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Effect of Ammonium Enrichment on Animal and Algal Biomass of the Coral *Pocillopora damicornis*[1]

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ABSTRACT: Algal and animal biomass parameters of colonies of the Pacific coral *Pocillopora damicornis* (Linnaeus) were measured as a function of time of exposure to elevated concentrations of seawater ammonium (20 and 50 μM [$(NH_4)_2SO_4$]) ranging from 2 to 8 weeks. Areal concentrations of zooxanthellae, chlorophyll, and protein increased with 20 μM ammonium addition. During the 8-week period of exposure to 20 μM ammonium, the population density of zooxanthellae increased from 3.5 to 7.5 $\times$ 10^5 cells cm^{-2}, chlorophyll *a* content of zooxanthellae increased from 5.7 to 8.6 pg, and animal protein concentration doubled (from 0.74 to 1.38 mg cm^{-2}). These data indicate that both the coral animal and the zooxanthellae respond to the addition of exogenous dissolved inorganic nitrogen provided as 20 μM ammonium. Growth of the symbiotic association in response to the addition of 20 μM ammonium adds further evidence to support the argument that growth of tropical symbioses is limited by the availability of nitrogen. However, the coral response is likely to depend on the concentration of ammonium provided, because the biomass parameters of corals held at 50 μM ammonium did not change significantly with time of exposure to the added nutrient.

SYMBIOTIC DINOFLAGELLATES (zooxanthellae) are found at high population densities in reef-building corals and other cnidarians living in the low nutrient concentrations characteristic of tropical seawater (Muscatine 1980). The abundance of these algal symbionts in their hosts, despite the external oligotrophic conditions, is attributed to conservation of nutrients. Nutrients acquired from ambient seawater and from metabolites resulting from host digestion are conserved via exchanges between the animal and zooxanthellae (Muscatine and Porter 1977, Rahav et al. 1989, Szmant et al. 1990). In spite of the inferred advantage of an abundant supply of nutrients for zooxanthellae in animal hosts, the actual nutrient status of the zooxanthellae is unknown. One approach to resolving the question of whether or not zooxanthellae are limited by the supply of nutrients is to experimentally manipulate the two sources of nutrients and to observe the response of the symbiotic partners. Nutrients are withheld from the symbiosis by maintaining the host in low-nutrient seawater for long periods of time without feeding (e.g., Cook et al. 1988). Nutrients are added to the symbiosis by intensive feeding or maintenance of the host in seawater enriched with dissolved inorganic nutrients. A positive growth response of the zooxanthellae to an added nutrient supports the hypothesis that growth of zooxanthellae may be limited by the supply of that nutrient under ambient seawater conditions (Cook

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and D'Elia 1987; but see Høegh-Guldberg [1994]).

Several studies have demonstrated that zooxanthellae population densities in corals increase with the addition of dissolved inorganic nitrogen supplied as ammonium to seawater flowing over the corals. Muscatine et al. (1989) found that 20 μM ammonium caused zooxanthellae population densities in *Stylophora pistillata* Esper to double after 2 weeks of exposure, but phosphate and particulate food (brine shrimp) additions had no effect on algal populations. Similar results were obtained with ammonium in *S. pistillata* and *Seriatopora hystrix* Dana in Australia by Høegh-Guldberg and Smith (1989) and in the coral *Pocillopora damicornis* (Linnaeus) in Hawaii (Stambler et al. 1991, Stimson and Kinzie 1991). Natural additions of nutrients, from excretion and defecation by haemulid fishes over coral colonies, also resulted in increased numbers of zooxanthellae within the tissues of Jamaican corals (Meyer and Schultz 1985).

Although an increase in zooxanthellae population density with the addition of ammonium has been well documented for *S. pistillata* and *P. damicornis*, the time course of the response and the long-term effects of sustained high inorganic nitrogen levels on the coral are unknown. This study describes the biomass parameters (animal and zooxanthellae) of *P. damicornis* exposed to 20 and 50 μM ammonium for periods ranging from 2 to 8 weeks. Corals maintained in ambient seawater were used as a basis for comparison (see Stambler et al. 1994). We measured the areal concentrations of zooxanthellae, chlorophyll, and animal protein as a function of length of exposure to ammonium-enriched seawater. Companion papers describe the changes in the elemental (C, N, and P) composition of *P. damicornis* under the various treatments (Muller-Parker et al. 1994) and in amino acid levels of the zooxanthellae (McAuley 1994).

