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PHOTOSYNTHESIS AND RESPIRATION IN *TRIDACNA GIGAS* AS A FUNCTION OF IRRADIANCE AND SIZE

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ABSTRACT

The effects of irradiance level and size on the rate of O_2 evolution and consumption was examined in *Tridacna gigas* using an oxygen electrode. Seven photosynthesis-irradiance (P-I) curves were generated for intact clams ranging from 1 to 23 cm in shell length. Both alpha and P_{max} decreased with increasing size of the clam. Oxygen evolution at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ and consumption in the dark were measured for an additional 9 clams ranging up to 38 cm in shell length. Oxygen evolved per gram clam tissue in the light, dark respiration, and the number of zooxanthellae per gram all decreased with increasing clam size. The average amount of shading experienced by the zooxanthellae in the clam tissues was estimated by comparing the P-I curves of different-sized clams to the P-I curves of zooxanthellae freshly isolated from these clams. Estimates of shading increased from negligible levels in small clams to 80% in the largest clams investigated. Use of these results to chose optimum light intensities for the mariculture of *T. gigas* is discussed.

INTRODUCTION

Bivalves of the family Tridacnidae are found throughout most of the Indo-West Pacific (Rosewater, 1965), although populations of the larger species have been declining in some areas in the face of intense overfishing (Pearson, 1977). One of the characteristics of tridacnid bivalves is their symbiosis with algae, and it has been suggested that the association may be a factor contributing to the large size of some tridacnids (Yonge, 1980), the largest of which, *Tridacna gigas,* has been observed to reach a length of 137 cm (Rosewater, 1965). The symbiotic alga *Symbiodinium microadriaticum,* occurs predominantly within the hemal sinuses of the hypertrophied siphon (commonly referred to as "mantle tissue" in the tridacnids) (Fankboner, 1971; Trench *et al.,* 1981). Only a few other marine bivalves are known to harbor symbiotic dinoflagellates, *e.g.,* the heart shell *Corculum cardissa* (Kawaguti, 1950).

The use of the larger tridacnids as food, and the resultant overfishing has generated an interest in the possibilities of commercial cultivation (see Munro and Heslinga, 1982). In the context of mariculture, studies of these clams have followed three directions. First, attempts have been made to gain an understanding of the spawning of clams with an aim towards controlled induction of spawning (Wada, 1954; LaBarbera, 1975; Jameson, 1976; Fitt and Trench, 1981); second, the artificial rearing of fertilized eggs through the developmental stages of metamorphosis and acquisition of algal symbionts (Beckvar, 1981; Fitt and Trench, 1981; Gwyther and Munro, 1981; Fitt *et al.,* 1984); third, an analysis of the importance of the contributions of the symbiotic algae to the nutrition of the clams (Muscatine, 1967; Goreau *et al.,* 1973; Trench *et al.,* 1981). This last parameter is important, since the potential for a significant proportion

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of the nutrition of the clams being provided by the symbionts renders these clams distinct from other bivalves that have been central to mariculture attempts in the past (Munro and Heslinga, 1982).

Previous studies (Wells *et al.*, 1973; Johannes *et al.*, 1972; Trench *et al.*, 1981; Mangum and Johansen, 1982) on the physiology of tridacnids and their zooxanthellae were not designed to yield data which could be directly applied to mariculture. Specifically lacking from the literature is information on the physiology of *T. gigas*, a prime candidate for mariculture (Munro and Heslinga, 1982) and information on how the physiology of the tridacnids change with age. *T. gigas* was chosen for this study not only because of its importance in the context of mariculture, but also because of some features which facilitated this work: the wide size range of individuals available makes possible a study of the relation between a variety of physiological parameters and the size of the clam; adults are not attached to the substrate (unlike *T. crocae* and *T. maxima*) and therefore collecting and placing them in a respirometry chamber can be accomplished with a minimum of stress; the majority of individuals of all sizes open soon after placement in a chamber and appear to behave normally throughout the experiment (unlike most other tridacnids).

P-I relations have been investigated in a number of associations between zooxanthellae and marine invertebrates (Barnes and Taylor, 1973; Scott and Jitts, 1977; Falkowski and Dubinsky, 1981; Fitt *et al.*, 1982). Similarly P-I relations of freshly isolated zooxanthellae have been described using zooxanthellae from a variety of invertebrates (Zvalinskii *et al.*, 1980; Dustan, 1982; Trench and Fisher, 1983). This study is one of only a few in which the P-I relations of the zooxanthellae *in situ* and *in vitro* are compared (Crossland and Barnes, 1977; Chalker and Taylor, 1978; Muller-Parker, 1984). Only Crossland and Barnes (1977) demonstrated shading of the zooxanthellae *in situ* (in the coral *Acropora acuminata*). In this study the effects of *in situ* shading on the zooxanthellae in a wide size range of clams are investigated. The large range in the thickness of the mantle (siphonal) tissue (where most of the zooxanthellae are situated) makes *Tridacna gigas* ideally suited for such a study.

Materials and Methods

Aquisition and maintenance of Tridacna gigas

This study was conducted at the Micronesian Mariculture Demonstration Center (MMDC) in the Republic of Belau (Palau), West Caroline Islands, Micronesia, between May and July, 1983. The three largest clams used in this study (wet weight 425–1700 g, shell length 23–38 cm) were collected on the reef at depths of 1.5 to 3.5 m. After collection the animals were maintained at 4 m depth on the reef adjacent to the laboratory. All of the smaller clams used in this study were reared at MMDC by G. A. Heslinga and F. E. Perron (Munro and Heslinga, 1982). Surfaces of the shells of the clams were cleaned using a scalpel and toothbrush to remove epiphytic and epizooic growth from the shell and then maintained for between two days and two weeks in unfiltered flowing sea water in a concrete raceway exposed to ambient insolation. These raceways have been used to rear giant clams for up to three years at MMDC (Heslinga *et al.*, 1984).

Intact clam oxygen exchange

The respirometer chambers were constructed of glass and clear lucite and fitted with clear lucite lids. The volume of the four chambers used in this study ranged from 20 ml to 61 l. All four chambers contained a stir bar mounted directly below the

oxygen electrode. During its operation, the stir bar maintained an adequate flow of water across the membrane of the electrode. This was sufficient to mix the water in the three smaller chambers, but in the largest chamber an additional larger stir bar was used. The three smaller chambers were water-jacketed in order to maintain a constant temperature of 28°C. The largest chamber was not jacketed, but no temperature variation was observed during any experiment.

A YSI model 53 oxygen electrode coupled to a Cole Palmer model 8377-15 chart recorder was used to record the change in oxygen tension within the chamber. Illumination was provided from above by a Mini-Cool movie light, and the irradiance was varied from 25 to 3000 $\mu E \cdot m^{-2} \cdot s^{-1}$ by adjusting the light-to-chamber distance. Irradiance was measured with a Li-Cor quantum photometer model LI-185 and a model LI-192S submersible quantum sensor.

