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The Influence of Symbiotic Dinoflagellates on Respiratory Processes in the Giant Clam *Tridacna squamosa*[1]

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ABSTRACT: Several aspects of respiratory gas exchange are distinctive in the giant clam *Tridacna squamosa*, which obtains nutrients from symbiotic dinoflagellates found in the mantle. During the day, when more oxygen is produced than consumed by the host and its symbionts, oxygen extraction is negative. Exhalant water P_{O_2} is higher than inhalant water P_{O_2}, and prebranchial blood P_{O_2} is higher than heart blood P_{O_2}. Ventilation of the mantle cavity and the gills continues, which rids the system of much excess oxygen and, possibly, prevents the formation of gas bubbles in the blood, which is supersaturated. In the dark, when the oxygen balance shifts to a rate of uptake that is unexceptional among lamellibranchs, the ventilation rate remains low and oxygen extraction high relative to species that rely exclusively on an exogenous food source. On a 24-hr basis, the total oxygen uptake exceeded the total oxygen production.

MEMBERS OF THE LAMELLIBRANCH genus *Tridacna* are believed to have solved the nutritional problem of limited plankton availability in tropical waters by "farming" large quantities of the dinoflagellate *Symbodinium microadriaticum* within the blood spaces of a vastly enlarged mantle (Yonge 1975). In sunlight, which appears to be focused on the algal symbionts by special hyaline organs in the mantle, labeled CO_2 taken up from the medium appears in the host tissue in the form of soluble end products of carbohydrate biosynthesis (Goreau, Goreau, and Yonge 1973; Muscatine 1967). As suggested by several authors, growth to the enormous size reached by some species may be possible in the tropics only because of this highly specialized mode of nutrition; Yonge (1975), for example, mentions a body weight of more than 1 megagram, a figure that probably includes the shell.

Respiratory gas exchange in tridacnids has been investigated largely from an ecological and nutritional point of view. Previous investigators agree that, in the presence of sunlight, more oxygen is produced than consumed by the clam and its zooxanthellae (Jaubert 1977; Johannes et al. 1974; Wells, Wells, and Van Derwalker 1973; Yonge 1936). However, the daily O_2 balance, or the ratio between net O_2 consumption and net photosynthetic O_2 production during a 24-hr period, is the subject of some disagreement, with estimates ranging from negative (Yonge 1936) to strongly positive (Johannes et al. 1974, Wells et al. 1973) to virtual unity (Jaubert 1977).

The shift from filtration of exogenous phytoplankton to symbiosis with endogenous algae as a mode of nutrition raises additional questions concerning the respiratory adaptations of tridacnids. As shown many years ago, the use of the gill as a feeding as well as respiratory organ is accompanied by extremely high velocity ventilation and low extraction of oxygen from the water current (Hazelhoff 1938). Food replaces oxygen as the critical variable in short supply in the water

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current (Jørgensen 1952). In the lamellibranchs, for example, ventilation rates often exceed 1 liter/g dry wt-hr (Booth and Mangum 1978) and oxygen extraction from normoxic water ($P_{O_2} > 135$ mm Hg) typically lies in the range 3–10%, which rises only to about 20–35% during hypoxia (Famme and Kofoed 1980; Mangum and Burnett 1975).

It was our intent in this study to investigate oxygen uptake in *Tridacna squamosa* Lamarck to determine whether the trophic adaptation is accompanied by respiratory features characteristic of animals that use their gills primarily in gas exchange rather than feeding. Our data also permit the calculation of a daily O_2 budget, which may be relevant to several controversial aspects of tridacnid biology.

MATERIALS AND METHODS

Animals were purchased from collectors at Bindoy, Negros Orientale, Philippine Islands (9° N), and held in natural light in running, unfiltered seawater (28–31°C) for 3–7 days prior to experimentation. On two occasions, clams were eaten by octopods in the aquariums, suggesting the possibility of natural predators other than those mentioned by McMichael (1974). Eight animals were used, six for the respiratory measurements and all eight for the chlorophyll determinations.

