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T. Maruyama · G. A. Heslinga

# Fecal discharge of zooxanthellae in the giant clam *Tridacna derasa*, with reference to their in situ growth rate

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**Abstract** The population dynamics of zooxanthellae living in the mantle of a giant clam, *Tridacna derasa*, was studied. The giant clams with shell lengths of 5 to 6 cm which had been reared in the Palau Mariculture Demonstration Center, in the Republic of Palau, were transferred to aquaria on deck of the R.V. "Sohgen-maru" and kept in running sea water at 29 to 30 °C. Two clams were removed from the aquaria, and zooxanthellae in the mantle were isolated every 2 h for 24 h. Numbers of the zooxanthellae in or not in the cell division stage were counted for calculations of the zooxanthellae population in the mantle and their mitotic index (MI). The MI increased after sunset and reached the maximum values of 6.1 to 11.5% at 03:00 to 05:00 hrs. The specific growth rate, μ, estimated from the MI was 0.083 to 0.14 $d^{-1}$. Five clams were kept in each of 2 Plexiglas containers in the aquarium for collection of the discharged feces every 3 to 4 h. The discharged zooxanthellae in the feces were counted. The zooxanthellae discharged in 24 h were 0.38 to 1.46% of the total zooxanthella population in the mantle, and 2.7 to 16.9% of the newly formed zooxanthella population in a day. Increase of zooxanthella population in the mantle was estimated from clam shell growth rate and from the correlation between zooxanthella population and clam shell size. Daily increase of zooxanthella population in the mantle was estimated to be approximately 7.6 to 19% of the newly formed zooxanthella population. Therefore, the sum of zooxanthellae populations accounting for daily increase in the mantle and discharge in the feces was 11 to 36% of the newly formed population. About 64 to 89% of the newly formed cells were missing; some of these may have been digested by the clam.

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T. Maruyama (✉)
Marine Biotechnology Institute, Shimizu Laboratories,
1900, Sodeshi-cho, Shimizu-shi,
Shizuoka 424, Japan

G.A. Heslinga[1]
Palau Mariculture Demonstration Center,
Koror 96940,
Republic of Palau

*Present address*:
[1]Indo-Pacific Sea Farms, P.O. Box 1206,
Kailua-Kona, Hawaii 96745, USA

## Introduction

Giant clams have a dense population of a symbiotic alga, *Symbiodinium* sp. (commonly called a zooxanthella), in their mantle tissues. Mansour (1946) reported that the zooxanthellae reside in a special tubular system in the mantle, and Norton et al. (1992) confirmed this observation. The tubular system originates at the stomach, branches and ends in the mantle, and may therefore be regarded as a part of the digestive tract of the clam. When the zooxanthellae multiply in the mantle, the excess zooxanthellae may pass into the stomach. The feces of giant clams contain intact zooxanthellae (Ricard and Salvat 1977; Trench et al. 1981). However, the fate of zooxanthellae in the giant clam is controversial. Fankboner (1971) and Yonge (1980) suggested that they are digested by amoebocyte in the clam mantle. They are, on the other hand, claimed to be resistant to digestion (Ricard and Salvat 1977; Fitt et al. 1986). Little information has been available on the in situ growth rate of zooxanthellae in the clam mantle or on its relationships to their discharge in the clam feces and to clam nutrition. In the present report, we have estimated the growth rate of zooxanthellae in the mantle by measuring the mitotic index and number of discharged zooxanthella cells in the feces. The importance of zooxanthellae in clam nutrition is discussed.

## Materials and methods

Living *Tridacna derasa* with shell lengths of 40 to 120 mm, reared at Palau Mariculture Demonstration Center (PMDC) in the Republic

of Palau, western Caroline Islands, were transported into aquaria on the deck of the R.V. "Sohgen-maru", and kept under natural sunlight with running sea water from Malakal port pumped from about 10 m depth. The water temperature was 29 to 30 °C throughout the experiment. Three separate experiments were carried out on 25/26 September, 29/30 September 1992 (sunset, 17:57 hrs; sunrise, 05:51 hrs), and 19/20 July 1994 (sunset, 18:06 hrs; sunrise, 06:20 hrs). The experiments started between 11:00 and 15:00 hrs, and ended at the same time on the next day.

