ESTIMATING CONTRIBUTION OFZOOXANTHELLAE TO ANIMAL RESPIRATION (CZAR) AND TO ANIMAL GROWTH (CZAG) OF GIANT CLAM *Tridacna maxima*

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ABSTRACT

Zooxanthellae are symbiotic dinoflagellate algae which live in association with marine invertebrates including giant clams. These algae are capable of translocating part of their photosynthetic products to the host. This translocation is one of the nutrition sources of the host. The present study aims to calculate the contribution of zooxanthellae on the energy requirements of adult giant clam (Tridacna maxima) during their respiration and growth processes. The result showed that zooxanthellae are capable of contributing 260.67% and 452.54% energy required by giant clams for respiration and growth during summer and 171.51% and 273.51 % during winter, respectively. It suggests that all the energy required for these two processes can be supplied by zooxanthellae.

INTRODUCTION

Giant clams are marine bivalves which live in coral reef ecosystems in the Indo-Pacific region. There are two genera and eight species of giant clams around the world, and seven of them can be found in Indonesian waters, except *Tridacna tevoroa* which only Lives in South Pacific countries (Romimohtarto *et al*., 1987; Knop, 1996). Traditionally, these clams were used by coastal people as a source of meat, building materials, and other purposes such as house hold equipments. Recently, these clams have become important export commodities by several countries mainly for aquarium animals (Lucas, 1994). Consequently, the condition of giant clams natural populations have been degrading rapidly including in Indonesia.

Giant clams are also known as marine invertebrate which live in association with single cell dinoflagelate algae commonly known as zooxanthellae. These algae which can be found in the mantle of the clams has an important role as a source of energy for the host. These algae are capable of translocating part of their photosynthetic products to the host which then be used for biological processes (Lucas, 1994).

So far there is no study available especially, on the estimation of the role of zooxanthellae on energy requirement by the clams. This study aims at estimating the

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contribution of zooxanthellae to animal energy requirements for respiration (CZAR) and for growth (CZAG).

MATERIALS AND METHODS

Giant clams, *Tridacna maxima* were collected from intertidal areas on the reef crest at One Three Island lagoon, Great Barrier Reef, Australia using permit at the depth of approximately 150 cm. These clams were grown in microatolls during winter and summer months. Isolation of zooxanthellae was done by using the method used by Ambariyanto and Hoegh-Guldberg (1997).

In order to calculate CZAR and CZAG several parameters are required. These are the specific growth rates of zooxanthellae, the carbon used for zooxanthellae growth and respiration, and the carbon used for animal growth and respiration (Muscatine *et al*., 1981; Ambariyanto, 2002).

Specific growth rate of zooxanthellae.

Zooxanthellae specific growth rate was calculated by using an equation first used by Wilkerson *et al*. (1983). This formula is based on the mitotic index (f = the percent of dividing cells in a population) and the duration of paired cell stage (t_d).

Mitotic index was calculated based on the number of dividing zooxanthellae in 1000 isolated zooxanthellae times 100 percent. While the value of t_d used in this calculation is 0.46 d based on several studies (Wilkerson *et al*., 1983; Muscatine *et al*., 1984; Hoegh-Guldberg *et al*., 1986). It is also noted that variation in t_d has only small impacts on CZAR (Muscatine *et al*., 1981).

$$\mu = 1/t_d \ln(1+f_{max})$$

where μ = zooxanthellae specific growth rate
t_d = 0.46 (Wilkerson *et al*., 1983; Muscatine *et al*., 1984).
f_{max} = mitotic index.

Carbon content of the zooxanthellae.

Carbon content of zooxanthellae was calculated using an equation that relates cell volume and elemental carbon (Strathmann, 1967). Cell volume of zooxanthellae was calculated base on the mean size of 100 zooxanthellae isolated from the giant clams. The equation to calculate carbon content is as follows:

log carbon = -0.314+0.712 log cell volume (Strathmann, 1967)

Carbon required for zooxanthellae growth.

Carbon used by zooxanthellae for growth can be calculated by multiplying zooxanthellae specific growth rate and carbon content, as the following:

$$C_{Zg} = \mu \times \textbf{Carbon per cell}$$

Carbon translocated by zooxanthellae

Carbon translocated can be calculated by the following formula, $T = P_Z - R_Z - G_Z$
where :
P_Z = carbon gained from zooxanthellaephotosynthesis
R_Z = carbon used for zooxanthellae respiration
G_Z = carbon used for zooxanthellae growth

Estimating CZAR and CZAG

CZAR can be calculated by the following formula : $CZAR = T / R_A$, where
T = carbon translocated and
RA = carbon required by the animal for respiration
While CZAG can be calculated by the following formula :
$$CZAG = (T - R_A)/\text{Carbon growth}$$

RESULTS AND DISCUSSION

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Specific growth rate of zooxanthellae.

