3 blank lines

Degradation of zooxanthellae in the coral *Acropora nasuta* during bleaching

2 blank lines

Takeshi MISE[1] and Michio HIDAKA*

Department of Chemistry, Biology, and Marine Science, University of the Ryukyus, Nishihara, Okinawa, 903-0213 Japan
[1]Present address: Seto Marine Biological Laboratory, Graduate School of Science, Kyoto University, 459 Shirahama-cho, Nishimuro-gun, Wakayama 649-2211, Japan
*Corresponding author: M. Hidaka
FAX: 098-895-8547, e-mail: hidaka@sci.u-ryukyu.ac.jp

Text: Times New Roman 10 point, two columns

Abstract To study morphological changes in zooxanthellae in bleached corals, we observed zooxanthellae in colonies of *Acropora nasuta* that suffered various degrees of bleaching. Colonies were collected from a shallow reef at Bise, Okinawa, from July to September in 2001. Zooxanthellae within the tissue of *A. nasuta* were classified into 3 types based on their coloration and shape: healthy-looking zooxanthellae (Hz.), pale zooxanthellae (Pz.) and transparent zooxanthellae (Tz.). The Pz. were pale yellow with more or less disorganized cellular content, while Tz. were swelled and vacuolated with no pigmentation. Tz. were the dominant zooxanthellae in colonies that suffered severe bleaching, while non-bleached colonies and those recovered from bleaching had mostly Hz. Some Tz. were ruptured with vacuoles spreading out of the cell. The density of zooxanthellae in severely bleached colonies was about 10^5 cells cm^{-2}. The present observation suggests that, during natural bleaching due to chronic stress, zooxanthellae remained in host tissue lose pigmentation and become swelled and vacuolated suggesting necrotic death of the zooxanthellae.

Keywords coral, bleaching, zooxanthellae, *Acropora nasuta*, symbiosis

Introduction

During the last two decades there has been an increasing incidence of coral bleaching. Bleaching is defined as a loss of coloration due to decreased number of symbiotic algae and/or their photosynthetic pigments. Coral bleaching has generally been attributed to high seawater temperatures and/or high solar radiation (e.g., Glynn 1993; Brown 1997; Hoegh-Guldberg 1999, 2000; Fitt et al. 2001). However, the mechanism by which zooxanthella density is reduced during bleaching is not yet well understood. A number of cellular mechanisms that would result in reduced densities of zooxanthellae have been proposed. Gates et al. (1992) reported that detachment of gastrodermal cells containing zooxanthellae occurs after a sea anemone or coral host is subjected to a temperature shock. Brown et al. (1995) did histological studies of corals sampled during a natural bleaching event and suggested that degradation of zooxanthellae within the gastrodermal tissue of host corals and release of zooxanthellae from the gastrodermis into the coelenteron are the major mechanism by which zooxanthellae density decreases during bleaching. On the other hand, loss of zooxanthellae can occur without decrease in chlorophyll concentrations per zooxanthella during warm-water bleaching (Jones 1997). Recently, Dunn et al. (2002) described that healthy-looking zooxanthaellae are expelled when a sea anemone host is exposed to acute high temperature stress, while, under chronic stress, zooxanthellae are degraded within the host tissue through necrotic or apoptotic death. In both cases, necrotic death of host gastrodermal cells always precedes or accompanies degradation of zooxanthellae. These studies suggest that there are multiple mechanisms causing zooxanthella degradation in naturally bleached corals as well as experimentally bleached cnidarians. However, only a few studies have been done on morphological changes in zooxanthellae in naturally bleached corals (Szmant and Gassman 1990; Brown et al. 1995; Kuroki and van Woesik 1999).

In order to study morphological changes of zooxanthellae in naturally bleached corals, we observed zooxanthellae in colonies of *Acropora nasuta* that suffered various degrees of bleaching in the summer of 2001. The relative abundance of zooxanthellae at various degradation stages and the zooxanthella density of branches sampled monthly from 14 colonies from July to September 2001 were examined. Zooxanthellae contained in bleached corals were swelled and vacuolated with loss of pigmentation, suggesting necrotic death.

Materials and Methods

Fourteen colonies of *Acropora nasuta* were tagged at the shallow reef at Bise, Okinawa, in July 2001. Underwater photographs of the colonies were taken using a digital camera (Olympus, Camedia C-2040 Zoom) with an underwater housing at monthly intervals from July to September 2001 to record the degree of bleaching. At the same time a branch of a few cm length was sampled from each colony and preserved in 10% formalin in filtered seawater.

