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Review

CALCULATING THE CONTRIBUTION OF ZOOXANTHELLAE TO GIANT CLAMS RESPIRATION ENERGY REQUIREMENTS

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ABSTRACT

Giant clams (Tridacnidae) are known to live in association with photosynthetic single cell dinoflagellate algae commonly called zooxanthellae. These algae which can be found in the mantle of the clams are capable of transferring part of their photosynthates which become an important source of energy to the host (apart from filter feeding activity). In order to understand the basic biological processes of the giant clams , the contribution of zooxanthellae to the clam's energy requirement need to be determined. This review describes how to calculate the contribution of zooxanthellae to the giant clam's energy requirement for the respiration process.

Key words: Giant clams, tridacnidae, zooxanthellae CZAR

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INTRODUCTION

Giant clams (Family: *Tridacnidae*) are large bivalves that are commonly found in coral reef habitats especially in the Indo-Pacific region. This family consists of two genera (*Tridacna* and *Hippopus*) and eight species: *Tridacna gigas, T. derasa, T. squamosa, T. maxima, T. crocea, T. tevoroa, Hippopus hippopus,* and *H. porcellanus* (Braley, 1992). As well as being prominent members of healthy coral reef ecosystems they are important to the people of South East Asia and the Pacific region as a source of food (meat) and building material (shells). These clams have recently become an important export commodity in several countries in this region (Tacconi and Tisdell, 1992; Tisdell *et al*., 1994).

One of the important aspects of the biology of giant clams is the existence of zooxanthellae which occupy the mantle of the clams as endosymbiotic dinoflagellate algae (Lucas, 1988). These zooxanthellae have a significant role, especially in the energy requirements of giant clams, since they are capable of translocating part of their photosynthetic products to their host. This is why as a member of bivalves giant clams do not only supply their energy demand from filter feeding process, but also from the energy translocation from zooxanthellae (Klumpps et al., 1992).

This review aims to describe how to calculate the contribution of zooxanthellae to the daily respiration energy requirements of giant clams.

Zooxanthellae-Giant Clams Association

Zooxanthellae are 'yellow-brown' dino-flagellate algae (Pyrrophyta) which live as endosymbionts in many marine invertebrate species (Brandt, 1881). Taylor (1974) listed four different species of zooxanthellae: (1) *Gymnodinium* (*Symbio-dinium*) *microadriaticum* (Freudenthal, 1962) which lives in protozoans, coelenterates, molluscs; (2) *Amphidinium chattonii* in some coelenterates; and (3) *A. klebsii* in platyhelminthes and (4) *Amphidinium* sp. in some protozoans.

Symbiodinium microadriaticum was originally isolated from the jellyfish *Cassiopeia* sp *(*Freudenthal, 1962). Later Taylor (1971) proposed that zooxanthellae be placed in the genus *Gymnodinium* because the free-living stage had great affinities to this genus. Loeblich and Sherley (1979) later suggested the use of *Zooxanthella microadriatica* when they found that the zooxanthellae isolated from *Cassiopea xamachana* differed slightly from *Zooxanthella nutricula* from the order Zooxanthellales (Brandt, 1881). Schoenberg and Trench (1980b), however, argued that the latter name was inappropriate, because the pyrenoids of the algae described by Brandt (1881) are traversed by chloroplast thylakoids (Hollande and Carre, 1974), which is not the case in *S. microadriaticum* from *Cassiopeia* sp.

Further work revealed that *Symbiodinium microadriaticum* are not monospecific. Morphological, physio-logical, biochemical and genetic differences among *S. microadriaticum* collected from different hosts are now more the norm than the converse (Schoenberg and Trench, 1980a,b; Blank and Trench, 1985 a,b). There are differences in the life cycle of zooxanthellae in symbiosis and in culture conditions. The coccoid, non-motile stage predominates in symbiosis, whilst in culture there is alternation between the motile and non-motile (coccoid) stage (Muscatine, 1980; Domotor and D'Elia, 1986). Motile stages are reported to be phototactic and occur only for a short period of approximately 0.5 hour (Taylor, 1969a; Domotor and D'Elia, 1986). Fitt *et al.* (1981) demonstrated different motility patterns of zooxanthellae collected from jellyfish (*C. xamachana, C. frondosa*), sea anemones (*Aiptasia tagetes, A. pallida*) and a giant clam (*T. gigas*) cultured in identical conditions.

