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Biology and Mariculture of Giant Clams

A workshop held in conjunction with the 7th International Coral Reef Symposium, 21–26 June 1992, Guam, USA

Editor: **William K. Fitt**

Contents

Introduction

FIFTY years ago in Palau, Wada (1942) described the hermaphroditic nature of reproduction in giant clams, followed by descriptions of spawning and methods of inducing spawning (Wada 1954). These observations set the stage for LaBarbera (1975) and Jameson (1976), who successfully raised larvae of tridacnids through metamorphosis. Once these major hurdles in raising giant clams were overcome, the door was open for the development of a mariculture industry, centred around the only cultured animal species not requiring additions of food. The first group through that door included Nancy Beckvar, Rick Braley and Jerry Heslinga, all working in the late 1970s at the Micronesian Mariculture Demonstration Centre (MMDC) in Palau, then directed by Bill Hamner. The historic clams spawned by this group are almost 15 years old today, and many are still alive on the reefs adjacent to the MMDC.

Since this time a number of hatcheries have sprung up throughout the Indo-Pacific, including Australia, Fiji, Indonesia, the Marshalls, Solomon Islands and Tonga. Major research facilities in Australia, Palau, the Philippines, and Solomon Islands have provided experimental data to facilitate development of successful hatchery and nursery techniques. The markets for products from giant clam hatcheries are just beginning to see the potential production, reflecting the combined effort of researchers, hatchery managers and workers, economists, and administrators.

The work published here is the result of recent research on giant clams from giant clam hatcheries throughout the Indo-Pacific. Many of the papers were presented at the Giant Clam Mini-Symposium at the 7th International Coral Reef Symposium (7ICRS) in Guam in June, 1992. This publication and much of the research presented here was supported by the Australian Centre for International Agricultural Research (ACIAR), and follows publication of previous research results (Copland and Jucas 1988). The Australian International Development Assistance Bureau, the International Centre for Living Aquatic Resources Management (ICLARM) and the University of the Philippines also contributed to the presentation of research included in this volume.

I thank Isobel Verecondi and Jenna Clark of James Cook University and Peter Lynch and Barney Smith of ACIAR for their help in making both the Clam Mini-Symposium at 7ICRS and this publication possible.

W.K. Fitt

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Genetics of Giant Clams: an Overview

J. A. H. Benzie*

GROWING interest in farming giant clams and restocking depleted wild populations with cultured animals has stimulated interest in the genetics of giant clams (Copland and Lucas 1988) for two reasons. Firstly to establish the nature of the genetic resources available in the wild. Secondly, to establish whether cultured stocks differ from wild populations. There was concern that genetically distinct populations occur in different parts of the Pacific and that this biodiversity might be threatened by the spread of cultured material throughout the Pacific. Naturally diverse populations could also be a valuable source of new strains for mariculture in the future. Considerable loss of production in aquaculture can result from reduced genetic variation (Sbordoni et al. 1987), and it is important to establish whether culture methods result in the significant loss of genetic variation. Similarly, the success of restocking programs rely on the vigour of the cultured stocks used and, if the aim is to augment local strains without changing their genetic makeup, the introductions need to approximate the genetic constitution of the local stock.

Surveys of genetic variation were therefore undertaken of the two species of greatest economic interest, *Tridacna gigas* and *T. derasa,* throughout the western Pacific (Benzie and Williams 1992a, 1993a, Macaranas et al. 1992). However, these species have become rare or extinct over large parts of their range as a result of overexploitation, and sampling was necessarily patchy. In order to better understand any patterns of variation that might emerge, surveys were also undertaken of *T. maxima*, a smaller species that is widespread throughout the Indian and Pacific Oceans, and for which a greater geographical coverage was expected (Benzie and Williams 1992b, 1993b). Cultured populations of *T. gigas* from Australia and Solomon Islands (Benzie and Williams 1993c) and of *T. derasa* in the Philippines have been examined.

The aim of this paper is to provide an overview of giant clam genetics, summarising the recent findings concerning the structure of wild populations, the genetic constitution of cultured populations, and the implications for mariculture and restocking programs in the Pacific.

Methods and Results

Genetic structure of wild populations

Several hundred individuals of *T. gigas, T. maxima* and *T. derasa* have now been examined from wild populations throughout the Pacific (Table 1, Fig. 1). All surveys used biopsies of mantle tissue that allowed clams to be sampled in situ without sacrificing them, and examined genetic variation using electrophoretically detectable protein variation in six to nine polymorphic loci (Table 1). Summaries of the techniques used are available in the respective papers, and full details of their development are given in Benzie et al. (1993).

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Compared with the average for mollusc populations of 0.10–0.20 average heterozygosity (Berger 1983), the values for all three clam species of 0.34–0.42 average heterozygosity were high (Table 1), and similar to that reported previously for *T. maxima* (Campbell et al. 1975). Values of other measures of diversity such as mean number of alleles per locus were also high, although those for *T. gigas* were less than the other giant clam species. One or two outliers with reduced genetic diversity were responsible for the lowest values of all diversity measures in *T. derasa* (the Fiji population) and *T. gigas* (the Kiribati and Philippines populations).

Dendrograms summarising the relationships between all populations for each species show the Great Barrier Reef (GBR), the Philippines and Solomon Islands, consistently cluster together, followed by Fiji and Tonga as outliers, in a 'West Pacific' group (Fig. 1). Samples from Cook Islands, Kiribati and Marshall Islands form a separate 'East Pacific' group. Genetic distances among the populations varied from species to species with those for *T. gigas* low, those for *T. maxima* three to four times greater, and those for *T. derasa* two to three times as much again for given island group comparisons. Nevertheless, the pattern of relationship among populations was almost identical.

Table 1. Summary of genetic diversity in wild populations of *T. gigas, T. maxima*, and *T. derasa* and of the sampling effort in the surveys

	T. gigas	*T. maxima*	*T. derasa*
Mean number of alleles /locus	2.2 (1.9–2.5)	4.2 (2.8–5.0)	3.1 (1.6–3.7)
Percentage of polymorphic loci	69 (50–100)	84 (67–100)	85 (44–100)
Direct count heterozygosity	0.42 (0.19–0.46)	0.39 (0.35–0.55)	0.34 (0.14–0.46)
Number of polymorphic loci screened	8	6	9
Number of populations screened	13	19	10
Total number of individuals screened	595	860	484

Source: Data abstracted from Benzie and Williams (1992a, 1993a) and Macaranas et al. (1992).

F-statistics were used by each study to partition genetic variation into that occurring within populations (F_{IS}), and that occurring between populations (F_{ST}). No experiment showed significant structuring within populations, and all reported general conformance of gene frequencies to those expected under conditions of random mating (conditions of Hardy–Weinberg equilibrium). All reported little differentiation among populations within local regions such as Solomon Islands or highly connected reef systems such as the GBR, but all species showed significant differences among populations on greater geographical scales (Table 2).

The pattern of gene flow among clam populations showed remarkable similarities among species, and demonstrated clearly that the increasing significance of population differentiation at the regional level was not simply the result of increasing genetic divergence with increasing geographical separation (Fig. 2). Fiji was as isolated from neighbouring Kiribati as it was from the Philippines. Gene flow was very high within local areas (usually $N_em>20$) and for *T. gigas* and *T. maxima* relatively high between the Philippines, the GBR and Solomon Islands ($N_em>10$).There appear to be major barriers to gene flow between the East and West Pacific

Table 2. Genetic differences among populations in different geographical regions (all values are F_{ST}, which describes genetic variation occurring among populations)

	T. gigas	*T. maxima*	*T. derasa*
Within local areas			
GBR	0.000^{ns}	-0.003^{ns}	0.012^{ns}
Solomon Islands	0.011^{ns}	-0.003^{ns}	–
Philippines		-0.002^{ns}	–
Kiribati		-0.003^{ns}	–
Within regions			
East Pacific	0.032^{a}	0.068^{b}	–
West Pacific	0.035^{b}	0.099^{b}	0.098^{b}
All populations	0.084^{b}	0.156^{b}	0.098^{b}

[a] $P<0.05$; [b] $P<0.001$; [ns] not significant

Notes: F-statistics were calculated using methods which explicitly take account of differences in samples sizes among the populations tested, and their significance tested using chi-square (Waples 1987).

Source: Data abstracted from Benzie and Williams (1992a, 1993a) and calculated from data in Macaranas et al. (1992).

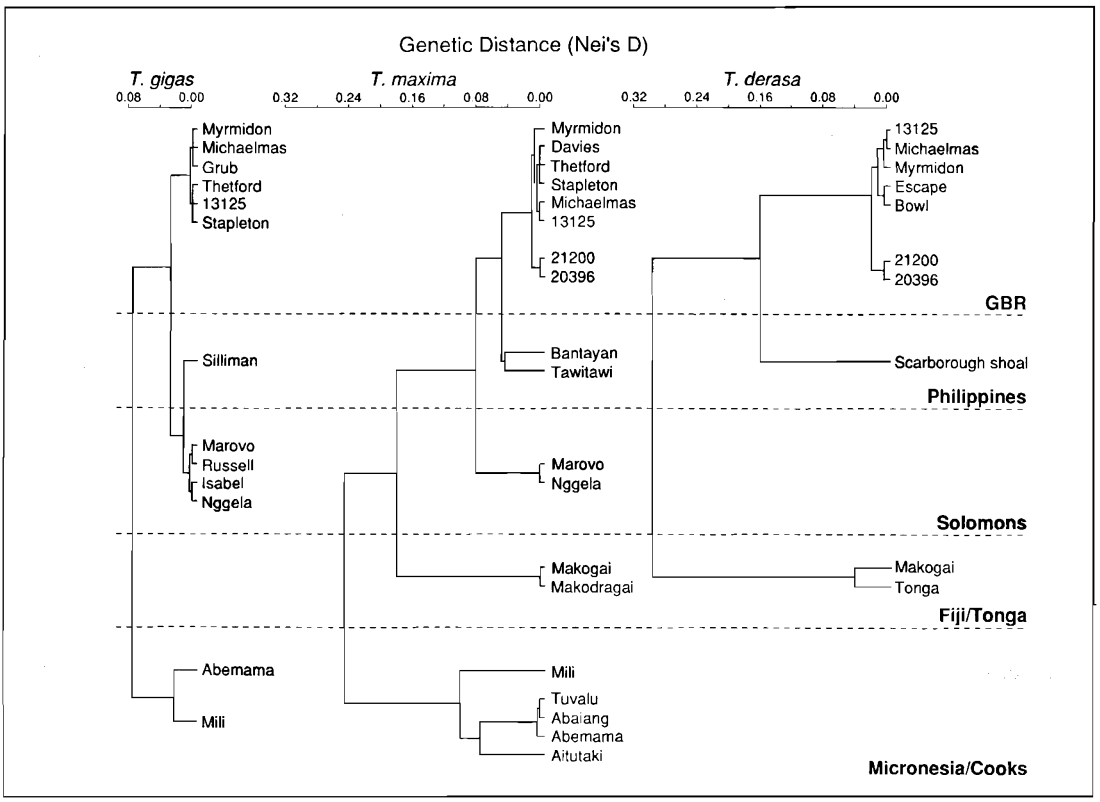

Figure 1. Dendrograms illustrating the similarity of the genetic relationships of *Tridacna gigas, T. maxima* and *T.derasa* populations from the Western Pacific. In each case Nei's unbiased genetic distances were clustered using the UPGMA method. Data from Benzie and Williams (1993a, 1992b) and Macaranas et al. (1992).

groups ($N_em<2$), and east–west between Australia, Solomons, Fiji, Tonga and Micronesia. The greatest connections follow the island chains connecting the Philippines through New Guinea to Australia, and separately to the Solomon Islands. Despite the South Equatorial Current connecting the Solomon Islands to Australia, and a 10-day planktonic lifespan for tridacnids, direct gene flow between these populations is limited. These patterns of gene flow are similar to biogeographical routes of dispersal suggested for fish faunas (Allen 1975) but also reflect the vicariant distributions of other marine faunas (Springer 1982). The NW–SE trend of connections runs almost perpendicular to major surface currents, but in Micronesia does match deeper flows (Fig. 3). It is not known whether these patterns reflect a continuing pattern of dispersal present day, or reflect historical fluxes of migration that no longer occur.

Cultured stocks

Samples of 90 individuals from each of three hatchery batches from both Solomon Islands and the GBR revealed lower average levels of genetic diversity within hatchery stocks of *T. gigas* than the natural populations from which the broodstock was derived (Table 3). This was not surprising in that very few individuals were used to produce each batch, and it was thought the Solomon's families were the product of single matings. The occurrence of more than four alleles for a given locus at a number of systems demonstrated clearly that more than two parents were involved in the production of each of these batches. It is not known whether the lower mean number of alleles per locus and lower polymorphism in the Palau material was a reflection of lower diversity of Palau *T. gigas* populations per se, or the result of additional selection either occurring later during the growth (these animals

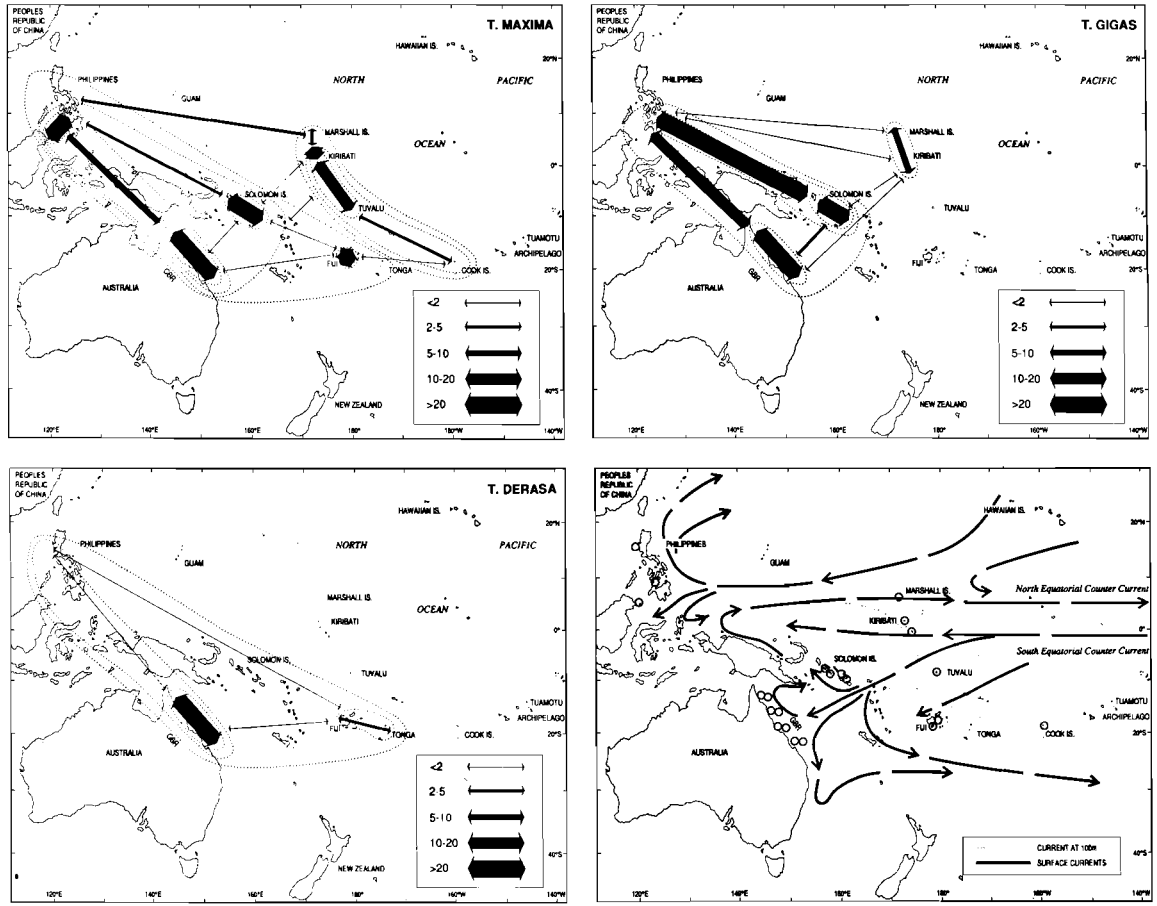

Figure 2. Gene flow among *Tridacna gigas, T. maxima* and *T.derasa* populations in the West Pacific. The thickness of the arrows represent different levels of dispersal, given by the average number of migrants per generation (N_em). N_em is the average number of migrants per generation, calculated from FST as follows: $N_em = ((1/F_{ST})-1)/4)$. Pairwise comparisons of population groups were made after pooling all the populations within each group so that no within group component of geneflow was included in the between group estimate. The dotted lines encircle populations within the groups defined by the dendrograms in Figure 1. Data abstracted from Benzie and Williams (1992a, 1993a) and calculated from data in Macaranas et al. (1992).

Table 3. Average genetic diversity in cultured batches of *T. gigas* compared with wild populations from the same region

	Great Barrier Reef		Solomon Islands		Palau
	Wild	Cultured	Wild	Cultured	Cultured
Mean number of alleles/locus	2.0 (1.8–2.1)	1.6 (1.4–1.8)	2.2 (2.0–2.3)	2.0 (1.8–2.0)	1.6 (1.6)
Percentage of polymorphic loci	50 (38–63)	38 (25–50)	53 (50–63)	50 (50)	38 (38)
Direct count heterozygosity	0.20 (0.19–0.22)	0.16 (0.10–0.20)	0.30 (0.25–0.36)	0.27 (0.18–0.34)	0.36 (0.35–0.36)
Number of populations or batches screened	6	3	4	3	2
Number of individuals screened/population		57–74	90		9–37

Notes: Cultured batches from the Great Barrier Reef and the Solomon Islands were about one year old and were still in the hatchery or in ocean grow-out nearby. Those from Palau were about 2 years old and had been translocated to reefs in Kosrae. Comparisons used eight loci for which data were available for both cultured and wild populations.

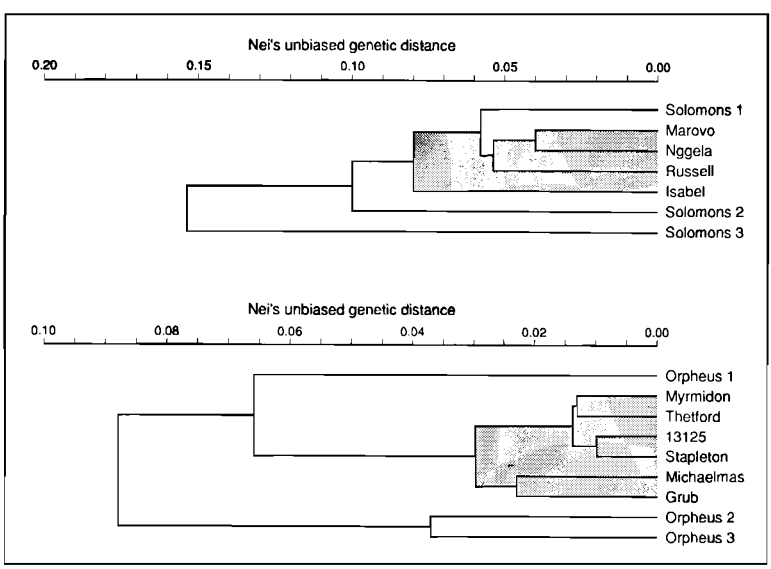

Figure 3. Dendrograms illustrating the considerable genetic divergence among cultured batches relative to each other and to the natural populations from which they were derived. The area between the natural populations is stippled. Data from Benzie and Williams (1993c).

were older than those from the other sites) or following translocation.

Gene frequencies of the cultured stocks were markedly different from the native populations, giving greater genetic distances among cultured batches and between cultured batches and natural populations, than among any of the natural populations (Fig. 3). Indeed, the level of differentiation among cultured batches was similar to that between populations from different regional groups (i.e. West and East Pacific).

No significant correlations were observed for either *T. gigas* between size at a given age within a batch and specific genetic markers (Benzie and Williams 1993c). Out of the six *T. gigas* batches, there were five positive correlations of size with individual heterozygosity, but only two of these were statistically significant (range of

r,–0.013 to +0.507; range of P, 0.90 to < 0.001) suggesting no strong and consistent effect of heterozygosity on growth as has been reported for other molluscs (Wilkins 1978). However, no specific experiments manipulating heterozygosity, and including fine environmental control during growth, were undertaken.

Discussion

Techniques recently developed to farm giant clams now provide a means of restocking depleted populations, and there is growing pressure to transfer cultured material throughout the Pacific (Braley 1989). The only published data available on giant clam genetics prior to the recent studies summarised in the previous sections concerned two populations of *T. maxima*, one from Marshall Islands and one from the Great Barrier Reef (Ayala et al. 1973, Campbell et al. 1975). Direct comparison of figures from these studies is not possible as they sampled different loci and included many monomorphic systems. However, they found small genetic differences over 4000 kilometres suggesting considerable dispersal by giant clams throughout the Pacific. Under these circumstances transfers of live material throughout the Pacific might be considered useful enhancements of local stocks by genetically similar introductions, irrespective of their source.

The recent studies, specifically aimed at analysing population structure, have each provided a very different picture and in combination, powerful evidence of fundamental genetic structuring of giant clam populations in the Pacific. The few large populations of giant clams that exist and which could be used as a source for broodstock differ in genetic constitution (e.g. GBR and Micronesian populations of *T. gigas*). Extinctions in particular places now represent a loss of diversity, rather than loss of part of a widely mixed population that might easily be replaced from elsewhere. The source of material to be transferred to a location is a now a critical issue if the aim is to enhance local stocks without endangering local genetic diversity.

Nor is the solution simply to apply current culture techniques to broodstock obtained locally. Mass producing animals from few adults, as happens at present, serves to reduce genetic diversity and creates major shifts in the gene frequencies of the cultured populations relative to their wild parents. A revision of hatchery techniques will be required to produce genetically diverse batches. Restocking programs may require several introductions over time, and include the progeny from many matings in order to produce populations whose gene frequencies approach those of natural local stocks. The source and genetic treatment of material for transfer needs to be carefully considered if plans for enhancing the survival of the giant clam and food production for Pacific nations, are not to destroy the resources upon which the future of the giant clam and its culture in the Pacific depend.

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The Zooxanthellal Tube System in the Giant Clam

J.H. Norton, M.A. Shepherd and H.M. Long*

THE giant clams, Tridacnidae, contain large numbers of dinoflagellate algae of the genus *Symbiodium*, living in their enlarged siphonal mantle (Yonge 1975). These symbiotic algae, commonly called zooxanthellae, provide much of the nutritional needs of the clam (Trench et al. 1981). However, since early this century (Yonge 1936), information on the location and the interaction of the zooxanthellae with the giant clam has been beset with uncertainties.

As part of a research project to document the anatomy and histology of the giant clam (Norton and Jones 1993), we confirm the existence of a zooxanthellal tube system, (Norton et al. 1990), elements of which were originally reported by Mansour (1945).

Materials and Methods

Tridacna gigas clams ranging from a few millimetres to 35 cm in shell length were dissected. Tissues were fixed in 10% seawater formalin, processed by routine histological methods and stained by haematoxylin and eosin. Additional stains included Masson's trichrome for muscle tissue and an Alcian Blue stain to delineate the tertiary zooxanthellal tubes. Pale (bleached) siphonal mantles were also examined histologically.

Histological sections from 50 *T. gigas* clams and serial sections on selected tissues of a 10 cm *T. gigas* clam were examined. The terminology is based on that used by Norton and Jones (1993). As giant clams orientate themselves with their hinge downwards in contrast to other bivalves, the orientation terminology of Rosewater (1965) was used. Briefly, the clam is orientated by calling the hinge and byssal gape edges ventral and the free, outer edges of the shell valves, dorsal. The mouth is anterior and is located in the 'hinge' end of the clam. The byssal gape and the incurrent siphonal orifice are posterior.

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Results

Zooxanthellal tube system—anatomy

The zooxanthellal tube system commences as a single primary zooxanthellal tube (Figure 1). It arises from one of the digestive diverticular ducts of the stomach, in the left of the digestive organ. It passes dorsally and posteriorly between the digestive diverticula, and then between the crystalline style sac and the muscular wall which encloses the digestive and reproductive organs. Above the digestive organ it divides into right and left tubes which run close to the dorsal aspect of the muscular wall. The paired tubes pass through this muscular wall into the kidney parenchyma close to the visceral–renal haemolymph portals. The tubes travel through the kidney in a more ventral direction until they leave the kidney. They then pass through the root of the middle ctenidial suspensory ligament close to the cerebrovisceral connective, the nerve which connects the cerebral ganglia to the visceral ganglion. The tubes continue to travel posteriorly until they reach the adductor muscle where they become embedded in the connective tissue sheath which encloses that muscle. While traversing the posterior of the adductor muscle, each tube gives off a branch which is embedded in the

floor of the excurrent water chamber. Each primary zooxanthellal tube passes toward the ends of the adductor muscle and travels dorsally into the root of the siphonal mantle. Each tube branches both anteriorly and posteriorly. One or more main branches run along in the root of the siphonal mantle close to the circumpallial artery, the circumpallial vein and the pallial nerves. Secondary zooxanthellal tubes branch into the upper levels of the inner fold of the siphonal mantle where they terminate in convolutions of thin tubes with blind ends, the tertiary zooxanthellal tubes, containing the zooxanthellae (Figure 2).

The tertiary zooxanthellal tubes are arranged approximately perpendicular to the exposed surface of the siphonal mantle. Other secondary zooxanthellal tubes branch off the primary zooxanthellal tube to form tertiary zooxanthellal tubes in other organs such as the dorsal and posterior surfaces of the adductor muscle, the bulbus arteriosus of the heart, the pericardium, the suspensory ligaments and demibranchs of the ctenidia and the lateral mantle. The concentration of zooxanthellae is higher in many of these tissues of small juvenile clams than in those of adult clams. This is presumably because light can penetrate further through thinner tissues and the translucent shells of small juvenile clams.

Zooxanthellal tube—histology

The primary zooxanthellal tube has an epithelial lining of cuboidal to low columnar cells with long cilia and is surrounded by a thin zone of muscle fibres. The secondary zooxanthellal tubes are thin-walled and are lined by tubular epithelial cells with thin cytoplasm and small, prominent dark nuclei. These cells also bear long cilia. Similar epithelial cells line the numerous tertiary zooxanthellal tubes which enclose the zooxanthellae (Figure 2). They do not appear to have cilia. In pale (bleached) mantles, a reduction in the concentration of zooxanthellae in the siphonal mantle is associated with a reduction or atrophy of the tertiary zooxanthellal tubes.

Discussion

The zooxanthellal tube system was partially described by Mansour (1945, 1946). However, its existence was rejected by Yonge (1953) and Fankboner and Reid (1990) believed that the presence of this duct system had not been convincingly verified.

Mansour (1945, 1946) did not emphasise that the fine tertiary zooxanthellal tubes have blind ends and that this tubular system does not communicate with the vascular system. The normal zooxanthellae do not live

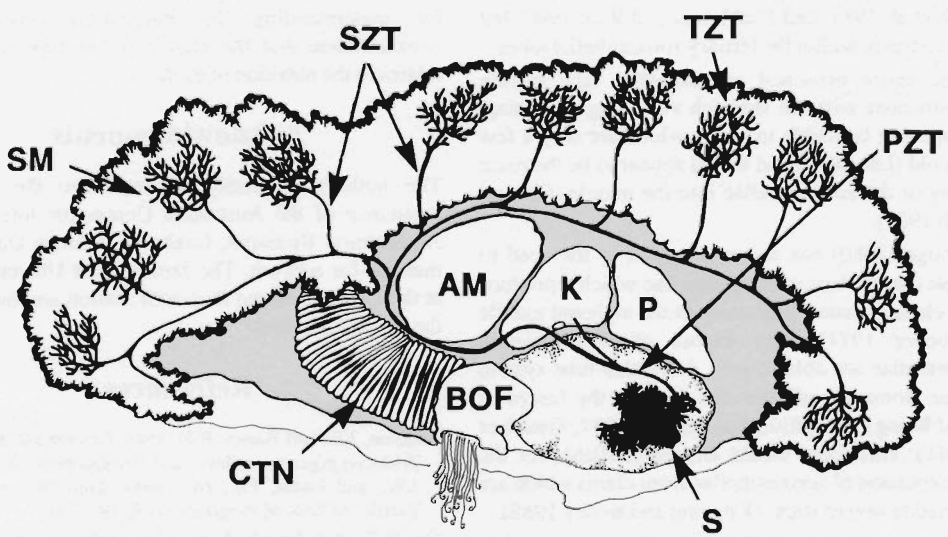

Figure 1. Diagrammatic view of the path followed by the zooxanthellal tube through one half of a clam. AM adductor muscle, BOF byssal organ/foot, CTN ctenidia, K kidney, P pericardium, PZT primary zooxanthellal tube, S stomach, SM siphonal muscle, SZT secondary zooxanthellal tube, TZT tertiary zooxanthellal tube.

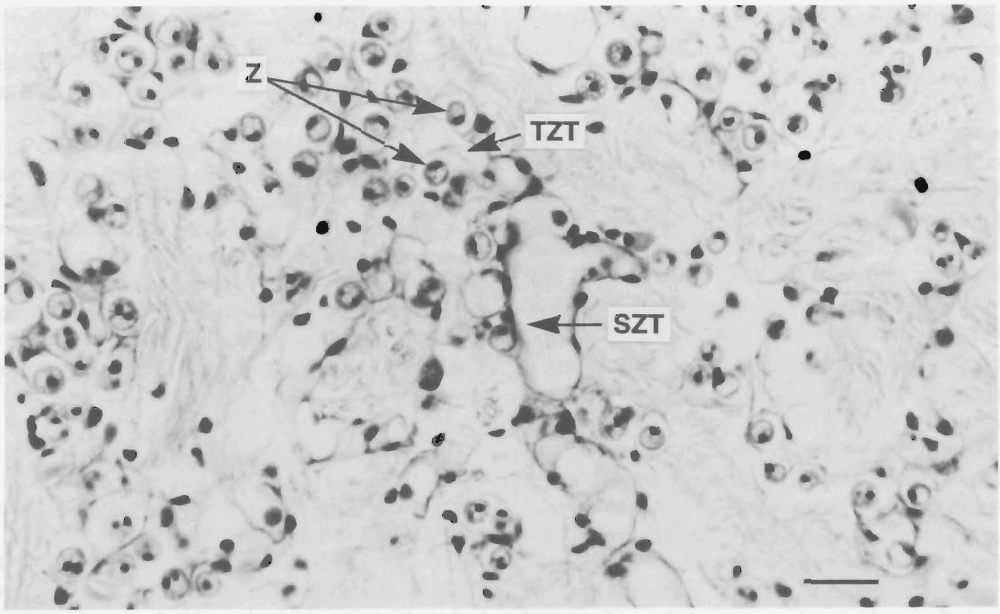

Figure 2. Secondary and tertiary zooxanthellal tubes in the siphonal mantle (transverse section, Alcian Blue stain, Bar = 23 µm,) SZT secondary zooxanthellal tube, TZT teritary zooxanthellal tube, Z zooxanthellae.

in the hemal or blood sinuses as stated by many authors (Yonge 1953, Kawaguti 1966, Fankboner 1971, Trench et al. 1981, and Fankboner and Reid 1990) but they live freely within the tertiary zooxanthellal tubes.

The entire branched zooxanthellal tube system communicates with the stomach via a single opening. This opening is visible in clams which are only a few weeks old (Lee 1990) and would appear to be the route of entry of the zooxanthellae into the mantle (Fitt and Trench 1981).

Yonge (1980) has drawn attention to the need to regulate the numbers of zooxanthellae which reproduce in the clams' tissues, especially in the siphonal mantle (Fankboner 1971). Any excess of these normal zooxanthellae are able to pass down this tube system into the stomach and leave the clam in the faeces as normal living algae (Ricard and Salvat 1977, Trench et al. 1981). This route would also be available for the mass expulsion of zooxanthellae from clams which are subjected to severe stress (Estacion and Braley 1988).

The entry of digestive enzymes from the stomach into the vascular system (Fankboner and Reid 1990) is unlikely to be a problem since the zooxanthellal tube system does not communicate with the vascular system.

The existence of the zooxanthellae within the tertiary tubes in the siphonal mantle has implications for understanding the interaction between the zooxanthellae and the clam's tissue especially as it relates to the nutrition of each.

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Parasites of the Giant Clams (Tridacnidae)

J.H. Norton*, M.A. Shepherd*, H.M. Long* and H.C. Prior†

WHILE the literature on diseases and parasites of molluscs is extensive, reports on diseases and parasites of giant clams are few. Humphrey (1988) and Anderson (1990) briefly reviewed these topics. In addition Goggin and Lester (1990) have recorded a rickettsial infection. The following is a summary of our findings on parasitic diseases in the giant clam over the period 1989–1992. Additional details are to be published elsewhere.

Materials and Methods

Sick clams showing abnormal clinical signs such as retracted mantle, or clams surviving from batches in which mortalities had occurred, were submitted for examination. Clinically normal clams were also processed in a search for pathogens. The clams were necropsied and the tissues were fixed in 10% seawater formalin. The tissues were processed by standard histological techniques and stained by haematoxylin and eosin. For examination by transmission electron microscopy, the formalin fixed tissue was refixed in glutaraldehyde, post fixed in osmium tetroxide, dehydrated through graded acetone and embedded in Spurrs' resin. Ultrathin (70 nm) sections were stained with lead acetate.

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Results

Rickettsia, a bacterial parasite which forms intracellular blue microcolonies, has been associated with mortalities of both juvenile and adult *Hippopus hippopus* clams. In the Philippines, hundreds of clams, 35–45 mm in shell length, died over several weeks in an aquaculture establishment. In the Federated States of Micronesia, mortalities have been reported in adult broodstock. In both cases, the ctenidia (or gills) had extensive lesions histologically (Fig. 1). Large numbers of blue microcolonies were present, usually unaccompanied by an inflammatory cell reaction. Also present were numerous red microcolonies which were degenerating rickettsial organisms. These were associated with a heavy infiltration of inflammatory cells, mainly granulocytes. Large areas of the ctenidia had been replaced by rickettsial cysts and inflammatory cells and little normal ctenidial tissue remained in many of the clams examined. The identification of the rickettsia was confirmed by electron microscopy.

An intracellular bacterium of unknown identity has been seen in the tissues of young juvenile *Tridacna gigas* and *T. maxima* which survived chilling as part of the winter mortality syndrome. The clams were usually less than six months of age and had been exposed to temperatures less than 20°C, in land-based culture tanks. Heavy mortalities occurred. Histologically, surviving clams had large numbers of banana-shaped or rod-shaped bacteria in the cytoplasm of the mantle epithelial cells and of cells of the ctenidia (Fig. 2). Granulocytic inflammatory cells were frequently associated with the lesions. These intracellular bacteria

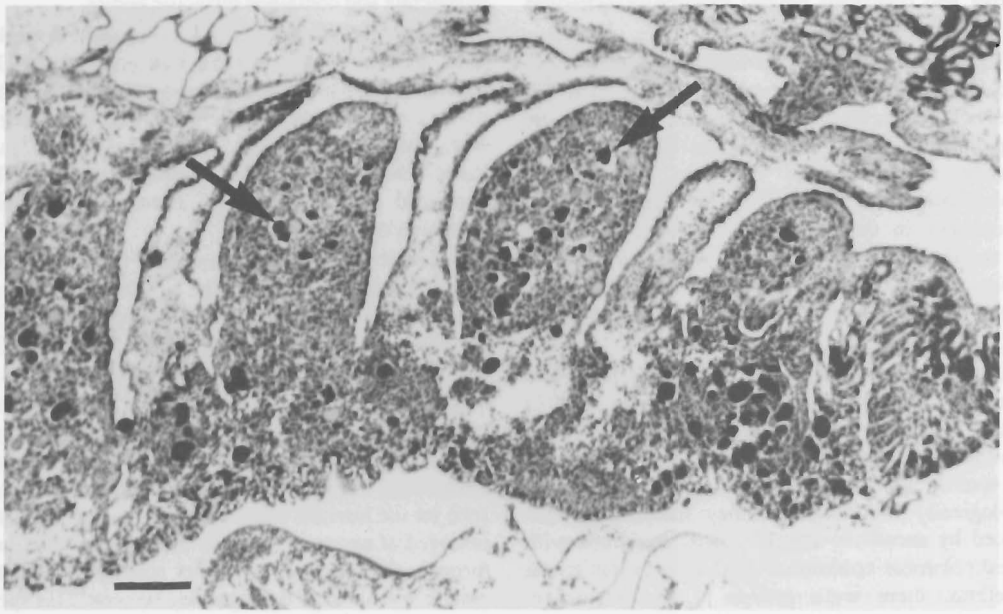

Figure 1. Ctenidia (gills) of an adult *Hippopus hippopus* heavily infected with *Rickettsia* sp. (arrows). Inflammation is widespread. Little normal tissue remains. (H & E stain, bar = 167 µm).

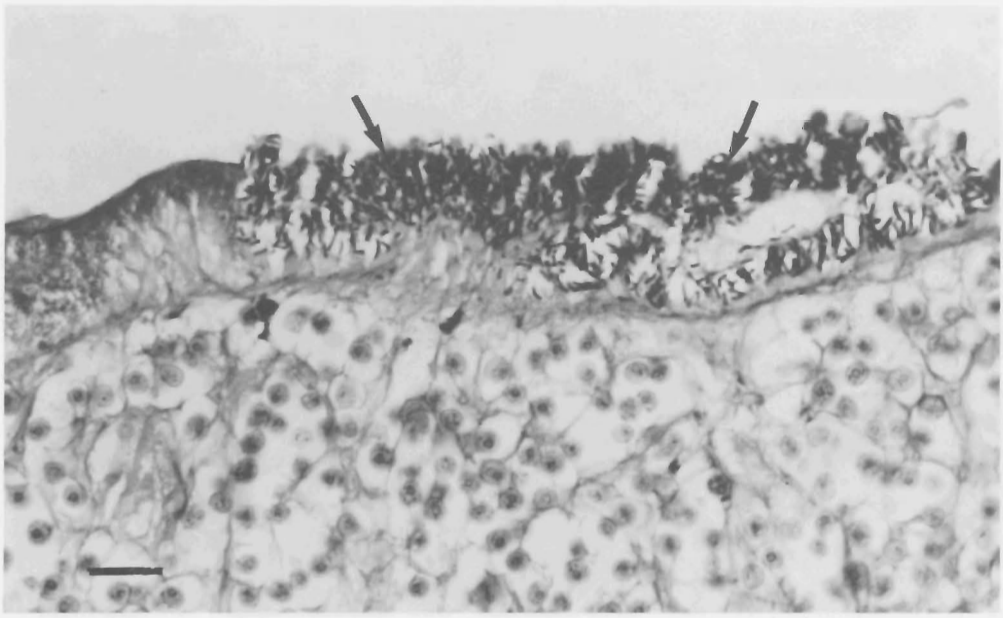

Figure 2. Banana-shaped bacteria (arrows) in the mantle epithelium of a young juvenile *Tridacna gigas* clam affected by the cold in an outbreak of winter mortality. (Periodic acid-schiff stain, shell length 1.0 cm, bar = 25 µm).

stained grey with haematoxylin and eosin were gram-negative and contained neutral mucopolysaccharides and glycoproteins.

The protozoan parasite, *Perkinsus* sp. is commonly found at low to moderate levels of infestation in the tissues of giant clams on the Great Barrier Reef, in the Philippines and Fiji. To date, no disease caused primarily by *Perkinsus* sp. has been seen. Histologically the organisms are seen in the macrophages in the macrophage centres associated with the digestive and reproductive organs (Fig. 3). *Perkinsus* sp. is readily cultured from the tissues of infected clams using thioglycollate medium (Ray 1966).

Another protozoan parasite, a *Marteilia*-like organism, has been found in a *T. maxima* in Fiji. No clinical signs were seen in the adult clam. However, when the clam was necropsied, numerous white spots were seen throughout the dark, mahogany-red kidneys. Histologically the normal kidney tissue had been replaced by numerous cyst-like structures lined with ciliated columnar epithelium (Fig. 4). Adjacent to the epithelium, there were groups of the protozoan. Towards the centre of the cyst-like structures, there were groups of the protozoan which appeared degenerate and contained refractile bodies.

Heavy infestations with a bucephalid fluke have been seen in *T. crocea* from one area of the Great Barrier Reef. Although the clams appeared normal, at necropsy the gonads had a yellowish-brown colour and were softer than normal. Histologically, the gonad tissue was replaced by bucephalid sporocysts. These contained cercaria (Fig. 5). There was a minimal inflammatory response only.

Turbellarians or flatworms may be seen as black dots against the white of the ctenidia. Microscopic examinations of wet mounts or histopathology of the ctenidia will confirm their presence (Fig. 6). These worms do not appear to be very harmful.

Boring sponges, *Cliona* sp, have been associated with damage to the internal lining of the shell valves in *T. gigas* from the Great Barrier Reef. The sponges tunnel from the outside of the shell and their presence is seen on the internal surfaces when the soft organs are removed at necropsy (Fig. 7). The sponge may tunnel through the internal lining to penetrate the lateral mantle where tissue damage may be seen. This sponge contains long, needle-like spicules made of silicon.

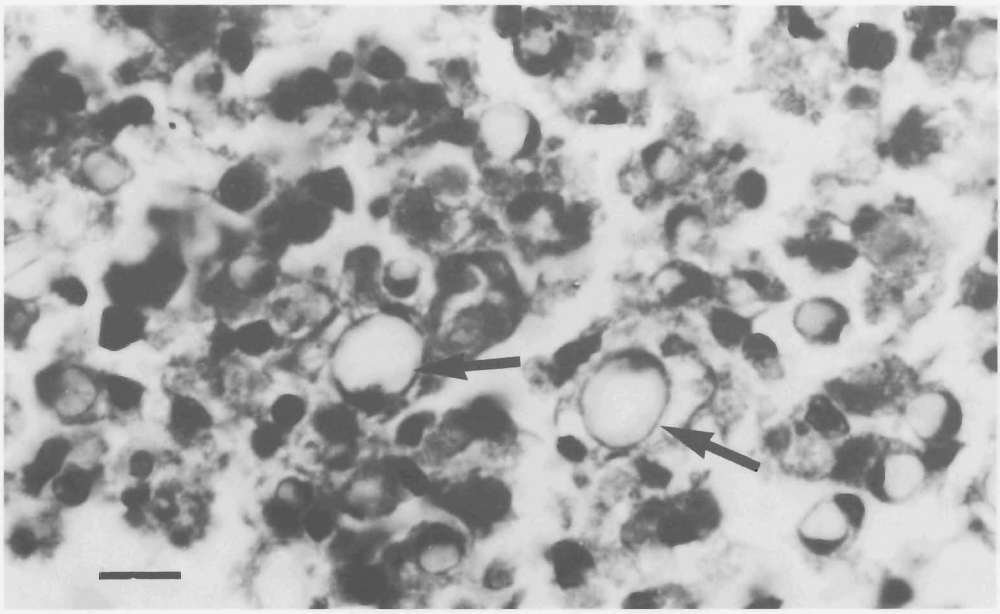

Figure 3. A close-up view of *Perkinsus* sp. organisms (arrows) in the connective tissue and macrophage centres of *Tridacna crocea*. (H & E stain, shell length 10 cm, bar = 6 µm).

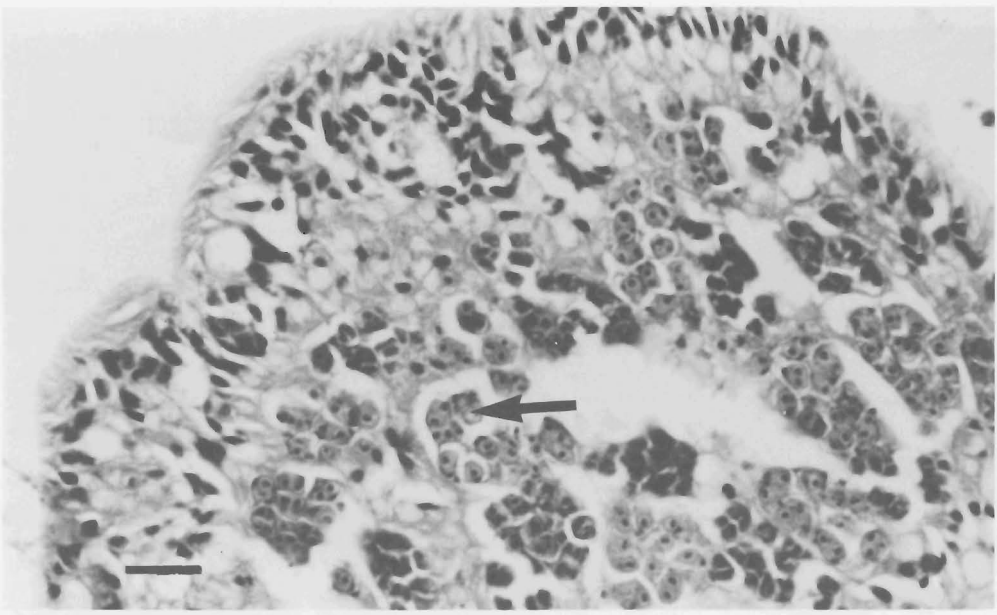

Figure 4. Section of kidney showing the cyst-like structure, top right hand corner, enclosing clusters of the *Marteilia*-like protozoa (arrow). (H & E stain, shell length 18 cm, bar = 14 µm).

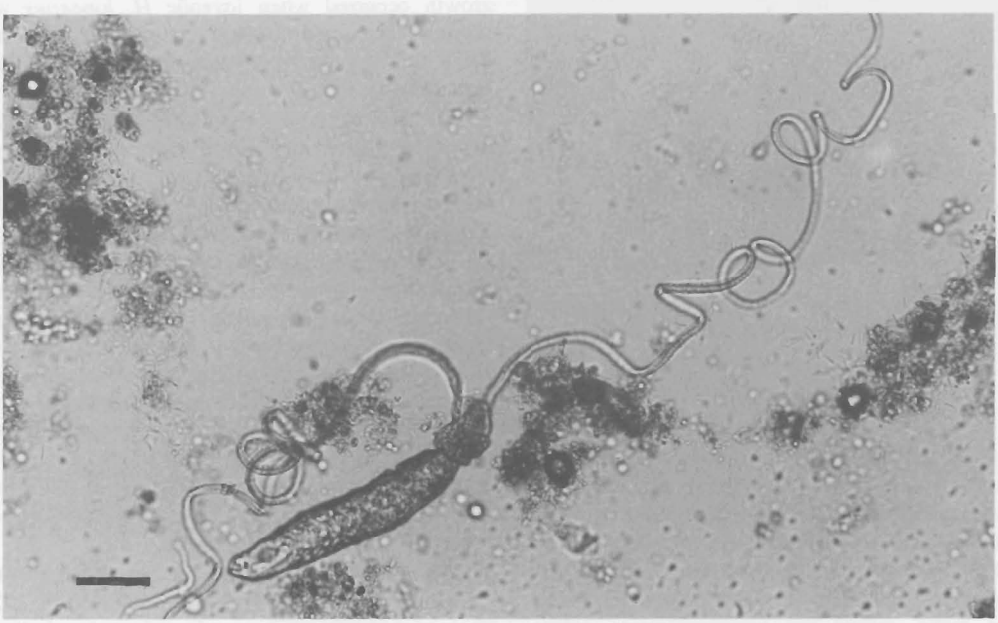

Figure 5. Bucephalid cercaria from the gonad of an infested *Tridacna crocea*. (Unstained, wet preparation, bar = 28 µm).

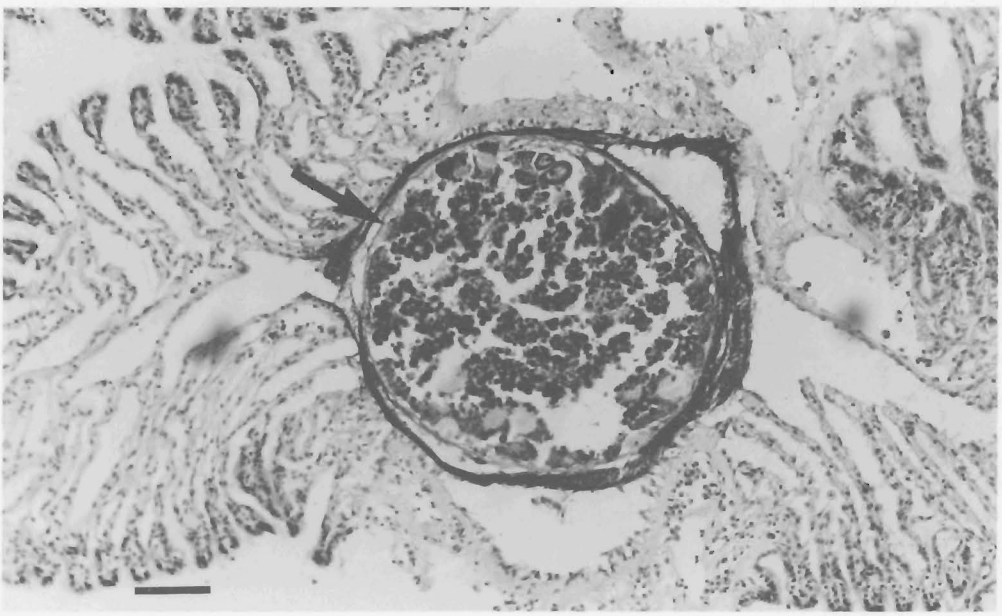

Figure 6. Section of a ctenidia (gill) of *Tridacna gigas* showing an immature tubellarian (arrow). (H & E stain, bar = 67 μm).

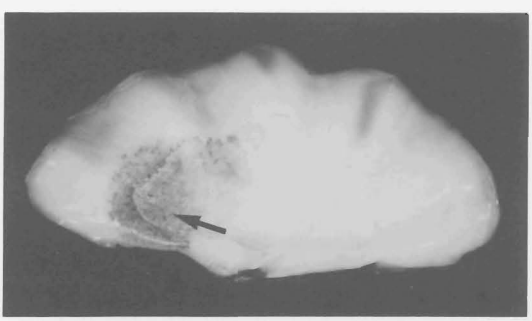

Figure 7. Inner surface of a *Tridacna gigas* shell showing the damage (arrow) caused by the boring sponge *Cliona* sp. (Shell length 30 cm).

Discussion

The losses attributed to rickettsial infection in both adult and juvenile cultured *H. hippopus* indicate a need to know more about the life cycle and epidemiology of this organism so that preventative measures can be employed. There are indications that low turnover rates of enclosed bodies of water e.g. in land-based culture tanks, makes the infection worse, while moving the clams into the open sea reduces the effects of this infection. Mortalities declined rapidly and growth occurred when juvenile *H. hippopus* were moved to the ocean nursery from crowded land-based tanks with low turnover rates of water (R.D. Braley, pers. comm.).

Losses from chilling and from secondary infections of surviving clams with intracellular bacteria associated with the winter mortality syndrome may be prevented by maintaining the temperature of culture tanks above 20°C by the use of heaters or by enclosing the culture tank under a plastic tent to retain the heat accumulated during the day. The wide distribution of *Perkinsus* sp. in giant clams together with reports of serious losses in other bivalve molluscs (Luckner 1983) indicate a need for further research. This should include the conditions under which *Perkinsus* sp. might be pathogenic and the clinical and necropsy signs that might be seen in giant clams. As many of the above organisms cannot be cultured in artificial media, there is need for the development of a giant clam cell line or a mollusc cell line to assist research.

Marteilia sp. has caused serious losses in other molluscs (Luckner 1983, Balouet 1979, Wolf 1979). The presence of a single case in a giant clam indicates the potential to cause losses in these bivalves also.

Bucephalid flukes have been found in an isolated population. This shows that some care needs to be exercised in selecting clams for culture.

There are likely to be many more as yet unrecorded pathogens and parasites of giant clams. There is need to know what pathogens and parasites are present in a given population, to use only healthy clams from disease-free populations for breeding and to translocate only young juvenile clams which have been certified by a quarantine protocol. As the giant clams are subjected to the stresses of intensive aquaculture, these pathogens and parasites will become more prominent and will require attention.

Acknowledgments

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A Quarantine Protocol to Prevent the Spread of Parasitic Diseases of Giant Clams (Tridacnidae) via Translocation

J.H. Norton*, R.D. Braley† and I.G. Anderson*

PACIFIC countries which no longer have wild stocks of giant clams because of over exploitation want to import clams to establish new breeding populations. For instance the Philippines, Fiji, Tonga, Cook Islands and Western Samoa approached the James Cook University with a request for clams from the University's hatchery at Orpheus Island on the Great Barrier Reef during 1990 and 1991. However there was some concern in these countries that parasites and other pathogens could be introduced with the imported clams. This concern was also held by Australian scientists in the Australian Centre for International Agricultural Research (ACIAR) funded Giant Clam Project. History has recorded some disastrous consequences of failing to take adequate precautions when translocating molluscs (Humphrey 1988; Sindermann 1988).

In an attempt to minimise the transfer of pathogens and other parasites, a quarantine and health testing protocol has been developed. The aim of the protocol was to attempt to limit the spread of any pathogen.

The purpose of the protocol was not simply to test a sample of clams from a given population, and if clear of pathogens and lesions, to endorse their translocation. This system could allow a pathogen at a very low prevalence or a pathogen in an early developmental stage, and possibly unrecognisable, to be translocated also. Information on the diseases of giant clams, the prevalence of each specific disease and the sensitivity and specificity of the testing procedures used to detect these pathogens was not available. This made the development of specific testing protocols impossible. Consequently, a quarantine protocol, based on good hygiene principles in rearing and examination of several samples of each distinct batch of clams, was tested to determine if a higher degree of certitude could be achieved.

Materials and Methods

The main features of the protocol are listed below:

1. The translocation of only young juvenile or larval clams so as to exclude 'carrier' states which are common in older animals.
2. Breeding only from clinically healthy clams.
3. The use of a separate batch system for each lot of clams i.e. a thorough cleaning, disinfection, rinsing and drying of the culture facilities prior to the rearing of each new batch of clam larvae.
4. The exclusion of all intermediate hosts including fish, gastropods and other molluscs from clam hatchery tanks, including a three metre high plastic screening around land-based tanks to exclude possible airborne marine organisms which might carry infectious or parasitic organisms.
5. The use of duplicate (1 μm) filters in the incoming water supply to exclude both intermediate hosts and infectious agents.

