

# Influence of Symbiont Strain on Early Growth of Tridacnids

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## Abstract

Symbiotic algae (zooxanthellae) from the mantles of fast- and slow-growing *Tridacna gigas*, from *T. maxima*, *T. crocea* and *Hippopus hippopus* were isolated and supplied to larvae of *T. gigas* grown under hatchery conditions. Significant differences in growth rates of the larvae and juveniles were seen between these various treatments. The larvae and juveniles which had been supplied with zooxanthellae taken from fast-growing *T. gigas* grew faster than those supplied with zooxanthellae taken from slow growers. No preference was noted in *T. gigas* for zooxanthellae from a conspecific source, and those given zooxanthellae from *T. maxima*, *T. crocea* and *H. hippopus* survived equally well for the duration of the experiment, 90 days. *T. gigas* juveniles were able to continue to take up zooxanthellae from the environment throughout the first 38 days of their lives. Freshly-isolated zooxanthellae taken from clams which are known to be fast-growers are therefore recommended for routine use in giant clam hatchery operations.

## Introduction

The mariculture of giant clams (Family Tridacnidae) is now a well-established technology, and hatcheries of varying degrees of sophistication are operating in the South Pacific. As in other bivalve species, enormous variation in growth rates is observed within cohorts (Pearson and Munro 1991).

Symbiotic algae in giant clam mantle tissues contribute photosynthetically fixed carbon to the host (Muscatine 1967; Griffiths and Streamer 1988). These algae (called zooxanthellae) are dinoflagellates (Yonge 1936); they were identified as *Symbiodinium microadriaticum* (Taylor 1969), but since then Trench and Blank (1987) have recognized four species within the genus *Symbiodinium* Freudenthal, and strains or subspecies have been identified by various methods including molecular systematics (Blank and Huss 1989; Rowan and Powers 1991a and b).

Eggs released from tridacnids do not possess zooxanthellae (LaBarbera 1975; Jameson 1976), and the larvae or juveniles must acquire their comple-

ment of algae from the environment. Tridacnids apparently are able to select strains of zooxanthellae that become established and maintain a symbiotic relationship (Fitt and Trench 1981). Fitt (1985) showed that different strains grew at different rates inside the host, some strains did not survive, and fast growing strains of zooxanthellae (inside the host) gave faster growth rates of the clams. Fitt et al. (1986) found that freshly-isolated zooxanthellae conferred higher growth and survival rates than did cultured zooxanthellae when given to veligers in the laboratory.

This paper describes investigations carried out under hatchery conditions to determine: i) whether the use of strains of zooxanthellae freshly isolated from fast-growing *Tridacna gigas* to inoculate batches of larvae in hatcheries will confer better growth and survival than zooxanthellae isolated from slow growing clams (Experiment 1); ii) whether *T. gigas* larvae will take up zooxanthellae from other tridacnid species, and what effect these will have on growth and survival of hatchery-reared *T. gigas* (Experiment 2); and iii) the effect of exposing *T. gigas* larvae/juveniles at increasing stages in their development to zooxanthellae (Experiment 3).

## Methods

The work in this study was divided into two phases: Phase 1 included the period of larval growth from age 8 to 24 days; and Phase 2 included the period from age 25 to 90 days. In Phase 1, larvae were cultured in 1  $\mu\text{m}$ -filtered and UV-sterilized seawater, while in Phase 2 the clams were moved into settlement tanks, and the seawater was not sterilized or filtered. In Phase 1 only the experimentally-added zooxanthellae were available to the clams. Symbioses were fully established by the time the experiments entered the second phase.

### *Spawning*

Adults were stimulated to spawn using 2 mM serotonin (Sigma, USA) intragonadal injection (Braley 1985). Eggs were collected in large plastic bags as they were vigorously expelled from the exhalant siphon, and kept in 20-l buckets. The eggs were fertilized by adding a small volume of sperm-laden water from another animal and stirred gently. Except for Experiment 2, the larvae used did not come from single parent spawnings. In Experiments 1 and 3 sperm came from more than one clam. The fertilized eggs were then transferred into 1,500 l fiberglass larval tanks at a stocking density of about 20 eggs/ml, maintained under ambient conditions of temperature in 1  $\mu\text{m}$ -filtered UV-sterilized seawater. Moderate aeration through an inline 0.45- $\mu\text{m}$  filter was provided for the first 36 hours, thereafter light aeration. Flocculated excess sperm and other organic matter was scooped off the surface using a 1-mm sieve cloth stretched across a wire frame.