MATERIALS AND METHODS

The collection and maintenance of *Pocillopora damicornis* under the different ammonium and seawater treatments are described by Stambler et al. (1994). Colonies from the various treatments were processed immediately for animal and zooxanthellae biomass parameters, and for the isolation of zooxanthellae for use by other investigators (see other papers in this issue). The number of colonies from each treatment that were processed ranged from eight for the ambient seawater controls to two for all ammonium treatments except the 6-week 50-μM ammonium treatment and the field sample, where only one colony of each was processed. The field colony was collected from the windward reef flat of Coconut Island, Kaneohe Bay, on 27 August 1991 and processed that same day.

Preparation of Animal and Zooxanthellae Fractions

Each coral colony was processed as shown in Figure 1. One branch of each colony was used for determination of biomass parameters; the rest of the colony was used to obtain zooxanthellae. Tissue was removed from coral skeletons using filtered (0.45-μm) seawater (FSW) and a Water Pik (Johannes and Wiebe 1970). The volume of the final homogenate solution was measured and sampled for hemacytometer counts of zooxanthellae. Homogenate samples were also frozen for biochemical and elemental analyses. The remaining homogenate was then separated into supernatant (soluble, or "animal fraction") and pellet (zooxanthellae) fractions by centrifugation at ca. 7000 rpm for 3–4 min. Zooxanthellae pellets were rinsed several times with FSW to remove any remaining animal tissue. The supernatants of pellet rinses were combined with the animal fraction. To remove animal particulate debris and skeletal $CaCO_3$ fragments, the zooxanthellae were then resuspended in FSW and passed sequentially through 73-μm and 20-μm Nitex screens held in a syringe filter apparatus (Gelman). Zooxanthellae numbers in the final suspensions were determined by hemacytometer counts. Samples were prepared from animal and zooxanthellae fractions and kept frozen until analyzed. The volume of each fraction, and that of the homogenate,