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6. The maintenance of a log (record) of water quality, management procedures and of any abnormalities and deaths of clams.
7. The use of only laboratory-reared algae and microencapsulated diets to feed the clams.
8. The use of only authorised staff who disinfect their boots, hands, etc. and who use a separate set of utensils and cleaning gear for each tank.
9. The use of regular laboratory tests to detect pathogens and lesions during the early life of the clams e.g. at zero weeks, three weeks of age and at every eight weeks thereafter for six months.
10. The statistical sampling of the clams to detect a 2% prevalence of a pathogen assuming that the test is 100% reliable i.e. 150 clams in a population of 100000 or more. These clams were fixed in 10% seawater formalin for histopathology. Another 150 clams were sent live for gross examination and for *Perkinsus* sp. culture in thioglycollate medium.
11. If any pathogen or lesion is detected, including those associated with a virus, chlamydia, rickettsia, protozoa or helminth, or if any unaccountable mortality occurs, the batch of clams is destroyed and the facilities thoroughly cleaned and disinfected. Since few pathogens or parasites have been recorded in giant clams, the batch is rejected if any lesion or pathogen is found since the risk associated with most organisms is unknown to allow any other decision.
12. Quarantine in the importing country in a land-based tank using a system similar to that used in the exporting country. This is a safe guard against any pathogen which might go undetected by the testing protocol in the exporting country either because the pathogen is present at very low levels (<2%) or because it is at an early developmental stage which may not be recognised. It allows early stages to develop and become more easily detected in laboratory tests. It also allows a pathogen time to multiply, spread and become more easily detected numerically.

Briefly, the importing country protocol included a separate batch system for each lot of clams, observation for three months, laboratory testing at six weeks and 12 weeks after arrival and disposal of water from the system into a land-based sump. Laboratory testing was limited to the histological examination of 150 clams which had been fixed in 10% seawater formalin. These fixed clams had been drained of formalin, packed and sent (usually posted) to a laboratory where facilities to process and examine histological sections were available.

Results

Two batches were prepared for export at less than six months of age, one of *T. gigas* (more than 64000 clams) hatched December 1990, and one of *H. hippopus* (more than 80000 clams) hatched January 1991. From these batches, four lots of *T. gigas* and four lots of *H. hippopus* were sent to five countries in the Pacific region. Specimens of both *T. gigas* and *H. hippopus* were received for histopathology from two countries.

Rickettsia sp. was detected in *H. hippopus* from two importing countries about 2.5–3 months after arrival. *Rickettsia* sp. was also detected in the *H. hippopus* remaining in the hatchery in Australia. This latter diagnosis was made during one of the routine tests on the remaining clams in the hatchery after the main group of clams had been exported.

Intracellular bacteria of the type associated with winter mortality (J.H. Norton, unpublished data) were detected in *T. gigas* approximately 2.5–3 months after arrival in two countries.

No *Perkinsus* sp. was detected in culture or on histopathology from any batch. No other parasites or pathogens were detected in the batches of clams for translocation.

Discussion

The need to have a period of quarantine both before and after the translocation of clams proved to be important. Whether three months in the importing country is sufficient to allow all pathogens to develop to a recognisable stage is uncertain. This interval may need to be extended as further knowledge of these agents is acquired.

The apparent failure of the quarantine protocol where two bacterial infections were detected in two lots of clams in the importing country needs further attention. The simultaneous detection of rickettsia in clams in both the importing and in the exporting country suggests that the rickettsia may have originated from the exporting country.

However, as rickettsia were also found simultaneously in locally produced clams in two of the countries, the infections could have originated from local rickettsia in these importing countries. The rickettsial infection of the ctenidia (gills) also suggests that it was water-borne. The use of filters smaller than 1μm or the use of ultraviolet treatment of incoming water might correct this problem.

No lesion indicative of any virus, protozoa, fungus or helminth was detected in any of the samples of clams examined for translocation.

This protocol will require modification as further research defines the number, range and epidemiology of parasites and diseases of giant clams. Meanwhile, it provides a basis on which to translocate clams with a minimal risk of also introducing unwanted parasites and pathogens.

Acknowledgments

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The Giant Clam as a Model Animal for Study of Marine Algal–Invertebrate Associations

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CORAL reefs are found in tropical waters and constitute 15% of the world's shallow sea floor (0–30 m). Their biogenesis is a result of calcification by either plants, or animals which harbour zooxanthellae; symbiotic unicellular dinoflagellates of the genus Symbiodinium. A major focus in recent reef research has been the study of this symbiotic association between algae and host at the cell biology and molecular level. This is important not only to gain a deeper understanding of the association and therefore the functional advantages inherent in the symbiosis but on a more practical level it is necessary to understand the response of these associations to nutrient loading and pollution and to explain coral bleaching.

While corals represent the most globally significant zooxanthellae symbiotic association, as far as scientific research is concerned corals have both geographic and technical drawbacks. As a result other organisms, such as sea anemones, have acted as model systems and there has been a trend to extrapolate results with these animals to all other zooxanthellate symbioses, the supposition being that, biologically and metabolically, their behaviour is similar or identical. While there is a recognition that the hosts themselves are dissimilar, it was believed until recently that there was a unifying factor in these symbioses, namely *Symbiodinium microadriaticum*. However the persistent research of Trench and co-workers (Trench and Blank 1987) has now demonstrated that there are several species of Symbiodinium. More recently Rowan and Powers (1991), using restriction fragment length polymorphisms and small subunit ribosomal RNA sequencing, have generated phylogenetic relationships between zooxanthellae isolated from a wide range of hosts. This shows that closely related algae are found in dissimilar hosts and that closely related hosts can harbour dissimilar algae. There is therefore no reason to assume that organisms, both host and algae, belonging to different taxonomic groupings, should exhibit the same or indeed similar properties.

Care, therefore, has to be exercised in extrapolating results obtained with one association to all other zooxanthellate symbioses. The literature contains several instances of differences between symbioses. For instance, the relative levels of the ammonium metabolising enzymes in host and zooxanthellae (Anderson and Burris 1987, Catmull et al. 1987, Miller and Yellowlees 1989, Rahav et al. 1989, Yellowlees et al. 1993). Any disparity in results between associations may be accounted for by the real differences that exist between these symbioses. Despite this, careful examination of the literature indicates most of these symbioses are very similar and consequently judicious extrapolation of results is warranted.

What then is the justification for using giant clams as a model animal for marine algal–invertebrate

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associations? At first glance, there appear to be significant morphological differences between clams and other symbioses. For instance, in the case of clams the zooxanthellae are extracellular and are found in diverticular tubes emanating from the stomach, while in corals and sea anemones the algae are intracellular and occur in the perialgal vacuole. In both these cases, however, the zooxanthellae are associated with the digestive system of the host and therefore may be considered functionally equivalent. Clams are more complex organisms than the cnidarian and protozoan symbioses, containing distinct organs, a blood supply and a primitive nervous system. However all depend on the in situ release of photosynthate from the zooxanthellae for their energy needs. The zooxanthellae, due to their containment, rely on the host for the supply of nutrients. There are therefore few substantial reasons why clams cannot be used as a model system for the functional aspects of algal symbioses.

Clams have many advantages over other associations. These include:

- ease of maintenance, handling and sampling ;
- provision of bulk host tissue permitting biochemical analyses and enzyme studies;
- rapid preparation of bulk zooxanthellae with no mucus;
- suitability for in vivo nuclear magnetic resonance (NMR) studies; and
- use for environmental monitoring.

Ease of maintenance, handling and sampling

Clams are easy to handle and maintain in an aquarium system. We have conducted experiments at the James Cook University Orpheus Island Research Station to determine the effect of elevated phosphate and ammonium concentrations on clams and corals. Such experiments are plagued by contaminating algal growth, however it is possible with clams to regularly remove algal growth from both the shell and aquarium surfaces. With corals this is not possible. Keeping algal growth under control is important in experiments of this nature since algae are major sinks for inorganic nutrients in the water column and therefore influence the ambient concentrations. While these experiments were terminated by sacrificing the clams, it has recently been demonstrated that certain metabolic aspects of the clam symbiosis can be monitored by analysing hemolymph samples removed from the animal (Rees et al. 1992). This method was used to demonstrate diurnal fluctuations in glucose levels. The technique has great potential for study of the clam symbiosis, its main advantage being that samples can be taken repeatedly from the same clam eliminating inter-animal variation. It also opens the possibility of monitoring clams in the field or in mariculture. In the latter instance it could be invaluable in determining whether clams are accessing nutrient supplements used in their culture.

Another approach to sampling is the use of tissue slices, a technique used extensively in early studies of mammalian biochemistry. Using 1 cm square tissue slices from clam mantle bathed in either seawater or hemolymph we have been able to demonstrate a diurnal fluctuation of glucose levels in the bathing fluid, the results paralleling those obtained with syringed hemolymph samples (Rees et al. 1992). The advantage in using tissue slices is that the membranes that separate cells are still intact and interactions between cells can be investigated whereas the use of cell homogenates destroys cell membranes making them inappropriate for looking at cell interactions. The use of tissue slices may be invaluable in the investigation of a number of important symbiotic processes including the action of the proposed 'host factor', the dependence of the algae on host nutrient supply and perhaps bleaching.

Availability of bulk host tissue

A knowledge of the biochemistry and cell biology of the symbiotic partners is necessary to our understanding of the association. This requires the application of basic techniques in these disciplines to the problem. One traditional approach to studying the biochemistry of organisms has been to purify particular enzymes of interest to that system. Glutamate dehydrogenase is, to our knowledge, the only enzyme from a cnidarian symbiosis that has been purified and studied in detail (Catmull et al. 1987). This paucity of information has been primarily due to the lack of tissue. Clams possess considerably more tissue than other symbioses and this will enable more detailed studies into the biochemical basis of the symbiosis. An illustration of how bulk tissue from clams can be an advantage is the investigation of the role of carbonic anhydrase in marine invertebrate symbioses. The work of Weis and co-workers (Weis et al. 1989, Weis 1991) has clearly demonstrated the crucial role played by this enzyme in marine symbioses. The localisation of carbonic anhydrase on the surface of the perialgal vacuole was determined using an antibody to the human enzyme which fortuitously cross-reacted with cnidarian carbonic anhydrase (V.M. Weis pers. comm.). No such cross-reactivity is evident with the clam enzyme. However because of the amount of tissue and enzyme present in the clam it is possible to purify clam carbonic anhydrase, prepare specific antibodies and use

these in localisation studies. One cannot envisage this being practical with cnidarians.

This paucity of material is a recurring problem, the magnitude of which is illustrated in Table 1. The figures relating to clams that are quoted in this table refer to soluble protein obtained from a brief homogenisation of clam mantle designed to release the zooxanthellae into suspension.

Rapid preparation of bulk zooxanthellae

Another major advantage of clams is that they are an abundant source of zooxanthellae (Table 1). While clams less than 5 cm in length contain few zooxanthellae, the number increases exponentially with clam shell length (Fitt et al. 1993). A 25 cm clam will yield 4×10^9 zooxanthellae from one mantle. Of perhaps greater significance to the study of symbiosis is the ease with which a clean preparation of zooxanthellae can be isolated. Starting from an intact clam the preparation of zooxanthellae, free from mantle tissue, takes a maximum of 30 minutes. The preparation of an equivalent amount of zooxanthellae from coral requires several hours and the quality of the preparation is very dependent on the amount of mucus released by the coral. This time-factor is crucial when basic metabolic processes such as photosynthesis or nutrient uptake are being examined. Physiological processes in zooxanthellae may well be modified during long preparation time. The microscopic examination of zooxanthellae preparations from clams shows no contamination with other algae. In a typical preparation from corals there is invariably a significant contamination with diatoms. This may not pose a problem in many instances, however it would be a distinct drawback to the use of modern molecular biology techniques such as PCR where any contaminating algal DNA would create the possibility of working with the wrong gene.

Table 1. Protein and zooxanthellae content of three symbiotic associations

	Protein	Zooxanthellae ($\times 10^6$)
Stylophora pistillata[a]	1.3 mg/cm^2	1.6 /cm^2
Aiptasia pulchella[b]	0.5–20 mg/anemone	0.5–5
Tridacna gigas (25 cm)[c]	705 mg	3800

[a]Muscatine et al (1989)
[b]Muller-Parker (1985), Cook et al. (1988)
[c]B. Baillie and D. Yellowlees, unpublished data

NMR in the study of clams

The use of NMR has great potential in the study of symbiosis. There are however some major drawbacks with the use of the technique. Firstly the small tissue volume of most symbioses limits its use as an in vivo technique to a limited number of organisms. On the technical side the presence of sea water is a major hurdle. The occurrence of paramagnetic ions in sea water is the major problem in the acquisition of spectra. NMR spectra have been obtained from sea anemones. However large numbers of tentacles (Rands et al. 1992) or whole animals (Steen 1986) have to be used. Attempts in our laboratory to collect spectra from intact corals, both soft and hard, have met with little success. We have, however, been more successful with clams which present the best opportunity to exploit the technique. They have bulk tissue and they can be readily examined out of their marine environment. The availability of wide bore magnets which allow experimentation on larger animals is a major advantage. Using this technique it is possible not only to acquire spectra of different nuclei, for example phosphorus 31, carbon 13 and proton, but also possible to localise the acquisition to a certain organ or part of the tissue. Using conventional NMR techniques it would be possible to inject bicarbonate, enriched with carbon 13, into the hemolymph and then observe its fate within different organs of the clam; for instance its incorporation into glucose and hence to glycogen. With the requisite attachments and software it is also possible to image the clam in the NMR in the same way that modern hospitals use magnetic resonance imaging for diagnosis. In the future it may be possible to image with different nuclei or look at the distribution of a single molecule and its fate within the organism.

Environmental monitoring

Marine organisms are bound to be affected by changes in water quality in their environment. Indeed many organisms have been used as indicators of pollution. The real time monitoring of water quality of a reef area as large as the Great Barrier Reef is impossible. However it may be possible to detect transient changes in water quality provided these perturbations result in some permanent alteration in the structure of the organism. Clams present such a possibility.

We have demonstrated that changes in various metabolic parameters occur when clams are exposed to elevated nutrients such as ammonia, nitrate and phosphate. These nutrients affect the hemolymph

concentration of that same nutrient. However when the seawater concentration reverts to its normal level the hemolymph concentration adjusts with it. Measurement of nutrient levels in hemolymph can not be used for retrospective nutrient monitoring, but it has been shown that the calcification pattern changes dramatically following exposure to both elevated phosphate and ammonia (Belda et al., unpublished results). The experiments of Belda and co-workers were over a period of three months during the maximum growth period. Clam extension is significant during this season and elevations in nutrient level will be detectable in the aragonite structure over much shorter periods. One would anticipate that an increase in nutrient levels for periods greater than two weeks would be detectable whereas more transient increases would not be evident. In addition since there is a difference in the perturbation caused by ammonium and phosphate it would be possible to differentiate between the two nutrients. Rasmussen and Cuff (1990) have previously demonstrated perturbations in calcification in corals following exposure to phosphate and this has been used to detect past nutrient elevations. Clams however present a logistically superior animal in that they could be placed in selected locations as a nutrient monitor and analysed at a later time for any change in calcification pattern. Since the clam lays down aragonite in an annual pattern, any change in calcification pattern can be traced to a fixed period in time.

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Nutrition of Giant Clams

W.K. Fitt*

THE relative importance of the different sources of nutrients to the nutrition of giant clams has been controversial for at least one hundred years. The first debate centred on whether the green cells in giant clams were actually algae or a special type of animal cell (Brock 1888). After confirmation of the presence of symbiotic dinoflagellates, the same type of brown algae found in reef corals and other cnidarians, attention shifted to whether nutrients from these symbionts originated from digestion of the algae by the clam, or from transfer of compounds from live and intact algae (Yonge 1936, 1953, 1980, Muscatine 1967, Goreau et al. 1973, Fankboner 1971, Trench et al. 1981, Reid and Fankboner 1990). While this argument has lingered in the literature for over 50 years, most of the major issues are now resolved, as details of the morphological, physiological and biochemical relationship between alga and host have been described. More recently the clam's ability to filter particulate material from the water has been assessed, and its potential importance in supplying essential nutrients to larvae, juvenile and adult clams characterised (Yonge 1936, Klumpp et al. 1992, Southgate, 1988). Other less studied aspects of nutrition of giant clams include uptake of dissolved inorganic and organic molecules from seawater (Goreau et al. 1973, Fankboner 1971) and a potential role for suspension feeding (Reid and King 1988).

This paper summarises what is known about acquisition of nutrients in giant clams, paying special attention to the unique aspects of the physiology of tridacnids. Whenever possible, a historical view of the research problems and their resolution to date is presented.

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Definitions

Interest in nutrition of giant clams originated from the unusual morphology of members of the Tridacnidae (Brock 1888, Grobben 1898, Yonge 1936, 1953, 1975, Stasek 1962). Adult clams are either attached to or sit on the substrate, umbo down, with the expanded fleshy siphonal tissues facing the surface of the water (Fig. 1). The distance between the incurrent and excurrent siphons is extended and the entire siphonal mantle area enlarged, compared to the other members of the Sub-Order Cardiacea. Both attributes are thought to have evolved to accommodate the algal symbionts (Yonge 1936). The algae are known by the trivial term 'zooxanthellae', a term referring to all brown, single-celled algae living in symbioses with animals.

The zooxanthellae found in giant clams are taxonomically most similar to those living in corals and other cnidarians and belong to the genus *Symbiodinium* (Trench 1979). Biochemical, genetic and morphological analyses have revealed that there are several species of *Symbiodinium* but only a few have as yet been formally characterised (Trench and Blank 1987, Rowan and Powers 1991).

Giant clams exhibit normal bivalve development. Trochophore larvae appear 8–24 hours post-fertilisation, developing into veliger larvae at 24–36

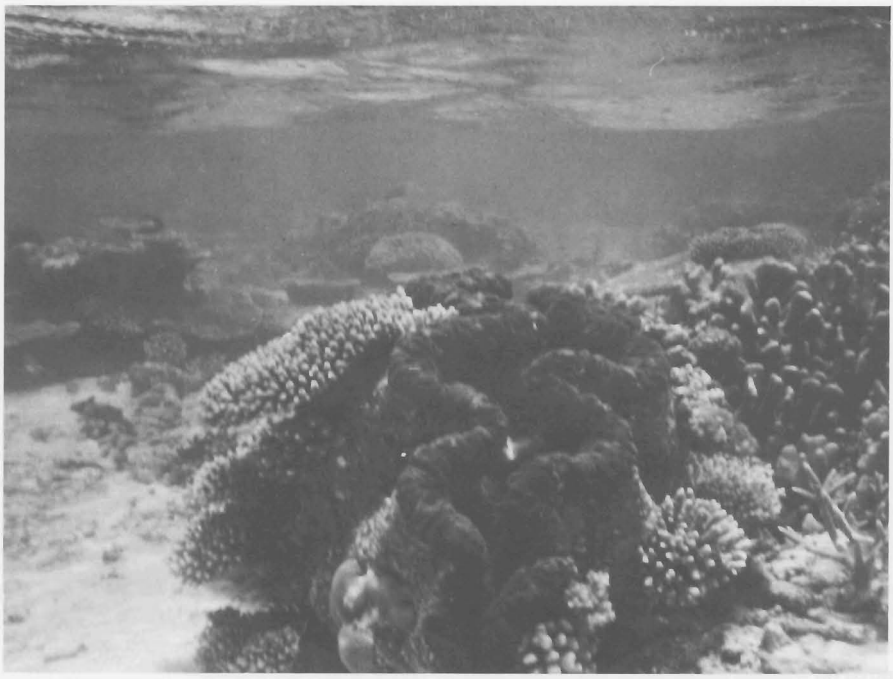

Figure 1. *Tridacna gigas* amongst symbiotic corals on a shallow patch reef in Palau, showing the expanded mantle extending over the margin of the shell.

hours. The length of the swimming veliger stage varies from 2–14 days, depending on clam species (Fitt et al. 1984) and most likely a variety of environmental factors (e.g. temperature, water quality). Prior to metamorphosis the veligers develop a foot and exhibit swimming and crawling behaviour with the foot extended, presumably sensing soluble and benthic cues important in settlement. Metamorphosis is a somewhat subtle process involving loss of the velum and initial development of ctenidia, and generally takes less than one week. During this time capture of particulate food and transfer into the stomach is perceived to be difficult, and the newly-metamorphosed clams are thought to survive on stored lipids acquired from the egg and/or previous veliger feeding. The resulting juveniles (Fig. 2) actively crawl on the substrate with their foot, filter feed with their gills, and typically within a week to ten days acquire and incorporate symbionts from seawater into tubular extensions of their stomach (Fitt and Trench 1981, Fitt et al. 1984, Norton et al. 1993). Though larvae may acquire symbionts from the surrounding seawater, few are apparently digested and a permanent symbiosis is not established until after metamorphosis (Fitt et al. 1986).

Planktotrophic larvae require an 'obligate period of planktonic feeding prior to their achieving competence successfully to metamorphose', while lecithotrophic larvae can 'achieve metamorphic competence and successful metamorphosis without ingesting planktonic organisms' (Kempf and Todd 1989). Lecithotrophic larvae are thought to depend primarily on nutrients originally stored in the egg. In practice, there is a planktotrophy–lecithotropy gradient, such that larvae of many species of bivalves exhibit some attributes of each nutritional mode. For instance, larvae of giant clams can survive through metamorphosis without any added food, providing the larval period is fairly brief (ca. less than a week). Hence by the definition they can be classified as 'lecithotrophic'. However, during this time they are capable of filtering food particles with their velar cilia following development of the digestive system about day 3 (LaBarbera 1975), so are functionally 'facultative planktotrophs'. When the larval stages extend beyond one week, veligers generally need to be fed in order to survive through metamorphosis, and under these conditions would be known as 'obligate planktotrophs'. The terminology is not as important as

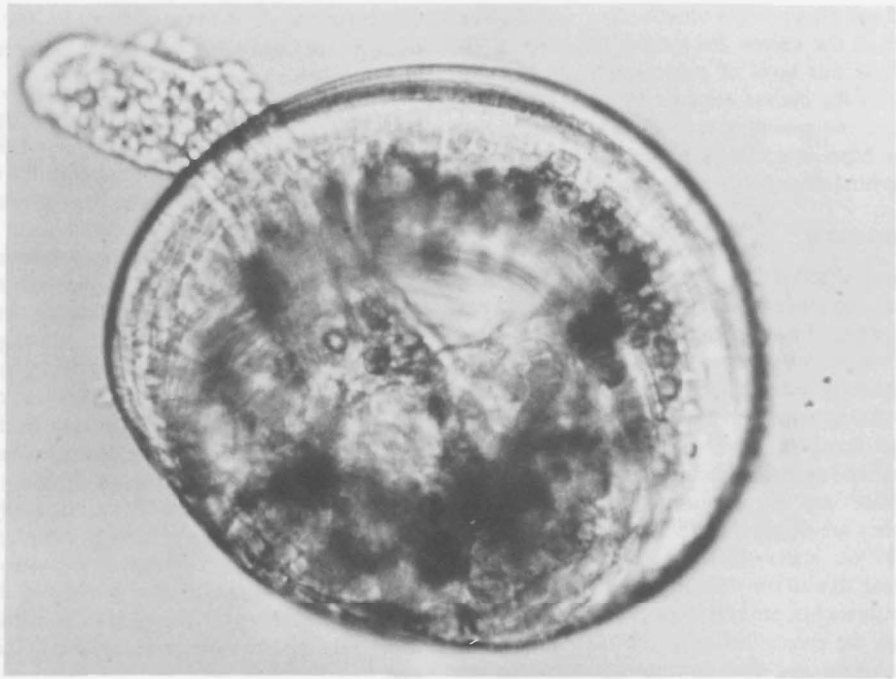

Figure 2. Newly metamorphosed *Hippopus hippopus*, characterised by an obvious foot and absence of the larval velum. Note zooxanthellae in the mantle region and statocyst at the base of the foot.

the concepts, and the fact remains that larvae of giant clams have different nutritional requirements depending on the location of the hatchery and conditions under which they are raised.

The term 'nutrition' generally refers to the means of obtaining organic substrates for growth, maintenance, and reproduction of an organism. The mechanisms for obtaining these substrates in giant clams includes filter feeding, suspension feeding, and uptake of soluble molecules from symbiotic algae and natural seawater.

Filter feeding

Giant clams, like most bivalve molluscs, are filter feeders. Larvae, juveniles and adults capture suspended particulate food via ciliated organs. While little is known about the nutrition of the trochophore larva, high mortality rates are often seen during this brief larval period. Culture with antibiotics has improved survival rates of trochophores, suggesting that the high mortality may be in part due to culture conditions (Fitt et al. 1992a). However, nutrition and genetics may also be important factors influencing larval survival. In contrast, veliger larvae have a ciliated velum, capable of capturing single-celled algae and other small particles (Jameson 1976, Klumpp et al. 1992). Both phytoplankton and zooxanthellae are ingested and can be seen in the stomachs of veliger larvae (Fitt et al. 1986). Artificial pellet foods, containing various combinations of protein, lipid and carbohydrates, are also ingested and digested by veliger larvae (Southgate 1988). Mortality during the veliger stage is extremely variable, and is thought to reflect physiological, intrinsic and culture factors.

Adult and juvenile giant clams have been described as having typical bivalve feeding apparatus, including ctenidia and palps, not differing fundamentally from those of other lamellibranchs (Yonge 1936, 1953, Reid and Fankboner 1990). Phytoplankton are ingested, and most digested, as indicated by the disappearance of ^{14}C-labelled *Isochrysis galbana* from seawater surrounding *Tridacna gigas* (Fitt et al. 1986). However, when ^{14}C-labelled freshly-isolated zooxanthellae (FIZ) were ingested by juveniles of this species, up to 80% were released over a 72-hour period following feeding, suggesting that zooxanthellae are somehow capable of resisting digestion in the stomach. In addition, *Tridacna gigas* retains approximately 75% of particles

between 2 and 50 μm while filter feeding, and absorbs about half of the carbon from them (Klumpp et al. 1992). While this level of particulate input can not supply all of the carbon required by giant clams for maintenance and growth, it is none the less potentially significant. More importantly, particulate feeding may supply essential micronutrients and trace metals.

Algal symbionts

The earliest detailed descriptions of zooxanthellae inside clams are fairly accurate, and have changed little over the years. However, the interpretation of these observations has varied tremendously. Unfortunately, much of the early interpretation, and some of the recent re-interpretation, appear to have been confined by the dogma that clams are herbivores and therefore they must digest and assimilate nutrients from algae. Since zooxanthellae are algae, many researchers have assumed they are digested by their hosts. For instance, Yonge (1936) states that 'the animals [in the Tridacnidae], like all lamellibranchs with the exception of the Septibranchia, are herbivorous, and thus capable of digesting the zooxanthellae'(p. 305). The following are observations and data concerning zooxanthellae symbioses in the Tridacnidae, followed by their interpretations.

Observation 1: Zooxanthellae are rarely, if ever, seen in the blood.

Though there has never been any convincing evidence of zooxanthellae in the blood, Yonge (1936) surmised that zooxanthellae are transported via the blood to the digestive diverticula where digestion occurs intracellularly. This avenue of transportation became a critical part of his 'farming' hypothesis, where certain zooxanthellae are 'pruned' from the mantle regions and digested in blood cells in the digestive gland. Yonge (1936) states that:

> fresh blood from the heart of *T. crocea* failed to reveal the presence of zooxanthellae ... The zooxanthellae are presumably carried from the mantle-tissues to the visceral mass by way of the blood stream. The process is probably rapid, because examination of fresh blood from the heart of *T. crocea* failed to reveal the presence of zooxanthellae in more than a very few of the numerous blood-cells (p. 310).

Trench et al. (1981) and others (e.g. Fitt, Yellowlees, Rees, unpublished data) have looked for zooxanthellae in the blood and not found them. Other studies on components of tridacnid blood have not noted the presence of zooxanthellae (Uhlenbruck et al. 1975, 1978, Baldo et al. 1978, Deane and O'Brien 1980).

Observation 2. Zooxanthellae in the siphonal tissues are enclosed in spaces defined by host membranes.

Brock (1888) was the first to describe zooxanthellae inside siphonal tissues of giant clams, where he found no evidence of host cells surrounding the symbionts. Yonge (1936, Fig. 11), however, showed zooxanthellae in the siphonal tissues in host cells, which he interpreted as blood cells. Since then researchers using electron microscopy, as well as standard histological techniques, have confirmed Brock's extracellular interpretation. Mansour (1946a, b), Kawaguti (1966), Fankboner (1971), Trench et al. (1981), and Fitt and Trench (1981) have all confirmed that zooxanthellae are not enclosed inside host cells while in the siphonal tissues. However the latter three studies clearly showed nearby host membranes similar to those drawn in Yonge (1936). Mansour (1946a, b), studying serial sections, found that the symbiotic algae are found in tubes which extended for great distances which he hypothesised connected the connected the mantle regions with the gut. Yonge (1953) dismissed these interpretations, somewhat ungraciously, commenting that:

> Such an extension of the stomach would be altogether without parallel in the Mollusca...In view of [Yonge's] personal difficulties in arriving at a correct interpretation of structure in the Tridacnidae, criticism of the conclusions reached by Mansour is tempered with much sympathy. It is certainly impossible to understand the structure of these animals without reference to that of typical eulamellibranchs from which they evolved (p. 559–560).

Yonge's (1936, 1953) view prevailed for many years, in spite of Mansour's detailed observations and contradictory evidence. It was not until almost 40 years later that Norton et al. (1992) confirmed the extraordinary siphonal tube system in tridacnids that contains the zooxanthellae, again on the basis of serial sections. They described a ciliated primary tube extending from a digestive diverticula duct, splitting above the digestive organs as described in Mansour's (1946) study, passing through the kidney and wrapping posteriorly around the adductor muscle before entering the siphonal tissues. Secondary tubes branch into the siphonal mantle, terminating in tangled convolutions of thin tertiary tubules containing the bulk of the zooxanthellae. The tertiary tubules are evidently what has been seen by virtually all researchers, their membranes originally interpreted as either blood cells or hemal sinus and connective tissues.

Observation 3. Zooxanthellae are seen in the stomach and digestive gland.

All investigators who have looked for zooxanthellae in the stomach and digestive gland of giant clams have found them. Yonge (1936) discovered, in examinations of the guts of tridacnids, that: '...there were a number of brown spherical algae corresponding in all respects to the zooxanthellae present in the tissues' (p. 295).

> Apart from these algae nothing of any significant food value was found in the stomach of the animals examined. In a solitary specimen of *T. derasa* which was opened for examination the stomach contained may of these zooxanthellae, the majority intact, but a few ingested within phagocytes. A few diatoms were also found and some fine filamentous threads of algae. In *Hippopus* many intact zooxanthellae were found in the stomach, some of them actually dividing; there were few phagocytes free in the lumen in any of the specimens examined, and in none of these were algae ingested. Some intact zooxanthellae were actually present in the faeces (p. 296).

Yonge concluded (p. 296) 'It would appear, therefore, that little food enters the gut except zooxanthellae...'. Other studies documenting zooxanthellae in guts of tridacnids include those of Goreau et al. (1973), Morton (1978), Trench et al. (1981), and Fitt (1984, 1985).

Yonge (1936, 1953) originally interpreted zooxanthellae found in the gut as those accidentally introduced from injuries sustained while collecting. This view has been upheld by a number of more recent investigators (Goreau et al. 1973, Reid et al. 1984a, Reid and Fankboner 1990), in spite of evidence to the contrary (Ricard and Salvat 1977, Morton 1978, Trench et al. 1981, Fitt et al. 1984, 1986) and Yonge's (1980) acknowledgment that 'Subsequent research has not supported these [= his early] views.' The conclusive physiological experiments were conducted over ten years ago when Trench et al. (1981) documented intact zooxanthellae released from guts of clams in the fecal pellets, on a daily basis for two weeks in an aquarium containing only 0.22 µM Millipore-filtered seawater, showing the only source of released zooxanthellae to be internal transfer from the mantle. The morphological data of Mansour (1946b) and Norton et al. (1993) have firmly established the integral relationship of zooxanthellae with the clam's digestive system, zooxanthellae entering and leaving the siphonal tube system by way of the gut. The close association of zooxanthellae with the host digestive system appears to be a fundamental theme of algal symbioses, also illustrated in corals, green hydra, many other cnidarians as well as some nudibranchs.

Observation 4. No zooxanthellae are found in the eggs of tridacnids.

The ubiquitous presence and fundamental importance of zooxanthellae to the nutrition of juvenile and adult clams suggests that tridacnids would have a direct mechanism of transferring their symbionts to the next generation. The initial studies on reproduction consisted of two sets of artificial fertilisations of eggs of *Hippopus hippopus*, both unsuccessful (Stephenson 1934). The 'early' fertilisations were thought to be 'unripe', while the second set of eggs were determined to be ripe (Yonge 1936, p317). Neither set of eggs contained zooxanthellae. It is remarkable that Yonge's interpretation of the fertilisation results was that 'the absence of zooxanthellae may have been responsible' and that the zooxanthellae 'possibly are carried into the eggs immediately before these are extruded, and are an essential factor in normal development.' Other researchers have also failed to observe zooxanthellae in eggs of tridacnids (LaBarbera 1975, Jameson 1976, Fitt and Trench 1981 and many others), and it is generally accepted that tridacnids, as in numerous species of reef cnidarians living in 'open' systems, do not pass their algal symbionts directly to the next generation ('closed systems') via the egg (Fitt 1984).

Observation 5. Zooxanthellae in clams are similar to those in corals.

Many authors have noted that zooxanthellae in giant clams are morphologically similar to those in corals (e.g. Boschma 1924, Taylor 1969, Trench 1979). Yonge (1936) originally thought that zooxanthellae were unable to live outside of the host (p. 311). However Kawaguti (1944) was able to culture symbiotic dinoflagellates from cnidarians and Freudenthal (1962) and McLaughlin and Zahl (1959) subsequently showed that many types of zooxanthellae thrive in culture conditions. They also must survive in seawater outside of the host, since they are acquired by juvenile clams (Fitt and Trench 1981, Fitt 1984, Heslinga and Fitt 1987).

Observations 6. Many varieties of zooxanthellae (genus Symbiodinium) are taken into symbiosis in tridacnids.

Since zooxanthellae are not transferred to the young via the egg, each generation must re-establish the algal symbiosis. All zooxanthellae of the genus *Symbiodinium* are taken into the mouth, and enter the tube system in the stomach (Fitt and Trench 1981, Fitt et al. 1986, Norton et al. 1993). If there is selection for specific type or species of zooxanthellae, the sorting apparently takes place after the symbioses are

established (Fitt 1985). The implication of having numerous species of zooxanthellae that can reside in symbioses with tridacnids is that each symbiotic combination may have its own ecological fitness in terms of factors such as light utilisation, transfer of metabolites, and algal and animal growth rates (see Schoenberg and Trench 1980). With such a wide range of habitats in nature, not to mention those conditions present in the hatchery, it is easy to see both the utility in symbiont diversity as well as the danger of specialisation. For instance, a clam growing in symbiosis with a particular species of *Symbiodinium* on the Great Barrier Reef may exhibit much higher growth rates than the same clam and zooxanthella transferred to and grown in Tonga. On the other hand, symbioses in clams with species of *Symbiodinium* found associated with rapid growth rates in the hatchery may do very poorly in the natural habitat, a possibility that needs to be kept in mind with projects designed to restock reefs (Benzie, these proceedings). However, it is apparent that one species or type of zooxanthella can be replaced by another. In experiments using clams with two morphologically different *Symbiodinium*, one of the symbionts outgrew the other (Fitt 1985), thereby showing that the competitive exclusion principle also exists in algal symbioses and is probably continually played out in nature (Schoenberg and Trench 1980).

Observation 7. The kidneys of tridacnids are relatively large compared to those of other bivalves.

Tridacnid clams, like most bivalves, have dark-coloured kidneys lying adjacent to the digestive system. Yonge (1936, 1953, 1980) and Reid et al. (1984b) have hypothesised that the kidneys of giant clams are involved in the ultimate fate of the zooxanthellae, presumably the place where remains of 'digested' zooxanthellae ultimately accumulate. Though the kidneys of the tridacnidae seem relatively large, there are no data to support this claim except Yonge's (1980) passing account of a 101 g kidney from a 1070 g clam. Equally evident from the literature is the lack of convincing argument that the size of the kidney has anything to do with zooxanthellae symbioses. Indeed, Trench et al. (1981) found virtually no evidence of photosynthetic pigments nor their break-down products in the kidney. Electron micrographs of intact zooxanthellae in tubules passing through the kidney (ibid.), look like the tubular extensions of the gut described by Norton et al. (1993). Yet Yonge (1953) stated that 'The indigestible residue [of zooxanthellae] is then disposed of by way of the kidneys, the great size of which is probably correlated with the additional need to deal with what is essentially faecal material.' 'It is difficult not to associate this enormous organ, 1/10th of the total tissue weight with the presence of symbiotic algae' (Yonge 1980). The danger of interpreting 'correlative' data is further illustrated by Reid et al. (1984b) contention that kidney concretions of tridacnids contain oxylate and are therefore related to presumative oxylate-containing zooxanthellae (Taylor 1969), in this case ignoring the fact that oxylate is a component of human kidney stones as well as other non-symbiotic bivalves. The correlations used to relate the kidney to the symbiotic zooxanthellae made little sense when first proposed, and make even less sense now that the zooxanthellae are known to be associated intimately with the clam's digestive system.

Uptake of dissolved nutrients

A key feature of zooxanthellae symbioses is the transfer of photosynthate from zooxanthellae to the host tissues. Qualitative studies using radioactively-labelled zooxanthellae from giant clams *Tridacna* sp. showed that the sugars glycerol and glucose, and amino acids, are among the compounds translocated from algae to the host (Muscatine 1967, Streamer et al. 1988). Carbon budgets have revealed that 90–95% of the carbon fixed daily by zooxanthellae is actually translocated to the host (Muscatine et al. 1983, Davies 1984, 1991, Fisher et al. 1985, Edmunds and Davies 1986). Translocated carbon is sufficient to meet the daily energy and growth requirements of the host (Fisher et al. 1985, Klumpp 1992), except in the case of newly-metamorphosed clams, which have fewer symbionts during the early stages of infection with zooxanthellae (Fitt et al. 1986).

Giant clams are capable of acquiring dissolved nutrients from seawater, in addition to those obtained from their zooxanthellae. Intact clams will deplete seawater of inorganic nitrogen (Wilkerson and Trench 1986) and phosphorous (Belda and Yellowlees pers. comm.). Additions of ammonia and nitrate lead to increased pigmentation and division of zooxanthellae, and subsequent increases in clam growth rate (Hastie et al. 1992, Fitt et al. 1993, Braley et al. 1992). Additions of phosphate in the presence of dissolved inorganic nitrogen increases growth rate of clams (Fitt et al. 1993). Decreased calcification and altered crystal formation of calcium carbonate are observed upon additions of inorganic nitrogen and phosphorous, which may be of concern to hatcheries intending on repopulation of natural stocks of clams on reefs. Phosphate, in particular, is known to inhibit calcium carbonate crystal growth (Simkiss 1964). It has been assumed that zooxanthellae are the component

responsible for uptake and assimilation of inorganic nutrients (Muscatine 1980). However recent documentation of high concentrations of enzymes in host tissue involved in assimilating nitrogen in corals (Dudler and Miller 1988) and clams (Rees et al. 1993), have led to alternate interpretations of such data. It appears probable that host tissues are at least partially involved in the uptake of these dissolved nutrients.

Uptake of dissolved carbon compounds can apparently supplement the major photosynthate input of carbon in adult clams, including dissolved organic compounds (Goreau et al. 1973, cf. Manahan 1990). The mantle epithelium is microvillated, an adaptation for increasing surface area thought to be important in uptake of dissolved organic matter (Fankboner 1971). The relative contribution of these sources to the nutrition of giant clams is yet to be determined.

Feeding clams in mariculture

One of the most controversial topics today concerning the mariculture of giant clams is whether to feed the non-symbiotic larvae. Some hatcheries cannot achieve high enough survival rates to stay in operation without feeding (Heslinga et al. 1990), while others routinely raise larvae without ever adding any particulate foods to the cultures (Southgate, these proceedings). While regional and local changes in water quality have often been cited as responsible, important energetic considerations probably form the basis of these differences. For some as yet undetermined reason, larvae of *Tridacna derasa* and *T. gigas* reach the pediveliger stage of development in about half the amount of time in Palau (ca. 5 days) than in either Australia or Solomon Islands. Obviously stored lipid contained in eggs will only provide energy for maintenance and development for a brief period of time, and shorter developmental times enable more larvae to survive through metamorphosis. The literature shows that larger veligers (day 2–4) metamorphose sooner and more of them survive longer than smaller day 2–4 veligers (Fitt et al. 1986), suggesting that metamorphosis occurs at a characteristic size (corresponding to developmental state) and that smaller veligers will necessarily take longer to reach that size. Large veligers probably originate from large eggs, as is implied from the expected correlation between egg size and survival documented for *T. gigas* (Southgate, these proceedings). This data suggests that the basis of the differences in developmental times between larvae in Palau and the same species in Australia and Solomon Islands is due to either different egg sizes or differences in larval development rates. The latter possibility could be caused by genetically different energetic and developmental pathways or regionally different water quality. All of these hypotheses are experimentally testable, and the answers would go a long way in determining where to build future hatcheries that will be competitively viable.

When developmental time through metamorphosis extends beyond the limit of expendable lipid and carbohydrate reserves, the larvae must either be fed or die. Larval foods used successfully in mariculture of giant clams include single-celled algae, high-protein pellets, yeast, and dissolved nutrients and vitamins such as those found in yeast extract (Fitt and Trench 1981, Fitt et al. 1984, Southgate 1988, Braley 1990). While increasing survival rates, feeding also adds to the basic costs of running a hatchery, up to 20% of the operating costs when using algal foods (Braley these proceedings). Pellet foods are markedly less expensive, but problems with their availability and even minimal training and manpower needed in their storage and use will detract from the long-term competitiveness with hatcheries that do not require larval feeding.

Besides economic considerations, the major drawbacks of feeding in any mariculture operation are two-fold. First, unless cultures are maintained axenically in a laboratory, bacteria are always co-cultured with both algal foods and animals. Additions of dissolved nutrients and particulate foods will increase the bacterial load in seawater, increasing the risk of disease and accumulation of toxic by-products. Second, virtually all photosynthetic and non-photosynthetic organisms normally consume oxygen in the dark, decreasing oxygen levels proportional to their biomass and density. Dense cultures of clams, algal contaminants, food algae, and bacterial 'soups' generated upon feeding with dissolved or particulate foods all contribute to increased oxygen demand.

Bacterial growth can be controlled in three basic fashions. Dilution with clean, fresh seawater will not only reduce densities of bacteria, but also dilute any toxic substances produced, as well as increase oxygen concentration. The problem with the 'dilution-solution' is retaining larvae in a system which is being drained. In addition, many facilities may not have the capability to add enough fresh seawater to actually keep up with bacterial growth and dilute the culture. The second method involves the addition of antibiotics to cultures of larvae. Though some scientists worry that antibiotics may negatively affect larval development and survival, as yet there is no evidence of adverse affects with additions of low concentrations of antibiotics, nor selection for survival of individuals that are not necessarily the most fit to survive in hatcheries or on the

reef (Fitt et al. 1992a). Third, and by far the easiest method of controlling bacterial growth, is to reduce nutrient additions (dissolved and particulate) to larval cultures.

All of these approaches are currently used, in various combinations, in raising giant clams. Most hatcheries now move fertilised eggs to relatively small aerated tanks, with no outflow, and allow development to continue at high densities (ca. 5–20 larvae/mL) with additions of antibiotics, until at least the first few days of the veliger stage (Braley 1990, Heslinga et al. 1990, Fitt et al. 1992a, Braley, these proceedings). Larvae are fed at some hatcheries, commencing about day 3 or 4, when the veliger digestive system is complete. Feeding with particulate foods can occur in the high-density stocked larval tanks, or after transfer to the lower density settlement and juvenile grow-out tanks, with or without antibiotics. The decisions on whether to feed or not feed larvae, whether to control bacterial growth with antibiotics, when to begin water flow and how to retain larvae, and when to transfer veligers to their settlement and juvenile grow-out tank are not easy to put into a formula answer. Instead, the basis for such decisions will inevitably involve compromises that, while designed to increase survival and growth rates, are dictated by factors which may not be very controllable, such as local economics, genetics of the clam stock, hatchery facilities and local water conditions.

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The Haemolymph and Its Temporal Relationship with Zooxanthellae Metabolism in the Giant Clam Symbiosis

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TRIDACNID clams form an endosymbiotic association with dinoflagellates of the genus *Symbiodinium*, which are often referred to as zooxanthellae. Experimental investigations of this symbiosis are important for two reasons. Firstly, the symbionts provide a major source of nutrition for the clam (Muscatine 1967, Goreau et al. 1973, Trench et al. 1981, Fisher et al. 1985, Fitt et al. 1986, Summons et al. 1986, Heslinga and Fitt 1987, Streamer et al. 1988) and, consequently, an improved understanding of the association is likely to be useful for mariculture of giant clams. Secondly, the hemolymph of the giant clam is in physical proximity to the symbionts and is likely to be involved in the exchange of metabolites between host and symbiont. The latter makes the giant clams unique among alga–invertebrate associations in the potential ease with which it is possible to obtain information on the nutritional relationship between host and symbiont (Yellowlees et al. these proceedings).

Earlier evidence suggested that the symbionts are located in the hemal sinuses. However, recent and elegant histology (Norton et al. 1993) has unequivocally located the symbionts in zooxanthellar tubes which ramify the mantle tissue. The tubes are situated adjacent to the hemal sinuses, with the zooxanthellae being separated from the hemolymph by a single layer of thin cells. It is likely, therefore, that the hemolymph is an important source of metabolites for the symbionts and that metabolic activity of the symbionts will be reflected by temporal changes in the characteristics of the hemolymph. This review will outline our current knowledge of hemolymph characteristics and the influence of symbiont metabolism on these characteristics.

Hemolymph Characteristics

The giant clams have an open circulation system with no true capillaries. Arterial hemolymph leaves the heart and drains into pseudovascular spaces or hemal sinuses in the various tissues, before travelling back to the heart via the gills.

In common with the hemolymph of most bivalves, giant clam hemolymph contains amoebocytes (Yonge 1953, Trench et al. 1981) and lacks respiratory pigments. As the function of respiratory pigments is the transport of oxygen, it is likely that this requirement is decreased in giant clams because of its population of photosynthetic symbionts. In *Tridacna squamosa*, hemolymph from the adductor muscle sinus sampled during the day is supersaturated with oxygen, whereas blood from the ventricle is close to 100% saturated (Mangum and Johansen 1982). The oxygen concentration in the hemolymph of *T. gigas* during the middle of the day is consistently close to 100%

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saturated (Fitt et al. 1993, unpublished data). Moreover, *T. maxima* and *T. crocea* have high superoxide dismutase and catalase activities (Shick and Dykens 1985), suggesting that excess oxygen is a greater problem than oxygen transport.

Hemolymph contains ions, metabolites and protein. The major compositional characteristics of giant clam hemolymph are outlined below.

Major ions

The concentrations of the major ions (Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, SO_4^{2-}) present in hemolymph are very similar to that of seawater (Deane and O'Brien 1980, Rees et al. 1993).

Inorganic carbon

Hemolymph from *T. gigas* contains dissolved carbon, the concentration of which is similar to seawater (1.8–2.2 mM) at night, but decreases to 1.6 mM during the day (Yellowlees et al. 1993). At the pH of hemolymph (see below) the predominant species of inorganic carbon is the bicarbonate (HCO_3^-) ion.

pH

The pH of hemolymph is usually slightly lower than that of seawater, with values of 7.6 for *T. maxima* (Deane and O'Brien 1980) and 7.4–7.9 for *T. gigas*, with hemolymph from smaller clams having higher values (Fitt et al. 1993). The values for *T. gigas* were obtained in the middle of the day, when pH values are greatest (see below).

Phosphate

Both total and inorganic phosphate are present in *T maxima* at concentrations about three times greater than seawater (Deane and O'Brien 1980). It is likely that the hemolymph acts as a reservoir of phosphate for the symbionts, but there is no information on whether phosphate concentrations fluctuate over a diel cycle.

Ammonia ($NH_4^+ + NH_3$)

Ammonia concentrations in reef waters are typically less than 1 mM, whereas in the venous sinuses of *T. gigas* they are in the range 2–9 mM (Fitt et al. 1993). However, we have calculated that the NH_3 concentration in hemolymph is close to equilibrium with that in seawater. The higher ammonia concentration in the hemolymph can be explained by the pH gradient between the hemolymph (pH 7.4 –7.9) and seawater (pH 8.3). The proportions of ammonia present as NH_3 and NH_4^+ are determined by pH, with the proportion of NH_4^+ increasing with decreasing pH. At equilibrium (assuming no active transport of NH_4^+ by animal tissues), NH_3 concentrations in both hemolymph and seawater will be identical, but NH_4^+ concentrations in the hemolymph will be greater than seawater values because of the lower pH. If *T. gigas* is maintained in raceways with elevated ammonia concentrations (mean 23 mM) hemolymph ammonia concentrations increase to 10–32 mM (Fitt et al. 1993). The hemolymph concentration is lower than would be expected if both were in complete equilibrium. However, it is obvious from other results not reported here, that the increase in the concentration of ammonia in the hemolymph has far reaching effects on the symbiosis, which help to explain this discrepancy. An interesting observation is that hemolymph from the pericardial sac has very high ammonia levels. The implications of this observation are not clear, but it is noteworthy that the pH of this hemolymph is low (Fitt et al. 1993).

Amino acids

The concentration of the 18 amino acids present in hemolymph from *T. maxima* is 873 mM, with taurine, glycine and alanine accounting for 68% of the total (Deane and O'Brien 1980). Urea, uric acid and creatine were not detected.

Protein

Hemolymph contains protein at concentrations in excess of 3.5 mg/mL in *T. maxima* (Deane and O'Brien 1980) and 250 mg/mL in *T. gigas* (Rees et al. 1993). The reason for this difference in protein concentration is not known, though the different methods of sampling, preparation and assay used may account for the difference. Alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase activities are present in hemolymph from *T. maxima* (Deane and O'Brien 1980). Carbonic anhydrase activity has been detected in the hemolymph from *T. gigas* (Yellowlees et al. 1993). Though the activity is less than that present in gill and mantle tissues, it is nevertheless significant. Whether the enzyme originates from one of these tissues or is specifically secreted into the hemolymph, however, is not clear.

The major protein in the hemolymph of giant clams is a lectin, tridacnin (Uhlenbruck et al. 1975). This protein interacts specifically with galactose residues in either polysaccharides or glycoproteins. The favoured epitope for tridacnin from *T. maxima* is adjacent, β-1,6-linked D-galactose residues (Eichmann et al. 1976). While the specificity for D-galactose is common to all tridacnins examined so far, different absolute specificity obviously exists, as demonstrated by their different hemagglutination patterns with a range of

mammalian erythrocytes (Uhlenbruck et al. 1978). The molecular weight of tridacnins is in the range 300 000 to 500 000, with the subunit molecular weight, based on SDS-polyacrylamide gel electrophoresis, being between 22 000 and 44 000 (Baldo et al. 1978). The tissue source of tridacnin is not known, nor is their biological role. While Uhlenbruck and Steinhausen (1977) have speculated that they may be involved in the symbiotic association, the physical separation of these large proteins from the zooxanthellar tubes makes this suggestion unlikely. Alternatively, they may, as has been postulated for other mollusc lectins, have a role in a defence-like mechanism.

Glucose and glycerol

Glucose and glycerol are present in hemolymph but the concentration of the latter in *T. gigas* does not exceed 4 mM (Rees et al. 1993). In contrast, glucose is present at 500 mM in *T. maxima* (Deane and O'Brien 1980) and reaches a maximum concentration in excess of 600 mM in *T. gigas* (Rees et al. 1993).

Effects of symbiont metabolism on hemolymph characteristics

There is a strong correlation between zooxanthellae density and hemolymph volume in *T. gigas*, with 4.5×10^6 algae/mL hemolymph (Fitt et al. 1993). This relationship suggests that there is a functional link between hemolymph and the symbionts in the zooxanthellar tubes. Moreover, the symbionts in the mantle are separated from the hemolymph by a single layer of cells. Therefore, it is likely that symbiont metabolism will affect hemolymph characteristics and recent experimental evidence which supports this assertion is outlined below.

Algal symbionts in the giant clams are actively photosynthetic and release photosynthate to the host (Muscatine 1967, Goreau et al. 1973, Trench et al. 1981, Fisher et al. 1985, Summons et al. 1986, Streamer et al. 1988). The relevant aspects of these characteristics, assuming glucose is the major photosynthetic end-product (Streamer et al. 1988, Rees et al. 1993), can be summarised by the following formula,

$$6HCO_3^- + 6H_2O \Leftrightarrow C_6H_{12}O_6 + 6O_2 + 6\,OH^- \quad (1)$$

As the pH of hemolymph is greater than 7, the major form of inorganic carbon is bicarbonate, but the species transported into the zooxanthellae (Yellowlees et al. 1993) and fixed by ribulose bisphosphate carboxylase is carbon dioxide. The net result is that symbiont photosynthesis should be accompanied by a decrease in the concentration of inorganic carbon and an increase in the pH of the hemolymph. There should also be an increase in the concentration of the major photosynthetic release product. Consequently, these changes in the concentration of metabolites in the hemolymph would be expected to show a diel cycle and to be prevented in continuous darkness.

During the day inorganic carbon concentrations in the hemolymph decrease from values similar to seawater (1.8–2.2 mM) to 1.6 mM (Yellowlees et al. 1993). This decrease occurs despite the ability of hemolymph to equilibrate rapidly with any changes in seawater inorganic carbon concentration (Yellowlees et al. 1993). Taken together, these lines of evidence suggest considerable utilisation of inorganic carbon by zooxanthellae in the giant clam symbiosis. A simple calculation serves to indicate the demand for inorganic carbon by symbionts in *T. gigas*. The gross photosynthetic rate varies from 2–10 pmole/zooxanthella per hour (Fisher et al. 1985). Assuming a PQ of 1, and 4.5×10^6 zooxanthellae per mL hemolymph (Fitt et al. 1993), this photosynthetic rate would correspond to 9–45 μmole inorganic carbon/mL hemolymph per hour. In other words, the symbionts would deplete 2 mM inorganic carbon in the hemolymph in 3–13 minutes, unless it is replaced by either host metabolic activity and/or seawater inorganic carbon.

