

## Nutrient Limitation in the Symbiotic Association between Zooxanthellae and Reef-building Corals: The Experimental Design<sup>1</sup>

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**ABSTRACT:** The question of nutrient limitation and of its regulatory effect on population densities of zooxanthellae in hospice was studied by an international team of researchers during an intensive 5-day workshop. Participants studied colonies of two coral species that were preincubated over different time periods ranging from 0 to 8 weeks under four different nutrient concentrations. A broad spectrum of parameters was measured simultaneously at the molecular, cellular, and colony levels of organization using a variety of techniques. This paper describes the overall experimental design.

THE WORKSHOP “Nutrient Limitation in the Symbiotic Association between Zooxanthellae and Reef-building Corals” was organized in response to scientific needs described in the preceding paper (Jokiel et al. 1994). The experimental approach chosen was to provide researchers with preincubated corals grown under different nutrient regimes for various lengths of time. These corals would be analyzed simultaneously by several teams using different techniques. All important parameters would be measured on the same corals. The result would be an extensive data set that might provide new insights into the dynamics of nutrient metabolism and resolve apparent conflicts between previous studies. Nitrogen was selected as the nutrient species to be investigated. Previous studies showed that the response of reef corals to nitrogen enrichment is more pronounced than that to phosphorus enrichment (Stambler et al. 1991). Further, it was important to keep the experimental design simple. Inorganic nitrogen was supplied in the form of ammonium, which has been shown to be rapidly taken up by reef corals (Kawaguti 1953, Muscatine and

D’Elia 1978, Burris 1983, Muscatine et al. 1984).

### MATERIALS AND METHODS

Two common species of Hawaiian reef corals were selected for this study. The highly branched imperforate species *Pocillopora damicornis* (Linnaeus) has been widely used for physiological studies throughout the Indo-Pacific (e.g., Richmond 1985). The perforate coral *Montipora verrucosa* Vaughan was selected as a second species for comparison. Corals were preincubated over different periods ranging from 0 to 8 weeks, under four different nutrient concentrations.

Colonies of *P. damicornis* about 10 cm in diameter and colonies of *M. verrucosa* were collected from Kaneohe Bay (Oahu, Hawaii). All of the colonies were initially collected from the same reef over a period of a few days and maintained thereafter in holding tanks under the same conditions as the control (ambient) treatment. At various time intervals, groups of corals were moved from the holding tanks into the experimental treatments.

Experiments were carried out in eight white fiberglass tanks with a water volume of ca. 400 liters (1.15 by 1.15 by 0.27 m). Each tank was supplied with unfiltered running seawater, at a rate of 4 liters min<sup>-1</sup>. All tanks were aerated. Tanks were located in sunlight and covered with neutral-density shade cloth

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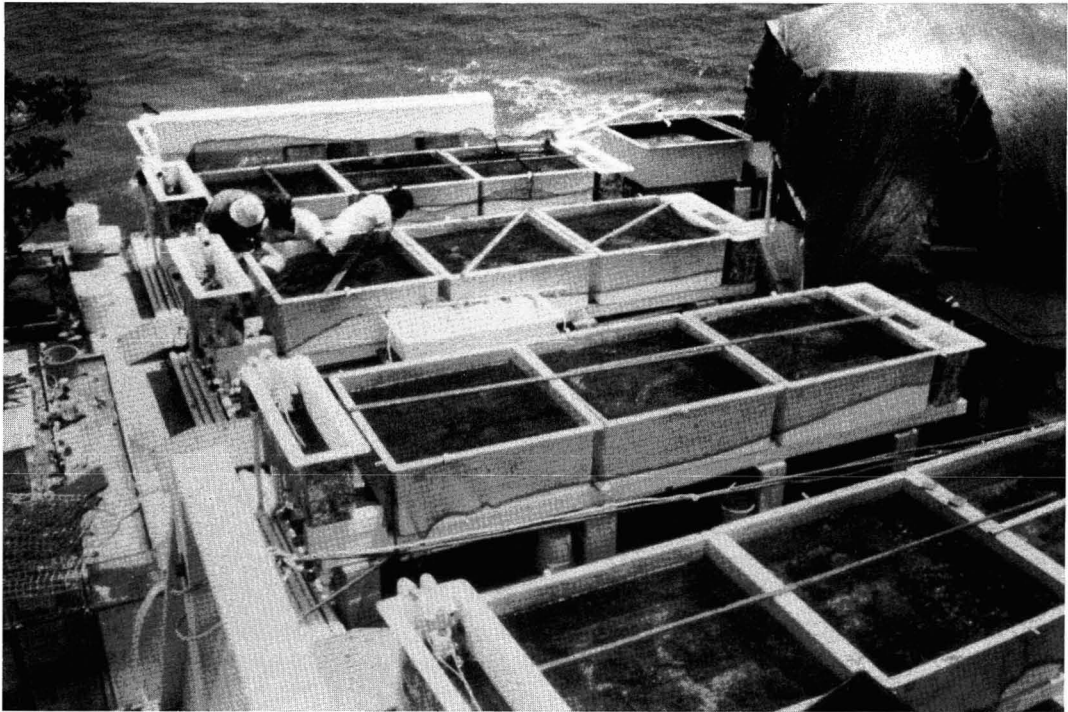


