Journal of **Coastal Development**
Vol. 1, No. 1, 1997: 37-42.

EFFECTS OF "EUTROPHICATION" ON THE SIZE AND NUCLEUS OF SYMBIOTIC ZOOXANTHELLAE

Ambariyanto

Faculty of Fisheries and Marine Science, Diponegoro University, Semarang - Indonesia

ABSTRACT

Zooxanthellae (Symbiodinium sp) from the giant clams, Tridacna maxima, were monitored for their responses to ammonium and phosphate addition in the field. Nutrient was added into ponded microatolls at every low tide using Nutrient Dispersal Units (NDUs) moored outside the atolls to reach final concentrations of 10μm for ammonium and 2μm of phosphate. Transmission electron micrograph showed that zooxanthellae size (as the maximum diameter) from nitrogen-treated clams was significantly than those from other treatments ($P<0.05$). However, the cross-sectional area of the nucleus was not significantly affected by the treatment smaller ($P>0.05$). The result suggests that the condition of zooxanthellae, such as the size of zooxanthellae from giant clams is influenced by relatively small changes in the concentration of nitrogen in the water column.

Key words : zooxanthellae, giant clams, nutrient enrichment

I. Introduction

Despite known as highly productive environment, coral reef ecosystem is also considered as environment with low concentration of nutrient. Therefore, the occurrence of eutrophication, which becomes an important problem world wide, would give significant impact not only to the corals, but also to the other organisms living in this habitat, such as giant clams. In the last few years, several scientists had attempted to investigate the effects of nutrient addition on the clams in the laboratory. The growth rate of clam, ***Hippopus hippopus***, was about three times faster under nutrient enrichment (Solis *et al.*, 1988). Similarly, Braley *et al.* (1992) reported that the growth of two different classes of ***Tridacna gigas***, were significantly improved by addition of dissolved inorganic nitrogen (DIN) into the surrounding waters. Ammonium and phosphate elevation also influences the biomass and shell calcification of giant clams. The total zooxanthellae density from ***T. gigas*** was significantly higher in

nutrient treated clams than in control. Furthermore, changes in the ultra-structure of the outer layer of the clam shells in response to the addition of ammonium and phosphate were reported by Belda *et al.* (1993 a,b).

Changes in the ultrastructure of zooxanthellae due to nutrient enrichment have also been reported in the coral, ***Pocillopora damicornis*** (Berner and Izhaki, 1994) and in the giant clam, ***Tridacna maxima*** (Ambariyanto and Hoegh-Guldberg, 1996). The present study attempts to further investigate effects of N and P enrichments on the cross-sectional area of nucleus and the size (as maximum diameter) of zoo-xanthellae from the giant clam, ***Tridacna maxima***.

II. Material and Methods

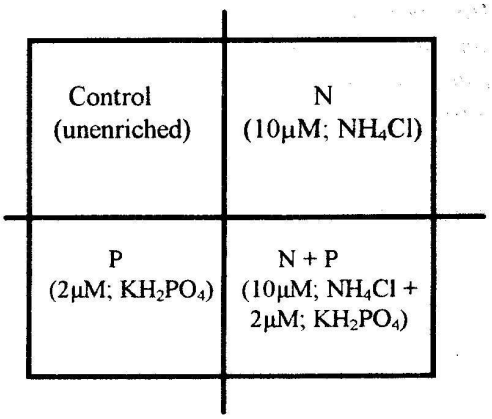
2.1. The ENCORE experiment

In response to the eutrophication problem in marine environment and lack of information in regard to the effect of nurient elevation to coral reef organisms, the ENCORE (Enrichment of Nutrient on Coral Reefs) experiment was proposed. It was initiated in 1991 by The University of Sydney and The Great Barrier Reef Marine Park Authority, and being held at One Tree Island Reefs ($23^{O}30'S$; $152^{O}06'E$) at the southern end of the Great Barrier Reef, Australia.

Twelve similar size microatolls were eutrophified by adding ammonium and phosphate, both separate and combined. There were four treatments in the ENCORE experiment (Figure 1.). The nutrient was added by using computer controlled nutrient dispersal units (NDUs, Steven and Larkum, 1993) which will disperse the nutrient at every low tide. The clams, ***Tridacna maxima***, were collected from natural population of the eastern side of One Tree Island. Giant clams (70 mm - 100 mm in shell length) were randomly assigned into 12 plastic cages and transferred to 12 micro-atolls in February 1993, eight months before the experiment began.

Figure 1.

Treatments in the ENCORE experiment.



