Induction of Symbiosis in *Tridacna crocea* (C. Bivalvia, F. Tridacnidae) Using Zooxanthellae from *T. gigas* and from *T. crocea:* Effects on Clam Survival and Growth

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ABSTRACT

Survival and growth of post-metamorphic *Tridacna crocea* juveniles were improved by inducing symbiosis with fresh or cloned zooxanthellae (Tg10) derived from *T. gigas*. Although clam growth was best with Tc4, survival was also poorest. Symbiosis with specific zooxanthellae was established at the pediveliger stage, with reinfection a few days after. It is suggested that while survival and growth may be easily monitored and may be used as indicators of good performance of a functional holobiont, other phenotypic traits such as resistance to disease, bleaching, etc. may also be considered in evaluating the effectivity of the selected zooxanthellae.

Key words: symbiosis, *Tridacna crocea*, zooxanthellae, *Tridacna gigas*, survival, growth

INTRODUCTION

Tridacnids are autotrophic organisms by virtue of their symbiosis with zooxanthellae (Trench and others 1981); at the same time, they are also heterotrophic filter-feeders equipped with large ctenidia (Klumpp and others 1994). Their capability for autotrophy distinguishes them from other cultivable filter-feeding bivalves (Munro and Gwyther 1981), and enhances their value as a mariculture species. Their zooxanthellae have been identified as *Symbiodinium* and belong to groups or clades A and C (Rowan and others 1996). Tridacnids are categorically of the "open symbiosis" type, i.e., aposymbiotic larvae acquire zooxanthellae from the environment (Trench 1987). As a result, they tend to be polymorphic symbioses, involving members of different *Symbiodinium* groups (Rowan and others 1996).

The clam host may establish symbiosis with *Symbiodinium microadriaticum* from different sources (e.g., different clam species or other invertebrate host) (Fitt and Trench 1981, Fitt 1985), which makes the clam-zooxanthellae symbiosis flexible. This allows holobiont evolution wherein the diversities of both clam and zooxanthellae must somehow merge (Iglesias-Prieto and Trench 1997, Rowan 1998). Even though the mechanism for selection may be passive, certain strains or types of zooxanthellae may persist and proliferate within the clam host more readily than others (Fitt 1985). From its photosynthetic zooxanthellae, the clam host acquires photosynthates sufficient for its metabolic carbon requirements (Trench and others 1981, Fisher and others 1985). On the other hand, zooxanthellae are able to propagate themselves within the mantle in large numbers, and are able to perform their various metabolic functions. Zooxanthellae are also able to absorb dissolved nitrogenous compounds (e.g., Braley and others 1992), including the clam host's nitrogenous waste products (Mingoa-Licuanan 1993). However, different factors such as irradiance, temperature, and

nutritional status of the holobiont may also determine the success of the clam-zooxanthellae symbiosis (Rowan 1998).

With increasing knowledge on zooxanthellae diversity and appreciation of the role of zooxanthellae in symbiotic associations, clam culture methods now employ additional approaches involving selection of zooxanthellae for symbiosis induction. Although growth rates of giant clams may vary with clam species, age, and environmental conditions, symbiotic zooxanthellae may also influence clam growth. Clam growth rates increase when larvae are infected with fast-growing zooxanthellae strains (growth rate based on algal cell doubling time, Fitt 1985) or with zooxanthellae extracted from fast-growing clams (based on clam shell length, Molea and Munro 1994).

This paper reports the results of symbiosis experiments on the slow-growing giant clam species, *Tridacna crocea,* infected with zooxanthellae from the fast-growing clam species *T. gigas*. This study aimed to investigate the effects of freshly isolated and cultured zooxanthellae from *T. gigas* and from *T. crocea* on the survival and growth of postmetamorphic *T. crocea* juveniles. The importance of this study lies in the fact that *T. crocea* is commercially important, its meat highly priced in the Asian market (Shang and others 1991), its shells commonly used in Philippine shellcraft (Juinio and others 1987), and live clams sought after in the international aquarium trade (Delbeek and Sprung 1994). Very small juveniles (less than 3 cm shell length) are quite mobile (Suzuki 1998), and in culture, they are easily lost from cages when transferred to the ocean nursery at such small sizes. This results in a lengthy period for rearing small juveniles in landbased raceways. It takes about two years to attain a size suitable for ocean rearing. Enhancing growth rates, therefore, would lessen the production cost of *T. crocea* and lower the amount of resources needed for rearing in the landbased nursery.