Schoenberg and Trench (1980a,b) reported differences in isoenzyme patterns, soluble protein, size, and the structure of the cell wall of zooxanthellae collected from different hosts. Using electrophoresis, Schoenberg and Trench (1980a) investigated 40 cultures from 17 host species representing 12 strains and found a unique combination of four isoenzyme patterns in each strain. Later Chang *et al.* (1983) found differences in the photoadaptive mechanisms of three strains of zooxanthellae collected from a clam (*T. maxima*), an anemone (*A. pulchella*) and a coral (*Montipora verrucosa*). Similarly, Blank and Trench (1985a,b) showed several differences in the number of chromosomes, chloroplasts, pyrenoid stalks, mitochondria, chromosome volumes, nuclear volumes and thylakoid arrangements of zooxanthellae isolated from jellyfish (*Cassiopeia xamachana* and *C. frondosa*), anemone (*Heteractis lucida*) and coral (*M. verrucosa*). These zooxanthellae were reported to maintain their differences when cultured in similar conditions.

The existence of these variations among zooxanthellae collected from different hosts suggests that zooxanthellae represent dozens, perhaps even hundreds of species. By investigating the biochemical, physiological, morphological, and behavioural

differences, Trench and Blank (1987) introduced three new species into the genus *Symbiodinium*. These are *S. goreauii , S. kawagutii* and *S. pilosum* isolated from the Caribbean sea anemone *Ragactis lucida*, stony coral *Montipora verrucosa* and the Caribbean zoanthid *Zoanthus sociatus* respectively. Rowan and Powers (1991, 1992) found 6 distinct differences in small subunit RNA (ssRNA) genes of zooxanthellae collected from 16 cnidarians using the polymerase chain reaction (PCR) method. They could not distinguish any differences in the symbionts from individual corals of the same species, but they found that different species of corals have algae with unique sRNA sequences. However, multiple populations of zooxanthellae have been reported from individual coral, *Montrastea annularis, M. faveolata*, and *M. Franksi* (Rowan and Knowlton 1995).

In more recent papers Baker and Rowan (1997) reported genetic differences among zooxanthellae isolated from various corals species collected from the Caribbean and Eastern Pacific. They found three different clades among those zooxanthellae which were then termed as clade A, B, and C.

Zooxanthellae-Giant Clam Relationship

Giant clams are known to live in association with symbiotic zooxanthellae (the term symbiosis being used throughout this paper is to describe the mutualistic interaction between zooxanthellae and the host). In giant clams these zooxanthellae are extracellular, unlike hermatypic corals where zooxanthellae are located intracellularly. Initially it was agreed that zooxanthellae in giant clams are located freely in the haemal sinuses of the siphonal tissue which expand along the dorsal surface of the clams (Yonge, 1953; Frankboner, 1971; Trench *et al.*, 1981). Norton *et al,.* (1992), however, found the existence of a tubular system associated with zooxanthellae within giant clams, previously reported by Mansour (1946). Norton *et al.* (1992) concluded that zooxanthellae in clams are located in a branched tubular structure, with a single layer of thin cells separating zooxanthellae and haemolymph (Rees *et al*., 1993).

There are two mechanisms by which zooxanthellae appear in the next generation of their symbiotic host. Zooxanthellae can be acquired by direct parental transmission *via* their egg, or by direct acquisition from the environment. In giant clams, however, zooxanthellae are not being passed to the larvae (LaBarbera, 1975; Jameson, 1976; Fitt *et al.*, 1984; 1986). This is proved by the fact that zooxanthellae are not found in the trochophore stage (Fitt and Trench, 1981). Giant clams larvae acquire zooxanthellae *de novo* from the environment, soon after metamorphosis (Jameson, 1976; Fitt *et al.*, 1984). In the hatchery, zooxanthellae isolated from adult clams are introduced to veliger stages in order to promote a rapid transition to the new stage (Gwyther and Munro, 1981; Fitt *et al.*, 1984; Trinidad-Roa, 1988).