The rate of O_2 depletion in a closed container was measured on deck, either in natural light and darkness or under a black plastic cover, with a polarographic electrode (Yellow Springs Instrument Co. Model 54) and chart recorder (Hewlett-Packard 680). Animals were placed mantle side up in the open, seawater-flushed containers and allowed to adjust for 2–3 hr prior to a measurement. Unlike many other species of lamellibranchs, they invariably opened their valves, exposed the mantle, and extended the exhalant siphon within a minute after induced closure. The seawater was mixed by a large (5-cm) magnet in the bottom of the container, which was placed on a submersible stirrer. The preparation was submerged in a large, seawater-flushed aquarium in order to maintain a constant temperature. The clam was about 0.5 m below the surface, simulating the shallowest depths in nature, and additional light reached the mantle through the walls of the aquarium, a distance of 0.05–1 m.

To determine oxygen uptake ($\dot{V}_{O_2}$) in normoxic water, the tip of the electrode was kept at a slight angle about 3 cm below the lid of the container (to prevent it from capturing the bubbles that quickly form in the sunlight). Also, to prevent the water from becoming highly supersaturated, it was necessary to slide the lid gently aside, opening the container and allowing thorough renewel of the medium from the surrounding seawater, at intervals no longer than 10–15 min. This operation, which was also used in measuring the normoxic rate in darkness, caused no perceptible movement or change in ventilation posture of the clam. To determine oxygen uptake ($\dot{V}_{O_2}$) in hypoxic water, the medium was not renewed and O_2 was monitored continuously for periods of several hours. Volume of the respirometers ranged from 1 to 3 liters, depending on the size of the clam (16–41 g wet wt).

Measurements were generally initiated before sunrise and terminated after midnight, since no chronological trends in the rates during darkness were noted.

Oxygen extraction was determined by measuring the P_{O_2} of point samples of excurrent water leaving the siphon and incurrent water in the surrounding medium (Radiometer BMS2 Blood Gas System). Values were obtained in normoxic, running seawater, and in moderately hypoxic, standing water. Repeated attempts to make manometric measurements of ventilation failed; the clam would not form a watertight seal around a cannula placed in the incurrent, and it ejected cannulae placed in the siphon by the powerful squirting contractions interpreted by McMichael (1974) as a defense response. Ventilation was calculated from the data on O_2 uptake or production and O_2 extraction.

Anaerobic samples of the blood were taken repeatedly from the adductor muscle sinus into a hypodermic syringe, within a few seconds of contact. Even though an individual was sampled more than once, no animal died from this treatment and, indeed, the valves

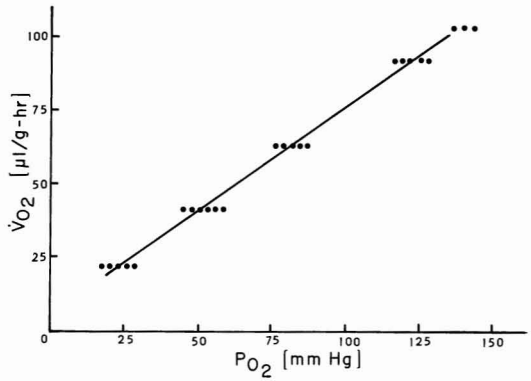

FIGURE 1. The relationship between oxygen uptake ($\dot{V}_{O_2}$) and water P_{O_2} in a 16-g wet wt *Tridacna squamosa*, at 30°C.

remained closed after sampling for less than 2–3 min, regardless of the time of day. Blood samples were also obtained from the ventricle within 5 sec of severing the adductor muscle.

Living tissue was dissected from the shell. The large sinuses in the foot and the visceral mass were drained and the animal blotted prior to weighing. Then the margin of the mantle was dissected and extracted with methyl alcohol repeatedly, until the absorbance of the extract (using an equal volume of tissue and solvent) declined to less than 0.05 (Zeiss DM4 recording spectrophotometer, 1-cm cell). The concentration of chlorophyll in the extracts was calculated from the extinction coefficients given by Jeffrey and Haxo (1968).