In addition to a diel cycle in dissolved inorganic carbon concentration, hemolymph in *T. gigas* also shows a similar, but inverse diel cycle in pH (Fitt et al. 1993). In the morning, the pH increases rapidly by 0.5 units to reach a peak at 12.00 noon, decreases in the afternoon and slowly regains the original pH value during the night. The increase in pH is probably due mainly to the decrease in inorganic carbon concentration, with the extent of the pH change being determined by the buffering capacity of the hemolymph. Assuming the changes in pH are solely a consequence of changes in inorganic carbon, they can be summarised by the following formula,

$$CO_2 + H_2O \Leftrightarrow HCO_3^- + H^+ \quad (2)$$

If CO_2 is taken up from the hemolymph by the zooxanthellae, the equilibrium of this reaction will be displaced and consequently HCO_3^- will react with a proton so that the equilibrium is adjusted to its original value. This will cause a net increase in the pH of the hemolymph. The rate of the reaction would be enhanced by carbonic anhydrase, which is present in high concentrations in the mantle (Yellowlees et al. 1993). Alternatively, HCO_3^- could be transported via the cells surrounding the zooxanthellar tubes. A combination of a sodium-coupled bicarbonate transport and maintenance of the electrochemical gradient by a Na^+/H^+ antiporter, would also result in an increase in hemolymph pH.

According to formula (1) symbiont photosynthesis should also be accompanied by an increase in the concentration of the photosynthetic release product(s). Previous research has indicated two possible release products: glycerol (Muscatine 1967); and glucose (Streamer et al. 1988). Glucose concentrations in the hemolymph of *T. gigas* show a diel cycle, with a 3.2-fold increase during the day; in contrast, glycerol concentrations are consistently low (Rees et al. 1993). Moreover, hemolymph from clams maintained in continuous darkness does not show any increase in glucose concentration (Rees et al. 1993). These data suggest that glucose is the major photosynthetic release product of symbionts in the giant clam symbiosis, but the evidence for this is not conclusive (see Rees et al. 1993 for a fuller discussion).

A summary of metabolites which show diel cycles in hemolymph of *T. gigas* is given in Table 1.

Table 1. Summary of metabolites whose concentrations show a diel cycle in *Tridacna gigas*

Metabolite	Increase/decrease during day
Glucose	Increase
Inorganic carbon	Decrease
pH	Increase

Implications for mariculture

There is a frequent requirement for methods of obtaining information on the health of any farmed organism. Obtaining samples of hemolymph from giant clams by syringe is a simple, effective and non-destructive method (Rees et al. 1993), with the potential for deriving useful information in the mariculture of clams. For example, hemolymph from healthy clams receiving adequate illumination should show a diel cycle in glucose concentration.

Conclusions

The giant clam symbiosis offers a number of experimental advantages in the study of alga–invertebrate associations (Yellowlees et al. these proceedings). These include the proximity of the hemolymph to the symbionts in the zooxanthellar tubes and the involvement of the hemolymph in the exchange of metabolites between host and symbiont. In addition, the ability to take samples of hemolymph by syringe (Rees et al. 1993) offers a useful approach to the study of temporal changes in the concentration of hemolymph metabolites. This review has highlighted a number of these temporal changes. The further experimental study of hemolymph and its role in the giant clam symbiosis should prove invaluable in enhancing our understanding of alga–invertebrate associations.

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Growth and Survival of *Tridacna gigas* Larvae: the Role of Exogenous Nutrition

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GIANT clams (Family Tridacnidae) are relatively new culture subjects. As such, initial larval rearing methods were based on those used for more traditional culture species, such as oysters. This protocol included the provision of micro-algae as a larval food source (Crawford et al. 1986, Heslinga and Fitt 1987). However, Fitt et al. (1984) showed that tridacnid larvae were able to complete metamorphosis in the absence of particulate food, although fewer spat resulted from batches of unfed larvae. More recently, following studies with *Tridacna derasa*, Heslinga (1988) questioned the necessity of exogenous particulate food for tridacnid larvae, and proposed that feeding may, in fact, be harmful to larvae. In Palau, larvae of *T. derasa* and *Hippopus hippopus* are now reared routinely without provision of particulate feeds (Heslinga et al. 1990).

In contrast, Gwyther and Munro (1981) showed that unfed *T. maxima* larvae were unable to complete metamorphosis unless provided with food, and similar results were found for *T. gigas* (Southgate 1988). At present, provision of particulate food is standard practice for rearing the larvae of *T. gigas, T. maxima, T. crocea* and *H. hippopus* in hatcheries in Australia, the Solomon Islands and throughout the south Pacific (Braley 1990, Usher 1990, J. Barker pers. comm.). Clearly, the issue of larval feeding has important economic implications for production of tridacnid seed.

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This study examines the importance of exogenous nutrition for larval growth and spat production of *T. gigas*.

Materials and Methods

Two aspects of larval growth were assessed, namely; shell and tissue growth of fed and unfed larvae, and the effect of larval size at settlement on production of 30-day-old metamorphosed juveniles.

Larval growth

Larval growth studies were conducted at both the ICLARM Coastal Aquaculture Centre (CAC) in Solomon Islands and James Cook University's Orpheus Island Research Station (OIRS), Australia. *T. gigas* broodstock were induced to spawn using serotonin injection (Braley 1985). Larvae were reared in 1 µm filtered water at a density of 5 /mL. Fed larvae received cultured micro-algae (*Isochrysis* aff. *galbana* clone T-ISO) at 2×10^4 cells/mL/day from day 3 to settlement (day 10). Water was changed every two days. At regular intervals during development, some larvae were preserved in 4% (v/v) formalin for subsequent shell measurements of 50 larvae. At three and 10 days post-fertilisation, some larvae were removed onto 53 µm nytex mesh, washed briefly with 0.9% (w/v) ammonium formate solution and freeze-dried for dry weight and ash determinations. Larval dry weights were measured on a Cahn 21 Electrobalance from triplicate determinations of 50–60 larvae. Ash was

determined as that remaining after heating for 4 hours at 500°C (Southgate et al. 1992b). Ash-free dry weight (AFDW) was calculated as the difference between dry weight and ash weight.

Relationship between larval size at settlement and spat production

Two cohorts of *T. gigas* larvae were reared at the CAC as described above, and either fed (as described above) or unfed. At 10 days post-fertilisation, when algae-fed larvae were pediveligers, both fed and unfed larvae were inoculated with zooxanthellae, counted, and placed into 1 litre plastic containers at a density of 1 larva per 1.6 mm^2 bottom area. The containers had 120 µm polyester mesh sides to allow through-flow of water. Two replicate containers for fed and unfed larvae from each cohort were suspended in shallow race-ways receiving 10 µm filtered sea water. The mesh was washed every two days to prevent algal fouling and maintain water flow. After 30 days, the number of juveniles in each container was counted and the mean percent survival calculated.

Results

Unfed larvae of *T. gigas* from both OIRS and the CAC were less well developed than fed larvae of the same age. The foot of unfed larvae was poorly developed and no settlement behaviour was evident at the time when fed larvae were showing settlement behaviour. Non-feeding appeared to slow larval development and extend the time to settlement. Shell growth of fed and unfed *T. gigas* larvae from OIRS and the CAC are shown in Figures 1 and 2. Morphometric data for *T. gigas* larvae are shown in Table 1. Larvae provided with micro-algae grew more rapidly and had greater shell length, dry weight and AFDW (organic content) at settlement (10 days) than unfed larvae of the same age. Although some unfed larvae from both CAC cohorts survived through metamorphosis to 40 days post-fertilisation, larvae fed micro-algae produced 10.8 and 2.7 times more juveniles than unfed controls. Survival to one month post-settlement was significantly ($P < 0.01$) correlated with both larval shell length and dry weight at settlement (Figs. 3 and 4); however, greater correlation was shown with shell length.

Discussion

A number of previous studies have shown that tridacnid larvae provided with micro-algae show greater shell growth than unfed larvae (Gwyther and Munro 1981, Fitt et al. 1984, Southgate 1988). The data presented above shows that larvae of *T. gigas* fed micro-algae also achieve greater dry weight and organic content (AFDW) than unfed larvae of the same age. Growth of larvae of *T. gigas* is maximised by providing exogenous particulate nutrition.

Larvae receiving micro-algae had greater organic content at day 10 than unfed larvae. This may indicate the presence of greater endogenous energy reserves which, in turn, allow greater survival through metamorphosis and during early spat growth. In contrast, unfed larvae must utilise a greater portion of their endogenous reserves to fuel development which, apparently, reduces survival over this period. It is likely that fed larvae, being larger than unfed larvae, may metamorphose into larger juveniles and they may also have greater energy reserves to dedicate to post-metamorphic growth.

Planktotrophic larvae require an obligate feeding period to achieve metamorphic competence. Larvae of

Table 1. Growth of algae-fed and unfed *Tridacna gigas* larvae from Orpheus Island Research Station (OIRS), Australia, and the Coastal Aquaculture Centre (CAC), Solomon Islands (mean ±SD).

Age (days)	Shell length (µm)	Dry weight (ng)	AFDW (ng)
CAC			
3	183.63 (±2.95)	635.0 (±22.1)	230.0 (±10.7)
10 (fed)	214.40 (±6.24)	1175.0 (±30.2)	368.0 (±23.2)
10 (unfed)	189.63 (±2.86)	750.0 (±41.8)	200.0 (±17.9)
OIRS			
3	174.30 (±3.57)	542.2 (±38.5)	208.7 (±12.8)
10 (fed)	190.20 (±5.28)	784.0 (±45.8)	275.0 (±15.6)
10 (unfed)	179.04 (±4.46)	679.0 (±28.7)	220.0 (±14.2)

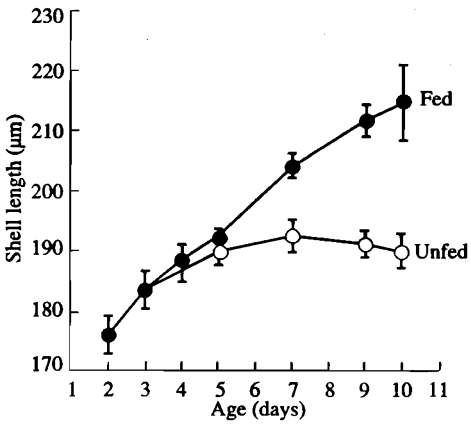

Figure 1. Shell growth (mean ± SD) of *Tridacna gigas* larvae from Orpheus Island Research Station (OIRS), Australia.

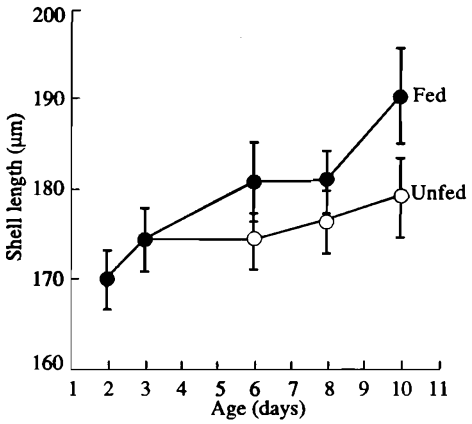

Figure 2. Shell growth (mean ± SD) of *Tridacna gigas* larvae from the Coastal Aquaculture Centre (CAC), Solomon Islands.

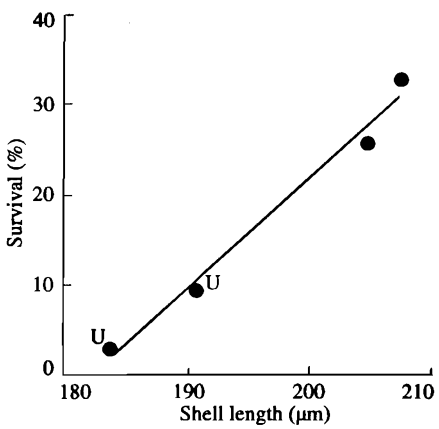

Figure 3. Relationship between mean larval shell length at 10 days post-fertilisation and mean survival to 40 days post-fertilisation. (Y = 1.2057 X–219.376, r = 0.9927). U signifies juveniles resulting from unfed larvae.

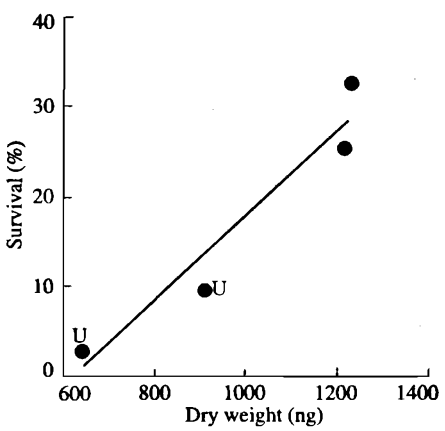

Figure 4. Relationship between mean larval dry weight at 10 days post-fertilisation and mean survival to 40 days post-fertilisation. (Y = 0.0474 X–29.769, r = 0.9614). U signifies juveniles resulting from unfed larvae.

T. gigas are demonstrable planktotrophs (Fitt et al. 1984) and are capable of digestion and assimilation of ingested nutrients (Southgate 1988), however, feeding appears to be facultative and, although not obligatory for successful metamorphosis, is required for maximal larval growth and spat yield. It is reasonable to assume that under natural conditions, tridacnid larvae utilise available particulate matter.

As well as particulates, tridacnid larvae also utilise dissolved organic matter (DOM), which may contribute significantly towards larval nutrition (Fitt et al. 1984). It is likely that unfed larvae may, in fact, receive an exogenous energy input in the form of DOM. The increase in the AFDW of unfed larvae from OIRS (Table 1) would seem to support this. Thus, the DOM load of the culture water may influence the number of spat resulting from a given batch of unfed larvae.

The major factor likely to determine the survival of unfed tridacnid larvae, is the extent of their maternally-derived energy reserves. This may differ between species, and between different larval cohorts due to natural variations in egg quality. Egg quality itself, will be influenced by factors such as broodstock holding protocol, season and latitude. Lannan et al. (1980)

proposed that within the gametogenetic cycle of bivalves, there exists a period when eggs of optimal quality are produced. In tridacnid culture, where many spawnings are chemically induced, the nature of this induction in over-riding natural chemical control of spawning may result in eggs of sub-optimal composition. Variations in egg/larval quality may explain the contrasting reports between studies in which unfed larvae were unable to complete metamorphosis (Gwyther and Munro 1981, Southgate 1988) and those in which unfed larvae successfully metamorphosed (Fitt et al. 1984, Heslinga et al. 1990).

In tropical countries at relatively high latitudes, such as Australia, the spawning season of tridacnid clams is largely restricted to summer months (Braley 1988, Nash et al. 1988, Shelley and Southgate 1988). As such, juvenile production in hatcheries must be maximised during this period. The data presented above shows that adequate larval nutrition is important in maximising production of *T. gigas* spat. This has important economic implications for tridacnid culture. Although micro-algae may cost up to A$413 /kg dry weight to produce (Southgate et al. 1992a), food costs can be reduced by using 'off the shelf' feeds such as microencapsulated diets, dried micro-algae and yeast (Southgate 1988, Braley 1990, Usher 1990). These products have a relatively low cost and require minimal labour input. For example, the cost of feeding a mixed diet of commercially available microcapsules (Frippak, U.K.) and dried *Tetraselmis* (Cell Systems Ltd., UK) to *T. gigas* larvae has been estimated at US$50 per cohort (Usher 1990). The reduced time to settlement and the greater number of spat produced, would seem to justify the cost of providing exogenous nutrition.

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Metamorphic Change in Tridacnids: Implications for Culture Methods

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THE time of settlement and metamorphosis of pelagic larvae is a critical stage in the life history of many benthic marine invertebrates, involving a dramatic, integrated series of behavioural, physiological, biochemical and morphological changes. Such major changes mean that functions such as feeding, are suspended during metamorphosis (Cole 1938, Hickman and Gruffydd 1971, Holland and Hannant 1973) with larvae reliant on stored energy reserves of neutral lipid (Holland and Spencer 1973) or protein (Utting 1986, Rodriguez et al. 1990). Bivalve metamorphosis is also characterised by a rapid decrease in larval condition, due to a decrease in tissue weight relative to shell growth (Lucas and Beninger 1985, Lucas et al. 1986). Because of the extent of these morphological and physiological changes metamorphosis is recognised as a time of extreme stress in bivalves.

Despite the overall similarities in metamorphic change in different species of bivalves, several different morphological criteria have been used to define the period of metamorphosis. King (1986 p.33) provides a general definition, reflecting the integrated nature of metamorphic change as that of the 'morphological, behavioural and physiological changes occupying a brief period between the onset of velar loss and secretion on the juvenile byssus and dissoconch shell'.

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Metamorphosis in cultured bivalves is typically a period of high mortality (Sastry 1965, Gwyther and Munro 1981). Reliance on stored energy reserves during metamorphosis means that nutritionally inadequate larvae are less able to survive the stress of metamorphosis than those in good condition. Nutritional condition is largely determined by the amount and type of food provided (Utting 1986, Whyte et al. 1987, Southgate 1988) but also varies with egg condition which is a function of broodstock condition (Helm et al. 1973, Bayne et al. 1975). Furthermore, an enforced delay in metamorphosis may occur due to detrimental culture conditions, such as low temperature (LaBarbera 1975, Sprung 1984) or the lack of a suitable substratum (Bayne 1965, Birkeland et al. 1971) thus reducing larval vigour and increasing mortality.

Studies of larvae of tridacnid clams during metamorphosis suggest similar morphological, physiological and behavioural changes occur in these species, as in other bivalves (LaBarbera 1974, 1975, Jameson 1976). One significant difference however, is the presence of symbiotic dinoflagellate, *Symbiodinium microadriaticum* (zooxanthellae) in post-larval tridacnids. Zooxanthellae are not present in the eggs of tridacnids and must be obtained exogenously (LaBarbera 1975, Jameson 1976, Fitt and Trench 1981). While larvae do not require zooxanthellae for successful metamorphosis (Fitt et al. 1984), cultured larvae are usually inoculated with zooxanthellae a few days prior to settlement (Fitt and Trench 1981, Fitt et al. 1984, Crawford et al. 1986). Ingested zooxanthellae reside in the gut until metamorphosis, when they are

observed to move to the developing mantle tissue (Fitt and Trench 1981, Fitt et al. 1984). As in other cultured bivalves, high mortality also occurs during metamorphosis in tridacnids (LaBarbera 1975, Jameson 1976, Gwyther and Munro 1981, Braley 1986, Crawford et al. 1986).

In order to reduce the high level of mortality that occurs at metamorphosis in cultured tridacnids, it is necessary to have a greater understanding of the changes occurring during this period. This study describes the physiological and biochemical changes in tridacnid larvae during metamorphosis and post-larval survival on different substrates.

Materials and Methods

Culture conditions

Larvae were obtained from serotonin induced spawnings of *Hippopus hippopus* (Braley 1985) and reared by the methods of Crawford et al. (1986). Post-larvae were reared in round polypropylene vessels (diameter 95 mm, height 80 mm). To facilitate water flow two windows, 50 mm × 120 mm were cut in the walls of the containers and fitted with 125 μm nylon mesh to facilitate water flow. Rearing vessels were placed in plastic freezer trays (Crawford et al. 1986) which were floated in 3000 L fibreglass tanks situated outdoors at Orpheus Island Research Station (OIRS) (18°40'S 146°30'E). Vessels were removed from the trays twice weekly and the window meshes cleaned with filtered seawater.

Biochemical analysis

Samples for biochemical and morphometric analysis were collected immediately pre-settlement (while normal larval swimming behaviour was evident) (day 10) and at approximately 14-day intervals after settlement. Pre-settlement larval samples comprised a sample of ca. 6000 live animals from the entire larval culture. Post-settlement samples were obtained by isolating 200–500 live individuals. Samples were washed on a 88 μm mesh sieve with 1 μm filtered sea water and distilled water, frozen, then freeze-dried. Dry weights of larvae and post-larvae were determined from triplicate weighings of 10–40 individuals on a Cahn 21 Automatic Electrobalance. Ash content was determined as that remaining after heating samples to 500°C for four hours. Ash free dry weight (AFDW) was defined as the difference between ash weight and dry weight.

Proximate biochemical composition of samples was determined from 0.5–10 μg of freeze-dried sample analysed by a sequential fractionation procedure based on the methods of Shibko et al. (1967). Carbohydrate was solubilised in 70% perchloric acid and determined by the methods of Dubois et al. (1956), using D-glucose as the standard. RNA was extracted from the precipitate by alkaline hydrolysis, using 0.3N NaOH and determined by the methods of Holland and Hannant (1973) using yeast RNA as the standard. The precipitate was then subjected to hot acid extraction of DNA in 1.5N perchloric acid at 90°C (Shibko et al. 1967) and DNA determined by the methods of Buckley (1979) using calf thymus DNA as the standard. Corrections for peptide contamination of nucleic acids, recommended by Munro and Fleck (1966) were also made. Lipid was then extracted from the precipitate by methods of Shibko et al. (1967) and determined by charring methods of Marsh and Weinstein (1966) using tripalmatin as the standard. Neutral lipid content was determined from an aliquot of the supernatant, following treatment with activated silicic acid (Holland and Hannant 1973). Protein content of the precipitate was then determined by the method of Lowry et al. (1951), using bovine serum albumen as the standard. The caloric content of post-larvae was estimated from the biochemical composition using caloric equivalents of 8.42 cal/mg, 4.3 cal/mg and 4.1 cal/mg for lipid, protein and carbohydrate respectively (Beukema and DeBruin 1979).

Substrate effects

The effects of six substrata on the survival of *Hippopus hippopus* post-larvae from settlement at day 10 to day 31 was also investigated. Rearing vessels of each substrate type were used and were stocked with 4000 larvae (0.63 larvae mm^{-2}). Duplicate vessels were used for each substrate. Cement sheet (fibro), fibro with attached carborundum (mullite) beads (Crawford et al. 1986), and plastic splasher pool liner—the material in routine use for larval settlement at OIRS—were presented as discs placed in the bottom of rearing vessels. Other treatments were the abrasion of the container base with medium grade sandpaper, similar to the treatment employed by Gwyther and Munro (1981) and provision of a 2.0 cm layer of coral rubble (0.5–2.0 cm pieces). The untreated bottom of the rearing vessel served as the control substratum. To reduce the likelihood of any settlement attractants remaining on previously used surfaces, all materials were washed in 1% chlorine solution, soaked in freshwater for 24 hours and air dried, prior to use. On day 31, post-larvae were collected by scrubbing the substrate with a soft plastic brush and washing the contents onto a 88 μm nylon mesh screen. In the case of the coral rubble, individual pieces of rubble were

washed in a 88 μm sieve then scrubbed. The contents of each container was preserved immediately in 4% formalin and survival estimated.

Results

Total dry weight of larvae from both spawnings increased approximately five-fold from day 10 to day 25 but growth rate declined after day 23 in spawning no.1 (Fig.1). Increase in ash content (shell weight) accounts for most of this increase, with little or no observed increase in AFDW (tissue weight). This is also reflected in a decrease in relative organic matter (AFDW/total dry weight) by more than 50% over the post-settlement period in both batches of larvae. An additional index of growth (the RNA:DNA ratio) calculated on a weight basis, also decreased, from 5–10 at day 10 to less than 1 in post-settlement samples, also consistent with a reduction in growth rate. These results are shown in Table 1.

Table 1. Biochemical composition and morphometric parameters of *Hippopus hippopus* post-larvae.

Spawning	#1			#2	
Age (days)	10	23	37	10	23
Total dry weight (g)	0.96	6.22	6.25	0.93	3.52
Ash free dry weight (AFDW) (μg)	0.59	1.78	1.42	0.39	0.50
Energy content (cal/post-larva × 1000)	3.63	8.44	6.96	1.91	2.62
Protein					
% Organic matter	36.41	29.92	65.23	36.25	56.17
% Caloric content	25.36	21.60	57.04	28.44	46.12
Carbohydrate					
% Organic matter	12.67	16.70	9.51	23.93	8.18
% Caloric content	8.42	11.49	7.93	17.90	6.40
Total lipid					
% Organic matter	48.54	47.33	20.45	34.92	29.55
% Caloric content	66.22	66.91	35.03	53.65	47.49
RNA					
% Organic matter	2.37	6.05	4.81	4.89	6.10
Neutral lipid					
% Organic matter	0.00	41.33	14.61	4.72	22.82
% Total lipid	0.00	87.33	71.43	13.51	77.24
Phospholipid					
% Organic matter	48.54	6.00	5.84	30.20	6.73
% Total lipid	100.0	12.67	28.57	86.49	22.76
Energy content (cal/mg organic matter)	7.16	6.85	6.01	5.62	6.30
% Ash	38.40	71.42	77.33	58.04	85.37
Relative organic matter					
(AFDW/total dry weight)	0.616	0.286	0.227	0.420	0.146
RNA:DNA	7.93	1.17	0.80	5.21	0.44

Biochemical composition

The amounts of each biochemical constituent were determined as a percentage of the total organic matter which was defined as the sum of amounts of protein, total lipid, carbohydrate and RNA (Holland and Spencer, 1973). These data were also used to estimate the composition on an individual basis, the changes in biochemical composition observed from day 10 to day 23 clearly differed between batches. Similarities exist between changes in the final sampling phases however, when tissue growth was minimal. During this time, relative protein levels increased from 35–40% to 60–70%, as did absolute values. There was a corresponding decrease in relative lipid levels, from 30–50% to 20–30%. However, in larvae from both spawnings there was an increase in the amount of lipid per post-larva due to an increase in the amount of neutral lipid and there were only minor changes in carbohydrate levels. There was a shift in partitioning of energy during post-larval development with a greater proportion of energy available as protein after settlement and a corresponding reduction in energy in lipids. Total energy content calculated on an individual or weight basis largely reflects the amount of lipid present (Table 1).

Substrate effects

Significant differences were found in the trial investigating survival of *H. hippopus* from day 10 to day 31 on six substrata (Fig. 2). The data were analysed by one way analysis of variance (ANOVA) and significant differences were found in survival between the treatments ($F_{6,11} = 65.76$ $P<0.0001$). A comparison of means by the protected least significant differences (PSD) method (Snedecor and Cochran 1971) showed that survival on carborundum was significantly greater than in any other treatment, also that survival in the rough treatment was significantly less than in both the carborundum and the liner treatments. No significant differences were found between any other of the treatments.

Discussion

The sharp increase in shell growth and decrease in relative organic matter (ROM) following settlement in *H. hippopus* has also been reported for other bivalves (Holland and Spencer 1973, Lucas et al. 1986, Utting 1986, Rodriguez et al. 1990). This phenomenon may thus be a feature of post-settlement growth in bivalves. However the subsequent decline in shell growth as observed here was not reported in other studies. In both batches of post-larvae there was a period of reduced tissue growth, concurrent with a reduction in stored neutral lipid levels, an increase in protein levels and a net reduction in energy content. These changes are consistent with those expected of individuals having undergone morphological changes at metamorphosis, when stored reserves are catabolised and new tissues

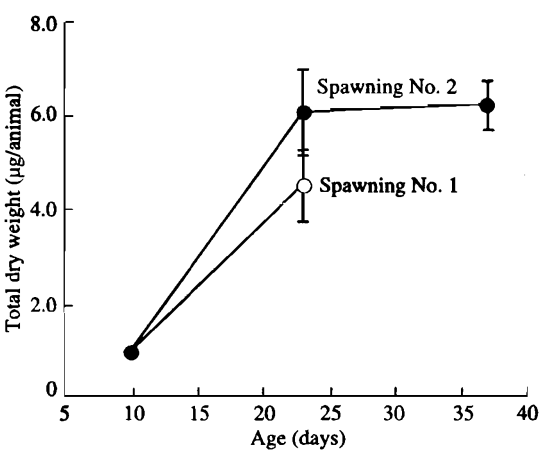

Figure 1. Total dry weight of *Hippopus hippopus* post-larvae from two spawnings.

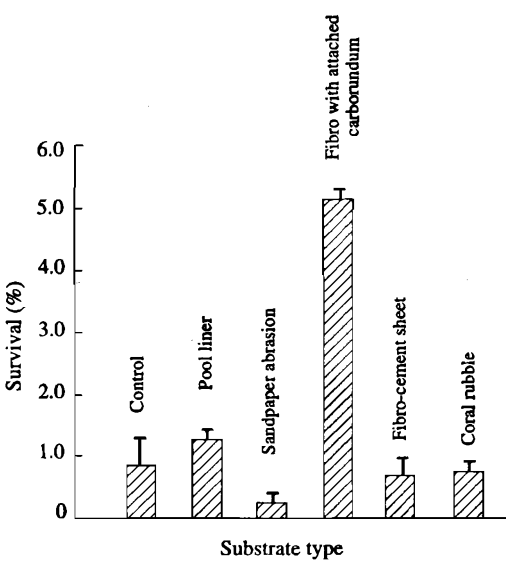

Figure 2. Survival of *Hippopus hippopus* post-larvae on six substrates from day 10 to day 31 (mean ± range).

produced. Despite such changes being therefore normal, they indicate that metamorphosed *H. hippopus* post-larvae then enter a period of nutritive stress.

The timing of these changes did vary between batches of larvae in that they were apparent earlier in post-larvae from spawning no.2. This may be a reflection of differences in levels of endogenous reserves between batches, indicating the larvae from spawning no.2 to be less nutritionally competent. Some evidence of this may be derived from lower survival of post-larvae from spawning no.2 (unpublished data). Such trends are consistent with the findings of Southgate (1990) who found a positive correlation between larval dry weight at settlement and survival of *T. gigas*.

While zooxanthellae are present in newly settled tridacnid larvae (Fitt and Trench 1981, Fitt et al. 1984) their energetic contribution is not known. Large numbers of zooxanthellae are not visible until two to three weeks after settlement (Fitt et al. 1984). It is therefore unlikely that newly settled larvae are significantly phototrophic. Newly settled tridacnid larvae may feed by using pedal movement and/or pedal and ctenidial cilia (Reid et al. 1992). and morphological data suggest that post-larvae should be competent to feed by ctenidial filter feeding within two to three weeks of settlement. Current standard rearing practice at OIRS involves cessation of feeding at settlement and rearing in filtered water for several weeks. Clearly if post-larvae are not phototrophic at this stage, there are likely to be adverse effects. Provision of particulate feed to newly settled tridacnid larvae may provide a means of reducing nutritive stress associated with settlement and metamorphosis and thus increase survival.

The type of substrates supplied to larval *H. hippopus* can significantly affect survival through metamorphosis and early post-larval life. This may occur by enhancement of settlement behaviour through the provision of appropriate stimuli for settlement, or by physical conditions affecting post settlement survival. Of the substrates tested in this study, carborundum (mullite) beads glued to the surface were the most suitable for *H. hippopus*. Significantly higher survival of *H. hippopus* on carborundum is consistent with the results of trials conducted at OIRS with *T. gigas* post-larvae (ACIAR 1986, 1987, Braley et al. 1988). Splasher pools are now standard for rearing post-larval and juvenile *T. gigas* at OIRS. Data presented here indicate that pool liner is, however, only a moderately successful surface in terms of survival.

The substrates tried here are not exhaustive nor do they provide data on specific characteristics which affected survival, therefore no clear criteria exist for new types of substrates that may be employed. Substances most effective for other cultured bivalves are based on data from natural settlement materials. However, the paucity of data on the distribution of newly settled, non-burrowing tridacnids preclude this option for these species. Based on the results of this study, it is possible that a granular substratum like carborundum may be suitable for *H. hippopus* larvae and a layer of fine sand on the bottom of splasher pools may be a cost effective means of increasing post-larval survival. Development of substrates suitable for other tridacnid species however, should be undertaken on an individual species basis, given the variety of habitats occupied by tridacnids.

The morphological, behavioural and biochemical changes that occur during settlement and metamorphosis in tridacnids reflect the similarities in larval biology between tridacnids and other bivalves. Proposed recommendations for rearing of the tridacnid post-larvae involve elements of standard rearing practices for other bivalve species. The development of heterotrophic techniques for the mass culture of newly settled tridacnids, involving particulate feed or dissolved nutrients is seen as necessary to improve survival and may also allow post-larval rearing in conditions of reduced light and alleviate problems of filamentous algal growth in tanks. Increased survival on certain substrates represents a non-laborious means of improving nursery production and the development of optimum substrates for tridacnids may significantly improve tridacnid rearing practices.

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Total Dehydrogenase Activity Reflects Oxygen Consumption Rates in the Giant Clam *Tridacna maxima* (Bivalvia: Tridacnidae)

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RESPIRATION is defined as an energy yielding process by living systems that degrade organic matter beyond the point of immediate biological utility (Packard et al. 1984). The rate and amount of energy extracted from the process varies with the type of organic material degraded, the degree of oxidation, the biological system and its environment. Energy derived from this process is utilised for survival strategies such as growth, reproduction, and locomotion. The usual way to evaluate respiration is to use oxygen consumption rate. This is the most convenient parameter to quantify and the only one determined in situ.

The use of oxygen consumption rates as an index of respiration is inadequate because of the dependence of such rates on the fluctuations in pH, temperature, tidal cycles, season, dissolved oxygen levels, organic pollution and organic content of the water (Pamatmat and Bagwat 1973). There is a need to complement the oxygen method with other methods advocated by various workers.

The use of total dehydrogenase or electron transport system (ETS) activity and translation of these to oxygen consumption is one alternative method. The basis of this approach, pioneered by terrestrial microbiologists Lenhard (1956) and Stevenson (1959), is the fact that by far the largest portion of energy turnover in aerobic organisms depends on the activities of dehydrogenases, which feed electrons into the respiratory chain. The activities of dehydrogenases must represent a measure of the electron transport capacity of the organism proportional, but not equivalent to, the actual rates of oxygen consumption.

To date, the method was found to provide a reliable estimate of the zooplankton component of oxygen consumption in the sea based on *in vitro* respiration of the copepod *Calanus pacificus* (Owens and King 1975). Other authors have established a direct correlation between ETS activity and oxygen consumption rates based on the study of pure cultures of various marine phytoplankton species (Kenner and Ahmed 1975 a,b). These studies assume ETS activity to be a measure of the maximum or potential oxygen consumption capability of the organism.

The results presented in this paper are from comparisons between oxygen utilisation rates and ETS activity in the giant clam *Tridacna maxima* from the same batch (see also Ablan 1991). Clams from different age groups were analysed.

Materials and Methods

Cultured clams

All clams used in this study were obtained from a single batch of *T. maxima* clams spawned in April 1990. Seventy one individuals were analysed in January 1991 when the clams were nine months old. Another set of analyses were conducted on 40 clams from the same batch a year later. The clams were all spawned and reared at the Silliman University Marine Laboratory in

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Dumaguete, Negros Oriental. A Manostat Dial caliper (± 0.1 mm) was used to measure shell lengths while a Mettler 160 AE balance (± 0.0001 g) was used to obtain flesh weights.

Respiration rates

Sea water used in each experiment was filtered and autoclaved prior to use. Methods for measuring oxygen consumption rates were based on those of Fisher et al. (1985). For the first group of 71 clams, respiration rates were recorded as the difference between initial and final dissolved oxygen content of a given volume of sea water as measured by a YSI Model 58 dissolved-oxygen electrode and meter. A single clam was enclosed in a previously blackened, airtight 50 mL flask and oxygen content measured before and after a 30 minute incubation period. The same water in the container without the clam was run as a blank prior to incubation. Respiration rates for the second group of 40 clams were measured using a Strath Kelvin oxygen meter and probe attached to a chart recorder. The water was constantly stirred using a magnetic bar and stirrer while temperatures were maintained at 25°C and salinity at 32 ppt in both setups.

ETS activity

ETS activity was measured according to the method of Owens and King (1975). Total protein content per ml homogenate sample from individual clams was determined using the modified Lowry method (Hartree 1979) and was used to calibrate ETS activity. The values obtained were translated to weight-standardised oxygen consumption rates and compared with values recorded through respiration experiments.

Results and Discussion

A summary of the characteristic parameters for each group of clams is listed in Table 1. Weight specific oxygen consumption rates, (O_{2W}), were calculated for a 'standard animal' 1 g in weight from values obtained from the respiration experiment (Garton et al. 1984). Oxygen consumption rates based on ETS activity (O_{2ETS}) were calculated from the formula of Owens and King (1975). The major addition involved standardising rates with total protein content of the homogenate and wet flesh weight of each clam.

A comparison of the oxygen consumption rates and ETS activity between the two groups of clams indicate a higher rate for clams from an older batch (Table 1). A T-test of these two values indicates the difference is significant at $p < 0.05$. This is consistent with larger clams requiring a greater amount of energy to maintain their biomass (Bayne and Newell 1983). As a result, metabolism as a whole increases .

It is interesting to note however, that within the same group, larger clams apparently consume oxygen at a lower rate than their smaller siblings (Tables 1 and 2). Higher growth rates have been associated with increased metabolic efficiency as indicated by decreased oxygen consumption and nitrate excretion rates in marine molluscs (Koehn and Shumway 1982, Garton et al. 1984, Garton 1984). 'Scope for growth', the term used to refer to the energy available to the clam for growth after all metabolic demands are met (Crisp 1984), must be associated with oxygen consumption rate and should increase with a decrease in respiration rate. The negative correlation between the measures of growth (shell length and flesh weight) and O_{2W} could be explained by an increase in scope for growth that occurs with a decrease in oxygen consumption rate.

A high correlation between respiration rate and ETS activity is generally observed in both groups of clams (Table 2). However, r values are higher for individuals in the first group than those in the second group of clams and O_{2W}/ O_{2ETS} (Table 1) ratios are higher in the clams analysed earlier. Chance and Williams (1956) define such a ratio as an index of

Table 1. Mean and standard deviations for variables in the experiments on *T. maxima*

Variable	Code	Age of clams	
		9 months	21 months
Shell length (mm)	SL	25.6 ± 6.6	42.2 ± 5.2
Flesh weight (grams)	W	0.4310 ± 0.2611	1.5801 ± 0.5438
Oxygen consumption (mg O_2/hr/g)	O_{2W}	0.77 ± 0.32	2.04 ± 1.10
ETS activity (mg O_2/hr/g)	O_{2ETS}	1.1 ± 0.7	4.2 ± 1.7
Ratio	O_{2W}: O_{2ETS}	0.7 ± 0.13	0.5 ± 0.2

Table 2. Correlation matrix with *r* values for variables (SL = shell length; W = flesh weight; O_{2W} = weight standardised oxygen consumption rate; O_{2ETS} = weight standardised ETS activity

	Age of clams							
	9 month old clams				21 month old clams			
	SL	W	O_{2W}	O_{2ETS}	SL	W	O_{2W}	O_{2ETS}
SL	1.000[b]				1.000[b]			
W	0.920[b]	1.000[b]			0.711[b]	1.000[b]		
O_{2W}	0.096	−0.700[b]	1.000[b]		0.144	−0.561[b]	1.000[b]	
O_{2ETS}	−0.147	−0.848[b]	0.826[b]	1.000[b]	−0.157[b]	−0.686[b]	0.608[b]	1.000[b]

[b]Significant at $p < 0.01$

respiratory control of the enzymes in the ETS. The results suggest that ETS enzyme control is greater in younger clams than older clams. As the clams grow older, oxygen consumption rates are influenced to a greater extent by factors apart from the inherent enzyme activity of the individual. Which factors, and to what extent each influences respiration rates, are subject to speculation.

Conclusion

Within the same group oxygen consumption rates are a function of the organism's size. Thus, larger clams consume oxygen at a lower rate. Between different groups, however, oxygen consumption is a function of the age of the clam. Therefore, the older clams require more energy for maintenance metabolism and thus respire at a higher rate.

ETS activity is, by and large, highly associated with oxygen consumption rate and is thus a useful tool for obtaining a holistic perspective of metabolism in the giant clam. The extent of the control of the ETS enzymes on respiration in *T. maxima* diminishes as the clams grow older.

Acknowledgments

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Effects of Immersion on Juvenile *Tridacna gigas*: Survival, Oxygen Consumption and Ammonia Excretion

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THIS study investigated physiological changes in juvenile *Tridacna gigas* under immersion stress, by determining survival, water loss, and respiration and ammonia excretion rates during or in relation to exposure. Results show that immersion tolerance (for periods up to 27 hours) relied on the clam's access to oxygen for gas exchange, rather than on humidity condition. This finding was supported by the gaping behaviour observed during immersion, the lack of dependence of clam survival on humidity condition and low water loss (5% water loss in dry conditions for a 27 hours immersion period).

Aerial respiration rate was 32% of the aquatic rate, reflecting a reduction in metabolic rate and a step towards energy conservation. Energy conservation is important for an intertidal bivalve since immersion means cessation of filter-feeding, thence starvation until tidal flooding. An oxygen debt from immersion was also incurred by the clam but was paid upon re-immersion. The size of debt was greater after 9 and 18 hours immersion than after 3 hours. Its interpretation, however, in relation to aerobic or anaerobic metabolism proves difficult for lack of calorimetric and biochemical studies.

Aerial excretion rate was 63% of the aquatic rate, again a manifestation of energy conservation, specifically of nitrogen reserves. Excretion rate depended on the immersion period, although no linear relationship was obtained for the immersion periods studied, i.e. 3, 9 and 18 hours.

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Growth and Survival of Giant Clam Spat in Floating Ocean Nurseries

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RESEARCH on the cultivation of giant clams has produced a variety of rearing protocols (Heslinga and Watson 1985, Crawford et al. 1986, Braley et al. 1988, Usher 1990, Heslinga et al. 1990), all of which rely on the use of a land-based nursery system in which the metamorphosed juveniles are reared to a size at which they are deemed to be sufficiently large for stocking into the ocean nurseries or for sale to consumers.

The size and age at which juvenile clams are moved to ocean nurseries has been variable, but successful movement to ocean nursery cages has usually been at a size of 2–4 cm and an age of 9–12 months (Heslinga and Watson 1985, Crawford et al. 1986). At the Coastal Aquaculture Centre in Solomon Islands we have attempted, over the past few years, to progressively reduce the size at which *Tridacna gigas* spat are moved to ocean nurseries. While some success has been achieved, mortality rates have usually been unacceptably high when spat of less than 2 cm shell length (SL) were transferred to the ocean.

Floating ocean nurseries (FONs) for giant clams were first suggested by Munro and Gwyther (1981) and a design proposed by Munro (1985) but, after negative results with a floating system were obtained by researchers in Australia (Crawford et al. 1988), the idea was not pursued. However, Neal Skinner of Marshall Islands Aquaculture Centre reported (pers. comm.) that a batch of *T. gigas* spawned by M. Buckingham at Mili Atoll, Marshall Islands, had been reared in cages suspended between floating oil drums over shallow seagrass beds in the lagoon and had suffered very low mortalities. This prompted a re-examination of the possibilities and a number of designs were formulated.

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Floating ocean nurseries

Construction details for floating ocean nurseries (FONs) are given by Munro and Hambrey (1992). The current 'standard' design is shown in Figure 1a and a more recent variant in Figure 1b. The essential components are a catamaran comprised of two lengths of 150 mm PVC waste piping with endcaps, joined by two hardwood crosspieces. Where they are available, there is an obvious opportunity for using bamboo floats, particularly if properly cured and epoxy-coated. Two hardwood or galvanised iron poles running parallel to the floats support two or more trays which contain the spat. Heavy monofilament nylon fishing line is used to lash the system together.

Each tray consists of a light cement base, reinforced with wire mesh and surrounded by a wall of 10 mm square 'copra wire' or 20 mm plastic 'oyster mesh' which extends to the water surface. It appears that the protective side-wall is unnecessary, in relatively deep (>3 m) water. Large (0.75 m^2) rectangular trays (Fig. 1a) are suspended from four trapezes constructed from lengths of heavy gauge galvanised wire with loops at each end, shackled to loops built into the ferrocement base and to the hardwood or galvanized iron bearers.

Recently an alternative design has been developed (Fig. 1b) in which the large 0.75 m^2 trays have been replaced by six small (0.42 m^2) trays which are attached to two weldmesh platforms suspended beneath the catamarans.

The catamarans are usually rigged in trains of three by polypropylene rope bridles and shackled to a mooring system consisting of individual anchor blocks, chain and a large float attached to an appropriate length (2.5 × depth) of heavy nylon line.

The cost of a 'standard' catamaran (Fig. 1a) is US$203 (SI$537) in Solomon Islands (Munro and Hambrey 1992).

Growth and survival rates

The first trials of the FONs were in June 1989, exclusively using *T. gigas*. Some batches of clams were lost as a result of structural failures and this has recurred when new designs have been tested. However, even in the absence of gear failures, survival of spat in the FONs has been very variable. This can be attributed to variations in the quality of husbandry, the sporadic incidence of attacks on spat by a species of flatworm *Stylochus* (Govan 1993) and by species of *Cymatium* (Gastropoda: Ranellidae). An increase in mortality rates was observed as the size at stocking into FONs was progressively reduced. The smallest sizes stocked

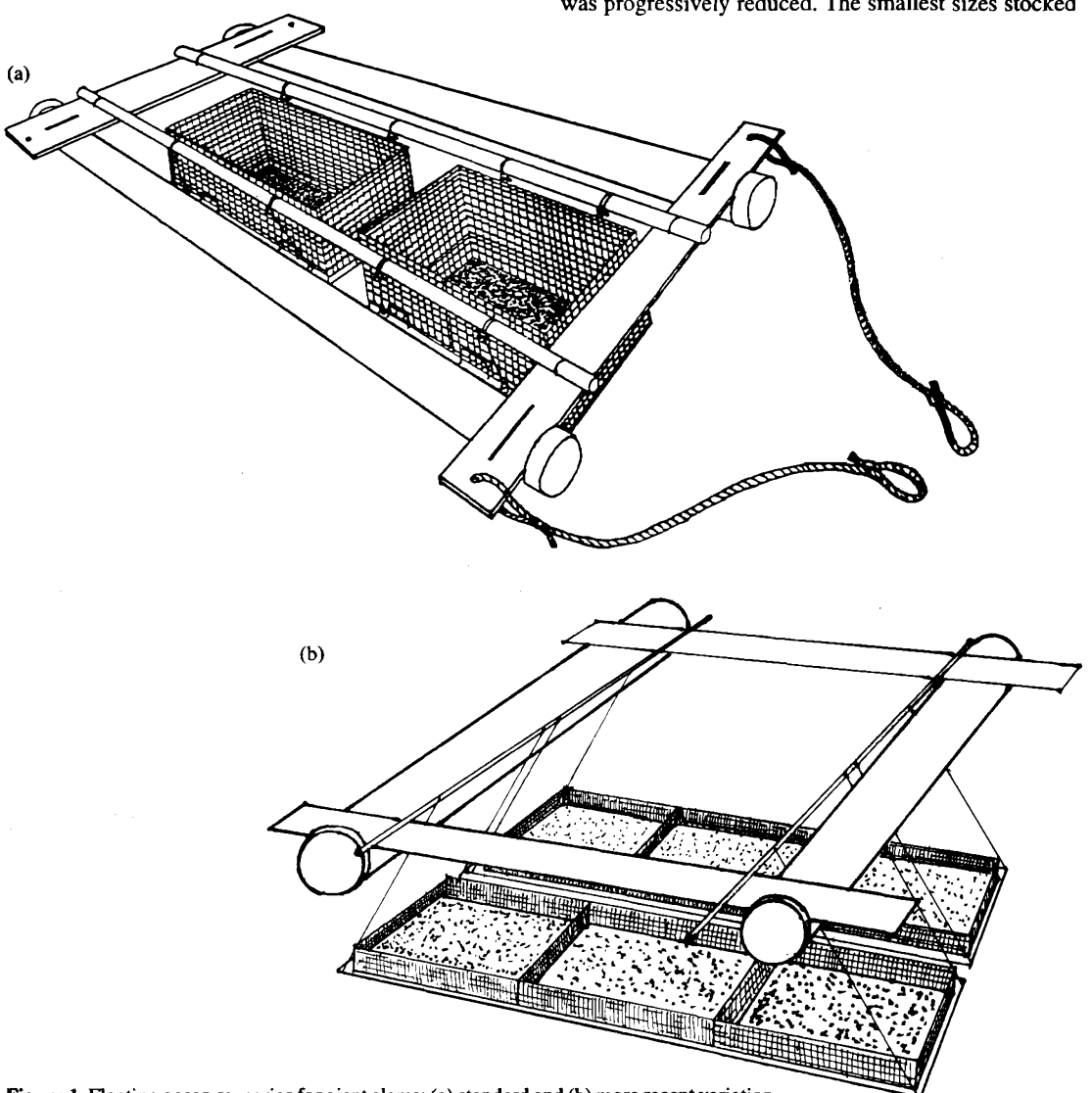

Figure 1. Floating ocean nurseries for giant clams: (a) standard and (b) more recent variation.

and remeasured to date have been of 2.6 mm mean SL at stocking. Stocking densities have ranged between 5000/m^2 (10 mm seed) and 15000/m^2 (3 mm seed).

High mortality of 60 mm *T. gigas* juveniles occurred in rough seas, when it appeared that the byssal attachments of the relatively large clams to the flat ferro-cement surface were inadequate to secure the clams and resulted in the clams being heaped at one end of the tray. Smaller clams were unaffected and it therefore appears that the spat should be transferred to benthic cages at a size of about 40 mm SL, depending on the degree of exposure of the site.

An important factor which emerged is that where nursery tanks were selectively harvested as spat attained a size of around 10 mm, the slower-growing batches of spat all grew at the same rate as their faster-growing siblings when they were transferred to FONs. Figure 2a shows the results from two representative cohorts. The other important feature shown in Figure 2a is that while the spat achieved no more than 2 mm/month (i.e. 10 mm at age 150 days) in nursery tanks, this rate changed to over 5 mm/month on transfer to the FONs. Figure 2b shows that mortality rates were independent of the ages of 10 mm spat stocked into FONs and that slow growth in tanks did not necessarily presage high mortality rates in the ocean nurseries. The implication here is that many or most clams regarded as being 'runts' were in fact stunted by environmental factors and, given improved conditions, responded accordingly.

In order to gauge the relative advantages, if any, of using FONs, a series of tests were executed between June and November 1991, to compare the growth (in terms of mean shell length and weight), survival and biomass attained by cohorts of clams of different initial sizes (range 2.5–5.5 mm) in land-based nursery tanks and square ocean nursery trays (Fig. 1b). Detailed results are given by Gilkes et al. (1992).

In the experiment, four batches of clams from two different cohorts were used, with mean starting sizes of 2.6 mm, 3.0 mm, 4.3 mm and 5.3 mm. Each batch was separated into six equal groups of 400–1000 clams and three of the groups were put into each of three FON trays which were left in a raceway for up to seven days until the clams had attached themselves. The remaining three groups were returned to a circular nursery tank (12 m^2 × 60 cm deep) in each of three cleared areas equivalent to the size of the FON trays. The tanks have an inlet system designed to force a moderate spiral water flow to a central standpipe.

Initial stocking densities in the trays and tanks ranged from 950–2380 spat/m^2, well below the levels at which any negative effects would be expected. Thereafter, at approximately 25 day intervals, counts were made of the surviving clams and 100 clams were removed from each tray or enclosure, measured and weighed and returned to their appropriate tray or tank. The sample clams were allowed to reattach themselves before they were returned to the sea. Durations of the four experiments were 77, 145, 74 and 51 days respectively.

The experimental results (Figs. 3; a–d) indicated substantially greater growth (both in shell length and weight/100 clams) for all groups of clams in the FONs compared with those in the nursery tanks; t-tests on the final mean shell size for each group showed clams in ocean nursery trays to be significantly larger in three of the four groups. In batch 4, the mean starting size in the FON was significantly smaller than that in the nursery tanks. The mean monthly increment in shell size was significantly greater in the FONs in all cases.

The range of survival in the four batches was very variable. The final percentage survival of the clams in the FONs in batches 2 and 3 was higher ($p<0.1$) than that of the clams in the nursery tanks. In batches 1 and 4 the survival of clams was very variable, although in batch 4, two groups of clams from the FONs showed the highest survival rates of 82% and 90% over a period of 51 days. In the smallest batch of clams, stocked at a size of 2.6 mm, mortality in the FONs was greater than in the nursery tanks.

Owing to varying survival, only batches 2 and 3 showed a significant difference between the final total biomass in the FONs and in the tanks, although the average biomasses attained by batches 1 and 4 were >2× and >3× more in the FONs. The percentage increase in biomass/100 initial clams was significantly greater in the FONs in groups 2, 3 and 4, but owing to variable survival this was not confirmed for group 1.

Discussion

The relatively poor growth rates attained in the land-based nursery tanks is a major factor in evaluating the relative merits of floating nursery systems. Heslinga et al. (1990) advocate the addition of inorganic fertilizers combined with heavy aeration to boost primary productivity of the symbiotic zooxanthellae and Fitt et al. (1993) report that spat of *T. derasa* provided with dissolved inorganic nitrogen attain 50 mm at an age of 12 months. This is similar to the growth rate being achieved by early transfer of *T. gigas* spat to the FONs. A clear alternative for the early juveniles would be the addition of supplementary foods to land-based nursery tanks to boost growth rates. This is currently the topic of further studies.

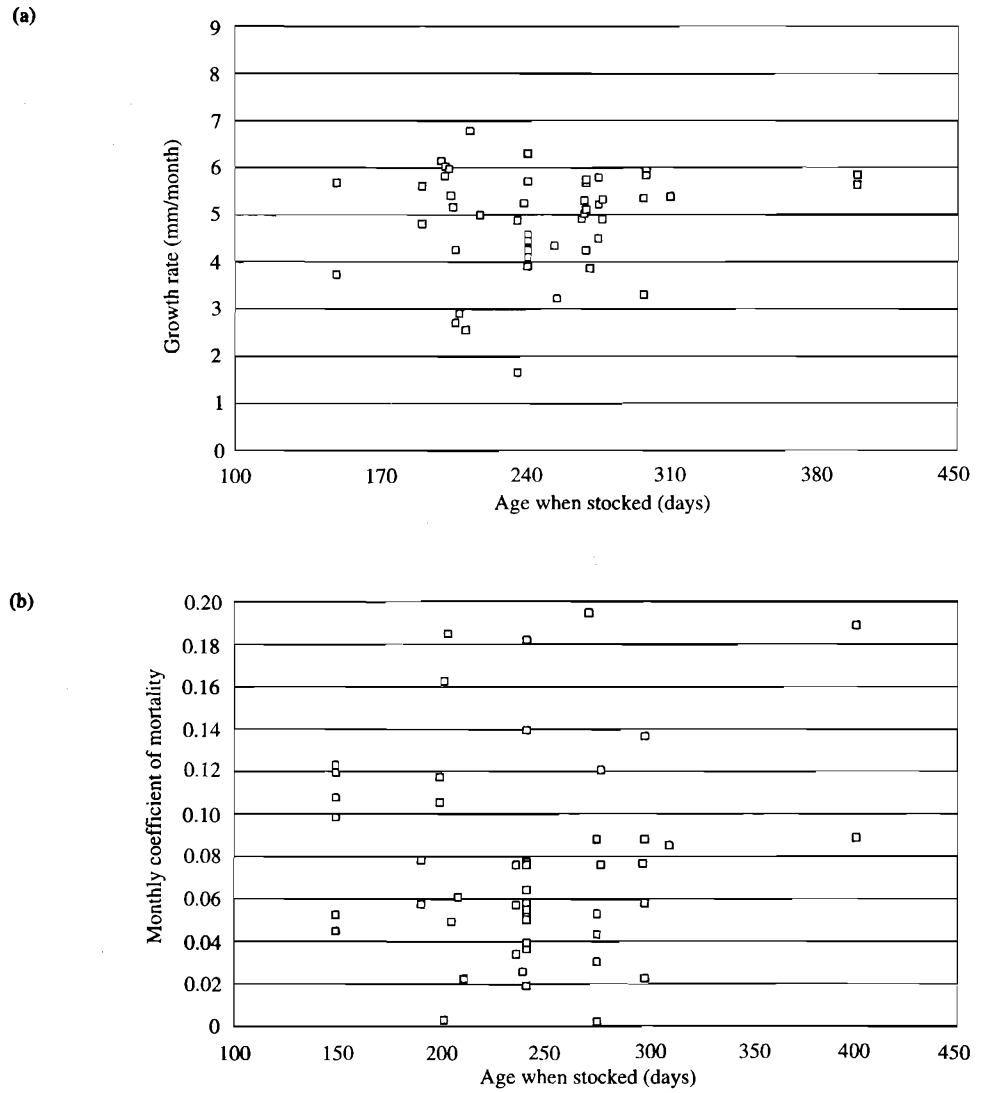

Figure 2. Comparison of (a) growth rate and (b) coefficient of monthly mortality of two cohorts of *Tridacna gigas* which were progressively stocked into floating ocean nurseries as they attained 10 mm shell length.