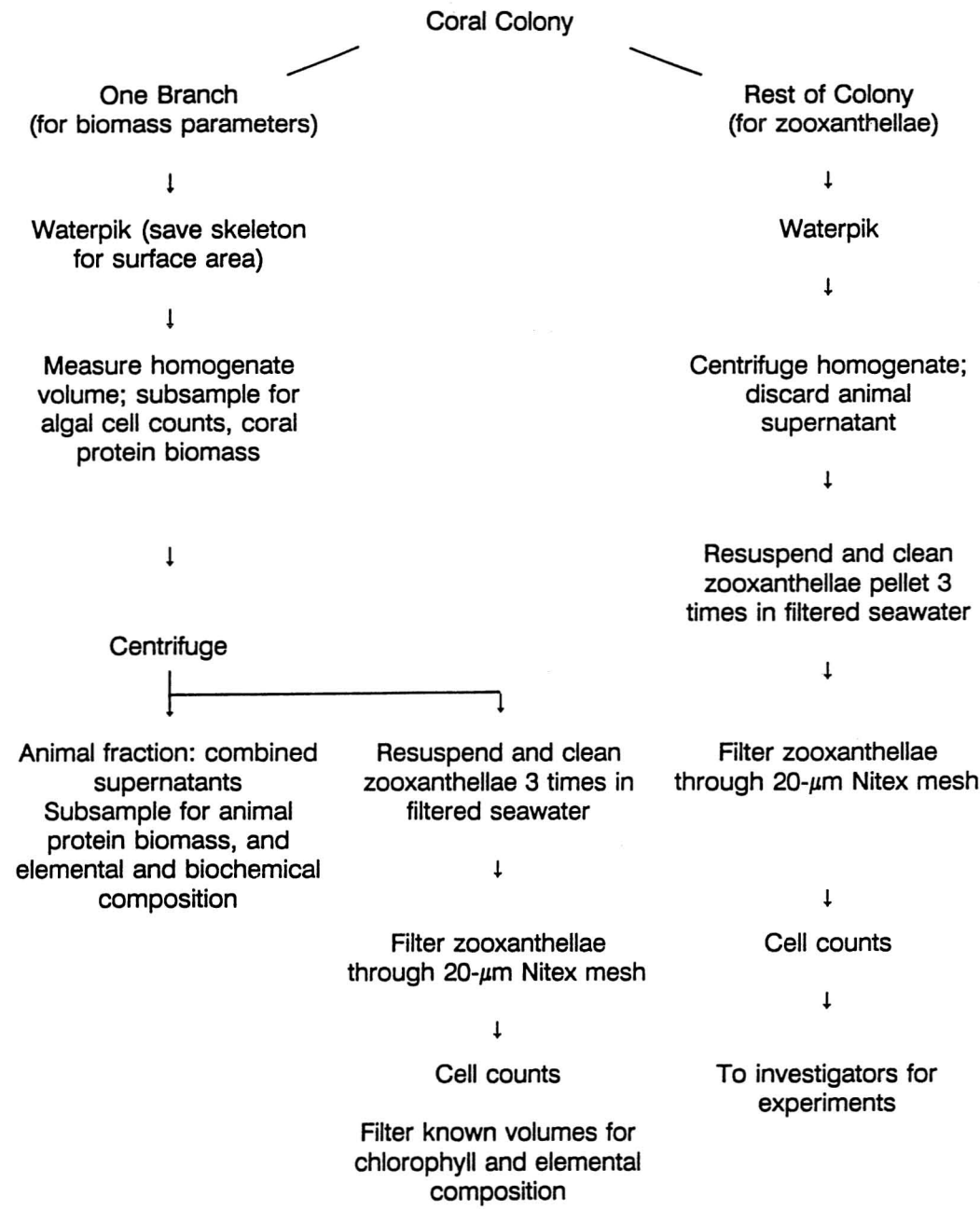

FIGURE 1. Summary of analytical procedures used to obtain animal and zooxanthellae biomass parameters of *Pocillopora damicornis*.

was related to the surface area of individual colonies.

Protein, Chlorophyll, and Coral Surface Area

Protein content of the homogenate and the animal fraction of each coral colony was determined by the method of Lowry (Lowry et al. 1951), using bovine serum albumin (BSA) as a standard. Samples were solubilized in 0.05 N NaOH at 40°C for 0.5 hr before proceeding with the protein analysis. After color development, samples were centrifuged to remove precipitate formed by the interaction of reagents with seawater, and the absorbance of the samples was read at 660 nm in a spectrophotometer (P-E Lambda).

For chlorophyll determinations, known numbers of zooxanthellae were filtered under vacuum (<250 mm Hg) onto 25-mm GF/C filters and stored frozen until analysis within 1 week of sampling. Filters were ground in ice-cold 100% acetone using a motorized tissue grinder. Chlorophyll was extracted for 18 hr at 4°C. The absorbance of acetone extracts was measured at 630, 663, and 750 nm on a diode-array spectrophotometer (Hewlett-Packard) after centrifugation to pellet filter material. The absorbance at 750 nm was used to correct for any turbidity at 630 and 663 nm. Chlorophylls *a* and c_2 of zooxanthellae were determined using the spectrophotometric equations of Jeffrey and Humphrey (1975).

The surface area corresponding to the amount of tissue removed from each coral specimen was obtained with a leaf area measuring device (Li-Cor Model 3100). Areas were calibrated by using branches of corals whose surface area was determined by the aluminum foil method (Marsh 1970). The branches of the coral skeletons were broken into lengths of relatively straight segments small enough to pass through the conveyor belt of the surface area meter. Multiple trial runs on the same collection of fragments from one coral produced results that varied <4%.

For statistical purposes, corals maintained in ambient flowing seawater for 8 weeks were considered "controls" and were presumed to represent corals at the start of the ammonium experiment. The effects of ammonium addition on the biomass parameters of corals maintained under the two ammonium enrichments were examined by linear regression over time, using the correlation coefficient (r) to indicate significance of treatments.

RESULTS

There was a significant increase in the areal population density of zooxanthellae in colonies of *P. damicornis* exposed to 20 μM ammonium ($P < 0.01$; Figure 2*A*). The number of zooxanthellae per square centimeter doubled over the 8-week period; zooxanthellae increased from 3.5 to 6×10^5 cells cm^{-2} within the first 2 weeks of ammonium enrichment (Figure 2*A*). Zooxanthellae densities in *P. damicornis* maintained in "N-stripped" seawater averaged 4.5×10^5 cells cm^{-2} and were not significantly different from those of the ambient seawater control corals. Although the increased zooxanthellae density of ammonium-enriched corals relative to seawater controls is apparent, the zooxanthellae density of a freshly collected colony from the reef was close to that of the N-enriched corals (6.79×10^5 cells cm^{-2}).

Zooxanthellae density expressed on the basis of animal protein biomass did not increase with time of exposure to 20 μM ammonium (Figure 2*B*). Although increases in zooxanthellae numbers were observed for corals maintained at 50 μM ammonium at the 2-week time point, there was no significant effect of time on population density of zooxanthellae for the 50 μM ammonium corals (Figure 2*A*,*B*).

