SOME RECENT ADVANCES IN STUDIES ON THE BIOLOGY OF GIANT CLAMS (TRIDACNIDAE)

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ABSTRACT

During the past 15 years an increasing number of publications on the biology of *Tridacna* have appeared in international journals. There are two reasons for this: firstly, the development of mariculture to counteract the heavy exploitation of natural populations and secondly, the uniqueness of these clams which depend on symbiosis with the zooxanthella *Symbiodinium microadriaticum*. Earlier studies focused on evolutionary explanations of the extraordinary size of giant clams and the function of the symbiotic relationship with zooxanthellae. Major advances have been made in the understanding of the symbiotic relationship and the nutrient requirements. Ecological studies are rare and the knowledge of reproductive- and population ecology is still fragmentary. The aim of this paper is to review some of the recent advances in the biology of giant clams and to point out areas where research may be conducted.

INTRODUCTION

The study of the biology of giant clams has attracted the interest of many biologists during the past 15 years and the number of publications has increased significantly especially in mariculture related aspects (*see* Vega 1990). Most authors emphasize the uniqueness of these eight species of clams belonging to the family Tridacnidae, their size and the symbiotic relationship with the dinoflagellate *Symbiodinium microadriaticum* Freudenthal, commonly known as zooxanthellae. The giant clams are confined geographically to the Indo-Pacific where they commonly inhabit coral-reef environments and many researchers express their concern over the extinction or rapidly diminishing clam populations mainly caused by exploitation or other anthropogenic activities (*see* Lucas 1988). In earlier zoological studies the immediate questions asked were "How can a mussel obtain such a large size?", and "What is the function of the symbiotic relationship?" (Yonge 1975, 1980) but the apparent simplicity of these questions is elusive and an increasing underlying complexity has been revealed. In spite of intensive research over the last decade these questions cannot be answered fully today although major advances have been made. The increasing and devastating exploitation of the clam populations has called for development of mariculture and extensive restocking programs. The development of mariculture techniques has been successful (Crawford *et al.* 1987) but our knowledge of the ecology of giant clams is at best fragmentary and may jeopardize efforts in population management and restocking programs.

The purpose of this paper is to review some of the recent advances in the biology of giant clams which have appeared in the major scientific journals as a complement to the Tropical Marine Mollusc Programme (TMMP) and to point out immediate areas where research is necessary.

The tridacnid clams are attractive species for mariculture since these molluscs become almost self-sustaining after metamorphosis and establishment of the symbiosis,

requiring only sunlight and warm, unpolluted sea water for growth. Recently, a status of the mariculture efforts has been presented by Crawford *et al.* (1987), Braley (1988, 1989), and Copeland & Lucas (1988). A substantial literature on mariculture of tridacnid clams has been published but only studies relevant to the general biology and ecology are chosen to be within the scope of this paper.

TAXONOMY

The taxonomy of giant clams has recently be reviewed by Lucas *et al.* (1991) who also described a recently discovered new species *Tridacna tevoroa* found exclusively in Fiji and Tonga waters of the tropical Pacific. According to Lucas *et al.* (1991) giant clams comprise eight species in two genera: *Tridacna* (six species) and *Hippopus* (two species). The taxonomy, species names and authors, and trivial names are shown in Table 1.

Based on the finding of the new species *T. tevoroa*, Lucas *et al.* (1991) revised the taxonomy of Rosewater (1965, 1982) and supplied a key to the tridacnid species. This key is shown in the appendix.

Kania *et al.* (1980) established a pattern of genetic relationship between six species of tridacnids using crude haemolymph samples analyzed in a two-dimensional electrophoretic technique using acrylamid gels. The objective was to provide so-called "haemolymph fingerprints" and estimate the genetic variation among individual species at a molecular level of the gene products present in the haemolymph. Not surprisingly, the haemolymph of *H. hippous* showed a different molecular weight distribution compared to other tridacnid haemolymphs, confirming the already taxonomic established distant relationship separating *Hippopus* and *Tridacna* at the genus level. The five species of the genus *Tridacna* showed a protein pattern of close resemblance between haemolymphs. Based on the haemolymph patterns, the authors established a line of relationship placing *H. hippopus* and *T. crocea* at the extreme ends. Next to *H. hippopus* was *T. gigas*, then *T. maxima*, *T. derasa*, *T. squamosa* and finally *T. crocea*. Comparing with the taxonomic diagnosis of Lucas *et al.* (1991) the patterns are not in perfect concordance, as *T. derasa* is taxonomically placed in the subgenus *Persikima* only relatively distantly related to *T. squamosa*, *T. maxima* and *T. crocea*. However, Kania *et al.* (1980) also found a haemolymph pattern in *T. crocea* with a threefold multiplication pattern in protein separation indicating interpretation problems about the origin of this difference relative to the rest of the species within the genus.

Table 1. The taxonomy, species names, authors and trivial names of giant clams, order Veneroida, superfamily Tridacnacea, family Tridacnidae.

Genus *Tridacna* Bruguiere, 1797 (6 species):
subgenus *Tridacna* s.s. Bruguiere, 1797
Tridacna gigas (Linnaeus, 1758);
subgenus *Chametrachea* Mörch, 1853
T. crocea Lamarck, 1819,
T. maxima (Röding, 1798),
T. squamosa Lamarck, 1819,
subgenus *Persikima* Iredale, 1937
T. derasa (Röding, 1798),
T. tevoroa Lucas, Ledua & Braley, 1991.

Genus *Hippopus* Lamarck, 1799 (2 species):
Hippopus hippopus (Linneaus, 1758),
H. porcellanus Rosewater, 1982.

Trivial names:
T. gigas: Giant Clam
T. crocea: Burrowing Clam
(Crocus Giant Clam)
T. maxima: Elongate Giant Clam
T. squamosa: Fluted Giant Clam
T. derasa: Great Clam
T. tevoroa: Devils Clam
H. hippopus: Horse's Hoof Clam
(Bear Paw Clam)
H. procellanus: China Clam

