

REVIEW: Symbiosis between the Giant Clams (*Bivalvia: Cardiidae*) and Zooxanthellae (*Dinophyceae*)

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ABSTRACT

Giant clams are the largest bivalves in the world that maintain a mutual relationship with zooxanthellae. Individual giant clam can harbor heterogeneous zooxanthellae, at least four taxa in genus *Symbiodinium*. The *Symbiodinium* lives in the zooxanthellal tubular system, a tube structure arising from one of the diverticular duct of the clam's stomach. Since the numbers of zooxanthellae is the one of some significant factors contributing to the clams growth and survival, the giant clams need to adjust the number of zooxanthellae for physiological reason with unclear mechanism. The important role of the symbiotic relationship to the clams can be seen on the survival, growth and nutrition of the clams. There are at least two significant factors determining the symbiosis, i.e. water temperature in related with level of light intensities and ammonium-phosphate rate. Some topic is still unclear, i.e. the determination of species in genus *Symbiodinium*, the mechanism for adjusting the population numbers of the algae and what kind of environmental factors determining the symbiosis. Thereby further research is still needed to clarify those missing.

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Key words: giant clams, zooxanthellae, *Symbiodinium*, symbiosis.

INTRODUCTION

There are many scientific interesting phenomenon occurs in marine ecosystems. Some of them are biota which maintains symbiotic associations with phototrophic dinoflagellates, commonly called zooxanthellae. The symbiotic dinoflagellates are the symbionts with a variety of marine biota as the hosts, included are the reef-building scleractinian corals, radiolarians, flatworms, jellyfish (Hackett et al., 2004), many alcyonarians, soritid foraminiferas, and a few bivalves (Maruyama et al., 1998; Lee et al., 2005). The giant clams (tridacnid bivalves) are also known as the host of symbiotic associations with certain zooxanthellae. They are different from most other bivalves, in that they contain large numbers of zooxanthellae, *Symbiodinium* (Carlos et al., 2000). It is the member of the class Dinophyceae, a group of single cell-protist (Coffroth and Santos, 2005).

Giant clams (*Cardiidae*, subfamily: *Tridacninae*) are the largest bivalves mollusks in the world that included on the IUCN red list of threatened species today (IUCN, 2007). Their most characteristic feature is the enlarged, upwardly directed and usually brightly colored mantle (Figure 1.), which is packed with zooxanthellae (Griffiths and Klumpp, 1996). They are presently restricted in association with coral reefs throughout the tropical Indo-Pacific region, with the center of diversity in the Indo-Malayan region (Lucas, 1988). Since their phototrophic characteristic, giant clams usually found in shallower habitats (1-20 m) (Chambers,

2007), except *Tridacna tevoroa* which inhabits in relatively deep waters (Klumpp and Lucas, 1994).

There are nine described species of the living clams, included only two genera, *Tridacna* and *Hippopus*, with seven and two species belong to the genera, respectively. Seven species were distributed in Indonesian waters, i.e. *Tridacna squamosa*, *T. gigas*, *T. derasa*, *T. crocea*, *T. maxima*, *Hippopus hippopus* and *H. porcellanus* (Newman and Gomez, 2002). Recently phylogenetic research have supported reduction in status of the *Tridacnidae* to a subfamily of the *Cardiidae* (*Tridacninae*), as suggested by recent cladistic analyses based on shell, anatomical, molecular characters, especially DNA gene sequences (Keys and Healey, 2000; Schneider and Foighil, 1999).

The symbiotic relationship have become attention many researchers since its important role in supporting the clam's life while stock depletion on the wild population of giant clam still appear today due to over-fishing and anthropogenic impacts (Ellis, 1999; Kinch, 2002) as well as natural process (Gomez and Mingo-Licuanan, 1998; Blidberg, 2004). It could make the clams more vulnerable if the relationship between host and algae is damaged (Blidberg et al., 2002). In many cases, mass mortality on giant clams population significantly caused by loss of zooxanthellae, a phenomenon called bleaching (Gomez and Mingo-Licuanan, 1998; Leggat et al., 2003). The acquisition of zooxanthellae is important factor which significantly determined the survival and growth of the juvenile (Fitt and Trench, 1981; Latama et al., 2001). This paper summarizes on what is known about the zooxanthellae, how the symbiosis does occurs, describes role of the symbiosis to the host and the environmental factors determining the symbiosis. Finally, it concludes the direction for future research on the giant clams-zooxanthellae symbiosis.