The investigations (Fig. 3c) showed that the size at which survival in the FONs became greater than in the tanks was between 3.0 and 4.3 mm and that this feature, in combination with the improved growth rates, led to substantial increases in the biomasses attained in the FONs. Spat attain about 4 mm at an age of about 140 days under current protocols at the Coastal Aquaculture Centre, having spent their first 21–28 days in larval rearing tanks and the remainder (up to 120 days) in the nursery tanks. Transfer to FONs at 4 mm would therefore allow up to three cohorts per year to be cycled through the tank system.

Our current data suggest that, in economic terms, the annual costs of operating FONs or a tank system are similar. If FONs are used, capital investment in shore-based tank systems can be reduced to less than one third

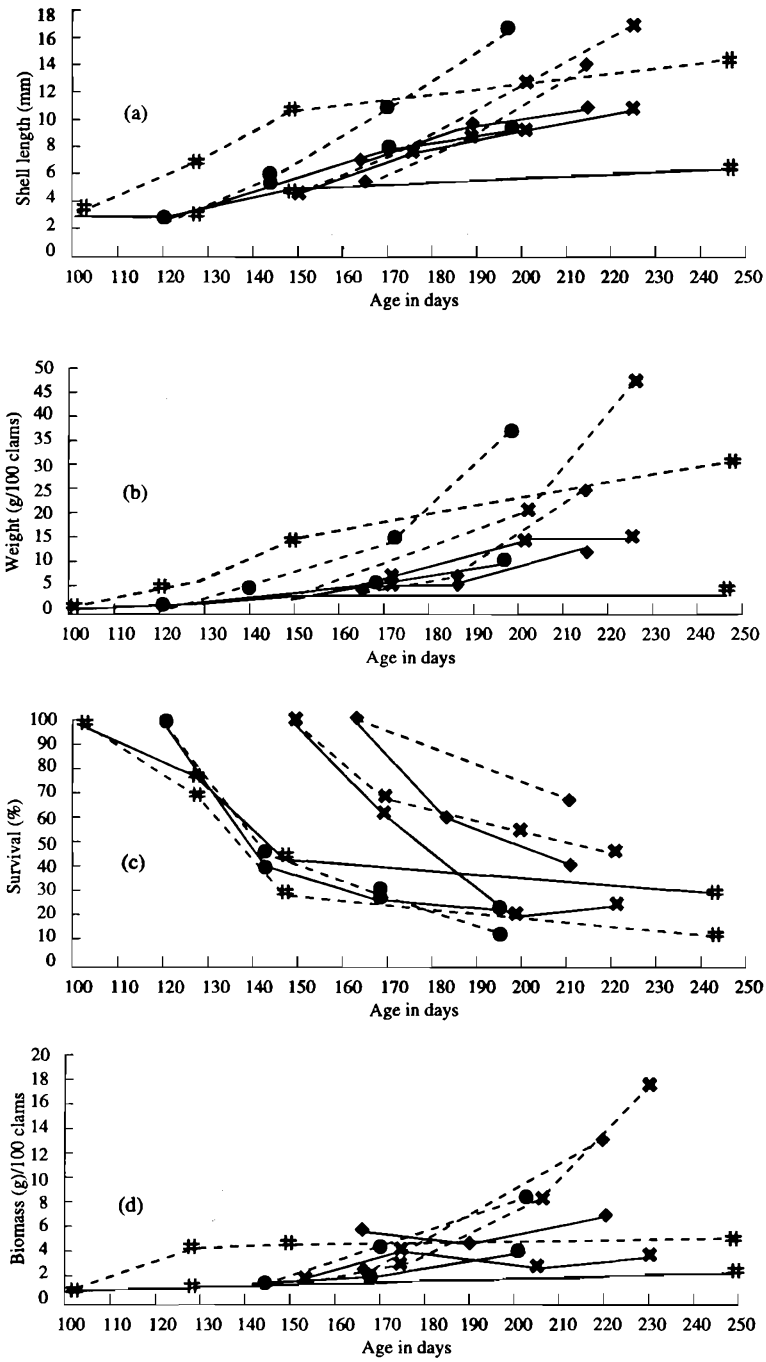

Figure 3. Paired comparisons of changes in (a) mean shell length (mm), (b) weight (g/100 clams), (c) percentage survival and (d) the biomass attained by *Tridacna gigas* spat in floating ocean nurseries (FONs) and in nursery tanks (NT), relative to the age and size at stocking into floating ocean nurseries. 910225 (NT) —●—; 910225 (FON) ---●---; 910318 (NT)—#—; 910318 (FON) ---#---; 910225 (NT) —X—; 910225 (FON) ---X---; 910318 (NT) —◆—; 910318 (FON)---◆---.

of the amount required for a system which relies entirely on tanks. Pumping and maintenance costs will be likewise reduced. However, the FONs are fairly expensive and also have relatively high maintenance costs, depending on the degree of exposure of the site. The higher production from the FONs justifies their use in economic terms.

Munro and Gwyther (1981) concluded that juvenile tridacnid clams were probably unable to acquire all of their nutritional requirements from their symbiotic algae, but would become progressively more phototrophic with increasing size. Klumpp et al. (1992) have shown that, at all sizes, *T. gigas* are remarkably efficient at clearing the water column of particulate matter. The enhanced growth rates in the FONs are therefore probably related to the constant interchange of sea water effected by waves and currents, coupled with maximum exposure to sunlight. One would therefore expect correspondingly fewer benefits to small clams in areas of sheltered water with slight currents, as clearance of the water column might be rapidly effected by the concentration of clams, leading to a shortage of food.

Conclusions

The main advantages of the FONs are that they permit spat to be moved into the ocean at a hitherto unfeasible size of about 4 mm SL and an age of five months, enabling more than three cohorts to cycle through the nursery in a year. The biomass attained at 224 days by each cohort of clams stocked into the 8 is six times greater than that attained in unfertilised nursery tanks. Additionally, the possibility is raised of placing the spat in the hands of specialised village ocean nursery operators at an early stage, thus adding value in the village, using unskilled and mostly under-employed labour, with potential economic benefits as a consequence.

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Density of Clams and Depth Reduce Growth in Grow-out Culture of *Tridacna gigas*

J.S. Lucas[*], S.R. Lindsay[†], R.D. Braley[§], J.R. Whitford[**] and K. Cowden[*]

RESEARCH into giant clam culture at Orpheus Island in the Great Barrier Reef region, NE Australia, developed methods for culturing *Tridacna gigas* (L.) (Bivalvia; Tridacnidae) in the inter tidal zone (Barker et al. 1988). Juvenile clams of approximately 15+ mm shell length were initially cultured intertidally with protective mesh above and below them (ocean-nursery phase) (Crawford et al. 1988). At approximately two years of age they were moved to 'covers' and 'exclosures' (Barker et al. 1988). The latter were suspended walls of mesh that exclude large predatory fishes. At about four years of age and 250 mm shell length the clams were put out on the reef-flat without protection (grow-out phase).

Large batches of juvenile *T. gigas* were reared in the intertidal zone at Orpheus Island and those resulting from the early spawnings in 1985 are now 0.3–0.4 m in shell length. They represent a substantial biomass per unit area of the grow-out site (Figure 1). Taking some approximate values for the large 'fields' of clams in 1990 (unpublished data), i.e., about 8 clams/m^2 at 5 kg whole wet weight each or 0.6 kg soft tissue weight, they represented about 400 t whole weight or 48 t meat weight per hectare, when extrapolated to this area. This is an exceptional stocking level compared to animal husbandry on land, where stocking rates for cattle or sheep grazing without feed supplements are 1–2t meat weight on prime pasture, and often much lower. Direct comparison, however, between stocking densities of grazing farm animals without food supplements and giant clams is not valid. Giant clams do not depend only on their 'internal pastures' of symbiotic algae. They also filter-feed on particulate organic matter suspended in the water column and this is potentially a significant part of their nutrition (Klumpp et al. 1992). So, giant clam stocking density may be influenced by the degree of water circulation and its particulate content.

Water circulation not only affects the filter feeding of giant clams, it may also influence the availability of dissolved nutrients, especially dissolved inorganic nutrients (DIN) required for the clam's autotrophic nutrition. As biomass per unit area of giant clams increases, competition for particulate food and DIN may lead to at least one of these becoming limiting to growth. Thus, the possibility of growth inhibition due to high stocking densities was investigated. Some evidence for this phenomenon appeared to come from a levelling off of soft tissue growth in these high-density clams from year four to five (unpublished data).

That the apparent growth retardation may be due to inter tidal culture was also considered. While growth of younger specimens of *T. gigas* was actually promoted by moderate periods of inter tidal exposure (Nash 1988, Lucas et al. 1989), larger clams may be less tolerant of emersion; or, growth may be inhibited due to the clam's

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mantle tissues being effectively higher in the inter tidal zone as the clam's shell height increases.

Thus, an experiment was conducted over a year to test for the effects on grow-out phase *T. gigas* of (a) culture within fields of clams versus outside fields of clams and (b) inter tidal versus subtidal culture. Growth parameters were compared for groups of giant clams growing either in the midst of fields of clams or in isolated groups and at different levels in the inter tidal zone or subtidally.

Materials and Methods

The *T. gigas* used in this experiment were part of a group that were spawned at Orpheus Island in October 1985. Data for whole wet weight, shell weight and drained soft tissue weight were obtained for batches of at least 10 clams at approximately yearly intervals since 1985. Thus a general growth curve was generated.

The growth experiment commenced in June 1990 when the clams were 4.7 years old. Three hundred specimens, individually tagged with numbered plastic strips attached by epoxy glue to their shells, were haphazardly assigned to 15 groups of 20 clams. These groups did not differ significantly in mean size or range for any of the measured parameters. Shell length, shell height, shell width, whole wet weight (WW) and underwater weight (UW) were measured for each specimen (see Lucas et al. 1991, Fig. 1, for illustrations of the linear dimensions measured). Total clam volume was determined from the formula:

$$\text{Total clam volume} = (WW-UW)/1.025^{*}$$

Because the soft tissues are essentially the same specific gravity as sea water, they have no underwater weight. UW is shell weight minus the weight of the sea water displaced by the shell. Thus, deducting UW from WW leaves the weight of sea water displaced by the total clam. Dividing this weight by the specific gravity of sea water gives the volume.

Three replicate groups of 20 clams were placed in quadrats containing 8 clams/m^2 density at each of five sites (Figure 2). Replicates at each site were spaced at least 20 m apart to take account of any local effects.

The sites were:

1. Within fields of grow-out clams (about 8 clams/m^2 density) at about 0.6 m height above Chart Datum in Pioneer Bay (see Parnell 1987) in the inter tidal zone (= clam fields, DI). This inter tidal level experiences about 1.5 hours mean emersion period per 24 hours (Lucas et al. 1989).
2. *and* 3. At approximately the same tidal height as DI to the north (NI) and south (SI) of the clam fields. Hereafter they are referred to as the inter tidal sites.

Figure 1. Six-year-old *Tridacna gigas* in dense grow-out culture at Orpheus Island, Great Barrier Reef, Australia. Shown at low tide.

4. At about Chart Datum, very low in the inter tidal zone (LI), on the reef-crest. This level is below Mean Low Water Springs (MLWS) and experiences about. 0.2 hours mean emersion per 24 hours (Nash 1988).
5. At about 3.5 m below Chart Datum and 5 m mean water depth in the shallow subtidal zone (ST) below the reef crest.

Sites 2–5 were natural fringing reef areas without any cultured *T. gigas* present before the addition of the experimental clams.

The clams were re-measured after a year, in June 1991. Growth increments in the measured parameters were analysed by ANOVA using Statistix 3.0.

Results

Growth of *T. gigas* over seven years in clam fields in the inter tidal zone at Orpheus Island is shown in Figure 3. Some reduction in the rate of soft tissue growth between years four and five is suggested.

Mean values (±1 SE) for the various parameters of the 300 *T. gigas* at the experiment's initiation in June 1990 were: shell length = 286.5 (± 0.9) mm; shell height = 170.5 (± 0.6) mm; shell width = 159.5 (± 0.6) mm; whole wet weight = 4.14 (± 0.04) kg; underwater weight = 1.49 (± 0.02) kg; whole volume = 2.66 (± 0.03) L.

Only four of the 300 tagged clams died during the year and thus data was available for 20 clams for most

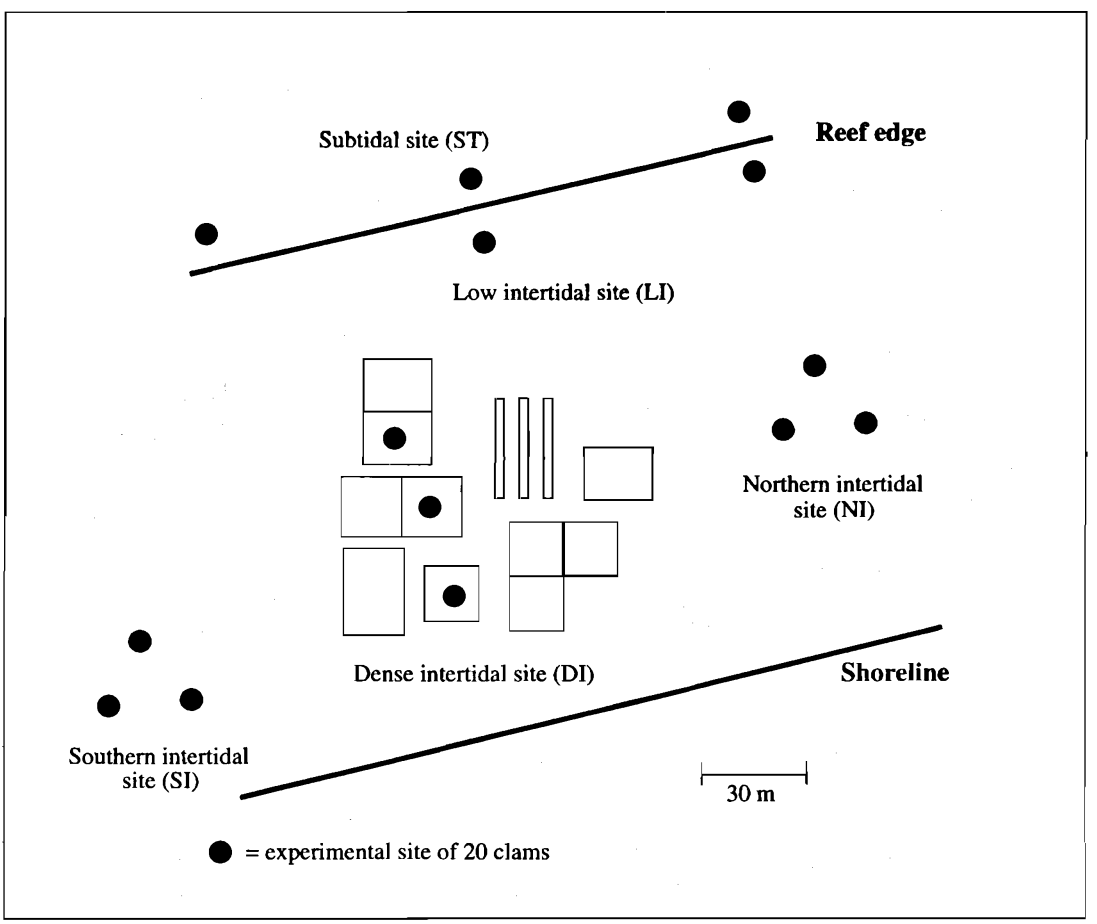

Figure 2. Diagram of the ocean-culture area in Pioneer Bay, Orpheus Island, showing the locations of the fields' of giant clams (large squares and rectangles). The elongate rectangles are 'lines' of nursery phase clams (see Barker et al. 1988). The five sites where measured clams were placed are indicated; each solid circle represents a group of 20 clams.

replicates in June 1991. Growth increments for the various parameters at the five sites are presented in Figure 4a–f. There was a consistent pattern of greatest growth at inter tidal sites, SI and NI, and lowest growth at the subtidal site, ST. Growth rates at the clam fields (DI) and reef-crest (LI) sites were intermediate.

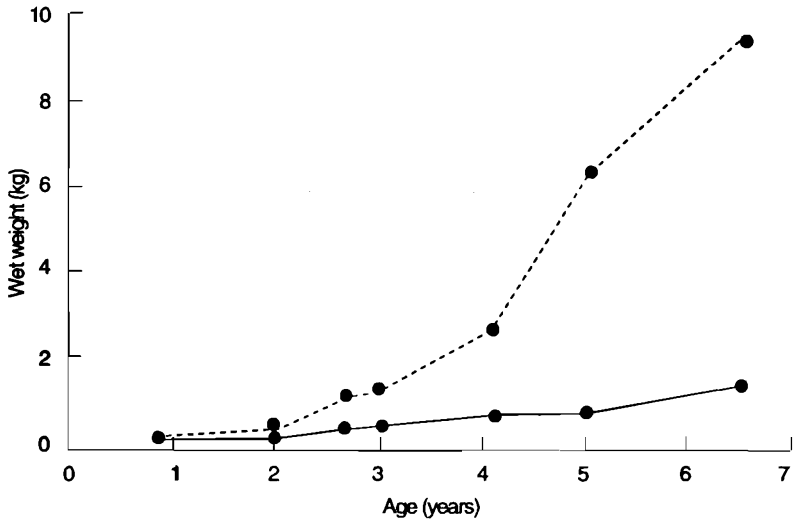

Figure 3. Growth in whole wet weight (dotted line) and drained wet tissue weight (solid line) of *Tridacna gigas* in the inter tidal zone at Orpheus Island.

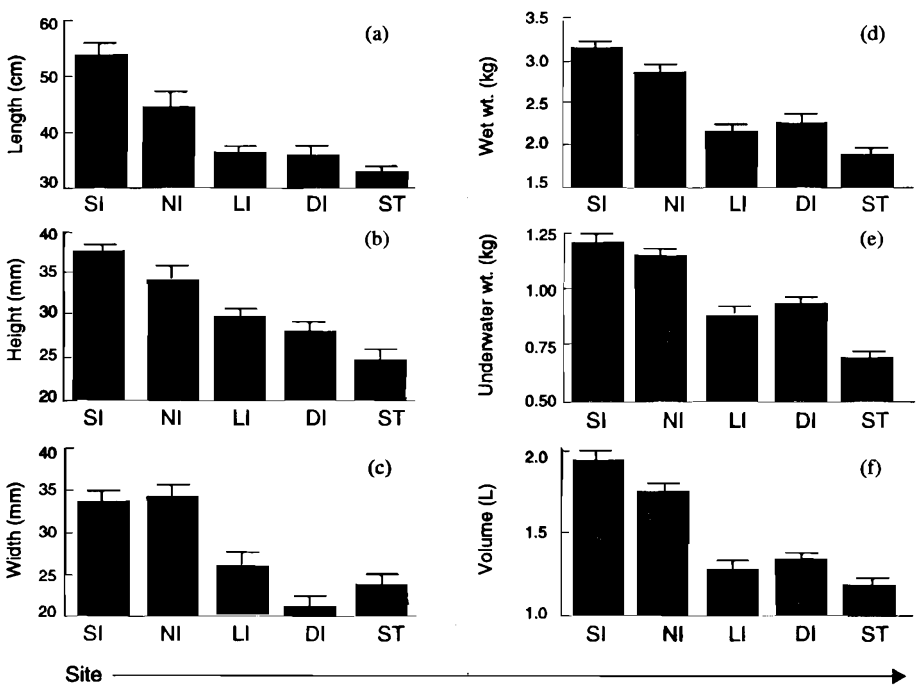

Figure 4. Mean growth increments over a year for *T. gigas* at five sites in Pioneer Bay, Orpheus Island. (a) shell length; (b) shell height; (c) shell width; (d) whole wet weight; (e) underwater weight; (f) whole clam volume. Sites: DI, clam fields; LI, very low inter tidal (reef crest); NI, northern inter tidal; SI, southern inter tidal; ST, subtidal.

ANOVAs for the six parameters showed that there were very significant differences ($P < 0.001$) between the sites, but no significant differences between the replicates at each site (Table 1). The data for each parameter were further analysed with Tukey tests to identify which means were significantly different (Table 2). Growth increments for the various parameters were always significantly greater at the two

Table 1. ANOVA analyses of mean growth increment data for *T. gigas* in Pioneer Bay, Orpheus Island. * = $p<0.05$; ** = $p<0.01$; and *** = $p<0.001$.

Source of variation	d.f.	M.S.	F
Shell length			
Sites	4	4370	27.2***
Replicates	2	120	0.7
Sites × replicates	8	370	2.3*
Residual	285	160	
Shell height			
Sites	4	1520	17.0***
Replicates	2	210	2.4
Sites × replicates	8	740	8.3***
Residual	285	90	
Shell width			
Sites	4	2050	19.7***
Replicates	2	120	1.1
Sites × replicates	8	290	2.8**
Residual	285	100	
Wet weight			
Sites	4	16	74.8***
Replicates	2	0.05	0.3
Sites × replicates	8	1.5	6.8***
Residual	269	0.22	
Underwater weight			
Sites	4	2.4	74.0***
Replicates	2	0.07	2.0
Sites × replicates	8	0.15	4.6***
Residual	279	0.03	
Volume			
Sites	4	6.1	58.8***
Replicates	2	0.15	1.4
Sites × replicates	8	0.69	6.7***
Residual	269	0.10	

Table 2. Tukey tests of significance between means for the various growth parameters and sites. Sites are ordered in decreasing order of growth from left to right. There is no significant difference ($p<0.05$) between sites connected by common underlining. Sites: DI—dense intertidal; LI—low intertidal (reefcrest); NI—northern intertidal; SI—southern intertidal; and ST—subtidal.

Shell length	SI	NI	DI	LI	ST
Shell width	NI	SI	LI	ST	DI
Shell height	SI	NI	LI	DI	ST
Wet weight	SI	NI	DI	LI	ST
Underwater weight	SI	NI	DI	LI	ST
Clam volume	SI	NI	DI	LI	ST

inter tidal sites adjacent to the clam fields (SI and NI) compared to the clam fields (DI). Growth also differed between SI and NI, being greatest at SI for all parameters except width (Figure 4).

Comparing growth at the four sites other than the clam fields, the higher inter tidal sites (SI and NI)(0.6 m above datum) had significantly greater growth increments than the very low inter tidal site (LI, reef-crest)(at datum) for all parameters except shell height. LI, in turn, had significantly greater growth increments than the subtidal site (ST)(–3.5 m below datum) for three of the five measured parameters. Thus, inter tidal height and water depth have strong effects on growth parameters, even over the narrow depth range used in this study.

Discussion

The significant differences in clam growth between different inter tidal heights, NI/SI versus LI, and between inter tidal and subtidal sites (Table 2) presumably result mainly from the attenuation of light intensity with depth. This affects growth through the somewhat linear relationship between gross photosynthesis rate per unit of clam tissue versus light intensity in clams of this size (Fisher et al. 1985).

Simple photometers making cumulative in situ readings were installed at the northern inter tidal (NI) and subtidal sites (ST). Over a year from June 1988 to June 1989, the subtidal photometer recorded 65.9% of the light at the inter tidal site (J.R. Barker, unpublished data). Thus, the approximately 4 m depth difference between the NI/DI/SI sites and ST was sufficient to reduce the light regime by one third in these somewhat turbid, inshore waters, where underwater visibility is typically in the 4–5 m range.

Greater growth at NI/DI/SI (0.6 m above datum) compared to the low inter tidal LI site (datum) confirms the findings of Nash (1988) and Lucas et al. (1989) that moderate levels of inter tidal exposure have a positive effect on growth of *T. gigas*. This effect was attributed by the previous authors to greater solar radiation and winter temperatures experienced by clams in this inter tidal zone. However, while these earlier studies were with juvenile clams, at least some of the animals in this study had reached male maturity (evident as spontaneous sperm releases), as part of their protandrous hermaphroditic maturation.

There was a strong effect on growth, related to stocking density, such that growth increments were significantly greater for all parameters at the inter tidal sites, SI and NI, adjacent to the clam fields, than within the fields of clams (DI) at the same tidal height. Thus, it appears that the availability of particulate foods for filter feeding or DIN for photosynthesis or both may have been sufficiently reduced in these dense fields of clams to significantly affect growth (cf. Klumpp et al. 1992). The effect, however, was only such that growth at DI was comparable to that obtained at the reef-crest and it was significantly greater than growth at the subtidal (ST) site for some parameters (Table 2).

There was no suggestion that the reduced growth in the clam fields was due to auto-pollution, such as from accumulations of pseudofeces or feces. Substrates within fields of giant clams did not differ noticeably from adjacent areas without clams. Furthermore, feces are never seen accumulating around clams in the field, even in areas of dense clams. Their feces are packed with zooxanthellae which disperse or are quickly eaten by fishes, etc. The reduction in growth at DI also cannot be attributed to some immediate physical effect of high density, e.g., the clams were too close to extend their mantles fully. The densities of the clams within the quadrats at each test site were identical (8 clams per m^2), so this effect would have influenced growth at all sites similarly.

A further suggestion of the possible effects of particulate nutrients and/or DIN comes from greater growth at the SI site than at NI. This may be related to the water circulation patterns in Pioneer Bay. The tides in this bay are semi-diurnal, mixed form, with spring ranges up to 3.5 m (Lucas et al. 1989). During flood tides the water circulation flows from south to north along the reef-flat, while during ebb tides this pattern is reversed (Parnell 1987). However, the northerly

currents on flood tides are stronger than the southerly currents on ebb tides. Thus, clams at SI should have experienced stronger flows of 'pristine' sea water (i.e., sea water that had not flowed across the fields of clams) than did NI, and may have benefited from higher levels of nutrients at these times.

These results have important implications for farming *Tridacna gigas* and other giant clam species during their grow-out phase. They highlight the need to culture giant clams as shallow as feasible where there is significant water turbidity and indicate the importance of good water circulation over the farm site. In terms of the economics of using a finite area for giant clam farming, they suggest that it is possible to stock high densities of clams with acceptable levels of growth reduction. At about a 2.3 kg increment in wet weight per clam in the fields and 8 clams/m^2, this represents about 180 t increment in clam weight per ha over a year. At 10% meat return from the wet weight this represents me at production of 18 t/ha/year—a very good return for an animal production system that requires no feed input and nothing more than occasional checks to see that there are no causes of mortality.

Acknowledgments

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Tridacna gigas and *T. derasa*: a Growth Comparison

E.D. Gomez and S.S.M. Mingoa*

TRIDACNID bivalves, or giant clams, have been shown to manifest variations in growth rates depending on the species and rearing conditions (see Munro and Heslinga 1983). Clams tend to have linear growth in the early years of life (McMichael 1974, McKoy 1980). They exhibit determinate growth, for instance growth rates begin to decline as they attain the maximum adult size (Yamaguchi 1977).

In tridacnid mariculture, particularly in the production of seed, high growth rates may expedite the rearing cycle. Having juveniles transferred from a land-based nursery to an ocean-based nursery is beneficial to the clam farmer or fisherman because the latter nurseries require minimal maintenance and support better clam growth compared to the former (Beckvar 1981). However, smaller clams are confined within protective cages that exclude predators until the clams reach the 'escape' size at shell lengths greater than 100 mm (Heslinga et al. 1984, Crawford et al. 1987). At this size they are essentially free from predation and hence can be removed from the cages and set unprotected on the seafloor. Until then, there will be a need for cages and the corresponding maintenance.

This present work focused on the enhancement of growth rates of giant clams in ocean nurseries, by comparing the growth of two species *Tridacna gigas* and *T. derasa*, reared at different sites. It is based on the premise that different species of giant clams may have specific ecological requirements. For instance, Munro and Heslinga (1983) have observed that *T. gigas* seems to thrive on shallow fringing reefs, while *T. derasa* appears to be oceanic, found less often on fringing reefs adjacent to land masses. However, it should be noted that generally most of the established ocean nurseries (e.g. at the Bolinao Marine Laboratory in the Philippines, and at the Micronesian Mariculture Demonstration Centre (MMDC) in Palau) have been found to be suitable for the rearing of most species. The point being made in this paper is that the potential to improve clam growth rates in ocean-based systems exists and must be explored. In so doing, the duration for cage culture in the ocean nursery may be shortened, hence reducing costs for materials and maintenance.

The general goal of the studies conducted was to evaluate the suitability of different sites for rearing different species of juvenile clams. Two growth experiments using *T. gigas* and *T. derasa* were conducted in succession. The purpose of the first study was to establish that clam growth rates differed depending on species, by measuring the growth rates of these two species at two shallow sites. The succeeding study was aimed at determining whether growth rates of different species would be enhanced by rearing at different sites having dissimilar hydrographic conditions.

Another objective of the study was to confirm the results of earlier studies in Bolinao (Gomez and Belda 1988) in which the *T. derasa* used had been stunted in the early stages of their growth before they were placed at the Silaqui ocean nursery.

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Materials and Methods

Experimental clams

Two species of giant clams, *T. gigas* and *T. derasa*, were used for rearing experiments. Two cohorts of *T. gigas* were used. The older cohort belonged to a batch of four-month-old juveniles imported from Australia in March 1990. This batch was spawned in November 1989 (hence referred to as Tg11/89) by the staff of the giant clam project at the Orpheus Island Research Station (James Cook University of North Queensland). Due to unfortunate circumstances, the number of Tg11/89 clams available for use in the experiments were limited. The younger batch of *T. gigas* was imported from Solomon Islands in April 1990 as 11-day-old larvae, spawned by the staff of the International Center for Living and Aquatic Resources Management (ICLARM)-Coastal Aquaculture Center in March 1990 (hence referred to as Tg3/90). Lastly, the *T. derasa* juveniles were part of a spawning conducted at the Bolinao Marine Laboratory by the giant clam project of the Marine Science Institute in April 1989 (hence referred to as Td4/89).

Prior to conducting the experiments, all clams were already being reared in similar constructed raised cages at the ocean nurseries at a bottom depth of 2.5 m.

Study sites

The study sites were three giant clam subtidal ocean nurseries situated north of Silaqui, a small islet north of Santiago Island (16°25'N, 119°55'E), Bolinao, Philippines (Fig. 1). The area was a sandy, seagrass bed. The bottom topography displayed a gradual slope eastward, such that shallow and deep sites could be

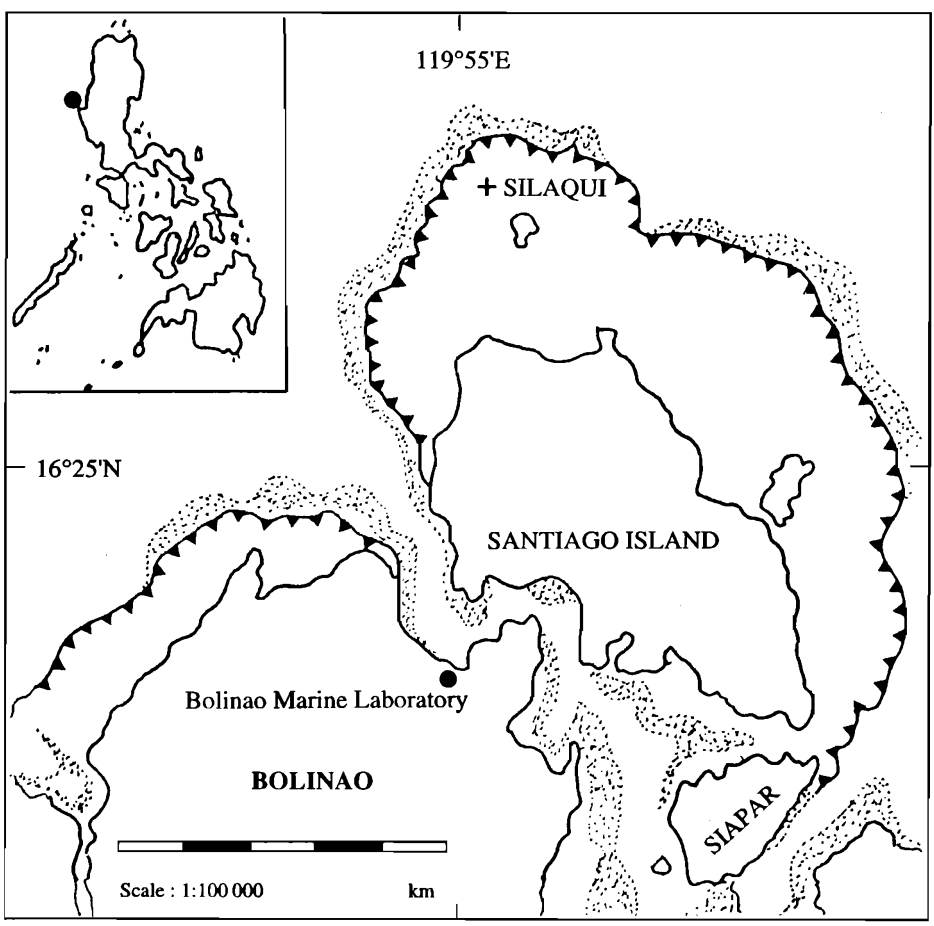

Figure 1. Map showing the study area (+) near Silaqui, Philippines.

established at relatively short distances and within close proximity to Silaqui. The sites were surrounded by a 5 cm mesh net serving as a territorial landmark or pen and as a filter against drift seaweed and seagrass debris.

The three sites were distinguished as either shallow or deep, the depths determined on several occasions with a depth gauge. The shallow sites were Sites 1 and 3, both not exceeding an average of 3 m depth. Site 3 was shallower than Site 1 by less than 0.5 m. Site 2 had an average depth of about 5.5 m.

Clams were kept in plastic mesh cages (155 cm × 95 cm × 25 cm) consisting of a rectangular box frame and lid made of grey polyvinyl pipes, and green polyethylene screen (2 cm mesh). Cages were raised about 0.5 m from the seabed, propped onto vertical angle bars that anchored them to the bottom. Additionally, the angle bars were fitted with bottomless plastic jugs serving as skirts to prevent crawling benthic predators from entering the cage via the legs. Cages were placed 0.5 m apart at each site.

Field experiments

Study 1 was initiated in December 1990, with groups of Tg11/89 (see Table 1 for initial sample sizes and lengths) and of Td4/89 placed in cages at Sites 1 and 3. Initially, clams were randomly grouped in trays within the cage to facilitate maintenance. Cage maintenance entailed cleaning the cage at least once a week by brushing to remove fouling benthic algae. Clams were occasionally thinned out to other cages to eliminate confounding effects of overcrowding.

Shell lengths of clams were measured with a vernier caliper (± 0.02 mm) initially in December 1990 and monthly thereafter. Although the experiment seemed to involve a two-way factorial design, initial mean shell lengths of Tg11/89 and Td4/89 at Sites 1 and 3 were belatedly analysed using One-Way Analyses of Variance (ANOVA). One-Way ANOVA was employed rather than Two-Way ANOVA because of the uncertainty that any effect of other factors not considered in this study was random (Zar 1984). Based on One-Way ANOVA ($P<0.05$): initial shell lengths of Tg11/89 at both sites were similar, lengths of Tg11/89 and Td 4/89 at Site 1 were similar, lengths of Tg11/89 and Td 4/89 at Site 3 were similar, and lengths of Td4/89 at both sites were significantly ($P<0.05$) different. Subsequent statistical analyses were conducted bearing these in mind. Table 1 shows initial mean shell lengths and standard deviations (± SD). Final lengths were recorded in September 1991 after a period of nine months.

Environmental parameters (light, temperature and water movement) were also monitored for the duration of the experiment. Underwater irradiance was measured once a week at noon with a Licor light meter equipped with a submersible probe. Light intensity records prior to March 1991 were not included in this study because the sampling methodology employed prior to March was inadequate. The protocol followed by this study was to position the probe at the level of the sand, at the central area of exclosure, free from obstruction within the exclosure. Three replicate instantaneous readings were averaged to get a mean value. On several occasions, light readings were taken inside the cages at noon. Comparisons with ambient underwater light readings showed that light within the cages was attenuated by about 20%.

At each site, subtidal temperature was determined with a minimum–maximum thermometer situated within a cage. Readings were recorded at least twice a week, with the thermometer being reset after each reading.

Water movement was measured using the clod card method (Doty 1971). This method, using small blocks made of plaster of paris, measures the degree to which diffusion or dissolution was enhanced by water movement where the clod card was exposed. Three replicate sets of clod cards were placed on the sand across the area of the exclosure at each site, in order to determine the general water movement conditions per site. All clod cards were retrieved the next day and

Table 1. Mean shell lengths of experimental clams

Study 1	Tg11/89	Td4/89
	Initial length±SD (mm)	
Site 1	63.63±16.67(24)	62.0±10.1(61)
Site 3	58.23±15.41(26)	59.72±16.37(59)
	Final length±SD	
Site 1	135.41±21.28(23)	82.86±19.38(44)
Site 3	124.12±22.64(24)	84.52±21.50(50)
Study 2	**Tg3/90**	**Td4/89**
	Initial length±SD	
Site 1	98.73±16.374(55)	100.60±14.93(55)
Site 2	99.45±13.23(55)	97.50±14.92(55)
	Final length±SD	
Site 1	150.43±20.16(53)	112.48±19.25(53)
Site 2	128.15±17.86(53)	119.51±18.37(53)

Note: Figures in parenthesis show the numbers of clams measured

processed following the methods of Doty (1971). For the period from December 1990 until March 1991, a modified 'slab' type of clod card suggested by Dennison and Barnes (1988) was used. Thereafter, the standard method of Doty was followed for reasons of economy.

Study 2 commenced in September 1991 when groups of Tg3/90 and Td4/89 were placed in cages at Sites 1 and 2 (see Table 1), following a Two-Way Factorial experimental design. This time the cages were better maintained ensuring the randomness of effect of any other factor not considered in this study. Data on clam sizes and environmental parameters were recorded regularly. The same protocol used for Study 1 to collect data and monitor the experiment was applied. Data for seven months were analysed using a Two-Way ANOVA.

Results

Study 1

Figure 2 shows the growth of Tg11/89 and Td4/89 at Sites 1 and 3 over a period of nine months. Final mean lengths are shown in Table 1. Highly significant differences in growth were obtained between Tg11/89 and Td4/89 at Sites 1 and 3 in shallow water over a period of nine months (One-Way ANOVAs, $P<0.0005$). For Sites 1 and 3, Tg11/89 yielded about 113% increase in size, measured as shell length. On the other hand, the shell length of Td4/89 increased by about 34% at Site 1 and by about 42% at Site 3.

One-Way ANOVAs on final shell lengths (Table 1) of Tg11/89 and Td4/89 at the shallow Sites 1 and 3 showed significant differences between: Tg11/89 at Sites 1 and 3 ($P<0.01$), Tg11/89 and Td4/89 at Site 1 ($P<0.0005$), and Tg11/89 and Td4/89 at Site 3 ($P<0.0005$). No significant difference was found with Td4/89 at Sites 1 and 3 ($P>0.05$).

Figure 3 shows trends in growth rates for the two species, with Tg11/89 having consistently higher growth rates than Td4/89 at both shallow Sites 1 and 3. There are apparent highs and lows in the growth rate, the former occurring around March till May, and the latter around January to February, and again in June.

Study 2

The growth data for Tg3/90 and Td4/89 are shown in Figures 2 and 3, and Table 1. The initial shell lengths indicated that Tg3/90 and Td4/89 at both Sites 1 and 2 were similar (two-way ANOVA $P>0.25$). Over an eight month period, lengths significantly increased (one-way ANOVAs, ($P<0.0005$) from initial values. Furthermore,

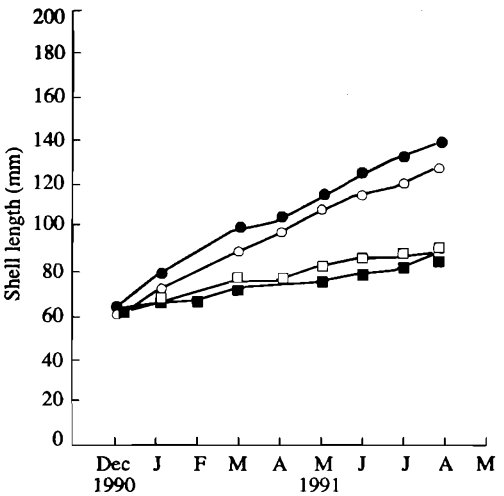

Figure 2. (a) Mean shell lengths of Tg11/89 in Sites 1 (●) and 3 (○), and Td4/89 in Sites 1 (■) and 3 (□) for Study 1.

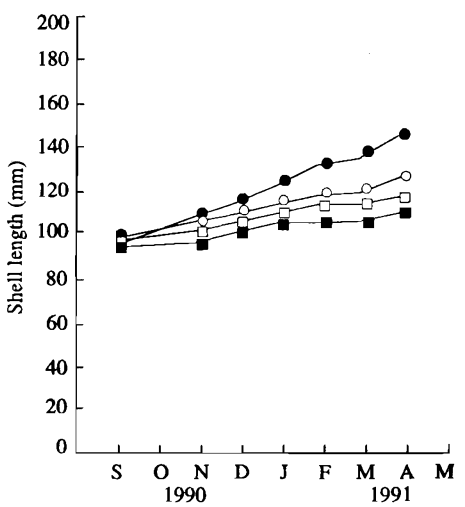

Figure 2. (b) Mean shell lengths of Tg3/90 in Sites 1 (●) and 3 (○), and Td4/89 in Sites 1 (■) and 3 (□) for Study 2.

growth for Tg3/90 was significantly greater that growth of Td4/89 (ANOVA $P<0.0005$).

Significant interaction was also found between species and sites ($P<0.0005$), indicating that growth differences may be attributed to difference in species as well as site (Zar 1984). From September 1991 to April 1992 (seven months), Tg3/90 grew by about 52% and by about 29% at Sites 1 and 2, respectively.

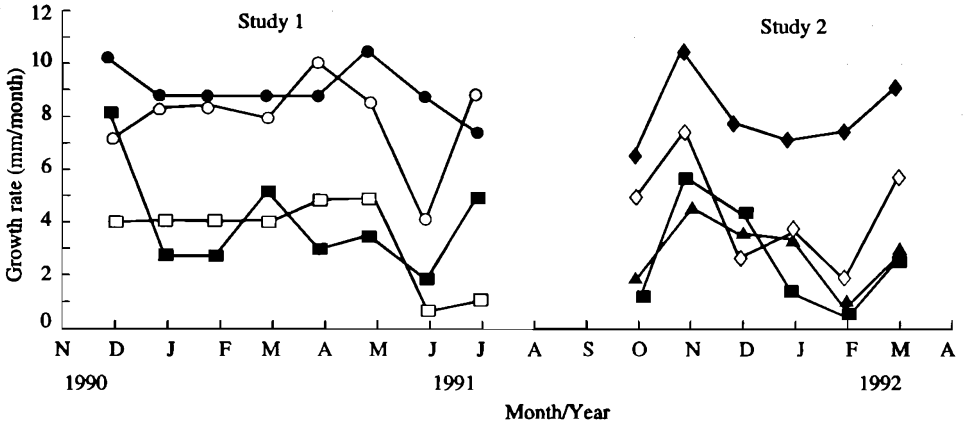

Figure 3. Monthly growth rates of Tg11/89 in Sites 1 (●) and 3 (○), Td4/89 in Sites 1 (■), 2 (□) and 3 (▲), and Tg3/90 in Sites 1 (◆) and 2 (◇).

This was followed by Td4/89 which grew by about 23% and by about 12% at Sites 2 and 1, respectively. Therefore, Tg3/90 attained its greatest growth at the shallow Site 1, while Td4/89 attained its best growth at the deeper Site 2.

Environmental parameters

Figure 4 shows underwater irradiance regimes at Sites 1 and 3 (ca 2.5 m) from March to August 1991 for the duration of Study 1, and regimes at Sites 1 and 3 from September 1991 to March 1992 for the duration of Study 2. Light intensity at Site 3 was typically higher than those at Site 1 (Fig. 4) reaching about 1200 μE/m^2/second in March 1991. By July, however, there was a steep downtrend, coinciding with the onset of the wet season, which usually begins in June, and ends in November. The light intensity values measured for Site 1 were still generally high, ranging from 1200 to 400 μE/m^2/second from March to August. Between Sites 1 and 2 (Study 2), light intensity regimes seemed to increase by November, marking the end of the wet season. The duration of the dry season is usually from December until April.

Minimum and maximum temperatures are shown in Figure 5, from January to August 1991 at Sites 1 and 3 (Study 1), and from September 1991 to March 1992 at Sites 1 and 2 (Study 2). Based on Mann-Whitney tests (Zar 1984) for similarities of minimum and maximum temperatures between Sites 1 and 3 for Study 1, and between Sites 1 and 2 for Study 2, results showed that: maximum temperatures at Sites 1 and 3 were similar ($P>0.20$); minimum temperatures at Sites 1 and 3 were

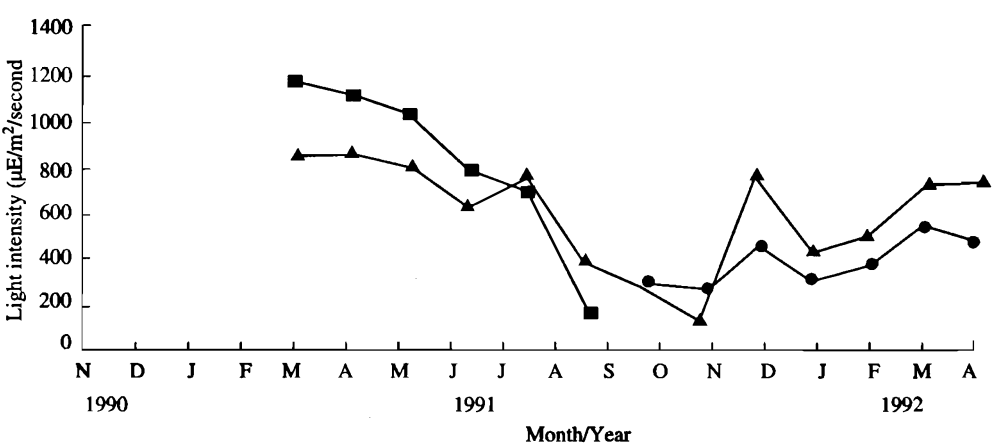

Figure 4. Average light levels at Sites 1 (▲), 2 (●) and 3 (■).

also similar (P>0.20); maximum temperatures at Site 1 were significantly greater than those at Site 2 (P<0.05); and minimum temperatures at Sites 1 and 2 were also significantly different (P<0.05).

For Study 1, from an apparent low around February in 1991, a rise in subtidal temperatures occurred up to June 1991. Then temperatures manifested a plateau until about September 1991, and then proceeded to decline. For Study 2, from September 1991, temperature values generally continued to drop, reaching a lowest point in January 1992, thereafter showing a rise. Minimum and maximum temperature values for the deeper Site 2 were consistently lower by approximately 2°C than those for Site 1. Overall, it would seem that, except for the temperature plateau in the middle of the year, subtidal temperature trends follow the dry–rainy seasons, as do underwater light regimes.

Water movement (based on the diffusion factor, DF) at Sites 1 and 3 (Study 1) from December 1990 to August 1991, and at Sites 1 and 2 (Study 2) from September 1991 to January 1992 are plotted in Figure 6. There was no apparent difference between the DF values obtained from different sites. Generally, high values or erratic trends, indicating more turbulence (e.g. from July 1991 to March 1992) were obtained during the rainy season.

Discussion

Growth rates among tridacnids being reared in ocean nurseries can be enhanced by the proper selection of a

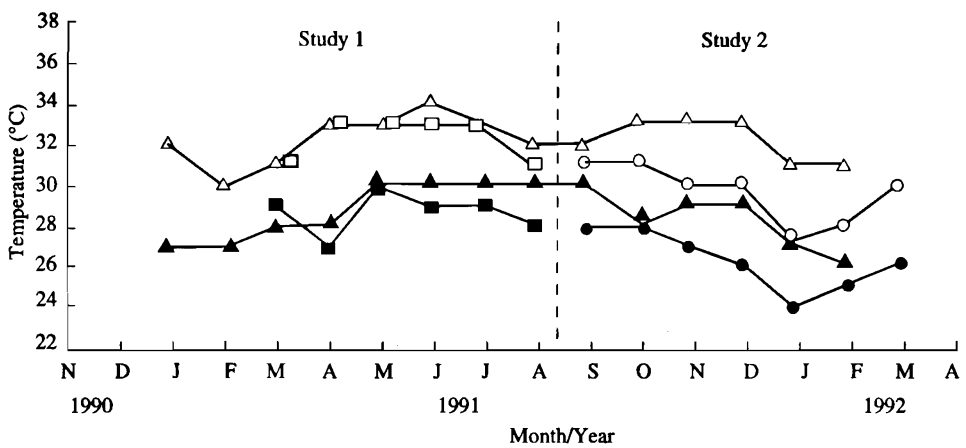

Figure 5. Average temperature (minimum and maximum) levels at Sites 1 (▲ min. Δ max), 2 (● min. O max) and 3 (■ min. □ max).

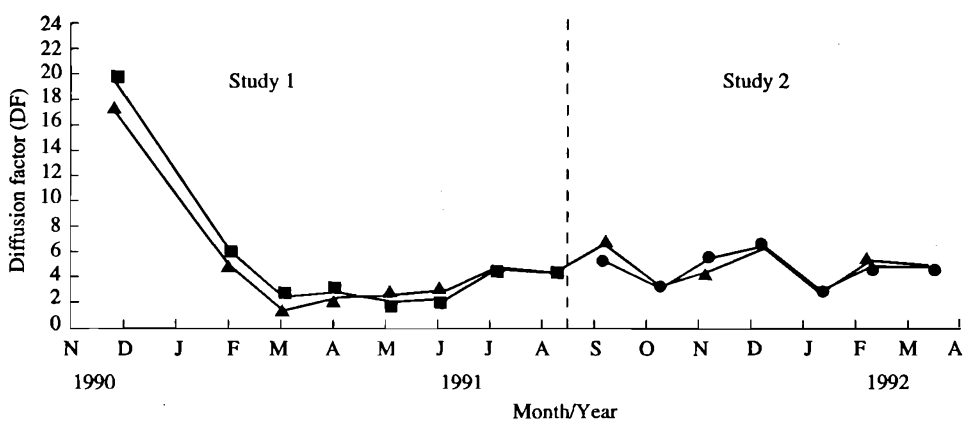

Figure 6. Average water movement (measured as diffusion factor using clod cards) at Sites 1 (▲), 2 (●) and 3 (■).

site suitable for the species. Growth rate estimates for ocean-reared *Tridacna gigas* based on the von Bertalanffy growth function vary from approximately 6 to 19 cm per year depending on the reef location. The rate of growth for *T. derasa*is approximately 4 cm per year (Munro and Gwyther 1981, Munro and Heslinga 1983). Such a wide range in growth rates, especially in *T. gigas*, may be attributed to genetic differences or differences in their environmental exposure.

The first study (Study 1) presented in this paper was initially conducted to answer the question: does *T. gigas* grow faster than *T. derasa*? The assumption here was that the rate of growth is an inherent genetic trait. The results of Study 1 showed that *T. gigas* had faster growth rates, 7.8–9.1 mm per month or 9.4–10.9 cm/year, than *T. derasa*, 3.4–4.2 mm per month or 4.1–5.0 cm per year. While these results show gross differences in growth rates between species, adequate comparisons and definitive conclusions were restricted since the results of Study 1 may have been confounded by the fact that their initial lengths were significantly different to start with.

To follow-up Study 1, the second study (Study 2) was designed to answer the question: Is growth rate dependent on the species, or is it dependent on the site? The results of Study 2 showed that the growth of *T. gigas* and *T. derasa* were dependent on both the species and the site. In shallow waters about 2.5 m deep (Site 1 values), the average growth rate of *T. gigas* was 7.9 mm per month or 9.5 cm per year, while in relatively deep water about 5.5 m depth (Site 2 values), *T. gigas* growth rates were 4.2 mm per month or 5.1 cm per year. On the other hand, *T. derasa* growth rates in the shallow site (Site 1 values) were 2.5 mm per month or 3.0 cm per year, while at the deep site, growth rate was 2.7 mm per month or 3.2 cm per year. Clearly, growth varied with exposure to different environments, as has also been shown in other growth studies on tridacnids (e.g. Crawford et al. 1988, Lucas et al. 1987).

In 1988, Gomez and Belda reported growth rates of *T. gigas* reared at three sites with characteristically different sediment loads. Two of their sites are silty, while the third site is actually Site 1 as in this study. They reported *T. gigas* having a growth rate of 7 mm per month (8.4 cm per year) at Site 1, while *T. derasa* had rates of 1.8 and 2.4 mm per month (2.2 and 2.9 cm per year) when grown in the silty sites, but 3.3 mm per month (4 cm per year) at Site 1. Hence, the results of the present studies indicate that growth rates of *T. gigas* and *T. derasa* as reported by Gomez and Belda in 1988 are comparable with those presented here.

The environmental parameters considered here were light, temperature and water movement, because of their importance in relation to tridacnid growth as expounded in other works (e.g. Lucas et al. 1989), and the ease by which they can be measured. Despite differences between sites in the levels measured of certain parameters, the general trends seemed to correspond with the dry and wet seasons. The temperature plateau from July to about September 1991 reflected the warm temperatures of the wet season, with the season's lower temperatures setting in by late September.

