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THE EFFECT OF TEMPERATURE ON ZOOXANTHELLAE OF *ISOPORA PALIFERA* AND *ACROPORA HYACINTHUS* FROM KARANRANG ISLAND, INDONESIA

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ABSTRACT

Climate change and global warming cause massive damage to the environment. One of the major events that are threatening the marine ecosystem is coral bleaching. Coral bleaching occurs when corals are exposed to above or below normal temperatures. The aims of this study are to compare the resistance of *Isopora palifera* and *Acropora hyacinthus* from Karanrang Island to temperature stress. Four treatment temperatures (28ºC, 30ºC, 32ºC, and 34ºC) were tested to assess the role of temperature stress and bleaching to *Isopora palifera* and *Acropora hyacinthus* for 48-hours. The abundance of zooxanthellae counted as the temperature stress variable. The results showed that there was a difference of coral response tothe treatment based on the time of experiment, after 48-hours experimentexposed at temperature treatment of 34°C the abundance of zooxanthellae from *Isopora palifera*was 0,06 $x10^5$ cm^{-2} and the abundance of zooxanthellae from *Acropora hyacinthus* is 0,18 $x10^5 cm^{-2}$. In comparison between species, *Isoporapalifera* taken from Karanrang Island was more resistant to temperature stress than*Acroporahyacinthus*.

Keywords:Climate change, coral bleaching, temperature rise, abundance of zooxanthellae.

INTRODUCTION

Coral reefs constitute some of the largest and most diverse ecological communities on earth and result from interactions between symbiotic organisms composed of photosynthetic dinoflagellate algae and cnidarian corals (Dustan, 1999, Stone 1999). The Coral Triangle as the heart of the world coral reefs is located across the coastal waters of Indonesia, Malaysia, Papua New Guinea, Philippines, Solomon Islands and Timor-Leste. Nearly 30% of the total coral reef area and 75% of all known coral species are found in this area, and it is home to over 3,000 species of fish twice the number found elsewhere in the world (Burke, *et al*, 2012). Indonesia with a total of 590 hard coral species represents more than 95% of the species diversity in the world. The greatest threats to today's coral reef ecosystems come from the anthropogenic pressures and global climate change that trigger a rise in seawater temperatures. In 2010, sea water temperature led to mass coral bleaching throughout Southeast Asia impacting many coral reefs in Indonesia. The worst affected areas are around Sumatra and Sulawesi, with 80-90% of coral reefs bleaching around Aceh (in the northern tip of Sumatra) (IPCC, 2007). The experiences of the last two decades suggest that bleaching happens when zooxanthellae are expelled from the coral tissue. There are numerous factors which are responsible for those events, with high temperatures and intense light being major contributors (Hoegh- Guldberg, 1999; Fitt *et al*., 2001).

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At high seawater temperatures, the damagetophotosynthetic and mitochondrial membranes in corals generates oxidative stress (Weis, 2008; Higuchi *et al* 2010), and this stress induces the loss of symbionts from coral tissues which gradually leads to coral bleaching (Jones, 1997).

Zooxanthellae are symbiotic dinoflagellates that form a mutualistic relationship with coral polyps and a wide range of marine invertebrates (Taylor 1974, Trench 1987). They have chlorophyll a and accessory pigments (e.g. other chlorophylls, carotenoids and phycobilins) for photosynthesis and provide products of photosynthesis to their host corals. It is estimated that there are about one million symbiotic zooxanthellae cells per square centimeter of coral tissues (S. Li, 2007), and up to 90% of the coral metabolic demand comes from the by-products of photosynthesis by the symbiotic zooxanthellae (R. Trench, 1979). Therefore, the growth of reef-building corals and the status of coral reef ecosystems are closely related to the photosynthesis of zooxanthellae.

A previous study observed preferential elimination of clade C zooxanthellae, which are associated with low irradiance, from multi-clade communities of *Symbiodinium* spp. in *Montastrea annularis* and *Montastre afaveolata* during bleaching (Rowan *et al*. 1997). Another study observed the selective release of symbionts with decreased photosynthesis at elevated temperatures from the same host species (Perez *et al*.2001). This study clarified that the algal partner is more susceptible to thermal stress than their coral hosts, suggesting that algal symbionts play a significant role in determining bleaching susceptibility of corals.