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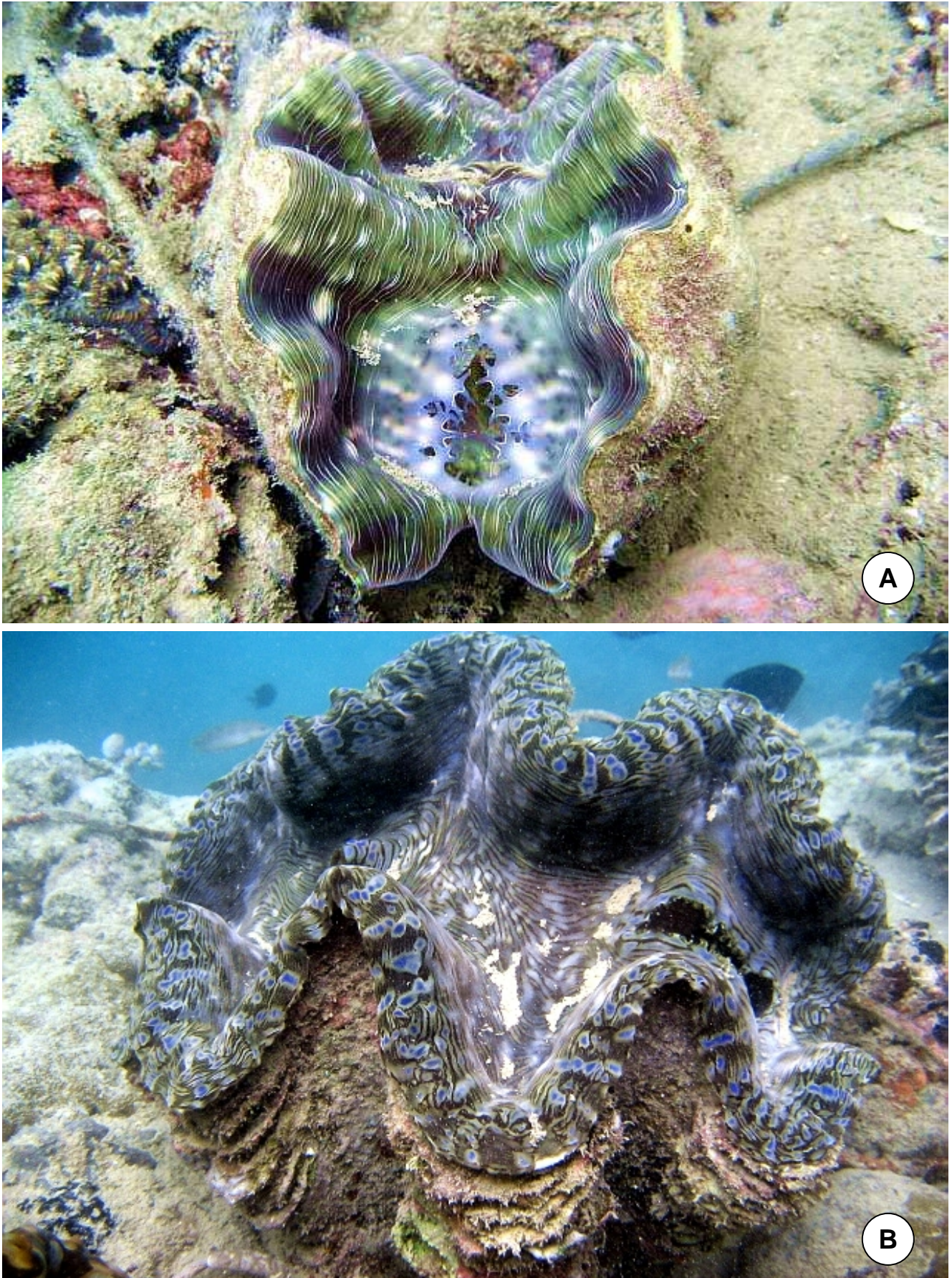


Figure 1. Giant clams from Kei waters Southeast Moluccas with brightly colored mantle. A. *Hippopus hippopus*, B. *Tridacna squamosa* (Photo: U.E. Hernawan).