The sites considered in Study 2 were distinctly different in terms of light and temperature. Although a multiple regression analysis would be desirable here, complete data sets (including growth rate, light, temperature and water movement) were too few to merit any sound conclusion. Nonetheless, by graphical comparison of the trends of growth rates and the measured environmental parameters, it was obvious that where there was a drop in light intensity and temperature, growth rates also decreased. Conversely, with a rise in the same environmental parameters, growth rates seemed to increase.

Trends obtained from Study 2 in growth rates of *T. gigas* and *T. derasa* at the shallow and deep sites showed some relation to certain unique environmental characteristics of the sites. That *T. gigas* clams grown at the shallow Site 1 manifested higher growth rates compared to those grown in the deep Site 2 indicate that the high light and/or high temperature regimes at the shallow site may be more suitable to the rearing of *T. gigas*. On the other hand, *T. derasa* clams reared at the deep site exhibited higher growth rates than those reared at the shallow site, showing that low light and/or low temperature conditions at the deep site were more favourable for rearing *T. derasa*.

With the development of different ocean culture methods at various tridacnid mariculture facilities, the unique needs of the culture species should also be considered. By this, the growth potential of the species may be close to being realised, as shown here with *T. gigas* and *T. derasa*.

Acknowledgments

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Growth and Survival of Cultured *Tridacna gigas* on a Patch Reef of the Great Barrier Reef

J.R. Barker* **and D. McKinnon†**

THE primary requirements for ocean culture of the giant clam, *Tridacna gigas*, are clean seawater with adequate current flow, sunlight and shelter (Lucas et al. 1988) Patch reefs, although occurring in oceanic water of apparently low nutrient concentration, may offer suitable conditions for the maturation of hatchery reared giant clams. A previous study compared growth at a continental island site and a site on John Brewer Reef, a platform reef (Barker et al. 1988a). It showed promising results and justified more detailed study into the use of mid-shelf reefs for giant clam ocean nursery culture.

This study considers the suitability of mid-shelf patch reefs for the culture of giant clams, and compares various locations within the reef for the optimisation of survival and growth rates.

Materials and Method

Davies Reef (18.8°S, 147°E) is a mid-shelf platform reef of the Great Barrier Reef (Fig. 1). We established three transects across the reef, each comprising four zones: the reef front, the back of the reef flat, a lagoonal bommie (large coral pinnacle) and a back reef bommie. Each of these zones had sites at three depths: reef flat (generally about 1 m), 5 m and 10 m (Fig. 1). This design allowed comparisons within zone by depth and between zones across the reef for the same depth.

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Two standard experimental units, each consisting of a plastic mesh tray with 25 mm plastic mesh lid, were established at each site, following the procedures of Crawford et al. (1988). A gravel substrate was placed into each tray along with 25 hatchery raised *Tridacna gigas* (mean shell length 17.4 ±0.6 mm), randomly selected. All sites were established in November 1987. Subsequent counts of survivors and measurements of shell length, using vernier callipers, were made in May 1988, February and June 1989 and March 1990.

We compared the light regimes at the reef front and lagoon sites by analysing the irradiance measured by in situ data loggers at these sites presented in the study of Klumpp and McKinnon (1989). Measurements of irradiance were scored in three categories: under the compensation point Ic (i.e. net respiration), between Ic and Ik (i.e. net photosynthesis but not saturated) and over Ik (saturated photosynthesis). Values of Ic and Ik for clams of similar size to those used in this study were 48 μE and 323 μE respectively (Klumpp et al. 1992).

Results

Survival

Severe loss of clams occurred at a number of sites from storm damage (Table 1). Entire trays were lost at some sites between November 1987 and May 1988 following Cyclone Charlie in March 1988 and between February 1989 and June 1989 following cyclone Aivu in April 1989. No clams survived on either the reef front or back reef bommie zones. The mean percent survival by zone and by depth, after subtracting the loss due to storm

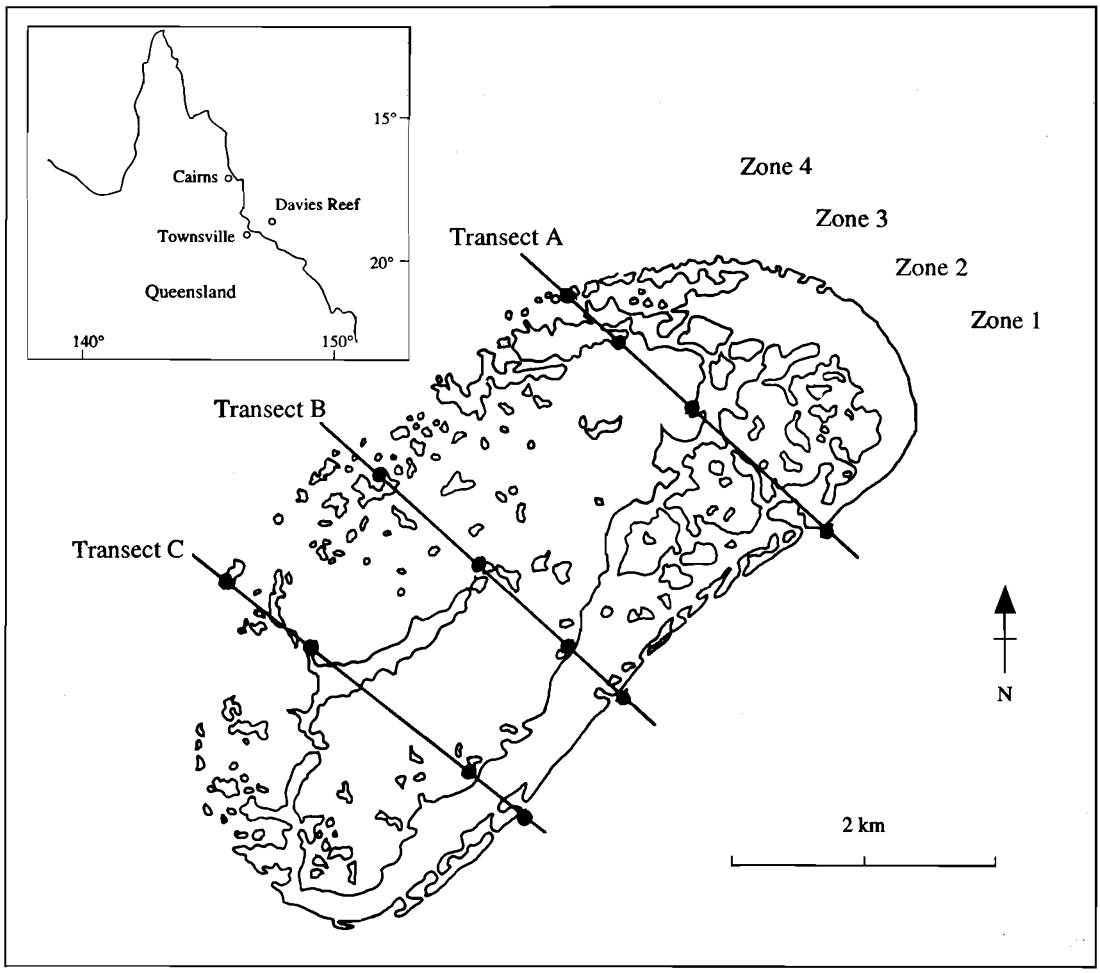

Figure 1. Davies reef showing site positions within zone areas 1–4: zone 1 = reef front; zone 2 = back reef; zone 3 = a lagoonal bommie; and zone 4 = a back reef bommie.

damage is shown in Figures 2 and 3. The final survival of clams at different depths was not significantly different ($p = 0.2825$). The mean numbers of clams per tray, averaged over all zones, were 5.0±1.4, 5.5 ±2.0 and 6.9±2.1 at reef flat, 5 m and 10 m depths, respectively. Average survival amongst zones was also non-significant ($p = 0.2950$).

Growth

Figures 4, 5 and 6 show growth for clams at 0 m (reef flat), 5 m and 10 m depths respectively. Table 1 outlines growth increments for the clams at sites that survived the study.

ANOVA comparisons of the final mean shell length showed a significant difference among clams living in the various zones ($p = 0.0195$). Tukey's analysis indicated that clams in zone 4, the back bommie zone, had a significantly lower mean shell length than clams living in the other three areas. Although not significant, the reef front (zone 1) clams tended to have a larger mean shell length than the other zones.

The analysis indicates no significant difference in growth rate among depths ($p = 0.0804$), although there appears to be a general trend of growth being inversely proportional to depth, Table 2.

Table 1. Shell length increase (mm) for clams surviving to 28 months. Initial samples were taken from a population with mean shell length of 17.44mm

Depth (m)	Site	Sample size	Growth increment (mm) ± SE
Reef flat	A2	10	174.5 ± 7.8
5	A2	5	148.6 ± 12.3
	A3	11	191.2 ± 6.4
	B2	3	179.3 ± 20.7
	B3	9	153.3 ± 9.0
	C3	5	188.8 ± 12.5
10	A1	11	140.7 ± 10.1
	A3	12	163.5 ± 10.8
	A4	11	144.9 ± 6.8
	B1	15	147.2 ± 7.1
	B2	4	140.9 ± 20.4
	B3	8	128.7 ± 5.6
	B4	13	171.4 ± 9.4
	C2	8	159.6 ± 7.1
	C4	13	183.8 ±5.3

Light climate

In December 1986 clams at the 10 m depth were saturated with respect to incident light for 47% of daylight hours at the reef front site (Table 3) as opposed to only 16% in the lagoon. In May 1987 there was a similar trend, these numbers being 32% and 12% for the reef front and lagoon sites, respectively.

Discussion

The use of a patch reef for the ocean culture of giant clams has the obvious disadvantage of susceptibility to heavy wave action. In our study at Davies Reef, storm damage apparently caused appreciable loss of trays especially at the shallow sites on the reef front and reef back. Substantial losses occurred following Cyclone Charlie in March 1988 and Cyclone Aivu in April 1989. To some extent, the degree of potential damage can be minimised by careful site selection and the use of suitably designed protection for the juveniles (Barker et al. 1988a, b). At Davies Reef, the best locations to minimise storm damage are at a depth of 10 m within the lagoon.

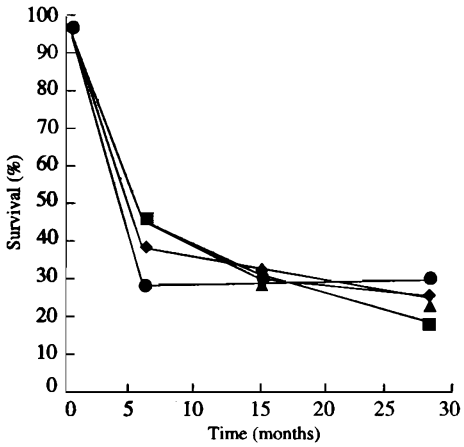

Figure 2. Survival of clams *Tridacna gigas* by reef zone corrected for loss of trays. Zone 1 ●; Zone 2 ▲; Zone 3 ■; and Zone 4 ◆.

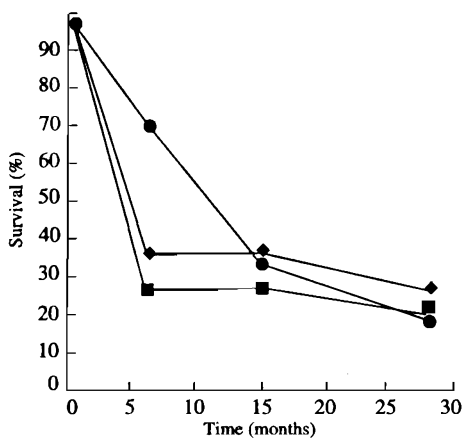

Figure 3. Survival of clams *Tridacna gigas* by depth corrected for loss of trays. Reef flat ●; 5 metres ■; and 10 metres ▲.

The survival of the clams within the cages that lasted for the full 28 months appeared to be independent of position on the reef or depth. However, when compared to the survival for similar aged clams on sheltered, mainland island fringing reefs, they are quite low. In these areas, expected average survival could be around 55–60% using similar technology to that used in this study, (Tisdell et al. 1991).

Best growth occurred at the reef front areas B4 and C4. This growth was not matched by A4, possibly because this area was affected by cyclonic activity and coral debris may have shaded the cages for some time during the trial. Dramatic shading would be expected to retard the growth of clams (Lucas et al. 1989). The high

Table 2. Mean shell length of *Tridacna gigas* in mm (± SE) at 28 months (sample sizes are given in parentheses)

Zone	Depth (m) 0	5	10	Overall mean
4	–	–	185.3 ± 4.9 (37)	
3	–	194.5 ± 5.9 (25)	167.0 ± 7.8 (20)	180.8
2	191.9 ± 7.8 (10)	177.5 ± 11.5 (8)	170.8 ± 8.1 (12)	180.1
1	–	–	161.8 ± 5.8 (26)	161.8
Overall mean	191.9	186.0	171.2	

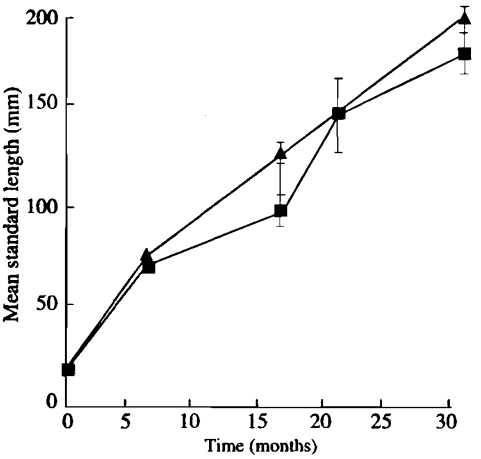

Figure 5. Growth of clams *Tridacna gigas* at 5 m on Davies Reef. Zone 2 ▲, Zone 3 ■.

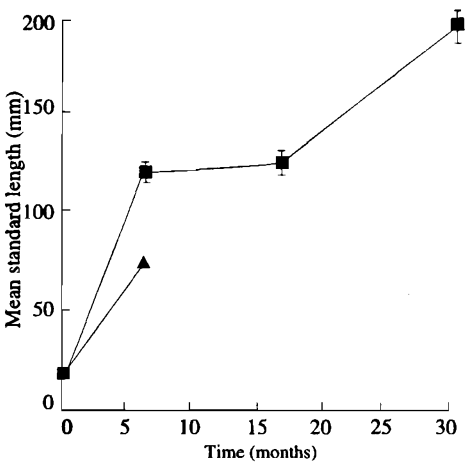

Figure 4. Growth of clams *Tridacna gigas* in shallow water (0 m) on Davies Reef. Zone 2 ▲, Zone 3 ■.

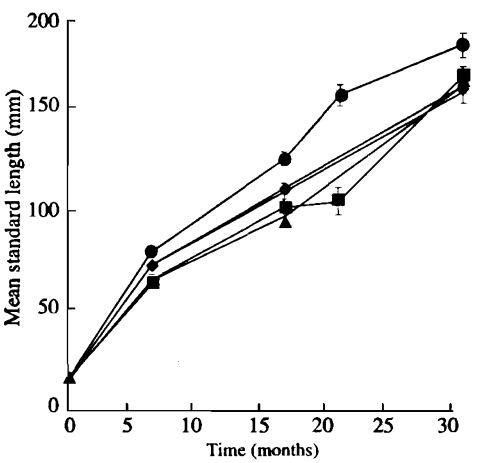

Figure 6. Growth of clams *Tridacna gigas* at 10 m on Davies Reef.Zone 1 ●, Zone 2 ▲, Zone 3 ■, and Zone 4 ◆.

growth rate of clams at the reef front as compared to the lagoon is most probably related to the differences in light climate. The clearer, oceanic water may allow the symbiotic zooxanthellae of the clams to be light saturated for a greater proportion of the day, as indicated by the higher Ik values.

Growth rate was lower within the reef lagoon and back-reef bommies than at the reef front. The generally more turbid conditions within the reef lagoon are due to particulate matter, which could be utilised in the clams heterotrophic nutrition (Klumpp et al. 1992). Our observations of highest clam growth at the reef front suggest that either the particle regime within the reef is not favourable for *T. gigas*, or that phototrophy is important in controlling the growth of this species. Unfortunately there are few data available on particle composition in reef waters, though detrital levels are known to be high in some areas (Roman et al. 1990).

The potential of patch reefs similar in overall size to Davies Reef for commercially growing giant clams is questionable. Preventing damage from storm activity is paramount, otherwise the ensuing mortality rate

Table 3. Light climate at reef front and reef lagoon, 10m sites. Data are minutes of irradiance falling within the light categories described in the text.

	Level of irradiance			
	Dark	i<Ic	Ic<i<Ik	i>Ik
Dec 1986				
Front	643	116	305	376 (n = 3)
Lagoon	638	151	525	126 (n = 3)
May 1987				
Front	748	127	345	220 (n = 2)
Lagoon	729	144	478	89 (n = 1)

Notes: Irradiance (i) was scored as under the levels for net respiration (i<Ic); between the level allowing for photosynthesis and that for saturated photosynthesis (Ic<i<Ik); or over the level for saturation of photosynthesis (i >Ik).

would make it less than attractive. Larger reefs would offer more protection to the lagoonal areas although less than maximum growth rate may occur. If a patch reef is the only option then it is necessary to find the most economic balance between growth rate and expected survival.

Acknowledgments

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Pros and Cons of Methodologies Used in the Hatchery and Land-based Nursery Phase of Giant Clam Culture

R.D. Braley*

GIANT clams (Bivalvia:Tridacnidae) are relatively new to the list of aquacultured marine species, the first successful small-scale larval culture being done in the mid-1970s (La Barbera 1975, Jameson 1976). Mass culture of giant clams began with the efforts of the Micronesian Mariculture Demonstration Centre (MMDC), Palau, in the early 1980s (Beckvar 1981, Heslinga et al. 1984, Heslinga and Watson 1985) and in the mid-1980s at the James Cook University of North Queensland Giant Clam Project in Australia (Crawford et al. 1986, Braley et al. 1988, Braley 1989). The latter project also involved the development of mass clam culture in the Philippines (Alcazar et al. 1987, Trinidad-Roa 1988), Papua New Guinea (Bell and Pernetta 1988), Fiji, Tonga and most recently the Cook Islands. The third major giant clam group in the Pacific region is sponsored by the International Centre for Living Aquatic Resources Management (ICLARM) Coastal Aquaculture Centre in Solomon Islands, which began operation in 1987 (Usher and Munro 1988).

Larval culture methods which have evolved out of the research groups vary in amount of effort expended in feeding and cleaning larvae from extensive to semi-intensive and intensive. There is some variation in methods used in the land-based nursery phase of young juvenile clams. All species of giant clams have been reared through the larval and land-nursery phases. Only the newly-described species, *T. tevoroa* (Lucas et al. 1990, 1991) still has F1 juveniles in the land-nursery phase of culture as of June 1992, while other species have F1 and F2 representatives in the ocean nursery phase of culture. This paper reviews advantages and disadvantages of using some recently developed methods in the culture of giant clams.

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Broodstock Spawning

Gametes of giant clams may be obtained for larval culture by induced or spontaneous spawning in tanks. The following three methods were used to induce successful spawning.

Gonad extract: an adult clam is sacrificed for the gonadal material and any excess material may be frozen (or freeze-dried) for future use. A small amount of the macerated gonad squirted into the vicinity of the gills typically results in sperm release within 1–5 minutes. Fresh clam gonad is ideal for a hatchery if a large number of good broodstock are available. This method does not require the purchase of chemicals and is therefore ideal in remote locations and stress to broodstock clams is minimised. This method is not suitable where only a small number of broodstock are available. The gonadal material in the water may contribute to contamination of fertilised eggs collected for larval culture by increasing the bacterial load.

Temperature shock: clams placed in seawater which has been allowed to heat up in the sun to 33–34°C, or clams placed on their side in the sun for 30–60 minutes, can be induced to spawn by moving the

warm clams into a spawning tank containing seawater at ambient temperature. While this temperature difference generally results in gamete release, the lethal temperature for tridacnid clams is about 35°C for the low latitude populations and may be lower for clams from higher latitudes. This technique is stress-inducing but not an invasive method for inducing spawning for the smaller species. The method is simple for remotely located hatcheries. However the technique is not suitable for large *T. gigas* and possibly very large *T. derasa*. Overheating and extended aerial exposure may be prove lethal to specimens which are overly stressed, though this may not be discernible prior or during this treatment. Excessive mucus may be produced by the clam when resubmerged in seawater, causing some contamination of the water into which eggs are spawned.

Serotonin (5-hydroxytryptamine creatinine sulfate complex): this neurotransmitter has been used successfully in inducing gamete release, especially in the smaller species of giant clams but also in *T. gigas* and *T. derasa* when they are ripe and ready to release eggs (Braley 1985, 1986, Crawford et al. 1986, Alcazar et al. 1987). When injected with serotonin and in combination with temperature shock the likelihood of a very large spawning of eggs is increased in tridacnids.

If moving broodstock into land-based tanks does not stress clams into spawning within a few days, they will most likely become acclimated to the tank within a couple of weeks and begin a pattern of sperm release which may eventually result in spawning of eggs. Most of the giant clam species exhibit a diel periodicity (mid–late afternoon) of spawning behaviour. Beckvar (1981) and Heslinga et al. (1984) found lunar phases of peak spawning activity in Palau but other data suggests that this lunar periodicity may vary with locality within the reproductive season (Braley 1984, 1986).

There are three types of culture for spawning and larval rearing of giant clams, these being extensive, semi-intensive and intensive. Extensive culture is simplest, where few materials are needed except outdoor seawater tanks which are filled only once during the entire larval stage and there may or may not be feeding of larvae involved. Semi-intensive culture involves more care of water filtration, care of early embryo stages, water changes through the larval phase and feeding of larvae. Intensive culture requires the most equipment and care by trained technicians and water quality is most important because of higher densities of larvae being cultured than in extensive or semi-intensive cultures; feeding of larvae is also practiced. Extensive or semi-intensive culture are more suited to remote Pacific Islands than intensive culture, because of the greater simplicity and less reliance on equipment, food and well-trained technicians. In intensive and semi-intensive culture there is necessarily more control over fertilisation and water quality for the developing embryos than in an extensive system. Fewer broodstock are required than in an extensive operation. The large number of broodstock required for extensive spawning may not be possible where extensive overharvesting has occurred. Likewise, relatively low numbers of an introduced species may curb use of extensive spawning. The egg water will be contaminated with feces, mucus and unwanted microorganisms associated with broodstock shells in the extensive system. In extensive operations, large numbers of broodstock are used to make sure a 'critical mass' of clams produces large numbers of eggs. This spawning normally takes place in large outdoor culture tanks and the eggs remain in the tank in which they were spawned (Heslinga et al. 1990). In intensive and semi-intensive culture, broodstock of the smaller species of giant clams are moved into containers of 1 µm filtered seawater (FSW) when egg release begins and repeatedly moved to new containers for further expulsions of eggs. In the case of large *T. gigas*, eggs are collected in plastic bags and put into separate containers of fsw for each expulsion. Sperm is added within 15 minutes of spawning; only 1–5 mL of concentrated sperm water per litre is required so as to avoid polyspermy.

Larval Culture Methods

Extensive

After the eggs have been spawned and broodstock removed from the tank, the water level is adjusted in this static culture so the initial stocking density is one egg/mL. Aeration should be provided.

Zooxanthellae may be added to the culture tank after several days post-fertilisation. This is done by either sacrificing a clam of the same species or cutting off small portions of mantle tissue without killing the clam. The mantle tissue is mascerated (by scalpel, razor blades or food blender) and rinsed with FSW through a screen (50 µm) to remove larger pieces of clam tissue and obtain zooxanthellae. This resultant brown suspension of zooxanthellae and mantle tissue is added to the larval tank for the swimming veligers to ingest.

Although the culture is considered static, the water level in the tank may be increased slightly each day until most of the larvae have ceased swimming and have settled (5–10 days). At this stage the seawater (whether raw or filtered) may be turned on.

This method of culture is the least complicated. It requires less skill and therefore the overall cost of

production is lower than an intensive method. Heslinga et al. (1990) recommended this method for remote hatcheries, low budget programs and where technical skills are lacking. The disadvantages of this extensive method include typically lower embryonic and larval survival, combined with often lower production. There is less control over the amount of sperm and unwanted microorganisms in the tank water compared to the semi-intensive or intensive methods of culture.

Semi-intensive

In this method eggs are collected by either plastic bags for larger species, or by moving spawning clams into clean bins of filtered seawater (FSW) to obtain eggs with little if any accompanying sperm. Count estimates are made of the fertilised eggs. A 'selection' technique was developed in Australia (Braley 1986, Braley et al. 1988) which requires the use of a 'hatching tank' for the embryonic and trochophore to D-stage veliger larvae. The stocking rate can vary between 20–40 mL when using 5–10 ppm antibiotic (streptomycin sulphate and/or neomycin are recommended), with moderate aeration and FSW. It is preferable for the tank to be kept out of the sun. After the veliger stage is attained (40+ hours) the larvae are transferred (on a 50–80 µm screen) to a larval and juvenile rearing tank (without further use of antibiotics) at a density of 1/ml where they remain until settlement and through the land nursery phase. A flow-through system (Fig. 1) may be used with the semi-intensive method (Braley et al. 1988). A cone of 80 µm mesh surrounds the standpipe and is held up by a polystyrene donut float. Aeration is provided around the standpipe base so the air bubbles brush away larvae which become trapped against the mesh. The large surface area of the cone causes little suction pressure at any one point where larvae may touch the cone mesh. A slow exchange of fresh FSW removes nutrients and wastes and helps retain the larvae. The screen needs to be changed every other day until most of the larvae have settled. Zooxanthellae are added more often, e.g. on days 4, 6 and 8. Also, as for the intensive method, the number of zooxanthellae added are more carefully calculated to be about 100–400/mL of tank culture water compared with the extensive method which may simply add the zooxanthellae suspension without determining density. If the species of clam being cultured is rare, zooxanthellae from a more common species may be used as the zooxanthellae donor. To ensure that the settled juvenile clams pick up zooxanthellae from their own species, some older juveniles (1+ years) are added to the tank, which release zooxanthellae in the feces.

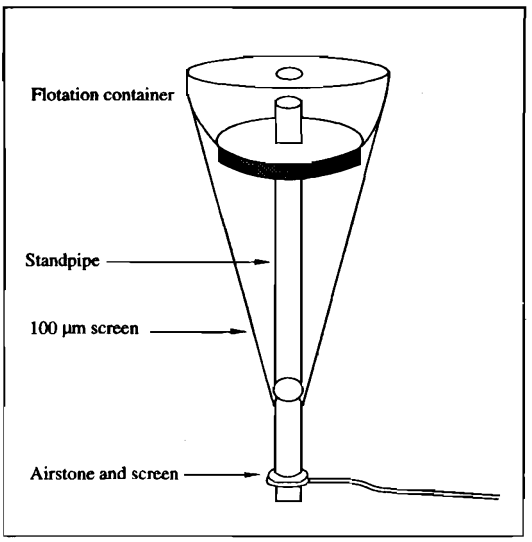

Figure 1. Flow-through cone screen (80–100 mm screen material) used in larval tank.

This selection method is more hygienic than the extensive method in which unfertilised eggs or dead/dying embryos remain on the bottom of the larval rearing tank which may cause increased bacterial loads for the healthy larvae. Although the semi-intensive method may not achieve survival rates as high as the intensive method, it is far less labour-intensive and more cost effective. It is virtually as simple as the extensive system and therefore recommended for remote hatcheries and those with low budget programs. The drawbacks of this system are the costs for antibiotics and aeration. At very remote locations this could be a problem. Additional tanks are required compared with extensive culture and there is a need for a microscope and haemocytometer to count zooxanthellae cells to obtain the correct dosage.

Intensive

The steps for the intensive culture are the same as those for the semi-intensive method, for the hatching phase to the veliger stage. However after retrieval and counts are made of day 2 selected veligers they are put into aerated fresh FSW at stocking rates up to 20/mL without antibiotics. The water may be changed every 1–2 days, or a conical flow-through screen may be used. If the water is changed it is preferable to drain larvae down onto an 80–100 µm screen and transfer to another tank already filled with FSW either every other day or on a daily basis if larvae are being fed. On day 5 the larvae are drained down on a screen, counted to

obtain survival rate and then transferred to settling tanks at a density of one larvae/5 mL (Heslinga et al. 1990). The settling tanks may be concrete raceways or circular tanks, or cheap above-ground splasher pools (PVC liners). Zooxanthellae are added often starting on day 4 as described above but with particular care taken to concentrate the zooxanthellae by centrifuge and to discard the clam tissue and haemolymph, in order to improve the hygienic condition of the culture. The concentrated pellet of zooxanthellae can be re-suspended in FSW for feeding to the larvae. At the ICLARM Coastal Aquaculture Centre in Solomon Islands the larvae are reared intensively but are not given zooxanthellae until they are pediveligers (days 9–100 in the Solomon Islands, Usher 1990).

The settling-tank water is filtered to one micron up to day 30 post-fertilisation. In some intensive operations a further tank transfer is made at day 28–30 and the post-metamorphic juvenile clams are graded and stocked into this final nursery tank (Usher 1990).

Clam larvae in intensive culture can be grown at relatively high densities, which reduces the tank volume needed to culture them through the larval stages. There is also better control of the water quality by this method than with the extensive or semi-intensive methods and generally one can expect a higher and more consistent survival of settled/metamorphosed larvae in the intensive method. The technique generally requires more highly trained technicians than for other methods and there is a need for more expensive equipment, clearly excluding remote locations or low-budget operations. The operational costs for intensive culture would be higher than the other two methods.

Larval feeding

Giant clam larvae are plantotrophic, as are the majority of bivalve mollusc larvae. However, Heslinga et al. (1990) in Palau claim that the eggs of at least *T. derasa* are lipid-rich and tend toward lecithotrophy. They do not feed their larvae with any microalgal or other particulate food, other than zooxanthellae and obtain good results. However, the importance of dissolved organic matter (DOM) and nanoplankton which pass through a hatchery's nominal one micron filter bags to the clam larvae has not been determined. Since there is a small amount of yolk material in each egg, the larval energy reserves until metamorphosis and the switch over to autotrophy is very limited and any amount of stress is likely to cause mortality of some larvae. Therefore the addition of food to provide extra energy reserves during stress periods, particularly the time between settlement and metamorphosis should improve survival and growth. In trials of dietary preference in giant clam larvae, naked flagellate algae were preferred to diatoms and growth was best with flagellate algae of 2–10 µm size (Fitt et al. 1984, Braley 1986, Southgate 1990). If an algal culture facility is considered feasible the three flagellate species of algae found to be suitable as food for giant clams are: *Isochrysis* sp. (Tahitian isolate), *Tetraselmis suecica* and *Pavlova salina*. Standard protocols exist for culture of these algal species for food. Quality of the algae can be monitored and fed to larvae at the most nutritious stage. Individual algal species can be tested for dietary preference and benefit to larvae. The amount of algae needed to feed large concentrations of larvae is in the range of 1000–15 000 cells/mL, less than that typically fed to oyster and mussel larvae. Densities of algae are adjusted daily using a haemocytometer for algal counts. Feeding should not begin until day 3 post-fertilisation because the gut has not yet fully developed when the larvae becomes a veliger on day two. Maintenance of the cultures requires both expensive equipment and technical expertise. At an island research station it was determined that 16–20% of the running costs were devoted to monospecific algal culture. The culture of these algae is therefore too complex for simple tropical hatcheries.

Greenwater culture is a term used to describe culture of the algae naturally found in seawater and is an alternative to monospecific unicellular algae. It is started by filtering seawater to 10–25 µm before adding medium for mass-algal culture. One such medium is termed 'Dix Mix', consisting of 520 g ammonium sulphate, 200 g superphosphate and 3.72 g thiamine HCl (vitamin B_1). This is crushed and mixed into 10 litres of warm tap water. The dosage is one mL/L of culture seawater for an algal culture already established but 0.25 mL/L for establishing a new culture. This method is easier to start and maintain in remote areas than monospecific unicellular algae. Only tanks, aeration and basic fertiliser are required. With this method, however, there is a much higher chance of contamination from ciliated protozoans, bacteria and unwanted blue-green algae compared with monospecific unicellular algae culture. Algae is added to larval cultures as described above.

Microencapsulated diets—Frippak booster for prawns and those made specifically for bivalves (Southgate 1988, 1990), and spray-dried algae (Algal 161, Celsys, *Tetraselmis suecica*)—have been used successfully as larval food in Australia and in partner countries in the ACIAR Giant Clam project and the ICLARM Coastal Aquaculture Centre, Solomon Islands. Southgate (1990) found that the micro-

encapsulated diet-fed larvae had identical survival to algal-fed juveniles at 35 days, though no controls survived to 35 days. There is considerable potential for 'off-the-shelf' larval food for remotely located hatcheries. To use these feeds requires only a refrigerator to maintain the opened containers. Small amounts of microencapsulated diets and spray-dried algae are required to feed larvae and they are easy to administer. However the containers of prepared foods are relatively expensive for low budget operations. Algal 161 is no longer available although there were good results reported from its use with bivalves. Baker's yeast has been used as a larval food but results so far indicate it is only marginally better than no food.

Feeding vs. no feeding

Studies have shown improvement in survival and growth of larvae given food when compared with unfed controls (Fitt et al. 1984, Braley 1986, Southgate 1990). However, additional expertise and equipment is needed for some foods to be cultured or maintained. If larvae can survive in numbers adequate to establish cultures of juveniles to settlement/metamorphosis there is no reason to culture or maintain expensive feeds. Also, feeding introduces some nutrients and therefore can promote bacterial growth.

Land nursery phase

This phase of rearing includes the period from larval settlement up to 8–12 months of age when the juveniles can be moved to the ocean nursery phase. Moving the juveniles out to the ocean nursery at an earlier age has been attempted experimentally but these are not yet standard practice. Table 1 shows the protocols used by the MMDC in Palau and those used by the ACIAR clam project in Australia. Other hatcheries in the Pacific Islands and the Philippines are generally variants of these two protocols however, there is usually no filtration of water beyond day 60 and aeration may not always be constant.

The major problem in the land nursery phase is algal fouling. The 50% shadescreen used in the Australian example (Table 1) keeps debris out of the tank and reduces light intensity from about 900–1000 $\mu E/m^2$/second to 400 $\mu E/m^2$/second (measurements at 0.7 m depth in tanks without and with shadescreen respectively). This restricts the growth of filamentous algae but because the small juvenile clams have a thin, translucent shell and mantle tissue the reduced light intensity due to the shadescreen does not proportionally reduce the photosynthetic rate of the clams (Fisher et al. 1985). In Palau no shadescreen is used on the tanks but the rapid seawater flow rates during the day may help

Table 1. Protocols for land nursery phase. Examples are shown for Palau (MMDC) and Australia (ACIAR— an associated project in the Pacific).

Palau (MMDC)		Australia (ACIAR)	
14–15	Heavy aeration seawater flow 20L/minute (12 hours)	2–90+	Moderate aeration, 10–25 mm FSW; 50% shadescreen canopy, seawater flow 7–10 L/minute (24 hours).
31–90	10 mm FSW in nursery tank at seawater flow 20L/minute (12 hours)		
90–150	No filtration of seawater; nitrogen supplements added, up to 100 mM N. Small trochus control algal fouling.	60–180+	Nitrogen supplements added; start with 5 mM concentrated Nitrogen, then 20–40 mM N. Manual algal fouling control.
120–150	Small rabbitfish (Siganidae) control algal fouling	90–180	Herbivore (cerithid snails, amphipods), algal fouling control.
150	Clams harvested and counted	180	Clams harvested and counted

Note: FSW = filtered seawater

keep filamentous algal growth to a minimum. In shallow tanks with a slower rate of seawater flow it is advisable to use shadescreen to avoid (solar) overheating of the water and algal fouling.

Control of algal fouling with herbivorous fish or invertebrates is common but species must be chosen carefully. These will generally be juveniles or small adults so as not to damage the juvenile clams. They are typically added after day 90. In Tonga, salt-acclimated mexican mollies (*Poecilia mexicana*) have been stocked in the nursery tanks to feed on the fouling algae and at the ICLARM Coastal Aquaculture Centre, surgeon fish (*Ctenochaetus striatus* and *Zebrasoma scopas*) salt-acclimmated tilapia (*Sarotherodon mossambicus*), ceriths, modulids and aplysids are used to alleviate the fouling overgrowth (Usher 1990). In Palau the gastropod *Trochus* is polycultured with juvenile clams (Heslinga et al. 1990).

Any appropriate method of reducing algal fouling is a positive step in the land nursery phase, since unrestricted fouling will contribute to higher mortality of juvenile clams. The method chosen will depend upon the budget of the hatchery (e.g. whether a hatchery can afford to pump more seawater or use shadecloth) and the availability of various herbivores.

Nutrient Addition

Despite the problem of algal fouling the clam juveniles require nutrients (for instance nitrogen and phosphate). Trials have shown growth is substantially improved with nutrients compared with controls (Fitt et al. 1984, Solis et al. 1988, Heslinga et al. 1988, Braley et al. 1993). The ammonium form of nitrogen is taken up more readily than the nitrate form but both can be used. While the additions of nitrogen have been shown to increase growth rates of clams the benefits of adding phosphate are not yet clear (Belda, pers. comm.). The optimum ratio of nitrogen:phosphate is not yet known but it is suggested that where 20–40 µM ammonium sulphate could be given daily in a spike, only 2–3 µM superphosphate should be given once per week (Braley 1990, Braley et al. 1993). In Palau the nursery tanks receive daily spikes of 100 µM ammonium nitrate.

Nitrogen addition has improved growth of juvenile clams and is being used as standard protocol in most hatcheries but the concentration of nitrogen ranges from 5–100 µM. About 20 µM nitrogen would be suggested as safe in virtually all cases. Fertilisers are relatively cheap and can be purchased and stored, even at remote locations. Higher concentrations can have harmful effects and it is important that calculations are carefully checked (Heslinga et al. 1990). Algal fouling increases with nutrient addition, therefore, manual cleaning or the addition of herbivores is necessary when using nutrient addition.

Temperature Effects/Recirculation

In the higher latitudes within the tropics the seawater temperature may drop to 21°C or lower during the winter months. Because the juveniles of some species of giant clams (especially *T. gigas*) are sensitive to low temperatures, high mortalities can occur. It is possible to maintain near summer water temperatures by direct solar heating in a greenhouse enclosing the tanks and recirculation seawater with biological filters (Braley 1990, Braley et al. 1993). When this system is used with careful nutrient addition growth of juvenile clams can be higher and mortality lower than without the greenhouse (Braley et al. in press). Therefore the culture of at least seed clams could take place in subtropical or temperate zones with greenhouse recirculation systems. This type of operation is potentially expensive to set up and the additional cost of pumping must be considered. These expenses are unnecessary if the site chosen is within the low latitude tropics.

Acknowledgments

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Constraints in Restocking Philippine Reefs with Giant Clams

H. P. Calumpong and E. Solis-Duran*

THE major objective of the Giant Clam Project in the Philippines when it was launched in 1984 was to develop mariculture techniques for giant clams for (1) food and (2) restocking of reefs. From 1985 to the present, Silliman University in Dumaguete City, Philippines has restocked over 20 areas in the Philippines (Figure 1). These areas possess some sort of protection from human disturbance and range from marine sanctuaries to private resorts. The area also consists of the natural range in habitats from coral reefs, seagrass beds, sandy bottom to rubble.

Materials and Methods

Clam juveniles, reared in the laboratory, were allowed to attach to substrates before transferring to the ocean. Substrates were generally made of cement but coral rubble, limestone and dead clam shells were also used. Size at transfer differed, ranging from 2.5–10 cm. The clams were placed inside cages made of bamboo, nylon or polyethylene at densities of generally 100 /m^2. Nearby sites were visited regularly (as often as every three months) where the clams were counted and measured.

Results and Discussion

A total of 26 sites were restocked (Figure 1). The sites were primarily chosen according to some degree of protection from humans. More than half of the sites were marine sanctuaries or potential marine sanctuaries. Potential sites refers to those where preparations (social and hydrobiological) are under way so that the site can be declared a sanctuary by the appropriate government agency. The marine sanctuaries are Apo Island, Pamilacan Island, Balicasag Island, Calauit Island, and Carbin Reef in Sagay. Two of the sites are resorts (Sugod and Samal Island) while Danahon is a seaweed farm. The rest of the sites are monitored either by institutions—Silliman University Marine Laboratory (SUML), University of the Philippines Marine Science Institute (UPMSI), Don Mariano Memorial State University (DMMSU)—or private persons. Of the 26 sites, 54% were coral reefs, 15% were seagrass beds, 27% were sandy and 4% consisted of rubble. All sites were subtidal generally between 2–6 m.

In 11 of the 26 sites, 9.8% (2067) of the total number of clams restocked survived (Table 1). The major cause of loss were typhoons (35.5%) followed by predation (28.6%). (Table 1 and Figure 2). Handling and transport rank third. This is because about 2000 clams sent by air to Pamalican Island got detached from their substrates and did not recover thereafter. Another 4.1% were unmonitored; the sites were too far for SUML to monitor and cooperators never responded to our inquiries. A very small percentage (1.2%) were observed covered with fine silt while the cause of death or loss for the rest was not known.

A comparison of survival in these habitats are given in Figure 3 with coral reefs yielding highest overall

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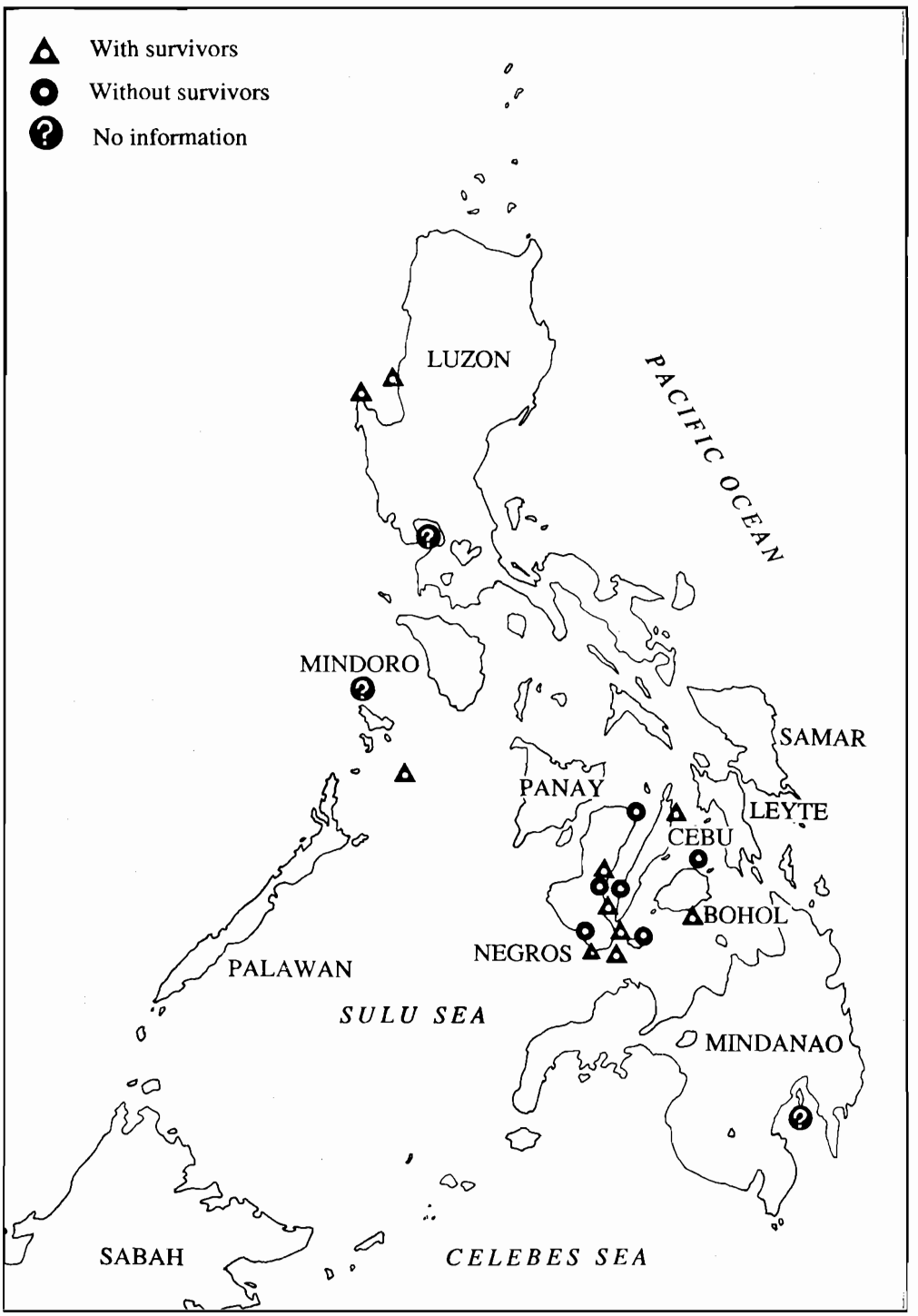

Figure 1. Map of the Philippines showing areas restocked with giant clams.

Table 1. Summary of fate of disseminated clams indicating total number at stocking (No), number of survivors as of May, 1992 and causes of deaths.

Site	No. at stocking	No. of survivors	Typhoons	Predation	Poaching	Handling/ Transport	Siltation	Unknown	Unmonitored
Apo I.	811	151	142	473				45	
Negros I.									
Bantayan	1813	23	1269	249			249	272	272
Tamao	1000	0	1000						
Cabulutan	700	14	500	186					
Calag-calag	2690	193	1000	1457					
Ayungon	1000	0	1000						
Tinaogan	2567	71		1739	757				
Kabugan	141	0	141						
Malaga	227	0	227						
Tigib	47					47			
Tambobo	55	0						55	
Campuyo	713	624						89	
Sagay	190	0	120	50	20				
Bohol									
Pamilacan	1156	16	839		301				
Balicasag	573	0	573						
Danajon	480	0	480						
Siquijor I.									
Villanueva	1535	0		1535					
Cebu									
Moalboal	50	0		50	50				
Sugod	150	106						44	
Palawan									
Pamalikan	3300	719				2300	2300	281	
Calauit	420								420
Davao									
Samal	416								416
Luzon									
Cavite	20								20
La Union	100	49						51	
Bolinao	533	67		417				49	
Total	20687	2033	7290	5907	1078	2387	249	886	856
%	100	9.8	35.2	28.6	5.2	11.5	1.2	4.3	4.1

survival (19.8%) followed by sandy areas (9.2%) and seagrass beds (4.8%). Survival decreases rapidly with only a few individuals reaching maturity (Table 2).

Growth rates are also compared in these three habitats (Table 3). Highest is *T. derasa* in sandy area (6.6 mm/month) followed by *T. squamosa* (4.7 mm/month) in coral reef. Growth rate of *H. hippopus* did not show big differences in the three habitats.

At present, potential breeding populations of clams exist in five restocked sites (Table 4) consisting of four species: *T. squamosa* in Apo Island and Cabulutan, Tayasan, Negros Oriental; *T. derasa* in Bolinao, Pangasinan; *H. hippopus* in Pamilacan Island and Bolinao; and *H. porcellanus* in Apo Island and Bolinao.

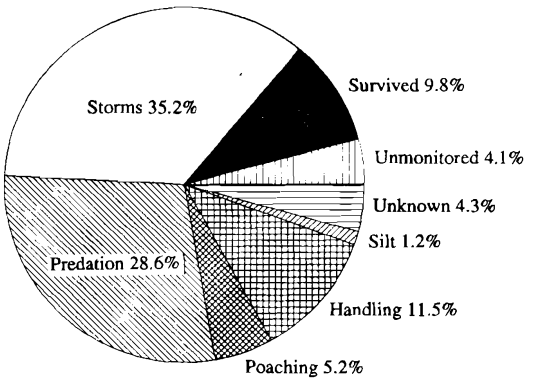

Figure 2. Fate of clams restocked in Philippine reefs.

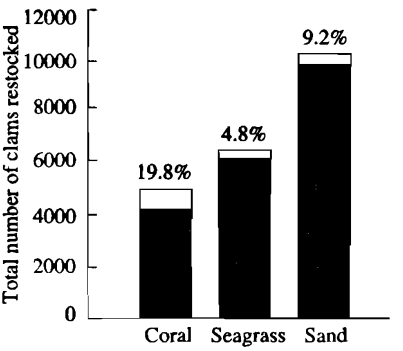

Figure 3. Survival of clams restocked in three types of habitat. Dead ■; and surviving □.

Table 2. Survival of clams (actual numbers) in restocked areas over time.

Species	Location	Year							
		0	1	2	3	4	5	6	7
T. derasa	Apo island	45	45	15	15	4	2	2	1
T. squamosa	Apo island	50	46	33					
H. porcellanus	Apo island	25	20	16	16				
	Bantayan	50	7	0					
H. hippopus	Apo island	20	5	1	0				
	Pamilacan island	64	9	9	9				
	Bantayan	50	45						

Table 3. Comparison of growth rates (mm/month) of clams restocked in different habitats.

Species	Seagrass		Coral reef		Sand		
	Pamilac	Bant	Apo	Tinaogan	Danajon	Pamali	Campuyo
T. derasa	3.3	2.37	1.69	–	6.6	2.78	
T.squamosa	–	1.4	1.8	4.7	–	–	2.52
H. hippopus	2.0	1.2	3.97	1.66	–	2.8	2.62
H. porcellanus	1.7	–	2.57	–	–	–	

Conclusions

This study has shown that raising clams and restocking reefs in the Phillipines can be successful. If the reef is protected from typhoons and poaching and one is careful with handling, then survival of as much as 60% or higher may be attainable.

The cause of highest clam mortality is nature: typhoons, monsoons, strong winds. This should be a major consideration in site selection. Protection from predators should be taken seriously as these can potentially cause high mortality. Mesh size of cages should always be smaller than clam size. Cage design should be appropriate to the type of habitat. The only reason why poaching is not the major cause of clam loss in this region is because most sites have some degree of protection or monitoring.

Table 4. Potential breeding populations of giant clams in restocked areas.

Site	Species	No.	Age (years)	Mean SL (cm)	Parentage
Apo island	*T. squamosa*	33	5.5	14	Carbin Reef
	H. porcellanus	16	4.6	16	Palawan
Pamilacan	*H. hippopus*	9	7	15.5	Manjuyod
Cabulutan	*T. squamosa*	14	4	11	Carbin Reef
Bolinao	*T. derasa*	34	5	>15	Palawan
	H. porcellanus	5	6	>15	Palawan
		4	4.6		Palawan
	H. hippopus	10	7	>15	Manjuyod
		24	7		

Although generalisations are difficult to make with regard to survival and growth in different habitats, certain trends are apparent:

- Areas such as the Danajon Bank—shallow, sandy with clear water seem to be best for *T. derasa;*
- *Hipopus* species seem to do well in most habitats and may be the easiest to restock.

In order to evaluate the success of the restocking program, not only in terms of survival but most importantly in terms of establishment of breeding populations, clams must be marked so that recruits can be traced. Genetic markers are important in this respect.

Finally, as in any restocking program, the cost is high and success very low. In order for us to establish effective breeding populations in the Philippines of the extirpated giant clam species such as *T. gigas, T. derasa* and *H. porcellanus*, restocking of juveniles should be in the thousands and should be carried out over many years. This requires substantial funding for the maintenance of clam hatcheries, restocking, marking and monitoring.

Acknowledgments

The following are thanked for their faithful monitoring of the sites and taking measurements of the clams: Janet Estacion, Dioscoro Inocencio, Gary Cuyugan, Grace Ozoa, Pablina Cadiz, Rick Braley, Rio Naguit, Sally Alcazar, Zaccharias Generoso, and clam cooperators. The Australian Centre for International Agricultural Research is gratefully acknowledged for the generous funding.

Notes on the Life Cycle of the Giant Clam *Hippopus hippopus* (Linn)

S. N. Alcazar*, Ma. R. A. Naguit* and G.A. Heslinga†

INDUCED spawning of *Hippopus hippopus* in the laboratory using freshly macerated or freeze-dried gonads has been reported by Gwyther and Munro (1981), Jameson (1976), and Wada (1954) while intragonadal injection of serotonin was reported by Alcala et al. (1986), Alcazar et al. (1987), Braley (1985) and Crawford et al. (1986). Fitt et al. (1984) studied the early development from fertilised eggs to the 58-day juvenile stage while Alcala et al. (1986) and Alcazar (1988) studied spawning and larval rearing.

Induced spawning of *Hippopus hippopus* has been conducted at Silliman University Marine Laboratory, Philippines (SUML) for the past eight years during the period from 10 December 1984 to 28 March 1992, with a total of 26 induced spawnings, 15 of which were successful. All these spawnings involved wild broodstock. This paper describes briefly the closed life cycle of *Hippopus hippopus*. The experiments were conducted at SUML, and the Micronesian Mariculture Demonstration Centre (MMDC) in Palau.

Materials and Methods

At SUML, two six-year-old F1 *Hippopus hippopus* (Fig. 1) coming from two different sets of parents were induced to spawn in the laboratory on 30 October 1991 using intragonadal injection of serotonin. The maternal parent, measuring 15.3 cm shell length (SL) came from a batch spawned on 14 August 1985 with parents collected from Cagayan Islands, Palawan. The clam releasing sperm (13.6 cm SL) was taken from a batch spawned on 30 August 1985 with parents collected from Campuyo, Majuyod, Negros Oriental (Alcala et al. 1986).

The clams were placed separately in 35 L shallow buckets containing double filtered (10 µm and 1 µm) seawater. Each clam was injected with 1 mL serotonin solution. During the sperm release, three to four containers (100 mL capacity each) of sperm water were collected from each clam and saved. Filtered seawater was allowed to flow through in the bucket to remove excess amount of sperm.

When eggs were first noticed, the clam was removed, rinsed thoroughly and transferred to another bucket filled with fresh filtered seawater, where it was allowed to release more eggs. About 100 mL of less dense sperm suspension from the 13.6 cm SL clam were used to fertilised the eggs. Number and density of eggs were estimated using the method described by Heslinga et al. (1990) and Braley (1990).

Fertilised eggs were placed in a one tonne circular hatching tank half filled with double-filtered seawater provided with gentle aeration. At two-days-old, the larvae were harvested and transferred to a three tonne concrete rearing tank and allowed to develop through to the juvenile stage. Supplemental food consisting of *Isochrysis galbana* (one litre at 10^5 cells/mL) was given three times a week for one month commencing after the veligers were harvested. Infection of

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Figure 1. SUML parent clams (F1).

zooxanthellae (about 5×10^6 cells) was administered on day four.

At MMDC, four broodstock were allowed to spawn spontaneously, two clams in each rectangular tank. In this case, the larvae were not infected with zooxanthellae.

