

EFFECT OF LIGHT ON THE ZOOXANTHELLAE NUMBERS AND CHLOROPHYLL LEVEL IN *TRIDACNA SQUAMOSA*

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ABSTRACT

This study investigated the effect of different light intensities on the zooxanthellae numbers and chlorophyll *a* level in *Tridacna squamosa*. The number of zooxanthellae and chlorophyll *a* level were calculated from 10 animals at each study location with different light intensities. The density of zooxanthellae increased when the exposure to sunlight decreased. The chlorophyll *a* level also increased with low levels of light intensity. Aspects concerning the physiology of zooxanthellae and chlorophyll, which have relevance to giant clams, are reviewed.

INTRODUCTION

Species of Tridacnidae are important constituents of the fauna of coral reefs in the Indo-Pacific region. The dependence of tridacnid bivalves upon the photosynthetic activities of endosymbiotic zooxanthellae (*Symbiodinium microadriaticum*) living within their hypertrophied mantle restricts the distribution of this group of bivalves to sunlit waters (Jaubert 1977).

The intensity of light decreases exponentially with depth, and is a major factor affecting the distribution of giant clams. The zooxanthellae will exhibit photoadaptive responses using various mechanisms, for example, by increasing the size of the photosynthetic unit (PSU), *ie* by increasing light-capturing chlorophyll *a* pigments (Chang *et al.* 1983) or the zooxanthellae numbers (Prezelin 1987). Such a change may affect the ratio of zooxanthellae photosynthesis to animal respiration, and hence the amount of photosynthetic products available for translocation.

In the present study, we analysed the photoadaptive response of *T. squamosa* placed at 3 to 25 m depths, (and isolated zooxanthellae of the same individuals) in order to clarify their adaptation to a lower light environment in a deeper habitat. To achieve this, we determined changes in the chlorophyll *a* concentration and the number of

zooxanthellae under low and high light intensities.

MATERIALS AND METHODS

This study was conducted from June 1996 to May 1997 in Teluk Pa'Kaleh, Pulau Pemanggil, Johor, Malaysia (Fig. 1). *T. squamosa* (shell lengths 45 to 55 cm) were collected from one specific location at depths from 3 to 7 m, and placed at 3, 8, 15 and 25 m depths. Each month, 10 clams were monitored at each depth. All clams used for these experiments had mantles fully extended in the light. Light intensity at each depth was measured by a LI-COR Quantum Sensor and an LI-1000 Data Logger (LI-COR inc./LI-COR Ltd., USA), which detect photons with a wavelength between 400 and 700 nm.

A small piece of the mantle tissue was cut by using SCUBA diving. The tissue was blotted briefly and weighed. Zooxanthellae were extracted from the mantle by maceration using a Teflon tube homogeniser. The homogenate was filtered through a 20 µm mesh nylon cloth to remove animal tissue debris. The filtrate was centrifuged repeatedly at about 280 G until the supernatant was clear. The supernatant was discarded and the freshly isolated zooxanthellae from each clam were resuspended in filtered sea water. Five 1 ml samples were counted with

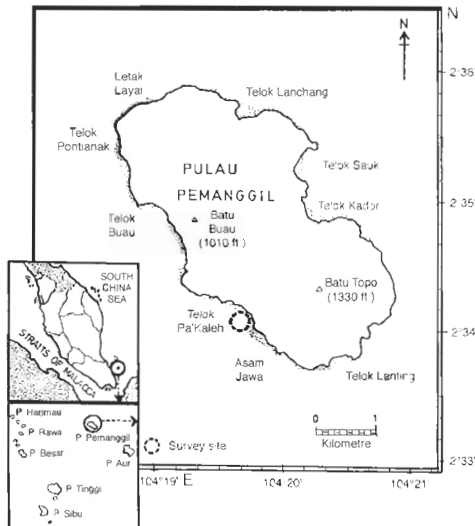


Figure 1. The location of Teluk Pa'Kaleh in Pulau Pemanggil, where *Tridacna squamosa* clams were placed at different depths for the study.

a Tohma haemocytometer under the microscope to determine the number of zooxanthellae. Microscopic observations of the prepared zooxanthellae indicated that this procedure did not disrupt the zooxanthellae.

Five samples of 1 ml suspension of zooxanthellae from each clam were filtered on glass fiber filters. The filters were cut into small pieces and immersed in 8 ml of 90 % acetone for 20 hours at 4 °C in the dark, for pigment extraction. Two ml 90 % acetone were then added and shaken vigorously. The chlorophyll extracted from each clam from each depth was centrifuged at $1100 \cdot G$, for 10 minutes. Absorbance of the supernatant was measured at 630, 647 and 664 nm with a NOVA II spectrophotometer. The chlorophyll *a* content was calculated from the standard equations of Jeffrey & Humphrey (1975).