DIVERSITY OF CLAM'S ZOOXANTHELLAE

In historical perspective, all species of giant clams harbor the zooxanthellae symbiont initially identified as *Symbiodinium microadriaticum* Freudenthal within their mantle tissue (Taylor, 1969; Kinzie and Chee, 1979; Fitt et al., 1986). At the moment, traditional biological methods have not provided useful information for identification and classification, though zooxanthellae have traditionally been difficult to classify. Thereby, little is known about the natural history of this symbiont (Rowan and Powers, 1991a). The poor status of zooxanthellae taxonomy in turn limits the study of the ecology and evolution of zooxanthellae (Rowan and Powers, 1991b). For that reason, further researches have been done to reveal the classification scheme of *Symbiodinium*.

Recent molecular genetic methods have solved many of the difficulties in zooxanthellae classification. Tremendous levels of diversity within the genus *Symbiodinium* have revealed using those methods. Numerous studies have shown that the genus is comprised of a group of diverse taxa (Carlos et al., 1999). Experimental evidence, however including behavioral, infectivity, physiological and ultrastructural, subsequently challenged the traditionally view about genus *Symbiodinium* (Coffroth and Santos, 2005). So, the formal description of the genus

Symbiodinium, which describes the in situ symbionts, should be revised (Wakefield et al., 2000).

The findings from subsequently studies by Rowan and Powers (1991a), Carlos et al. (1999), LaJeunesse and Trench (2000), Pochon et al. (2001, 2004), and Takabayashi et al. (2004) have led to the development of a new classification scheme for genus *Symbiodinium* that divides the genus into several large group, called clades, i.e. *Symbiodinium* clade A, B, C, D, E, F, G, and the last clade H. Recently, Hunter et al. (2007) has shown phylogenetic relationship between the major clades of *Symbiodinium* with the other eukaryotes and free living dinoflagellates using Internal Transcribed Spacer region 2 (ITS2) of nrDNA operon as the genetic marker (Figure 2.).

The traditional view of zooxanthellae-invertebrate symbioses, particularly on giant clams, suggests that one host species harbors taxonomically homogeneous symbiont population (one host-one symbiont). In fact, recently molecular studies have altered that view. Individual giant clams can maintain a symbiotic relationship with heterogeneous *Symbiodinium*, at least 4 zooxanthellae taxa (Carlos et al., 2000). Preliminary molecular genetic study using RAPD patterns demonstrated that giant clams associate with a large number of genetically distinguishable algal symbionts (Baillie et al., 2000b). Phylogenetic study using small-subunit rRNA has shown that there were

Symbiodinium clade A and C isolated from six species of giant clams (Carlos et al., 1999). Baillie et al. (2000a) has shown the same finding using allozyme and RAPD patterns. The last finding, *Symbiodinium* clade D has been isolated from giant clam, i.e. *Tridacna crocea* (Ishikura et al., 2004). Recently, isolation by Lee et al. (2005) characterized 4 symbionts, i.e. *Symbiodinium*, *Aphidinium*, *Tetraselmis*, and unknown chlorophyte.

However, the determination of species in genus *Symbiodinium* still not yet been specified. There is one question remains unresolved: which molecule(s) differentiates a species within this genus? So, the direction for future research within that genus is to solve the "species problem".

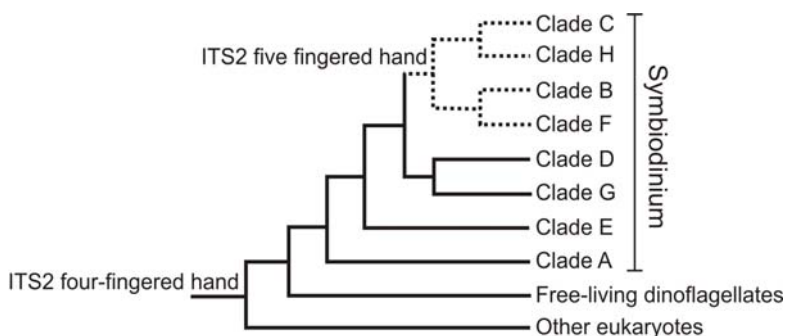


Figure 2. The phylogenetic relationship between the major clades of *Symbiodinium*. The dashed lines leading to *Symbiodinium* clade C, H, B and F represent these clades possessing the secondary structure of ITS2, the five-fingered hand structure (Hunter et al., 2007).