Results

Spawning, larval development and growth

At SUML, approximately, 1 194 000 eggs were spawned by one clam (15.3 cm SL) after the first injection with serotonin. Injections of serotonin were administered at ten minute intervals. The second clam released sperm after the second injection and stopped 25 minutes after the last injection. The newly fertilised eggs had a mean diameter of 103.4 µ ± 204 (n = 50). About 1 164 000 eggs were fertilised normally (about 97.48%); some underwent polyspermy.

The chronology of larval developmental events of F2 *Hippopus hippopus* is presented in Table 1. The eggs underwent normal development. Trochophore larvae emerged after 20 hours, becoming straight-hinged veligers in 20 hours and pediveliger after 4–9 days. Metamorphosis was complete in 10 days. At MMDC a total of 6 449 052 eggs were released during a spontaneous spawning.

Larval and juvenile survival and growth

Survival rates for each developmental event are presented in the Table 1. Survival rate from fertilised eggs to trochophore at 20 hours was 42.96% while survival rate to straight-hinged veliger at 30 hours was 8.59%. At day five, 7.75% of the veligers reached the pediveliger stage out but only 1.72% metamorphosed at day 10. At 6 weeks only 0.52% of the metamorphosed juveniles survived and 0.013% reached six months of age (150 individuals).

At 20 hours, trochophores reached a mean shell length of 112.0 µm ± 1.37 (n= 25). At 30 hours, veligers were 170.6 µm SL ± 1.37, (n= 50) while five day old pediveligers reached 191.72 µm SL ± 9.6 (n= 50). The larvae metamorphosed at day 10 with a mean shell length of 230.86 µm ± 15.0 (n= 20) and at 6 weeks the juveniles attained shell length of 1.2 mm ± 0.54 (n= 50). The six-month-old F2 juveniles (Fig. 2) reached a mean shell length of 6.175 mm ± 3.05 (n= 50). Six-month-old juveniles produced at MMDC reached the sizes ranging from 12.3 mm to 15.2 mm.

Table 1. Chronology of developmental events in F2 *Hippopus hippopus*.

	Sulimar University Marine Laboratory			Micronesian Mariculture Demonstration Centre		
Post-fertilisation time	Mean length ±SD[a] (µm)	No. of individuals	Survival from fertilised eggs (%)	Length	No. of individuals	Survival from fertilised eggs (%)
0 hours (fertilised eggs)	103.40 (±2.04)	1 164 000	–		6 449 052	
20 hours (trochophore)	112.00 (±1.37)	500 000	42.960	–	–	
30 hours (veliger)	170.60 (±11.6)	100 000	8.590	–		–
5 days (pediveliger)	191.72 (±9.6)	90 000	7.730	–	–	
10 days (metamorphosed juvenile)	230.86 (±15.05)	70 000	1.720		–	–
6 weeks(juvenile)	1.20 (±0.54)	600	0.520	–	–	
6 months (juvenile)	6.17 mm	150	0.013	12.30–15.20 mm	1188 and 1295	0.018–0.020

[a]Minimum sample size = 20

Figure 2. SUML 6-month-old F2 juveniles.

Discussion

The mean size of the F2 fertilised eggs (103.4 µm ± 2.0 n= 30) was very much smaller compared to those observed by Alcala et al. (1986), (143.16 µm ± 7.26) and Alcazar (1988) (143.5 µm ±3.7) for F1 *H. hippopus*. However, Jameson (1986) observed F1 *H. hippopus* eggs that were also small in size (93.1 µm ±32). Egg size has been reported to vary even in the same species (Alcazar, 1988). The appearance of veligers at 30 hours closely coincided with the observations of Fitt et al. (1984), Alcala et al. (1986)

and Alcazar (1988). It took 48 hours for all the larvae to reach the veliger stage. Alcala et al. (1986) and Alcazar (1988) reported that F1 *H. hippopus* larvae developed into straight-hinged veligers in 22–27 hours. Pediveligers developed on the fifth day which is comparable to the data obtained by Alcala et al. (1986) and Alcazar (1988) on F1 *H. hippopus* larvae. Fitt et al. (1984) however, reported pediveligers developing 9–10 days after fertilisation. Metamorphosis occured 10 days after fertilisation.

Generally, the larvae underwent normal development from trocophores to juvenile stage. The mean size of trocophore larvae was 112.0 µm ± 1.37 (n=25). Mean size of veligers was 170.6 (µm ± 1.37, n=50).

Six-month-old F3 *H. hippopus* juveniles at MMDC reached 12–15 mm SL while those at SUML are only measured 6.2 mm ± 4.0 (n =20).

To compare the survival of six-month-old juvenile with that of laboratory-reared F1 juveniles from wild parents, the F1 juveniles showed better survival (0.26 %) compared to that F2 (0.013 %). These percentages are comparable to those obtained at MMDC, 0.018 %–0.020 %.

It is evident from this report that survival among F2 juveniles was very low. This may be a result of genetic degeneration in F2 offsprings. It must also be noted that growth of F2 juveniles varied significantly between the two hatcheries. At SUML, six month old F2 juveniles measured 6.2 mm ± 4 (SD) mm in shell length on average (n=50) while those at MMDC measured 12.3–15.2 mm in shell length.

Acknowledgments

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Biochemical Evidence of Self-fertilisation in *Hippopus* Species

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IN 1987, Alcazar (1988) attempted to cross-breed the two *Hippopus* species of giant clams using *H. porcellanus* egg and *H. hippopus* sperm. The ensuing larvae developed just like those of *H. porcellanus* in that they spent less time in the trocophore stage (9.5 hours vs. 20 hours for *H. porcellanus*) and metamorphosed a day earlier (Alcazar 1988). Alcazar (1988) further observed that some shells of 3-month old juveniles had fine scales at the posterior end of the valves just like *H. hippopus* but were paper-thin just like those of *H. porcellanus*. A few older juveniles looked like *H. porcellanus* with smooth shells but exhibiting yellowish-pink flecks on their shells (Figure 1a). Because of the presence of these juveniles which looked intermediate between the two species, the cross-breeding experiments were repeated, the second one the same as the first except that the parents came from different sources, the third one a reverse of the first two, with *H. hippopus* as egg source and *H. porcellanus* as the sperm source. Juveniles produced from the first and third spawnings (no F1 from the second spawning survived after 3 months) were analysed using horizontal starch gel electrophoresis utilising seven genetic markers.

This paper presents the results of the electrophoresis experiments and discusses their implications.

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Methodology

Spawning

Three spawnings were conducted in the laboratory. The first (S1) was carried out on October 29, 1987 with a 38 cm *H. porcellanus* from Cagayan Island, Palawan (egg source) and a 23 cm *H. hippopus* (sperm source) from Campoyo, Manjuyod, Negros Oriental. The second spawning (S2) was carried out on May 20, 1989 with a 46.6 cm *H. porcellanus* from Cagayan Island, Palawan as the egg source and a 24.6 cm *H. hippopus* from Marigondon, Cebu as the sperm donor. The third spawning (S3) occurred on December 20, 1990; this time a 26.1 cm *H. hippopus* from Marigondon, Cebu was the source of eggs and a 38.2 cm *H. porcellanus* from Palawan was the source of sperm. All spawnings followed the same procedure described by Alcazar et al. (1987). Clams were each placed in 60 L glass aquaria containing double-filtered (10 µm and 1 µm) seawater. Each clam was injected with 1 mL serotonin solution and observed for gamete release. Sperm released were collected in three 100 mL containers and saved. Filtered seawater was allowed to flow through the aquarium to remove excess sperm.

When the clam started to release eggs, it was removed from the aquarium, rinsed thoroughly with filtered seawater to remove adhering sperm and transferred to another bucket with freshly-filtered seawater where it was allowed to release eggs. The eggs were fertilised using one container (100 mL) of saved sperm. Details of these spawnings are given in Table 1.

Table 1. Spawning success, veliger and juvenile survival

	29 October 1987 (S1)	20 May 1989 (S2)	20 December 1990 (S3)
Egg source	*H.porcellanus*	*H.porcellanus*	*H. hippopus*
Locality	Cagayan Is. Palawan	Cagayan Is. Palawan	Marigondon,Cebu
Sperm source	*H. hippopus*	*H. hippopus*	*H. porcellanus*
Locality	Manjuyod Negros Oriental	Manjuyod Negros Oriental	Cagayan Is., Palawan
No. of eggs released	3132000	5900000	5000000
No. of eggs fertilised	288000	1900000	3000000
No. of trochophores	60000	1850000	256700
No. of veligers	22000	2870	28200
No. of pediveligers	12000	2800	21000
No. of juveniles	1500 (3 months-old)	1500 (1 month-old)	7510 (3 months-old)
Survival from fertilised eggs (%)	0.52%	0.078%	0.25%
No. of juveniles at present	10	0	515

Electrophoresis

Samples of macerated mantle were obtained from 5 S1 individuals (4.5 years old, 11–17 cm shell length [SL]), 20 S3 individuals (1.5 years old, 3–6 cm SL), 10 hatchery-bred (F1) *H. porcellanus* (5.5 years old, 11–14 cm SL) and 10 individuals of *H. hippopus* (1 year old, 3–5.5 cm SL). The mantles containing zooxanthellae were homogenised in homogenisation buffer (75 µM magnesium sulfate, 0.2% Triton X-100, 0.13% w/v) polyvinyl pyrrolidone (PVP) in 50 µM sodium phosphate buffer, pH 8.0 and 0.1 M NaCN added prior to use to a final concentration of 2 µM to exact proteins. Exudates were adsorbed onto strips of filter paper (Whatman No. 8). These were inserted into cooled moulded 12% starch gels (Sigma starch, Sigma Chemical Company, USA) made with Tris-EDTA-Borate (TEB pH 8.4), Tris-EDTA-Citrate (TEC pH 7.9) and Tris-Citrate (TC pH 7.0) for horizontal gel electrophoresis. The gels were run at a constant current setting of 40 mA per gel for 6–7 hours. Protein patterns for seven enzyme systems: glucose phosphate isomerase (GPI, E.C. = 5.3.1.9); malate dehydrogenase (MDH, E.C. = 1.1.1.37); phosphoglucomutase (PGM, E.C. = 2.7.5.1); diaphorase (DIA, E.C. = 1.6.*.*); leucyl-glycylglycine (LGG, E.C. = 3.4.11/13.*); and glutathione reductase (GSR, E.C. = 1.6.4.2) were scored after adding substrates and co-factors. Details of the buffer systems used, electrophoretic and staining procedures are given in Benzie et al. (1993) and Macaranas (1991).

Results

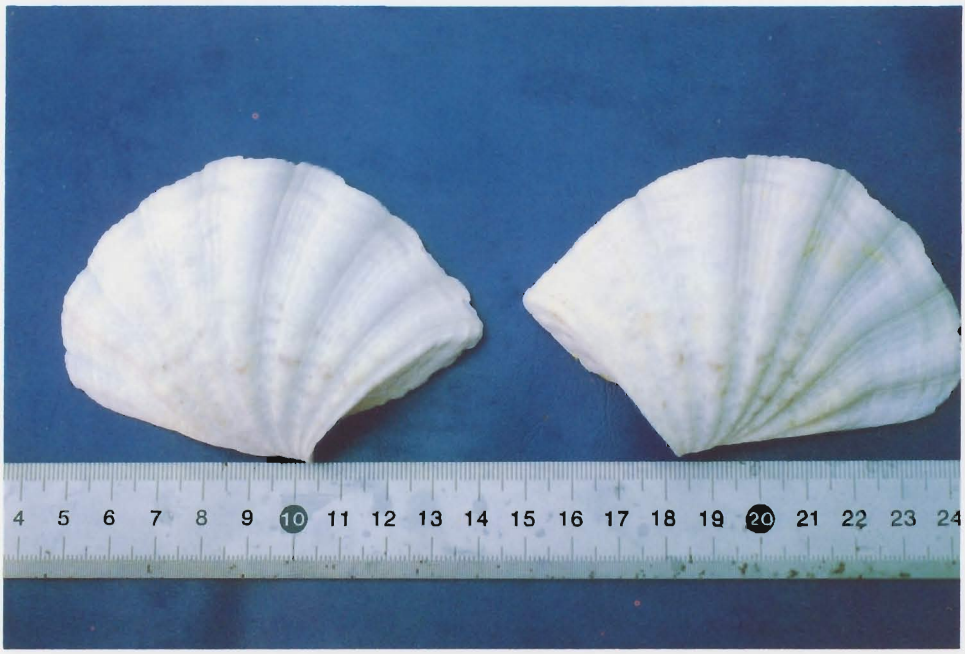
Morphologies

Morphologically, S1 juveniles showed similarities with *H. porcellanus*, the egg source. The shells were characteristically those of *H. porcellanus*, generally smooth without strawberry flecks on the surface. However, a few shells had light yellowish-pink strawberry flecks (Figure 1a). Papillae were present in the inhalant siphon characteristic of *H. porcellanus* (Figure 1b). S3 juveniles resembled the egg source, *H. hippopus* in having brown mantles, scutes and prominent strawberry flecks on the shell (Figure 2).

Enzyme Patterns

Results of electrophoresis on seven enzyme systems are shown in Table 2. The enzymes investigated gave clear banding patterns in each of the samples sampled (Figure 3).

(a)



(b)

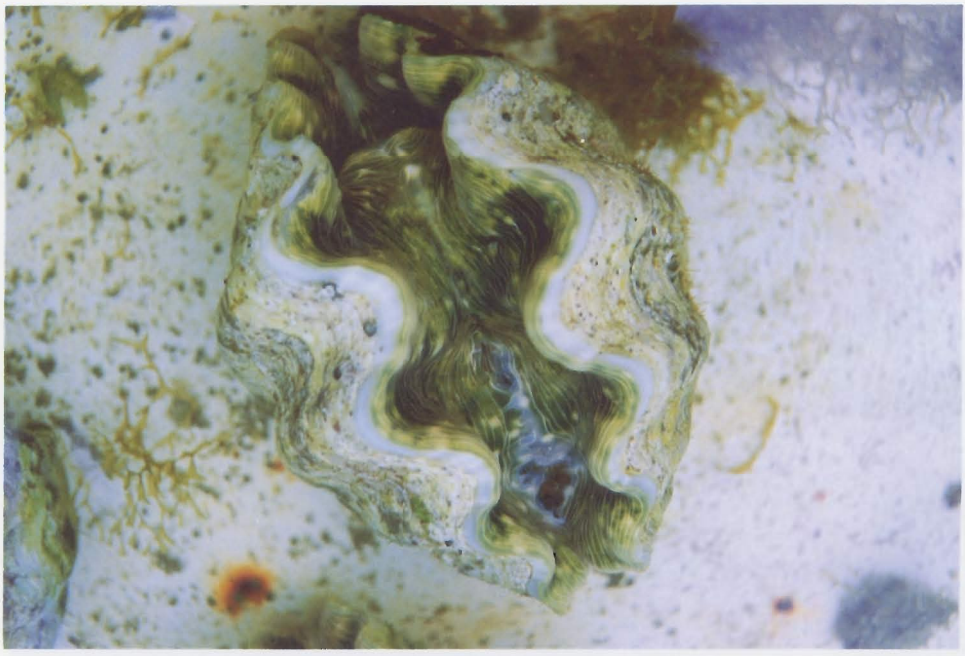


Figure 1. Morphology of S1 juveniles: (a) cleaned shells showing yellowish-pink flecks; and (b) live juvenile showing prominent papillae lining the inhalent siphon.

Figure 2. Morphology of S3 juveniles.

Table 2. Gene frequencies of alleles for 7 enzymes of 4 populations of *Hippopus* species.

Enzymes				Population			
	Locus	Allele		*H. porcellanus*	S1	*H. hippopus*	S3
			(N)	9	5	10	20
Glucose phosphate isomerase (GPI)	GPI-1	A		0	0	0.150	0.350
		B		0.389	0.400	0.450	0.150
		C		0	0	0.400	0.475
		D		0.611	0.600	0	0.025
			(N)	9	5	10	20
Malate dehydrogenase (MDH)	MDH-1	A		0	0	1.000	1.000
		B		1.000	1.000	0	0
			(N)	8	5	10	19
	MDH-2	A		0.125	0	0.800	1.00
		B		0.250	0.200	0.150	0
		C		0.438	0.600	0	0
		D		0.188	0.200	0.050	0
			(N)	7	1	10	20
Phosphoglucomutase (PGM)	PGM-1	A		0	0	0.800	0.950
		B		0.143	0	0.100	0.050
		C		0.857	1.000	0.100	0
			(N)	9	5	10	18
Diaphorase (DIA)	DIA-1	A		0	0	0.600	0.694
		B		0	0	0.400	0.306
		C		1.000	0.600	0	0
		D		0	0.400	0	0
			(N)	9	5	9	19
Leucyl-glycylglycine (LGG)	LGG-1	A		0.500	0.100	0	0.026
		B		0	0	0	0.079
		C		0.500	0.900	0.722	0.316
		D		0	0	0.167	0.289
		E		0	0	0.111	0.132
		F		0	0	0	0.026
		G		0	0	0	0.053
		H		0	0	0	0.079
			(N)	7	4	10	9
Glutathione reductase (GSR)	GSR-1	A		0.714	0	0.500	0.333
		B		0.286	1.000	0.500	0.667

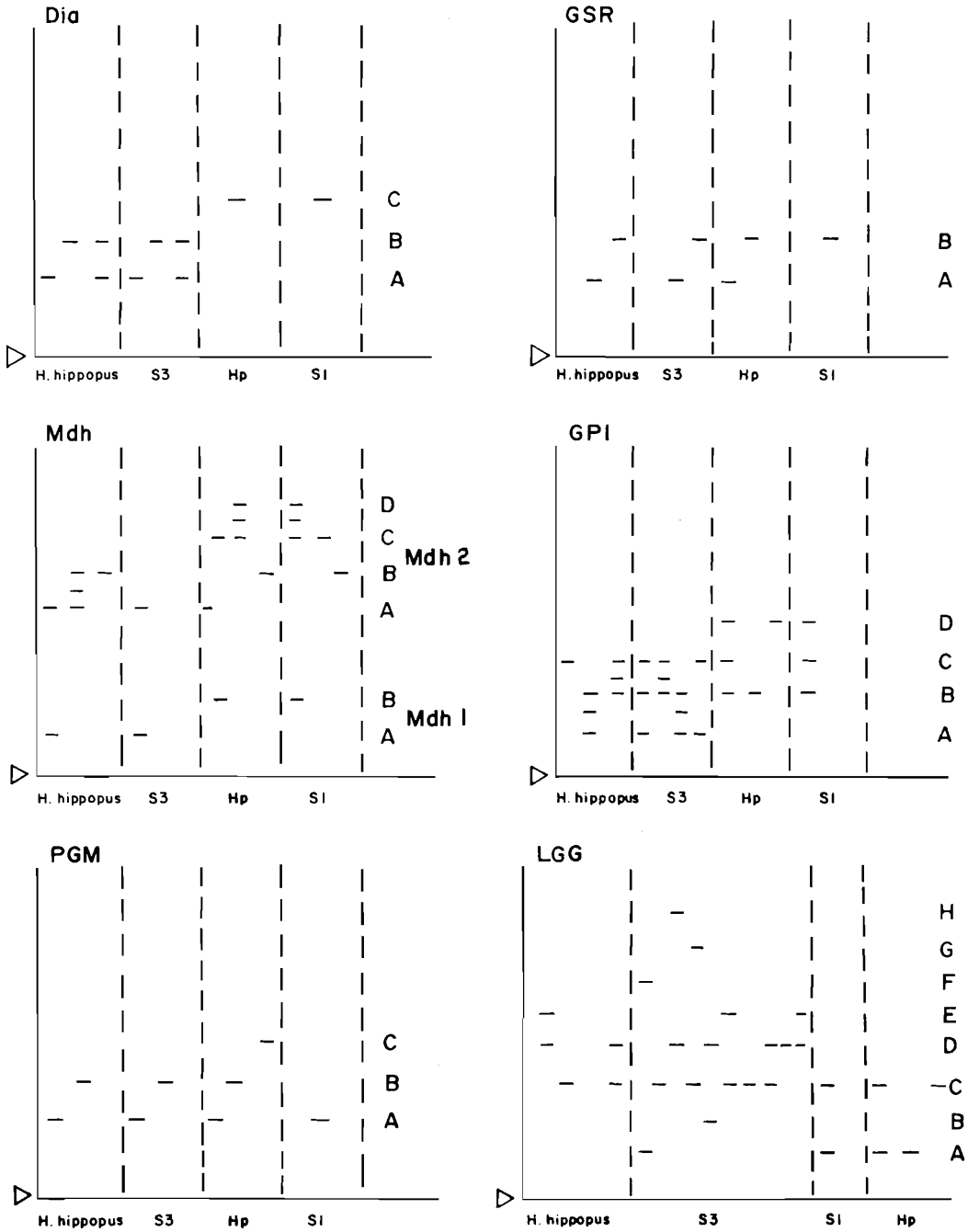


Figure 3. Electrophoretic analysis of mantle samples of *H. hippopus* and *H. porcellanus* (Hp) individuals showing allelic expressions of 7 genetic markers: DIA = Diaphorase; GSR = Glutathione reductase; MDH = Malate dehydrogenase (1 and 2); GPI = Glucose phosphate isomerase; PGM = Phosphoglucomutase; and LGG = Leucyl-glycylglycine.

Among the seven enzymes analysed, malate dehydrogenase-1 (MDH-1) seemed to be monomorphic with a fixed difference between *H. hippopus* and *H. porcellanus*.

Correspondence of alleles were observed between *H. porcellanus* and S1 and between *H. hippopus* and S3 at glucose phosphatase isomerase (GPI-1), phosphoglucomutase (PGM) and diaphorase (DIA) (Fig. 3). Although leucyl-glycylglycine (LGG-1) had eight alleles, two of which were shared between the two pure species (B and C), the strong similarity between *H. porcellanus* and S1 was demonstrated by the absence of alleles which were only present in *H. hippopus* and S3 (Fig 3; d–h). The most remarkable similarity, however, was observed at MDH-1 where S1 and S3 were fixed for the same allele as their respective egg sources.

These similarities between juveniles with their maternal species were further quantified using Nei's Genetic Distance (Table 3). The smallest distances were observed between *H. hippopus* and S3 and between *H. porcellanus* and S1. The distance between both species was 1.216 and between S1 and S3, 0.713. A clustering method (UPGMA) using the genetic distance values in Table 3 clearly shows the genetic relatedness of the juveniles to their maternal species (Fig. 4). The results provide strong evidence for self-fertilisation in the two sets of spawning experiments.

Table 3. Genetic similarity/distance coefficients using Nei's (1978) unbiased genetic distance.

Population	Hh	Hp	S1	S3
Hh	–	0.627	0.645	0.164
Hp	1.216	–	0.260	1.728
S1	1.142	0.156	–	1.545
S3	0.032	0.707	0.713	–

Discussion

It is very interesting to note that although the shell characteristics of the juveniles were generally similar to those of their respective egg sources, two morphological characters, thickness of the shell and coloration, did overlap indicating their unreliability as taxonomic markers.

Two enzymes, GPI and LGG exhibited some unexpected patterns in the S1 (for GPI) and S3 (for LGG) juveniles—banding patterns not otherwise seen in the Hp or Hh samples. This can be explained by the fact that samples of Hp and Hh used for comparison were not the original parents (since these had died) but were juveniles from wild broodstock taken from the same locality spawned and reared in the laboratory.

The possibility that the juveniles were still carrying proteins from their respective egg sources (maternal effects) at the time of testing only exists if the samples tested were very young. In this case, all samples tested were one year old or older and it is less likely that they still exhibited maternal effects.

The probability of self-fertilisation occurring under the spawning protocol observed is high. Giant clams are hermaphroditic and the simultaneous release of both gametes in one clam is common.

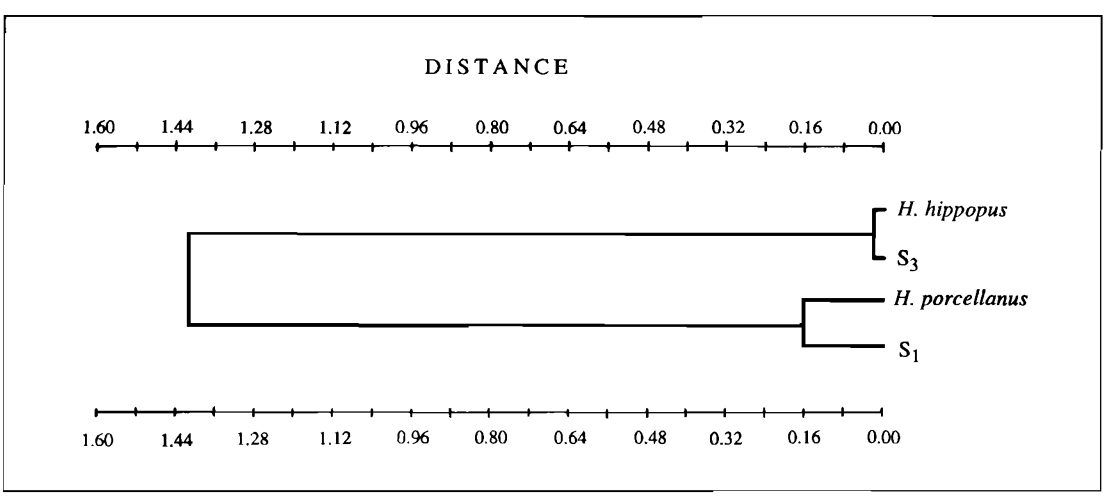


Figure 4. Cluster analysis of Nei's genetic distance of 4 samples of *Hippopus* species.

The presence of any hybrid pattern especially between fixed loci like MDH-1 would establish the success of hybridization. A possibility that hybrids may have been produced but were not viable under the spawning conditions is another possibility. On the other hand, strong isolating mechanisms between giant clam species may be present preventing species from hybridising, which could explain why there are only a few species (total = 8) of giant clams in the world.

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Controlling Predators of Cultured Tridacnid Clams

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SURVIVAL of cultured juvenile giant clams in the ocean nursery phase is reported to be relatively low, ranging from as low as 40% over the first 2.5 years for *Tridacna gigas* stocked at six months of age (Barker et al. 1988) to 50–75% over four years for one year old *T. derasa* (Heslinga et al. 1990). Much of this mortality can be attributed to predation.

Many animals are known to be capable of preying on juvenile tridacnids (Govan 1993) but most are not usually abundant or are excluded by properly closed ocean-nursery cages and therefore are not serious obstacles to giant clam culture.

Ranellid gastropods of the genus *Cymatium*, parasitic pyramidellids and, possibly, stylochid flatworms are the most serious pests found so far as they are difficult to control (Govan 1993). Other predators commonly reported include muricid gastropods and xanthid, portunid and diogenid crabs.

Cymatium muricinum (Gastropoda: Ranellidae) has previously been reported as a serious pest of cultured tridacnid clams (Perron et al. 1985). This species and also *C. aquatile*, *C. nicobaricum* and *C. pileare* are found throughout the tropical Pacific and are serious pests at almost all locations where tridacnids are cultured in ocean-nurseries (Govan 1992). They are difficult to control because they are capable of settling out of the plankton in clam cages at an early stage in their life cycle. Larger individuals represent a serious threat because of their nocturnal habits, ability to locate clams easily and ability to kill rapidly and consume even relatively large clams.

As part of an international collaborative study of predators of cultured giant clams some aspects relevant to the reduction of clam losses due to predation have been studied at the Coastal Aquaculture Centre of the International Centre for Living Aquatic Resources Management (ICLARM CAC), Silliman University Marine Laboratory (SUML) and the Fisheries Division of Western Samoa (WSFD). The aspects discussed in this paper are: the effects of cage location and design and the vulnerability of different clam species to predation by the gastropods *Cymatium pileare*, *C. muricinum* (Ranellidae), *Chicoreus palmarosae* (Muricidae) and *Bursa granularis* (Bursidae) and the crustaceans *Atergatis floridus* (Xanthidae), *Carpilius convexus* (Xanthidae), *Thalamita danae* (Portunidae) and *Dardanus pedunculatus* (Diogenidae).

Methods

Prey choice experiments

In the first series of prey choice experiments (performed at SUML) three gastropod and four crustacean predators were offered four species of clam (*Tridacna derasa*, *T. maxima*, *T. crocea*, and *Hippopus hippopus*). Three similar-sized individuals of each predator were tested individually in 80 litre flow-

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through aquaria. Five byssally attached individuals of each prey species were placed in aquaria with individual predators. Dead clams were counted and removed daily but not replaced. Two size classes of clam prey were tested; small (10–30 mm shell length, SL) and medium (30–60 mm SL). Control aquaria were also maintained containing clams but no predators. No mortality was observed in these aquaria. Most experiments lasted for eight weeks.

Prey consumed were not replaced, thereby progressively requiring the predator to consume subsequent prey in order of preference over the duration of the experiments. Data from this experiment were expressed as an accumulative ranking similar to the method used by Morton (1990). Data from the three replicates for each predator were pooled. The first individual prey chosen by a predator species was allotted a number equivalent to the total number of prey consumed by that predator species, the second individual prey was allotted that number minus one and so on until the last prey individual chosen which was accordingly allotted the number one. Summing of the numbers for each species gives an approximate indication of overall prey preference as shown by Morton (1990).

In the second series of preference experiments (performed at ICLARM CAC) two tridacnid species, *T. gigas* and *H. hippopus* (40–50 mm SL) were offered to the predator, *C. muricinum*, in 24 litre flow-through aquaria. Five byssally attached specimens of each clam species were placed in aquaria with individual predators. Dead clams were counted, removed and replaced daily, experiments lasted from 2–3 weeks. Predators in these experiments were starved for one week prior to the start of the experiment.

Two experiments were conducted, one with four adult specimens of *C. muricinum* (mean size 43.8 mm) and one with eight juveniles (mean size 20.6 mm). No controls were maintained but similar or higher densities of clams were kept in similar aquaria during the course of the experiment and no mortalities were observed.

Use of trestles in ocean-nurseries

Eight 0.7 m^2 cages containing 400 juvenile *H. hippopus* (mean length 32 mm) and 400 *T. gigas* (mean length 41 mm) were placed in an ocean-nursery at Namu'a, Western Samoa. Four cages (two containing *H. hippopus* and two containing *T. gigas*) were placed directly on the sand bottom. The remaining cages were raised 0.8 m off the seabed on trestles in an attempt to reduce the incidence of benthic predators, principally *Cymatium* spp. and *Chicoreus* sp. Clams were measured and counted every month for three months and predators were collected during routine, usually nocturnal patrols.

Exclusion of ranellids from cages

Cage mesh size: Laboratory observations of the genus *Cymatium* at ICLARM CAC suggested that these snails could penetrate cage meshes provided the minimum diameter of the snail was less than the mesh aperture. In order to determine the optimum mesh sizes needed to exclude predatory snails from cages, measurements were made of four species commonly found preying on clams in ocean-nurseries, namely *C. muricinum*, *C. aquatile*, *C. pileare* and *C. nicobaricum*. The length and minimum diameter of 15 individuals of each species ranging from early juvenile to adult were recorded.

Trestle leg excluders: A variety of devices were designed to prevent snails from climbing the legs of trestles used to raise cages above the seabed. Designs included: inverted cones of various materials, bundles of tangled mesh, bundles of sharp bristles, groups of horizontal disks at different spacings, cones with polythene skirts, suspension of cages on fine wires and combinations of the above. These devices were tested in 2000–5000 L tanks. Starved *C. muricinum* were placed in the tanks with clams in trays on trestles. Trestle legs were fitted with various devices. Five prototypes of the most successful device were further tested in similar tanks. Clams were placed on top of the devices which were suspended in the tanks. Snails were placed in trays attached to the bottom end of a rod simulating a trestle leg passing through the device. The snails had no option other than staying in the tray or climbing up into the device. In all these trials dead clams were replaced daily.

Results

Prey choice experiments

During the first series of prey choice experiments the methods of attack of the different predators were observed. The crustaceans; *Atergatis floridus* (purple and grey colour morphs), *Carpilius convexus*, *Dardanus pedunculatus* and *Thalamita danae* crush or chip the valves of their prey although the latter species also attacks through the byssal orifice if this is exposed. The gastropods *Cymatium pileare* and *Bursa granularis* attack by inserting their proboscii between the valves of their prey and at least in the former species toxic salivary secretions may be used. *Chicoreus palmarosae* often drills through the valves of the prey although attacks may take place through the valve gape or byssal orifice.

The results of the first series of prey choice experiments expressed as accumulative rankings are shown in Table 1 for small clams, and in Table 2 for medium-sized clams. Of the species offered *T. derasa* was generally the preferred or most susceptible prey and *H. hippopus* was the least consumed prey for both small and medium-sized clams. Although there is variation between predator species this trend is apparent for both gastropod and crustacean predators and is more marked for small clams. This overall order of preference or susceptibility is maintained whether accumulative ranking, total number of clams consumed or simple ranks are considered (Table 3). *T. crocea* and *T. maxima* occupy an intermediate position with no clear ranking being apparent.

Results of the *H. hippopus* and *T. gigas* prey choice experiments are shown in Tables 4 and 5. Both large and small *C. muricinum* consumed far more *T. gigas* than *H. hippopus*. Variation in numbers of clams consumed by the snails was high. Adult snails consumed on average 0.5 clams/day regardless of species, whereas small snails consumed an average of 0.3 clams/day. On occasion some of the *T. gigas* became byssally detached but all attacks were observed to take place between the valve gape and not through the byssal orifice.

Table 1. Results of a tridacnid prey choice experiment in which predators were offered four species of 10–30 mm SL clams, expressed as sum of accumulative rankings[a].

Predator	Size (cm)	*T. derasa*	*T. crocea*	*T. maxima*	*H. hippopus*
Atergatis floridus (grey)	4.0 CW	203.5 (12)	213.0 (11)	154.0 (7)	24.5 (4)
Atergatis floridus (purple)	3.3 CW	417.0 (15)	314.5 (13)	321.5 (13)	378.0 (12)
Carpilius convexus	4.8 CW	343.5 (14)	248.5 (12)	242.5 (10)	200.5 (9)
Dardanus pedunculatus	3.2 CL	270.0 (14)	365.0 (11)	286.0 (12)	114.0 (8)
Thalamita danae	4.9 CW	417.0 (14)	411.0 (14)	448.5 (14)	319.5 (14)
Bursa granularis	3.8 SL	418.5 (15)	268.5 (11)	248.0 (10)	241.0 (12)
Chicoreus palmarosae	9.2 SL	529.0 (15)	235.5 (9)	249.0 (12)	261.5 (14)
Cymatium pileare	6.7 SL	347.0 (13)	295.0 (14)	74.0 (7)	103.5 (6)
Totals		2945.5 (112)	2351.0 (95)	2023.5 (85)	1642.5 (79)

[a]Numbers in brackets are total number of clams consumed. Mean predator sizes are shown. CL = cheliped length; CW = carapace width; SL = shell length.

Table 2. Results of a tridacnid prey choice experiment in which predators were offered four species of 30–60 mm SL clams, expressed as sum of accumulative rankings[a]

Predator	Size (cm)	*T. derasa*	*T. crocea*	*T. maxima*	*H. hippopus*
Atergatis floridus (grey)	4.0 CW	27.0 (5)	64.0 (8)	0.0 (0)	0.0 (0)
Atergatis floridus (purple)	3.3 CW	187.5 (10)	42.0 (4)	116.5 (9)	215.0 (10)
Carpilius convexus	4.8 CW	308.5 (13)	308.5 (12)	426.0 (13)	85.0 (9)
Dardanus pedunculatus	3.2 CL	39.0 (5)	59.0 (4)	16.0 (4)	96.0 (7)
Thalamita danae	4.9 CW	487.0 (15)	286.5 (14)	379.5 (13)	122.0 (8)
Bursa granularis	3.8 SL	7.5 (3)	13.0 (2)	7.5 (2)	0.0 (0)
Chicoreus palmarosae	9.2 SL	328.5 (11)	78.5 (5)	305.5 (14)	233.5 (10)
Cymatium pileare	6.7 SL	104.5 (8)	157.0 (9)	38.5 (5)	135.0 (7)
Totals		1489.5 (70)	1008.5 (58)	1289.5 (60)	886.5 (51)

[a]Numbers in brackets are total number of clams consumed. Mean predator sizes are shown. CL = cheliped length; CW = carapace width; SL = shell length.

Table 3. Order of preference of seven species of predator for four tridacnid species[a].

	30–60 mm SL clams				1–30 mm SL clams			
	Td	Tc	Tm	Hh	Td	Tc	Tm	Hh
Atergatis floridus (grey)	2	1	3.5	3.5		2	1	34
Atergatis floridus (purple)	2	4	3	1		1	4	32
Carpilius convexus	2.5	2.5	1	4		1	2	34
Dardanus pedunculatus	3	2	4	1	1	3	1	24
Thalamita danae	1	3	2	4		2	3	14
Bursa granularis	2.5	1	2.5	4		1	2	34
Chicoreus palmarosae	1	4	2	3		1	4	32
Cymatium pileare	3	1	4	2		1	2	43
Totals	1	3	2	4		1	2	34

[a] Td, *T. derasa;* Tc, *T. crocea;* Tm, *T. maxima;* and Hh, *H. hippopus*, based on accumulative rankings presented in Tables 1 and 2.

Table 4. Consumption of two species of tridacnid clam, *Tridacna gigas* and *Hippopus hippopus*, offered to adult *Cymatium muricinum* over a 17-day period.

C. muricinum	No. consumed	
(mm)	*T. gigas*	*H. hippopus*
44	9	0
45	7	1
42	5	4
43	8	1
Totals	29	6

Table 5. Consumption of two species of *Tridacna gigas* and *Hippopus hippopus*, offered to young Cymatium muricinum over a 21-day period.

C. muricinum	No. consumed	
(mm)	*T. gigas*	*H. Hippopus*
23	9	0
20	4	0
21	4	1
21	9	1
22	8	0
22	5	0
17	5	0
18	2	3
Totals	46	5

Use of trestles in ocean-nurseries

Mortality was lower amongst clams raised off the seabed on trestles than in benthic cages (Table 6). *H. hippopus* experienced less mortality than *T. gigas* both on trestles and in benthic cages. Observations of the clams in the ocean-nursery suggested that the principle cause of mortality was predation as opposed to environmental factors as none of the batches grew slower or appeared more stressed. About twice as many predatory snails (*Cymatium* spp. and *Chicoreus* sp.) were collected from benthic cages. Predators were observed to be more abundant in cages at night. The ranellids were not recently settled juveniles.

Exclusion of ranellids from cages

Cage mesh size: The relationship between the length of four species of Ranellidae and their minimum shell diameter is plotted in Figure 1. The minimum shell diameter of these ranellids is roughly half the shell length ($Y = 0.45\ X$, $r^2 = 0.97$). It is apparent from these data that all but exceptionally large snails may pass through 20 mm aperture square mesh.

In order to estimate the expected efficiency of various mesh sizes in excluding *Cymatium* spp. Size-frequency data provided by Steve Lindsay for *Cymatium* spp. recovered from benthic cages with a mesh size of 25 mm in Kosrae during 1991 are plotted in Figure 2. The mean size of the 91 snails collected was 31 mm. Based on the calculated length/diameter relationship approximateley 67% of these snails would have been excluded by 12.5 mm aperture square mesh.

Table 6. Mortality over three months of four batches of juvenile tridacnids in the Namu'a ocean–nursery[a].

	Benthic cages	Trestle cages
	Mortality (%)	
H. hippopus (30–40mm)	6.7	1.0
T. gigas (40–65mm)	12.0	8.0
	Predators—(numbers collected)	
Cymatium	19	9
Chicoreus sp.	5	3

a400 H. hippopus and 400 T. gigas were placed in four trestle cages and another 400 clams of each species were placed in four benthic cages.

Trestle leg excluders: *C. muricinum* had little difficulty in by-passing, sometimes in a question of minutes, all but two of the designs tested. Snails were highly active and perseverant whilst searching for prey and capable of maintaining contact with the devices under most circumstances. Snails were observed to experience difficulty crawling on vertical sheets of thin polythene (such as carrier bags) but the polythene soon fouled, stiffened or became detached rendering the devices unreliable.

The only design which showed promise functioned along the lines of a trap and is shown in Figure 3. This design has a removable base allowing trapped *Cymatium* to be collected and destroyed. Trials using this excluder showed that snails only successfully circumvented these devices on average 0.03 (range 0–0.07) times per day compared to 0.59 (range 0.33–1) for trestles with all other designs of excluder. Snails invariably killed clams within one day on trestles without excluders.

Discussion

Overall *H. hippopus* is the least vulnerable to predators of the clam species tested although not consistently in the case of larger clams. The range of attack methods used by predators suggests that the combination of strong shell, reduced byssal orifice, sharp valve edges and capacity to tightly close the valves confers on *H. hippopus* its greater resistance to most predators. Conversely, the relative susceptibility of *T. derasa* to these predators is probably a function of the thin valves with possibly weaker closure.

The less strongly defined order of preference detected in the case of the larger clams may in part be due to the lower numbers of clams consumed overall, which reduces the efficiency of the numerical technique employed, and also to the fact that *T. maxima* and *T. crocea* have relatively stronger shells and byssal attachment at this size than *T. derasa* and to a lesser extent *H. hippopus*.

Perron et al. (1985) showed that juvenile *H. hippopus* are significantly less vulnerable to *C. muricinum* predation than *T. gigas* or *T. derasa* for 80–95 mm SL clams. The results of the present study confirm this for *T. gigas* in the case of both small and

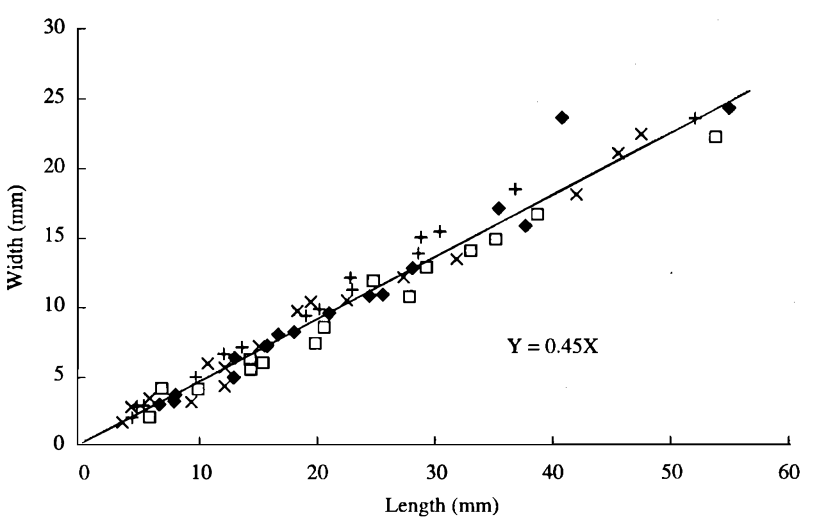

Figure 1. Plot of measurements of length against minimum diameter of 15 individuals of four species of *Cymatium* gastropod. *C. aquatile* □ ; *C. muricinum* + ; *C. nicobaricum* × ; and *C. pileare* ◆.

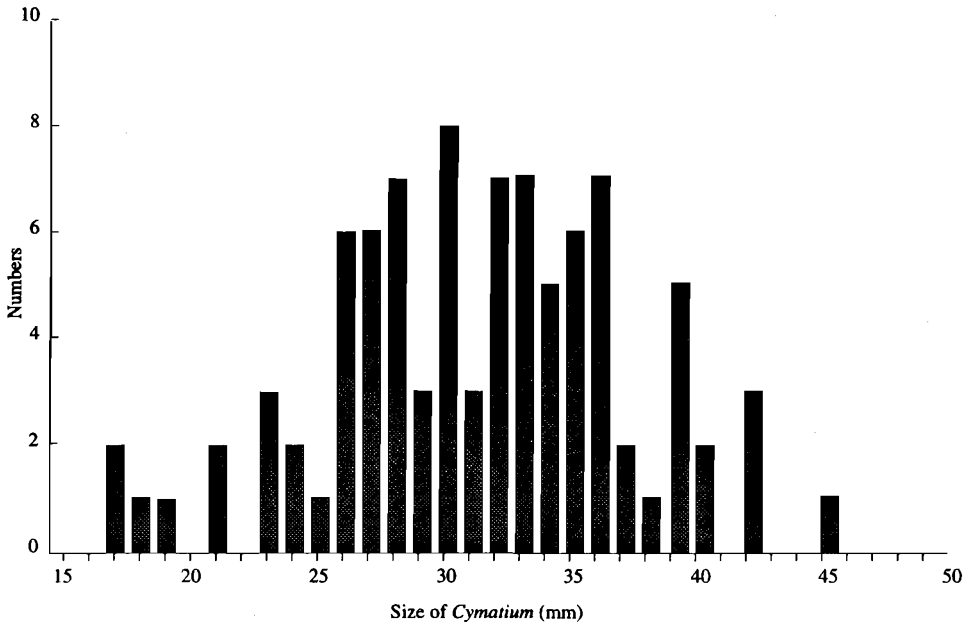

Figure 2. Size frequency of *Cymatium* snails recovered from benthic clam cages in Kosrae during 1991 (data courtesy of S. Lindsay).

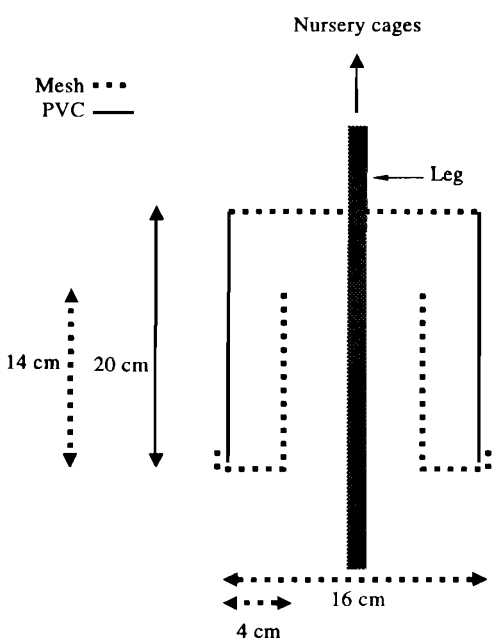

Figure 3. Cut-away diagram of a device designed to reduce the number of gastropod predators successfully climbing the legs of trestle cages in ocean–nurseries for giant clams. The excluder is made of PVC pipe with a fine mesh covering and removable base also made of mesh. The base allows trapped snails to be removed.

large *C. muricinum* although the smaller *H. hippopus* used in this study were not totally immune to attack and indeed two out of twelve snails showed no clear preference. No attacks were observed through the byssal orifice suggesting that the reason for the lower vulnerability of *H. hippopus* is not the zipper-like byssal orifice of this species as suggested by Perron et al. (1985), but more likely its capacity to close its sharper valves tightly, or possibly differences in the location of the internal organs which are the target of ranellid attacks.

The ocean-nursery trial in Western Samoa supports the contention that *H. hippopus* is less vulnerable to predation than *T. gigas*. The use of trestles apparently improved the survival of both species of clams and reduced the incidence of predatory gastropods.

Trestles do not necessarily reduce the settlement of larval ranellids although raising the cages may present more favourable hydrographic conditions for clams and less favourable ones for these larvae. Trestles do appear to hinder the attempts of adult and sub-adult ranellids to reach clam cages and these factors may account for their relative success, as found when trestles were used in Cook Islands (Sims and Howard 1988).

Trestles may be particularly useful where clam farmers determine that ranellids are entering cages from the surrounding seabed and not from the plankton.

In regularly maintained cages this is reasonably easy to determine; recently settled individuals of these species of *Cymatium* are small, thin-shelled and fragile whereas older snails resident in the ocean-nursery area may be similar in size but have thicker, heavier shells and may even possess a greatly thickened lip at the shell aperture (H. Govan, pers. comm).

Mesh sizes of about 25 mm are commonly used in ocean-nursery cages around the Pacific (Heslinga et al. 1990, Calumpong 1992) and meshes up to 50 mm square have been adopted in some locations (G. Heslinga, pers. comm.). Richardson (1991) recommended the use of 25–50 mm square meshes in ocean-nursery cages based on results obtained at Orpheus Island, Queensland. However no *Cymatium* spp. have ever been found in ocean-nurseries at this location (J. Lucas, pers. comm.). The main benefit of these large mesh sizes is the reduced surface area available to algal fouling which reduces the labour input required for its control.

The size distribution of ranellids (mainly *C. muricinum*) recovered from benthic cages in Kosrae is similar to that observed for most benthic cages in Solomon Islands. As shown in the second prey choice experiment larger snails are capable of killing significantly more clams than smaller snails.

The possibility of excluding approximately two thirds of the more voracious predators by using smaller mesh sizes would appear to merit more attention. When selecting meshes for ocean-nursery cages a variety of factors will have to be considered, including the expected abundance of ranellids, degree of algal fouling expected, availability of labour for fouling and snail control and the cost of the meshes.

The increased input of labour required to control algal fouling on smaller meshes may be offset by a reduction in the usual frequency of checks required for ranellid control. Another possible solution is the use of rectangular meshes which would be narrow enough to exclude larger *Cymatium* while providing a reduced surface area for algal fouling.

A less obvious consideration is the access to cages of naturally occurring biological control agents of predators. Portunid, xanthid and diogenid crabs and some fish have been observed feeding on pyramidellids, juvenile *Cymatium* and flatworms (H. Govan pers. comm.). The reduction of mesh size may have the undesired effect of excluding such organisms. The relative importance of all these factors can be expected to vary a great deal from site to site. If biological control agents are introduced into clam cages it may be that smaller meshes will be required to contain them.

Cymatium spp. are capable of climbing on to trestles supporting clam cages, the excluder device (Fig. 3) shows potential in reducing the numbers of snails entering such cages. This design has not yet been tested in the field but drawbacks may include the exclusion of naturally-occurring biological control agents and the cost of the devices.

Little information is available on the impact of wild biological control agents on clam predators. Work in progress suggests that it may be more effective to introduce known biological control agents into cages but more work is required on this topic.

Conclusions

H. hippopus appears to be less vulnerable to predators than other species although *H. porcellanus* and *T. tevoroa* were not tested. The relatively thin-shelled *T. gigas* and *T. derasa* were apparently the most vulnerable to predators.

Raising ocean-nursery cages above the seabed on trestles is recommended as it is likely to increase survival of clams and reduce the incidence of predators. Subject to site-specific variables and considerations of cost, several measures are available to reduce the impact of predation on clam farms, including smaller mesh sizes, different mesh shapes and devices to prevent predators ascending the legs of trestles.

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Heavy Metal Uptake and Loss in the Burrowing Clam, *Tridacna crocea*: Implications from a Public Health and Mariculture Viewpoint

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ANTHROPOGENIC disturbances of the natural environment frequently result in heavy metal enrichment of coastal waters and the organisms that inhabit them. Bivalve molluscs, in particular, are well known for their ability to concentrate certain metals in response to elevated levels in the water column. As a consequence, representatives of this group are often used as bio-indicators of aquatic heavy metal pollution because their elemental body loads tend to reflect the degree of ambient contamination.

Although poor metabolic regulation of a pollutant is an essential prerequisite of any bio-indicator, it is not a particularly desirable attribute for bivalves eaten by man. This is because specimens taken from metal enriched waters may be so contaminated that they are rendered unfit for human consumption even though the organisms themselves are unaffected and apparently healthy. For shellfish farming ventures, the impairment of edible quality and commercial potential of such resources could have devastating economic repercussions.

Considerable scientific attention has been focused upon the heavy metal accumulating capacity of temperate bivalve species, especially those of economic importance (see reviews by Phillips 1977, 1980, 1990, Prosi 1983). In contrast, very little complementary information is available for tropical species of the world. The tridacnid clams, in particular, are a little known group in this regard, despite the enormous commercial potential that they represent and the unprecedented economic growth and industrial development (and pollution potential) currently underway in many countries throughout their range. Thus, an understanding of the kinetic processes describing the uptake and loss of potentially toxic heavy metals in tridacnid clams is of considerable importance both from a public health and mariculture viewpoint.

For these reasons a series of field translocation and relocation experiments between a clean and relatively polluted environment, together with controlled laboratory exposure studies, were undertaken to determine heavy metal kinetics in the burrowing clam, *Tridacna crocea*. The primary objectives of these studies were to gain insight to the vulnerability of giant clam nurseries to elevated heavy metal inputs of natural and anthropogenic origin and assess the bio-indicator potential of *T. crocea* for monitoring heavy metal pollution in the reef environment. We report here on the uptake and loss of zinc (Zn), copper (Cu), lead (Pb) cadmium (Cd), and mercury (Hg) in the edible tissues (gills, mantle, visceral mass, posterior adductor muscle) and kidney of *T. crocea* in relation to appropriate public health standards recommended by the Australian National Health and Medical Research

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Council (NHMRC) and subsequently adopted by the Australian Food Standards Council (Rayment 1991). These metals were selected for study because of their relatively high pollution potential based on their toxicity and diversity of industrial uses (Bryan 1976). Information relating to the bio-indicator potential of *T. crocea* has been published separately (Denton and Heitz 1991).

Materials and Methods

Tridacna crocea (shell length 4–7 cm) were taken from Orpheus Island, a clean coastal site, located approximately 10 km offshore, 60 km NNW of Townsville, north Queensland, Australia. They were collected subtidally, from partially eroded coral boulders, using a hammer and chisel and were permitted a three week recovery period prior to experimentation. Both field and laboratory investigations were incorporated into the study as outlined below.

Field transplant studies

Clams used in the transplant studies were transferred from Orpheus Island to Townsville Harbour in plastic cages and suspended from a floating pontoon to a constant depth of 0.5 m. Located thus, they were unshaded for 6–8 hours each day and received a maximum light intensity of around 1000 $\mu E/m^2$/second at mid-day. Following a 15-week exposure period to the harbour waters, remaining clams were deployed back to Orpheus Island for a depuration period of 13 weeks. Clams and seawater were sampled at regular intervals during the exposure and depuration periods and analysed for Zn, Cu, Pb, Cd and Hg by methods previously described (Denton and Burdon Jones 1986a, b).

The major advantage of this type of experimental approach was that clams were exposed to elevated metal levels from realistic sources under natural conditions. However, complementary laboratory investigations were also necessary for variable dose/response experiments and because the harbour waters were not adequately enriched with all metals of interest.

Laboratory exposure studies

T. crocea were exposed to Cd, Pb and Hg at concentrations representative of severely polluted waters. These metals were of particular interest because they accumulate in living organisms, have no known biological function and rank among the most toxic of heavy metals. Two treatments (5 and 30 µg/L for Cd and Pb, and 5 and 20 µg/L for Hg) were used to determine whether uptake rates where dependent upon ambient metal concentrations. All experiments were conducted out of doors to facilitate sufficient light for the clam's algal symbionts. The uptake experiments were carried out in polyethylene lined, glass tanks (60 × 40 × 40 cm) covered with clear acrylic tops. The tanks were shielded from direct sunlight with 50% shadecloth in order to reduce radiant heating during the day, and were individually equipped with 300 W aquarium heaters to minimise cooling at night. This arrangement was successful in maintaining a relatively stable experimental water temperature of 26± 2°C.