RESULTS

The mean value for oxygen uptake in six animals at night and in normoxic water is 108 ± 3 SE μl/g wet wt-hr ($N = 25$; see Figure 1), the individual values showing no clear relationship to body size within the small range (16–41 g) examined. This figure does not differ from the rates obtained using the same animals during the daytime but in darkened chambers ($P = 0.80$ according to Student's t test for paired observations, $N = 9$). An experiment performed after a long period of uninterrupted ventilation demonstrated that the *Tridacna squamosa* system is an oxyconformer in the ambient P_{O_2} range 5–172 mm Hg, below which oxygen uptake ceases (Figure 1). After 1–3 hr of anaerobiosis the O_2 uptake was regulated in the ambient P_{O_2} range 90–159 mm Hg. An elevated rate of posthypoxic oxygen uptake was observed in only one of three animals tested, and the increase was only 7%. None of the three died. On 29 October 1979, a day on which the sun rose in Bindoy at 0606 (U.S. Nautical Almanac), net oxygen production (the excess of photosynthesis over aerobic metabolism) was detected first at 0725. The rate climbed quickly at first and then more slowly for several hours (Figure 2), under little cloud cover. The rate then increased rapidly again to peak shortly before noon (Bindoy is located in the eastern portion of its time zone). The peak was followed by an equally rapid decrease to a rate which then declined slowly to the compensation point, reached at 1635; the sun set at 1722. The afternoon had been cloudier than the morning but not overcast; the compensation point on an overcast day occurred at 1542. Thus, the daily period of net O_2 production in Bindoy Bay (9° N) during the late fall is about 9 hr; this is 2 hr shorter than at Enewetak (11° N) during the summer solstice (Johannes et al. 1974).

The duration of balanced O_2 production and uptake is very brief—just 5 min following sunrise and 10 min before sunset. When the shift from net production to net uptake was induced suddenly at midday by darkening the container, the duration of balance was the same, but the rate of oxygen uptake in normoxic water remained low for about 15 min, suggesting the utilization of O_2 stores in body fluids. When the cover was removed after 2 hr, the duration of balance was longer (10.5 min) than in the morning and the rate of O_2 production was lower than expected at midday for about 20 min, suggesting the replenishment of O_2 stores. Thus, the longer duration of balance in the morning than in the afternoon may reflect the filling of empty internal O_2 stores.

After 1–3 hr of hypoxia (water $P_{O_2} < 40$ mm Hg), the rate of O_2 production in the afternoon rose from the prehypoxic value of 157 to 175 μl/g wet wt-hr, at a time when the light intensity was decreasing. The mechanism