Field studies on mass bleaching have reported differences in bleaching susceptibility among coral species. It was reported that corals with faster growth rates (e.g. acroporids and pocilloporids) have been more severely affected by bleaching than slower growing species (e.g. poritids and faviids) in the Indo-Pacific region (Brown and Suharsono, 1990).

The aim of this study is to examine and compare temperature stress responses in corals from the family Acroporidae (*Isopora palifera*,Lamarck 1918 and *Acropora hyacinthus*, Dana 1984) from Karanrang Island.

MATERIAL AND METHOD

The experiment about temperature stress on corals*Isopora palifera* and *Acropora hyacinthus*was conducted at Hasanuddin University Marine Station from September to Oktober 2016. Mitotic index and abundance of zooxanthellae counted as temperature stress parameter.

Sampling Collection.

Samples were collected from Karanrang Island E.119.37690^{0}S.04.85259^0 (Figure 1).Samples of *Isopora palifera and Acropora hyacinthus* were collected in September 2016 from water depths between 1-3 m. One colony per species was collected and placed in separate plastic bag, which filled with sea water. Insitu water temperature was 29.8^{o}C, measured with a HANNA multiparameter water quality checker series HI 98194. The coral colonies were placed in the same outdoor tank filled with ambient seawater (29-30^{o}C) for two weeks to recover from damage during fragmentation and transportation from sampling time.

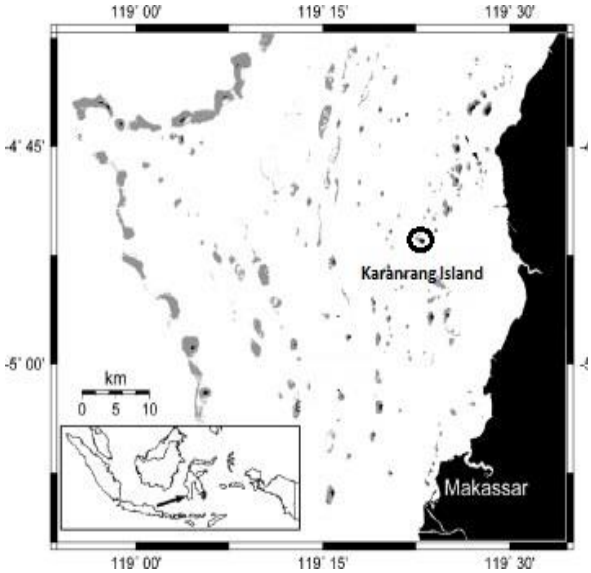

Figure 1. Map of the Spermonde Archipelago in southern Sulawesi. The Karanrang Island is circled. Map modified from Cornils *et al*. (2010).

This acclimation to reduce the possible effect of thermal or irradiance history on the stress susceptibility of the corals (Brown *et al*., 2000, 2002)

Experimental Design.

Four treatment groups were tested to assess the roles of temperature stress and bleaching on *Isopora palifera* and *Acropora hyacinthus*: 28^o, 30^{o}C (control), 32^{o}C and 34^{o}C, each with three replicates. Visual data of coral bleaching was collected for every 4hours by photographing the samples,density of zooxanthellae and counting per 12hours for period of 48hours.

Coral Surface Measurement.

The coral surface area was measured based on the method of Marsh (1970). Aluminum foil (1, 2, and 3 cm^2) were cut from a roll of standard kitchen foil and weighed to determine the weight per unit area of the foil. The procedure was repeated three times to provide an average of 2.90 ± 0.03 mg/cm^2 (mean ±SD) (Veal *et al*, 2010). Each coral skeleton was then carefully wrapped in the foil to minimize the overlapping of the foil. The weight of the foil required to cover each coral was then used to estimate the surface area of the coral skeleton.

Zooxanthellae Density Counting.