Before placing a clam in a respirometer, the shell was lightly brushed to remove any growth accumulated since the original cleaning. It was then rinsed with 0.45 μm (pore size) filtered sea water and placed in an appropriate respirometer containing 0.45 μm filtered sea water. All rate measurements were made between 80–100% of air saturation using a linear portion of the trace after at least 10 minutes of equilibration time under each condition. Only data obtained from clams with fully expanded mantles are reported in this study. When investigating clams smaller than 2 cm in shell length (wet weight < 0.05 g), 2–6 individuals of the same size were placed in the chamber in order to achieve easily measurable rates of oxygen exchange. These experiments were always conducted between 10:00 a.m. and 5:00 p.m., and the order of the different irradiances was random. Dark respiration rate measurements were obtained between every irradiance level by turning out the light and covering the chamber with black plastic.

As a control for oxygen flux not due to *T. gigas*, measurements were made with both empty chambers and chambers containing clam shells from which the animals had been removed. No oxygen change was detected at any light level in the two larger chambers or at irradiances up to 600 $\mu E \cdot m^{-2} \cdot s^{-1}$ in the smaller chambers. A small change was observed in the smaller chambers at the higher irradiances, both in the presence and absence of empty shells, and was subtracted in the rate calculations. This small change was presumably due to the effect of temperature on the oxygen electrode when the movie light was in close proximity to the small volume chambers.

Clam vital statistics

Shell dimensions (length, width, height) were measured to the nearest millimeter with calipers and total volume, by displacement. The clams were dissected from their shells and their total wet weight (blotted dry) measured to the nearest milligram. The clams larger than 2 cm in shell length were then further separated into three tissue classes (muscle, mantle, and remains) and weighed. The mantle and remains were homogenized separately in known volumes of filtered sea water (0.45 μm pore size millipore filter) in a Virtis tissue homogenizer. Replicate samples of each homogenate were removed and prepared for analysis of chlorophyll as described below. In the case of clams smaller than 2 cm, the entire clam was homogenized.

Isolation of zooxanthellae

Zooxanthellae from larger clams were isolated from a portion of the mantle homogenate described above, following the methods of Trench (1971a) (in the case of clams smaller than 2 cm, zooxanthellae were isolated from the whole animal homog-

enate). The homogenate was first filtered through three layers of cheese cloth to remove pieces of animal debris. Zooxanthellae were then isolated from the resultant slurry by repeated 2-minute centrifugations at approximately 1000 rpm in a Beckman table top centrifuge. The cells were resuspended in filtered sea water between each centrifugation. This procedure was repeated three times, after which the supernatant was clear and very little debris was observed upon microscopic examination of the pellet. The cells were resuspended in filtered sea water to approximately 10^6 cells/ml and the concentration determined by 6 replicate counts in a hemacytometer. Two aliquots (usually 5 mls) of this suspension were removed for analysis of chlorophyll and the remainder was used for analysis of photosynthetic and respiratory oxygen exchange by isolated zooxanthellae.

Quantification of chlorophyll

The samples were first pelleted at approximately 2500 rpm for 2 min and the supernatant discarded. The pellets were then resuspended in 0.4 ml of distilled water and frozen and thawed three times over a 24-hour period. The thawed samples were then ground in a Tenbroeck ground-glass tissue grinder with 3.6 ml of acetone and allowed to extract overnight in the dark at 0°C. The samples were clarified by centrifugation at approximately 2500 rpm and the absorbance of the supernatants were measured at 630 and 663 nm in a Turner model 330 spectrophotometer. The pellet was refrozen in 0.4 ml of distilled water, and re-extracted with 3.6 ml of acetone and the absorbance measured as before. The total amounts of chlorophylls *a* and c_2 in the samples were calculated using the equations of Jeffrey and Humphrey (1975). Replicate samples usually agreed within 10% and the average of the two replicates was used for further calculations.

Oxygen exchange by isolated zooxanthellae

Measurements of photosynthetic oxygen evolution were made in a YSI stir bath chamber using a YSI model 53 oxygen electrode coupled to a Cole Palmer model 4377-15 chart recorder. Temperature was maintained at 28 ± 0.5°C with a recirculating water bath. Illumination was provided by a Viewlex model V-25-P slide projector and irradiance was varied from 15 to 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$ by placing plastic window screen over the projector lens and varying the projector-to-chamber distance. Irradiance levels were measured using a LiCor Quantum photometer with a submersible quantum sensor. Dark respiration rates were determined when the incubation chamber was covered with black plastic. Freshly isolated zooxanthellae were suspended in filtered sea water at densities of 10^6 cells/ml in order to minimize self shading by the algal cells. Incubation volume was 3.0 ml as suggested by the instrument manufacturer. In experiments where photosynthetic oxygen evolution at more than one irradiance level was measured (for the photosynthesis *versus* irradiance curves), fresh cells were placed in the chamber after every other measurement to minimize any deleterious effects of the stir bar on the cells. In order to reduce variation due to possible photosynthetic rhythms (Prézelin *et al.*, 1977; Chalker and Taylor, 1978; Muller-Parker, 1984), experiments were conducted between 10:00 a.m. and 4:00 p.m. and the order of the different irradiances was random.

RESULTS

The 33 clams used in this study ranged in shell length from 0.86 to 38 cm, and in wet weight from 0.014 to 1700 grams (Table I). The allometric relation between

TABLE I

Daily P/R and CZAR in Tridacna gigas

Wet weight w/o shell (grams)	Shell length (cm ± S.D.)	Daily P/R 500 $\mu E \cdot m^{-2} \cdot s^{-1}$	Daily P/R 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$	%CZAR 40% trans.	%CZAR 95% trans.
0.014	1.0 ± 0.08 (4)	1.4	1.5	63	149
0.02	1.15 ± 0.05 (6)	2.4	2.5	109	259
0.041	1.55 ± 0.01 (4)	3.0	3.2	134	318
0.045	1.84	1.05	1.1	60	142
0.052	1.54 ± 0.05 (6)	2.7	2.8	120	284
0.08	2.12	1.4	1.5	64	152
0.84	4.0	1.55	1.7	72	169
1.0	4.3	1.65	1.8	76	179
1.25	4.8	1.55	1.7	68	164
1.325	6.5	1.4	1.6	67	160
14.79	10.0	1.3	1.7	70	167
18.2	10.3	2.3	3.1	128	312
290	23.5	1.5	2.0	105	249
424.6	23.0	0.82	1.1	44	112
1700	38	1.05	1.4	60	142
Average ± S.D.		1.67 ± 0.6	1.9 ± 0.65	83 ± 27	197 ± 65

P/R was calculated assuming 10 hours of sunlight at either 500 or 1000 $\mu E \cdot m^{-2} \cdot ^{-1}$ and 24 hours of respiration. CZAR was calculated using the following equation and the translocation values indicated in the table.