The areal animal protein content of the corals increased significantly ($P = 0.05$) with time of exposure to 20 μM ammonium (Figure 2*C*). Protein content of these corals almost doubled over the 8-week period, paralleling the increase in zooxanthellae numbers in these corals (Figure 2*A*) and accounting for the lack of change in zooxanthellae density normalized to animal protein (Figure 2*B*). The animal protein content of corals exposed to 20 μM ammonium was 0.96 mg

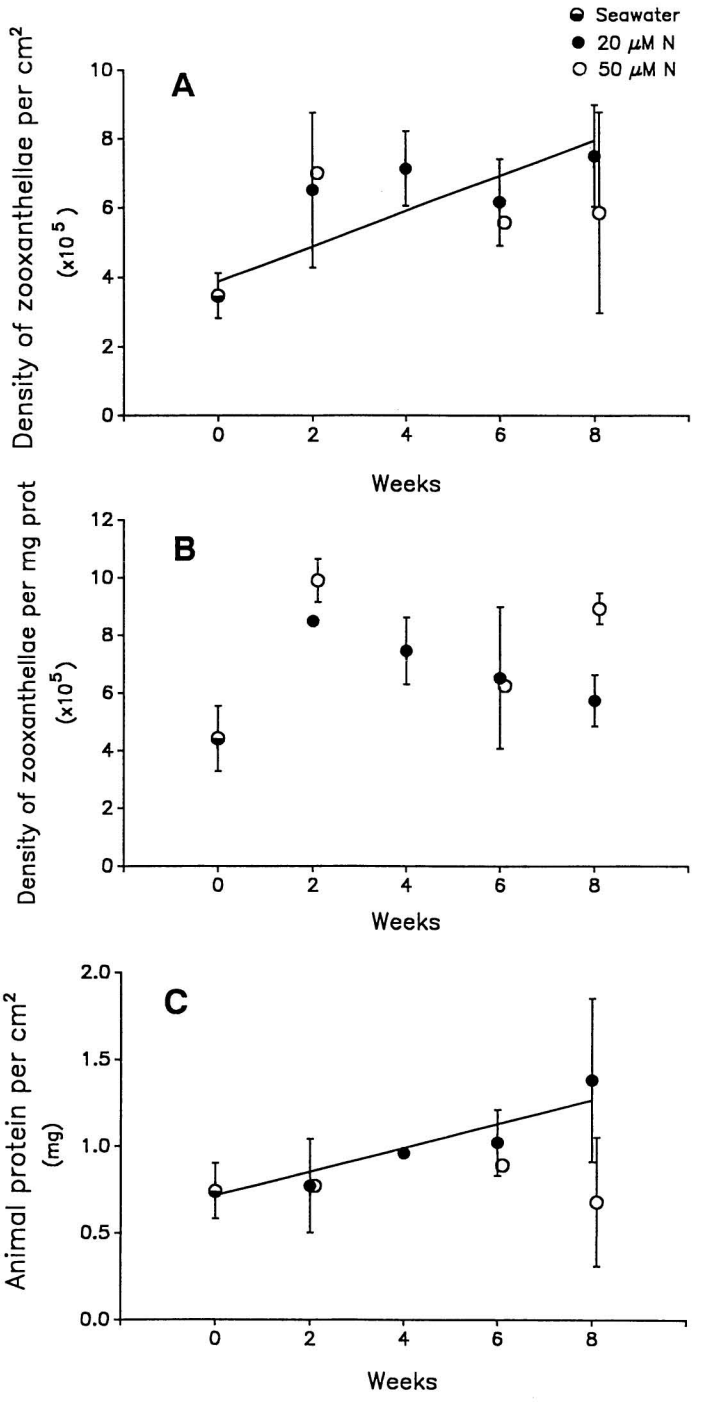

FIGURE 2. Density of zooxanthellae and animal protein in different colonies of *Pocillopora damicornis* as a function of time of exposure to 20 μM and 50 μM ammonium additions in seawater. Ambient seawater control colonies maintained under the same light and water flow conditions were used for the zero time point. Data are expressed as numbers of zooxanthellae normalized to coral surface area (*A*) and to protein biomass (*B*). Animal protein biomass per unit surface area is included in *C*. Error bars are ± 1 SE. The x axis for the 50 μM ammonium data is shifted slightly to the right for clarity. Regression lines are provided for significant effects observed with 20 μM ammonium (*A*: $r = 0.613$, $P < 0.01$; *C*: $r = 0.511$, $P = 0.05$).