Coral tissues were airbrushed into a plastic bag filled with 50 mL of filtered seawater until all tissue was removed (the time for this process varied depending on the size of the coral branch; from five to ten minutes). Each of the samples was shaken vigorously; then, using a clean pipette, the sample was placed onto a Neubauer Improved (0.100 mm) haemocytometer, and viewed under 40x magnification with a light microscope. To mitigate 'edge effects' (i.e. counting cells lying on quadrat margins more than once) only the cells which touched the top and left-hand side of each square were counted. There were three replicate counts from each branch (McCowan *et al*. 2011). Zooxanthellae densities were calculated by following formula:

$$D = \frac{P \times Q \times 10000}{L}$$

Where D is the abundance of zooxanthellae, P is dilution, Q is number of zooxanthellae cells counted, L is number of coral fragment surface area and 10000 is convert 0.1 mm³ to 1 cm³.

Statistical Analyses.

The results of the experiment were analyzed using a two-way analysis of variance (ANOVA; factors: treatment and time) using GraphPad Prism 5 for Windows.

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RESULT AND DISCUSSION

Tests of between-subject effect of the experiment show that the time of experiment significantly affected the density of zooxanthellae (two-way ANOVA, $p < 0.01$; Table 1). There is no significant difference in all temperature treatment. This result indicates that the coral samples affected by temperature due to prolonged exposure to high temperature.

The Abundance of Zooxhantellae.

Samples from 28°C temperature treatment, showed a dramatical decreased of zooxanthellae abundance after 12 hours of temperature experiment (Figure.2a) since anin-situ water temperature from sampling site was 29.8°C so the treatment of 28°C was 1°C lower than the ambient seawater.These results were consistent with the previous study, who have reported that bleaching of *Acropora* spp. was occurred when the temperature was increasing or decreasing slightly (Williams and Bunkley-Williams 1990; Marshall and Baird 2000). Hoegh-Guldberg (1994) studied a variety of bleached scleractinian corals from French Polynesia and found that low temperatures caused bleaching in numerous species, and *Acropora* spp. showed the greatest susceptibilityto low temperature. Our finding contradicted with a study on *Montastrea annularis*, *M. cavernosa*, *Agaricia lamarcki*, *A. agaricites*, and *Siderastrea radians*that reported that those corals were not stressed at temperatures <30°C (Fitt and Warner, 1995; Warner *et al.* 1996).