CZAR = [PG(1000 $\mu E \cdot m^{-2} \cdot s^{-1}$) − 0.05R](10 h)(%Translocation)(100) + R(24 h).

the wet weight of the clam (without its shell) and the length of the shell is described by the following equation:

$$\text{Shell Length (cm)} = 4.246\ (\text{wet weight in g})^{0.30}$$

This equation was calculated in the log form:

$$\log(\text{shell length}) = 0.3 \log(\text{wet weight}) + 0.628 \qquad (r = 0.995,\ n = 16)$$

(Correlation coefficients for all allometric equations refer to the log transformations.)

The amount of chlorophyll *a* per gram of clam, as well as the number of zooxanthellae per gram of clam decreased with increasing size of the clam (Fig. 1). Chlorophyll *a* ranged from 2.0 to 3.1 pg/zooxanthella cell in the various clams but did not vary as a function of clam size. Weight of the "mantle" tissue ranged from 27 to 55% of the wet weight of the larger clams (clams smaller than 0.1 g wet weight were not dissected into the three tissue classes). An average of 87% (±6.2%, n = 8) of the chlorophyll *a* was found in the "mantle" tissue of the larger clams.

Initial observations indicated that while the smallest size-class of clams (<2.0 cm) demonstrated saturation of oxygen production at 600 $\mu E \cdot m^{-2} \cdot s^{-1}$, the larger clams did not saturate at any ambient light intensity (up to 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$) (Fig. 5). Since oxygen evolution measurements could not therefore be made at "P_{max}" for all clams, we chose 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ as the experimental irradiance because this is well above the saturation irradiance for isolated zooxanthellae (200 $\mu E \cdot m^{-2} \cdot s^{-1}$) (Fig. 6), and in the top of the range experienced by the clams *in situ*. In order to determine the relation between oxygen production and the size of the clam, oxygen flux rates in the dark and at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ were measured for a wide size range of clams. Photo-

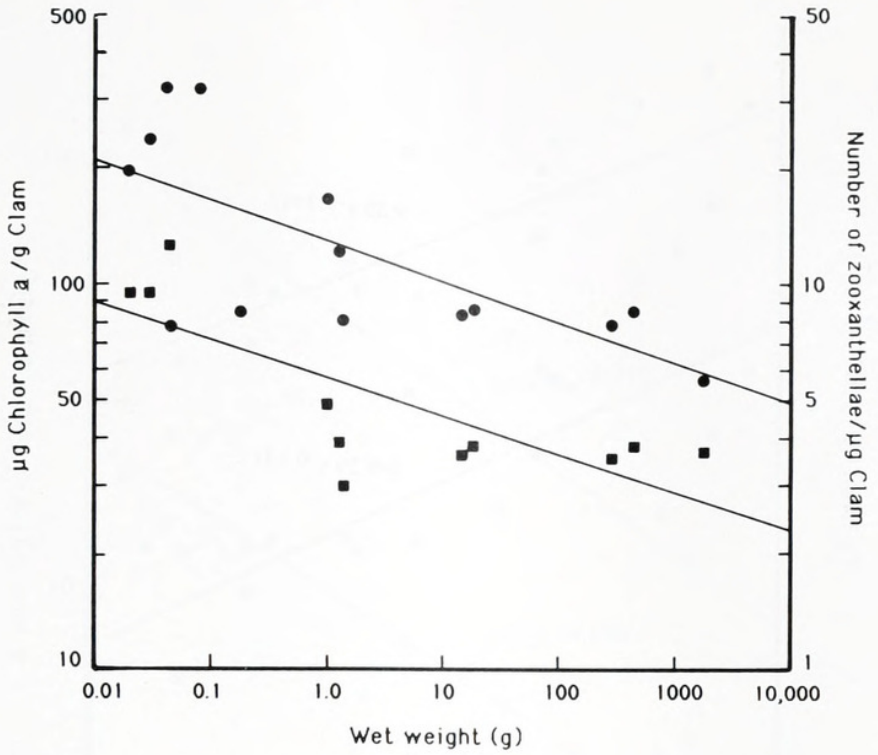

FIGURE 1. Number of zooxanthellae and amount of chlorophyl *a* per gram (wet weight) of *T. gigas* as a function of the wet weight of the clam (without shell). Squares represent the number of zooxanthellae per µg clam ($y = 5.67x^{-0.0966}$, n = 11, r = 0.77, $P < 0.01$). Circles represent the µg Chl. *a* per gram clam ($y = 127x^{-0.1035}$, n = 14, r = 0.69, $P < 0.01$).

synthetic oxygen production rates of the zooxanthellae *in situ* were inversely related to the size of the clam (Fig. 2).

The decrease in number of zooxanthellae per gram of clam (Fig. 1) and in *in situ* productivity of those zooxanthellae with increasing size of the clam (Fig. 2) results in a strong inverse relation between oxygen production rate and size of the clams (Fig. 3). The rate of respiratory oxygen consumption is also inversely related to the size of the clams (Fig. 3).

The relation between photosynthetic oxygen evolution and irradiance (P-I) was determined for 7 clams ranging from 1.15 to 23.5 cm in shell length (0.02–290 g wet weight). Four representative curves are presented in Figure 5. Both alpha/g (the slope of the initial light limited portion of the P-I curve) and "P_{max}" (assumed maximum photosynthetic rate) decrease with increasing size of the clam. The smallest clams showed light saturation of photosynthesis by 600 $\mu E \cdot m^{-2} \cdot s^{-1}$, but the larger clams were not saturated even at 2000 $\mu E \cdot m^{-2} \cdot s^{-1}$.