The uptake experiments ran for 32 days. At the start of each experiment all tanks contained 20 clams in 80 L of seawater giving an approximate biomass of 6 g/L. Metal levels in each test tank were monitored daily, as previously described (Denton and Burdon Jones 1981), and the test water was changed every two days.

Cadmium levels in the test tanks remained fairly stable between water changes whereas substantial losses of Pb and Hg occurred within a few hours of being added. To maintain dissolved levels, a series of polyethylene 'drip' bottles containing either 1 mg Pb/L or 9 mg Hg/L for the low metal treatments, and 6 mg Pb/L or 36 mg Hg/L for the high metal treatments were arranged centrally above their respective tanks. The flow rate from each bottle was adjusted to deliver approximately 3 mL/minute. Aeration in the centre of each tank ensured rapid mixing of the dispensed solutions. The levels of metal found daily in each tank over the entire exposure period are summarised in Table 1.

At the end of the exposure period, surviving clams, subjected to the higher concentration of each metal, were transferred to clean seawater to depurate for 32 weeks. Tissue analysis was conducted on batches of 10 clams removed at intervals during both the exposure and post-exposure periods.

Seawater used throughout the experiments was collected from a clean coastal site and stored in an 11000 litre, epoxy-lined, concrete reservoir. Heavy metals in the reservoir were maintained at low levels by an algal–turf scrubber system. The same system was also used to scavenge metals released by clams during the depuration period.

Results and Discussion

Heavy metal concentrations in the edible tissues of *T. crocea*, from Orpheus Island, are shown in Table 2 together with their respective maximum permitted concentration (MPC) established by the National

Health and Medical Research Council (NHMRC) of Australia (Rayment 1991). The levels shown are similar to those reported for other species of giant clam collected from non-polluted waters from the Great Barrier Reef (Burdon Jones and Denton 1984a, b, Denton and Heitz 1991) and elsewhere in the Indo-Pacific (Khristoforova 1980, Khristoforova et al. 1979, 1983, Murphy et al. 1991). Clearly, giant clams from non-polluted waters do not pose a public health threat for any of the metals of interest.

Table 1. Concentration of lead (Pb), cadmium (Cd) and mercury (Hg) (µg/L) in the test seawater during the 32-day exposure period.

Test tank[a]	Nominal concentration			Mean daily concentration ± 1SD		
	Pb	Cd	Hg	Pb	Cd	Hg
1	5	5	5	5.4±1.7	6.4±1.9	4.8±1.6
2	5	5	5	5.5±1.9	5.5±1.7	5.0±2.0
4	30	30	20	29.8±6.9	31.6±5.3	19.5±7.2
5	30	30	20	27.2±9.8	31.8±3.3	21.4±6.9
6	30	30	20	32.7±6.8	30.7±3.2	19.4±7.1
3	30	30	20	31.6±7.2	32.9±6.0	21.6±6.9
7	30	30	20	34.6±6.5	31.4±3.2	20.5±6.7
8	0	0	0	all <1.0	0.3±0.1	all <0.2

[a]clams exposed to each metal separately

Notes: *Pb and Cd analysis:* 250 mL (0.45 µm filtered) seawater, acidified (pH 3) with silica-distilled conc. HNO_3 and extracted with 4% ammonium tetramethylene dithiocarbamate (2 mL) and 5-methyl-2-hexanone (5 mL) for 5 minutes and allowed to stand for a further 10 minutes. Organic phase dried (80°C) and digested with HNO_3 (130°C) and analysed by atomic absorption spectrophotometry (AAS). *Hg analysis:* 2 mL (glass-fibre filtered) seawater, acidified with silica-distilled, conc. HNO_3 (1 mL), preserved with 5% potassium dichromate (0.25 mL), and made up to volume (25 mL) with double-distilled water. Solutions analysed by flameless AAS adopting the method of Stainton (1971).

Field transplant studies

Clams translocated to Townsville Harbour from Orpheus Island were subjected to a two order of magnitude increase in dissolved Zn, at least one order of magnitude increase in dissolved Cu and Pb, and a 2.5–5 fold increase in dissolved Cd (Table 3). Ambient enrichment factors for Hg in the harbour waters (if any) were unable to be determined as levels of this metal were consistently below the limits of analytical detection.

Table 2. Heavy metals in the edible tissues of *T. crocea* from Orpheus Island in relation to the appropriate maximum permitted concentrations (MPC) recommended by the Australian National Health and Medical Research Council (NHMRC).

Metal	Concentration range[a] (µg/g wet weight)	NHMRC MPC (µg/g wet weight)
Zinc	2.2–4.4	150
Copper	0.17–0.23	70
Lead	<0.01–0.16	2.5
Cadmium	0.13–0.48	2.0
Mercury	<0.01–0.03	0.5

[a]analysed according to procedures described by Denton and Burdon Jones (1986a). N=40.

Table 3. Heavy metals in surface waters from Orpheus Island and Townsville Harbour during the study period (March 5–September 12, 1984).

Metal	Orpheus Island[a] (µg/L)	Townsville Harbour[b] (µg/L)
Zinc	0.10–0.20	13.25–17.87
Copper	0.17–0.23	2.15–4.21
Lead	<0.06	0.33–0.66
Cadmium	0.02–0.04	0.10–0.14
Mercury	<0.001[c]	<0.001[c]

Notes: Data are range of values determined ([a]N=15, [b]N=21) according to procedures described by Denton and Burdon Jones (1986b) and are representative of the 'dissolved' (0.45 µm Millipore membrane filtered) metal fraction.

[c]analysed according to procedures described by Topping and Pirie (1972).

The uptake and depuration curves for those metals accumulated by *T. crocea* during exposure to the Townsville Harbour waters are illustrated in Figures 1–3. The biological half-lives (B1/2) shown were obtained from a semi-logarithmic plot of the depuration data over time (Spacie and Hamelink 1985).

Only Zn and Cu levels accumulated in the clam's edible tissues (Figs. 1 and 2) whereas Zn, Cu and Pb were taken up by the kidney (Fig. 3). The importance of the kidney as a central repository for these and other metals, and the major role it plays in their detoxification and excretion, has previously been discussed (Reid et al. 1984, Denton and Heitz 1991).

Cd and Hg did not accumulate in any of the tissues examined. In fact, edible tissue levels of Cd actually

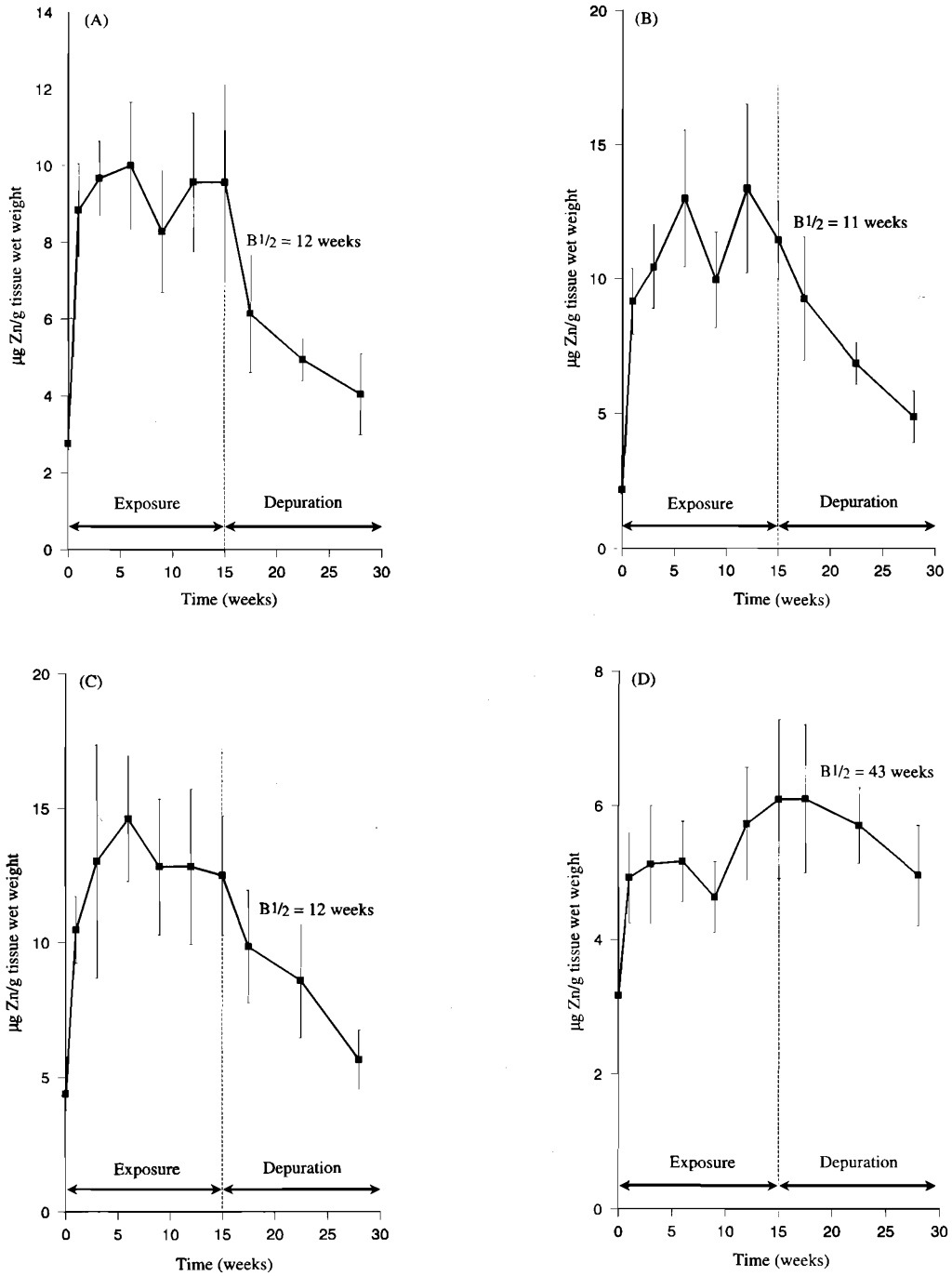

Figure 1. Zinc levels in the edible tissues of *T. crocea* during exposure to Townsville Harbour waters for 15 weeks and depuration at Orpheus Island for 13 weeks. (A) = Gills; (B) = Mantle; (C) = Visceral Mass; (D) = Adductor. All data plots are arithmetic means± 95% confidence limits. N = 10.

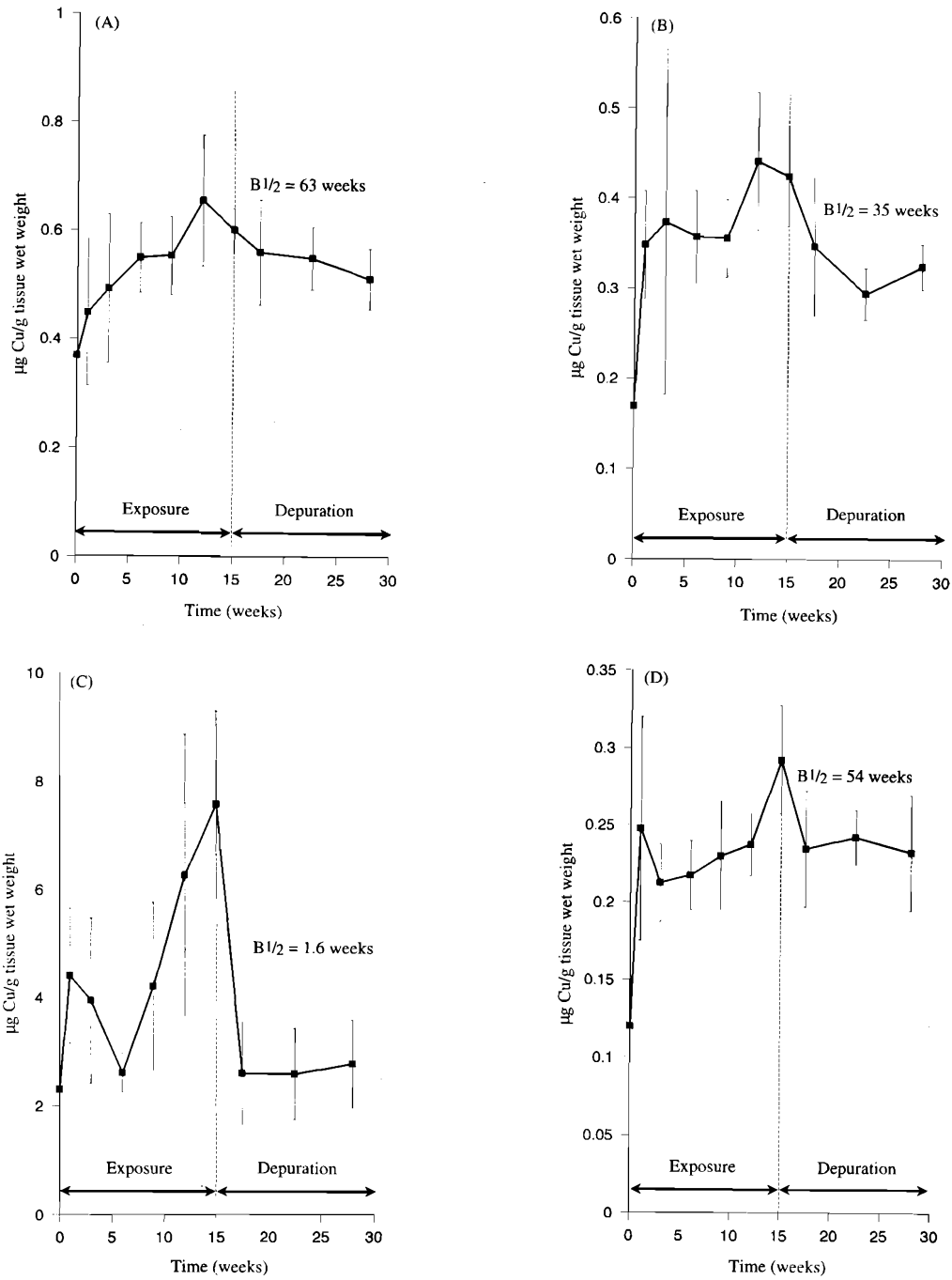

Figure 2. Copper levels in the edible tissues of *T. crocea* during exposure to Townsville Harbour waters for 15 weeks and depuration at Orpheus Island for 13 weeks. (A) = Gills; (B) = Mantle; (C) = Visceral Mass; (D) = Adductor. All data plots are arithmetic means± 95% confidence limits. N = 10.

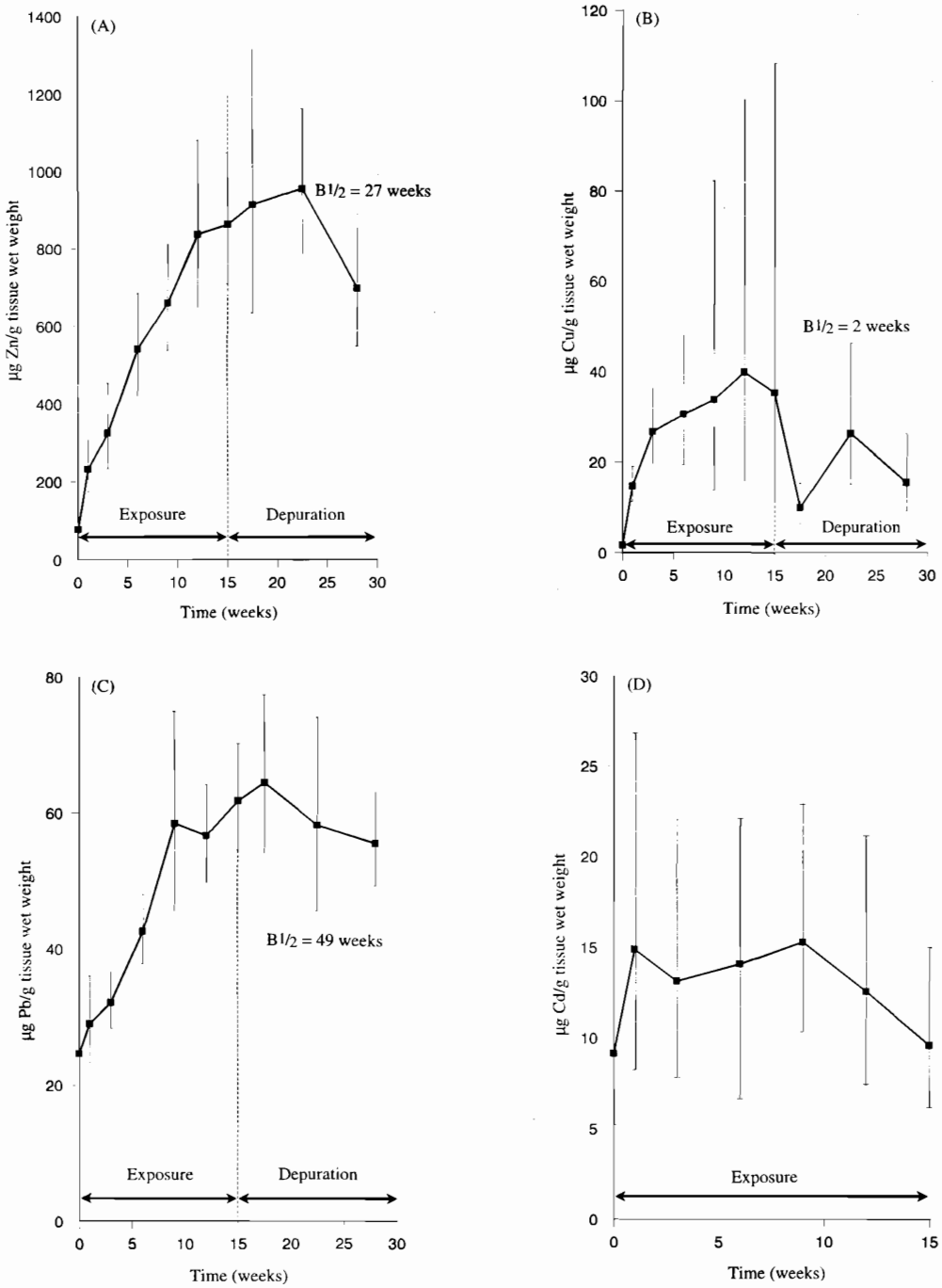

Figure 3. Zinc, copper, lead and cadmium levels in the kidney of *T. crocea* during exposure to Townsville Harbour waters for 15 weeks and depuration at Orpheus Island for 13 weeks. (A) = Zinc; (B) = Copper; (C) = Lead; (D) = Cadmium (exposure only). All data plots are geometric means± 95% confidence limits. N = 10.

declined over the exposure period (Fig. 4). Interestingly, suppressed Cd accumulation has been noted in other organisms collected from Townsville Harbour (Burdon-Jones et al. 1982, G. Denton et al. unpublished data). This phenomenon strongly suggests that much of the dissolved Cd at this site is in a form which is biologically unavailable.

Although both Zn and Cu were accumulated by the edible tissues of *T. crocea* (Figs. 1 and 2), such increases were rather modest considering the degree of ambient enrichment, and remained well below the respective National Health and Medical Research Council (NHMRC) levels of recommended maximum permitted concentration (MPC) for each metal. Tissue levels also paled in comparison to those observed in the oyster, *Saccostrea amassa,* from the harbour. On a wet weight basis, for example, *S. amassa* from this site contained a mean of 907 µg Zn/g and 178 µg Cu/g compared with 9.3 and 8.2 µg/g respectively in specimens from Wistari Reef in the southern section of the Great Barrier Reef. Moreover, the enrichment factors portrayed by *S. amassa,* for these metals, were very much higher than those shown by *T. crocea* and closely approximated those identified from seawater analysis in Table 3.

Further inspection of the edible tissue uptake curves, for Zn and Cu (Fig. 1), generally indicates that steady state concentrations were established within the first few weeks of exposure, and implies the presence of relatively efficient regulatory mechanisms for both metals. The notable absence of Pb accumulation in the edible tissues, coupled with a significant and continued increase in renal Pb concentrations over the exposure period (Fig. 4), is strong evidence for the efficient metabolic regulation of this element as well.

The loss of metals accumulated by *T. crocea* was found to be both metal and tissue dependent with B1/2s ranging from a few weeks to several months (Figs. 1–3). The implications are thus, that uptake, storage, transportation and excretion mechanisms differ between metals and between tissues.

Laboratory exposure studies

The uptake and depuration curves obtained for Pb, Cd and Hg during the controlled laboratory exposure experiments are depicted in Figures 5–8. In most tissues, uptake rates and steady state concentrations (where attained) were higher in the high metal treatments indicating a concentration-dependent relationship. Biphasic depuration kinetics was evident in most edible tissues (Figs. 5–7) and may be conceptualised as the existence of two compartments, one of which rapidly loses its accumulated metal load during depuration while the other retains it for an appreciable period of time. In such instances, the corresponding metal B1/2 for the 'fast' and 'slow' phases are shown.

It will also be noted that the renal concentrations of each metal continued to rise for some time after exposure had ceased and presumably reflects transportation to the kidney, from the other tissues, during clearance (Fig. 8). Interestingly enough, others have reported this phenomenon although no explanation has been offered. For example, Ueda et al. (1982), also working with *T. crocea*, observed a continued rise in renal cobalt-60 for 10 days after exposure had finished. Similarly George and Pirie (1980) found that the kidney of the mussel, *Mytilus edulis*, continued to accumulate zinc-65, and showed no sign of equilibrating, eight days after exposure had terminated.

In keeping with our field observations, *T. crocea* demonstrated a marked ability to metabolically regulate Pb in its edible tissues during this part of the study (Fig. 5). Although some Pb accumulation was certainly evident in both high and low treatments, steady state concentrations were attained relatively quickly in the majority of tissues and in no instance exceeded the NHMRC recommended MPC of 2.5 µg/g wet weight. Furthermore, once transferred to clean water, the bulk of the accumulated Pb was eliminated within the first three weeks of depuration.

Cadmium concentrations in the clam's edible tissues closely approximated those for Pb by the end of the exposure period. This inferred that both metals were accumulated at similar rates, although a comparison of renal Cd and Pb concentrations indicated that actual rates of uptake and mobilisation in the edible tissues were appreciably slower for the former metal (Fig. 8). This was somewhat surprising in view of the relatively high Cd concentrations normally found in clams from non-polluted waters (Table 2). Noteworthy in this regard, however, is the fact that steady state concentrations were only convincingly demonstrated in the gills. Thus, Cd levels would, in all likelihood, have continued to accumulate in all other tissues had the exposure period been extended.

From a public health viewpoint, the mantle and visceral mass (especially the digestive gland) are target tissues for Cd uptake. However, prolonged exposure to severe Cd pollution would seem to be required before the NHMRC recommended MPC of 2.0 µg/g wet weight is exceeded in these tissues. In the present study, for example, only clams held in the high Cd treatment for 32 days approached this value and levels diminished rapidly once they were returned to clean

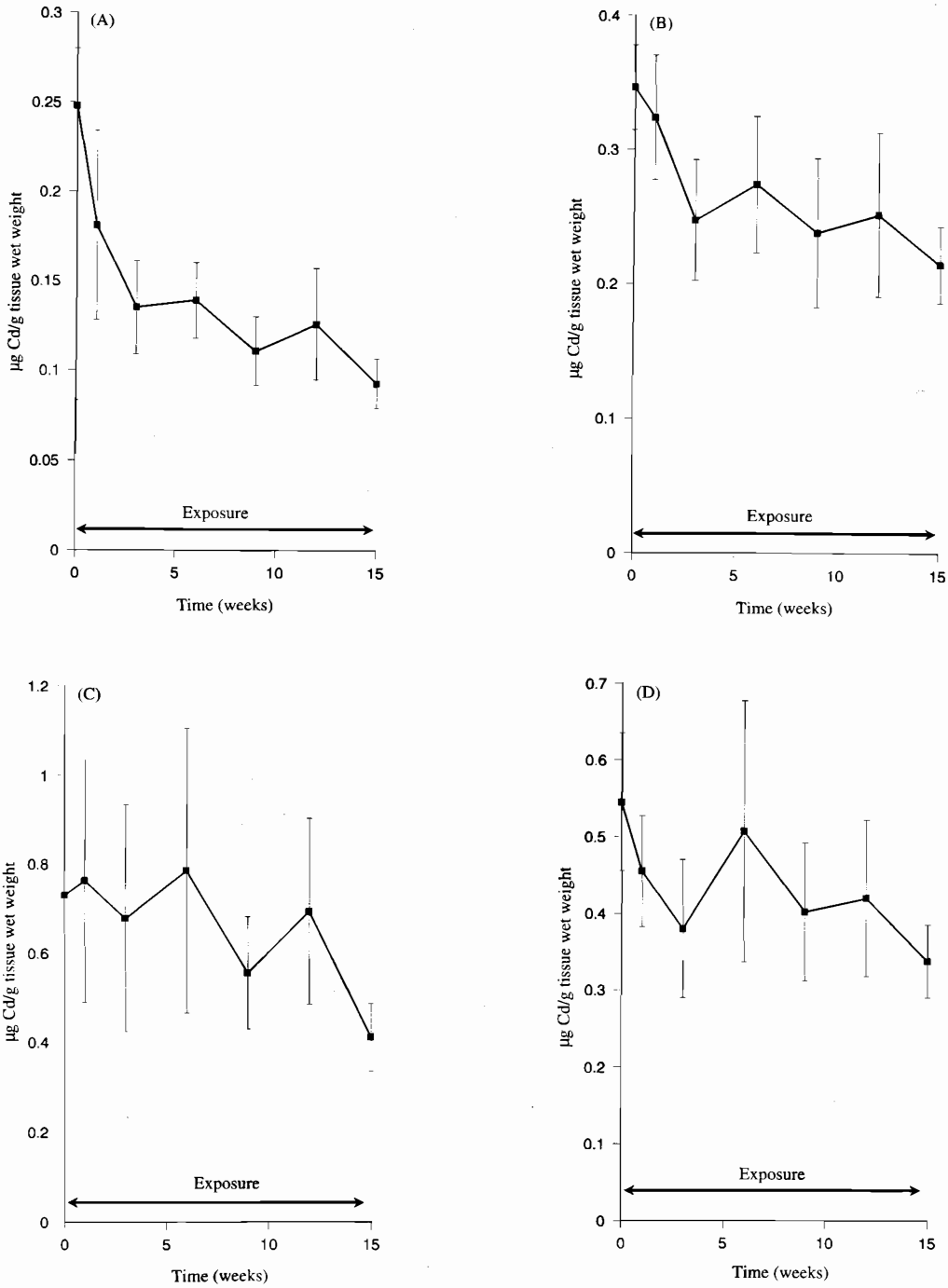

Figure 4. Cadmium levels in the edible tissues of *T. crocea* during exposure to Townsville Harbour waters for 15 weeks. (A) = Gills; (B) = Mantle; (C) = Visceral Mass; (D) = Adductor. All data plots are arithmetic means± 95% confidence limits. N = 10.

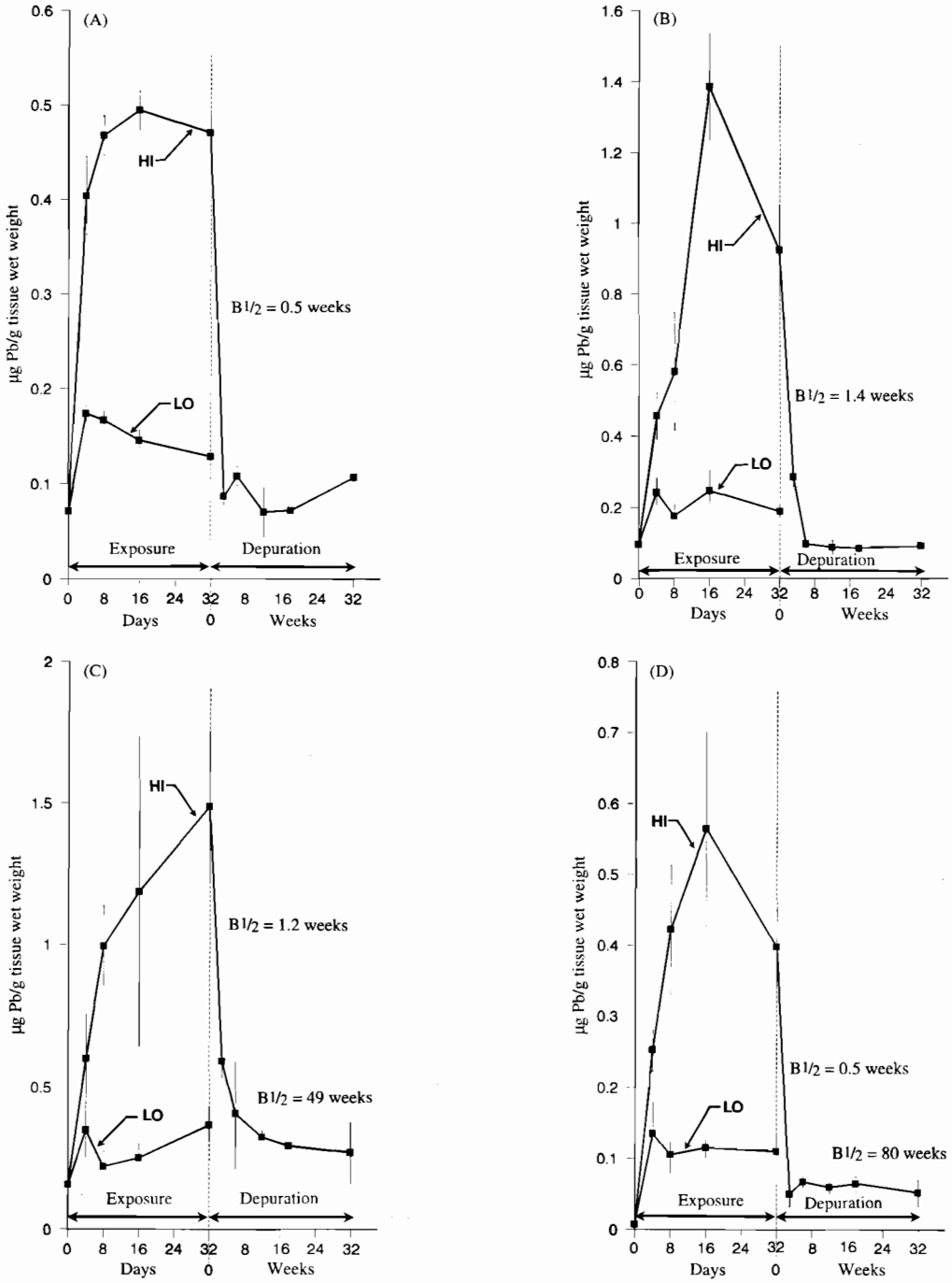

Figure 5. Lead levels in the edible tissues of *T. crocea* during laboratory exposure to low (5 µg/L) and high (30 µg/L) Pb concentrations for 32 days and depuration (high treatment only) for 32 weeks . (A) = Gills; (B) = Mantle; (C) = Visceral mass; (D) = Adductor. All data plots are arithmetic mean and range. N = 2 pools of tissues each from 5 clams.

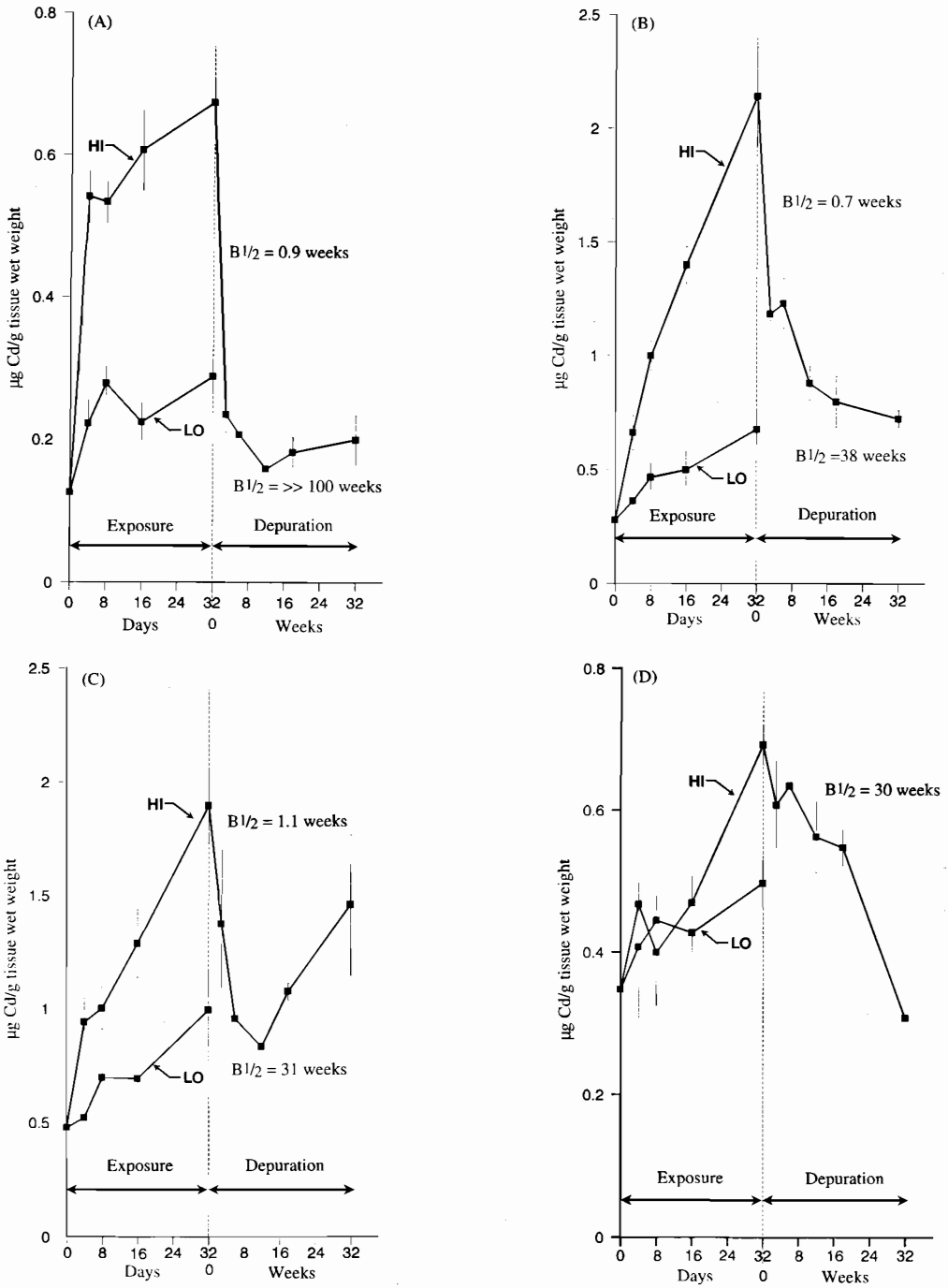

Figure 6. Cadmium levels in the edible tissues of *T. crocea* during laboratory exposure to low (5 µg/L) and high (30 µg/L) Cd concentrations for 32 days and depuration (high treatment only) for 32 weeks. (A) = Gills; (B) = Mantle; (C) = Visceral Mass; (D) = Adductor. All data plots are arithmetic mean and range. N = 2 pools of tissues each from 5 clams.

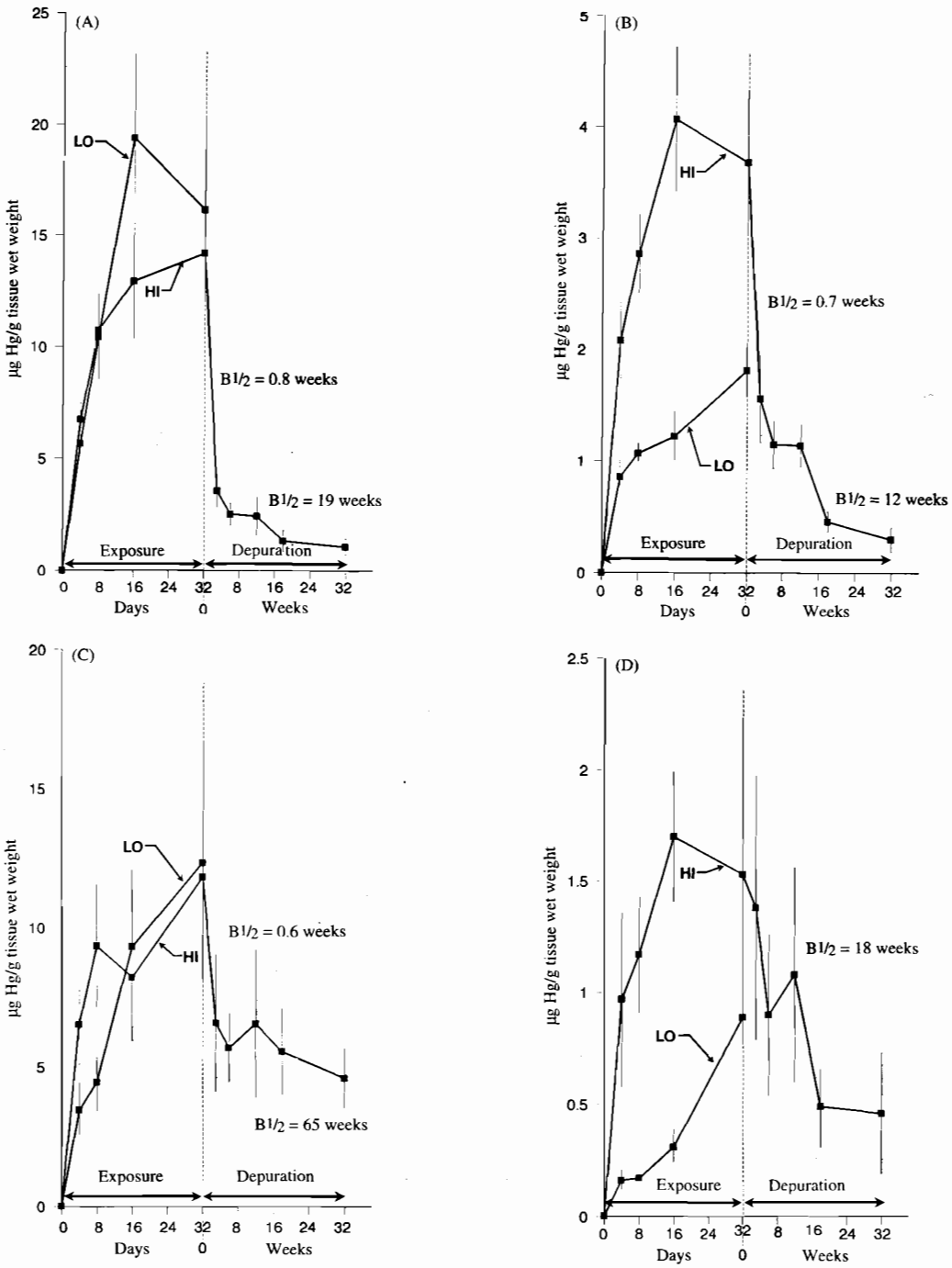

Figure 7. Mercury levels in the edible tissues of *T. crocea* during laboratory exposure to low (5 µg/L) and high (20 µg/L) Hg concentrations for 32 days and depuration (high treatment only) for 32 weeks. (A) = Gills; (B) = Mantle; (C) = Visceral Mass; (D) = Adductor. All data plots are arithmetic means ± 95% confidence limits. N = 10.

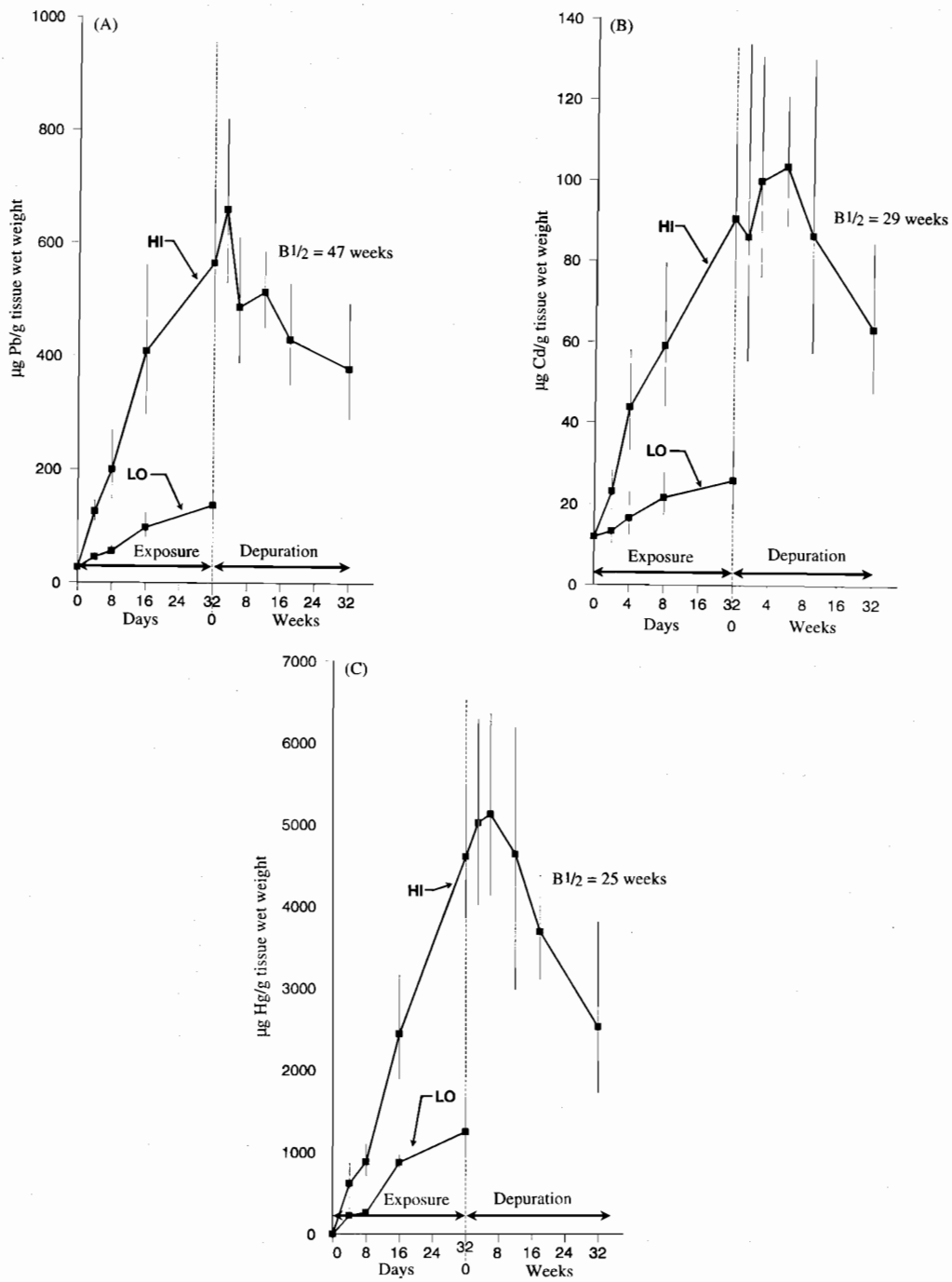

Figure 8. Lead, cadmium and mercury levels in the kidney of *T. crocea* during exposure to low and high concentrations of: (A) = Lead at 5 and 30 µg/L, (B) Cadmium at 5 and 30 µg/L, (C) Mercury at 5 and 20 µg/L for 32 day and a depuration (high treatments only) for 32 weeks. All data plots are geometric means ± 95% confidence limits. N = 10.

seawater (Fig. 6). It should be mentioned here that the apparent Cd concentration increases in the visceral mass during depuration were attributed to weight loss during spawning and not a net gain in absolute amounts of this metal.

Of the three metals tested, Hg was accumulated by *T. crocea* to the greatest extent. In fact, all edible tissues exhibited a very high affinity for this metal (Fig. 7) and quickly accumulated it to levels often greatly in excess of the NHMRC recommended MPC of 0.5 µg/g wet weight in both treatments. Uptake rates in the gill and visceral mass were not significantly different between high and low treatments suggesting that saturation of external binding sites and/or internal transportation mechanisms had occurred at these levels of exposure.

The enormous increase in renal Hg concentrations, over the exposure period (Fig. 8), indicates that a significant proportion of the total Hg taken up by the edible tissues is readily mobilised to the kidney. The initial sharp decline in Hg levels from the gills, mantle and visceral mass during depuration (Fig. 7) is further evidence for this and, in turn, reflects the existence of regulatory processes. However, the clear indication of a persistent, residual Hg component in the latter tissues also means that clams exposed to severe Hg pollution may require a cleansing period of several months or even years, before they are acceptable for human consumption.

Concluding Remarks

It is clear from the foregoing study, that *T. crocea* possess a marked capacity to confine Zn, Cu, and Pb to a relatively narrow range that is most unlikely to exceed NHMRC food standards even in waters that are substantially enriched by these metals. Brief exposure to elevated Cd concentrations also seem unlikely to affect the quality of edible clam tissues owing to slow accumulation rates for this metal. However, prolonged exposure over a month or more, may require clams to be purged in clean seawater prior to marketing. In contrast, the clam's relatively high affinity for Hg suggests that exposure periods of relatively short duration, even under conditions of mild Hg enrichment, could have potentially disastrous effects on the commercial viability of this bivalve.

Acknowledgments

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Economics of Giant Clam Production in Australia and Fiji and Sensitivity Factors

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THIS paper gives an overview of our main research results relating to the economics of production of giant clams for meat. This research was sponsored by the Australian Centre for Agricultural Research as part of its project 'Economics of Giant Clam Mariculture' (ACIAR Project No. 8823). Results are reported for the mariculture of *Tridacna gigas* in Australia (both for the economics of the hatchery/nursery phase and for the ocean phase) and for Fiji for the ocean phase of mariculture of *Tridacna derasa*.

Results for *Tridacna gigas* in Australia

Australian estimates are based upon the experience of scientific researchers at James Cook University and extrapolation, rather than a time-series for an actual commercial nursery, because of lack of data for the latter. The culture methods developed at the Orpheus Island Research Station of James Cook University (JCUOIRS) were assumed to be used by the hatchery/nursery. Estimates were made for both the operating and full cost of producing clam seed of one year of age in quantities of 100000, 500000 and a million seed per year. Full cost includes an allowance for all the funds tied up in clam production. Details of estimation of the costs are set out in Tisdell, Lucas and Thomas (1990) and the results are summarised in Table 1.

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Substantial economies of scale are evident from Table 1. When the commercial volume of production is 100000 seed at the end of one year and full cost is accounted for at a 5% rate of interest on funds, clam seed on average would be expected to cost about A$1.50 each. If only operating cost is taken into account, then the average cost per clam seed is about A$1.10. But cost per seed is much lower when 500000 clam seed are produced annually. Full cost, at 5% interest, averages A$0.425 per clam and for a production of 1 million clams annually falls to A$0.275. Hambrey (1991a) also found, on the basis of experience at the International Centre for Living Aquatic Resources Management (ICLARM) hatchery in Solomon Islands, that economies of scale are important in the production of clam seed.

Returns on the Ocean Phase of Mariculture of *T. gigas*

To determine whether the ocean growout of *Tridacna gigas* could be profitable under Australian conditions, internal rate of return analysis was applied to a hypothetical farm placing 10000 one-year-old seed in the ocean each year and using the intertidal culture techniques developed at JCU. Data from JCUOIRS on costs was supplemented by information from Reefarm Hatcheries, Cairns (see Tisdell, Barker et al. 1991, Tisdell, Tacconi et al. 1991 for details) and growth and mortality rates were those found at JCUOIRS and by the observation of Munro (1988). Details are available in (Tisdell, Barker et al. 1991). After an initial period (two years) of higher mortality rates, a mortality rate of 5% per annum is assumed to apply.

Table 1. Per unit cost of producing giant clam seed at a function of volume of annual output[a].

Type of cost	Number of seed clams per year		
	100000	500000	1000000
Operating cost			
	1.01–1.22	0.29–0.35	0.18–0.23
	(1.115)	(0.32)	(0.205)
Full cost (5% interest)			
	1.31–1.77	0.37–0.48	0.24–0.31
	(1.54)	(0.423)	(0.275)
Full cost (10% interest)			
	1.43–2.01	0.41–0.54	0.26–0.35
	(1.72)	(0.47)	(0.305)

[a]All dollar values used in this article are Australian dollars. Figures in parenthesis are averages of upper and lower bounds of per unit costs of production.

Seed clams of one year old were assumed to cost A$0.75 each and the aim of the farm was assumed to be to hold seed clams long enough in the ocean to maximise its internal rate of return on resources employed, that is the profitability of its operations. The end product is assumed to be the clam meat only, although it is possible also that there could be a market for the shell. Clam meat could sell at the alternative prices listed in Table 2 at the farm-gate in Australia. A price of around A$5 seems highly probable given that clam meat in Australia should retail for A$10–12 per kilogram, a price comparable to that for fish, according to surveys by Tisdell and Wittenberg (1990a,b and cf. Shang et al. 1991).

The internal rates of return listed in Table 2 are all positive. While this is encouraging from a commercial point of view, the industry will only be truly economic if the rate of return exceeds the rate of interest on funds. The most likely situation of those shown in Table 2 could be that involving a farm-gate price of $A5 per kg, a post-harvest drip loss of 40% and an internal rate of return of 11.25%. Therefore, this type of clam farming is likely to be profitable under Australian conditions but not highly profitable.

The number of years which *T. gigas* has to be held in the ocean to maximise returns in supplying the meat market might seem relatively long, e.g. 11 or 14 years in the intermediate price case. However, examination of the internal rate of return function indicates that after the ninth year of the ocean phase only a very slight increase in returns is achieved by holding the clams longer. Thus taking into account cash flow considerations and impatience, harvesting in the ninth year of grow-out is likely to be optimal. The clams would then be 10 years old. Nevertheless, this is a longer period of ocean grow-out than that recommended by Watson and Heslinga (1988) for *T. derasa*. They recommend a grow-out period of five years for one year old *T. derasa*, that is, their harvest at six years old, based on the fact that *T. derasa* has significant decrease in growth at onset of the female phase of reproduction.

Preliminary Estimates of Economic Returns from Ocean Farming of *T. derasa* in Fiji

Prospects for subsistence production and commercial mariculture of giant clams in Fiji, and domestic and export markets for clam meat have been considered (Tacconi and Tisdell 1992 a,b,c). Fiji has had a substantial export market for giant clam meat in the past and there is also a domestic market for the meat. Harvest of natural populations supplied past demand from clam meat, but has led to the near extinction of the larger species.

Subsistence Prospects

Fijian data indicates that giant clam farming at the village level is unlikely to be economic for subsistence purposes. From surveys of villagers, it is clear that they

Table 2. Number of years for which *Tridacna gigas* should be held in ocean phase to maximise economic returns and the resulting internal rate of return (IRR).

Farmgate price/kg of meat in A$	Without drip loss		With 40% drip loss	
	No. of years to hold	IRR%	No. of years to hold	IRR%
3	14	11.25	17	6.03
5	11	18.00	14	11.25
7	9	23.50	12	15.40

are mainly interested in whether giant clam farming can provide them with cash income rather than means for subsistence (Vuki et al. 1991). Estimates of Tacconi and Tisdell (1992a) indicate that the cost per kilogram of producing giant clam meat by mariculture is likely to exceed the price at the village level of substitutes for clam meat such as fish. Also the capital outlay required for the purchase of seed is comparatively high for a village household (cf. Veitayaki 1990) There are also risk factors to consider, such as loss of clam stocks due to predators or disease.

Commercial Prospects for Fiji and Profitability

T. derasa seed stocks are at present held by the Fijian Fisheries Division and this is likely to be the first species released for commercial farming in Fiji. It will be recommended by the division that giant clams be held for five years in the ocean phase before harvest, the optimal length of time to hold *T. derasa* according to the estimates of Watson and Heslinga (1988).

Taking account of the possible cost of seed (F$0.50 or F$1.00) and the cost of protective cages, alternative prices for muscle and other edible meat and different mortality rates, as well as different levels of drip weight loss, internal rates of return are as set out in Table 3. More detail is available in Tacconi and Tisdell (1992a). Hambrey (1991b) points out that a drip weight loss in clam seed of up to 50% is possible.

These estimates should be regarded as upper limits for returns since no allowance for labour cost has been made mainly because no reliable data for these in the Fijian village situation was available. Implicitly they are therefore assumed to be zero but even on an opportunity cost basis, they are likely to be positive in practice. The range of farm-gate prices considered for meat and mantle are believed to be realistic taking into account previous market prices and margins necessary for transport, for processors and for middlemen. Possibly the intermediate set of prices in Table 3 is most likely in the long run but in the short run when supply would still be limited, the higher set of prices would be realistic.

Table 3. Internal rates of return from ocean farming of *T. derasa* in Fiji.

	Farm-gate price/kg		
	Muscle F$10 Other tissues F$3	Muscle F$7 Other tissues F$2	Muscle F$5 Other tissues F$2
Drip loss 5%			
Low mortality			
(25% first year, 5% afterwards)			
—seed F$1 each	5.4	Negative	Negative
—seed F$0.5 each	20.4	11.5	8.9
High mortality			
(25% first year, 10% afterwards)			
—seed F$1 each	0.9	Negative	Negative
—seed F$0.5 each	15.3	6.7	4.2
Drip loss 40%			
Low mortality			
—seed F$1 each	Negative	Negative	Negative
—seed F$0.5 each	14.2	5.85	2.6
High mortality			
—seed F$1 each	Negative	Negative	Negative
—seed F$0.5 each	9.3	1.3	Negative

Source: Based on Tacconi and Tisdell (1992a).

Table 3 indicates that the rate of return to ocean growout of *T. derasa* is very sensitive to the price of clam seed. If the price for clam seed can be kept to F$0.50 each, a positive rate of return can be obtained in all the alternative situations highlighted in Table 3, except one. On the other hand, if clam seed costs F$1 each, a negative rate of return is to be expected in most cases.

A positive rate of return from the farming of *T. derasa* in Fiji under the conditions considered seems to depend on the price of clam seed being kept at low levels, and there being low labour costs at the village level in tending clams. At F$0.50 each for seed, and given the intermediate price set, returns are all positive but none are exceptionally high.

Concluding Observations

The economic prospects for farming *T. gigas* profitably under Australian conditions seem on the whole to be more favourable (or at least no less so) than those for farming *T. derasa* in Fiji under the conditions specified. This indicates that an efficient Australian industry could be competitive with Fiji despite higher labour costs.