## **Larval Rearing**

### **PHASE 1. AGE 8-24 DAYS**

The larvae were reared following standard *T. gigas* larval rearing protocols (Usher 1990) in tanks of lightly-aerated, 1  $\mu$ m-filtered, UV-sterilized seawater. Routine water changes and feeding on alternate days started on day 2. The standard feed consisted of a 50:50 mix of Frippak Booster microcapsules and freeze-dried *Tetraselmis suecica* and was given to the larvae at a rate of 0.16 g/100 l. For Experiment 1, 1 ppm chloramphenicol was applied after every water change; for Experiments 2 and 3, 12.5 ppm streptomycin was used until day 14 when overflow began. Veligers/pediveligers were sieved on day eight and stocked into outdoor circular culture bins. In Experiment 1, each of the three treatments was replicated six times; a total of eighteen 50-l bins were stocked with 100,000 larvae each. In experiment 2, fifteen 50-l bins were used; each of the five treatments was replicated three times by stocking each bin with 100,000 larvae.

**Sampling methods:** Veligers/pediveligers were sieved onto an 80- $\mu$ m mesh every two days, and juveniles sieved every four days starting day 16. The animals from each bin were then concentrated into 1-l volumes, from which six replicate 0.5-ml samples were taken with a Gilson P-1000 automatic pipette. In order to randomize sampling as much as possible, the larval/juvenile suspension was stirred gently while samples were being taken.

**Growth and survival measurements:** Growth was monitored by measuring the lengths of 30-50 individuals from each 0.5-ml sample every fourth day from day eight until day 24. Survival was monitored by counting the percentage of live animals in each 0.5-ml sample.

The veligers/pediveligers continued to be fed on alternate days until day 13 on which day they were given half the quantity of feed, and then no longer fed. Incoming seawater continued to be filtered and UV-sterilized until day 24. On day 24 the juveniles were sieved and total numbers in each bin counted, to give the final survival result for Phase 1. All the juveniles from the replicates of each treatment were then pooled, and restocked for the beginning of Phase 2.

### **Isolation and Introduction of Zooxanthellae**

Zooxanthellae were given on day nine. The zooxanthellae were collected by scraping a small piece of mantle tissue to release them into suspension. The freshly isolated zooxanthellae were given directly to the veliger/pediveliger larvae after washing and filtering the suspension through six different size sieves: 300, 200, 132, 80, 53 and 25  $\mu$ m in series.

For Experiment 1, zooxanthellae were taken from mantle tissue of a 2-year old cohort of *T. gigas*, consisting of individuals raised under identical conditions since their spawning. The 2-year old cohort was divided into three groups: large, medium and small clams. It was assumed that their sizes reflected fast, medium and slow growth rates. Two clams from each phenotypic group were sacrificed to provide zooxanthellae termed "fast", "medium" and "slow" strains.

For Experiment 2, zooxanthellae were isolated from four different species, *T. gigas*, *T. maxima*, *T. crocea* and *Hippopus hippopus*. The number of zooxanthellae added to the larvae was in the order of 100 cells/ml of larval culture suspension.

For Experiment 3, a cohort of larvae (2.5 million) was reared in 1 µm-filtered UV-sterilized seawater following the standard protocol, including feeding. Every fourth day from day 14 the veligers were sieved onto an 80-µm mesh and a sample of 20,000 stocked into two replicate buckets at 10,000 per bucket. Suspensions (in the order of 100 cells/ml) of zooxanthellae freshly isolated from juvenile *T. gigas* were introduced to the buckets. Clams were observed and measured before and 24 hours after zooxanthellae introduction. Length measurements were calculated from the means of 30 clams from random samples of each replicate, taken as described above. A growth curve was drawn from length measurements of the clams which had not yet received any zooxanthellae, and which thus provided baseline growth data.