### Estimation of the growth rate of zooxanthellae in the mantle

The clams were placed in an aquarium of 40 × 60 cm with running sea water, 40 cm deep, for 24 h. Two clams were sampled every 2 h. Adductor muscles were severed with a surgical knife, and the fleshy mantle was then cut longitudinally in half. One half of the mantle was removed from the shell, cut in pieces with a pair of scissors, placed in a 50-ml plastic centrifuge tube with about 15 ml natural sea water (NSW), and homogenized with a Polytron homogenizer (PT10-35, Kinematica, Switzerland) for 10 s. The homogenate was then strained through gauze and centrifuged at 700 ×*g* for 2 min. After removal of the supernatant, pelleted algal cells were re-suspended in about 10 ml of NSW and fixed by adding 1 ml of formalin. The final volume was adjusted to 30 ml with NSW. Samples were kept at room temperature until use. The population of the zooxanthellae was determined with a modified Neubauer haemocytometer under a light microscope (Nikon, Co., Japan). Ten replicates were counted for each sample, and total zooxanthellae population in a whole mantle (incorporating the other half of the mantle) was calculated. Approximately 600 to 1200 zooxanthellae in total were counted for each sample; dividing cells and cells with division furrows (see Fig. 3) were scored separately. The mitotic index (MI) was calculated as follows:

$$\mathrm{MI} = \frac{\text{number of dividing cells}}{\text{total cell number}}.$$

The growth rate of zooxanthellae was estimated by the following formula (Weiler and Chisholm 1976):

$$\mu = \frac{1}{t}\ \ln(F_{\text{total}} + 1) \qquad (1)$$

$$F_{\text{total}} = \frac{t_s}{t_d} \sum_{t=0}^{t=1} \mathrm{MI}\ , \qquad (2)$$

where μ, $t$, $t_s$ and $t_d$ are specific growth rate, time in days, sampling interval in hours, and duration of the cell division process in hours, respectively.

### Estimation of the numbers of zooxanthellae discharged in the feces

Five clams with 40 to 60 mm shell lengths were placed on a stainless steel wire shelf 3 cm above the bottom in each of 2 Plexiglas chambers with a diameter of 15 cm in each of 3 experiments. The chambers were covered with Plexiglas tops 1 to 2 mm above the chamber to leave a space for some water exchange. The chambers were then immersed in an aquarium with running sea water at 29 to 30 °C. Fecal pellets of the clams were collected every 3 to 4 h for 24 h. The clams in the chamber were removed, and the fecal pellets were allowed to sink to the bottom of the chamber. After removal of the upper layer of sea water through gauze with a siphon, the bottom layer of the sea water which contained the fecal pellets was transferred to a plastic 50-ml centrifuge tube (Corning, USA). The feces were collected by centrifugation at 280 ×*g* for 1 min. Then they were homogenized in ca. 7 ml sea water for 10 s at room temperature with a Polytron homogenizer. The resulting fecal suspension combined with washings of the homogenizer tip (in total ca. 10 ml) was transferred to a 15-ml plastic centrifuge tube and fixed by adding 0.5 ml formalin until the cells were counted. Zooxanthellae both in the feces and in the mantles of the clams from which the feces were collected were counted as described above.

### Morphology of the feces and zooxanthellae in the feces

Freshly discharged feces of *Tridacna derasa* with 80-mm shell length (imported from PMDC to Japan) were collected with a rubber-capped pipette and observed under a binocular dissecting microscope (Nikon, Japan) or a light microscope (Nikon, Japan).

