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Final Report to the U.S. Agency for International Development

on Grant DPE-5542-G-SS-1035-00

The following represents the final technical report to the U.S. Agency for International Development on Grant DPE-5542-G-SS-1035-00 entitled "Spawning and Developmental Physiology of Tridacnid Bivalves (Mollusca: Bivalvia).

The major goals of this study were stated in the initial proposal as: .... "to gain a better understanding of the biology of Tridacna with particular emphasis placed on the following two areas; a) the early stages of development and the mode of 'infection' by the symbiotic algae and b) quantification of the nutritional contribution of the symbiotic algae to the nutrition of the animals." This report will indicate the extent to which those goals were achieved.

The field portion of the study was conducted in the Republic of Belau (Western Canolines) during the summers of 1982 and 1983. In addition to the Principal Investigator, the following were participants from UCSB: William K. Fitt, Charles R. Fisher, Frances P. Wilkerson. The P.I. acknowledges the co-operation of the Director and staff of the Micronesia Mariculture Demonstration Center on the island of Malaka. In particular we wish to mention the following Palauans: S. Derbai, E. Quiterong, U. Ikesakes, B. Madraisau, K. Matreklai, M. Ngiramengior and O. Orak. Mr. J. Heslinga and Dr. F. Perron were kind enough to allow experimental use of aquarium-reared specimens of Tridacna gigas, and we express gratitude for these, as such small specimens were not readily available on the reefs.

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We also wish to express our gratitude to North West Airlines, Continental Airlines and United Airlines for their co-operation and assistance in facilitating air transportation of rather large volumes of equipment back and forth across the Pacific.

1. The larval stages of *Tridacna*.

a. Spawning

During this study, the time we could spend in Palau was restricted to the summer months. Hence, in 1982 we were there from July through September, and in 1983 from May to July. Based on information available in the literature (e.g. see Fitt and Trench, 1981), *Hippopus hippopus* and *Tridacna gigas* are the two clams that naturally spawn at this time of year. However, J. Heslinga (personal communication) maintains that *T. gigas* spawns all year round, but to my knowledge, the viability of 'unseasonal' spawn is not known. Regardless of whether tridacnids are seasonal spawners or not, it is abundantly clear that the spawning of viable eggs and sperm will only occur, under either natural or artificial conditions, when the clams are mature and the gonads 'ripe'. If clams are ready to spawn, they do so under the influence of several stimuli, e.g. H_2O_2, elevated temperature or macerated gonads spread through the water (sea Beckvar, 1981; Fitt and Trench, 1981; Gwyther and Munro, 1981). Forcing the clams to spawn before gonadal maturity simply results in the release of immature eggs with very low survival potential. Based on our experiences, *H. hippopus* spawned, under the reproducible influence of gonadal macerates, in association with the new

moon. Under those conditions we always recovered viable eggs and sperm. Collecting eggs and sperm, and effecting fertilization was routinely performed as described for I. squamosa (Fitt and Trench, 1981).

Under ideal conditions for mariculture purposes, it would probably be desirable to control the maturation of gonads in Tridacna, but in practical terms, it is my considered opinion that a viable program can be launched without this level of control. Each spawn will release several million eggs which can potentially produce a vast number of juveniles which would have to be cared for over a period of time. Overproduction could easily cause any system to become overwhelmed.

2. The Trochophore and veliger stages.

In most mariculture attempts on tridacnids currently being conducted, the larval biology of the clams is treated as a 'black box'. Eggs and sperm are brought together, and the result is regarded as successful some few weeks later when the metamorphosed juveniles are recovered from the so-called 'massculture containers'. During this period most investigations concur that mortality is very high indeed, such that the recovered spat is about 1% of the fertilized eggs.

Because of this high mortality, we determined to assess the larval stages under more controlled conditions of laboratory culture. Questions asked were: what is the influence of density on survival? What is the influence of an exogenous supply of nutrients? How does either factor affect growth rates of larvae?

These questions were essentially answered during the first summer, and were reported at the Sea Grant Meetings in San Diego in December, 1982. (The proceedings of that meeting are still to appear in Mariculture). Briefly, we found that, as with other molluscan larvae, maintenance at low densities promoted better survival (optimal densities were 1-5 larvae/ml sea water). Since the trochophores do not feed, the larvae must rely entirely on nutrients deposited in the eggs. It is not surprising then, that eggs spawned before maturity do not fare well.