All strains of *Symbiodinium* sp. are ingested by clams. Only a specific strain, however, will eventually dominate a particular host (Fitt and Trench, 1981; Fitt, 1985b; Fitt *et al.*, 1986). Strain selection by the clams entirely depends on how a particular strain can grow, survive and compete with other strains within the host tissue (Fitt *et al.*, 1986).

Contribution of Zooxan-Thellae to Giant Clams Respiration

Zooxanthellae have been found in the stomach, digestive gland (Morton, 1978; Frankboner and Reid, 1981; Heslinga and Fitt, 1987) and faeces of clams (Trench *et al.*, 1981; Fitt *et al.*, 1986). Since they were morphologically intact, photo-

synthetically functional and viable, Trench *et al.* (1981) suggested that the zooxanthellae cannot be digested by clams as was previously thought (Frankboner, 1971; Frankboner and Reid, 1981).

Zooxanthellae are capable of transferring part of their photosynthetic products to the host (Muscatine, 1967; Goreau *et al.*, 1973; Taylor, 1974; Streamer *et al.*, 1988; Fitt, 1993). Their contribution depends primarily on the light intensity (*via* photosynthesis) and on clam size, both of which affect the proportion of zooxanthellae reached by the light (Heslinga and Fitt, 1987).

Several approaches to the study of the photosynthetic contribution of zooxanthellae have been used. Muscatine *et al.* (1981) outlined CZAR (the Contribution of Zooxanthellae to Animal Respiration). CZAR can be calculated as follows:

$$CZAR = \frac{[P_Z \text{ net } (24 \text{ h})]\ (\%Tr)}{R_a\ (24 \text{ h})}$$

where:

P_Z net (24 h) = net carbon assimilated by zooxanthellae during photosynthesis over 24 h

% Tr = the percentage of the photosynthates translocated by zooxanthellae to the host

Ra (24h) = carbon respired by animal over 24 h

CZAR is based on the measurement of the production (photosynthesis) and consumption (respiration) of oxygen which are then converted into units of organic carbon (Muscatine, 1980a; Muscatine *et al*., 1981). Respiration is the sum of the respiration of the host and zooxanthellae. Although the respiration rate under total darkness can be easily measured, respiration in the light is currently difficult, if not impossible to measure accurately (Muscatine *et al*., 1981). Therefore, the first assumption that has to be made is that respiration in the dark and in the light are the same. In addition, zooxanthellae respiration must be distinguished from animal respiration. Zooxanthellae respiration can be measured directly *in vitro*, but may not represent the real respiration rate of zooxanthellae within the host (Hoegh-Guldberg and Hinde, 1986; Muscatine, 1990). Morphological and physiological changes in zooxanthellae occur soon after they are isolated from their host (Trench, 1979). The second assumption proposed by Muscatine *et al*. (1981), is that the ratio of zooxanthellae and host respiration is proportional to their respective biomass. Although this method has been used for a large number of studies it should be noted that the assumption that the host and symbiont have similar metabolic intensities (that is, respiration at the same rate per gram of tissue) has not been directly tested (Hinde, 1989).

In order to calculate the value of CZAR for a symbiosis, several parameters have to be measured. These include the zooxanthellae specific growth rate, zooxanthellae carbon content, zooxanthellae and host protein content, daily carbon fixation by zooxanthellae and carbon respired by zooxanthellae and the host.

Zooxanthellae specific growth rate can be calculated by first isolating zooxanthellae from the host and determining the mitotic index of the algae (the ratio of dividing cells in 1000 cells at the time when the sample is taken). This index then can be converted to specific growth rate using the following equation, for phased mitotic indices (Wilkerson *et al.,* 1981; Muscatine *et al*., 1984):

$$\mu = 1/t_d \ \ln(1+f)$$

where:

μ = specific growth rate
td = duration of paired cell stage in hour
f = the maximum value of mitotic index

td is difficult to measure directly from symbiotic algal populations. A range of studies, however, have estimated that td lies somewhere around 0.46 d (Wilkerson *et al.,* 1983; Hoegh-Guldberg *et al.,* 1986). It can also be shown that CZAR is relatively insensitive to quite large variation in td (Muscatine *et al*., 1981).