## Results

### Population of zooxanthellae in the mantle

The population of zooxanthellae in the mantle increases with the clam shell length in the range between 40 and 120 mm (Fig. 1). This correlation was calculated by a software program, Graph III (Computer Associates, USA for Macintosh), on the assumption that the population of zooxanthellae in the mantle was proportional to the surface area of the mantle, which in turn was proportional to the square of the shell length. The regression curve was:

$$P_{zx} = 6.15 \times 10^4 \times L^2 + 8.58 \times 10^5 \times L - 5.14 \times 10^7, \quad (3)$$

where $P_{zx}$ was the population of zooxanthellae in the mantle and $L$ was the shell length of the clams in millimeters. The coefficient of determination, $r^2$, was 0.86.

### Mitotic index of the zooxanthellae

Figure 2 shows the diurnal change of the mitotic index (MI) of zooxanthellae. While it was less than 3% during the daytime, it increased after sunset and reached the maximum value in the early morning. The maximum mean values of MI, calculated from the mean of 2 clams

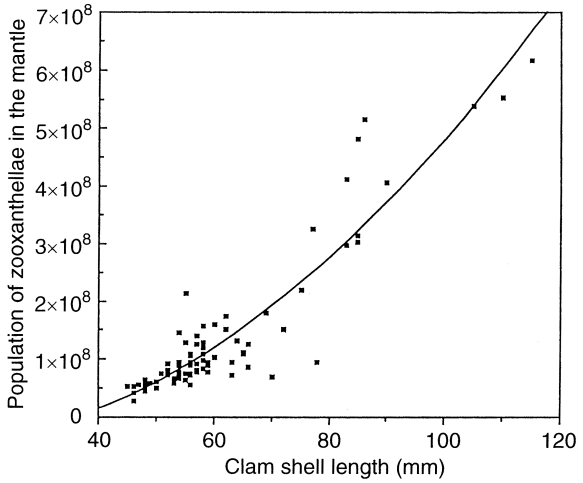


**Fig. 1** *Tridacna derasa*. Relationship between shell length and zooxanthella population in the mantle

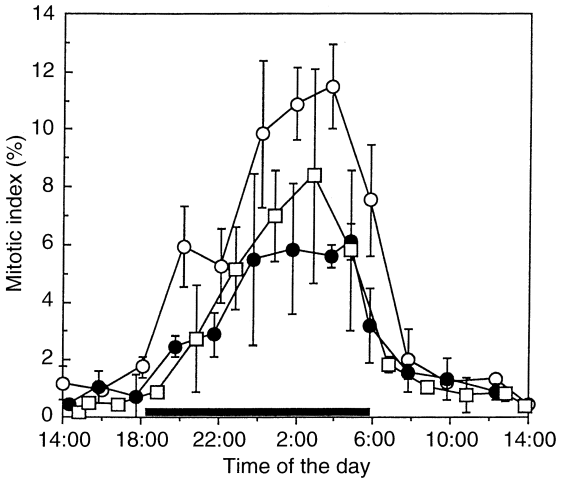


**Fig. 2** *Tridacna derasa.* Diurnal pattern of mitotic index of zooxanthellae in the mantle. *Bar on abscissa* indicates night (from sunset to dawn). *Open* and *closed circles*, and *open square* indicate mean mitotic index of zooxanthellae from 2 clams in each of 3 experiments. *Error bar* = deviation of duplicate data from mean value