Small piece of tissue was peeled off from each of the preserved samples and squashed under a cover slip. The squashed samples were observed under a microscope

(Nikon Optiphot) with differential interference contrast optics. One hundred zooxanthellae were examined and categorized as either healthy-looking zooxanthellae (Hz.), pale zooxanthellae (Pz.), or transparent zooxanthellae (Tz.) (see Results). In order to measure zooxanthellae density (*ZD*), the number of zooxanthellae (*n*) within three microscopic fields (3 x 0.071 mm^2) was counted for each sample using a 40X objective lens. The area of the isolated tissue (S_0) and the area of the tissue after squash (S_1) were measured by drawing the contour of the tissue before and after squashing under a stereomicroscope equipped with a camera lucida. The drawings were input to a computer using a scanner and the area was measured using a NIH image software. The zooxanthellae density (*ZD*) was calculated using the following formula.

$$ZD = \frac{n}{0.071 \times 3} \cdot \frac{S_1}{S_0} \cdot 100 \text{ (cells cm}^{-2}\text{)} \qquad (1)$$

Results

Out of 14 colonies of *Acropora nasuta* observed, 4 colonies (NB) remained non-bleached during the observation period, 3 colonies (BR) were bleached in August but recovered normal brown color in September, 3 colonies (BPD) were bleached in July and August and partially died but the remaining part of the colonies recovered in September, and 4 colonies (BD) died of bleaching in August (Fig. 1).

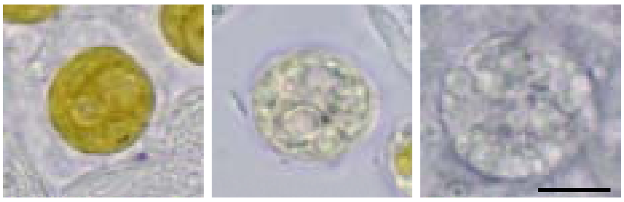

Fig. 3. Three types of zooxanthellae observed in non-bleached and bleached colonies of *Acropora nasuta*. **A**, Healthy-looking zooxanthella (Hz.) in a non-bleached colony. **B**, Pale zooxanthella (Pz.) in a bleached colony. **C**, Transparent zooxanthella (Tz.) in a bleached colony. Scale bar = 5 µm.

While non-bleached colonies contained mostly healthy-looking zooxanthellae (Hz.), bleached colonies contained pale or transparent zooxanthellae (Pz. and Tz.) (Fig. 2). The Hz. possessed brown color and normal cytoplasmic organization. The Pz. were pale yellow with more or less disorganized cellular content, while Tz. lacked pigment and were swelled with their cytoplasm filled with vacuoles (Fig. 3). Occasionally zooxanthellae undergoing rupture were observed (Fig. 2C).

Figure 4 shows the relative abundance of Hz., Pz., and Tz. in colonies that suffered various degrees of bleaching. Non-bleached (NB) colonies had mostly Hz. throughout the observation period of 3 months (Fig. 4A). Bleached but recovered (BR) colonies had many Pz. and Tz. in addition to Hz. in August but they contained mostly Hz. after they recovered in September (Fig. 4B). In BPD colonies that were bleached in July and August and partially died in September, Tz. were the major zooxanthellae in July and August, but Hz. became dominant in the recovered portion of the colony in September (Fig. 4C). Bleached colonies (BR and BPD) had a significantly higher percentage of Pz. and Tz. than non-bleached colonies in August (LSD multiple comparison test, $P<0.01$) (Fig. 4B, C). However, the survived and recovered part of the colonies contained mostly Hz. in September and there was no significant difference in the relative abundance of Pz. and Tz. between the recovered portion of bleached colonies and non-bleached colonies. In colonies (BD) that were bleached in July and dead in August, Tz. and Pz. were the major zooxanthellae and Hz. were less than 10% (Fig. 4D). Degraded zooxanthellae (Dz.) with shrunk cytoplasm and a gap within the cell wall, which are commonly observed in corals exposed to stress in laboratory experiments (Fukabori, 1998; Mise and Hidaka in prep.), were rarely observed in the naturally bleached colonies of *A. nasuta* (Fig. 4, category of 'others').