The result of this study showed that maximum value of mitotic index is 0.025, thus the specific growth is :
$$\mu = 1/0.46 \ln (1+0.025) = 0.054 \text{ day}^{-1} \dots (1)$$

Carbon content of the zooxanthellae.

The results of the present study showed that the cross-sectional area of zooxanthellae in the clams was 47.9 μm^2. The cell radius (r) is hence:

$$r = \sqrt{47.9/\pi} = 3.9 \ \mu m, \text{ and the}$$
$$\text{Cell volume} = 4/3 \text{ x } \pi \text{ x } r^3 \qquad = 248.47 \ \mu m^3$$

Carbon content of zooxanthellae can be calculated as follows
log carbon = -0.314+0.712 log cell volume
Carbon per cell = 24.628 pg C $cell^{-1}$........(2)

Carbon required for zooxanthellae growth.

$$\begin{aligned} C_{Zg} \quad &= \mu \text{ x Carbon. } cell^{-1} \\ &= 0.0054 \text{ day}^{-1} \text{ x } 24.628 \text{ pgC } cell^{-1} \\ &= 1.3299 \text{ pgC } cell^{-1} \text{ day}^{-1} \end{aligned}$$

Carbon required for growth of the entire population of zooxanthellae (=3.8 10^8 cell $clam^{-1}$) is

$$\begin{aligned} C_{Zg} \quad &= 3.8 \ 10^8 \text{ cell x } 1.3299 \text{ pgC } cell^{-1} \text{ day}^{-1} \\ &= 0.502 \text{ mg C day}^{-1} \dots (3) \end{aligned}$$

Respiration of giant clam and zooxanthellae.

The respiration due to the zooxanthellae within a clam is a fraction of the total respiration. In previous studies the ratio of zooxanthellae and animal biomass has been used to calculate the portion of the total respiration due to zooxanthellae (Muscatine *et al.*, 1984; Hoegh-Guldberg *et al.*, 1986).

Protein content per zooxanthellae is 77.7 pg $cell^{-1}$. This value is based on an average of values reported from the literature for similar size of cells. These were 58.1 pg $cell^{-1}$ (zooxanthellae from nudibranch

Pteraeolidia ianthina; Hoegh-Guldberg *et al.*, 1986), and 88.85 and 86.5 pg $cell^{-1}$ (zooxanthellae from light and shade adapted coral *Stylophora pistillata* respectively; Muscatine *et al.*, 1984). Using the average value (77.7 pg $cell^{-1}$), the protein content in zooxanthellae is

$$= 3.8 \ 10^8 \text{ cell x } 77.7 \text{ pg } cell^{-1} = 29.53 \text{ mg}$$

The total protein content of zooxanthellae and the animal host (giant clam protein content) was calculated by multiplying the protein content per gram mantle (5.329 mg g^{-1}) by the total wet tissue weight (47.311 g; Ambariyanto, 1996). It is assumed that protein content within clam mantle and other internal parts is similar. Hence, the total protein content in zooxanthellae and animal is

$$= 5.329 \text{ mg } g^{-1} \text{ x } 47.311 \text{ g} = 252.12 \text{ mg}$$

The percent of the total respiration due to zooxanthellae was

$$=29.53 \text{ mg}/252.12 = 0.117, \quad \text{or } = 11.7 \text{ \%}$$

The mean value of total respiration by intact clam (R_C) in this model was

$$R_C= 0.172 \text{ mmol } O_2 \text{ clam}^{-1} \text{ h}^{-1}$$
(Ambariyanto, 1996), which is
$$= 0.172 \text{ x } 32 = 5.504 \text{ mg } O_2 \text{ clam}^{-1} \text{ h}^{-1} = 5504 \ \mu g \ O_2 \text{ clam}^{-1} \text{ h}^{-1}$$
$$= 5.504 \text{ x } 24 = 132.09 \text{ mg } O_2 \text{ clam}^{-1} \text{ day}^{-1}$$

This value can be converted into carbon using RQ_A (respiratory quotient for the animal = 0.8; Muscatine *et al.*, 1981) and the ratio of the masses of C and O_2 (*ie.* 12/32 or 0.375), as the following

$$\begin{aligned} R_C &= 5504 \ \mu g \ O_2 \text{ clam}^{-1} \text{ h}^{-1} \text{x } 0.375 \text{ x RQ} \\ &= 5504 \ \mu g \ O_2 \text{ clam}^{-1} \text{ h}^{-1} \text{ x } 0.375 \text{ x } 0.8 \\ &= 1651.2 \ \mu g \ C \text{ clam}^{-1} \text{ h}^{-1} \\ &= 39.63 \text{ mg C clam}^{-1} \text{ day}^{-1} \dots (4) \end{aligned}$$