#### PHASE 2. AGE 25-90 DAYS

Equal numbers of juveniles at a stocking density of 1.2 juveniles/cm<sup>2</sup> were placed in replicated containers for settlement in running, unfiltered and unsterilized seawater outdoors. In Experiment 1, the settlement containers were miniraceways, and in Experiment 2, circular bins were used as well as miniraceways. During this period, survival of the clams was monitored regularly by taking samples, and regular visual checks for algal growth were made. Grazers used in the miniraceways to control algal growth included cerithid snails and seahares *Stylocheilus* spp. On day 90, the clams were harvested and a random sample of 50 clams from each replicate was measured. The total harvest of clams was dried using blotting paper, weighed and the final total counts determined as follows:

$$\text{Final total count} = \frac{\text{total mass (TM)} \times \text{sample count (SC)}}{\text{sample mass (SM)}}$$

where

TM is the total mass of clams in each raceway/bin;

SC is the total number of clams in a sample and

SM is the total mass of clams in a sample.

#### DATA ANALYSES

Growth data from all experiments were analyzed by analyses of variance. The significance level specified was 0.05 to compare the means of length measurements between treatments.

The means of the replicate survival counts for the final days of each phase were analyzed in the same way.

## Results

Figs. 1a and 1b show mean shell lengths (in microns) of three groups of a cohort of *T. gigas*, respectively fed zooxanthellae isolated from *T. gigas*, fast, medium and slow growers. By the end of the experiment, day 90, the clams which had received zooxanthellae from the fast growers, were significantly larger than the other two groups. This experiment was repeated using another cohort of *T. gigas* larvae, and showed the same significant differences at the 0.05 level. The zooxanthellae used in this experiment were not from the same source as those in Experiment 1, but were again taken from fast (F), medium (M) and slow (S) growers of a cohort of *T. gigas* kept under identical conditions since birth. This data set is given in Table 3.

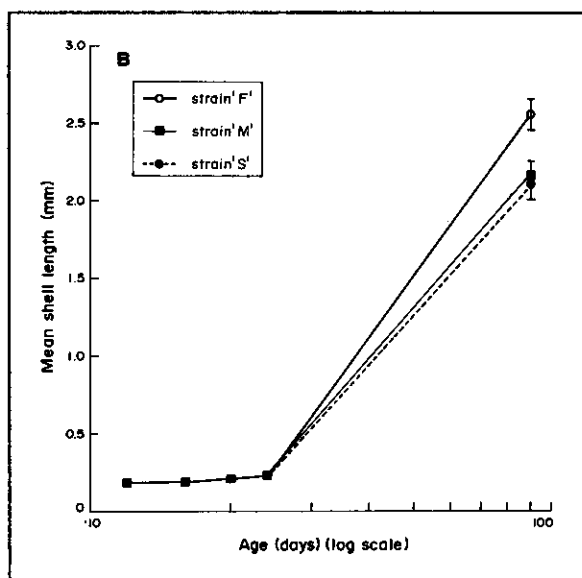
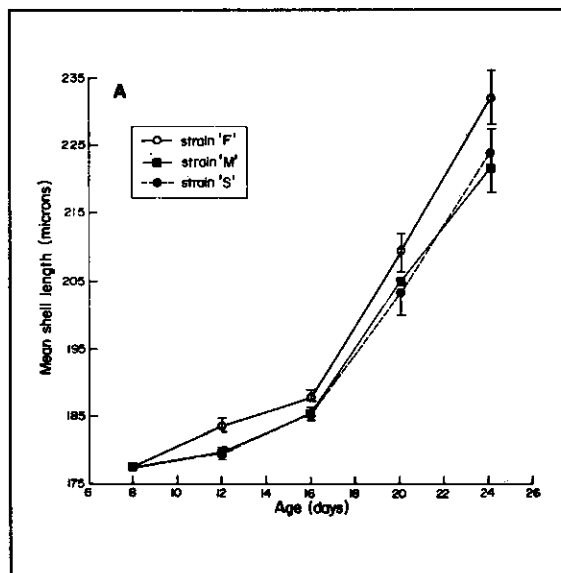
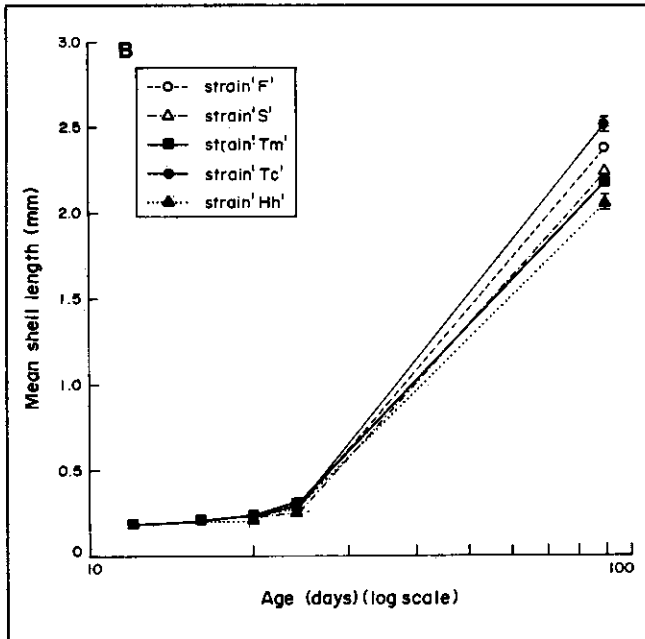
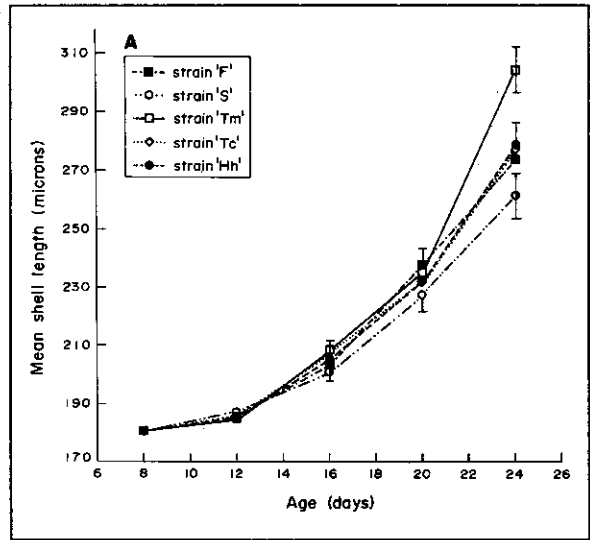