MATERIALS AND METHODS

At the outdoor culture facility of the Bolinao Marine Laboratory, nine *Tridacna crocea* brood stock were induced to spawn in the tank using serotonin on 7 April 1998. While all clams released sperm, only three proceeded to release eggs after 22 to 25 minutes. The eggs were collected and placed in a bin filled with UV-treated 0.2 μm-filtered seawater (UVFSW). Egg count reached a total of 666,000. The eggs had an average diameter of 86.64 μ. They were fertilized with sperm from other clams within two hours of collection. The fertilized eggs were transferred to 250-liter larval tanks filled with gently aerated UVFSW and subjected to routine seawater changes and microalgal feeding (*Isochrysis galbana* at 15,000 cells per ml).

On Day 8 of postfertilization (pf), a total of 55,000 pediveligers (pv) were harvested and transferred to replicate 60-liter bins lined with cement slabs for substrates. Pediveliger stocking density was 6,875 pv per bin (or 40 pv per 10 sq cm). They were infected with zooxanthellae at a density of 200 cells per ml. The following zooxanthellae treatments were used: *T. gigas* Freshly Isolated Zoox (=TgFIZ), *T. gigas* clone No. 10 (=Tg10), *T. crocea* Freshly Isolated Zooxanthellae (=TcFIZ) and *T. crocea* clone No. 4 (=Tc4). On Day 13, clams were reinfected with zooxanthellae accordingly. After two weeks, when the zooxanthellae had migrated from the clam's gut to its mantle, the clams in bins received flowing seawater serially filtered down to 1 μm.

The bins were randomly placed in a large tank serving as a water bath of running seawater to minimize large fluctuations of seawater temperature. For the duration of the experiment, they were periodically rotated clockwise weekly to eliminate differences in physical location (e.g., variations in shade) within the water bath. Seawater temperature of the water bath was recorded with an underwater Seamon recorder.

Three months after fertilization, the bins were harvested and the live clams counted. The stocking density was standardized to 600 juveniles per bin (or 3 juveniles per 10 cm^2). Survival of post-metamorphic juveniles was determined monthly by counting the number of live clams per bin. The shell lengths of 30 clams selected randomly were measured using a caliper, and their growth rate was determined. Data was collected for eight months.

RESULTS AND DISCUSSION

At first harvest after metamorphosis, mean percent survival was higher for clams infected with *T. gigas* zooxanthellae. TgFIZ and Tg10 had 51.4% and 55.7 % survival, respectively, while TcFIZ (37.1 %) and Tc4 (41.5 %) exhibited lower mean percent survival (Table 1). Thereafter, mean percent clam survival from 3 to 8 months old was plotted per treatment in Fig.1.

It may be noted that clams infected with cultured zooxanthellae Tg10 exhibited the best survival rate compared to the other treatments for up to eight months in the landbased nursery. However, there was a continuous decline in survival of all clams which may be attributed to temperature effects. Temperature data collected from the tank were tabulated showing monthly extremes of water temperature (Table 2).

The highest value of 35.0°C was obtained on 27 August 1998. In July, there were serious mortalities of giant clam stocks (brood stock and sub-adults) in the field in relation to high seawater temperature anomalies (Gomez and Mingoa-Licuanan 1998). A similar effect may have caused the mortalities in the experimental tank. These mortalities reflected in the steeper rate of decline in survival from June to August compared to the succeeding months.