The carbon required for zooxanthellae respiration (R_Z) was calculated by multiplying (4) by the proportion on the total respiration due to the zooxanthellae (that is 0.117, see above) :

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R_Z = 39.63 mg C clam^{-1} day^{-1} x 0.117 = 4.64 mg C day^{-1} .... (5)

Therefore, carbon required for animal respiration (R_A):

R_A = 39.63 - 4.64 = 34.99 mg C day^{-1}.. (6)

The carbon translocated (T) by zooxanthellae to the host was calculated by subtracting the carbon used for zooxanthellae respiration (R_Z) and growth (G_Z) from the carbon gained from zooxanthellae photosynthesis (P_Z) This is :

$T = P_Z - R_Z - G_Z$

P_Z is based on the oxygen output resulted from the gross photosynthesis (P_g) of zooxanthellae within the clam host. P_Z is calculated using the relationship between P_g and irradiance and light data collected every 10 minutes for 24 h at One Tree Island at twice i.e. in the summer and winter.

$$P_Z = P_g^{max} (1-e^{-\alpha I/Pgmax})$$

a. P_Z during summer= 221.12 mg O_2 clam^{-1} day^{-1}

Converted into carbon, P_Z = 221.12 mg O_2 clam^{-1} day^{-1} x 0.375 x PQ^{-1}

= 221.12 mg O_2 clam^{-1} day^{-1} x 0.375 x 1.1

= 91.21 mg C clam^{-1} day^{-1} ....(7)

b. P_Z during winter= 145.48 mg O_2 clam^{-1} day^{-1}

Converted into carbon, P_Z = 145.48 mg O_2 clam^{-1} day^{-1} x 0.375 x PQ^{-1}

= 145.48 mg O_2 clam^{-1} day^{-1} x 0.375 x 1.1

= 60.01 mg c. clam^{-1} day^{-1} ....(8)

The carbon translocated (T) by zooxanthellae to the host was calculated by subtracting (3) and (5) from (7) for summer and (8) for winter respectively. That was

a). T_{summer} = 91.21 mg C day^{-1}- 0.502 mg C day^{-1} - 2.32 mg C day^{-1}

= 88.39 mg C day^{-1} ....(9)

%T_{summer} = (88.39/91.21) x 100 %

= 96.9 %

b). T_{winter} = 60.01 mg C day^{-1} - 0.502 mg C day^{-1} - 2.32 mg C day^{-1}

=57.19 mg C day^{-1}...........................(10)

%T_{winter}= (57.19/60.01) x 100 % = 95.3 %

Contribution of zooxanthellae to animal respiration (CZAR)

CZAR was calculated by dividing the carbon translocated by the zooxanthellae to the host during summer and winter (T; 9 and 10) by the carbon required by the animal for respiration (R_A; 6).

$CZAR = T / R_A$

$CZAR_{summer}$ = (91.21 mg C day^{-1} / 34.99 mg Cday^{-1}) x 100 %

= 260.67 %

$CZAR_{winter}$ = (60.01 mg C day^{-1} / 34.99 mg Cday^{-1}) x 100 %

= 171.51 %

Contribution of zooxanthellae to animal growth (CZAG)

CZAG was calculated by first determining the carbon used for clam growth. This calculation is based on the average total carbon content of the soft tissue of the model giant clam (that is 3.689 g clam^{-1}) and the percent change in buoyant weight per day (0.32 % in summer and 0.22 % in winter; Ambariyanto, 1996).

a). CZAG for the model clam growing in the summer was

Carbon growth = 3.689 g clam^{-1} x (0.32 % / 100%) = 11.8 mg C day^{-1}

CZAG = (T - R_A)/Carbon growth

= (88.39 mg C day^{-1} - 34.99 mg Cday^{-1}) / 11.8 mg C day^{-1} x 100%

= 452.54 %

b). CZAG for the model clam growing in winter was Carbon growth = 3.689 g clam^{-1} x (0.22 % / 100%) = 8.12 mg C day^{-1}

CZAG = (T - R_A)/Carbon growth

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$= (57.19 \text{ mg C day}^{-1} - 34.99 \text{ mg Cday}^{-1}) / 8.12 \text{ mg C day}^{-1} \times 100\%$
$= 273.40 \%$

The result of this analysis is summarized in **Table 1.**

Table 1. Summary results of CZAR and CZAG calculation on untreated giant clam, *Tridacna maxima*, for summer and winter periods.