P-I relations were also determined for zooxanthellae isolated from the mantles of three different clams. One of these curves, together with the P-I curve for the intact clam from which the zooxanthellae were isolated, is shown in Figure 6. The freshly isolated zooxanthellae did not show any evidence of photoinhibition up to maximum ambient illumination (2000 $\mu E \cdot m^{-2} \cdot s^{-1}$), although the P_{max} for freshly isolated zooxanthellae was well below that of the intact clam. The dark respiration rates averaged 8.5% (±1.8, n = 9) of P_{max}. The alpha values for freshly isolated zooxanthellae ranged from 0.0019 to 0.0038 ($\mu MO_2 \cdot \mu gChl a^{-1} \cdot \mu E^{-1} \cdot m^2$), but were not correlated with the size of the clam from which the zooxanthellae were isolated. P_{max} (photosynthetic rate at 600 $\mu E \cdot m^{-2} \cdot s^{-1}$) was measured for algae isolated from eleven different clams. The rates varied over a two-fold range when calculated on the basis of either chlorophyll

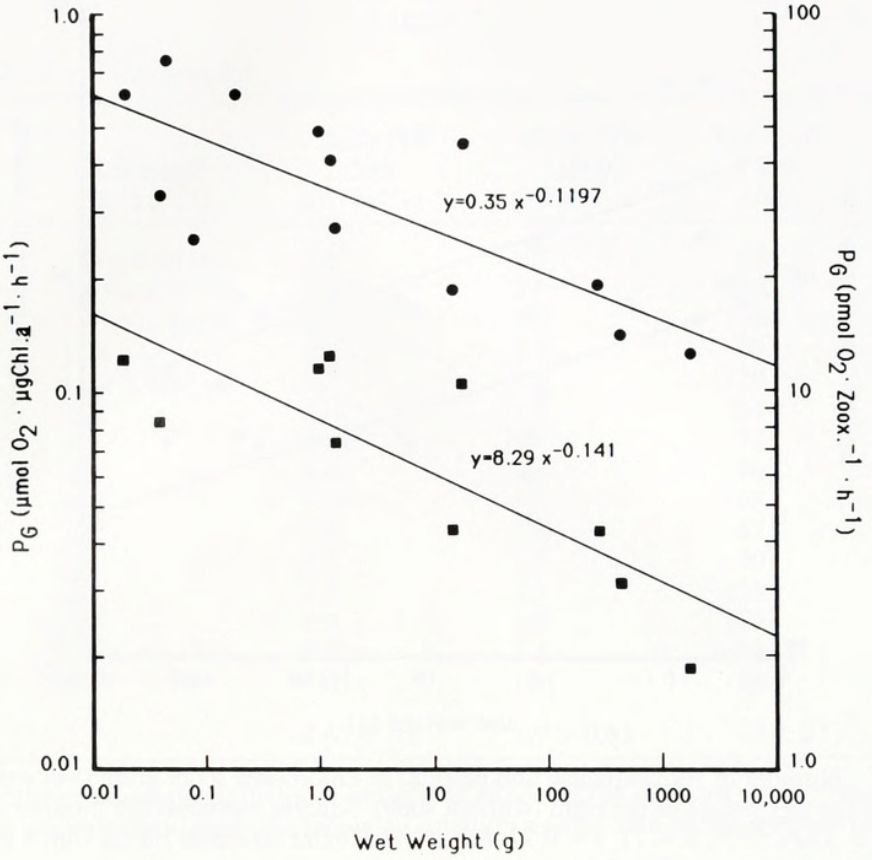

FIGURE 2. Gross photosynthetic oxygen production (P^G) in *T. gigas* at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ as a function of the wet weight of the clam. Squares represent pmol oxygen produced per zooxanthella per hour ($y = 8.29x^{-0.141}$, n = 10, r = 0.82, $P < 0.01$). Circles represent μmol oxygen produced per μg Chl. *a* per hour ($y = 0.35x^{-0.1197}$, n = 13, r = 0.78, $P < 0.01$).

a or algal cell number. The values were independent of the size of the clam from which the zooxanthellae were isolated (Fig. 7).

Using these data, an envelope enclosing all possible P-I relations for the freshly isolated zooxanthellae was constructed and then superimposed on P-I curves of the same four clams shown in Figure 5, with oxygen production rates presented per μg Chl. *a* so that the direct comparison can be made between the performance of intact clams and freshly isolated zooxanthellae (Fig. 8). The alpha values of the smallest clams are within the range of values of the freshly isolated zooxanthellae. However, the alpha values of the larger clams decrease progressively as the size of the clams increases. The P_{max} values of all but the largest clams are higher than the highest P_{max} measured for freshly isolated algae.

The ratio of gross oxygen production to consumption over a 24-hour period (P/R) and the percent contribution of carbon translocated from the zooxanthellae to animal respiration (CZAR) was calculated for each clam (or group of clams), using a variety of assumptions. The daily P/R assumed 10 hours of sunlight at either 500 or 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ and 24 hours of respiration at the same rate as that measured in the dark. CZAR was calculated using the methods of Muscatine *et al.* (1981). The theoretical equation for this calculation is: CZAR = (P_{Gross}Zoox. for 24 h) (%translocation) ÷ (R Animal for 24 h). The specific assumptions we made for our calculations of CZAR are the following; algal biomass and the algal contribution to the overall respiratory rate of the clam is approximately 5% (Trench *et al.*, 1981). Daily photo-