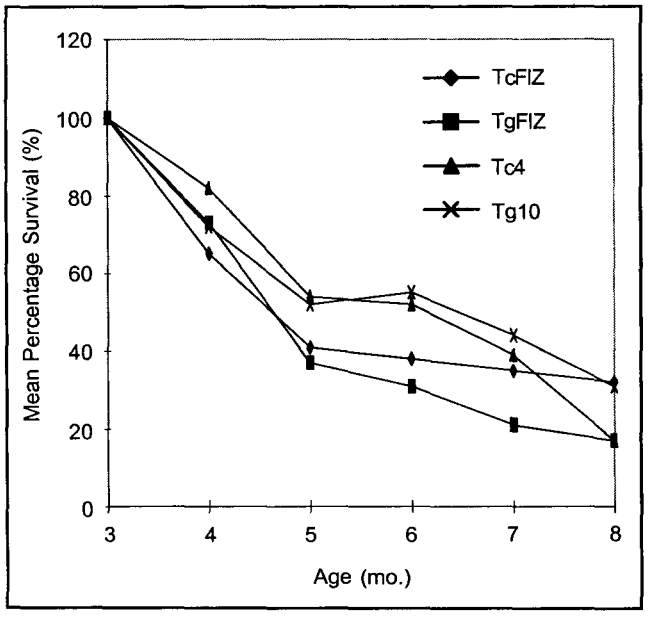

Fig. 1. Mean percentage survival of *Tridacna crocea* after 8 months in the hatchery

Although shell length mean values were highest (9.8 mm) for Tc4 clams, followed by Tg10 clams (9.3 mm), TgFIZ (9.1 mm), and TcFIZ (8.2 mm) (Table 3), ANOVA ($p<0.10$) on shell lengths showed no significant difference between treatments over a period of eight months. A study by Estacion et al. (1986) has shown that there may be a growth delay in *T. maxima*, which showed significant differences in shell length after 2-3 months. For the present experiment, while the 8-month observation period may, in general, be relatively long enough to detect any difference between treatments, perhaps the slow growth rate of *T. crocea* requires a

Table 1. Mean per cent survival of juvenile *Tridacna crocea* infected with zooxanthellae (clones and freshly isolated zooxanthellae, FIZ) from *T. crocea* and *T. gigas*, over a period of 8 months

Treatment	Jun*	Aug**	Sept	Oct	Nov	Dec
TgFIZ	51.4	73	37	31	21	17
Tg10	55.7	72	52	55	44	31
TcFIZ	37.1	65	37	31	21	32
Tc4	41.5	82	54	52	39	17

* with initial stocking density, S.D. at 40 pv/10 sq.cm.
** with initial S.D. at 3 juv/10 sq.cm.

Table 2. Monthly range of water temperature in the raceways from April to September

Month	Range (°C)
April	26.9 - 33.3
May	28.0 - 33.8
June	28.8 - 34.8
July	29.7 - 34.1
August	28.9 - 35.0
September	29.4 - 34.1

Table 3. Mean shell length measurements (mm) of *Tridacna crocea* over a period of 8 months

Treatment	Month						
	Jun	Jul	Aug	Sept	Oct	Nov	Dec
TgFIZ	2.6	3.7	4.7	5.5	7.1	8.5	9.1
Tg10	2.5	3.2	4.3	5.3	7.4	8.3	9.3
TcFIZ	2.9	3.9	4.5	5.7	7.2	8.2	8.2
Tc4	2.9	3.6	5.1	5.9	7.4	9.0	9.8

longer duration of study. *T. crocea* has a reported growth rate of 1.5 cm per year (Murakoshi 1986).

Apparently, zooxanthellae may affect the survival and growth of clams in different ways. Overall, TgFIZ and Tg10 showed improvement both for clam survival and clam growth. Although growth of Tc4 was highest, its survival was poorest. This study showed that the survival and growth of *T. crocea* in culture may be improved by the kind of zooxanthellae that was used for inoculating the larvae. In this case, zooxanthellae from the fastest-growing giant clam species *T. gigas* were used and compared to those from the slow-growing *T. crocea*. Good shell growth and survival were obtained with *T. gigas*-derived zooxanthellae. With Tc4, growth was best, but survival was poorest.

The evaluation of the effectiveness of specific strains or kinds of zooxanthellae need to consider the overall performance of the clam as a functional holobiont. Survival and growth are parameters that are easily monitored, but other phenotypic characteristics such as resistance to disease, to bleaching, etc. need to be evaluated as well.

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