Fig. 1. Growth of *T. gigas* larvae/juveniles given zooxanthellae from fast (F), medium (M) and slow (S) growers of a cohort of *T. gigas*. Confidence limits at  $P < 0.05$  level are indicated. 1a shows the growth in the first phase, up to day 24, and 1b shows both phases of growth, up to 90 days.

Figs. 2a and 2b show the results of giving zooxanthellae from various sources to a third cohort of *T. gigas* larvae, and the growth curves obtained over the two phases of the experiment. At the end of Phase 1, the clams which had received zooxanthellae taken from the mantle of *T. maxima* were significantly larger than any of the others. Those which had received zooxanthellae from the slow-growing *T. gigas* were smaller, but not significantly so ( $P > 0.05$ ), than those which had zooxanthellae from fast growers or any of the others. By day 90, there was more overlap between the various treatments, and although there were significant differences, no pattern consistent between the two phases emerged. Juveniles which had been given "fast" zooxanthellae were still larger than those given "slow" zooxanthellae, but not significantly so.

Fig. 2. Growth of *T. gigas* larvae/juveniles given zooxanthellae from various sources: fast (F) and slow (S) growers of a cohort of *T. gigas*, *T. crocea* (Tc), *T. maxima* (Tm), and *Hippopus hippopus* (Hh). Confidence limits at  $P < 0.05$  level are shown. 2a shows the first phase, up to 24 days, and 2b shows both phases up to 90 days.



Tables 1 and 2 give survival numbers for the replicates of "treatments" in Experiments 1 and 2. None of the treatments differed significantly in percentage of survivors by the end of either Phase 1 or Phase 2 in either experiment, analyzed by ANOVA.

The effect of adding zooxanthellae to *T. gigas* larvae/juveniles of increasing age is shown in Fig. 3. On day 14, all clams looked healthy and active, but by day 18, aposymbiotic clams were relatively inactive. By day 30, these were completely inactive and on day 42 all aposymbiotic clams were dead.