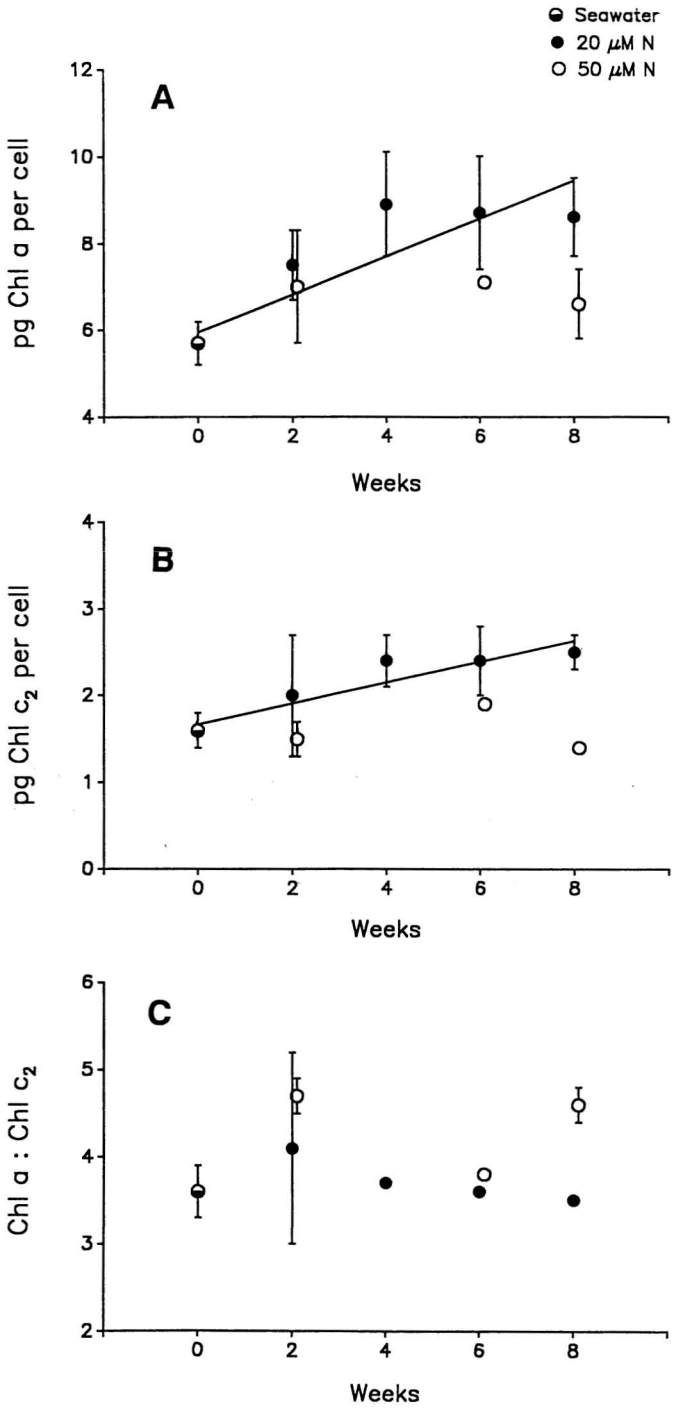

FIGURE 3. Chlorophyll of zooxanthellae isolated from different colonies of *Pocillopora damicornis* as a function of time of exposure to 20 μM and 50 μM ammonium additions in seawater: chlorophyll *a* cell^{-1} (*A*), chlorophyll c_2 cell^{-1} (*B*), and the ratio of chlorophyll *a*: c_2 (*C*). Ambient seawater control colonies maintained under the same light and water flow conditions were used for the zero time point. Error bars are ± 1 SE. The x axis for the 50-μM ammonium data is shifted slightly to the right for clarity. Regression lines are provided for significant effects observed with 20 μM ammonium (*A*: $r = 0.663$, $P < 0.01$; *B*: $r = 0.572$, $P < 0.05$).

cm^{-2} after 4 weeks and increased to 1.38 mg cm^{-2} by week 8; the animal protein content of corals exposed to 50 μM ammonium declined from 1.8 at 2 weeks to 0.68 mg cm^{-2} at 8 weeks (Figure 2*C*).

The relationship between density of zooxanthellae expressed on the basis of surface area and animal protein content was explored further by regressing these two parameters for the pooled coral colonies, including colonies maintained in ambient seawater, 20 μM ammonium, 50 μM ammonium, "N-stripped" seawater, and a field colony. The correlation between these two measures of algal density was significant ($r = 0.391$; $P = 0.048$), showing that there is generally good agreement between the two measures of algal population density.

The increase in numbers of zooxanthellae with 20 μM ammonium enrichment was accompanied by a significant increase in chlorophyll *a* and chlorophyll c_2 of zooxanthellae (Figure 3*A*,*B*). The chlorophyll *a* of zooxanthellae from corals maintained at 50 μM ammonium was consistently lower than that of zooxanthellae from the corals maintained at 20 μM ammonium (Figure 3*A*,*B*) and was not significantly different from that of the zooxanthellae in the seawater controls (Figure 3*A*). The chlorophyll *a* : c_2 ratio remained unchanged with exposure to 20 and 50 μM ammonium (Figure 3*C*) and averaged 3.7 for the corals maintained at 20 μM ammonium. The chlorophyll content of zooxanthellae isolated from the field colony (6.0 pg chlorophyll *a*, 1.6 pg chlorophyll c_2, 3.8 chlorophyll *a* : chlorophyll c_2) was identical to the mean values obtained for zooxanthellae from the corals in ambient seawater.