THE PHOTO-NUTRITIONAL SYSTEM

Iridophores of the giant clam

Giant clams have spectacular colourful siphonal tissues. The evolution of the protective pigmentation may have been a result of the hazards of exposure to harmful wavelengths or to ensure that the optimal light frequency will reach the zooxanthellae, but it may also provide patterns of camouflage, as suggested by Griffiths *et al.* (1992). Scattered all over the mantle but aggregated along the edge in rows of decreasing density with increasing distance from the edge, the so-called iridophores can be observed. The iridophores give rise to an almost infinite variety of brilliant colour patterns, especially in the smaller species. The iridophores consist of groups of cells (iridocytes) each containing a laminated body (iridosome) composed of regularly stacked flattened platelets, the iridosomal platelets. According to Griffiths *et al.* (1992), iridocytes are characteristically grouped together with 50-100 iridocytes in one region of the mantle. The iridocytes occupy the same region of the mantle as the photosynthetically active zooxanthellae, which are confined in the narrow tubular system anastomosized between the iridocytes. Griffiths *et al.* (1992) found single or small groups of iridocytes surrounded by zooxanthellae or, alternatively, one or a few zooxanthellae surrounded by a number of iridocytes. According to Kamishima (1990), two types of iridophores have been identified in molluscs. The first type, which has been observed in some opisthobranchs, contains small granular or vesicular organelles by which incident light is split. In giant clams, and in the heart clam (*Corculum cardissa*), a second type of iridophore has been observed. This type shows colour by reflection and interference effectuated by multilayered platelets arranged uniformly in the cytoplasm. A similar type of iridophore has also been observed in cephalopods (*see* Kamishima 1990). The spectacular thing about the iridophores of the giant clams is that they show a clear monochromatic colouration in various spectral ranges caused by multiple rows of reflecting platelets of uniform thickness. The platelets are arranged with high precision reflecting only a narrow range of the spectrum and thereby producing the unique and prominent colouration of the mantle. Only a few studies of iridophores of giant clams have been made. Recently, Kamishima (1990) studied the organization of the iridophores of *T. crocea* collected in Japanese waters. He found that iridophores platelets are 80 to 120 nm in thickness, differing from cell to cell, and the distance between individual platelets are kept constant by intervening cisterns, thereby forming a lattice aligned parallel in one direction. The reflecting body of the platelet is electron dense and probably of a paracrystalline protein structure, a finding which is in agreement with Griffiths *et al.* (1992). The distance between the reflecting masses of the platelets was estimated at 80 to 100 nm but constant within cells. Kamishima (1990) concluded that the various colouration of the giant clam mantle is determined by the variability of platelet distance between cells. If the optical path of the reflecting layer equals a quarter wavelength of the reflecting spectrum, which in giant clams is blue to red, the refractive index (n) is about 1.5. Griffiths *et al.* (1992), however, found that the platelets making up the iridosomal lamellae were uniform in thickness and in *T. gigas* measured 74.8±10.2 nm, in *H. hippopus* 71.1 ± 6.0 nm and in *T. crocea* 71.4 ± 9.86 nm which were more variable in breadth and length. In face view the platelets are flattened structures about 100-150 nm wide and over 1000 nm long with slightly rounded ends. The disagreement in the thickness measurements between Kamishima (1990) and Griffiths *et al.* (1992) may be due to different fixation procedures.

Since the refractive index of the material of the platelets in giant clam iridophores is not known, it is not possible to accurately determine the optical thickness of the layers. Griffiths *et al.* (1992) assumed a refractive index of 1.42 for the platelets of *G. gigas* and 1.33 for the interlamellar space. They found that first-order maximal interference would occur at a wavelength of approximately 411 nm (range 355.2 - 476.6 nm). Griffiths *et al.* (1992) concluded that at the lower end of the interference spectrum, the iridosomes of giant clams may contribute to protection against harmful irradiation in the ultraviolet-A (320-400 nm) range, enhancing the protection in the ultraviolet-B range (280-320 nm) provided by ultraviolet-absorbing compounds. Considering the possible shrinkage of the interlamellar space during tissue preparation, Griffiths *et al.* (1992) further concluded that the maximum interference probably will be into the blue and perhaps into the green part of the spectrum, consistent with the observed interference colours displayed by clams *in situ*. It is, however, not possible to determine the exact orientation of the platelets relative to the mantle surface in fixed tissues. Observations by Kamishima (1990) and Griffiths *et al.* (1992) showed a general orientation of the iridosomal lamellae of giant clams to be stacked with the broad face of the upper platelets parallel with the mantle surface, thereby obtaining maximum interference. This pattern is apparently not uniform and Griffiths *et al.* (1992) suggested that iridosomes may also function as diffraction gratings and the presence of well-developed muscle tissue in the mantle may effect optimum exposure of the zooxanthellae but also significantly control orientation of the iridosomes relative to the surface.

The symbiont zooxanthella

The zooxanthella symbiotic with tridacnids was described by Kawaguti (1944) as a *Gymnodinium*-like dinoflagellate and believed to be taxonomically and morphologically similar across the range of host animals. Freudenthal (1962) redescribed the zooxanthella as *Symbiodinium microadriaticum*, a classification adopted by recent workers. However, the question of whether a single species is found in all marine invertebrates has still not been satisfactorily investigated as there is some evidence that different strains of zooxanthellae obtained from different hosts differ in their ability to enhance growth when exchanged between species (*see* Kinzie & Chee 1979; Chang *et al.* 1983). In giant clams, zooxanthellae are found in numbers of 2 x 10^8 per gram *Tridacna* mantle. They may constitute from 3 to 14 % of the protein biomass (Muscatine 1980).

Acquisition of zooxanthellae

The zooxanthellae in tridacnids are most numerous in the hypertrophied siphonal tissues, but may also be found in the heart, stomach, digestive gland, and intestine (*see* Fitt & Trench 1981). The natural questions of interest are how the zooxanthellae are acquired and what are the methods of acquisition? Fitt & Trench (1981) reviewed previous studies and various hypotheses; they emphasized problems of whether the algae were transferred from the parent or obtained after metamorphosis. Several hypotheses have been advanced explaining the route of infection. These include "invasion through the mantle", and the more likely route through the digestive system. This latter hypothesis seemed plausible since a number of early studies have shown that algae pass unharmed through the digestive system and are extruded in faecal pellets. LaBarbera (1975), and Jameson (1976) have clearly shown that zooxanthella are not found associated with eggs or sperms released from tridacnids, and thus must be acquired from the environment. Fitt & Trench (1981) found that *S. microadriaticum* was obtained during the veliger stage by larval feeding and transferred to the siphonal tissues after metamorphosis by an unknown mechanism. They concluded that primarily motile zooxanthellae are ingested

and that growth of veligers and juveniles was greater with zooxanthellae than without. The symbiont algae were observed in the siphonal tissue one to three weeks after ingestion. Fitt *et al.* (1986), studied veliger larvae and juveniles of *T. gigas* and *H. hippopus* cultured with different strains of zooxanthellae and found that freshly isolated zooxanthellae can contribute to the nutrition of tridacnid larvae thereby increasing growth and survival. The presence of zooxanthellae was not a prerequisite for growth, survival or metamorphosis of larvae in contrast to newly metamorphosed, juvenile and adult clams (Fitt *et al.* 1986).