Period	P_Z (mg O_2 d^{-1} clam^{-1})	R (mg $O_2 d^{-1}$ clam^{-1})	P/R ratio	Translocation (%)	CZAR	CZAG
Summer	221.12	132.09	1.67	96.9 %	260.67 %	452.54 %
Winter	145.48	132.09	1.10	95.3 %	171.51 %	273.40 %

As can be seen in **Table 1.**, there is seasonal influence on the estimated values of CZAR and CZAG. This result is in agreement with Hoegh-Guldberg *et al.* (1986) who predicted that the contribution of zooxanthellae to the host, *Pteraeolidia ianthina* (Nudibranchia) was also substantially influenced by season. Hoegh-Guldberg *et al*. (1986) predicted that in animals with high zooxanthellae population densities, the symbionts contribute to the host's respiratory needs during winter, spring and summer at rate of 79%, 121% and 173%, respectively. Hoegh-Guldberg *et al.* (1986) suggested that these differences is primarily due to differences on the light availability during different seasons. The decreasing light with depth also influences CZAR as shown by the variation in values for *Stylophora pistillata* at different depth (78% at 35 m and 157% at 3 m depth; McCloskey and Muscatine, 1984). Light history can also have photoadaptive influences as shown for CZAR in *S. pistillata*. Light adapted corals had higher CZAR than shade-adapted corals, 143% and 58% respectively (Muscatine *et al.,* 1984).

The results of the present study show that CZAR values for *Tridacna maxima* range from 260.67% in summer to 170.51% in winter. The percent translocation remains similar at 96.9 % and 95.3 % for winter and summer respectively (see **Table 1**.). The contribution of zooxanthellae to animal growth (CZAG) ranged between 452.54%

(summer) and 273.40% (winter). It is interesting that CZAG both during summer and winter are above 100% suggesting that zooxanthellae are able to contribute all the requirements of animal growth. The CZAR values of giant clams calculated during this study were higher than those reported by Trench *et al.,* (1981). These authors reported that the contribution of zooxanthellae to the respiratory carbon requirements of *T. maxima* range from 62% on cloudy days to 84% (assuming 40% translocation) on sunny days. The differences in CZAR values was probably due to differences in percentage translocation used by Trench *et al*. (1981). The CZAR values from the present study are comparable to those from larger clams *Tridacna gigas*. Fisher *et al.* (1985) reported that CZAR of *T. gigas* was 83% and 197% by using the values of 40% and 95% translocation respectively. Similarly Fitt *et al.* (1986) found that CZAR value in *Hippopus hippopus*, was between 7% and 137% using translocation values of 40% and 98% respectively.

It should be kept in mind, however, that the organic carbon lost through calcification and mucus production is not included in this calculation, since no experiment was conducted during the present study to quantify the amount of carbon used for these processes. Secondly, the values of percent translocation, CZAR

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and CZAG of nutrient treated clams are probably different from the values calculated for the control clams here. Differences due to the fact that zooxanthellae isolated from ammonium treated clams reduced zooxanthellae size and starch accumulation within the cell are likely to have significant effect on the energy budgets calculated here.

The calculation of CZAR which was proposed by Muscatine *et al*. (1981) has been widely used for corals (Muscatine and Porter, 1977; McCloskey and Muscatine, 1984; Hoegh-Guldberg *et al*., 1986 *etc*) and giant clams (Fisher *et al.*, 1985; Mingoa, 1988; Klumpp *et al*., 1992; Klumpp and Lucas 1994 *etc*). The results of this method, however, have to be interpreted cautiously since some assumptions, and estimated values of some parameters, have been used in the calculation. The first assumption is that respiration rate in the dark and in the light are the same, while the second assumption is that the respiration rate of zooxanthellae and their host is proportional to their biomass. By using these assumptions, the result of the CZAR calculation may not represent the real contribution of zooxanthellae to animal respiration. For example, the respiration rate of zooxanthellae within the clams is impossible to measure, although it can be measured *in vitro* after isolation. The respiration rate of isolated algae, however, is not necessarily similar to the respiration rate of zooxanthellae within the host. Changes in zooxanthellar morphology and physiology occur soon after isolation (Trench, 1979). Secondly, the respiration rates per biomass of zooxanthellae and the clams may not be the same. Hoegh-Guldberg and Hinde (1986) estimated that the respiration rate of zooxanthellae is higher than that of the host, based on their protein biomasses. In addition, the calculation of the specific growth rate of the zooxanthellae is based on the duration of the paired cell stage (t_d) and the mitotic index of the zooxanthellae. Since the value of t_d is difficult to measure, the value of 0.46 d has been used (see Wilkerson *et al.*, 1983).

It is not clear, however, whether the t_d value of zooxanthellae in giant clams is also 0.46. Moreover, during this calculation the carbon content of the zooxanthellae was not measured, but was estimated using the equation proposed by Strathmann (1967). This equation, however, may underestimate the carbon content of zooxanthellae (Hinde pers. Comm). Finally, in this calculation it was also assumed that there are no changes in the photosynthetic and respiratory rates of giant clams in different seasons

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