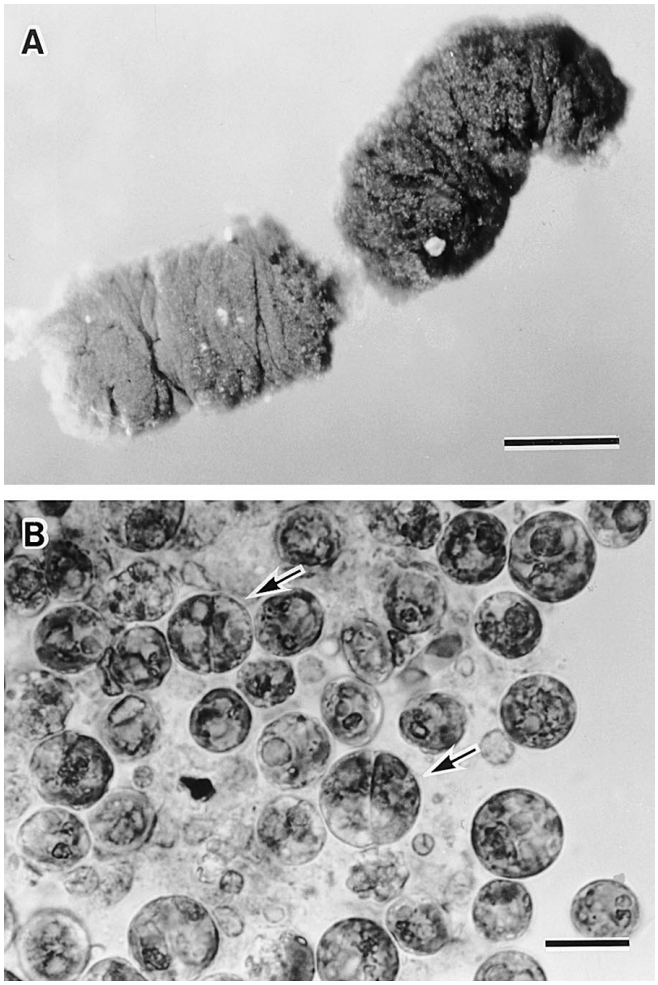


**Fig. 3** *Tridacna derasa.* Feces of the specimen with 80-mm shell length. **A** Low-power magnification. *Scale bar* = 500 μm. **B** Higher-power magnification. *Scale bar* = 10 μm. *Arrows* indicate dividing cells. Note existence of amorphous debris which may be decomposing material

in 3 experiments, were 6.1 to 11.5% at 03:00 to 05:00 hrs. The duration of the cell division process ($t_d$) was postulated to be 8 h, because from 22:00 to 06:00 hrs MIs were higher than half the maximum values (Fig. 2). This value, 8 h, was used as $t_d$ for further calculations in the estimation of the growth rate of the zooxanthella population.

### Morphology of the feces

Feces of *Tridacna derasa* containing zooxanthellae are shown in Fig. 3A, B. Most of the discharged zooxanthellae were indistinguishable from intact algal cells freshly isolated from the mantle. Amorphous debris and possible degraded materials were also observed in the feces (Fig. 3B). While dividing cells were frequent, no swimming form was observed. Filamentous cyanobacteria, diatoms and ciliates were also sometimes observed.

### Zooxanthellae discharged in the feces

The numbers of zooxanthellae discharged in the feces in the 3 separate experiments (6 chambers, 5 clams in each chamber) are shown in Fig. 4. No obvious diurnal rhythm was observed in these experiments. The number of discharged algae was exceptionally high in Chamber f in Experiment 3 (July 1994, Fig. 4), in which the cover had accidentally fallen off between 18:00 and 21:00 hrs, but there seemed no obvious reason for this to have influenced the result. Number of zooxanthellae discharged were estimated from mean discharge rates of algae in the feces to range between 3.1 and $9.8 \times 10^5$ [mean ± SD = $(4.9 \pm 2.6) \times 10^5$] cells clam$^{-1}$ d$^{-1}$ (Table 1). These 3.1 to $9.8 \times 10^5$ cells were equivalent to 0.38–1.46% (mean ± SD = 0.63 ± 0.41%) of the total zooxanthella population in the mantle (Table 1).

## Discussion

The present results clearly showed that zooxanthellae divided synchronously in the clam mantle, though the degree of synchrony was low (Fig. 2). Belda et al. (1993) likewise reported synchronous division of zooxanthellae in *Tridacna gigas*. Wilkerson et al. (1983) reported that the extracellular algal cell in cnidarian symbioses, as in our present studies, divided synchronously, but the intracellular algal cell did not.