Site of zooxanthellae

Based on the work of Yonge (1936, 1953) it was previously believed that the zooxanthellae were maintained intra-cellularly in blood amoebocytes located in the haemal sinuses of the siphonal tissues and periodically digested after transport to the viscera. It was further hypothesized that the indigestible remains were accumulated as concretions in the enlarged kidney (*see* Trench *et al.* 1981a). However, some authors have argued that the zooxanthellae were found intercellularly (*see* Trench *et al.* 1981a for review). Trench *et al.* (1981b), in a light- and electron microscopic study, showed that the zooxanthellae indeed were found intercellularly and lie free in the haemal sinuses. They were also unable to support the view that zooxanthellae were digested by amoebocytes. The observed pycnotic zooxanthellae in the siphonal tissues which have previously been interpreted as evidence of digestion, were suggested to be a result of autolytic degradation (Trench *et al.* 1981b). This was supported by the observation that no evidence was found of amoebocytes or any other cell type in the haemal sinuses (Trench *et al.* (1981a). Furthermore, Trench *et al.* (1981a) were also unable to support the view that undigested remains were deposited in the kidneys, as no degraded zooxanthellar pigments were identified in kidney extracts. They found the kidney concretions to be phosphorite, which is common for other bivalves which do not harbour symbiotic algae (*see* also Reid *et al.* 1984b).

Pathways of zooxanthellae

The pathways of zooxanthellae in the giant clam was enigmatic for many years. Zooxanthellae are found in tridacnid faecal pellets, even when clams are kept in filtered sea water, and thus the excreted algae cannot originate from the surrounding environment but must have originated from the clam itself (Fankboner & Reid 1981). However, the mechanism by which the algae pass from the haemal sinuses to the alimentary tract was unknown until recently. It was previously known that zooxanthellae were not only confined to the siphonal tissues but were also observed in other tissues where blood circulates. A tubular system connecting the siphonal tissue with the stomach was partly described by Mansour (1946) but this was disputed by Yonge (1953) and not investigated further. However, Norton *et al.* (1992) convincingly confirmed the presence of an elaborate tubular system by which zooxanthellae are able to migrate between the stomach and the hypertrophied siphonal tissues. The entire branched tubular system originates as a single primary tube with one opening, arising from a digestive diverticular duct of the stomach, and is further divided into secondary and tertiary sections (Norton *et al.* 1992). The primary zooxanthellar tubes have an epithelial lining with ciliated cells, and are surrounded by muscle fibres. The secondary tubes are thin-walled with cilia but no outer muscle fibres are present, while the cells of tertiary tubes are similar to secondary tubes but lack cilia. The zooxanthellae are confined to the tertiary tubes which have blind ends and no communications with the haemolymph system. The blind ends prevent digestive enzymes from passing from the stomach to the haemolymph.

These findings answer a large number of unsolved questions and simplify explanations of how the symbiosis functions. The zooxanthellae are taken up by newly metamorphosed clams and through this pathway passed to the hypertrophied siphonal tissues aided by beating cilia. The mass expulsion of zooxanthellae from heat stressed clams (Fankboner & Reid 1981), may be effectuated through the tubular system by forceful contractions of the viscera and muscles. Whether the zooxanthellae in the adult clams are supplemented from outside sources by similar pathways is not clear. Zooxanthellae have been observed in the stomach and to pass through the intestine and rectum and subsequently released unharmed in the faeces (Fitt *et al.* 1986; Trench *et al.* 1981a). It is therefore not unlikely that zooxanthellae may both be acquired and expelled dynamically through the tubular system.

Photosynthesis

The majority of studies on the productivity and photosynthesis of zooxanthellae have been carried out *in vitro* under artificial conditions in incubation chambers. Effects of the interposition of animal cells in the clam obviously must constitute an important biological factor in zooxanthellae function which cannot be disregarded. Studies of productivity of zooxanthellae in symbiosis with giant clams must take into account not only the amount of oxygen produced and carbon fixed per unit time, but also the symbiotic interactions which influence the availability of algae-fixed carbon to the host.

Pigments

The photosynthetic pigments of zooxanthellae from five species of tridacnid clams have been characterized as identical to those of the dinoflagellate *Amphidinium sp.* These mainly consist of chlorophyll a and c in addition to a number of other pigments (*see* Muscatine 1980 for review). The ratio of chlorophyll a and c_2 varies between zooxanthellae from different hosts. An *in vitro* action spectrum for zooxanthellae isolated from *T. maxima* is given by Scott & Jitts (1977).

Scott & Jitts (1977) found that clam zooxanthellae were shade adapted, and zooxanthellae saturated at lower irradiance showed greater photosynthetic efficiency at subsaturation levels than do free-living phytoplankton. This phenomenon may be a result of the clam/symbiont interactions where zooxanthellae are densely packed in the tubular system of the mantle tissue. Zooxanthellae of larger clams are more shaded than those in smaller clams since there is an inverse relationship between size of clam (*T. gigas*) and photosynthetic oxygen production rates (Fisher *et al.* 1985). Furthermore, the number of zooxanthella per gram of clam tissue also decreases as a function of clam size. Fisher *et al.* (1985) found that the smallest clams showed light saturation of photosynthesis at an irradiance level of 600 $\mu E\ m^{-2}\ s^{-1}$ while the larger clams were not saturated even at 2000 $\mu E\ m^{-2}\ s^{-1}$. Apparently, light reaching the surface of the mantle of a clam must penetrate increasingly thicker tissue as a function of clam size. Additionally, the zooxanthellae themselves are stacked, resulting in variable light intensities reaching the individual zooxanthellae. The mantle of smaller clams is relatively thin, and shading effects are negligible. In larger clams, however, only about 25 % of the incident light reach the zooxanthellae on average. Fisher *et al.* (1985) concluded that at a light intensity of 500 $\mu E\ m^{-2}\ s^{-1}$, which is the intensity that a clam may experience on a cloudy day or in deeper water, the photosynthetic rate of the smallest clams is only reduced 4 % as compared to the rate at a light intensity of 1000 $\mu E\ m^{-2}\ s^{-1}$. Larger clams, however, may experience a reduction of 25 %. Light is probably a contributing limiting factor for how large giant clams can grow, if sufficient planktonic food is limited.

