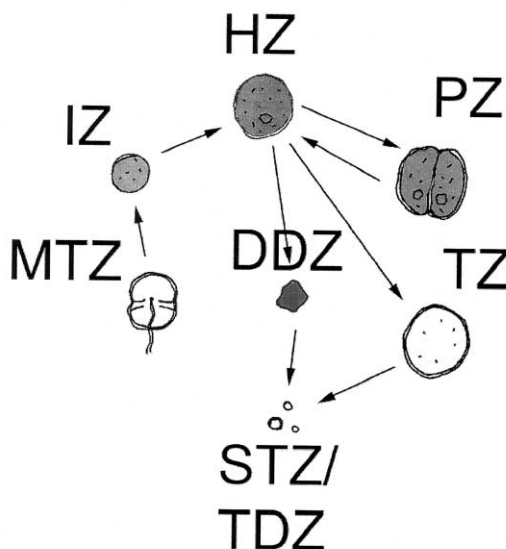


Fig.1. Body types of endosymbiotic zooxanthellae adapted from previous research (MCLAUGHLIN et al., 1966; TITLYANOV et al., 1996; KUROKI & van WOESIK, 1999). Definition of body types: 1) HZ - healthy zooxanthellae, characterized by their large (6-8 μm in diameter) circular shape, and greenish-brown color 2) PZ - proliferating zooxanthellae, HZ undergoing mitotic division 3) DDZ - dark degraded zooxanthellae, smaller in size and darker than HZ (usually approximately 3 μm in diameter) and more irregular in shape 4) TZ - transparent zooxanthellae, zooxanthellae that are characterized by a near or complete loss of color (photosynthetic pigments) 5) STZ/TDZ - small transparent zooxanthellae and transparent degraded zooxanthellae, very small in shape (less than 3 μm in diameter) and colorless 6) MTZ - mobile transparent zooxanthellae, characterized by their two flagella and bell-like shape, and 7) IZ - immature zooxanthellae, zooxanthellae with photosynthetic pigments but not yet full in size (approximately 4 μm in diameter).

Materials and Methods

Sampling

Samples of *Zoanthus pacificus* were collected each month from four field sites (Kokubu-Wakamiko (K), Sakurajima-Hakamagoshi (S), Yakushima-Kurio (Y), and Amami-Oshima-Kasari (A)). The four field sites are separated by a distance of almost approximately 400 kilometers

north-south. At each field site 5 locations at different depths and exposure were selected and one sample from each consistently collected. Collected samples were immediately placed in a cooler for transport.

Environmental data (pH, ocean temperature, salinity, conductivity, and dissolved oxygen content) were collected with a YSI Inc. Aquameter. Data was collected from each location at each site every month. The data was also supplemented by UV and temperature data from local weather stations.

Zooxanthellae Body Type Research

Immediately after sampling, endosymbiotic zooxanthellae were isolated from each *Zoanthus* sample by use of a 0.1-ml syringe and examined under a microscope at 400X using a hemocytometer. Zooxanthellae were classified into different body types (see Fig. 1 for a detailed explanation), and the results compared with environmental parameters (seawater temperature, salinity, DO, pH, etc.) over time. Counts were performed on each sample from each location at each site for five fields of view.

Transect Study

At both the Y and A sites, one inter-tidal location of dimensions 50 cm by 50 cm was selected. Photographs of each transect site were taken each month, and the percentage cover calculated using a computer-drawn grid. These data were compared over time against environmental data collected.

DNA classification of *Zoanthus* and zooxanthellae

Zoanthus and zooxanthellae both have been the subject of controversy with regards to classification, and no classification study of *Zoanthus* in southern Japan has yet been performed. Colonies of *Zoanthus* of oral disk color not reported in literature are numerous at all four sites. To confirm that our focus species is indeed *Zoanthus pacificus* and to shed light on the endosymbiotic zooxanthellae, classification to the DNA level is scheduled.