The combined effect of increased chlorophyll per cell and numbers of zooxanthellae on changes in the areal chlorophyll content of 20 μM ammonium-enriched corals is shown in Figure 4. The average amount of chlorophyll ($a + c_2$) per square centimeter was three times higher at 8 weeks (Figure 4*C*). There was no consistent increase in the areal distribution of chlorophyll with length of exposure to 50 μM ammonium (Figure 4). Because chlorophyll *a* per cell showed a significant positive correlation with algal density ($P = 0.038$; Figure 5), it was not possible to separate the effect of ammonium addition from self-shading due to increased zooxanthellae density.

DISCUSSION

The increase in the density of zooxanthellae in *P. damicornis* with ammonium enrichment was evident within the first 2 weeks after the initial addition of ammonium. The first few days after the start of ammonium addition seem not to have received adequate attention in coral studies; they may reveal significant information about nutrient-induced zooxanthellae population dynamics. Most studies have determined the effect of ammonium on the population density of zooxanthellae in corals after periods ranging from 13 days (Stambler et al. 1991) and 14 days (Muscatine et al. 1989) to 19 days (Høegh-Guldberg and Smith 1989). All of those studies reported increased densities of zooxanthellae with ammonium addition during periods that coincide with our first measurement of increased algal density in *P. damicornis* (Figure 2*A*).

This is the first study to examine the effect of time on the response of a coral to sustained elevated ammonium. Although the surface area–based density of zooxanthellae in corals maintained in 20 μM ammonium increased with time, it is important to distinguish between long-term responses (months to years) and short-term responses (days to weeks) to addition of ammonium. *P. damicornis* exposed to 17 μM ammonium for 2–4 months had algal densities in the branch tips that were three times those of branch tips in controls (Stimson and Kinzie 1991). Because that study was conducted during the winter season and algal densities are provided for tips (not whole colonies) and for one time point only, it is not possible to compare algal densities directly and infer that the differences in densities are related to the duration of exposure to high concentrations of ammonium. Future studies should concentrate on changes in biomass of corals during the first few days of exposure to nutrients, as well as