Assimilation of carbon

Under *in vitro* laboratory conditions, assimilation of zooxanthellae isolated from coral ranges from 1.0 to 3.9 mg C h^{-1} mg Chl a^{-1} (Muscatine 1980). These values fall within the normal range of one to ten for free-living phytoplankton. Carbon fixed by free-living phytoplankton is ultimately used for growth, but a small amount may be released as dissolved organic carbon. However, in symbiosis the zooxanthellae cannot grow at the maximum rates while a substantial part of the carbon is translocated and taken up by the host. The source of inorganic carbon is primarily the surrounding sea water where carbon dioxide is abundant, but the mechanism whereby zooxanthellae photosynthetically fix carbon dioxide is not clear (*see* Trench 1987). However, Yellowlees *et al.* (1993) recently showed that the photosynthesis rate of clams (*T. derasa*) varies as a function of the concentration of inorganic carbon, preferable as carbon dioxide, in the haemolymph. The haemolymph of the clam is in equilibrium with the surrounding sea water, and the gill tissue contains high levels of carbonic anhydrase which contribute to the regulation of inorganic carbon between the two media. Unlike free-living marine microalgae, the zooxanthellae showed low levels of carbonic anhydrase on the external cell membrane. The high enzyme activity was found in mantle extracts, indicating that this enzyme plays an important role in maintenance of the symbiosis. Yellowlees *et al.* (1993) suggested that the enzyme is probably associated with the zooxanthellae tubes in the mantle.

Assimilation of nutrients

Zooxanthellae cannot live on carbon alone, but require dissolved inorganic nutrients comparable to those of free-living dinoflagellates, notably ammonium, nitrate, and phosphate. In a study of the kinetics of nutrient uptake in zooxanthellae isolated from *T. crocea*, D'Elia *et al.* (1983) found that freshly isolated zooxanthellae exhibit nutrient-uptake kinetic responses similar to those of free-living marine microalgae. In a medium enriched with nitrate, nitrite or ammonium, light did not affect the uptake of any of the substrata tested. However, Wilkerson & Trench (1986) reported that light was necessary to sustain DIN (dissolved inorganic nitrate) uptake by the clam zooxanthellae in darkness which reduced ammonium uptake but had no effect on nitrate uptake (*see* also Fitt *et al.* 1993a). In their study, both clam and coral zooxanthellae showed a surge in the uptake of these nutrients with high uptake rates indicating that the zooxanthellae were nutrient-limited (*see* also Summons *et al.* 1986). Furthermore, Wilkerson & Trench (1986) found that ammonium repression of either nitrate uptake or reduction which occurs in many free-living algae, does not occur in zooxanthellae of *T. gigas*. They suggested that the zooxanthellar enzyme nitrate reductase may not be repressed in the presence of ammonium, but this is unlikely to be the case in coral zooxanthellae. However, Fitt *et al.* (1993a) found that NO_3 uptake was repressed in the presence of NH_3. When ammonium nitrate (50 µM) was added to a raceway with juvenile *T. derasa*, NH_3 was taken up at a higher rate than NO_3 and nitrate was depleted from the raceway only after the NH_3 concentration dropped below 2.5 µM. It is not clear, however, how nutrients are taken up by the clam host and transported to the zooxanthellae (*see* Fitt *et al.* 1993b). Most of the nutrients required by the clams are probably taken up from sea water through the gills and transported by simple diffusion to the zooxanthellae, because haemolymph levels of both ammonium and inorganic carbon are in balance with the sea water concentrations (*see* Belda *et al.* 1993a).

Nitrogen flux in *T. gigas* has been shown to shift with ontogeny (Fitt *et al.* 1993b). Larvae and newly metamorphosed juveniles with relatively few zooxanthellae release ammonium while other juveniles and adult clams, which contain high numbers of zooxanthellae, take up dissolved inorganic

nutrients. The uptake is highest in juveniles and decreases with increasing clam size. However, this phenomenon is not entirely correlated with zooxanthellae density but is suggested by Fitt *et al.* (1993b) to be due to a combination of both high zooxanthellae density and high rates of assimilation by those zooxanthellae living at high densities.

Release of photosynthates to the host

The evidence available for transfer of exudates from zooxanthellae to the clam host is surprisingly limited and primarily originates from studies of isolated cells. According to a review by Trench (1979), isolated zooxanthellae may release from 20 to 60 % of the total carbon fixed, mostly as glycerol but also as small amounts of glucose and alanine. However, by adopting a syringe method for taking *in vivo* haemolymph samples, Rees *et al.* (1993) found glucose and not glycerol to be the major *in vivo* release product of the clam zooxanthellae, which confirmed similar results obtained by Streamer *et al.* (1988) using radioactive labelling.

Control of zooxanthellae

The association between the clam host and the zooxanthellae is complex and in order to maximize the supply of photosynthate, the host clam must exercise some control over the zooxanthellae number. One way to control zooxanthellae number is to regulate the nutrient supply (*see* Cook & D'Elia 1987; Rees 1991). From a mariculture perspective it is important to maximize growth and performance of zooxanthellae, so an understanding of the regulating mechanism is important. Attempts to improve growth of clams by adding nutrients have been successful (*e.g.*, Wilkerson & Trench 1986; Solis *et al.* 1988; Hastie *et al.* 1992; Fitt *et al.* 1993b; Belda *et al.* 1993a). It is generally believed that increasing the nutrient supply causes an increase in the number of zooxanthellae in the host. However, Belda *et al.* (1993a) found that the amount of chlorophyll *a* per clam is directly related to zooxanthellae number but chlorophyll *a* per zooxanthella is inversely related to zooxanthellae number. Nevertheless, by increasing nutrient supply, the total chl *a* content in the clam tissues increases and consequently increases the photosynthetic capacity of the host-alga association (Hoegh-Guldberg & Smith 1989; Fitt *et al.* 1993b).

To elucidate the relative importance of nutrients, Belda *et al.* (1993a) investigated the effects of elevated nutrient levels on both clam and zooxanthellar biological parameters. Belda *et al.* (1993 a,b) found that inorganic nitrogen and phosphorus altered the calcification pattern of *T. gigas* and that these nutrients also affected the clam soft tissues by enhanced growth. Enhanced growth was obtained with added N or N+P but not P alone, and correlates directly with increase in shell length. The increase in number of zooxanthellae following N-supplement is evidence for N-limitation. However, the observed C:N ratio was not typical for N-deficient cells. Belda *et al.* (1993a) concluded that control of zooxanthellar growth may be regulated through P-limitation since zooxanthellae in clams can utilize increases in the availability of ammonium, while any increase in ambient phosphate is not accessible. However, zooxanthellae sustain a significantly higher mitotic index (MI = proportion of dividing zooxanthellae x 100) in the presence of a combination of both N and P. A summary of the responses of clam and zooxanthellae to nutrient supplement is shown by Belda *et al.* (1993a).