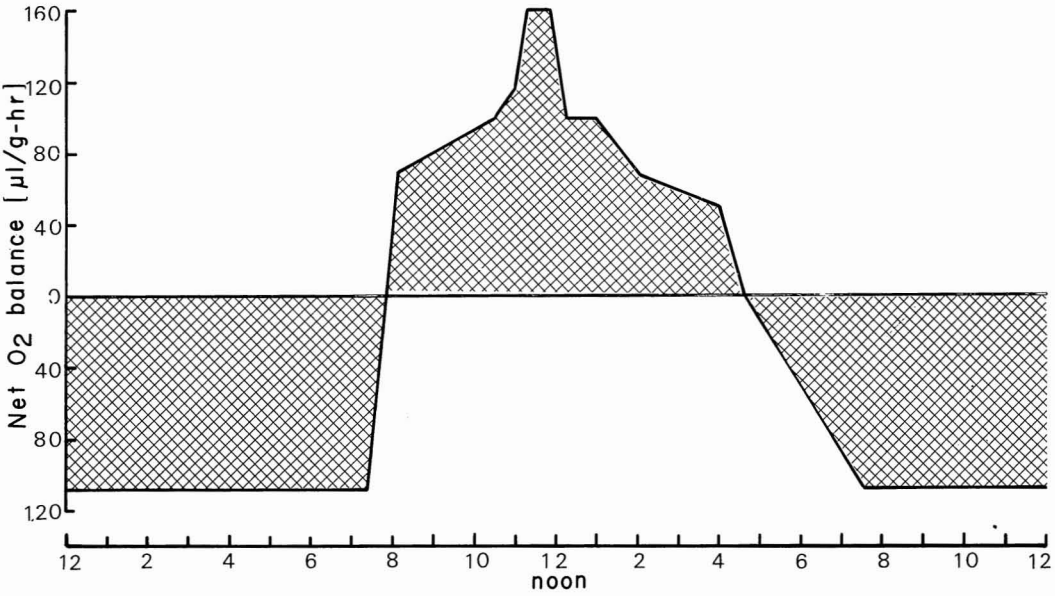

FIGURE 2. The oxygen balance of a 20-g wet wt *Tridacna squamosa*. Temperature 29–30°C, water P_{O_2} = 150–160 mm Hg. Maximum and minimum rates not significantly different from those in three larger individuals on which prolonged measurements were made ($P > 0.05$ according to Student's t test). A single set of measurements made on an overcast day showed very little net O_2 production.

that permits this compensatory response is not known.

During the dissection, we learned that the figures for O_2 uptake represent the clam and animal symbionts as well as algal symbionts. One species of shrimp and two crabs, weighing 2–3% of the total biomass, were found in the mantle cavities of four of the six clams for which O_2 uptake values were obtained.

Oxygen Extraction from or Addition to Ambient Water

When animals were placed in normoxic water (average $P_{O_2} = 140 \pm 7$ SD mm Hg) and kept in the dark, the P_{O_2} difference between ambient water and exhaled water averaged 58.7 mm Hg, giving an O_2 extraction of 41.4 ± 10.2 SD% ($N = 9$). Calculations according to the Fick principle, using the $\dot{V}_{O_2}$ value measured in the dark (108 µl/g wet wt-hr), give a ventilation rate of 66.8 ml/g wet wt-hr.

In daylight during the middle of the day and in normoxic water, the P_{O_2} difference between ambient and exhaled water averaged -21.9 ± 7.2 SD mm Hg ($N = 11$). If $\dot{V}_{O_2}$ under these conditions were the same as in the dark and in normoxic water, then total O_2 production would be about twice the average shown in Figure 2. The volume of O_2 added to the water is only half that extracted in the dark; thus, the Fick principle predicts that ventilation goes down during the day, and the prediction is likely to underestimate the change due to the incorrect assumptions of the same $\dot{V}_{O_2}$ in air-saturated as in supersaturated water (Figure 1) and the same rate in the light as in the dark. Glycolate synthesis, which is favored by high levels of O_2 and light, is known to occur in *Gymnodinium* (Hellebust 1965), and it is likely to have an appreciable effect on total $\dot{V}_{O_2}$. Nonetheless, our measurements and visual observations clearly indicate that ventilation does not cease in daylight.

At night and in hypoxic water (P_{O_2} = 22–47 mm Hg), oxygen extraction decreased to 23–35%, averaging 29% ($N = 5$). At an O_2 uptake rate of about 33 µl/g-hr (Figure 1), this result suggests a pronounced decrease in ventilation, to about one-fourth of the normoxic rate.

Blood P_{O_2}

The P_{O_2} of blood taken from the adductor muscle sinus of animals held in normoxic or only slightly supersaturated water (141–172 mm Hg) during the day was greater than 159 mm Hg, averaging 162 ± 3 SE mm Hg and ranging up to 177 mm Hg ($N = 10$). No bubbles were noted in the blood as it passed into the syringe, but they formed on standing. Presumably, the valves exert enough pressure on body fluids to keep O_2 in solution. The P_{O_2} of blood taken from the ventricle was lower (139 ± 2 SE mm Hg, $N = 4$), as would be expected if the ventricle contains mixed postbranchial and the adductor muscle unmixed prebranchial blood.