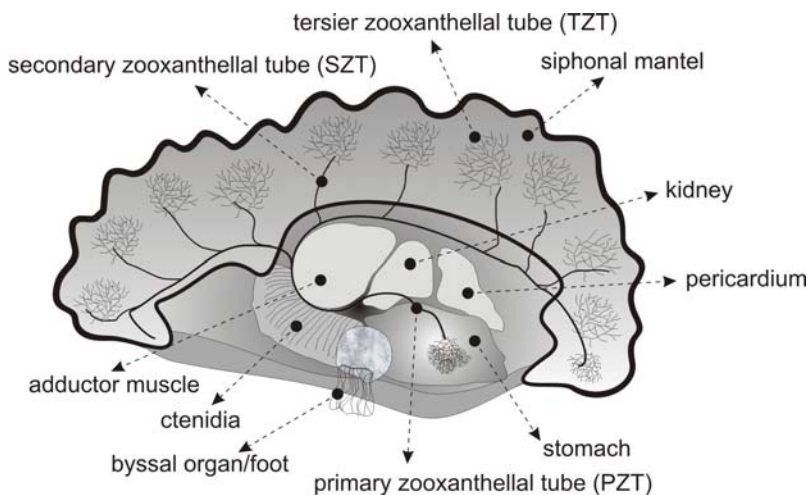


Figure 3. Diagrammatic view on the medial side of the zooxanthellal tubular system (Norton et al., 1992).

THE ZOOXANTHELLAL TUBULAR SYSTEM

Subsequently studies by Fitt and Trench (1981) and Fitt et al. (1986) have shown the zooxanthellae not inherited from parent to offspring. Neither eggs nor sperm released from adult tridacnids contain zooxanthellae and so after fertilization, the symbiotic algae were not present in either the fertilized eggs or trocophore stages. It implied that each generation must acquire their complement of symbionts from the environment. This pattern is called horizontal ("open" system) transmission (Coffroth and Santos, 2005). The acquisition of zooxanthellae by larvae of the clams takes place after the trocophore stage. Initial uptake of zooxanthellae is through the mouth and move into the stomach.

For many years before 1992, the zooxanthellae have been assumed to pass and live in the haemal sinuses of the hypertrophied siphon after ingested and move into the stomach (Fankboner, 1971; Fitt and Trench, 1981; Trench et al., 1981; Fitt et al., 1986). But in the next, Norton et al. (1992) has found that the symbiotic algae live in a tubular structure which no direct connection with the haemolymph. In fact, the existence of this tubular structure within clam's body has been indicated by Mansour (1946). His observation partially indicated that there was a tubular system arising from the clam's stomach, extending into the mantle and containing large number of zooxanthellae.

Through an anatomical and histological study, Norton et al. (1992) has revealed that the symbiotic relationship take place in a tubular system indicated by Mansour (1946), namely zooxanthellal tubular system (Figure 3). One of the digestive diverticular ducts of the stomach develops to be a single primary tube, known as primary zooxanthellal tube (PZT). It passes posteriorly to the dorsal side of the digestive organ. Above the organ, this tube divides into left tube and right one. Each tube travels through the kidney to enter the root of the middle ctenidial suspensory ligament and rounds the adductor muscle wall on posteriorly direction. On the posterior side of the adductor muscle, the tube runs dorsally into the root of siphonal mantle, before branching both anteriorly and posteriorly. Each branch travels inside the root of siphonal mantle and gives off branches, namely secondary zooxanthellal tube (SZT). It travels to the upper level of the inner fold of siphonal mantle and gives off many thin branches, namely tertiary zooxanthellal tube (TZT). The TZT terminates in a convolution with blind ends. The other secondary tubes form tertiary tubes not only in the siphonal mantle but also in other organs, such as in the connective tissue of the bulbus arteriosus of the heart, the pericardium, the ctenidia, and the lateral mantle (Norton et al., 1992).

The tertiary tubes contain the large numbers of zooxanthellae. In bleaching giant clam, mantle lacking zooxanthellae, the tertiary zooxanthellal tube atrophy. No zooxanthellae were observed living free in the hemal sinuses since the anatomical and histological evidence showed that the zooxanthellal tubular system has no connection with the haemolymphatic system (Norton et al., 1992). The morphological pattern is similar to the other member of Cardiidae, *Corculum cardissa* that also known has a tubular system that was seen in tridacnid clams. The zooxanthellae occurred intercellularly within the tertiary tube cell and separated from the haemolymphatic system by a tissue that is one cell layer thick (Farmer et al., 2001). This finding has explained and resolved the confusing discussion about the location and fate of symbiosis between the clam and zooxanthellae.

The existence of the zooxanthellal tubular system has also simplified the view on the way of bleaching. In response to the environment condition, i.e. increasing environmental temperature, the algae leave the clam by unknown mechanism, move from the tertiary tubule to the stomach, pass through the intestine and are released within feces via rectum (Norton et al., 1992).