Energy budget

The relative contribution of photosynthates and filter feeding to the energy budget for smaller *T. gigas* clams (up to 206 mm) has been estimated by Klumpp *et al.* (1992). They found that respiration levels of *T. gigas* were typical of those for suspension-feeding bivalves. Previous light history had no effect on dark respiration, and thus daytime respiration rates were the same as those measured in darkness. The particles available for ingestion in coral reef environments

are relatively small (< 10μm). *T. gigas* cleared particles from 3 to 40 μm relative constantly at an efficiency of approximately 80 %. Klumpp *et al.* (1992) calculated that a 1-gram clam cleared the water at a rate of 1.85 $l\ h^{-1}$, and in the process ingested 4.3 mg C d^{-1}. The ingested particulate organic matter was absorbed at an efficiency of 54 %, resulting in an absorbed food intake of 2.32 mg C d^{-1} for a 1-gram clam. However, rates of uptake are size-dependent. Klumpp *et al.* (1992) found that absorbed particulate organic carbon potentially makes a significant contribution of about 65 % to the total carbon demand of small clams, but this value declines with size. Accordingly, photosynthates makes an increasing contribution with increasing clam size. Klumpp *et al.* (1992) concluded that other potential sources of carbon, such as uptake of dissolved organic matter, were not important for clams. Autotrophy is a vital component of clam nutrition, and young clams loose their zooxanthellae or die when kept in darkness (*e.g.*, Fisher *et al.* 1985). Klumpp *et al.* (1992) confirmed previous observations that zooxanthellae of giants clams are able to provide most, if not all, of the daily respiratory demand.

Regulation of growth of adult clams in the field seems to be controlled by the availability of light (*see* Lucas *et al.* 1989). Larval and juvenile clams, however, do not seem to be dependent on exudates from associated zooxanthellae, although evidence is available that translocation does occur (Fitt *et al. 1986*). Veliger larvae fed isolated zooxanthellae show higher growth and survival rates but the symbionts appear not to be required for growth, survival, and metamorphosis if adequate particulate food is available.

Feeding and digestion

The feeding behaviour of the giant clam *T. gigas* has a marked circadian rhythm consisting of a diurnal phase of active filtration and a nocturnal torpor which cues a gastric digestive rhythm. According to Reid *et al.* (1984a) and Morton (1978), *T. gigas* and *T. crocea* exhibit nocturnal inactivity. The inhalant and exhalant currents are weak or undetectable and no response to light and tactile stimuli is shown. Vigorous prodding produces only a slight and gradual adduction of the valves. During daylight hours, however, shadow effects, water turbulence and tactile stimuli causes rapid phasic adductions of the valves and the expulsion of large volumes of mantle water. Intracellular digestion does not exhibit a digestive cycle corresponding to the feeding cycle.

The function of the digestive system of *T. gigas* has been investigated by Reid *et al.* (1984b) who concluded that the relative proportions of the digestive system, with exception of the kidney, are typical of other bivalves. In the tridacnids, the kidneys form a single, prominent mass of tissue constituting up to 10 % of the wet weight of the soft tissue, which is considerable greater than in other bivalves (Yonge 1980). It is reasonable to assume that the enlarged kidney is somehow physiologically related to siphonal and mantle tissue functions, particularly to their role of zooxanthellae symbiosis. It has previously been suggested that the kidney is involved in digestion of degenerated zooxanthellae transported by means of amoebocytes. This view, however, has been challenged by Trench *et al.* (1981b) and seen in the light of the recent rediscovery of the zooxanthellar tubular system (Norton *et al.* 1992), this function seems less probable, because excess zooxanthellae are passed directly to the stomach where digestion may eventually occur. Other hypotheses, explaining the relatively large size of the kidney, have been advanced suggesting that kidney size is associated with the massive shell and the supply of shell components, or the regulation of pH of body fluids, or, alternatively, have some exceptional function related to nitrogen metabolism (Reid *et al.* 1984b). Nevertheless, the role of the kidney and the problem of its relatively large size remain unsolved and needs further study.

Growth

Growth of *Tridacna* clams can be measured as growth of shell and soft tissues. By increasing the area of the mantle exposed to sunlight and stimulating zooxanthellar performance by adding nutrients, the total production of exudates increases and may consequently enhance clam growth.

Growth enhancement can be achieved in juvenile clams by regular exposure to nutrient enriched sea water (*see* Solis *et al.* 1988; Wilkerson & Trench 1986). Hastie *et al.* (1992) treated juvenile *T. derasa* (mean length 27.55 mm) with nitrate and ammonium (DIN) enriched sea water (50 µM potassium nitrate and ammonium sulphate added compared to 0.1-0.3 µM nitrate, 0.2-0.5 µM ammonia and < 0.3 µM phosphate usually found in tropical reef environments; *see* Crossland 1983) in closed tanks during night but kept the juveniles in raceways with fresh sea water during daytime. The result after a 60 day trial was a mean length improvement of 17-21 % and a wet weight increase of 288-375 % compared to untreated controls. No obvious detrimental effect of DIN exposure was observed. Similarly, Fitt *et al.* (1993a) found that the individual growth rate of 2-3 mm long juvenile *T. derasa* increased about 60 to 75 % when 50 µM DIN was added to the sea water during a 10 day experiment. Phosphates seem not to have any effect on clam growth and clams exposed to phosphates alone (2 µM) had growth rates considerable lower than control clams with no added nutrients. These results confirm the observations by Wilkerson & Trench (1986) that zooxanthellae in field conditions are probably nutrient-limited. After the 60 day DIN-treatment, Hastie *et al.* (1992) transferred the clams to raceways with fresh sea water and observed growth for another 90 days. The transferred DIN-treated clams showed the same growth rates as non-treated clams but maintained their size advantage throughout the observation time. Hastie *et al.* (1992) also observed that after 15 days of treatment, the mantle of the DIN-treated clams became darker and appeared to be more vividly coloured, and that gape size was more pronounced, giving the clams an appearance of larger size. Hastie *et al.* (1992), did not perform any further investigations of the causes of variation in colour of the mantle. However, Fitt *et al.* (1993b) reported that juvenile *T. derasa,* which were DIN-treated for 10 days with 50 µM ammonium nitrate, doubled their zooxanthella density, and zooxanthella division rates were a third to two times higher than in control clams.

Belda *et al.* (1993b) found that inorganic nitrogen and phosphorous influenced the calcification pattern of *T. gigas*, and Belda *et al.* (1993a) found that nutrient concentrations also influenced the soft tissues. Addition of N or N+P, but not P alone, increased shell length. This finding is in concordance with the results of Fitt *et al.* (1993b) and Hastie *et al.* (1992) who studied juvenile growth as a function of nutrient enrichment. However, Belda *et al.* (1993b) found that by addition of N (ammonium), the shell-extension rate increased while shell-weight decreased due to a reduction in calcification. The reduction in calcium-carbonate deposition was found to be greatest at mixed concentrations of ammonium and phosphate and both of these nutrients appear to depress calcification in clams (Belda *et al.* 1993b).