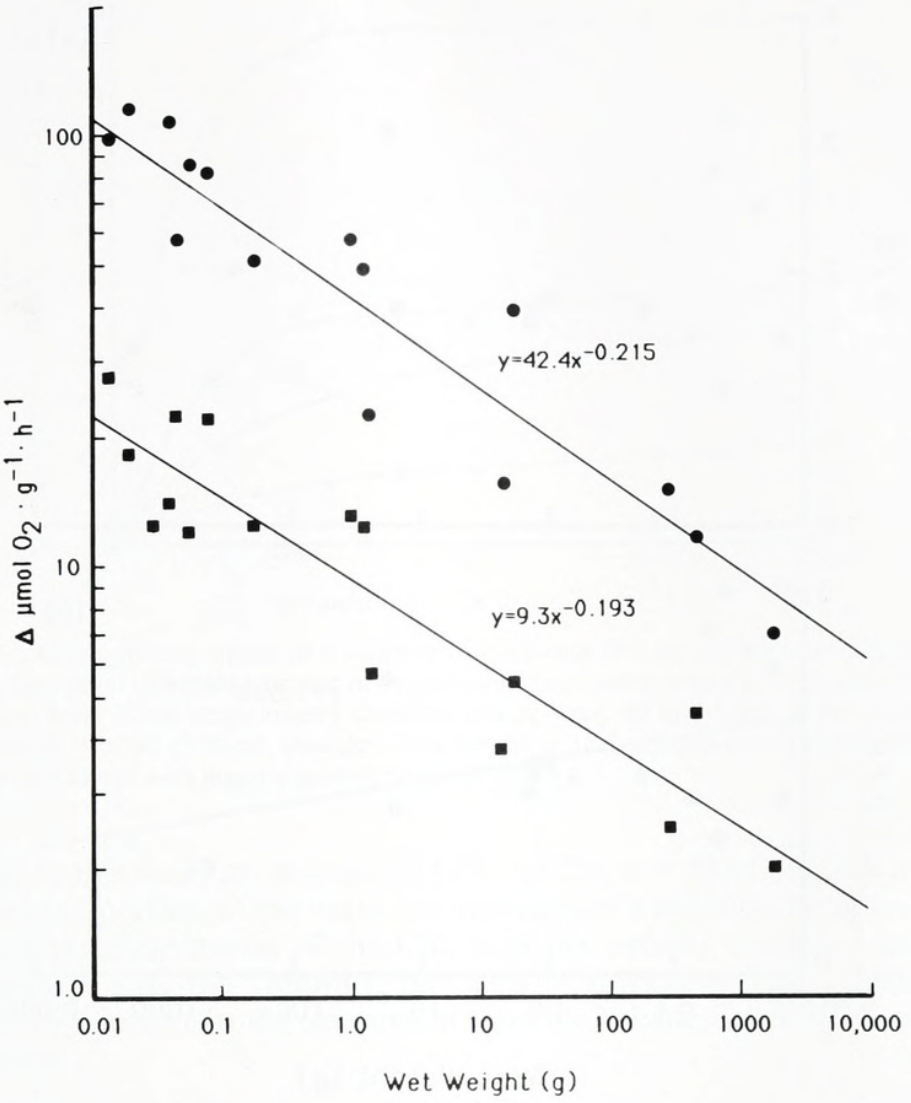

FIGURE 3. Oxygen evolution (P_g) at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ and respiration (R) per gram clam per hour as a function of the wet weight of the clam. Circles represent the gross photosynthetic rate ($y = 42.4x^{-0.215}$, $n = 15$, $r = 0.94$, $P < 0.01$). Squares represent the respiration rate ($y = 9.3x^{-0.193}$, $n = 16$, $r = 0.93$, $P < 0.01$).

synthesis is approximately equal to 10 hours of sunlight at an irradiance of 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$. The dark respiration rate is equal to the light respiration rate. Both the PQ and RQ are approximately equal to 1.0. We calculated CZAR twice assuming two different values for translocation; 40% (Trench *et al.*, 1981) and 95% (Muscatine *et al.*, 1984). For an in-depth discussion of these and other assumptions inherent in these calculations, see: Muscatine and Porter (1977); McCloskey *et al.* (1978); Muscatine (1980); and Trench *et al.* (1981); Muscatine *et al.* (1984).

Daily ratios of photosynthesis:respiration (and therefore CZAR) at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ were not correlated with the size of the clam (Fig. 4a), but at 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ there was an inverse relation between P/R and increasing size of the clams (Fig. 4b). Daily P/R and CZAR values are presented in Table I. Assuming 10 hours of sunlight at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$, P/R values ranged from 1.1 to 3.2 and averaged 1.9. Assuming either 40% or 95% translocation the calculated values of CZAR range from a minimum of 44% to a maximum of 318%.

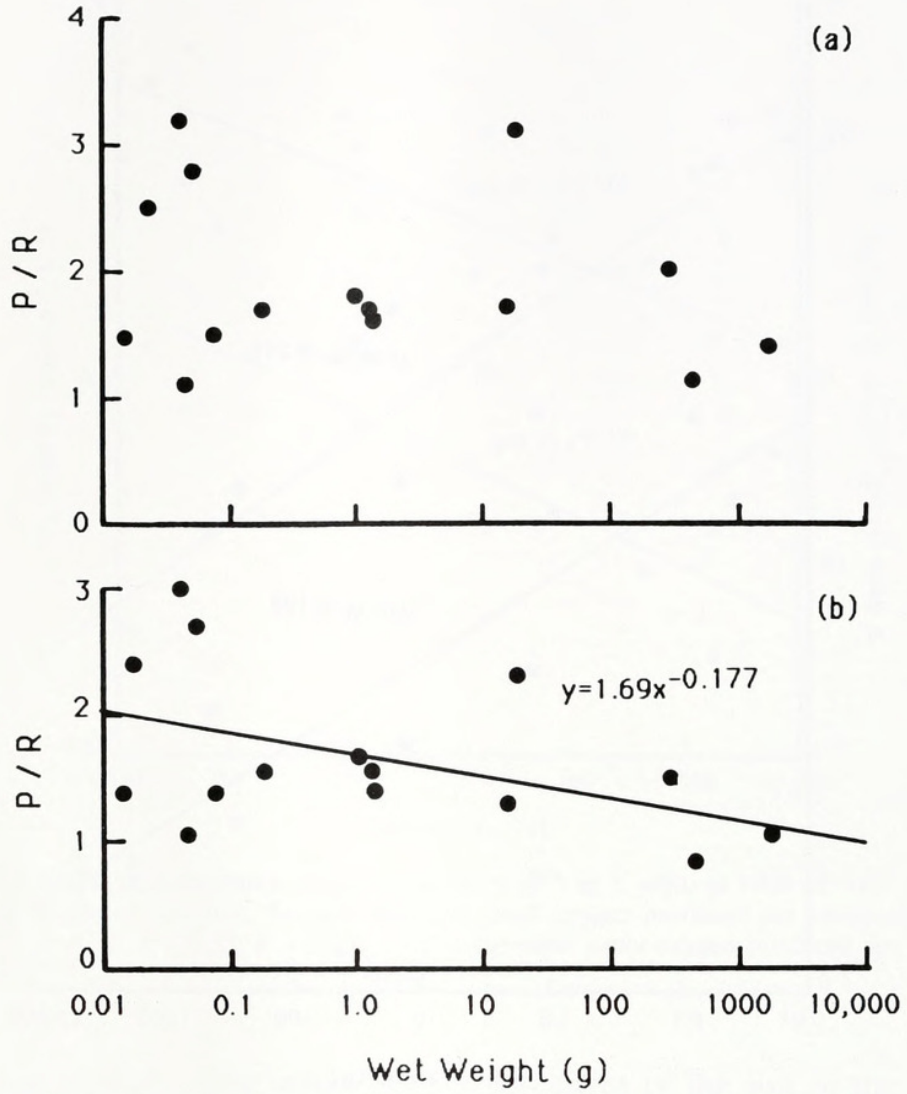

FIGURE 4. The ratio of gross photosynthesis to respiration (P/R) as a function of the wet weight of the clam at two different levels of irradiance. (a) P/R as a function of wet weight at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ (n = 15, r = 0.27). (b) P/R as a function of wet weight at 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ ($y = -0.177 \log x + 1.69$, n = 15, r = 0.468, $P = 0.1$).