The veliger stages are capable of feeding. In controlled experiments, we provided veligers with particulate food in the form of a variety of phytoplankton species (Isochrysis, Platymonas and Phaeodactylum) and vitamins. We found that Isochrysis galbana (Tahitian strain) was the best particulate food, producing maximum survival and growth of veligers. Platymonas was barely adequate and Phaeodactylum was a poor food source. Despite some reports in the literature to the contrary, the larvae of tridacnids do not inherit the symbiotic algae harbored by their parents. Therefore, during the veliger stage of development, tridacnids are not unlike any other larval clam. They have a requirement for an exogenous supply of food. Vitamins and dissolved organics (amino acids, glucose) also enhance growth and survival of veligers which appear to be able to absorb organic compounds across the integument. However, caution should be excercised in using these as their addition to containers always results in the blooming of bacteria which lower the O_2 tension of the water and can kill the larvae. Most of these bacteria are gram-negative, and rather resistant to antibiotics.

Providing veligers with either a) symbiotic algae freshly isolated from adult animals or b) from stock cultures, did not appear to have a very significant effect on either growth or survival. The reason for this appears to be that the symbiotic algae are not digested by the veligers, and simply pass through the alimentary system. The freshly isolated algae may have some effect (though not statistically significant) by virtue of their release of photosynthate. Cultured symbionts do not release photosynthate. A symbiosis between the algae and the clams is only established morphologically and biochemically, with attendant nutrient transport, after metamorphosis from pediveligers to juvenile clams. The acquisition of symbiotic algae and the formation of a functional symbiosis is not a prerequisite for metamorphosis, as pediveligers not exposed to symbiotic algae do metamorphose. This is in contrast to at least one report in the literature.

Using a technique involving ^{14}C-labelled algal food, we estimated that 6 week old H. hippopus had a digestive efficiency of 93% with respect to Isochrysis and Platynonas, and less than 2% with respect to different strains of the symbiotic Symbiodinium microadriaticum. By contrast, analysis of gas exchange using the polarographic O_2 electrode illustrated that the photosynthetic contribution of the symbiotic algae could, under optimum conditions, theoretically satisfy all the respiratory carbon requirements of the juvenile clams. (See further information on photosynthesis below).

Adopting a system of careful control of the density of the larvae, providing adequate nutrition (as particulate or dissolved organic matter) and providing the pediveligers with algal symbionts at the most opportune period resulted in an increased survival. This increase represents a change from

some 99% mortality to about 40-50% mortality. Hence, I believe that with an appropriate nutritional program, the production and rearing of Tridacna is feasible. Several specimens reared entirely under laboratory conditions have been transported back to Santa Barbara and have been maintained here.

3. The contribution of the algal symbionts to clam maintenance.

This aspect of the study was conducted with two aims. First, an analysis of the photosynthetic contribution of the algae (S. microadriaticum) to the animals, and secondly, analysis of the photosynthetic biology of the algae themselves. This latter aspect included the sources and mechanisms of inorganic nutrient uptake, with the emphasis on nitrogen.

a. Photosynthetic contribution of algal symbionts to nutrition of juvenile and adult clams.

The method used in this study was respirometry. To measure gas exchange in juvenile clams, we had to construct very small respirometer chambers. These turned out to be more than adequate.

The data in O_2 exchange (photosynthetic production versus respiratory consumption) over 24 h periods indicated that juvenile clams, prior to the period when the mantle was maximally populated with algae were not independent of an exogenous food supply. The likely reason for this is that during the period of algal proliferation within the clam tissues, the

photosyntate produced is directed to algal growth, and translocation to the animals is low. In addition, the clams themselves undergo a period of active growth, thereby increasing their nutritional demands.

By contrast, when the algal population reaches an asympotote in the clam, and algal growth is retarded, translocation of algal photosynthate to animal tissues increases, and as a result, the animals become largely independent of exogenous nutrients.

We conducted a study intended to gain information on the photosynthesis-size relations in I. gigas. We found a relation described by the following equation:

$$\mu M\ O_2/\mu g\ Chl\ a/h = -0.195\ (L)^{-0.4} \quad \text{where } L = \text{shell length.}$$

Because of the phenomenon of self-shading by algae 'close-packed' in the mantle tissue, there is a reduction in the average amount of light that reaches the algal population. There is also a reduction of the number of algae/g clam as the size of clams increase.

Since the clams are dependent on light by virtue of the symbiotic algae, maintaining the clams in an optimum light regime during growth is of obvious importance. Thus, a small clam, less than 5 cm in length saturates photosynthetocally at light levels as low as $100 \mu\xi \cdot m^{-2}\ sec^{-1}$ while a clam of 20 cm shell length needs at least $1500 \mu\xi \cdot m^{-2}\ sec^{-1}$ to achieve photosynthetic saturation. Hence, maintaining young clams on an open reef environment after removal from a hatchery without appropriate concern for

levels of illumination does not provide them with the optimum growth conditions. This is definitely an aspect that should be considered when open water maintenance is anticipated for mariculture.