The carbon content of the zooxanthellae can be determined based on the cell diameter and volume using the following equation (Strathmann, 1967):

$$\log C = - 0.314 + 0.712 \log V$$

where:
C = carbon
V = volume

The carbon content of zooxanthellae can then be used to convert information about the number of cells produced each day into a measure of the carbon retained by zooxanthellae.

Algal protein content can be measured by analysing the protein content of a known number of zooxanthellae isolated from the host. The total protein content of the host is usually analysed using the Lowry method (1951).

The daily net carbon fixation of zooxanthellae can be calculated from the photosynthetic rates of the algae and the daily irradiance. The oxygen produced by photosynthesis then can be converted to carbon multiplying the weight of oxygen produced with 0.375 per PQ (photo-synthetic quotient; Muscatine *et al*., 1981).

CZAR in the coral *Pocillopora damicornis* can be as high as 86.8%, by assuming 40% translocation from algae to the hosts (Muscatine and Porter, 1977). Muscatine (1980, review) found that with a mean translocation of 63% in *P. damicornis* and of 69% in *Fungia scutaria* with different values of photosynthetic and respiration quotients and respiration ratios of algae, coral and animal, CZAR could range from 41% - 136% and 36% - 180% respectively. Hoegh-Guldberg *et al.* (1986) reported potential seasonal differences in the contribution of zooxanthellae to the host, *Pteraeolidia ianthina* (Nudibranchia) and found that high densities of symbionts contributed 79%, 121% and 173% to the host in winter, spring and summer respectively. Muscatine *et al.* (1984) reported a higher CZAR in light-adapted *Stylophora pistillata* (143%) compared with shaded colonies (58%). Similarly, McCloskey and Muscatine (1984) showed that CZAR in *S. pistillata* was 78% at 35 m and 157% at 3 m depth.

The CZAR for zooxanthellae in giant clams also depends on the percentage translocation by zooxanthellae. Using the value of 40% and 95% translocation, the mean values of CZAR of *Tridacna gigas* were 83% and 197%, respectively (Fisher *et al.,* 1985). Moreover, CZAR values in *Hippopus hippopus* were reported to vary between 7% and 137% depending on the photosynthetic and respiratory rates and the percentage translocation (assumed to be 40% and 98%, respectively; Fitt *et al.,* 1985). When the percentage translocation value of 95% is used, the CZAR values are likely above 100% for *T. gigas* (Fisher *et al.,* 1985; Mingoa, 1988; Klumpp *et al.,* 1992), and *T. derasa* and *T. tevoroa* (Klumpp and Lucas, 1994).

Trench *et al.* (1981) showed that the zooxanthellae from *Tridacna maxima* can contribute more than 50% of the clam's respiratory carbon requirements, which range from 62% up to 84% (assuming 40% translocation) on cloudy versus sunny days respectively. The importance of the availability of light was

also reported by Mingoa (1988) who found that the CZAR value of shade-reared juveniles of *T. gigas* (mean value of 91.9%) was significantly higher than the CZAR from unshaded clams (mean value of 72.9%). The lower value of CZAR from unshaded clams can be explained by the lower PR ratio, indicating less fixed carbon produced and available to the host. Mingoa (1988) also found that the value of photosynthetic efficiency (a) and maximum photosynthetic rate (Pmax) from shade-reared clams were significantly lower than those of unshaded clams.

The phototrophic and heterotrophic contributions, through the translocation of photosynthate by zooxanthellae and filter feeding, respectively, to the nutrition of giant clams have been investigated by Klumpp *et al.,* (1992), Klumpp and Griffiths (1994) and Klumpp and Lucas (1994). These contributions are size- and species-dependent. Filter feeding becomes less important with increasing size of the clams. For example, filter feeding provides 65 % and 35 % of total carbon needed by small and large *Tridacna gigas* respectively (Klumpp *et al.,* 1992). In all species the value of CZAR increases with increasing the size of the clams (Klumpp and Griffiths, 1994). These authors concluded that phototrophy is an important source of energy to the host for any size and species of giant clams.

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