POPULATION DYNAMICS OF ZOOXANTHELLAE

The numbers of zooxanthellae have been considered as the one of some significant factors contributing to the clams growth and survival. The clams lacking large number of zooxanthellae were known possessed significantly

decrease in fitness, resulting reduced growth, fecundity and survival (Leggat et al., 2003). On that relevance, further studies about population of zooxanthellae are suggested to focus on the relationship between the zooxanthellae population and various physiological factors in giant clams, such as nutrition and growth performance, rates of filter feeding, respiration, and photosynthetic responses.

The population number of zooxanthellae related with allometric parameters, such as projected mantle area and body size, is believed as the best indication of the relative contribution of phototropic production. Griffiths and Klumpp (1996) showed logarithmic increases in total zooxanthellae numbers with increasing clam length, for *Tridacna squamosa*, *T. gigas*, *T. derasa*, *T. crocea*, and *H. hippopus*. At the small body size (2 cm length), the zooxanthellae numbers were much lower in *T. squamosa* and *T. gigas* than *H. hippopus*, *T. crocea* and *T. derasa*. But by 30 cm, *T. gigas* and *T. squamosa* had the largest zooxanthellae population, indeed reflected high relative photosynthetic rates.

Maruyama and Heslinga (1997) have studied the daily population dynamics of zooxanthellae living in the mantle of a giant clam. Their population number and Mitotic Index (MI) were calculated by counting the numbers of the zooxanthellae in or not in the cell division stage. The MI increased after sunset and reached the maximum at 03.00 to 05.00 hours. There were population dynamics of zooxanthellae daily took place on the giant clam. Increasing number of zooxanthellae population correlated with the growth of the clam (mantle and shell) and followed with the number of zooxanthellae discharged in the feces.

The giant clams need to adjust the number of zooxanthellae for physiological reason, in response to ambient condition. To date, mechanism adjusting the population number is still unclear. Initially, Fankboner (1971) hypothesized that the zooxanthellae were selectively culled from the population in the mantle edge by amoebocytes and are intracellularly digested via amoebocyte lysosomes both in the circulatory system and the interdiverticular spaces of the digestive gland. This process considered as "the low systematic removal and utilization of degenerate zooxanthellae".

As has been stated before, there is no enough evidence supporting hypothesis proposed by Fankboner (1971). According to the hypothesis, there should no healthy zooxanthellae discharged in the feces. In contrast, Trench et al. (1981) showed that many of defecated zooxanthellae were morphologically intact and photosynthetically functional. Even Maruyama and Heslinga (1997) estimated number of the fecal zooxanthellae was up to 1.46% of the population in the mantle. About 64 to 89% of the newly formed zooxanthellae from the population were missing and still unclear. Further research is needed to clarify this missing.

ROLE OF THE SYMBIOSIS TO THE HOST

The important role of the symbiotic relationship to the clams could be seen on the survival, growth and nutrition of the clams. Initial observation on the contribution of the zooxanthellae to the survival and growth of the clams has done by Fitt and Trench (1981). The study showed survival and growth of larvae with zooxanthellae was greater than those without zooxanthellae. Juveniles with zooxanthellae can survive with light as the sole energy source for over 10 months. The presence of zooxanthellae in the stomach of the veligers was assumed to increase the survival and growth of the larvae after metamorphosis (Fitt et al., 1986).

Subsequently, Molea and Munro (1994), Belda-Baillie et al. (1999) and Latama et al. (2001) showed that different symbiont strains taken from various sources provided significantly different effect to the survival and growth of the tridacnid larvae. The larvae which had been supplied with zooxanthellae taken from the same host species (homologous zooxanthellae) more survive and growth faster than the larvae with zooxanthellae from different host species (heterogonous zooxanthellae). Freshly isolated zooxanthellae from the fast-grower (*T. gigas*) are recommended for routine use in giant clam hatchery operation.

On the nutritional aspect, the giant clams-zooxanthellae relationship is actually shown a mutualistic symbiosis. Giant clams as the host, provide a protected habitat for zooxanthellae. These symbiotic algae live in the mantle tissue, especially the zooxanthellal tubular system (Norton et al., 1992). Simply, the giant clams have an advantage on the nutritional aspect since the zooxanthellae supply energy to their host, mainly in the form of complex sugars (Fitt et al. 1986). The algae represent as the major source of metabolic carbon. An estimated 95% of the carbon fixed by these algal symbionts is translocated to the host. Those amounts are sufficient to fulfill at least the metabolic energy requirement (Klump et al., 1992; Klump and Griffiths, 1994; Hawkins and Klump, 1995).