Using Eqs. 1 and 2, and 8 h as $t_d$, specific growth rates, μ, of zooxanthellae in the clam mantle were estimated to be between 0.083 and 0.14 (mean ± SD = 0.10 ± 0.03, $n$ = 3) d$^{-1}$ (Table 1). This value was higher than that reported for zooxanthellae in *Tridacna gigas* in natural sea water (0.04), but about the same as that

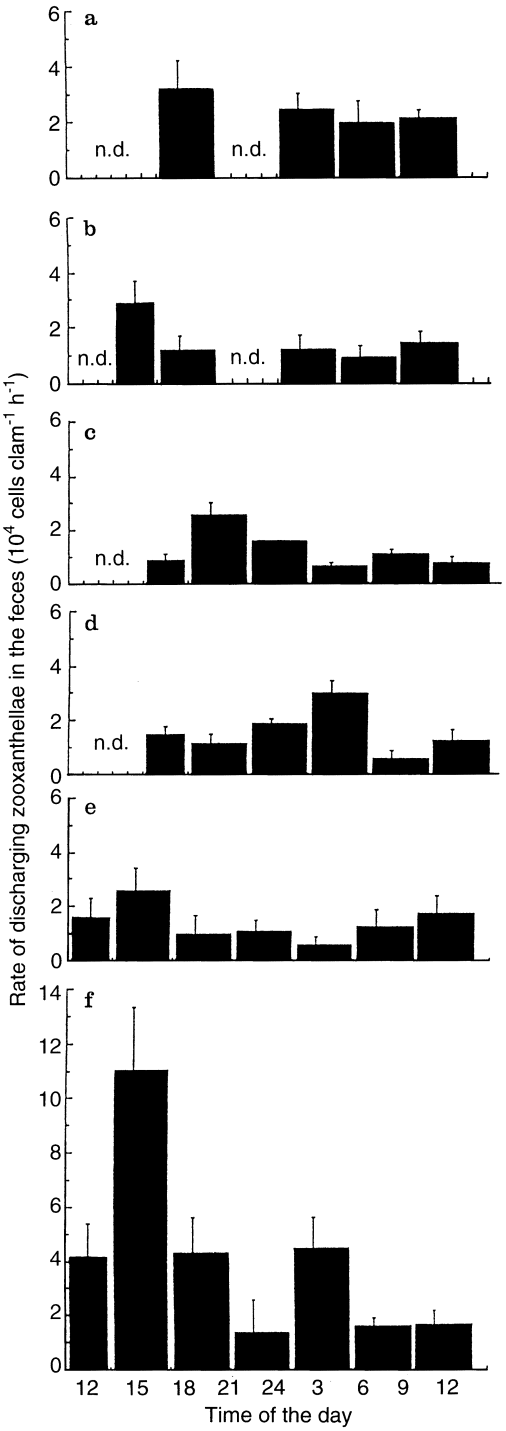


**Fig. 4** *Tridacna derasa.* Rate of discharge of zooxanthellae in feces. [**a–f** indicate the experiment chambers] *Bars* = mean rate in 3 or 4 h incubation; *error bars* = standard deviation (*n.d.* not determined)

observed in clams kept in enriched sea water (0.1) (Belda et al. 1993). An in situ doubling time was calculated to be 5.0 to 8.3 d. The present results indicate that approximately 10% of the zooxanthella cells in the mantle divide every day. Cells in the division stage, based on our 3 experiments, were calculated to be between 5.7 and 14.5 $\times$ $10^6$ [mean $\pm$ SD = (8.7 $\pm$ 4.0) $\times$ $10^6$] cells clam$^{-1}$ d$^{-1}$ (Table 1).