DISCUSSION

The total number of zooxanthellae in *Tridacna gigas* increased with the size of the clam, however the number of zooxanthellae per gram wet tissue decreased with increasing clam size (Fig. 1). Intuitively, one might expect that this relation occurs because the weight of the clam increases as a cube function while the area of the mantle increases as a square function. If this were true, then the allometric equation relating the total number of zooxanthellae (or total Chl. *a*) to the weight of the clam would have an exponent of 0.67. However, the allometric equations relating these parameters are;

$$\#\text{ of Zoox.} \cdot 10^{-6} = 5.623\ (\text{weight})^{0.9} \qquad (r = 0.996,\ n = 11,\ P < 0.01)$$

and

$$\mu\text{g Chl. } a = 128.8\ (\text{weight})^{0.89} \qquad (r = 0.996,\ n = 14,\ P < 0.01)$$

The exponents are considerably greater than the expected 0.67, which is probably a reflection of two facts. First, not all of the zooxanthellae are found in the "mantle"

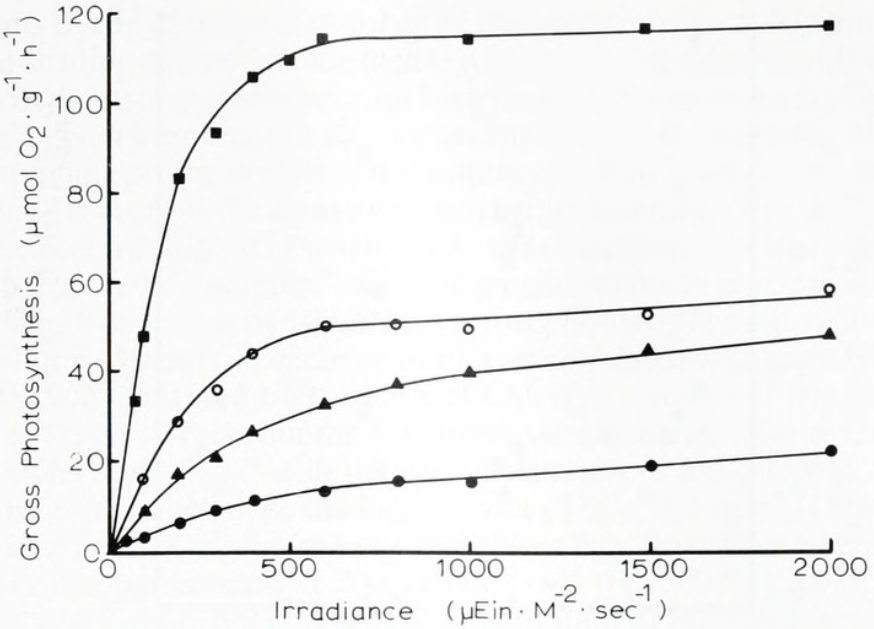

FIGURE 5. Gross photosynthesis as a function of irradiance (P-I curves) for four different size classes of clams. Each data point represents the rate of oxygen evolution over at least a ten-minute period measured when the oxygen level in the respirometry chamber was between 80 and 100% of air saturation; closed circles—wet weight = 1700 g: closed triangles—wet weight = 18.2 g: open circles—wet weight = 1.25 g: closed squares—six clams with an average wet weight of 0.014 g.

of the clams. In this study an average of 13% (±6.2%, n = 8) of the Chl. *a* was found in the "remains" portion of the clam (no correlation was found between the size of the clam and the distribution of the Chl. *a* in the various tissues). Second, as the clam increases in size, the "mantle" increases in thickness. Thus, there is a third dimensional component in the amount of "mantle" tissue, where 87% of the zooxanthellae reside.

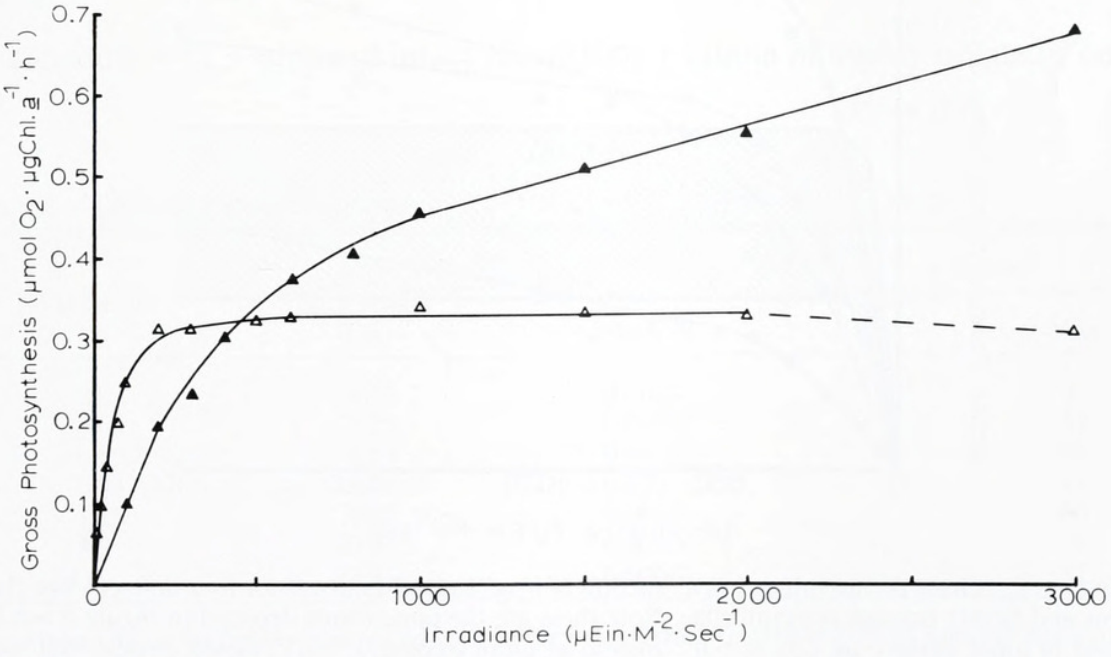

FIGURE 6. Photosynthesis as a function of irradiance (P-I curves) for the 18.2 g clam and zooxanthellae freshly isolated from it, calculated on the basis of Chl. *a* content: open triangles represent the freshly isolated zooxanthelle; closed triangles represent the intact clam.