At night, the P_{O_2} of blood taken from the heart of a normoxic clam was 60 mm Hg, and that of adductor muscle blood was 38–42 mm Hg ($N = 3$). The mean chlorophyll ($a + c$) content of eight animals was 0.59 mg/g wet wt of the soft tissue of the clam plus its algal symbionts. Wells et al. (1973) mention a figure of 4% for the mantle alone, perhaps 40 mg/g dry wt.

DISCUSSION

With the exception of the positive character of the O_2 balance sheet during the daytime, the outcome of respiratory oxygen exchange in *Tridacna squamosa* resembles that in lamellibranchs that use the gill primarily as a feeding organ. Considering the large body size, the high experimental temperature, and the likelihood that some of the measured oxygen uptake was due to epibiota on the shell, the rate of oxygen uptake is unexceptional and, in the dark, blood P_{O_2} is similar (e.g., Booth and Mangum 1978). On the other hand, O_2 extraction approximates the range usually found in animals with primarily respiratory gills (Hazelhoff 1938), quite unlike the values found in filter-feeding mollusks (Booth and Mangum 1978, Mangum and Burnett 1975). Assuming that dry weight is 20% of wet weight, the ventilation rate in the dark is one-fifth the value found in the filter-feeder *Modiolus* (Booth and Mangum 1978) and two-thirds that in the hemoglobin containing *Noetia* (Deaton and Mangum 1976), in which feeding has not been investigated. The low ventilation rate supports the conclusion that the requirement of an exogenous food source is small (Johannes et al. 1974, Ricard and Salvat 1977). It seems somewhat anomalous that the gill of another species (*T. derasa*) appears to be modified for an increased filtration efficiency (Purchon 1968), unless the increase proves to be qualitative rather than quantitative. Although the size of the digestive structures is reduced (Purchon 1968), no data are available on gill surface areas, which appear to be relatively small.

From a purely respiratory point of view, there remains the following question: Why does ventilation continue at all during daylight when the ventilatory requirement, $\dot{V}_w/\dot{V}_{O_2}$, becomes negative? Several possible explanations come to mind, including the CO_2 requirement for photosynthesis, the need to flush away metabolic wastes, and, as pointed out by Yonge (1975), a requirement for nutrients (such as the substances utilized in protein synthesis) that is not filled by the liberation of photosynthetic end products into the clam's tissues. A less obvious but important explanation is the need to remove excess O_2 and prevent supersaturation of the tissues, which would otherwise result in internal embolisms and disrupt the convection of body fluids, as well as expose the tissues to possibly toxic effects of excess O_2.

None of our data clearly indicate a daily O_2 balance of either zero or a surplus, as reported by Jaubert (1977) and Johannes et al. (1974), and the basis of the discrepancy cannot be discerned from the previous reports. Although the shorter day length and, possibly, greater cloud cover undoubtedly limited O_2 production in our experiments, these factors seem unlikely to be responsible for the observed O_2 deficit of about 50%. The balance would become positive if some combination of the following erroneous assumptions were made: (1) the net O_2 production day is 11 hr long (Johannes et al. 1974) throughout the year, (2) net O_2 production occurs at the peak rate throughout the day, or (3) net O_2 uptake occurs at a rate measured in hypoxic water. Alternatively, our use of shipboard instead of in situ experimentation could have introduced an inhibitor of O_2 production,

such as excess light intensity (Downton et al. 1976). Finally, the relationship between O_2 uptake, O_2 production, and body size in these animals is unknown. Our clams were relatively small for the species, and it is possible that O_2 uptake, but not O_2 production, was relatively high. We cannot compare our data with those of previous investigators, who do not give figures for the absolute rates, body size, chorophyll content, etc.

Regardless of the source of the discrepancy, it will be interesting to evaluate the significance of O_2 production of the population on an annual basis, taking into account growth and natural disturbances of sunlight such as the number of cloudy days.

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