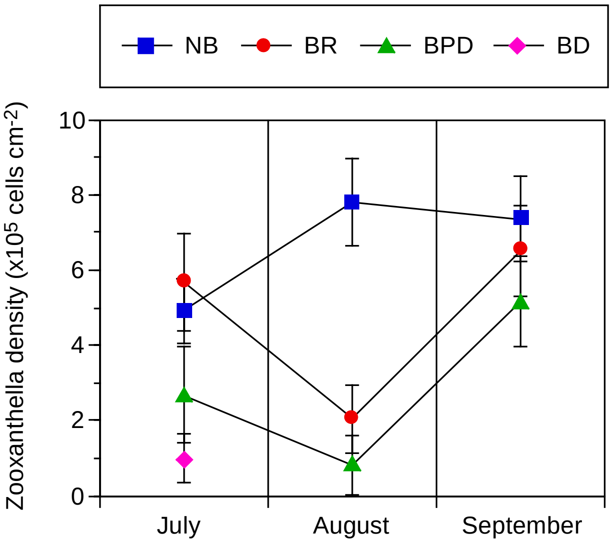

Fig. 5. Zooxanthella density in colonies of *Acropora nasuta* that suffered various degrees of bleaching. Samples were collected and fixed on 7-20th July, 18th August, and 16th September, 2001. NB, non-bleached colonies; BR, bleached but recovered colonies; BPD, bleached and partially died colonies; BD, bleached and died colonies. Means ± SE (n=3-4).

The zooxanthellae density of bleached colonies (BR and BPD) was significantly lower than that of non-bleached colonies (NB) in August (Fig. 5) (LSD multiple comparison test, $P<0.01$). However, after they recovered in September, there was no significant difference in zooxanthellae density among NB, BR, and BPD colonies ($P>0.05$). The zooxanthellae density of the BD colonies sampled in July was about 10^5 cells cm^{-2}, while that of the BPD colonies sampled in August was slightly less than 10^5 cells cm^{-2}.

Discussion

This study shows that transparent zooxanthellae, Tz., were dominant in the tissue of bleached colonies of *Acropora nasuta* sampled in July and August 2001 (Fig. 4). Most transparent zooxanthellae were swelled with conspicuous vacuolization. Zooxanthellae in the process of cell rupturing were also observed. These observations are consistent with the previous studies on naturally bleached corals (Szmant and Gassman 1990; Brown et al. 1995) and a sea anemone exposed to chronic stress (Dunn et al. 2001) and suggest that zooxanthellae are degraded via necrosis within the tissue of naturally bleached corals.

This study also revealed a negative correlation between algal density and the percentage of Tz. and Pz. (Fig. 4 and 5). Bleached colonies (BR and BPD) had significantly higher percentage of Tz. and Pz. and significantly lower algal density than non-bleached colonies in August 2001. In September 2001, some of the bleached colonies recovered and both the algal density and percentage of Pz. and Tz. returned to the level of non-bleached colonies.

The swelled, vacuolated zooxanthellae observed in this study were different from degraded zooxanthellae or degraded zooxanthella particles observed in non-stressed corals (Titlyanov et al. 1996, 2001) or in corals exposed to acute stress in laboratory experiments (Fukabori, 1998; Mise and Hidaka in prep.). In these cases, the degraded zooxanthellae had shrunk cytoplasm and gaps within the cell wall and degraded zooxanthella particles were small, irregular shaped particles without cell wall. It is likely that these degraded zooxanthellae are produced by host digestion (Titlyanov et al. 1996; Jones and Yellowlees 1997), though it is also possible that they are degraded via apoptosis. Kuroki and Woesik (1999) observed pale-transparent zooxanthellae with cell organelles and small-transparent zooxanthellae in bleached colonies of *Stylophora pistillata* and suggested that pigment degradation in zooxanthellae and subsequent cell shrinkage rather than zooxanthellae expulsion were the mechanism involved in bleaching of the coral. Dunn et al. (2002) observed both swelled, vacuolated zooxanthellae and shrunk zooxanthellae with condensed organelles and cytoplasm in the gastrodermis of the sea anemone exposed to chronic (7 d) high temperature stress, and suggested that zooxanthellae undergo in situ degradation via two pathways, that is, necrosis and programmed cell death (apoptosis). Thus it is likely that the pathway of zooxanthella degradation may differ depending on the intensity and duration of stress and also among the host species.

The vacuolated, transparent zooxanthellae observed in this study were also different from the transparent zooxanthellae observed in polyps of *Galaxea fascicularis* exposed to strong light (Fukabori 1999; Mise and Hidaka in prep.). The transparent zooxanthellae induced by strong light retained their cellular structure though they lost pigmentation completely. In this case, pigment degradation occurred while cellular organelles appeared intact.

It is interesting that 14 colonies observed in this study showed different bleaching responses, although they occurred in a small area of a shallow reef. Whether these differences are due to differences in the microenvironment or in bleaching susceptibility of different zooxanthella genotypes associated with these colonies remains for further studies.