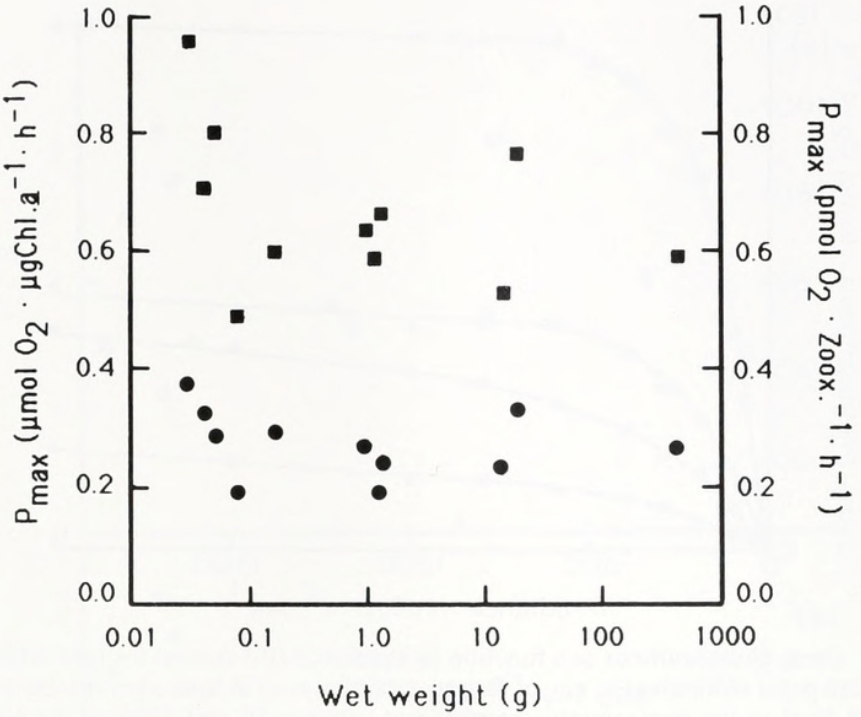

FIGURE 7. Gross photosynthetic oxygen evolution by freshly isolated zooxanthellae at a saturating light intensity of 600 $\mu E \cdot m^{-2} \cdot s^{-1}$ (P_{max}) as a function of the wet weight of the clam from which the zooxanthellae were isolated. Closed squares represent P_{max} in pmol oxygen produced per zooxanthella per hour; closed circles represent P_{max} in μmol oxygen produced per μg Chl. *a* per hour.

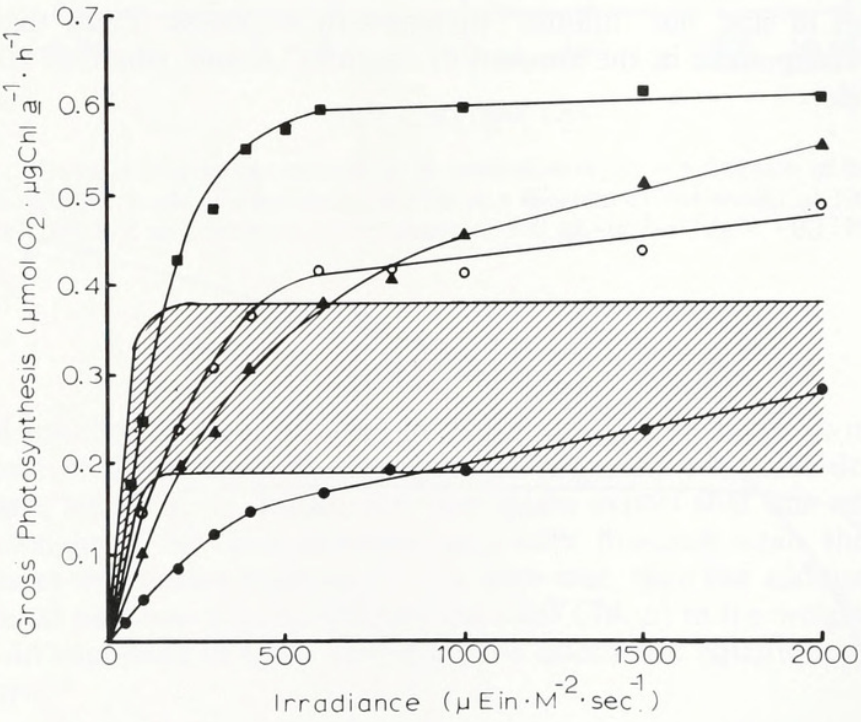

FIGURE 8. Gross photosynthesis as a function of irradiance (P-I curves) for four different size classes of clams and freshly isolated zooxanthellae. Note these are the same clams depicted in Figure 6 but P_g is expressed in μmol oxygen · μg Chl. $a^{-1} \cdot h^{-1}$ instead of μmol oxygen · $g^{-1} \cdot h^{-1}$: closed circles—wet weight = 1700 g: closed triangles—wet weight = 18.2 g: open circles—wet weight = 1.25 g: closed squares—six clams with an average wet weight of 0.014 g. The hatched area represents a composite of all P-I curves obtained for zooxanthellae freshly isolated from *T. gigas*.

The effects of the decreasing number of the zooxanthellae per gram of clam and the increased shading of those zooxanthellae with increasing clam size (discussed below) are reflected in the strong inverse relation between decreasing productivity (O_2 evolved/g clam/h) at a given illumination and increasing size of the clam (Fig. 3).

Light impinging on the surface of the mantle of a clam must penetrate increasingly thicker tissue as the size of the clam increases, before reaching all of the zooxanthellae, which are themselves stacked (Trench *et al.*, 1981). Evidence of increased shading of the zooxanthellae in the "mantles" of the larger clams comes from two different series of experiments. In one series of experiments gross photosynthesis at 1000 $\mu E \cdot m^{-2} \cdot s^{-1}$ was measured for 13 clams. A decrease in P_G was correlated with increasing clam size, even when P_G was expressed on the basis of Chl. *a* or number of zooxanthellae (Fig. 2). In another series of experiments P-I curves were generated for seven clams. The decrease in alpha and "P_{max}" with increasing clam size is indicative of the increased shading of the zooxanthellae in the larger clams (Table II, Fig. 8). Only the smallest clams show saturation of photosynthesis at ambient light intensities whereas the freshly isolated zooxanthellae saturate at 200 $\mu E \cdot m^{-2} \cdot s^{-1}$ (Figs. 5, 6) (also Scott and Jitts, 1977; Trench and Fisher, 1983). The lack of saturation of photosynthesis in the larger clams can be explained by the observation that as light intensity is increased, saturating levels of irradiance are reaching deeper into the mantle tissues. In order to put a value on this shading phenomenon, one can compare the P-I curves of intact clams to P-I curves of freshly isolated zooxanthellae. Superimposed on the P-I curves of the four clams in Figure 8 is a hatched area which represents all of the possible P-I curves obtained for freshly isolated zooxanthellae. When we speak of the shading of the zooxanthellae in a certain sized clam, what we mean is the average amount of shading throughout all of the zooxanthella-containing tissues. For example, in a large clam the zooxanthellae on the surface of the mantle may be light-saturated at 400 $\mu E \cdot m^{-2} \cdot s^{-1}$, while those deep in the mantle are perceiving only a small fraction of the incident light. Because of this, it is necessary to compare the alpha values of the P-I curves; this is the only portion of the curves where all of the zooxanthellae are light limited. We therefore propose an equation to estimate the degree of shading in a clam:

$$\% \text{ shading} = (1 - \text{alpha of intact clam})(100) \div (\text{alpha of freshly isolated Zoox.})$$