2.2. Tissue preparation

Three clams per atoll were dissected before and nine months after the beginning of nutrient additions. They were dissected within 24 hours of collection. Three small pieces of mantle tissue were cut from each of the clams and were fixed by using glutaraldehyde solution (3%) mixed with 0.1 M phos-

phate buffer. Post-fixing was done by using 1 % of Osmium Oxalate (OsO_4). Dehydration was done by putting the sample through a series of ethanol solutions (30 %, 50 % and 70 %) for 10 minutes each, followed by final dehydration i.e. in a 100 % ethanol solution for 30 minutes which was done twice. Infiltration on the samples was done in a mixture of resin and 100 % ethanol (1:1) overnight, followed by embedding and baking in 100 % resin overnight at 65° C. Embedded samples were cut and stained with a solution of uranyl acetate, ethanol and distilled water mixture, followed with lead citrate.

2.3. Transmission electron micrograph.

TEM (Transmission Electron Micrographs (Phillips 301 E) was used to investigate the ultrastructure of zooxanthellae. Only zooxanthellae chose stalk of the pyrenoid was visible were taken in order to avoid any bias of the cell size due to the position of the section through the algal cells (Trench, 1979). Five photos were taken per sectioned block and three blocks prepared per clam. A Tracor Northern Imaging Computer was used to measure the cross-sectional area of nucleus and the largest diameter of zooxanthellae.

2.4. Statistical analysis.

The differences between the three microatolls within each treatment were tested using one way ANOVA (Analysis of Variance). No significant difference was detected in the data from microatolls within each treatment ($P>0.05$), therefore, the data from each set of three microatolls were pooled. ANOVA was also used to test the effect of the treatment on the cross-sectional area of nucleus and the maximum diameter of zooxanthellae. This was also done on the pretreated giant clams in order to test for differences between treated atolls prior to nutrient addition. Student Newman Keuls test was used to compare between means. Data were tested for normality and the homogenity of variances, and no violations on the ANOVA assumptions were detected.

III. Results

There was no effects of the treatment on the cross-sectional area of nucleus of zooxanthellae from giant clam, ***Tridacna maxima***. The maximum diameter of zooxanthellae from N-treated clams, however, was significantly smaller than those from P and N+P treatments and control clams, demonstrating that the addition of 10 μM NH_4^+ reduced cell size ($P<0.05$). The maximum diameter occurred among zooxanthellae from control clams (10.4 μm), and the minimum diameter was found in N-treated clams (6.3 μm) (Figure 2).

IV. Discussion

One important factor influencing the growth, morphology and physiology of marine algae is the availability of nutrients in their immediate environment, especially inorganic nitrogen and phosphorus. The size of fast growing cells

also tends to vary with nutrient concentration, with smaller cells being associated with faster growth due to higher nutrient concentration. The result of this study shows that the size (as maximum diameter) of zooxanthellae was significantly reduced by N addition. This is in agreement with Ambariyanto and Hoegh-Guldberg (1996) who reported that the size (as cross-sectional area) of zooxanthellae of N-enriched clams was smaller than control clams. In their papers, Lehman (1976) and Berdalet *et al.* (1994) reported that the cell size of the dinoflagellate ***Heterocapsa*** sp and ***Pediastrum duplex*** (Chlorophyceae) increased under nutrients starvation (N and P). They suggested that the cells were unable to divide during limited availability of nutrients. The smaller size of zooxanthellae from N treated clams from the present study probably could be related to higher growth rates compared to those from other treatments. This was supported by our other results (Ambariyanto and Hoegh-Guldberg, 1995) which showed that the density of zooxanthellae from ammonium treated clams was higher than in other treatments.

The results of the present study also showed that there was no effects of nutrient enrichment on the cross-sectional area of nucleus of zooxanthellae. Similar result was found by Berner and Izhaki (1994) that the relative volumes of the nuclei, mitocondria and other organelles were not significantly influenced by high concentration of nitrogen.

Are zooxanthellae from giant clams nutrient limited?

It has been shown that intact giant clams and isolated zooxanthellae are capable of taking up nutrients from seawater (Wilkerson and Trench, 1986; Domotor and D'Elia, 1984). The addition of nutrients in seawater increases the growth rate and density of symbiotic zooxanthellae in corals and giant clams (Hoegh-Guldberg and Smith, 1989; Belda *et al.*, 1993b; Ambariyanto and Hoegh-Guldberg, 1995). Moreover, nutrient addition in seawater also changes the ultrastructure of zooxanthellae in the coral ***Pocillopora damicornis*** and giant clam ***Tridacna maxima*** (Berner and Izhaki, 1994; Ambariyanto and Hoegh-Guldberg, 1996). It is obvious that these reports support the possibility that the symbiotic zooxanthellae in giant clams are nutrient limited. More studies, however, need to be done before conclusion which support that hypothesis can be taken.