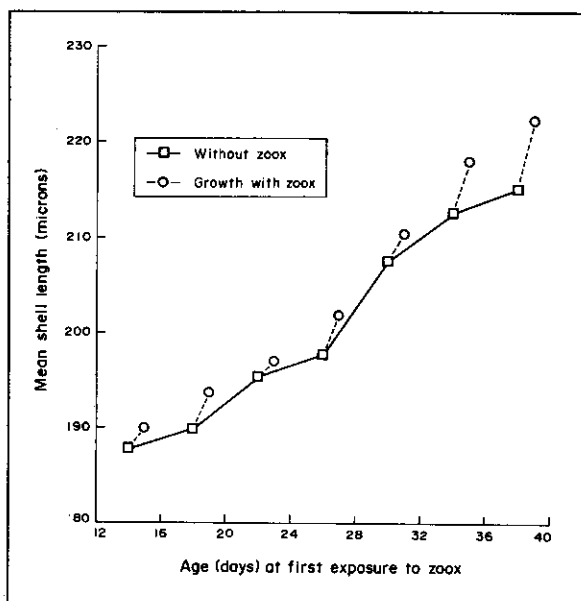


Fig. 3. Growth curve of aposymbiotic *T. gigas* from day 14 to 38, fed on a microcapsular and dried algal diet. The points above the growth curve show mean lengths ( $n=30$ ) from two replicate buckets of juveniles which were withdrawn and given zooxanthellae on days 14, 18, 22, 26, 30, 34 and 38, and measured 24 hours later.

Table 1. Mean number ( $n=36$ ) of clams surviving in three treatments, Experiment 1. Means were calculated from numbers of live animals in six 0.5-ml random samples taken from each of six replicates in Phase 1. In Phase 2, all surviving juveniles from each treatment were pooled and restocked at 9,000 juveniles per raceway, four raceways per treatment. Values in brackets are numbers of survivors on day 90, values in square brackets are standard errors.

Age (days)	Numbers surviving (thousands)		
	Strain F	Strain M	Strain S
8	Six replicate bins for each strain stocked at 100,000/bin		
12	54.8 [15.1]	54.6 [14.8]	67.9 [12.0]
16	25.9 [5.9]	29.6 [9.3]	31.2 [6.7]
20	14.9 [3.9]	21.2 [8.1]	21.4 [4.7]
24	12.6 [3.9]	14.8 [8.0]	18.1 [3.3]
Restocked, 2nd phase, 9,000 juveniles/raceway			
90	(3,557) (1,726) (3,298) (1,412)	(1,712) (3,759) (2,332) (1,793)	(4,253) (2,204) (2,945) (1,015)
Mean	2,498 [1,085]	2,399 [947]	2,604 [1,356]



Table 2. Mean numbers (n=18) of clams surviving in five treatments, Experiment 2. Means were calculated from numbers of live animals in six 0.5-ml random samples taken from each of three replicates in Phase 1. In Phase 2, all surviving juveniles from each treatment were pooled and restocked at 8,000 juveniles per raceway/bin, two raceways and one bin per treatment. Values in brackets are numbers of survivors on day 90, values in square brackets are standard errors.

Age (days)	Numbers surviving (thousands)									
	Strain F		Strain S		Strain Tm		Strain Tc		Strain Hh	
8	100,000 per bin*									
12	80,000 per bin*									
12	80.0	[0]	80.0	[0]	79.9	[5.5]	78.6	[1.0]	67.9	[4.5]
16	28.0	[4.6]	29.2	[11.7]	22.0	[8.0]	17.6	[5.4]	25.7	[3.5]
20	19.6	[3.4]	18.7	[8.1]	12.2	[3.2]	11.9	[5.7]	16.7	[1.4]
24	14.2	[0.85]	12.0	[5.8]	8.9	[3.6]	8.5	[3.9]	9.8	[1.1]
Restocked, 2nd phase, 8,000 juveniles per raceway or bin										
90	(1,421)		(1,968)		(4,832)		(2,147)		(3,648)	
	(3,916)		(814)		(277)		(171)		(1,990)	
	(2,894)		(2,859)		(1,544)		(3,427)		(3,360)	
Mean	2,744		1,880		2,218		1,915		2,999	
	[1,254]		[1,025]		[3,251]		[1,640]		[886]	

\*Treatments Tm, Tc and Hh were set up on day 8 with 100,000 larvae per replicate bin; F and S were set up from the same stock on day 12 with 80,000 per replicate bin.