TABLE II

Shading of Tridacna gigas *zooxanthellae*

Wet weight (g)	Shell length (cm)	Alpha/Chl. *a*	% Shaded	
			Min	Max
0.02	1.15	0.0025	0	34
0.041	1.55	0.0018	5	53
0.052	1.54	0.0018	5	53
0.84	4.07	0.002	0	47
1.25	4.82	0.0015	21	60
18.2	10.3	0.0010	47	74
290	23.5	0.0005	74	87

Alpha/Chl. *a* for freshly isolated zooxanthellae ranged from 0.0019 to 0.0038 and the minimum and maximum % shading experienced by zooxanthellae in the clam was calculated by using these values in the following equation:

$$\% \text{ shaded} = [1 - (\text{alpha clam}) \div (\text{alpha zooxanthellae})](100)$$

In this equation the ratio of alpha values is equal to the ratio of photosynthetic rates at a given limiting light intensity. The range of alpha values calculated for the seven clams for which P-I curves were generated are shown in Table II. In the smaller clams the mantle is relatively thin and shading would not significantly reduce the maximum photosynthetic rate the zooxanthellae can achieve. The zooxanthellae in the larger clams are estimated to receive, on the average, only 25% of the incident light. Muller-Parker (1984) found no significant shading of zooxanthellae *in situ* in *Aiptasia pulchella.* This is not surprising because, as the author pointed out, *Aiptasia* is a very small, unpigmented anemone. On the other hand, Crossland and Barnes (1977) determined that the algae in the coral *Acropora acuminata* were significantly shaded *in situ.* Application of the % shading equation to the data of Crossland and Barnes (1977), indicates that on the average about 20% of the incident light reached the zooxanthellae *in situ.* This low value is probably a reflection of both the position of the algae in the coral's tissue and the geometry of the staghorn coral. The zooxanthellae on the underside of a coral branch could be shaded by the coral skeleton itself.

The P_{max} of freshly isolated zooxanthellae is significantly lower than the P_{max} of all but the largest clam measured (Fig. 6, 8). This phenomenon, while difficult to interpret, could be attributable to a number of causes: (1) physical damage to some of the zooxanthellae during the isolation procedure. This damage might not be discernible at the level of light microscopy, and could result in partial photosynthetic inactivation; (2) zooxanthellae in isolation could be producing superoxide anions (O_2^-) and therefore hydrogen peroxide (H_2O_2), which is not detectable with an oxygen electrode. The deleterious effects of O_2^- and H_2O_2 would normally be controlled by superoxide dismutase and catylase present in host tissue (Dykens and Shick, 1982; Dykens, 1984; and E. M. Tytler, pers. comm.); (3) the host milieu could stimulate high rates of photosynthesis in zooxanthellae. This could be due to *in situ* pH or to some other "factor" in the host (Trench, 1971b, Deane and O'Brien, 1980). None of these possibilities would increase the alpha values for freshly isolated zooxanthellae. The only effect on alpha that any of these possibilities would have is to lower the alpha value for freshly isolated zooxanthellae which would make our estimates of shading conservative minimum values.

When comparing Figures 5 and 8 it is apparent that the trend of decreasing alpha and P_{max} with increasing clam size becomes somewhat blurred when photosynthesis is expressed on the basis of chlorophyll *a* (Fig. 5). This can easily be accounted for by the well known effects of photoadaptation on Chl. *a* content in zooxanthellae (Zvalinskii *et al.,* 1980; Dustan, 1982; Chang *et al.,* 1983). In this study the variation in chlorophyll *a* content per zooxanthella was not correlated with the size of the host clam. Chlorophyll *a* per cell was not determined for the smallest clams because the small amount of material present in those clams did not allow that determination as well as assaying total Chl. *a* per clam. In the larger clams the zooxanthellae in the hemal sinuses of the "mantle" are shaded to different degrees depending on their position in the mantle. Therefore, any changes in Chl. *a* content of the zooxanthellae deep in the mantle are obscured by the presence of "surface" algal cells in the same extract.

The relation between photosynthesis and size discussed above would yield a similar relation between P/R and size were it not for the fact that respiration rate also decreases with increasing body size (Fig. 3). The exponent of the equation relating body weight to respiration falls between 0 and 1 for most organisms (R is expressed as total oxygen consumed per unit time) and is a function of the allometric increase in metabolism with growth (Zeuthen, 1953; Schmidt-Nielsen, 1974). This value ranges from 0.595 to 0.93 for *Mytilus edulis* (Kruger, 1960; Read, 1962). Bayne *et al.* (1976) averaged

the values obtained in a large number of studies on marine mussels and found a value of 0.71. The value for *T. gigas* of 0.81 found in this study ($R = 8.0\ W_{0.81}$) is higher than this average, but well within the range of values found for *Mytilus,* and while difficult to interpret may reflect a difference in temperature, feeding, season, or stage in life history of the animals (Zeuthen, 1953; Kruger, 1960; Kuenzler, 1961; Widdows, 1978; and Walsh and Somero, 1981; *cf.* Read, 1962; Ansell, 1973).

The calculated values of daily P/R and especially of CZAR vary greatly depending upon the assumptions one makes in the calculations (Table I). Nevertheless, it is clear that the zooxanthellae do play a very significant role in the nutrition of *T. gigas* and in fact may be able to supply as much as 100% of the respiratory carbon requirements of the clam. At high light intensities ($\geq 1000\ \mu E \cdot m^{-2} \cdot s^{-1}$) the variation in the P/R for the intact symbiosis shows no strong correlation with the size of the clam (Table I, Fig. 4a). However, if one assumes an average daily illumination of $500\ \mu E \cdot m^{-2} \cdot s^{-1}$ such as the clam would experience on a cloudy day or in deeper water, an inverse relation between P/R and clam size is evident (Table I, Fig. 4b). The difference between Figures 4a and b can be understood by inspecting the P-I curves for the four different sizes of clams shown in Figure 5. Lowering the light intensity from 1000 to 500 $\mu E \cdot m^{-2} \cdot s^{-1}$ lowers the photosynthetic rate of the smallest clams by only 4%, while it results in a 25% reduction of the photosynthetic rate of the two largest clams. This reduction is due to the increased shading in the larger clams as described above.

These physiological parameters of *Tridacna gigas* are of special import to a mariculture operation. Juvenile clams can be grown optimally at light intensities that are significantly below ambient. This should help reduce algal fouling of the growth tanks. On the other hand, since zooxanthellae freshly isolated from *T. gigas* showed no evidence of photoinhibition at even the highest ambient light intensities ($2000\ \mu E \cdot m^{-2} \cdot s^{-1}$) (Fig. 6), and since the photosynthetic rates of the larger clams are not saturated at ambient levels of irradiance (Fig. 5), the higher the intensity at which one grows the larger clams, the better.

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