Table 1 summarizes the present results. Average values were calculated including the exceptionally high value of zooxanthellae discharge in Chamber f. We estimated that (4.9 $\pm$ 2.6) $\times$ $10^5$ cells, which was 2.7 to 16.9% (mean $\pm$ SD = 6.7 $\pm$ 5.1%) of the number of newly formed cells in a clam, were excreted in the feces every day. Growth rate of *Tridacna derasa* with 5 to 6 cm shell length at PMDC was 0.14 mm shell length d$^{-1}$ (Heslinga 1989). Using Eq. 3, the increasing rate of zooxanthella population in these clams was estimated to be 1.1 $\times$ $10^6$ cells clam$^{-1}$ d$^{-1}$. This value is 1.1 to 1.6% (mean $\pm$ SD = 1.4 $\pm$ 0.2%) of the total zooxanthella population living in the mantle, and 7.6 to 19.3% (mean $\pm$ SD = 14.7 $\pm$ 5.3%) of the newly formed zooxanthella population in a day. Therefore, the percentage of daily increased zooxanthellae in the mantle and discharged in the feces was equivalent to 11–36% (mean $\pm$ SD = 21 $\pm$ 9%) of the newly formed zooxanthella population. This indicates that a zooxanthella population equivalent to approximately 64 to 89% (mean $\pm$ SD = 79 $\pm$ 9%) of the newly formed zooxanthella was missing. Some zooxanthellae might be lost from the feces by differentiation into swimming cells. Fitt et al. (1981) reported that motile zooxanthellae appear only for a limited time at the end of the light period in a light/dark cycle, and no motile zooxanthella was observed in the dark period. No obvious lower discharging rate of zooxanthellae was observed (Fig. 4), and no swimming form of zooxanthella was observed in the feces. Therefore, although some swimming algal cells might be lost during feces collection, the number is not likely to be great.

Digestion of zooxanthellae by tridacnid clams has also been suggested (Fankboner 1971; Yonge 1980). Fitt et al. (1986) reported that juvenile *Hippopus hippopus* larvae ingest zooxanthellae, but no trace of their digestion was recognized, and approximately 76% of the $^{14}C$ labelled zooxanthellae was detected in the feces. Trench et al. (1981) observed zooxanthella cells in various stages of disorganization in addition to intact ones in the rectum of *Tridacna derasa*. This may indicate the digestion of zooxanthellae in the clam. Digestion of zooxanthellae was observed in symbiotic nudibranchs (Kempf 1984); incubation in the dark (starvation) increased the percentage of degenerated zooxanthella cells in the feces of nudibranchs.

More than 50% (Trench et al. 1981) or most (Klumpp and Lucas 1994) of the organic substances required by a giant clam is supplied in the form of photosynthetic products secreted by zooxanthellae, while filter-feeding also contributes to giant clam nutrition (Klumpp et al. 1992; Klumpp and Lucas 1994). Although zooxanthellae are discharged in the feces of the giant clams, many must obviously pass intact through the digestive tract. The present data, which fail to account for approximately 79 $\pm$ 9% of the newly formed zooxanthella, indicate that these algal cells may be digested by the clam.

**Table 1** *Tridacna derasa*. Summary of zooxanthella population dynamics

| | Averages (mean ± SD) |
|---|---|
| Shell length | 5.6 ± 0.1 cm |
| Zooxanthella population in mantle | (8.2 ± 1.6) × $10^7$ cells $clam^{-1}$ |
| Specific growth rate of zooxanthella population in mantle[a] | 0.10 ± 0.03 $d^{-1}$ |
| Discharging rate of zooxanthellae in the feces | (4.9 ± 2.6) × $10^5$ cells $clam^{-1}$ $d^{-1}$ |
| Percentage of daily discharged zooxanthellae in total zooxanthella population in mantle | 0.63 ± 0.41% |
| Percentage of discharged zooxanthellae in newly formed zooxanthella population in mantle | 6.7 ± 5.1% |
| Increasing zooxanthella population in mantle[b] | 1.1 × $10^6$ cells $d^{-1}$ $clam^{-1}$ |
| Percentage of increasing zooxanthella population in newly formed zooxanthella population in mantle | 14.7 ± 5.3% |
| Percentage of missing zooxanthellae in the newly formed zooxanthella population | 78.7 ± 9.3% |

[a]Calculated from mitotic index
[b]Calculated from clam shell growth rate and the correlation between clam shell length and zooxanthella population in the mantle

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