Table 3. Mean lengths (n=50) of clams in repeated Experiment 1, using a different cohort. At the end of Phase 1 (24 days), the six replicates for each treatment were pooled and the survivors restocked in four replicate miniraceways for Phase 2 (90 days). Values in brackets are standard errors.

Age (days)	Shell length [µm]					
	Strain F		Strain M		Strain S	
12	180.8	[4.8]	178.4	[5.2]	180.5	[5.9]
	178.7	[4.1]	178.9	[5.1]	178.9	[4.9]
	180.0	[5.3]	180.0	[4.6]	180.1	[4.9]
	179.9	[5.0]	180.5	[4.9]	179.7	[4.0]
	179.5	[5.6]	180.6	[6.1]	182.3	[6.7]
	184.1	[5.8]	184.8	[6.3]	181.9	[4.6]
Mean	180.5	[1.9]	180.5	[2.3]	180.6	[1.3]
16	194.7	[7.2]	186.6	[7.7]	186.5	[7.2]
	193.8	[6.8]	186.7	[7.0]	188.9	[6.1]
	189.5	[6.2]	188.5	[7.1]	188.1	[10.7]
	188.6	[8.4]	186.9	[7.1]	184.0	[5.6]
	184.9	[4.9]	187.8	[7.4]	189.6	[8.9]
	192.1	[6.9]	188.2	[8.4]	190.2	[6.5]
Mean	190.6	[3.7]	187.5	[0.8]	187.9	[2.3]

Continued

Table 3. Continuation

Age (days)	Shell length [ $\mu\text{m}$ ]					
	Strain F		Strain M		Strain S	
20	201.0	[8.9]	200.6	[16.3]	196.1	[15.7]
	208.5	[11.9]	197.6	[9.0]	191.7	[8.0]
	203.5	[15.7]	196.9	[11.7]	210.2	[21.8]
	214.8	[16.4]	194.8	[10.4]	188.9	[8.4]
	200.7	[10.2]	195.9	[13.6]	206.7	[19.1]
	208.8	[14.1]	195.1	[11.3]	196.2	[14.3]
Mean	206.2	[5.5]	196.8	[2.1]	198.3	[8.4]
24	232.7	[20.6]	215.5	[21.2]	219.9	[23.6]
	246.4	[23.7]	209.9	[21.1]	206.6	[16.5]
	232.2	[22.3]	222.7	[25.8]	222.8	[23.6]
	252.4	[25.5]	217.7	[21.5]	204.9	[13.3]
	233.0	[22.7]	220.5	[23.7]	230.9	[31.6]
	234.2	[17.7]	210.9	[16.3]	201.6	[11.6]
Mean	221.8	[34.4]	216.2	[5.1]	214.5	[11.7]
90	2,700.0	[509.1]	2,400.0	[816.0]	2,200.2	[653.8]
	3,100.0	[649.5]	2,400.0	[625.3]	2,000.0	[490.9]
	2,700.0	[808.6]	2,100.0	[387.4]	2,300.0	[631.5]
	2,300.0	[637.9]	2,000.0	[482.7]	1,700.0	[378.6]
Mean	2,700	[326.6]	2,225	[206.2]	2,050	[264.6]

## Discussion

Two different cohorts of *T. gigas* larvae/juveniles showed significantly better growth rates under hatchery conditions when supplied with zooxanthellae taken from fast growing clams (Experiment 1). The extreme variability in growth rates exhibited by giant clams within cohorts can therefore perhaps be regarded, at least partly, as a result of acquisition of different strains of zooxanthellae. Other explanations of this variability in bivalves include aneuploidy and heterozygosity effects (Thiriou-Quévroux et al. 1992).