The present results point out that bleached colonies of *A. nasuta* can recover from bleaching in one month if the temperature returns to normal. In September, the 10-d average sea surface temperature in front of the Sesoko Station of Tropical Biosphere Research Center, Sesoko Island, Okinawa, returned to below 30^{o}C and was about 28^{o}C in mid and late September (Nakano, personal communication). Within one month zooxanthella density of the recovered colonies increased four fold. This is not unexpected since the doubling time for zooxanthellae in three species of *Acropora* has been estimated to be 7.7-15.3 days (Wilkerson et al. 1988).

This work showed zooxanthellae in naturally bleached colonies of *Acropora nasuta* were swelled and vacuolated without pigmentation and that some showed cell rupturing, suggesting necrotic death. This study also points out the necessity of further work on the degradation pathway of zooxanthellae in hosts exposed to various stresses as other degradation pathways, such as via programmed cell death or host digestion, are suggested in other coral species under different stress conditions. Morphological changes of zooxanthellae might be useful for assessing sub-lethal damage on coral reefs (Salih et al. 1995).

Acknowledgements

We would like to thank anonymous reviewers for helpful comments to improve the manuscript. The authors also thank the staff of Sesoko Station, Tropical Biosphere Research Center of University of the Ryukyus, where part of this study was done. This study was partly supported by the grant Scientific Research from Sasakawa Grant.

References

Brown BE (1997) Coral bleaching: causes and consequences. Coral Reefs 16 Suppl: S129-S138

Brown BE, Le Tissier MDA, Bythell JC (1995) Mechanisms of bleaching deduced from histological studies of reef corals sampled during a natural bleaching event. Mar Biol 122: 655-663

Dunn SR, Bythell JC, LeTissier MDA, Burnett WJ, Thomason JC (2002) Programmed cell death and cell necrosis activity during hyperthermic stress-induced bleaching of the symbiotic sea anemone *Aiptasia* sp. J Exp Mar Biol Ecol 272: 29-53

Fitt WK, Brown BE, Warner ME, Dunne RP (2001) Coral bleaching: interpretation of thermal tolerance limits and thermal thresholds in tropical corals. Coral Reefs 20: 51-65

Fukabori Y (1998) Mechanism of bleaching of hermatypic corals: degradation and expulsion of zooxanthellae. Umiushi-Tsushin 18: 2-4 (in Japanese)

Gates RD, Baghdasarian G and Muscatine L (1992) Temperature stress causes host cell detachment in symbiotic cnidarians: implications for coral bleaching. Biol Bull 182: 324-332

Glynn PW (1993) Coral bleaching: ecological perspectives. Coral Reefs 12: 1-17

Hoegh-Guldberg O (1999) Climate change, coral bleaching and the future of the world’s coral reefs. Mar Freshwater Res 50: 839-869

Hoegh-Guldberg O (2000) Global climate change and the thermal tolerance of corals. Galaxea, JCRS 2: 1-11

Jones RJ (1997) Changes in zooxanthellar densities and chlorophyll concentrations in corals during and after a

bleaching event. Mar Ecol Prog Ser 158: 51-59
Jones RJ, Yellowlees D (1997) Regulation and control of intracellular algae (=zooxanthellae) in hard corals. Phil Trans R Soc Lond B 352: 457-468
Kuroki T, Van Woesik R (1999) Changes in zooxanthellae characteristics in the coral *Stylophora pistillata* during the 1998 bleaching event. Galaxea 1: 97-101
Salih A, Cox G, Hinde R (1995) Autofluorescence imaging of symbiotic algae in corals using confocal microscopy – a potential tool for environmental monitoring. Zool Stud 34 Supplement 1: 53-55
Szmant AM, Gassman NJ (1990) The effect of prolonged "bleaching" on the tissue biomass and reproduction of the reef coral *Montastrea annularis*. Coral Reefs 8: 217-224
Titlyanov EA, Titlyanova TV, Leletkin VA, Tsukahara J, van Woesik R, Yamazato K (1996) Degradation of zooxanthellae and regulation of their density in hermatypic corals. Mar Ecol Prog Ser 139: 167-178
Titlyanov EA, Titlyanova TV, Yamazato K (2001) Formation, growth and photo-acclimation of colonies of the hermatypic coral *Galaxea fascicularis* under different light conditions. Symbiosis 30: 257-274
Wilkerson FP, Kobayashi D, Muscatine L (1988) Mitotic index and size of symbiotic algae in Caribbean Reef corals. Coral Reefs 7: 29-36