The significance of correlation between the parameters and the collection depths was evaluated by analyses of variance (ANOVA). A linear model and the overall response mean were compared at a confidence level of 5 % ($p < 0.05$).

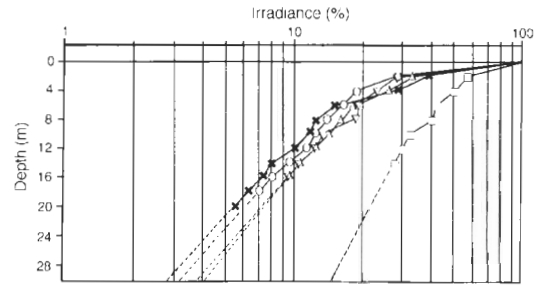


Figure 2. Light intensity profiles at sampling site (Teluk Pa'Kaleh). ∇ - June 96; \square - September 96; Δ - December 97; \circ - March 96; \ast - May 97. Dashed lines are extrapolations.

RESULTS

Light intensity profiles

The surface irradiance ranged from $1,750 \mu\text{mol m}^{-2} \text{s}^{-1}$ to $2,555 \mu\text{mol m}^{-2} \text{s}^{-1}$ when measured on sunny days around noon from June 1996 to May 1997 (Fig. 2). Light intensities at 25 m, ie the greatest depth of *T. squamosa* in this study, were between ca. 3 % (May 1997) and 20 % (September 1996) of the surface irradiance.

Zooxanthellae count

Fig. 3 shows the zooxanthellae count of *T. squamosa* at different depths. In the first 3 months (June to August 1996), there were no significant differences in zooxanthellae count between the giant clams at different depths. Significant differences between the clams were first detected in September 1996 (4th month). Zooxanthellae counts from clams at 25 m depth had increased significantly to $83 \cdot 10^6$ zooxanthellae g^{-1} mantle tissue, compared to clams at 3, 8 and 15 m depth (average $50 \cdot 10^6$ zooxanthellae g^{-1} mantle tissue).

Clams at 15 m depth started to show significant differences in zooxanthellae counts in October 1996 (5th month). The counts at 15 m and 25 m depth had increased significantly compared to those from 3 and 6 m depths. However, the counts from clams at 15 and 25 m were consistent (no significant changes) from October and onwards until the end of the study period (12th month).

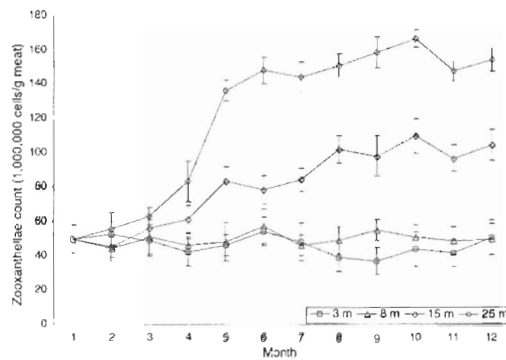


Figure 3. Zootaxanthellae count of *Tridacna squamosa* at different depths.

Chlorophyll *a* level

Fig. 4 shows the chlorophyll *a* level of the clams from various depths. In the first two months (June to July 1996), there were no significant differences in the chlorophyll *a* level between clams from different depths. Significant differences were first detected in August 1996 (3rd month), where the chlorophyll *a* level from clams at 25 m depth had increased significantly from 23 to 35 $\mu\text{g g}^{-1}$ mantle tissue, whereas the chlorophyll *a* level from clams at 3 m had decreased significantly from 23 to 18 $\mu\text{g g}^{-1}$ mantle tissue. Clams from 8 m and 15 m did not show any changes in their chlorophyll *a* level at this stage.

The chlorophyll *a* level in clams from 25 m depth was quite consistent from August (3rd month) until the monsoon months November 1996 to January 1997 where chlorophyll *a* concentrations were slightly lower.

The chlorophyll *a* level in clams from 3 m depth continued to decrease significantly until September (4th month). Then the level became consistent throughout the rest of the study period. No significant changes occurred during the monsoon period.