ECOLOGY

Only a few papers on the ecology of giant clams have been published. The majority of these deals with distribution and abundance (stock assessments) on the Great Barrier Reef and focus mainly on the two largest species *T. gigas* and *T. derasa* (Braley 1987 a,b; Alder & Braley 1989; Pearson & Munro 1991).

Distribution and abundance

Large scale investigations of the distribution and abundance of *T. gigas* and *T. derasa*

have been carried out along the Great Barrier Reef by Braley (1987a) in order to make comparisons with previous stock assessments. Braley found that *T. gigas* has it southernmost limit at 19°S, while *T. derasa* were found at all localities along the reef, but with bimodal peaks in density in the northernmost and southernmost areas. The observed distribution patterns were suggested to be regulated by temperature for *T. gigas,* and by salinity or turbidity for *T. derasa*. The mean densities per hectare of clams were highly variable ranging between 236.6 ± 196.7 S.D. of *T. gigas* on high density reefs to 0.6 ± 1.4 S.D. on low density reefs. Similar data for *T. derasa* is 5.9 ± 7.4 and 2.9 ± 7.3 clams per hectare. Mortality (empty shells) was estimated to 10.4 % for *T. gigas* and 12.7% for *T. derasa*. These density data are within the range reported for *T. maxima* by Sims & Howard (1988) at surveys around Cook Islands (*see* also Copeland & Lucas 1988). However, large scale data has to be treated with caution and may not be particularly useful in calculating production, because the local environment and fishing pressures most likely differ between sites.

The small scale spatial distribution of adult *T. gigas* and *T. derasa*, and juvenile *H. hippopus* has been investigated by Braley (1987b). He found that *T. gigas* and *T. derasa* were significantly aggregated or clumped and occurred more frequently among branches of *Acropora* corals. However, the factors causing these patterns were not investigated. Braley (1987b) additionally tested substratum preference of juvenile *H. hippopus* and was able to reject the hypothesis that juvenile *H. hippopus* showed no preference for any of the offered substrata. The results showed that the majority of juveniles preferred *Acropora* corals. However, the significance of these experiments is unclear in explaining adult distribution since movements of transplanted juveniles may be secondary to actual settlement (*see* Bayne 1964).

Aggregation of the burrowing clam, *T. crocea*, has also been suggested as evidence of gregarious settlement by Hamner (1978), but patterns of adult distribution most likely reflect a combination of settlement and juvenile mortality. Hamner (1978) demonstrated patterns of intraspecific competition in patches of *T. crocea* occurring on coralline substratum, and found that competition was linearly related to clam density. Evidence of competition in this species is strongly visualized by shell damage and deformation caused by the boring activity (chemical and mechanical) of the clams which may cause up to 40 % mortality of neighbours. An original aggregated distribution of one-year old clams eventually became random due to intraspecific competition (Hamner 1978).

Giant clams are found at depths within the tidal range as proximity to the surface facilitates availability of light to the symbiotic zooxanthellae, and clams may consequently be exposed at low tides (*see* Fisher *et al.* 1985). Mingoa-Licuanan (1993) found in laboratory experiments that juvenile *T. gigas* exhibit physiological adaptation to emersion; clams showed aerial oxygen consumption and nitrogen conservation during emersion. Air-exposed juvenile clams lost only 5% of their body water after 27 hours exposure and mortality was generally found to be low. However, growth rates may depend on emersion time. Nash (1988) transplanted juvenile *T. gigas* (+1 and +2 year class) to platforms at different tidal levels (0, 50, 80, 105 and 120 cm above tidal datum) and monitored growth and mortality. He found highest mortality in the +1 clams at the highest levels (100 %), while the +2 clams experienced low mortality at all levels. Growth of the +1 clams was similar among all the survivors at the three levels, while +2 clams showed the fastest growth at the three lowest levels. Nash (1988) found that at low levels of emersion (<3.75 h/d), growth rates were positively correlated with temperature while at higher levels of emersion, growth rates and temperatures were

negatively correlated. These results are in concordance with Crawford *et al.* (1988), who showed that rearing of *T. gigas* on trays was most successful in the benthic intertidal compared to trays suspended at subsurface levels, in subtidal racks and those placed in the benthic subtidal (*see* also Lucas *et al.* 1989). Estacion & Braley (1988) also found high survival but slow growth of juvenile *T. gigas* reared in intertidal ponds, as did Barker *et al.* (1988) transplanting juvenile *T. gigas* to different reefs at different tidal levels. Barker *et al.* (1988) found an average growth rate which was 1.3 times faster at an intertidal site as compared to subtidal sites.

Growth

Growth in natural populations of *T. crocea* and *T. maxima* has been recorded by McMichael (1974), Hamner & Jones (1976), and more recently for *T. gigas* and *T. derasa* by Pearson & Munro (1991). In culture, initial growth rates of juveniles are low until a size of 20-40 mm in length is obtained, then the growth rates increase, probably due to increased zooxanthella performance. Growth then proceeds linearly but eventually growth rates diminish with increased adult size (see Hastie *et al.* 1992). Larger species show higher growth rates than smaller ones (see Lucas 1988). This pattern is in concordance with the observations of Pearson & Munro (1991) who studied populations of *T. gigas* and *T. derasa* on a 2.7 ha reef area on central Great Barrier Reef, but great variability among individuals was found. The authors compared empirically derived growth curves with calculated curves (Fabens and von Bertalanffy) and found a relatively poor fit for *T. gigas* but a good fit for *T. derasa,* especially for smaller animals. The maximum growth rate in shell length recorded for *T. gigas* was 8.2 cm per year and for *T. derasa* 4.52 cm per year. These rates were obtained in the size classes of 20.1 to 25 cm and 12-15 cm, respectively.

Mortality

Causes of mortality in natural populations are largely unknown, with the exception of fishery (*e.g.*, Pearson 1977) and intraspecific competition (*T. crocea*, Hamner 1978). A large number of invertebrates have been found to prey on juvenile *T. gigas* in ocean-nurseries on the Solomon Islands (Govan 1994). Gastropods of the genus *Cymatium* were the most voracious and abundant predators. At the same time they were most difficult to control. Other predators were muricid gastropods, decapods, and polyclad flatworms. Major pests were ectoparasitic opisthobranchiate snails (Pyramidellidae) and shell-boring sponges. Pyramidellidae have been reported as causing mortality among cultured clams, but have not been reported to be abundant in natural populations (Cumming 1988, 1993). The reported parasites and diseases have recently been reviewed by Humprey (1988). Mass mortality in populations of giant clams (*T. gigas* and *T. derasa*) has been reported by Alder & Braley (1989) where about 50 % of the populations have died over a two year period. The cause of mortality was related to the occurrence of a protozoan (*Perkinsus* spp.). Mortality in natural populations of *T. gigas* and *T. derasa* on the Great Barrier Reef has been reported by Braley (1987a) who reported mean annual mortality rates among adults of 1.65 ± 0.54 % for *T. gigas* and 2.07 ± 2.59 % for *T. derasa*.