FIGURE 1. View of the experimental tank facility.

so as to expose the corals to 80% of full solar radiation (Figure 1).

Six to eight colonies of *P. damicornis* from the holding tanks were transferred to the experimental tanks every 2 weeks. Thus, colonies preincubated for 0, 2, 4, 6, and 8 weeks were available to the investigators for simultaneous analysis during the 5-day workshop.

Ammonium  $[(\text{NH}_4)_2\text{SO}_4]$  solution was pumped into the intake flow of the tanks with a peristaltic pump at a rate sufficient to raise the nutrient level to either  $20 \mu\text{M}$  or  $50 \mu\text{M}$ . Water entering the "ambient" control tanks was not altered, so values in those tanks remained the same as nutrient concentrations on reefs in Kaneohe Bay ( $<1 \mu\text{M}$ ). Water supplied to the "nutrient stripped" treatment was first passed through a flume (4 m long, 40 cm wide, 40 cm deep) filled with the macroalga *Gracillaria salicornia* (C. Agardh) Dawson (Figure 2). Water leaving the flume had undetectable ammonium concentration. Water within the "stripped" tanks, how-

ever, showed ammonium concentrations approaching that found in the ambient tanks. Possible sources of this nitrogen include nitrogen excreted and lost from the experimental corals added to the "stripped tanks" and nitrogen fixed by algae growing within the tanks.

From an ecological point of view, levels of nitrogen in the  $20\text{-}\mu\text{M}$  and the  $50\text{-}\mu\text{M}$  treatments are clearly above values encountered in nature. However, to investigate the dynamics of the symbiosis, it is useful to load the symbiotic relationship above the normal limits and observe the outcome.

In the course of the workshop the following parameters were determined: (1) coral growth rate; (2) density of the zooxanthellae within their host; (3) photosynthesis rate of the zooxanthellae, in hospice and after isolation; (4) dark respiration rates of the intact colony and of freshly isolated zooxanthellae; (5) division rate of the zooxanthellae; (6) levels and activity of the key enzymes involved

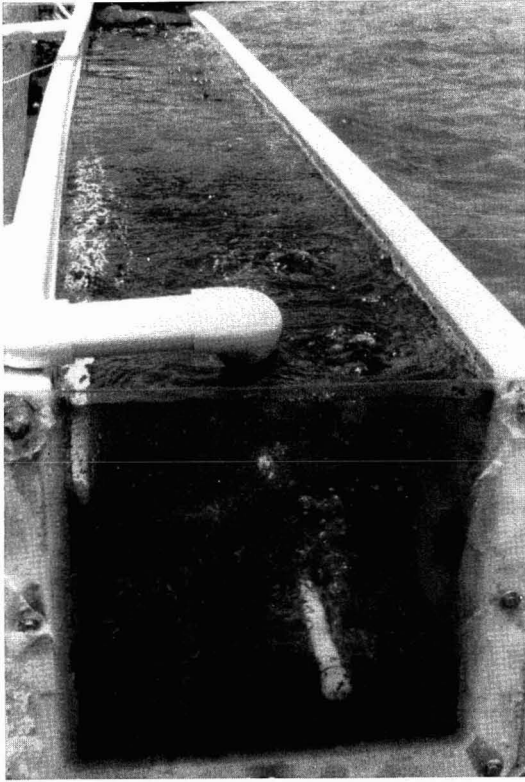


FIGURE 2. Flume containing macroalgae used to remove nutrients from the "stripped" treatment.