Comparison of clams from 8 and 15 m depths did not reveal significant differences in chlorophyll *a* from June to November 1996 (1st to 6th month). In December, clams from 15 m depth showed higher chlorophyll *a* level. There was a slight decrease in Janu-

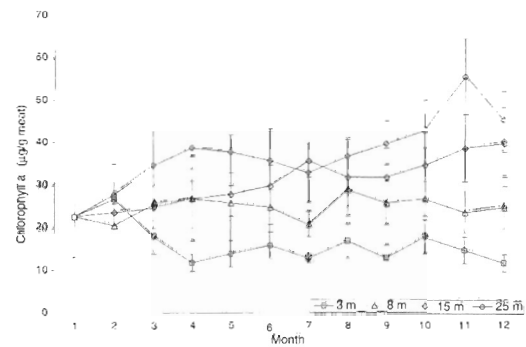


Figure 4. Chlorophyll *a* level of *Tridacna squamosa* at different depths.

ary (8th month) followed by a steady increase from February to April (9th to 11th month). The chlorophyll *a* level in clams from 8 m depth decreased significantly in December (7th month). It increased in January (8th month) before becoming consistent from February and onwards.

Overall changes in chlorophyll *a* concentrations during the monsoon season from October to February, did not reflect any specific trends.

DISCUSSION

The importance of light for algal symbiotic relationships depends on the ability of the zooxanthellae to adapt to short- or long-term changes in their photic environment, and the ability of the animal to oscillate between autotrophy and heterotrophy.

This study showed that *T. squamosa* adapted to the lower light levels when placed in deeper water. The adaptation could be measured as an increase in the number of symbiotic zooxanthellae, and an increase in the concentration of chlorophyll *a* per g mantle tissue. Similar results were reported in juveniles of *T. gigas* where the chlorophyll *a* concentration in shade-adapted juveniles was more than twice that of light-adapted clams (Mingoa 1988). The present trend was similar to that obtained with *T. maxima* zooxanthellae grown in culture at different light intensities (Chang *et al.* 1983). The

chlorophyll *a* level also increased with depth in the coral *Fungia* spp. but in that study, the density of zooxanthellae (estimated per unit surface area) decreased with depth (Masuda *et al.* 1993).

In this study, the adaptation of *T. squamosa* indicated a sequence of adaptation starting with an increase in chlorophyll *a* concentrations, which then is followed by an increase in density of zooxanthellae when clams are exposed to lower light levels.

There were no specific trends in the chlorophyll *a* concentrations of *T. squamosa* during the monsoon months. This may be due to conditions of the surface water (turbidity and wave action), which affect the amount of light penetrating into the water column during that period.

The chlorophyll *a* concentrations in clams located at 3 and 25 m depth were about 12 and 56 $\mu\text{g g}^{-1}$ mantle tissue respectively (an increase of 5 times with depth). The zooxanthellae counts in the same clams were $51 \cdot 10^6$ and $155 \cdot 10^6$ zooxanthellae g^{-1} mantle tissue respectively (an increase of 3 times with depth). Based on these figures, we calculate the concentration of chlorophyll *a* per zooxanthella to be about 23.5 pg at 3 m, and 36.1 pg at 25 m depth. This result is similar to the results reported for *T. gigas* juveniles (Mingoa *op. cit.*).

The light intensity in water decreases logarithmic with depth. But our results showed that there is only a double increase in the chlorophyll *a* per zooxanthella in the clams when the depth decreased from 3 to 25 m depth. Dubinsky *et al.* (1984) pointed out that there is a reduced efficiency in chlorophyll *a* molecules in harvesting incident light with decreasing intensity (*in casu* increasing depth).

Obviously, the observed compensation measures of increased chlorophyll *a* and increased density of zooxanthellae with depth, are inadequate for the clams. The consequence of reduced light intensity could be a reduced growth rate, or a shift to other methods of nutritive acquirement. A shift between

autotrophy to heterotrophy has been demonstrated in corals. In giant clams, other sources of nutrition from plankton, dissolved organic substances (DOM), and particulate matter (detritus) need to be identified.

Mechanisms of photoadaptation in zooxanthellae of different host species have been studied *in vivo* (Wetthey & Porter 1976; Dustan 1982) and *in vitro* (Chang *et al.* 1983). Some of these mechanisms involve changing the PSU size, the PSU number, or the activities of the electron transport system (Chang *et al.* 1983; Prezelin 1987). These mechanisms need to be identified in *T. squamosa* clams.

Mingoa (1988) has demonstrated fatality of *T. gigas* juveniles after 6 days in darkness. This can explain observations made in Pulau Pemanggil reefs where no giant clams were found at depths exceeding 12 m. The varied habitats occupied by tridacnid species may also be characterized by different photic conditions, possibly influencing their photoadaptability. However, this hypothesis requires further investigation.

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