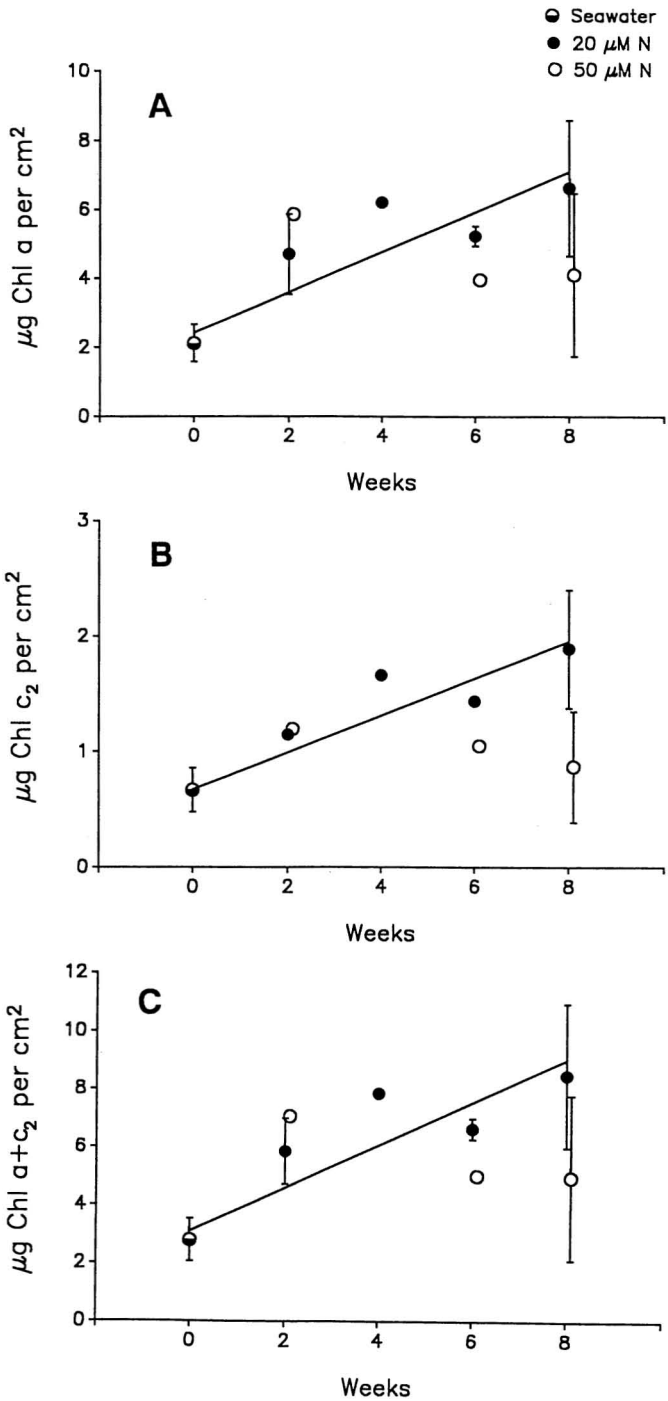

FIGURE 4. Areal chlorophyll content of *Pocillopora damicornis* colonies as a function of time of exposure to 20 μM and 50 μM ammonium additions in seawater: chlorophyll a/cm^2 (*A*), chlorophyll c_2/cm^2 (*B*), and total chlorophyll (a and c_2)/cm^2 (*C*). Ambient seawater control colonies maintained under the same light and water flow conditions were used for the zero time point. Error bars are ± 1 SE. The x axis for the 50-μM ammonium data is shifted slightly to the right for clarity. Regression lines are provided for significant effects observed with 20 μM ammonium (*A*: $r = 0.756$, $P < 0.001$; *B*: $r = 0.762$, $P < 0.001$; *C*: $r = 0.761$, $P < 0.001$).