Another aspect of our study, relating more to basic aspects of photosynthetic biology of the symbiotic algae was concerned with the pathway and mechanism of CO_2 fixation. There has been debate in the literature (see Trench and Fisher, 1983) on whether these algae use the classical C_3 or a C_4 pathway. We have now demonstrated the following enzyme systems to be present and functional: pyruvate Pi dikinase; phosphoenolpyruvate carboxylase; NADP malate dehydrogenase and 'malic' enzyme (decarboxylating). These are the complete enzyme systems involved in the C_4 pathway.

Another problem has been whether these algae demonstrate photorespiration, a process whereby no net carbon is fixed in photosynthesis. Again we have demonstrated that all the enzyme systems involved in the photorespiratory pathway exists in these algae.

From the point of view of N-assimilation, the algae in Tridacna were also found to be unusual. To illustrate: S. microadriaticum in the sea anemome Aiptaisa pulchella use only NH_4^+ as a nitrogen source. When these algae are cultured on media containing an NO_3^-, the enzyme nitrate reductase is induced. By contrast, the algae in Tridacna utilize NH_4^+ and NO_3^- simultaneously, hence the implication that in A. pulchella NO_3^--reductase is an inducible enzyme system while in Tridacna the same enzyme is constituitive. This is a totally new and unexpected finding.

Students who have been assisted in their research by this grant, and their current dispositions are as follows:-

Dr. S.S. Chang completed the Ph.D. and is now a postdoctoral fellow at the Brookhaven National Labortories in Upton, N.Y. Dr. Chang studied the photoadaptive capabilities of the algae from I. maxima as well as the biochemistry of the light-harvesting apparatus, the peridinin-chlorophyll a-protein complexes.

S.S. Chang and R.K. Trench (1982) Peridinin-chlonophyll a-proteins from the symbiotic dinoflagellate Symbiodinium microadriaticum. Proc. R. Soc. Lond. (B). 215: 191-210.

S.S. Chang, B.B. Prezelin and R.K. Trench (1983) Mechanisms of photoadaption in three strains of the symbiotic dinoflagellate Symbiodinium microadriaticum. Mar. Biol. 76: 219-231.

S.S. Chang and R.K. Trench (1984) The isoelectric forms, quaternary structure and amino acid composition of peridinin-chlorophyll a-proteins from the dinotlagellate Symbiodinium microadriaticum. Proc. R. Soc. Lond. (B) [In Press].

Dr. William K. Fitt completed the Ph.D and is currently a postdoctoral fellow at Ruhr University, Bochum, West Germany. His papers, published and in preparation, include:

W.K. Fitt and R.L. Trench (1981) Spawning, development and aquisition of zooxanthellae by Tridacna squamosa (Mollusca: Bivalvia). Biol. Bull. 161: 213-235.

W.K. Fitt and R.K. Trench (1983) The relation of diel patterns of cell division to diel patterns of motility in the symbiotic dinoflagellate Symbiodinium microadriaticum. New Phytol. 94: 421-432.

W.K. Fitt and R.K. Trench (1983) Infection of invertebrate hosts with the symbiotic dinoflagellate Symbiodiniam microadriaticum. Pages 659-681 in H.E.A. Schenk and W. Schwemmler, (eds). Endocytobiology. Walter de Gruyter Co. Berlin.

W.K. Fitt and R.K. Trench (1983) Endocytosis of the symbiotic dinoflagellate Symbiodincum microadriaticum by endodermal cells of the scyphistomae of Cassiopeia xamachana and resistance of the algae to host digestion. J. Cell Sci. 64: 195-212.

W.K. Fitt, C.R. Fisher and R.K. Trench (1984) The larval biology of tridecnid clams. Aquaculture.

Charles R. Fisher will file his dissertation in July, 1984. He has been studying the photosynthetic relations between Tridacna and the symbiotic algae. Publications with Fisher include:

R.K. Trench and C.R. Fisher (1983) Carbon dioxide fixation in *Symbiodinium microadriaticum*: Problems with mechanisms and pathways. Pages 659-674 in H.E.A. Schenk and W. Schwemmler (eds), *Endocytobiology*. Walter de Gruyter & Co. Berlin.

C.R. Fisher and R.K. Trench (1984) Photosynthesis and respiration in *Tridacna gigas* as a function of irradiance and size. Biol. Bull. (In Press).

Reprints of all these papers will be forwarded to the US-AID and to the MMDC as soon as they become available.

Finally, on behalf of my graduate students and myself, I would like to thank the Officers of the US-AID for their co-operation and patience in the administration of this grant.

Respectfully submitted,

23 May, 1984

Robert R. Trench

Professor of Biology