in the uptake and assimilation of nitrate and ammonium by the zooxanthellae; (7) levels and activity of carbonic anhydrase, postulated to facilitate  $\text{CO}_2$  uptake under limiting conditions; (8) the chemical composition of both the zooxanthellae and animal fractions of the symbiosis; (9) release rate of zooxanthellae; (10) ultrastructure of zooxanthellae.

#### RESULTS AND DISCUSSION

During preliminary experiments, concentrations of  $100 \mu\text{M}$  ammonium were shown to be toxic to the two species of Hawaiian corals, although this concentration was previously used successfully with the coral *Stylophora pistillata* Esper (Muscatine et al. 1989). Treatment levels of  $50 \mu\text{M}$  ammonium,  $20 \mu\text{M}$  ammonium, ambient ( $<1 \mu\text{M}$

ammonium), and "stripped" ( $\ll 1 \mu\text{M}$ ) treatments were selected for the experiment. The colonies in all four treatments remained alive throughout the course of the experiment. Colonies grown in the highest ammonium concentration appeared to be stressed. Often the polyps were contracted, and many of the colonies lost tissues from the lower portion of the branches.

The most apparent effect of the ammonium concentration on the colonies was the color change. Colonies at the high ammonium concentration were darker than the control colonies. The colonies in the stripped treatment were lighter in color than the control corals. The color changes developed gradually during the first 2 to 3 weeks of incubation. After that time the color remained unchanged.

The workshop participants succeeded in applying the various measurement techniques to the same experimental corals in a coordinated and systematic manner. The critical factor in the success of this venture was the sequential flow of the same sample from one "work station" to the following one (Figure 3). We began with the nondestructive procedures, proceeded through the cellular procedures, and concluded with biochemical studies and analyses of preserved samples. These, in turn, ultimately led to data reduction and analysis. This approach also allowed each participant to observe and participate in an unusually diverse array of methods. Ongoing discussions centered on the merits and pitfalls of the various methodologies and research philosophies. The application of all these techniques to the very same samples eliminated possible differences in results and conclusions stemming from differences among different species, regions, and seasons. Results of research conducted by the various research teams of this workshop are presented in papers contained in this volume. Other papers are still in preparation, so additional results are forthcoming. Collaboration and follow-up discussions were an important result of this workshop. For example, a session involving the participants of the workshop was held at the Seventh International Coral Reef Symposium in Guam in 1992.