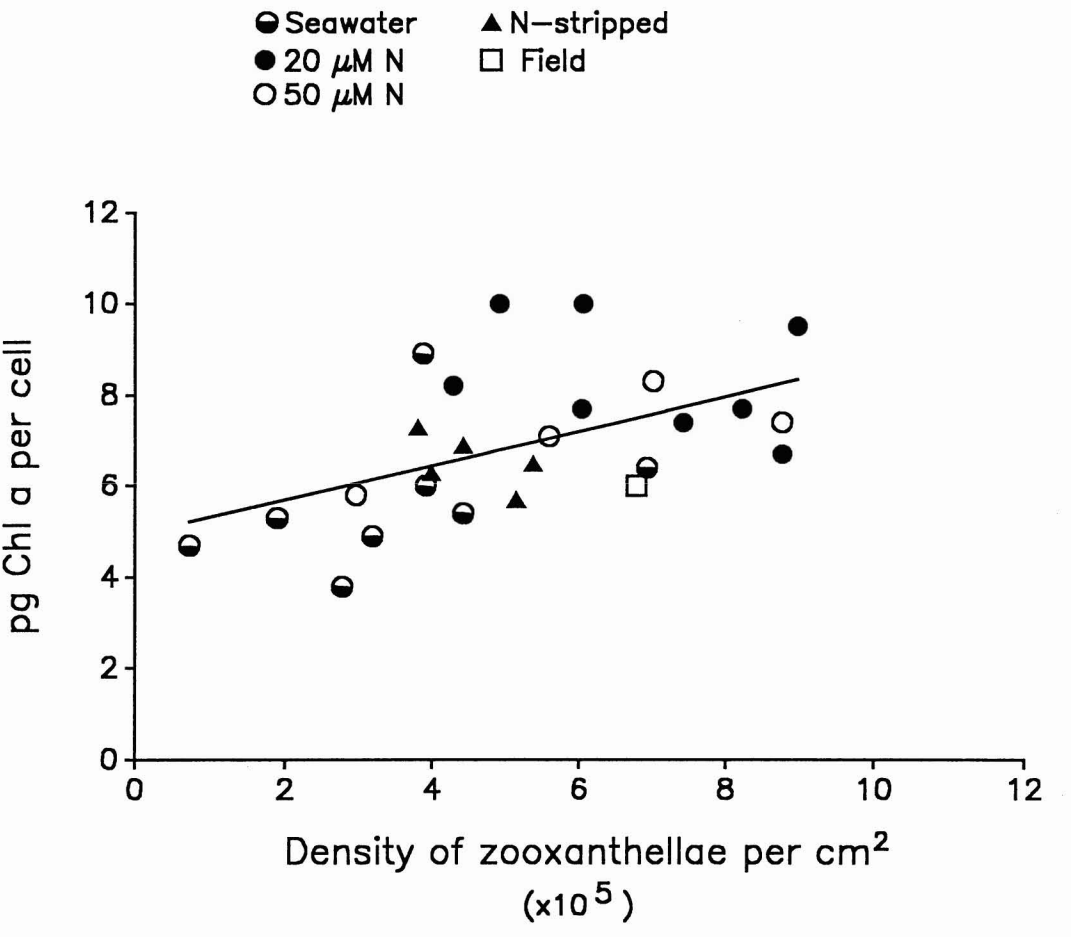

FIGURE 5. Chlorophyll *a* per zooxanthella versus population density of zooxanthellae in individual colonies of *Pocillopora damicornis* exposed to three experimental treatments: 20 μM ammonium, 50 μM ammonium, and "N-stripped" seawater, or maintained in ambient seawater. Data for all time points and a field-collected colony are included. The correlation between these two parameters was significant ($r = 0.40$; $P < 0.05$).

those obtained with corals subjected to high nutrient levels for periods exceeding 2 months (e.g., Stimson and Kinzie 1991).

Zooxanthellae density in the single freshly collected coral field colony was relatively high in comparison with densities in the ambient seawater control colonies. This difference may be related to the prolonged maintenance of the ambient seawater corals in shallow seawater tanks. However, the chlorophyll contents of zooxanthellae from the field colony were the same as those of zooxanthellae from the ambient seawater corals, indicating similar exposures to light and nutrients (see also Figure 5).

The increase in chlorophyll of zooxanthellae in *P. damicornis* exposed to 20 μM ammonium (Figure 3) may result from a combined response to ammonium and a photoadaptive response to increased self-shading of zooxanthellae at high population densities (Figure 2*A*), because there is a significant correlation between chlorophyll *a* per zooxanthella and density of zooxanthellae (Figure 5). Muscatine et al. (1989) did not obtain a significant increase in chlorophyll *a* per cell in *Stylo-*

phora pistillata with ammonium, in spite of increased density of zooxanthellae. Our results suggest that a significant increase in chlorophyll with ammonium enrichment of *S. pistillata* might have occurred in Muscatine et al.'s (1989) study if they had extended their experiment beyond 2 weeks. However, chlorophyll content of zooxanthellae may be independent of algal density under conditions where N supply limits photoadaptation (Dubinsky et al. 1990).

Addition of 20 μM ammonium caused a significant increase in animal protein biomass (Figure 2*C*). This suggests that either ammonium is directly assimilated into protein by the host animal or zooxanthellae are translocating a greater quantity of N-rich compounds. Support for the lack of direct incorporation of ammonium into animal protein is provided by Ferrier (1992), who found that intracellular free amino acid pools in the host tissue of both symbiotic and aposymbiotic anemones (*Aiptasia pallida* [Verrill]) maintained in 20 μM ammonium were not significantly different from those of anemones maintained in low-nutrient seawater. It would be worthwhile to determine the effect of ammonium enrichment on the quality and quantity of products translocated from the zooxanthellae to the animal. Our results clearly show that the addition of 20 μM ammonium caused a significant increase in animal protein with time, but that 50 μM ammonium had no effect. Single time point comparisons by Muscatine et al. (1989) and Achituv et al. (1994) showed that the protein of coral animal tissue does not vary significantly with ammonium addition.

Algal density based on animal protein biomass (Figure 2*B*) showed no significant effect of time, but density based on surface area increased significantly for the corals held in 20 μM ammonium-enriched seawater. This suggests that there are differences in the rate of animal tissue growth and skeletal extension in these corals. However, the significant correlation between zooxanthellae density based on animal protein and surface area for the pooled coral colonies shows that there is generally good agreement between the two methods of measuring population density of zooxanthellae.

No significant trends with time were observed for any biomass parameter of corals maintained exposed to 50 μM ammonium. In some cases, this may be attributed to the smaller sample size for this group of corals. However, it is clear that animal protein (Figure 2*C*), cell-specific chlorophyll (Figure 3), and areal distributions of chlorophyll (Figure 4) were reduced for the colonies in the 50-μM ammonium treatment. This suggests that 50 μM ammonium may be less stimulating than 20 μM ammonium to the growth of zooxanthellae or perhaps even stressful to the coral. The latter suggestion is supported by a decrease in the specific growth rate of zooxanthellae in corals exposed to 50 μM as compared with 20 μM ammonium (Høegh-Guldberg 1994).

The data show generally that the addition of 20 μM ammonium results in a time-course change in zooxanthellae and animal biomass of *P. damicornis*. The response of *P. damicornis* to the addition of inorganic nitrogen adds further evidence to the argument that growth of tropical symbioses, such as that between zooxanthellae and their coral hosts, is limited by the availability of inorganic nitrogen.

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