Acknowledgments

This work was supported by the Indonesian Marine Science Education Program to A and the grant from the Great Barrier Reef Marine Park Authority to O, H-G and D. Yellowlees (ENCORE Project). Thanks are also due to the staff members of Electron Microscope Unit, Sydney University for their help and to the manager of OTI Research Station (G. and P. Carter).

References

Ambariyanto and Hoegh-Guldberg O. 1995. Impact of External Inorganic Nutrients on Giant Clams and their Zooxanthellae Under Field Conditions. Proc. Aust Coral Reef Conference.

Ambariyanto and Hoegh-Guldberg O. 1996. Nutrient Enrichment and the Ultrastructure of Zooxan-thellae from the Giant Clam ***Tridacna maxima***. Mar. Biol. 125:359-363

Belda C.A, Cuff C, Yellowlees D. 1993a. Modification of Shell Formation in the Giant Clam ***Tridacna gigas*** at Elevated Nutrient Levels in Sea Water. Mar Biol 117: 251-257.

Belda C.A, Lucas J.S., Yellowlees D. 1993b. Nutrient Limitation in the Giant Clam-zooxanthellae Symbioses: Effects of Nutrient Supplements on Growth of Symbiotic Partners. Mar Biol 117: 655-664.

Berdalet E, Latasa M, Estrada M. 1994. Effects of Nitrogen and Phosphorus Starvation on Nucleic Acid and Protein Content of ***Heterocapsa*** sp. J. Plankton Res. 16 (4): 303-316.

Berner T, Izhaki I., 1994. Effect of Exogenous Nitrogen Levels on Ultrastructure of Zooxanthellae from the Hermatypic Coral ***Pocillopora damicornis***. Pac. Sci. 48 (3): 254-262.

Braley R.D., Sutton D, Mingoa S.S.M., Southgate P.C. 1992. Passive Greenhouse Heating, Recirculation, and Nutrient Addition for Nursery Phase ***Tridacna gigas***: Growth Boost During Winter Months. Aquaculture 108: 29-50.

Domotor S.L. and D'Elia C.F. 1984. Nutrient Uptake Kinetics and Growth of Zooxanthellae Maintained in Laboratory Culture. Mar. Biol. 80: 93-101.

Hoegh-Guldberg O and Smith G.J. 1989. Influence of the Population Density of Zooxanthellae and Supply of Ammonium on the Biomass and Metabolic Characteristics of the Reef Corals, ***Seriatopora hystrix*** and ***Stylophora pistillata***. Mar. Ecol. Prog. Ser. 57:173-186.

Lehman J.T. 1976. Photosynthetic Capacity and Luxury Uptake of Carbon During Phosphate Limitation in ***Pediastrum duplex*** (Chlorophyceae). J. Phycol. 12: 190-193.

Solis E.P., Onate J.A., Naguit M.R.A. 1988. Growth of Laboratory-reared Giant Clams Under Natural and Laboratory Conditions. In: Copland, J.W., Lucas, J.S. (eds) Giant Clams in Asia and the Pacific. ACIAR Monograph No. 9 Canberra, pp 201-206.

Steven A. and Larkum T. 1993. ENCORE: The Effect of Nutrient Enrichment on Coral Reefs. Search 24(8): 216-219.

Trench R.K. 1979. The Cell Biology of Plant-animal Symbiosis. Ann. Rev. Plant. Physiol. 30:485-531

Wilkerson F.P. and Trench R.K. 1986. Uptake of Dissolved Inorganic Nitrogen by the Symbiotic Clam ***Tridacna gigas*** and the Coral ***Acropora*** sp. Mar. Biol. 93: 237-246.

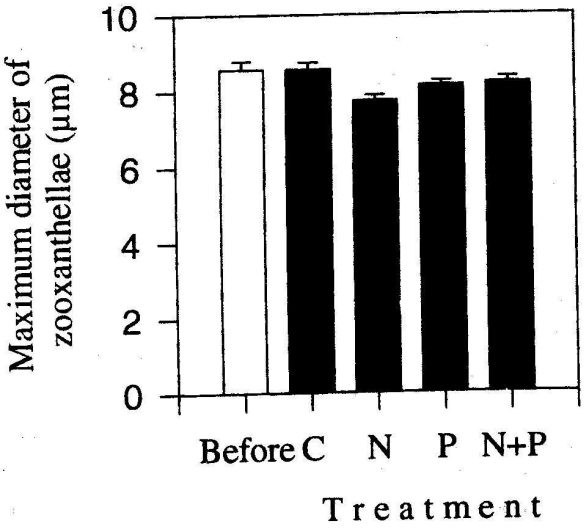


Figure 1.

The mean maximum diameter of zooxanthellae (μm±SE) from giant clams from different treatments. C=control; N=ammonium; P=phosphate.