Samples of *Zoanthus* were collected from field sites in late August 2002. Collected samples were placed in marked bottles, and placed immediately into coolers away from direct sunlight exposure. Upon return to Kagoshima University labs, samples were wrapped in aluminum foil to prevent exposure to light, and placed in a deep freeze at -80 °C.

DNA analysis of both *Zoanthus* and zooxanthellae is currently being performed at the JAMSTEC headquarters in Yokosuka, Japan, starting from September 2002. While initial results are known, it is expected that conclusive results will not be ready until spring/summer 2003.

Results

The results of the zooxanthellae body type analysis when compared with seawater temperatures are shown in Figure 2. A close examination of the graphs shows that there are two major patterns, a northern pattern with winter 2001~2 decreases in healthy zooxanthellae (NZ) shown by K (a) and S (b), and a southern pattern displayed by Y (c) and A (d) with healthy zooxanthellae decreases in abnormally hot summer 2001. As well, the southern group shows either only a slight drop (Y) or no drop (A) in NZ ratios in winter. All sites showed little or no decrease in NZ ratios in summer 2002, when ocean temperatures did not rise much above average expected levels.

Figure 2(a) - Monthly Changes in NZ Ratio and Ocean Temperature, Wakamiko, Kokubu

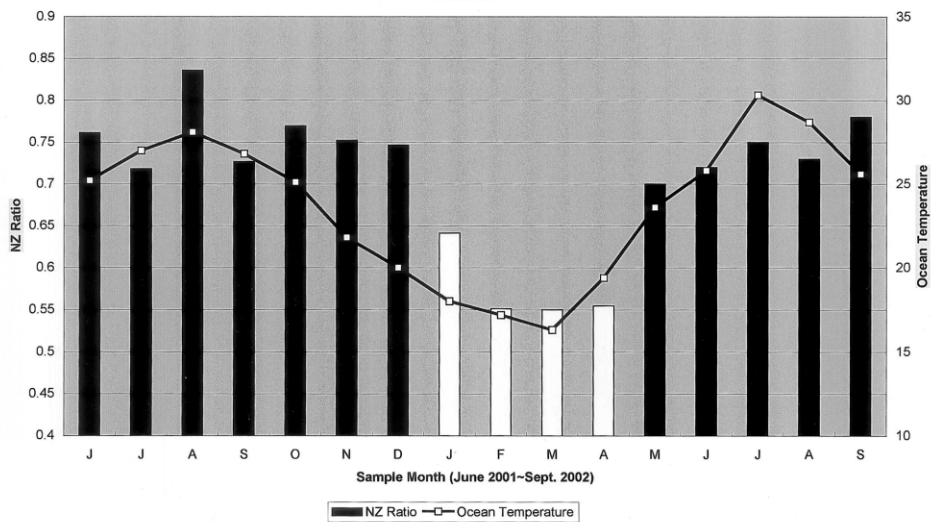


Figure 2(b) - Hakamagoshi, Sakurajima

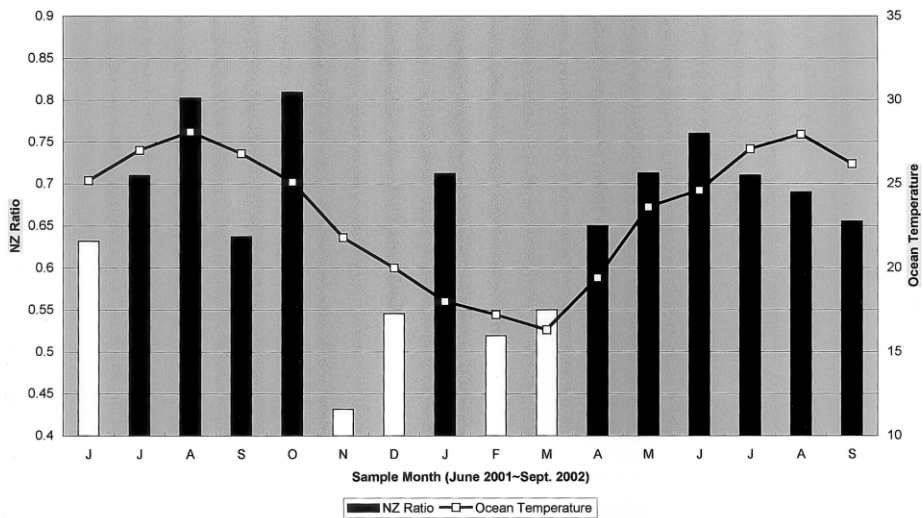


Fig.2. Monthly changes in NZ ratios at the four field sites. (a) Wakamiko, Kokubu, (b) Hakamagoshi, Sakurajima, (c) Kurio, Yakushima, and (d) Tomori Beach, Kasari, Amami-Oshima. Please note that black bars represent NZ values greater than 0.65 (thus 65% or higher NZ content), while white bars represent NZ values lower than 0.65, indicating stress.

Transect data of percentage cover of *Zoanthus* at Y and A are shown in figure 3. Both locations show a winter decrease in percentage cover that was not reflected in the zooxanthellae data. Summer data from 2002 is still being analyzed at the time of writing.

Figure 2(c) - Kurio, Yakushima

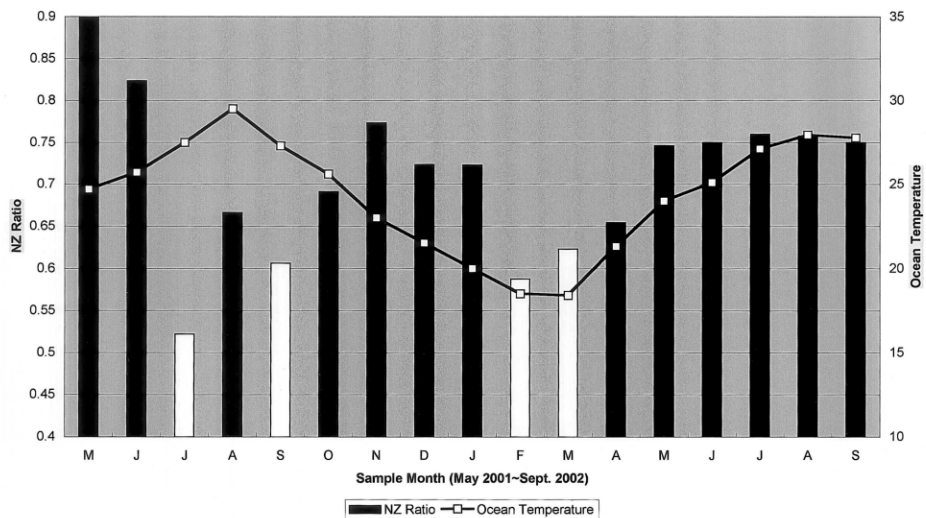
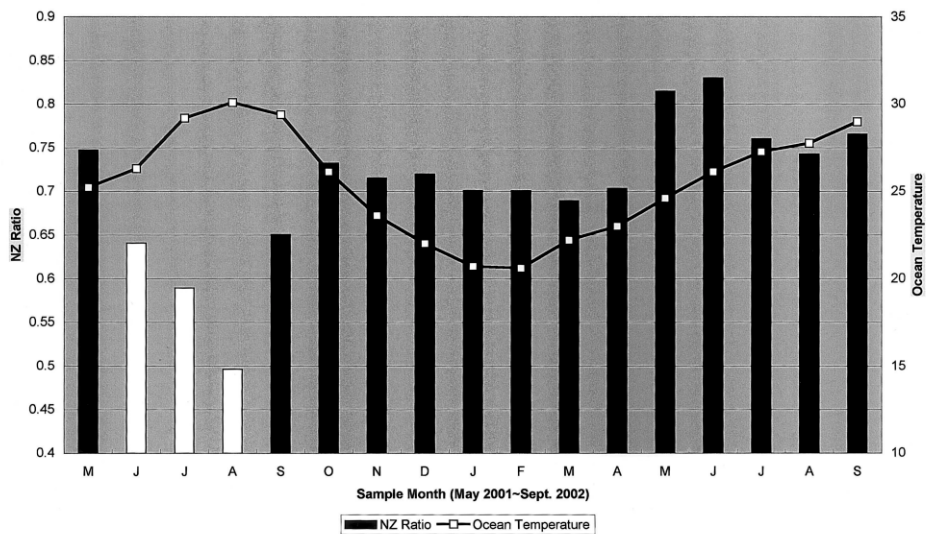