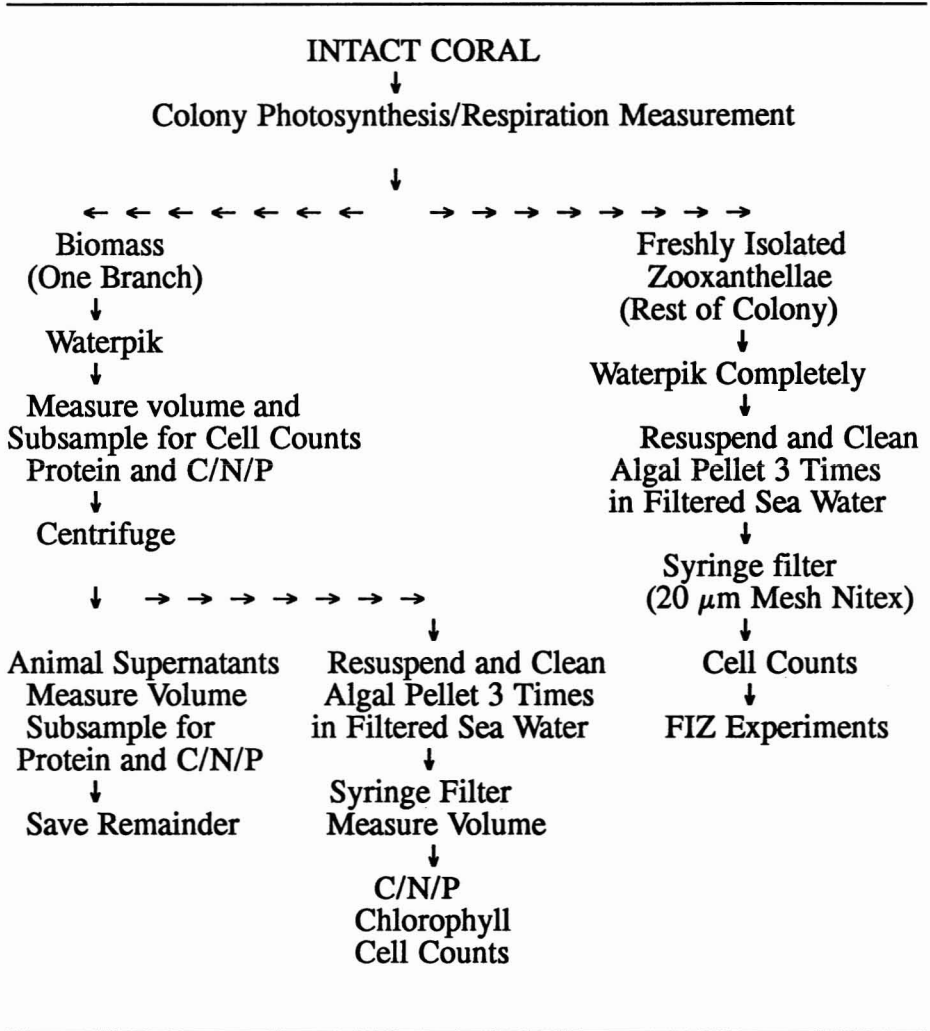


FIGURE 3. Procedures and routing of samples during final analysis of the corals.

Further, other workshops based on this successful approach are now in the planning stage.

#### LITERATURE CITED

- BURRIS, R. H. 1983. Uptake and assimilation of  $^{15}\text{NH}_4$  by a variety of corals. *Mar. Biol. (Berl.)* 75:151-155.
- JOKIEL, P. L., Z. DUBINSKY, and N. STAMBLER. 1994. Results of the 1991 United States-Israel Workshop, "Nutrient Limitation in the Symbiotic Association between Zooxanthellae and Reef-building Corals." *Pac. Sci.* 48:215-218.
- KAWAGUTI, S. 1953. Ammonium metabolism of the reef corals. *Biol. J. Okayama Univ.* 1:171-176.
- MUSCATINE, L., and C. F. D'ELIA. 1978. The uptake, retention and release of ammonium by reef corals. *Limnol. Oceanogr.* 23:725-734.
- MUSCATINE, L., P. G. FALKOWSKI, Z. DUBINSKY, P. A. COOK, and L. McCLOSKEY.

1989. The effect of external nutrient resources on the population dynamics of zooxanthellae in a reef coral. Proc. R. Soc. Lond. B Biol. Sci. 236:311–324.
- MUSCATINE, L., P. G. FALKOWSKI, J. W. PORTER, and Z. DUBINSKY. 1984. Fate of photosynthetically fixed carbon in light and shade adapted colonies of the symbiotic coral *Stylophora pistillata*. Proc. R. Soc. Lond. B Biol. Sci. 222:181–202.
- RICHMOND, R. H. 1985. Variations in the population biology of *Pocillopora damicornis* across the Pacific. Proc. 5th Int. Coral Reef Symp. 6:101–106.
- STAMBLER, N., N. POPPER, Z. DUBINSKY, and J. STIMSON. 1991. Effect of nutrient enrichment and water motion on the coral *Pocillopora damicornis*. Pac. Sci. 45:299–307.