The above assumes that all the edible clam meat is marketed. This usually is the case in the Pacific Islands. In the past, Fiji had a sizeable export market in giant clam meat (see Table 4). It's export peaked at 38.5 tonnes in 1988, after which an export ban was imposed because of depletion of natural stocks. About half of the export was muscle and the remainder, other soft edible tissues such as mantle. The main export destination for muscle was Taiwan and for mantle New Zealand, where it was probably consumed by immigrants from the Pacific Islands. In addition, the 'normal' Fijian domestic market for clam meat is about 13 tonnes annually.

Table 4. Quantity of giant clam meat exported from Fiji, 1984–1988.

Year	Weight (kg)
1984	7276
1985	20794
1986	16806
1987	5490[a]
1988	38493

[a]Data available only for the period September–December.
Source:Based on Fiji Fisheries Division (1986, 1987), Files of Fiji Fisheries Division.

If peak exports were to be regained by Fiji, 275 000 *T. derasa* of six years old would be needed annually to supply exports, the estimate being based upon volume of muscle needed. Depending upon survival rates, this would require half a million one year old giant clams to be grown out in the ocean each year, and at 600 seed per household, about 800 households being supplied. However, the Fijian Fishery Division hatcheries capacity is about 200000 eight month old seed per year (Batibasaga and Ledua 1992) so even under very favourable economic conditions, availability of seed may be limiting to expansion of Fijian commercial production. However, Fiji has had a substantial market for clam meat, with its export market being much larger in the past than its domestic market, and could look to recapturing a part of its former export market as a result of clam farming. Tonga has similar prospects but it has never had commercial exports of clam meat on the scale of Fiji (Tacconi and Tisdell 1992c).

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The Economic Potential of Village Based Farming of Giant Clams (*Tridacna gigas*) in Solomon Islands

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INTEREST has been shown throughout the Pacific in the possibility of developing giant clam mariculture as an export industry and it has been suggested that such an industry might be particularly suitable as a part-time activity for Pacific Island villagers.

Much of the research effort has concentrated on the production of large *Tridacna gigas* or *Tridacna derasa* (e.g. Munro 1988, Barker et al. 1988). This paper considers prospects for the farming of large *Tridacna gigas* as a village based industry in Solomon Islands. It is based on two detailed working papers on market value and production costs for *T. gigas* in Solomon Islands (Hambrey 1991a and b). A third working paper on the economic potential of farming smaller giant clams (either young *T. gigas* or smaller species) is in preparation.

Other studies on the economic potential of farming large *T. gigas* or *T. derasa* have been undertaken by Tisdell et al. 1991 (for the Australian mainland) and Tacconi and Tisdell 1992 (for Fiji, Tonga and Western Samoa).

Unless otherwise stated, all costs given below are in Solomon Island dollars (= US$0.37).

Production Parameters

The production system is still undergoing development and different researchers favour different systems or production strategies. The production parameters are also still rather poorly defined: the survival in hatchery tanks and floating cages is still highly variable; the effectiveness or otherwise of grading and discard at various stages is poorly established; and there are still very little data available on the survival rates of larger clams on the open reef.

For the purposes of this analysis the production process is assumed to be as follows: four month old hatchery grown spat at 4 mm (unselected) or 6 mm (selected) are stocked in floating ocean nurseries alongside a hatchery. These are grown for six months to a size of around 35 mm and then sold to villagers for grow-out. At village sites they are grown in cages for the first year and net exclosures for the second. Thereafter they are transferred to the open reef (unprotected) for final grow-out to a size of 45 cms +.

The range of production parameters used in the economic models is given in Table 1. For the most important or ill-defined parameters, three values are used in the economic models: worst case, corresponding to a production situation where regular bouts of poor performance are experienced, e.g. as a result of algal fouling in hatchery, flatworm or predatory snail infestation in ocean nursery; most likely case, corresponding to what research staff believe could be consistently achieved with well trained and motivated staff using the best currently available technology and husbandry practices—i.e., rather better than present average performance; and best case, representing what might be possible if the factors resulting in occasional excellent performance in individual tanks or cages could be understood and reproduced consistently.

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Table 1. Production parameters.

(a) Hatchery (production of four month old 4–6 mm spat)	
Yield per tank (10 m^2)	20 000, 40 000, 80 000
Age at harvest	Four months
Production cycles/year	3
Percentage discard	60, 0
Mean size (mm) selected, unselected	6,4
(b) First stage ocean nursery (production of 10-month-old 35 mm seed)	
Harvest/grade after three months; restock and hold for further three months; sell to on-growers.	
Stocking density phase 1	10000/m^2
Stocking density phase 1	2000/m^2
Survival phase 1 (selected)	0.3, 0.5, 0.6
Survival phase 2	0.5, 0.7, 0.8
Survival phase 1 (unselected)	0.2, 0.4, 0.5
Survival phase 2	0.5, 0.5, 0.7
No. of production cycles/cage/year	3
(c) On-growing at village sites (production of seven to nine-year-old 45 cm clams)	
Stocking density phase 1 (cage on trestle)	800/m^2
Stocking density phase 2 (cage on bottom)	200/m^2
Stocking density phase 3 (exclosure)	30/m^2
Stocking density phase 4 (open reef)	5/m^2
Overall survival (%)	15,22,30
Production cycles/year	2
Time to harvest (years)	6,7,8

Cost of hatchery spat (3–6 mm)

Estimates for capital and operating costs of a hatchery based on 300 m^2 of growing tank space are presented in Table 2. Operating costs are presented in three categories: fixed, which would correspond to the essential overheads for any reasonable sized hatchery; semi-variable, which would vary in approximate proportion to the physical size of the hatchery; and variable, which would vary in approximate proportion to the output from a hatchery. The relatively high fixed and semi-variable costs (between $109000 and $143000 for hatcheries with up to 300 m^2 of growing tank), imply that spat production costs will be highly sensitive to scale of production (when considering hatchery design) and even more sensitive to the actual output rate for any established hatchery.

The basic hatchery described could produce 1.8–7.2 million ungraded spat of approximately 4 mm (worst and best cases) in size with a most likely production of 3.6 million. Corresponding estimates for cost/spat would be 8.5, 2 and 4 cents. If 60% of these were considered runts and discarded, unit costs for the remaining (ca. 6 mm) spat would be in the range of 5–21 cents, with a most likely value of 11 cents. Figure 1 shows the way in which these values would vary (approximately) with scale of production in terms of hatchery size. Costs would be even more sensitive to the actual output of a hatchery of any given size.

Cost of seed (35 mm) production

The capital and operating costs for a first stage ocean nursery, assuming shared management and overhead costs with a hatchery and growing spat from 4–6 mm to 35 mm in six months in floating cages, are given in Table 3. Costs are again divided into fixed, semi-variable and variable. Spat costs (variable) dominate the production costs for such a nursery (76%). A specialist producer not directly associated with a hatchery and supplying widely dispersed growers would suffer much higher fixed (overhead) costs and would therefore experience important economies of scale. Villagers might be able to undertake this stage themselves but because of the relative unimportance of labour in operating costs, their production costs are unlikely to be significantly lower; indeed it is probable that survival rates would be lower (and spat costs correspondingly higher) than those achieved in a centralised or specialist facility.

Using the most likely cost for selected 6 mm spat (11 cents), unit productions costs for 35 mm seed would vary between 30 and 88 cents with a most likely value of 40. Approximately 10 cents of this is associated with labour, capital (floating cages) and overheads; the highly variable balance is contributed by the spat costs. Assuming that all the hatchery output is kept in Solomon Islands, such a cost would correspond to a total annual Solomon Islands production of 504 000 35 mm seed.

Other researchers have estimated production costs for seed of 25–30 mm direct from the hatchery at A$ 0.37–0.48 (= SIB $0.74–0.96) for 500 000 production (Tisdell et al. 1990). The differences derive from the Australians' higher estimates for capital and labour costs and the different production process. Using their

Figure 2. Capital and operating costs for *T. gigas* hatchery.

Capital costs		Operating costs	
Fixed		Fixed	
Land and buildings	77600	Manager	28000
Pumphouse and generator	21000	Assistant/engineer	12000
Intake line	4300	Office	7000
Spawning tanks (2)	1200	Maintenance (7% capital)	12748
Freshwater system	2600	Insurance.(3% capital)	5464
Truck	35000		
Equipment	15420	Capital charge	40977
Misc.	5000		
Installation	20000		
Subtotal	182120	**Subtotal**	106189
Semi-variable (design variable)		**Semi-variable** (design variable)	
Tanks (30 at 10 m^2)	18000	Labour/technicians	24600
Pipes, drains, shade	12500	Maintenance/insurance (10% on cap)	7555
Aeration	4000	Capital charge	16999
Pumps	28600	Subtotal	49154
Broodstock	2450	Variable	
Installation	10000	Fuel/lubricants	11500
		Consumables	5570
Subtotal	75550	Subtotal	17070
Total capital costs	257670	**Total operating costs**	172413

Cost/spat		Yield (spat/m^2)		
		2000	4000	8000
Discard	0	0.09	0.04	0.02
	60	0.22	0.11	0.05
	Hatchery output	1800000	3600000	7200000

Notes: it is assumed that management, workshop and office space is shared with the ocean nursery. Age at harvest would be 4 months; each tank is restocked on average every 4 months; the total growing tank area is 300 m^2; and the capital charge = sum (capital cost/life+ 0.1* capital cost).

Table 3. Seed (35 mm) production costs: central specialist facility. Example for 35% overall survival: production 504000.

Capital costs (SIB$)			Life	Operating costs	SIB$	Percentage
Variable	Cages	49140	5	Fixed		
Fixed	Canoe	700	6	Office	4000	2
Fixed	Tools	2000	3	Transport	5000	2
Fixed	Masks	1000	4	Semi-variable		
Fixed	SCUBA	2000	5	Maintenance/insurance[a]	5484	3
Total		54840		Capital charge	16745	8
				Variable		
				Spat	162720	79
				Packaging	230	0
				Labour	12850	6
				Total	207029	
				Production cost	0.41/35 mm seed	

Notes: It is assumed that management and overheads are shared with the hatchery. The above assumes a 6-month production cycle in two three-month stages. Clams are stocked and harvested regularly at approximately monthly intervals. The stocking density at stage 1 was 1000/m2 and 200/m2 at stage 2; survival at stage 1 was 0.5 and at stage 2 was 0.7; runt cull at end of stage 1 was 0; and the spat cost was 0.11.

[a] Maintenance/insurance was charged at 10% of capital.

production parameters with CAC estimates for hatchery costs would give a hatchery produced (one year old) seed cost of SIB$0.6.

The delivered cost of seed to the village grower will be higher according to transportation costs. For sites other than those in the immediate vicinity of the seed producer, these could be anywhere in the range of SIB$0.01–0.1 per seed clam for Solomon Island growers, according to the location and size of shipment.

Returns to village growers (grow-out to 45 cm+)

Returns to village growers will depend critically on seed costs, survival and growth rates and market value. 'Most likely values' for these are extremely difficult to establish at this stage and deserve some discussion.

The most likely value for seed cost derived above was SIB$0.40 + delivery costs. It must be remembered that this corresponds to production (and therefore sales) of 500000 seed per annum. Costs would be very much higher at lower rates of output. Such a cost would therefore only be possible once a village based industry was well established and well organised in the Solomon Islands, with a total output of perhaps 150000 45 cm clams, (corresponding to around 33 tonnes of adductor muscle, 247 tonnes of mantle meat and more than 3000 tonnes of shell). Clearly, many years of investment in hatchery production, village extension and market identification, development and supply would be required before this became a reality.

Data from the best performing and longest established village trials in Solomon Islands suggest by extrapolation from current growth rates that a size of 45 cm (average) could be achieved in 6.5–7.5 years (from stocking at 35 mm) using high quality (highly selected) seed. Growth rates quoted for Orpheus Island (Barker et al. 1988) of 5–6 mm per month for sub-tidal cultivation (clams between 30 and 225 mm) tend to confirm these figures. Studies on wild clams in New Guinea and on the Great Barrier Reef (Munro and Gwyther 1981, Munro 1986, 1989, Pearson and Munro 1991) suggest that the age of 45 cm clams averages something in the range of 7–9 years, which corresponds well with the above. For the purposes of this analysis, it is assumed that the average growout from 35 mm–45 cm using moderately selected stock at good sites will take between six and eight years with a most likely value of seven years.

Survival from stocking (using highly selected 10–12-month-old seed) through to around 230 mm after 30 months, at the best and longest established village trials, stands at between 28% and 40%. At more

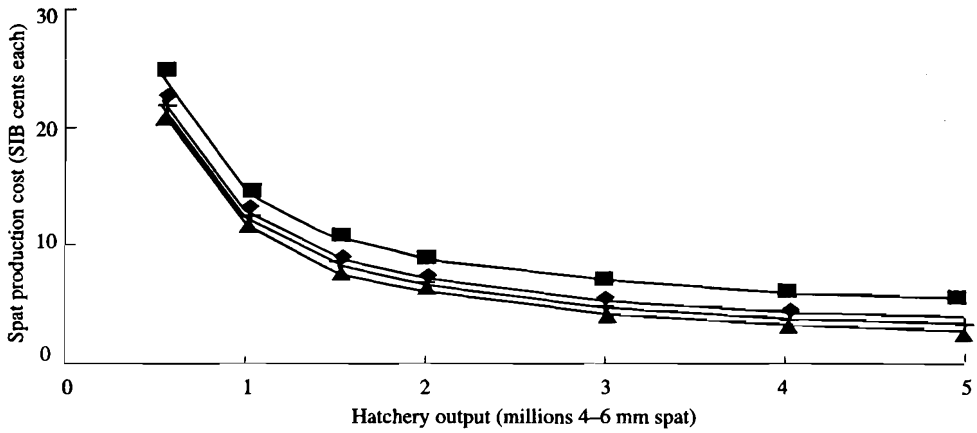

Figure 1. Economies of scale for spat production. ■ = 60% discard; 4000/m2; ◆ = 60% discard; 8000/m2; + = no discard; 4000/m2; and ▲ = no discard; 8000/m2.

typical sites neglect and/or serious snail (*Cymatium* spp.) infestation may result in very much worse performance. At Orpheus Island the average survival in 'lines' over a 19 month period from stocking at 11 months was 41% (Barker et al. 1988). For clams of 20 months + the same authors suggest an annual survival rate of >84% and for clams over 30 months more than 90%. Very little data are available on the survival of the large clams on the open reef. The data of Pearson and Munro (1991) for wild *Tridacna gigas* on Michelmas reef show that survival is very variable but in their study the average annual survival rate increased from 0.6 at 20 cm to 0.95 at 45 cm, which would correspond to 45% to 55% overall survival between 20 and 45 cm. On the basis of this rather limited data it is suggested that overall survival between stocking at 35 mm and marketing at 45 cm is likely to lie in the range 15–30% with a most likely value of 22%. Survival in the earlier stages (35–100 mm) might be improved substantially if some effective means of controlling predation, (especially by *Cymatium* snails) can be developed.

Numerous market and processing studies have been undertaken for large giant clam products (Carlton 1984, Dawson 1986, Sanders 1987, Trachet 1989, Phillipson 1989, Tisdell 1989, Tisdell and Wittenberg 1990, Shang et al. 1990, Parry 1990, Cowan 1991) but very few of these have examined collection, processing and exporting costs—the main component in seafood and marine product value, especially for small island nations. Table 4 gives the estimated return for different products from a 45 cm *Tridacna gigas* to a clam farmer at a typical Solomon Island village site. The adductor muscle is likely to bring a return of no more than SIB$3.6–4.6 on a 45 cm clam and the return from frozen or processed mantle meat products is likely to be negative or insignificant, apart from some possible pickled products. The shell has not been included because this size (45–50 cm) of shell is not and has never been internationally traded in large volumes and although there is considerable interest in it for a variety of applications in southeast Asian markets, it has so far been impossible to estimate a likely future export value for significant volumes.

For grow-out it is appropriate to use cash flow techniques for the analysis, since cash flow will be crucial for a long term production cycle. An example cash flow over a 15-year period is presented in Table 5. Using a delivered seed cost of 41 cents (i.e. most likely cost + 1 cent distribution costs, corresponding to a relatively accessible and large scale operation or group of growers), overall survival of 22% from stocking at 35 mm to harvest at 45 cm, an average production cycle of seven years and a farm-gate value of SIB$5/clam (corresponding to sales of adductor + mantle only), the cash flow generates a negative internal rate of return (IRR—labour charged at $10/day) and a negative return on labour (ROL—cost of labour giving a zero internal rate of return). For a farm-gate value of $7.5 (i.e. assuming $4–$5 for adductor and mantle and $2–$3 for the shell), IRR would be 7% and ROL $14/day and for a farm-gate value of $10 (i.e. assuming a value of around $5 for the shell), IRR would be 14%

Table 4. Giant clam (large *T. gigas*) products: potential farm-gate value in the Solomon Islands based on most likely retail, wholesale, or CIF value.

	Product/market	retail price	wholesale price	port value	import tax	CIF	freight type	freight cost	FOB Hon	export tax	net of tax	export process cost	process yield %	value raw	less process cost	Internal freight	price/kg to producer	value/ clam $US	Value/ clam $SI
1	frozen adductor, 2-300g, Taiwan		17.0	17.0	25.0	13.6	air	4.2	9.4	10.0	8.5	1.4	97.0	8.2	6.8	0.8	6.0	1.3	3.6
2	frozen adductor, 2-300g, Taiwan		16.0	15.0	25.0	11.3	sea	0.5	10.8	10.0	9.7	1.4	97.0	9.4	7.9	0.8	7.1	1.6	4.3
3	frozen adductor, 3-400g, Taiwan		24.0	25.0	25.0	18.8	air	4.2	14.6	10.0	13.1	1.4	97.0	12.7	11.3	0.8	10.5	3.5	9.3
4	frozen adductor, 3-400g, Taiwan		23.0	23.0	25.0	17.3	sea	0.5	16.8	10.0	15.1	1.4	97.0	14.6	13.2	0.8	12.4	4.1	11.0
5	frozen adductor 2-400g, Hong Kong					12.0	air	3.5	8.5	10.0	7.7	1.4	97.0	7.4	6.0	0.8	5.2	1.2	3.2
6	Dried adductor , Hong Kong (Top subst.)					50.0	air	3.5	46.5	10.0	41.9	1.6	18.0	7.5	6.0	0.6	5.4	1.2	3.3
7	Dried adductor, Hong Kong					25.0	air	3.5	21.5	10.0	19.4	1.6	18.0	3.5	1.9	0.6	1.3	0.3	0.8
8	smoked adductor (vacpak, Pac. Isl.)	50.0				20.0	air	2.2	17.8	10.0	16.0	2.1	45.0	7.2	5.1	0.6	4.5	1.0	2.7
9	soft adductor (vacpak, Pa. Isl.)	40.0				16.0	air	2.2	13.8	10.0	12.4	2.0	50.0	6.2	4.2	0.6	3.6	0.8	2.2
10	canned adductor (S E Asia)					7.0		0.2	6.8	10.0	6.1	2.0	45.0	2.8	0.8	0.6	0.2	0.0	0.1
11	whole meat/mantle, Pacific is./NZ	4.0				2.0	air	2.0	0.0	10.0	0.0	1.4	57.0	0.0	-1.4	0.6	-2.0	-3.4	-9.1
12	whole meat/mantle, Australia	6.0				3.0	sea	0.3	2.8	10.0	2.5	1.4	57.0	1.4	-0.0	0.6	-0.6	-1.0	-2.8
13	whole meat/mantle, Australia	6.0				3.0	air	0.6	2.4	10.0	2.2	1.4	57.0	1.2	-0.2	0.6	-0.8	-1.3	-3.6
14	dried mantle, HK/Tokyo					10.0	sea	0.5	9.5	10.0	8.6	1.6	6.5	0.6	-1.0	0.6	-1.6	-2.6	-7.2
15	clam crackers					10.0	sea	0.5	9.5	10.0	8.6	1.7	6.0	0.5	-1.2	0.6	-1.8	-2.9	-7.8
16	pickled mantle (uncooked, japan)	24.0		9.6	15.0	8.2	sea	0.5	7.7	10.0	6.9	2.1	40.0	2.8	0.6	0.6	0.0	0.1	0.2
17	pickled mantle cooked	24.0				9.6	sea	0.5	9.1	10.0	8.2	1.5	17.0	1.4	-0.5	0.6	-1.1	-1.8	-4.8
18	smoked mantle, vacpak	40.0				16.0	sea	0.5	15.5	10.0	14.0	1.9	15.0	2.1	0.2	0.6	-0.4	-0.6	-1.6
19	minced mantle frozen, vacpak					3.0	sea	0.3	2.7	10.0	2.4	2.0	45.0	1.1	-0.9	0.6	-1.5	-2.5	-6.9
20	minced mantle, cooked in brine					3.0		0.5	2.5	10.0	2.3	1.8	18.0	0.4	-1.4	0.8	-2.2	-3.7	-10.0
21	canned mantle					3.0		0.3	2.7	10.0	2.4	1.8	18.0	0.4	-1.4	0.6	-2.0	-3.3	-8.9

Notes:

1) a CIF-retail mark-up of 2 is assumed for bulk frozen product, and 2.5 for all other products.

2) products 3 and 4 assume a 50 cm (ca 9 yr old) clam.

3) Products 2,4 : at present there is no refrigerated delivery, Solomon to Taiwan. Would have to go via Australia (not recommended by exporters) or refrigerated container bought or hired, and delivery costs payed both ways

All values in $US/kg Unless otherwise stated values/clam refer to a 15cm clam aged approximately 7.5 yrs

Table 5. Cash flow and returns to village grower aiming at annual production of 1000 45 cm clams. Assumes seed cost of SIB$0.4; survival to market 22%; and farm-gate value $8/clam.

	year 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
COSTS															
SEED	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818	1,818
CAGES	185			185			185			185			185		
EXCLOSURES		702			702			702			702			702	
EQUIPMENT+ MEDICINES	500			500			500			500			500		
TOTAL before labour	2,503	2,520	1,818	2,503	2,520	1,818	2,503	2,520	1,818	2,503	2,520	1,818	2,503	2,520	1,818
LABOUR	739	739	739	739	739	739	739	739	739	739	739	739	739	739	739
TOTAL incl. labour	3,241	3,258	2,557	3,241	3,258	2,557	3,241	3,258	2,557	3,241	3,258	2,557	3,241	3,258	2,557
REVENUE							2,000	6,000.00	8,000.00	8,000.00	8,000.00	8,000.00	8,000.00	8,000.00	8,000.00
NCF before labour and interest	(2,503)	(2,520)	(1,818)	(2,503)	(2,520)	(1,818)	(503)	3,480	6,182	5,497	5,480	6,182	5,497	5,480	6,182
CNCF before labour and interes	(2,503)	(5,023)	(6,841)	(9,344)	(11,863)	(13,682)	(14,184)	(10,704)	(4,522)	975	6,455	12,637	18,134	23,614	29,796
INTEREST	(300)	(603)	(821)	(1,121)	(1,424)	(1,642)	(1,702)	(1,284)	(543)	0	0	0	0	0	0
NCF incl. interest, no labour	(2,803)	(3,122)	(2,639)	(3,624)	(3,943)	(3,460)	(2,205)	2,196	5,639	5,497	5,480	6,182	5,497	5,480	6,182
CNCF incl. interest, no labour	(2,803)	(5,926)	(8,565)	(12,189)	(16,132)	(19,592)	(21,797)	(19,601)	(13,962)	(8,465)	(2,985)	3,197	8,694	14,175	20,356
NCF inc. labour, no interest	(3,241)	(3,258)	(2,557)	(3,241)	(3,258)	(2,557)	(1,241)	2,742	5,443	4,759	4,742	5,443	4,759	4,742	5,443
CNCF incl. labour, no interest	(3,241)	(6,500)	(9,057)	(12,298)	(15,557)	(18,113)	(19,355)	(16,613)	(11,170)	(6,411)	(1,670)	3,773	8,532	13,274	18,717
NCF incl. labour and interest	(3,542)	(3,861)	(3,378)	(4,363)	(4,682)	(4,199)	(2,944)	1,457	4,901	4,759	4,742	5,443	4,759	4,742	5,443
CNCF incl. labour and interest	(3,542)	(7,403)	(10,781)	(15,143)	(19,825)	(24,024)	(26,967)	(25,510)	(20,610)	(15,851)	(11,110)	(5,666)	(908)	3,834	9,277

SUMMARY OF RETURNS		VARIABLES		INPUT PARAMETERS		PRODUCTION AND SIZE	
NPV before labour and interest	4,937	Labour (SIB$/day)	10.00	cage cost	25.00	seed requirements	1,545
IRR before labour and interest	0.151	delivered seed cost	0.40	tools, masks etc	500		
						cage requirement	6
NPV incl. labour, no interest	(1,243)	survival to 60mm	57	annual production	1,000	buffer	2
IRR incl. labour, no interest = IRR	0.088	survival to 100mm	44	time to harvest	7.0 yrs	Exclosure area(sq.m)	67
		survival to market	22	size at harvest	45 cm		
NPV incl. interest, no labour	(1,297)						
		Market value (SI$)	8.0	Stock density 0-6 m	400		
NPV incl. labour and interest	(7,477)	Discount rate	0.10	Stock density 6-12 m	100	labour (days/yr)	74
IRR incl. labour and interest =0 for ROL	0.038	Interest rate	0.12	Stock. density, excl	30		
				cage inspections/week	3		
				time/cage (mins)	10		
				Exclosure net cleans/month	1		
				Predator checks (hrs/excl/week	1		

and ROL $33/day. The accumulated investment requirements (mainly on seed, cages, exclosures) are substantial, rising to around SIB$22000 in year seven for a village grower aiming at an annual production of 1000 market clams, with a payback period of 11 years (farm-gate value $10) to 13 years (product value $7.5). Given the rather low labour requirements for production (around 70 man/days per year for 1000 output), it is evident that giant clam farming is capital rather than labour intensive.

Since likely seed cost and survival during grow-out are so poorly established, it is useful to examine the market value which would be required for different seed costs and survival rates, in order to achieve a 20% rate of return on investment, or a $20 return on labour. These are shown in Figures 2 and 3 for a seven year grow-out period and permit a rapid assessment of economic potential and sensitivity to production and marketing assumptions. Each line or isobar corresponds to a 20% return on capital or a $20 return

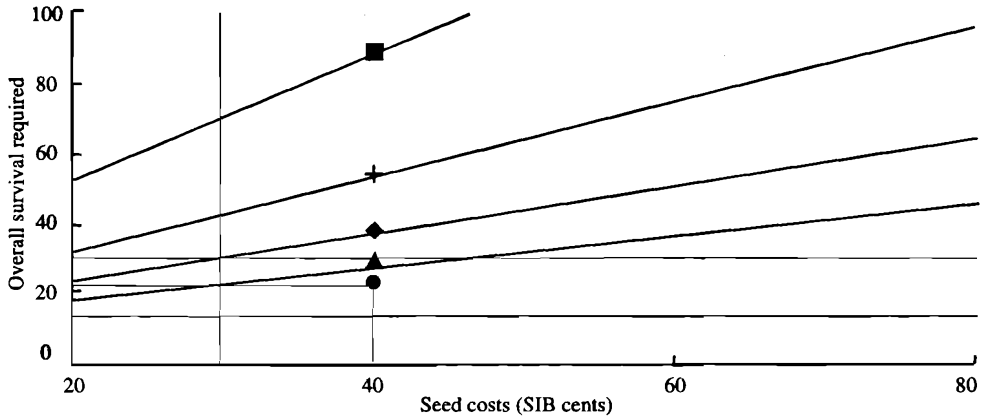

Figure 2. Seed costs and survival rates needed for 20% IRR at a range of 'farm-gate' values. ■ $5/clam; + $7/clam; ◆ $9/clam; and ▲ $11/clam.

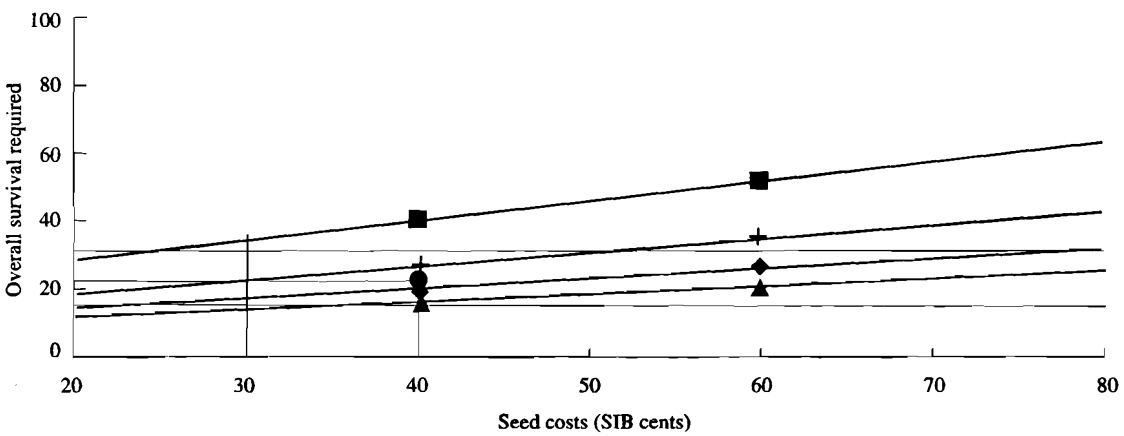

Figure 3. Seed costs and survival rates needed for SIB$20 ROL for a range of 'farm-gate' values. ■ $5/clam; + $7/clam; ◆ $9/clam; and ▲ $11/clam.

on labour for a particular market value. The 'likely zone' on the basis of the above discussion is marked and shows that a 20% return on capital would only be possible under optimistic assumptions about seed costs and survival rates, coupled with a farm-gate shell value of at least $5 (ca. $0.3/kg). A $20 return on labour could be achieved under rather less optimistic assumptions.

Conclusions

It is apparent from the foregoing analysis that the adductor muscle and mantle are unlikely to contribute more than half of the farm-gate value which would be required for the financial viability of giant clam farming (large *Tridacna gigas*) in Solomon Islands and that high value/high volume markets for the shell (at least $0.3/kg at the village site, for a total Solomon Islands production of around 3000 tonnes), will need to be developed if such farming is to become a viable industry in Solomon Islands.

Unfortunately several features of this potential industry make its suitability as a part-time village based activity highly questionable. These include the high investment requirements (>$22000 per 1000 clams annual production), the long payback period (11 years or more) and the very high production and marketing risks, which are compounded by the long growout period and the fixed production ratio of meat to shell

(which is likely to lead to a mismatch between supply and demand in any new developing markets).

The production of smaller clams may have greater potential because of the more rapid returns (and associated lower risks); the possibility of selling whole frozen meat to a single market; the larger numbers of seed that would be required for any particular output allowing for greater economies of scale in the hatchery; and the well established international markets in smaller (bowl sized) shells.

In the light of these considerations, it may be appropriate to place at least as much emphasis on the marketability of the shell as on meat yield or growth rate, when considering species choice or optimum market size for future research or development on giant clam mariculture.

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Distribution, Habitat and Culture of the Recently Described Giant Clam *Tridacna tevoroa* in Fiji and Tonga

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A recently described eighth series of tridacnid clam, *Tridacna tevoroa* Lucas, Ledua, Braley (Lucas et al. 1990, 1991) exhibits interesting characteristics which may change the taxonomy of the family. Few specimens have been found live in Fiji and only recently larger numbers of this species have been found in Tongan waters. The clam first came to the notice of Fiji Fisheries Division officials. This locally known 'tevoro' or 'devil' clam was proposed as a new species at an international meeting in Australia (Lewis and Ledua 1988). It was subsequently described (Lucas et al. 1990) and a more detailed description, together with tissue and shell morphometric comparisons with *T. derasa* were made (Lucas et al. 1991). The shell of *T. tevoroa* was significantly ($p<0.05$) thinner than *T. derasa* in terms of shell width, umbo thickness and shell weight versus shell length. In the central Ha'apai Group of Tonga this clam is known as the 'vasuva ngesi manifi', translated directly to 'clam shell thin'. Data on the habitat of this new species is sparse, since few have been seen by Fisheries officials or scientists just prior to the description. Lucas et al. (1991) state that '*T. tevoroa* appears to be along outer slopes of leeward reefs, in very clear, oceanic water at 20–30 m.' More than fifty *T. tevoroa* have been found in Tonga, especially in the central Ha'apai group, allowing us a clear description of the habitat of this rare species.

A concentrated effort was made to collect as many broodstock specimens of this new species in Tonga as possible and to transport these to the hatchery at Sopu, Tongatapu for attempted spawning and larval culture. An account of the first successful spawning and culture of the larvae and juveniles will be described.

Methods

Field collection—habitat

The first *Tridacna tevoroa* was collected in 1986. About 20 individuals were collected in the eastern Lau group of Fiji in 1989, 1990 and 1991. Although most clams were collected by Self-Contained Underwater Breathing Apparatus (SCUBA), a few were gathered by a traditional Pacific islands method. Here, a weight tied to the end of a long rope is lowered into the mantle of the open (and unsuspecting clam) which subsequently closes its valves tightly and is lifted up to the boat at the surface. A fisheries research vessel (20 m length) was used for the collection expeditions. During SCUBA collections, two divers went to areas where this locally named 'tevoro' clam had been spotted and marked by

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local free divers. New areas were surveyed in later collections by the Fisheries division divers for presence of the tevoro clam. Clams were returned in seawater tanks to the Ministry's giant clam hatchery at Makogai Island about 24 steaming hours from the Lau group. They were held in cages on sand at about 13–14 m.

The first *Tridacna tevoroa* were collected in the Ha'apai Group in Tonga in mid-1989 and some clams were transported by ship in seawater tanks to the Fisheries Department's giant clam hatchery at Sopu, Tongatapu Island. Further collections were made in Vava'u and Ha'apai in 1990, 1991 and early 1992 and shipped to the hatchery at Tongatapu where they were held offshore at 18 m. A few tevoro clams were even purchased from a fisherman on Tongatapu Island who was going to sell the clams in the market. All collections were done by SCUBA, except for several specimens collected by free divers in Vava'u and Tongatapu. An 8 m Fisheries Department skiff was used in other cases. In February 1992 two of the authors carried out a two day SCUBA search at reefs in northern Ha'apai to find live tevoro clams in their natural habitat and further detail of the habitat.

Spawning and culture

The first successful attempt to spawn *T. tevoroa* took place at the Tonga Fisheries Department at Sopu on 30 October 1991. Ten mature *T. tevoroa* were placed in a clean, empty cement raceway on their sides for 45–50 minutes to heat up. This temperature shock induction method has worked well with *T. derasa* in Tonga and Fiji in the past. Filtered seawater was then quickly added to the tank to a level just above the shell edge of the clam when standing upright. Sperm was collected in plastic containers and when egg release began, eggs were collected with plastic buckets or bags. About 1 ml concentrated sperm was added per litre of egg water to obtain fertilisation. However, some of the egg water already contained sperm from the spawning tank. Standard protocol development at James Cook University–Orpheus Island for semi-extensive culture were used in the hatching and larval phases of culture (Braley et al. 1988, Braley 1990). During the larval phase a flow-through screen was used to provide the best quality seawater to these larvae. On day 2 (about 40 hours post-fertilisation) the swimming veligers were 'selected' from the hatching tank and placed in the larval-nursery tank. The stocking density was very low (0.05 veligers per ml) due to poor survival of veligers through the hatching phase, despite the use of 7 ppm antibiotic (streptomycin sulfate). Also, it was thought wise to maintain larvae in good quality seawater to increase the chances of a higher production of juveniles. This required a greater depth of water and a shadecloth cover directly over the cement raceway to approximate the light levels found at greater water depth. Larvae were fed baker's yeast every second day at a rate of 1.4 g of yeast/10000 L seawater. One of the adult tevoro clams was sacrificed to give to the larvae zooxanthellae during their pre-settlement phase. On another day zooxanthellae were provided from a sacrificed *T. derasa*. After settlement, adult *T. tevoroa* were placed on the bottom of the tank to provide the right variety of zooxanthellae to the metamorphosing larvae, through feces.

The fouling algae was first cleaned by siphoning off from the bank bottom at day 90. Juvenile clams were heavy enough to fall to the bottom of a bucket while the algae could be floated off the top of the bucket. Juveniles were measured and examined under a dissecting microscope.

A second attempt at spawning *T. tevoroa* was made at Tonga Fisheries Department's hatchery in January–February 1992. Twelve new broodstock had been collected from Ha'apai in January 1992 and transported to the hatchery at Sopu for induced spawning. Solar heated seawater (to 32°C) and injection of 2 mM serotonin (1.5–2.0 mL dosage) were used. No spawning was observed this time. One of the 12 broodstock died on arrival at the hatchery and was dissected to check gonad condition.

Results

Field collection/habitat

Figure 1 shows a map of the islands where tevoro clams have been collected. The coordinates are listed in Table 1 for the location of collection. Table 1 details the collection of all the known specimens from 1986 through January 1992 associated with the government Fisheries from both Fiji and Tonga. Table 2 lists location and habitat information from discussions with Ha'apai and Tongatapu fishermen who have seen and collected tevoro clams. An estimate of the number of clams found per man hour of search on SCUBA showed that an average of about one clam per man hour was collected in Tonga. The average from Fiji is about 0.26 clams/man hour. It should be noted that the divers doing the searching are particularly fit divers and have an eye for clams in the habitat. About half of the clams in Tonga were found on the leeward and half on the windward side of reefs. However, windward side of reefs were still somewhat protected within barrier islands or reefs. No search has yet been made on outer windward reefs. In Tonga the mean depth for the clams is 26 m, varying from 9–33 m. Table 2 lists a response

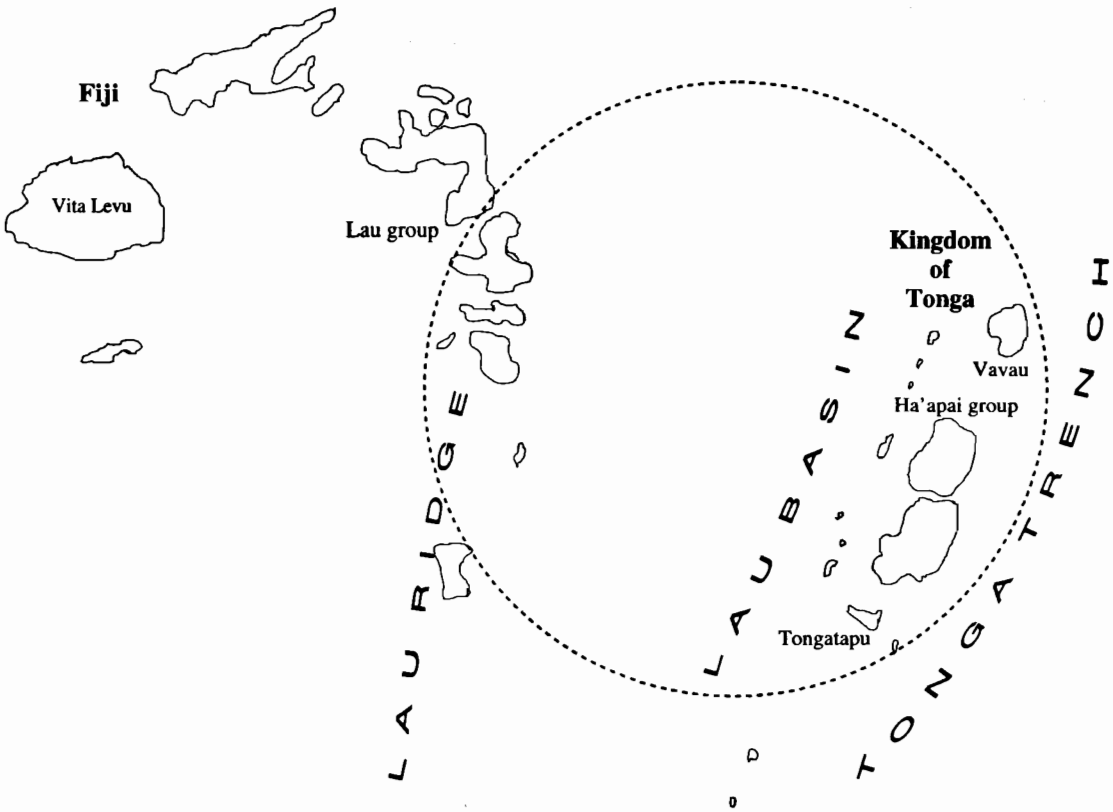

Figure 1. The main island groups of Tonga and the eastern Fiji Lau group of islands showing the known species distribution within the circle of *Tridacna tevoroa*.

from an old diver from Uiha Island, Ha'apai, suggesting that he saw or collected tevoro clams in 3 m of water in the 1940s; and clams were seen in small clumps at that time. The mean depth of clams collected in Fiji was 27.4 m, varying from 20–33 m and all were found on the leeward side of reefs and islands. Many of the clams found in Tonga were adjacent to the edge of a sand patch and cradled against rocky outcrops, rubble or bare rock with steep slopes. It is significant that during the SCUBA search in February 1992 in Ha'apai two of the authors found a considerable number of tevoro clams on live coral. However, in Fiji these clams have not been found on live coral, possibly because little live coral was found at this depth in the Lau group. Figure 2 shows profiles of the bottom in areas where tevoro clams have been found.

Larger, older specimens do not travel as well as younger tevoro clams and such deaths were recorded. Other deaths have occurred in Fiji after being held in the ocean nursery for two weeks or longer, or were eaten by turtles. Tevoro clams held at the 18 m deep site in Tonga looked healthy.

Spawning and culture

Five of ten clams released eggs, while seven released sperm. However, the number of eggs were small for four clams. A total of 28.2×10^6 eggs were collected. The fertilisation rate determined after 2 hours averaged 90.6%. Only 90000 veligers were retrieved from the hatching tank. This represents 0.3% survival from fertilised eggs. On day 90 there were about 2000 juvenile *T. tevoroa* found live on the bottom of the tank. This is about 2.2% survival from veligers, but only 0.08% survival from fertilised eggs to day 90. Juvenile clams have scutes like the other *Tridacna* species. The mantle does not reach over the edge of the shell in these small juveniles. The average shell length of 100 clams was 6.5 mm ± 1.2 mm (SD).

The second attempts at spawning *T. tevoroa* in January–February 1992 did not result in any successful sperm or egg release, nor any other spawning behaviour other than inflated excurrent siphons in some of the broodstock. The specimen which died on arrival from Ha'apai and was dissected had no gonad visible.

Table 1. Location–habitat description of *Tridacna tevoroa* specimens in Fiji and Tonga.

Collection date	Location coordinates	Depth (m)	Habitat	Number collected	Estimate of clams/man hrs search on SCUBA (m/h)
Early August 1989	Lofanga, Ha'apai 174°33'W and 19°49'S	33	T,L,RO(AA-20)	1	1 clam
Late August 1989	Auhangamea channel, Uiha Is., Ha'apai 174°20'W and 19°52'S	24–30.5	T,L,RO(AA-O adjacent to 40 slope R)	12	2.5 clam
May 1990	Kahefahefa Is., Vava'u 174°03'W and 18°48'S	18	T,W(inside barrier lagoon) on S(AA-O) next to R(AA-70)	5	0.35 clam
February 1991	Kahefahefa Is., Vava'u 174°03'W and18°48'S	27.5–35	T,W(Inside barrier lagoon), R(AA-30); on S(AA-)) next to R(AA-30-70)	6	0.04 clam
June 1991	Kahefahefa Is., Vava'u 174°03'W and 18°48'S	9	T,W(inside barrier lagoon), on S(AA-O) next to C(AA-20)	1	2.0 clam
January 1992	Faka'osi reef, Pangai, Ha'apai 174°26'W & 19°43'S	27.5–30.5	T,L(7 clams),W(inside barrier of isl., 5 n.mi. away; 9 clams), on S(AA-O) next to RO(AA-20-60) & some C	16	0.33 clam
February 1992	Luahoko Is., W & NW sides, Ha'apai 174°28'W & 19°41'S	24.4–27.5	T,L(18 clams)W(3 clams), 2 on S(AA-O)next to C;19 on C(AA-0-20); clumps of 4,3,2 seen, others singly	21	9.1 clam
January 1986	Cakau Tabu reef, Lau 178°32'W 17°40'S	20	F,L,S slope (AA-30)	1	0.25 clam
November 1989	Vatoa Is., Lau 178°13'W 19°56'S	33	F,L,RO next to R(AA-20-60)	6	0.30 clam
February 1990	Vatoa Is., Lau 178°13'W 19°56'S	0	F,L,RO next to R(AA-20-60)	5	0.20 clam
April 1991	Vatoa Is., Lau 178°13'W 19°56'S	26.6	F,L,RO next to R(AA-20-60)	2	0.30 clam

Notes: F—Fiji, T—Tonga, W—windward reef (forereef), L—leeward reef (backreef or lagoon), S — sandy, R—bare rock, C—live hard coral, RO—rocky outcrops between sand/rubble, AA—approximate angle of the substrate to the horizontal (degrees).

Discussion

Field collection—habitat

The relatively close proximity of the eastern Fiji Lau group and the Tongan Ha'apai group make the species distribution understandable. The presence of the clams both north (Vava'u) and south (Tongatapu) of Ha'apai in Tonga follows, since the ocean bottom topography is deep only for short distances between the rises of each group. Ha'apai has large areas of suitable reefs and shoals with typical habitat for *T. tevoroa*. It is suggested that this area may be the centre of distribution of this newly-described species and it is probably the largest repository of the species.

The features of *T. tevoroa* typical of both *T. derasa* and *Hippopus hippopus* were discussed in Lucas et al. (1991) and it was suggested that *T. tevoroa* could

Table 2. Location and habitat information on *T. tevoroa* from information from local fishermen.

Name	Village (Island)	Comments
Mr Peni Iketau (31 yr)	Faleloa (Foa Is.)	He has seen/collected about 20 tevoro clams since 1982; he often uses SCUBA. Clams 18–27 m), leeward side of reefs; clams scattered, not clumped together, but highest number (7) seen around Luahoko Is., west of Foa Is. Clams are almost always found next to flat sand but touching rocky outcrops or rock slope next to the sand.
Mr Sione Punai Sr and Jr (66 yr and 38 yr)	Uiha (Uiha Is.)	They named three areas good for clams, including *T. tevoroa*—Hakau Ata reef, Limu Is., Luataua shoal. Elder Sione saw and collected many tevoro clams in the 1940s; these were on sand near the reef usually and as shallow as 3 m down to 27 m. They were found in groups in shallower water many years ago. These people told us another Tongan name for the tevoro clam, *Vasuva ngesi sio ata* (window pane shell clam).
Mr Oueni	Tolonga (Tongatapu Is.)	The clams were collected from the reef between Tau and 'Ata islands (the same reef). He sold seven of these to Tonga Fisheries Dept. He collected the clams from13.7–16.7 m, some on sand and some on coral.
Mr Peni Tuicake	Lau (Vatoa Is.)	He collected tevoro clams from 9.1 m, amongst a branching *Acropora* thicket. This sighting was from 1984 on Vatoa. He and another fisherman also found two tevoro clams on a nearby reef (Vuata Vatoa) in shallow water.

represent a transitional stage between the genera *Tridacna* and *Hippopus*. However, without further information, a separate subgeneric or generic status has not been proposed.

It could also be suggested that *T. tevoroa* speciated in this area of its known distribution, perhaps even from a successful hybridisation of *T. derasa* and *H. hippopus* (found in both Fiji and Tonga prior to local extinction 20–30+ years ago). Although this is highly speculative without the support of genetic evidence, it is most interesting to note that seven of the eight members of the family Tridacnidae have their centres of distribution around Indonesian and southern Philippines, as is typical of many Indo–Pacific families of reef vertebrates and invertebrates. The smallest distribution of any tridacnid is that of *T. tevoroa*, located far from the Indo–Pacific 'source' of speciation. This will remain highly speculative until fossil evidence, new records for species distribution and genetic work support or refute this.

Spatial distribution appears to be very sparse with single individuals being found at most locations. However, clumps of four individuals were seen twice and other smaller clumps were seen in Tonga. These could represent small breeding groups for this species. Evidence of former greater abundance and distribution in shallow water in Ha'apai would indicate that fishing pressure has also contributed to the rarity of this species.

Spawning and culture

The lack of success in spawning of the *T. tevoroa* broodstock during the latter part of January indicates that this may be the resting period of gametogenesis. This was supported by the dissection of one specimen which died and from a specimen which was killed by Tonga Fisheries staff in December 1991 showing no gonad visible. The successful spawning in late October 1991 indicates that this species has a breeding season not unlike that of *T. derasa*. On the Great Barrier Reef in Australia the breeding season of *T. derasa* is from late winter–early spring to early summer and virtually all are spent by mid-December (Braley 1984, Braley 1988). In Fiji the breeding program for this species is from July to October and in Tonga from September to late November. It must be noted that the examples of the breeding season of *T. derasa* given here are from higher latitudes within the tropics 17°–21°S) while there is evidence from hatchery spawnings that at lower latitudes (Palau, 7°N) this species has an almost full-year breeding season (Heslinga et al. 1984).

The poor success of veligers resulting from the hatching phase may be attributed to polyspermy or self-fertilisation (see Calumpong et al. these Proceedings). Recent quantitative evidence of polyspermy in oysters (Stephano and Gould, 1988) further supports the likeliness of the results in this spawning. Normally, the clam releasing eggs is removed from the spawning tank, rinsed with filtered seawater and placed into

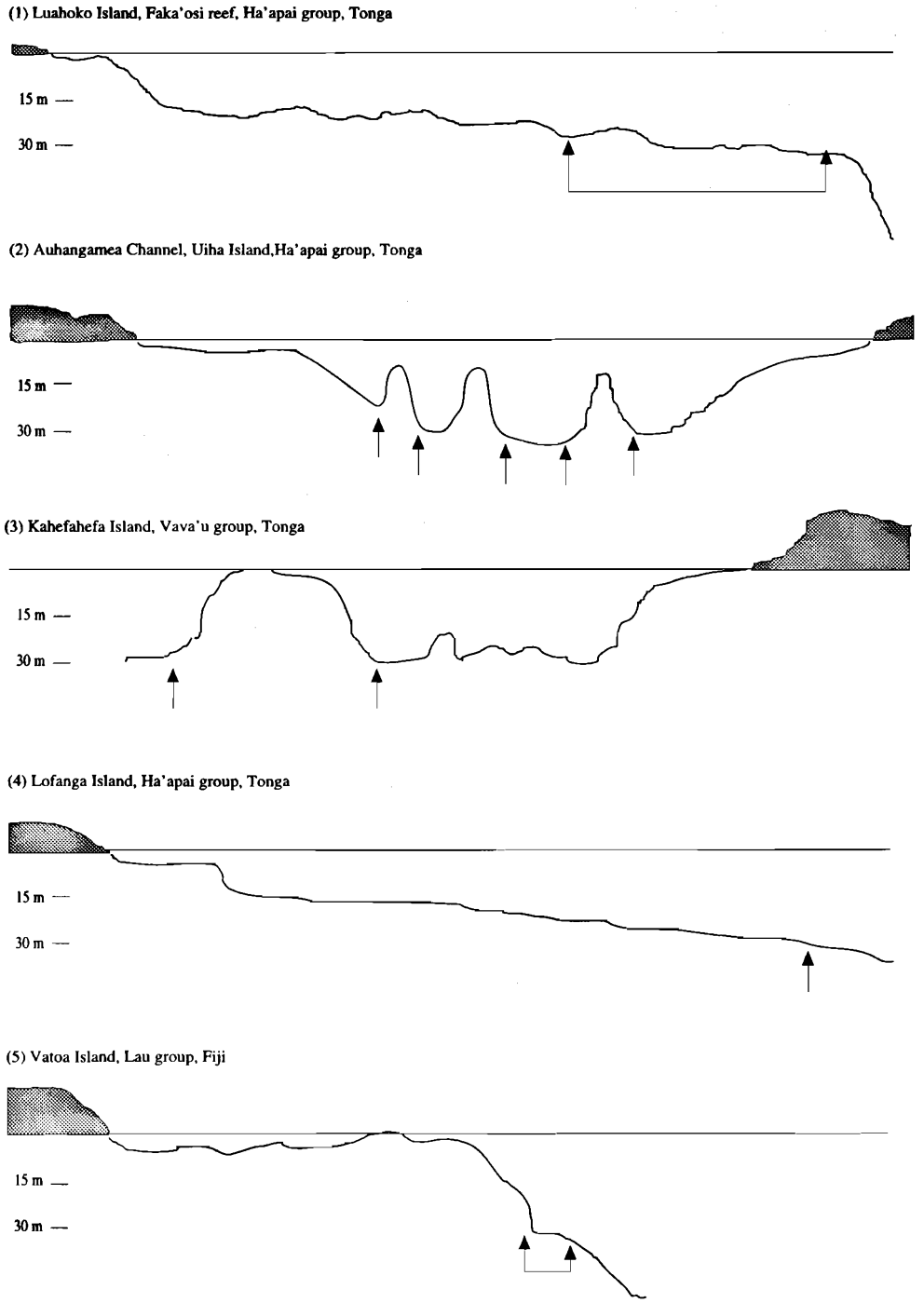

Figure 2. Profiles of sea bottom topography where tevoro clams have been found in Tonga and eastern Fiji. Depth markers for 15 m and 30 m are shown and names of islands or reefs are given where the clams have been found.

successive bins of filtered seawater before sperm is added to effect fertilisation. Here, due to lack of some equipment, the eggs were collected as spawned in the spawning tank plus the addition of the normal amount of sperm. The fertilisation rate was high but number of veligers surviving at 40 hour post-fertilisation was very low. Given the use of antibiotics in the hatching phase, the implication would be polyspermy as a cause. If one considers the sparse distribution and abundance of *T. tevoroa* it would appear that polyspermy could present a problem in the fertilisation of eggs of this species.

Acknowledgments

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Some Responses of the Giant Clam to Elevated Nutrient Levels in Sea Water

C. Belda, C. Cuff, J. Lucas and D. Yellowlees*

THE addition of dissolved inorganic nutrients to sea water during the early stages of growth of cultured tridacnid clams is now common practice. This prompted us to examine the implications on the physiology and morphology of the clam and their symbiotic algae. Our studies revealed that elevated dissolved inorganic nutrients effect many aspects of the structure, biology, physiology and biochemistry of these animals. Some of these changes are beneficial for clam mariculture. However, when applied to wild stock, as may be the case where anthropogenic input into adjacent waters is significant, then the effects may not be so beneficial. Our results have been submitted to Marine Biology in the following two manuscripts:

Belda, C.A., Lucas, J.S., Yellowlees, D. 1993. Nutrient limitation in *Tridacna gigas* symbiosis: Effects of nutrient supplements on growth of the symbiotic partners.

Belda, C.A., Cuff, C., Yellowlees, D. 1993. Modification of shell formation in the giant clam *Tridacna gigas* at elevated nutrient levels in sea water.

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