Figure 2(d) - Tomori, Kasari, Amami-Oshima



Discussion and Conclusions

The results in figure 2 and 3 indicate a low-end critical temperature for *Zoanthus pacificus* at approximately 15 °C, which is the winter minimum seawater temperature for the K and S sites. *Zoanthus* survives at this temperature, but the ratio of healthy NZ zooxanthellae decreases, and *Zoanthus* colonies experience winter contraction even at the warmer Y and A sites further south.

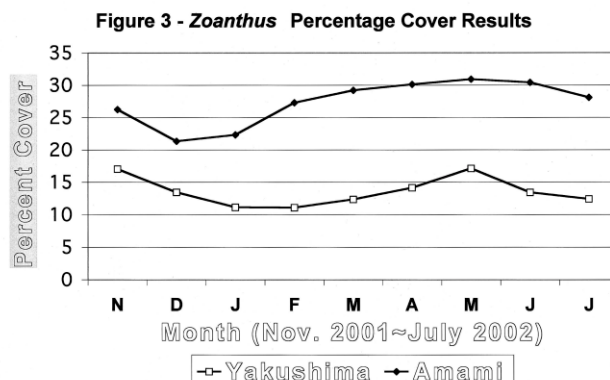


Fig.3. Monthly changes in percentage cover of *Zoanthus* at Yakushima and Amami-Oshima field sites.

Maximum seawater temperatures appear to be in the area of 30 °C for *Zoanthus* at all sites, as *Zoanthus* NZ ratios at Y and A sites decreased during the abnormally hot 2001 summer. K and S sites showed no summer decrease despite being further north and seawater temperatures being only slightly cooler.

One factor not shown in these calculations is the fact that length of exposure is also important. While seawater temperatures may fluctuate between 15 and 30 °C, *Zoanthus* at the Y and A sites are exposed during extreme tides, with many colonies not even in tidepools. A temperature of 13 °C has been recorded in a tidepool at Y during January 2002, and outside air temperatures reached as low as 2 °C. Conversely, at the A site in August 2002, a tidepool seawater temperature of 37.8 °C was recorded, and air temperature at all sites regularly can hit 35-36 °C in summer. It appears that *Zoanthus* and zooxanthellae can survive short (up to 6 hour) exposures to very extreme temperatures, but long-term differences of even 0.5 °C can greatly impact their survival. Summer 2002 data from Y and A further supports this, as the normal seawater temperatures observed (up to 27.8 °C) did not cause any decrease in NZ ratios, as opposed to summer 2001 data.

An important question that remains to be answered is whether or not the summer decrease in NZ at Y and A is a recent phenomenon or not. Coral bleaching and associated problems are assumed to be a result of global warming yet our research shows that, at least at K and S sites, some stress in winter appears to be naturally occurring. With ocean temperatures expected to continue to rise in the future, the naturally occurring pattern of winter stress may switch to the summer stress pattern seen in Y and A samples in the summer of 2001. How these trends impact cnidarians and their zooxanthellae remains to be seen, but the overall prognosis given the sensitivity of *Zoanthus* and NZ ratios to even a small change (as seen in summer 2001) is not good.

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