A third cohort (Experiment 3) failed to show significant differences in growth between those supplied with zooxanthellae from fast and slow growing *T. gigas*, but those given the "fast" strain were larger throughout the experiment, and by the end of 90 days they were still ahead of those given the "slow" strain. It is worth noting that this cohort was from a single-parent spawning, whereas the clams used in the other two experiments were derived from multiple-parent spawnings.

Selection of zooxanthellae extracted from fast-growing clams for introduction to cohorts of cultured larvae may thus enhance growth, and is certainly worth application in hatchery operations.

Although the epithet "strain" is used here to denote differences among the zooxanthellae, we do not mean to imply defined differences. The difficulties

pertaining to zooxanthellae taxonomy are under investigation (Rowan and Powers 1992).

Mortality was highest between days 8 and 12. This mortality is associated with the metamorphosis from pediveligers to juveniles (Fitt et al. 1984). Differences in numbers of survivors between the treatments were usually not significant, and no conclusions are drawn here about the effect of zooxanthellae strain on survival. Survival was highly variable both within and between experiments, and because it is usually low, it can be very difficult to obtain accurate survival rates from experiments of this kind. To obtain sufficient numbers for statistical analysis, it would be necessary to have more replicates than can be physically handled. It is therefore not possible to say which of the different strains tested had an impact on survival. If survival is correlated with size, faster growth of larvae and juveniles under both hatchery and natural conditions would be advantageous.

Zooxanthellae strains isolated from *T. maxima*, *T. crocea* and *H. hippopus* were taken up by larvae of *T. gigas* within 24 hours of their introduction to the larval cultures. This is consistent with the observations of Fitt and Trench (1981) that tridacnid veligers were capable of ingesting strains of *S. microadriaticum* isolated from sea anemones (*Aiptasia tagetes*, *Zoanthus sociatus* and *Heteractis lucida*), from jellyfishes (*Cassiopeia xamachana* and *Mastigias papua*) and from other species of tridacnid clams. Although we found significant differences in size in the clams given zooxanthellae from various sources, the rankings changed after settlement, thus no clear pattern emerged. Fitt (1985) found that some zooxanthellae strains (taken from *T. gigas* and *Zoanthus sociatus*) did not survive in the mantle of juvenile *Hippopus hippopus*, and the clams died, although *H. hippopus* given zooxanthellae from *T. maxima* grew well.

Unfortunately, it was not technically feasible to supply the juvenile clams with filtered seawater during Phase 2. It is possible that after 24 days when the clams were moved into the settlement containers, they took up different strains of zooxanthellae from the unsterilized, unfiltered seawater, although their symbioses were already established by then. The zooxanthellal tube system remains open to the alimentary canal throughout the clam's life (Norton et al. 1992).

Observations on clams without zooxanthellae in Experiment 3 confirmed those of Gwyther and Munro (1981) and Fitt and Trench (1981), that newly metamorphosed juveniles can survive and grow without symbionts if nutrients are present. However, as they mature, their response to added zooxanthellae increases (Fig. 3), implying that they become increasingly dependent on a photosynthetic translocate to supply their nutritional needs. Klumpp et al. (1992) have shown a shift from heterotrophy to increasing dependence on autotrophy as the clams grow.

The results of Experiment 3 mean that it is possible to export juvenile clams without zooxanthellae in them until they are about one month old. This would eliminate a possible source of infection; an important consideration in quarantine regulations. Zooxanthellae could then be supplied as soon as the juveniles arrived at their destination.

## Conclusions

Zooxanthellae taken from various sources confer significantly different rates of growth on tridacnid larvae/juveniles. There is no evidence to show that only conspecific sources should be used. On the contrary, *T. gigas* larvae and juveniles grew better with zooxanthellae supplied from species other than *T. gigas*. The ability of different types of zooxanthellae to enhance growth in the host has far-reaching implications in mariculture of tridacnids. Until more is known about the various types, the symbiotic relationship, and the selection process by the host, the best procedure would be to use freshly-isolated zooxanthellae taken from clams which are known to be fast-growing.

There appears to be no "window" of uptake time of symbionts in *T. gigas*, but the need for photosynthetically translocated carbon increases as they mature. The unanswered question now is: are zooxanthellae exchanged once a symbiosis is established or are they merely turned over inside the clam? In other words, can symbiotic recombination occur?

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