Survivorship curves (survivorship as a function of size rather than age) of natural populations of *T. gigas* and *T. derasa* have been published by Pearson & Munro (1991), who monitored growth and mortality during a five year period at Michaelmas Reef, central Great Barrier Reef. These data are unique and show that *T. gigas* had an increasing survival rate until a size of about 40 cm after which survival was close to 100 %. A slight decrease in survival rate was observed at sizes larger than about 70 cm.

Similar estimates for *T. derasa* were also presented, but Pearson & Munro (1991) found these data inconclusive due to small sample sizes. However, mortality between years for both species was highly variable.

Reproduction

Recent studies of reproduction of tridacnid clams are few. A reason for this may be that a study using conventional techniques requires sampling of a representative number of animals at different intervals throughout at least one year (*see* Giese & Pearse 1974). Such sampling is not warranted for the larger *T. gigas* and *T. derasa* on the Great Barrier Reef because this would require destruction of the sampled specimens (Braley 1988).

Tridacnid clams are protandrous hermaphrodites; small individuals are predominantly males, but larger sizes become simultaneous hermaphrodites successively (Braley 1988; Nash *et al.* 1988). Dolgov (1992) studied populations of *T. squamosa* in the South China Sea and found that the relative proportion of the female gonad increases with increasing age. Clams at a weight of 0.1 to 1.8 kg gross body weight were found to be phenotypic males, while larger individuals of 0.9 to 9.2 kg were simultaneous hermaphrodites. Dolgov (1992) suggested that the reason why tridacnid clams have earlier been classified as either sequential or simultaneous hermaphrodites is due to difference in population structure among the studied populations. In old size-structured populations sequential hermaphrodism may prevail while in young populations of uniform size and age composition, functional simultaneous hermaphroditism prevails and the sequential pattern may not appear at all.

Shelley & Southgate (1988), determined the gonad index for *H. hippopus* and *T. crocea* by sampling 3 to 4, and 4 to 5 specimens monthly of each species, respectively. These species showed annual reproductive patterns most pronounced in *T. crocea* and less so in *H. hippopus* (Shelly & Southgate 1988). However, large variations between specimens were found and the reproductive season extended over several months, with maximum activity during the austral spring and early summer. Similarly, Nash *et al.* (1988) examined 129 *T. gigas* specimens histologically, and found evidence of a major spawning during the austral summer (January-March).

Natural spawning of sperm of small groups of *T. gigas* and a single *T. derasa* have been observed in the field by Braley (1984). In *T. gigas*, Braley observed diel periodicity and found spawning generally coincided with incoming tides near full and new moon phases. Sperm-spawning was observed to last for 30 minutes to two and a half hours with spawning contractions every 2-3 minutes. However, spawning of eggs was never observed. Additionally, Braley (1984), using an egg-catching device, reported natural spawning at Lizard Island occurring 3 to 7 days from the new moon in late October. The results indicated that spawning of eggs did not follow spawning of sperm. Nevertheless, the factors regulating spawning in the field are not clear and require further research.

Spawning may be induced in clams kept in cultures by injecting serotonin directly into the gonad or squirting gonad extract into the inhalant aperture (Crawford *et al.* 1986). Ripe clams respond by releasing sperm, and occasionally eggs after a short period (*see* also Fitt *et al.* 1984). According to Gwyther & Munro (1981) sperm are never observed to be spawned after egg release within a single individual. Braley (1988) found in an earlier study that egg release in the field does not necessarily follow sperm release, and suggests that specific environmental cues are probably necessary.

Through the use of a gonad biopsy technique (Braley 1988) the reproductive pattern of the two larger species *T. gigas* and *T. derasa* has been investigated in the field by Braley

(1988) at two sites on the Great Barrier Reef, by sampling the same individuals over a two year period. Previous data (Gwyther & Munro 1981) suggest that the reproductive condition is highly variable between individuals, species and populations. Braley (1988) confirmed this and found that a significant number of individuals were in a regressive stage of gonad development throughout the year, but that spawning predominantly occurred during summer.

Our knowledge of the reproductive ecology of tridacnid clams is fragmentary and it is evident that much ecological research is required to understand how the reproductive patterns regulate dispersal, distribution and abundance.

Development and larval ecology

Recent studies on fertilization and larval development in tridacnid clams have focused on the applications for mariculture, and emphasized questions regarding the induction of spawning and of larval survival as a function of density and food supply under laboratory conditions (*e.g.,* Crawford *et al.* 1986). Larval development in tridacnid clams seems to be fairly uniform among the investigated species, but variation in laboratory conditions between studies complicates comparisons. In general, fertilized eggs (approximately 100 µm in diameter) develops into trochophore larvae within 24 hours at about 28 °C. The proceeding development is somewhat variable depending on nutrition and larval density (*see* Fitt *et al.* 1984). After an additionally 24 hours, the veliger stage is reached and after one week the pediveliger stage may be reached. Metamorphosis takes place shortly thereafter. There is no evidence of feeding in the trochophore stage and feeding has been observed to commence in the veliger stage (Fitt *et al.* 1984). The acquisition of the zooxanthella *S. microadriaticum* occurs by feeding during the veliger stage. In spite of the availability to the tridacnid of numerous other phytoplankton species in the reef environment, only the dinoflagellate *S. microadriaticum* is ingested and subsequently establishes a symbiosis. The transition from the heterotrophic veliger stage to symbiotic juvenile and adult stages may take from a few days to several weeks (*see* Fitt & Trench 1981). Mortality during the larval stages and through metamorphosis in the laboratory has been found to be high. According to Fitt *et al.* (1984), less that 1 % of the spawned eggs survive larval development. However, Crawford *et al.* (1986) was able to obtain sur-vival rates to the pediveliger stage of 40 % and 26 % at larval densities of 2 ml^{-1} and 10 ml^{-1}, respectively, and suggested that the poor results of previous authors might be due to bacterial contamination of the cultures.

Larval behaviour at settlement and substratum preferences have not been investigated in detail. According to Fitt *et al.* (1984), after 9-14 days of development, pediveligers alternately crawl on the bottom of Petri dishes by extension and retraction of the foot, or swim just off the bottom. Gwyther & Munro (1981) tested larval substratum preference at settlement by placing a number of different substrata in the rearing tanks (wood, asbestos, sand, glass with alga film, shell, PVC). The results showed that the larvae were able to settle on all the offered substrata but less often on painted wood. However, since no rigorous statistical tests on larval preference were made and no information on larval age at settlement is available, interpretation and application to field conditions seems difficult. After metamorphosis juveniles attach themselves by byssus treads to the substratum.