The zooxanthellae also assimilated nitrogen excreted by host tissues. Nearly 100% of the nitrogen product subsequently released from zooxanthellae was incorporated in host tissues, with no significant loss from the clam over at least 10 days. Zooxanthellae therefore conserve and recycle essentially all nitrogenous end-products within the clams, affording giant clams a nutritional advantage over non-symbiotic bivalves (Hawkins and Klump, 1995). They can also produce amino acids and fatty acids, a portion of which are translocated through the algal cell wall directly into the blood circulation of the host (Ellis, 2003).

ENVIRONMENTAL FACTORS DETERMINING THE SYMBIOSIS

There are three environmental factors limiting the mutualistic relationship between the giant clams and zooxanthellae. The first factor is temperature and light intensities, especially elevated water temperature. Globally elevated sea water temperatures (28-34°C) are believed to lead a breakdown in the zooxanthellae photosynthetic apparatus, either in photosystem II (Warner et al., 1996) or the dark reactions of photosynthesis (Jones et al., 1998) and eventual expulsion of the algae from the host, a phenomenon called bleaching (Leggat et al., 2003). Gomez and Minguo-Licuanan (1998) have reported high mortalities phenomenon on giant clams during El-Nino. Seven species (*Tridacna gigas*, *T. derasa*, *T. squamosa*, *T. maxima*, *T. crocea*, *H. hippopus*, and *H. porcellanus*) have been reported bleached and died at the same time as unusually elevated water temperatures and coral bleaching during 1997-1998. The 1998 bleaching event was found to decrease the zooxanthellae population 30-fold when comparing bleached to non-bleached clams (Leggat et al., 2003).

There are physiological changes that occur in the giant clams influenced by elevated water temperature (Blidberg et al., 2002) since the photosynthesis of the zooxanthellae is impaired above 30°C and ceases completely at 34-36°C (Iglesias-Prieto et al., 1992). Buck et al. (2002) showed that increased light intensity and temperature of 4-6°C from the

ambient are the main causes for bleaching in giant clams. Thus, the study confirmed the four major aspects involved in bleaching: (i) loss of symbiotic algae, (ii) decrease of *chl a/c1* in the remaining symbiotic algae, (iii) retention of small zooxanthellae in the tissue, and (iv) release of ammonium (NH₄⁺) into the water column while nutrient uptake of ammonium was largely blocked.

The second factor is the ammonium (N) and phosphate (P) rate. While the zooxanthellae in giant clam are ammonium and phosphate-limited, the limiting factor is a function of the availability of ammonium and phosphate to the symbiosis (Belda et al., 1993). Nitrogen addition significantly changed the ultrastructure of the zooxanthellae inhabiting the clams (Ambaryanto and Hoegh-Guldberg, 1996). Exposure of the clams to elevated N (10 µm) increased zooxanthellae density, reduced zooxanthellae size, down-regulated N uptake by zooxanthellae freshly isolated from their hosts, and reduced glutamate in the clam haemolymph, with increased pools of some free amino acids (methionine, tyrosine) in the zooxanthellae. These results confirm that the zooxanthellae in giant clams are N-limited *in situ* and have free access to inorganic N from the sea water. There is also corroborating evidence that the zooxanthellae are P limited *in situ* as well, possibly due to host interference. While the N-P ratios of the animal host reflected ambient N and P concentrations in the sea water, those of the zooxanthellae did not (Belda-Baillie et al., 1998).

Of course, the elevated temperature, light intensities, ammonium and phosphate are not the only factor determining the symbiosis, which may also influenced by such factors as reduced salinity, marine pollution (Blidberg, 2004), parasitic infection (Shelley et al., 1988) and bacterial or virus infection, etc. Further studies are needed to understand the environmental limiting factors and stress tolerance in giant clam for restocking the wild population and providing useful information in conservation management.

CONCLUSION

The symbiosis between giant clams and zooxanthellae actually shows a mutualistic relationship. Many studies revealed what about the symbiosis, the host and the symbiont. There are three directions for future research: (i) the determination of species in genus *Symbiodinium* since the tremendous level of that genus, (ii) the population dynamics of zooxanthellae, focusing on the mechanism for adjusting the population numbers of the algae, and (iii) what kind of environmental factors determining the symbiosis.

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