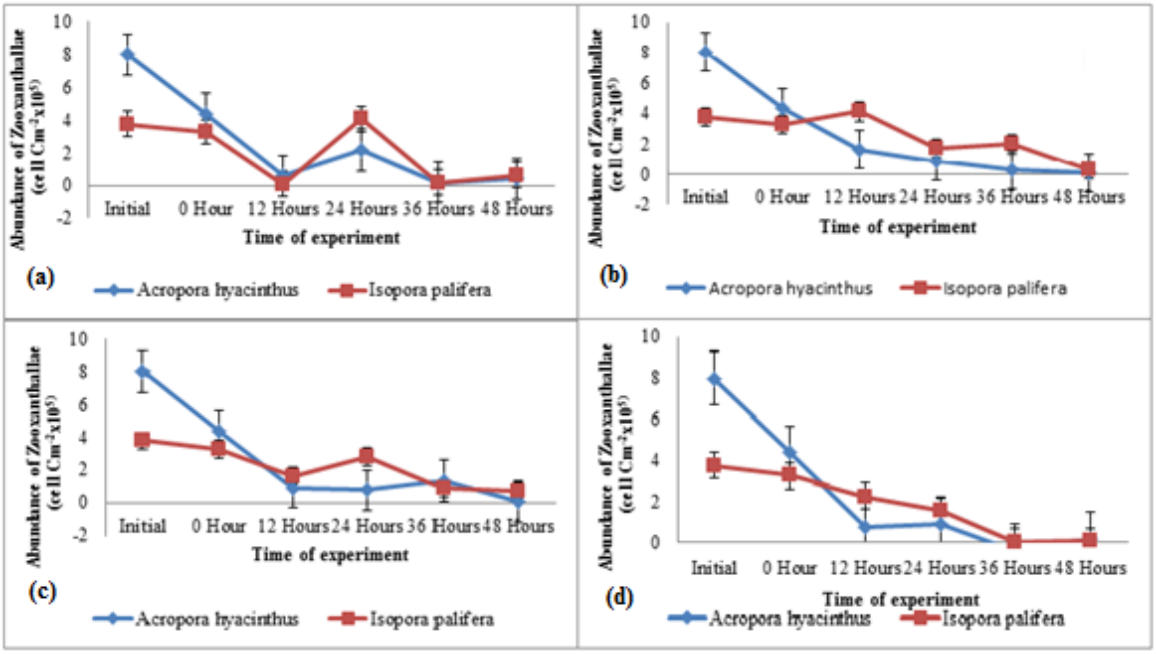

Figure 2. Abundance of zooxanthellae; (a). 28°C, (b). 30°C, (c). 32°C, (d). 34°C

An interesting fact appearedat 24-hoursof 28°C temperature exposure where the abundance of zooxanthellae was increasedsignificantly (Figure. 2a).This finding indicated that the temperature stressed caused zooxanthellae actively divided, which led to a high mitotic index (data unpublished).This finding lined with the previousstudy by Suharsono (1992) who found that algae released into the coelenteron from their intracellular location in the endoderm, during temperature increase, must subsequently divide at a faster rate than those remaining in the tissues. It is suggested that higher mitotic index is the result of the inclusion of released and dividing algae in the host coelenteron. Interestingly we found that after 24-hours of treatment,both high and low temperature would trigger the stress of *Isopora palifera* and *Acropora hyacinthus*. Furthermore, the highest abundance of zooxanthellae in *Isopora palifera*was found at 30°C after 12-hours of treatment (Figure. 2b). Zooxanthellae of *Isopora palifera* tended to decrease slowly after 12-hours of treatment, while *Acropora hyacinthus* decreased dramatically from the beginning of the experiment. Marshall and Baird (2000) also recorded similar observations of bleaching sensitivity in *Acropora* spp. in the Great Barrier Reef at 30–31°C. *Acropora palifera*, *A. hyacinthus*, and *A. cytherea* suffered high mortality, with <8% of some populations remaining alive.

Stress responses of *Isopora palifera* under 32°C temperature treatmentwere recorded after 24-hours treatment (Figure. 2c). Furthermore, both examined coral species that were exposed to the temperature of 32°C showed a lower zooxanthellae abundance

than 28^{o}C and 30^{o}C temperature treatments. This finding indicated that the upper thermal limit of stress for *A. hyacinthus* and *Isoporapalifera* from Karanrang Island was 32^{o}C. This was consistent with the previous study by Coles *et al.* (1976), who reported that the upper thermal tolerance for *A. formosa* and *A. hyacinthus* at Enewetak, Marshall Islands, was approximately at 31^{o}C. Wilkerson (1988), and Marshall and Baird (2000) also found a similar result where *Acropora* spp was sensitive to a heat stress at 32^{o}C.

At the end of the experiment (48 hours after temperature treatment), the abundance of zooxanthellae in *Isopora palifera* and *Acropora hyacinthus* at the highest temperature treatment (34^{o}C) was the lowest accounting for 0.06x10^{5}cells cm^{-2}and 0.18x10^{5} cells cm^{-2}, respectively. This finding indicated that the longest period of temperature stress for *Isopora palifera* and *Acropora hyacinthus*, the severe impact on the physiology of zooxanthellae. This finding is supported by Weis (2008) and Higuchi *et al* (2010) who found that at high sea water temperatures with long exposure will damage the photosynthetic and mitochondrial membranes of corals, as well as cause an oxidative stress condition.

CONCLUSION

Temperature stress of tropical coral (*Isopora palifera* and *Acropora hyacinthus*) could be occurred either at high temperature and low temperature.*Isopora palifera* from Karanrang Island was more resistant to temperature stress than *Acropora hyacinthus*.

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