Dispersal

Attempts have been made to describe the genetic variability and differentiation of giant clam populations in the Indo-Pacific and along the Great Barrier Reef. In a study of *T. gigas* along the Great Barrier Reef, the only place in the world with relatively large numbers of this species, Benzie & Williams (1992) found high levels of genetic variabil-

ity but no significant differentiation among populations. The genetic distance was calculated to D = 0.0007. Benzie & Williams (1992) concluded that the reef population is effectively one large panmictic population. Obviously, dispersal of larvae seems to occur effectively along the entire reef although larval life is only 10 days. Similarly, Cambell *et al.* (1975) studied populations of *T. maxima* from Heron Island, Great Barrier Reef, and from Enewetak Atoll (Marshall Islands; a nuclear test site) about 2,400 miles away, and found a high genetic variability and a high genetic similarity (D = 0.032). However, Macaranas *et al.* (1992) studied the genetic structure of *T. derasa* populations from Fiji, the Philippines and the Great Barrier Reef, and found large genetic differences. They estimated the genetic distance from 0.137 to 0.341 which is considerably higher than previously reported for *T. maxima* and *T. gigas*. The Great Barrier Reef populations were found to be separated from the Philippine population by mean genetic distances about 8 times greater and from the Fiji population about 15 times greater than among Great Barrier Reef populations. However, the genetic distance among the Great Barrier Reef populations was small (D = 0.013). The genetic differentiation among *T. derasa* populations was found to increase with increasing geographic separation and was thus consistent with an "isolation-by-distance model" (Macaranas *et al.* 1992). The genetic variability (mean heterozygosity) in all the studied populations was found to be high; in *T. derasa* about 40 %, in *T. gigas* and *T. maxima* about 20 %. A 20 % value is within the limits generally found in molluscs but the 40 % value reported for *T. derasa* is high and the reason for this is not clear. The conclusion seems to be that within populations, strong genetic mixing occurs over long distances even though local recruitment has been reported to be low and sporadic (Pearson & Munro 1991). In *T. derasa* the genetic differentiation among populations in the west Indo-Pacific may be explained by historical isolation of the Philippines from Australia which may have increased the genetic divergence (Macaranas *et al.* 1992). However, very little is known about larval dispersal and further studies of the factors regulating genetic variability is required.

Recruitment

Recruitment to populations of giant clams seems highly variable both in space and time. Based on an analysis of the population size structure, Pearson & Munro (1991) found that recruitment was low for populations of *T. gigas* and *T. derasa* on the Great Barrier Reef. Individual growth rates were also variable and they concluded that it was possible that the majority of the individual *T. gigas* were all derived from a single spawning season. Braley (1988) surveyed four sites on the Great Barrier Reef and also found low recruitment. However, during one particular period (1987), recruitment of *T. gigas* was extraordinary high but exhibited large variations between sites.

CONCLUSIONS

The major efforts in studies of the biology of giant clams have been directed towards development of mariculture with the purpose of optimizing growth. This has resulted in a relatively large number of papers which focus on the photo-nutritional system and emphasize effects of nutrients on growth. Our understanding of the reproductive ecology of giant clams is fragmentary and factors regulating gonad development and subsequent spawning require further studies. Factors which regulate larval dispersal, settlement and adult distribution and abundance are largely unknown. An understanding of the ecology of giant clams is important if restocking programs are to be successful and is a prerequisite if management of populations and their coral reef environments are to be carried out effectively to prevent destructive exploitation and species extinction.

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APPENDIX 1
Key to the Tridacnid species

1 Byssal orifice region of opposed valves with interlocking teeth; distinct region of shell around byssal orifice, outlined by ventral-most pair of prominent radial ribs; mantle, when fully extended, not projecting laterally beyond shell margins..... *Hippopus*... 2
- Byssal orifice of opposed valves without interlocking teeth; no distinct ventral region of shell outlined by prominent radial ribs; mantle, when fully extended, usually projecting laterally beyond shell margins..... *Tridacna*.. 3

2(1) Shells thick and strongly ribbed, with reddish blotches in irregular bands; incurrent apertures without guard tentacles..... *H. hippopus*
- Shells in specimens less than about 200 mm shell length not thick nor strongly ribbed and with only faint reddish blotches; incurrent apertures with guard tentacles..... *H. porcellanus*

3(1) Shell length of large specimens >550 mm, sometimes greater then 1 m; with about four elongate, interdigitating projections of each distal shell margin, being most elongate and acute in large specimens; shell without scutes, except for some tubular projections near umbo in very small juveniles; mantle brownish, with numerous iridescent blue-green circles..... *T. gigas*
- Shell length rarely >550 mm; without elongate, interdigitating projections on each distal shell margin; mantle variably coloured, without iridescent blue-green cirles..... 4

4(3) Shell length up to 550 mm, occasionally larger; upper region of large shells plain, without scutes or strong ribs; hinge usually longer than half shell length..... 5
- Shell length usually <400 mm; upper shell region with scutes or eroded scutes; hinge equal to or less than half shell length..... 6

5(4) Rib-like radial folds on shell without coloured patches; mantle without protuberances; incurrent aperture with inconspicuous guard tentacles..... *T. derasa*
- Rib-like radial folds on shell usually striped with coloured patches near umbo; mantle with protuberances; incurrent aperture with conspicuous guard tentacles..... *T. tevoroa*

6(4) Shell approximately symmetrical about umbo in lateral view, with hinge about half shell length; scutes large and well-spaced both within and between radialrows; lateral distance between scutes in adjacent rows usually about the same as scutes width; byssal aperture narrow to moderately wide; not embedded into substrate; mantle usually of subdue and mottled collar; incurrent aperture with distinct guard tentacles..... *T. squamosa*
- Shells usually asymmetrical about umbo in lateral view, with hinge less than half shell length; scutes usually low and often eroded, set close together both within radial rows and between rows; byssal aperture moderately wide to wide; embedded or partly embedded into substrate; mantle brightly coloured; incurrent aperture with indistinct guard tentacles..... 7

7(6) Shell length <150 mm; shells not strongly asymmetrical about umbo in lateral view; byssal aperture wide; scutes eroded away except near shell margin; occurs deeply embedded in reef substrate..... *T. crocea*
- Shell length of large specimens often >150 mm; shell often strongly asymmetrical about umbo in lateral view; byssal aperture moderately wide to wide; scutes present in substantial part of upper shell region; occurs partially embedded in reef ubstrate..... *T. maxima*