Ammonium enhancement of dark carbon fixation and nitrogen limitation in zooxanthellae symbiotic with the reef corals *Madracis mirabilis* and *Montastrea annularis*

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Abstract. The nutrient status (limitation vs sufficiency) of dinoflagellates (zooxanthellae) symbiotic with reef corals in Bermuda was assessed in 1989 and 1990 by measuring the enhancement of dark carbon fixation with 20 μM ammonium by isolated symbionts. A colony of *Madracis mirabilis* was kept in the laboratory and fed daily or starved for one month. Symbionts from fed portions of the colony had ammonium-enhancement ratios ($NH_{4\ dark}^{+}$; SW_{dark}; SW = seawater without added ammonium) similar to those of the original field population (1.2 to 1.3). Ammonium-enhancement ratios increased with starvation of the host ($\bar{x} \geq 1.7$) as did values for $V_{D'} : V_L$ [(ammonium dark rate – seawater dark rate): light rate in seawater]. Both parameters indicated decreasing nitrogen sufficiency of the algae when the host was not fed, but starvation appeared to affect these algae less than symbionts of sea anemones. Field samples of zooxanthellae from *M. mirabilis* (Three Hill Shoals and Bailey's Bay Flats) yielded results similar to those for fed corals, but those taken from Bailey's Bay Flats in May 1990 yielded exceptionally high values for enhancement (>3) and $V_{D'} : V_L$ indicating pronounced nitrogen limitation at the time of sampling. We sampled zooxanthellae from populations of *Montastrea annularis* at 8 m (Three Hill Shoals) and 24 m (Soldier's Point) depths. Enhancement and $V_{D'} : V_L$ values for zooxanthellae from the 8 m corals were density-dependent: symbionts from corals with "normal" symbiont densities displayed the most nitrogen limitation (enhancement values = 1.4 to 2.0), while those from bleached corals with lower density exhibited enhancement and $V_{D'} : V_L$ values typical of nitrogen-sufficient algae. Symbionts isolated from the 25 m corals yielded the highest values, and appeared to exhibit the least nitrogen-sufficiency for this species.

Introduction

Reef corals typically live in tropical environments with low concentrations of dissolved nutrients. The issue of nutrient limitation or sufficiency of dinoflagellates (zooxanthellae) symbiotic with marine invertebrates has become a focus of attention since nutrient supply directly affects and may regulate symbiont growth (Cook and D'Elia 1987, Cook et al. 1988, Miller and Yellowlees 1989, Muscatine et al. 1989a, Rees 1991).

There is indirect evidence that the growth of zooxanthellae in reef corals is limited by the availability of nutrients, particularly nitrogen, under natural conditions. Long-term additions of ammonium produced increased symbiont densities in *Stylophora pistillata* (Hoegh-Guldberg and Smith 1989, Muscatine et al. 1989a) and *Pocillopora damicornis* (Stambler et al. 1991, Stimson and Kinzie 1991). In addition to dissolved inorganic inputs, the feeding history of the host may affect the nutrient status of these algae, as it does in sea anemones (Cook et al. 1988, 1992, D'Elia and Cook 1988). It has been difficult to directly measure the nutrient status of algae from field populations of corals. One complication is that the supply of available nutrient sources (host feeding, and dissolved inorganic and organic seawater nutrients) may be temporally and spatially variable on the reef. It is thus important to develop and apply techniques to conveniently assess the nutrient status of zooxanthellae from field populations of individual hosts.

One test of nitrogen sufficiency in microalgae is the effect of ammonium addition on dark carbon fixation, since ammonium stimulates heterotrophic carbon fixation in nitrogen-deficient cells (Morris et al. 1971, Yentsch et al. 1977, Flynn 1990). We have used this technique to assess nutrient sufficiency of the symbiotic zooxanthellae from laboratory and field populations of the sea anemone *Aiptasia pallida*; starvation of the host produced a pronounced effect, indicating nitrogen limitation of the symbionts (Cook et al. 1992). In this paper, we apply the technique to zooxanthellae isolated from two common species of Bermudan reef corals.

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Materials and methods

Corals and zooxanthellae

Coral specimens were obtained with SCUBA from patch reefs on the North Shore and from a fringing reef on the South Shore of Bermuda during 1989 and 1990. Two patch reefs (4 to 6 m depth) were located within 2 km of the North Shore of Bermuda (Bailey's Bay Flats, and reefs north of Whalebone Bay; Fig. 1). The third patch reef, Three Hill Shoals, was located farther offshore; corals at this site were collected at 8 m depth. Corals were collected from the South Shore site (Soldier's Point) at 24 m depth. *Madracis mirabilis* was collected from all sites except Soldier's Point. *Montastrea annularis* was collected from Bailey's Bay, Three Hill Shoals and Soldier's Point.

Seawater samples for inorganic nutrient determinations were collected in situ on the reef. Empty acid-washed plastic syringes were filled with seawater in the vicinity of coral colonies. Upon return to the boat, the seawater samples were filtered through Whatman GF/F filters (0.7 μm) and placed on ice. All samples were stored frozen until analysis. Dissolved inorganic nutrients were measured using standard procedures on autoanalyzers at the Chesapeake Biological Laboratory (University of Maryland) as described by Muller-Parker et al. (1990).

As neither specific nor generic names have been applied to the dinoflagellate symbionts of either *Madracis mirabilis* or *Montastrea annularis*, we will refer to them simply as "zooxanthellae". Zooxanthellae were isolated from field corals within 2 to 4 h of collection. Individual branches of *Madracis mirabilis* or small (~6 × 6 cm) pieces of *Montastrea annularis* were used as sources of symbionts. The algae were isolated from homogenates of coral tissue obtained with a Water-Pik (Johannes and Wiebe 1970) using 0.22 μm-filtered Sargasso Sea surface water (FSSW). Coral homogenates were centrifuged at ~1000 × g for 2 or 3 min. Algal pellets resulting from the same specimen were combined and re-washed with FSSW three or four times. The final pellet was resuspended in FSSW and passed sequentially through 70 and 20 μm Nitex mesh to remove skeletal debris, animal material and clumps of algae. Cell suspensions were adjusted to 0.5 to 1.2×10^6 ml^{-1} for ^{14}C incubations; densities were determined from 8 to 10 replicate hemocytometer counts.

Ammonium enhancement of dark C fixation

The ammonium enhancement of dark carbon fixation by isolated zooxanthellae was measured with ^{14}C in light and dark scintillation vials as described by Cook et al. (1992). All experiments were performed in 0.22 μm-filtered FSSW. Control vials contained cells in seawater only, and ammonium vials contained either 20 or 50 μM NH_4Cl. All samples were run in duplicate, and were corrected for specific activity and background. Background counts were determined from vials containing formalin-killed cells. All vials were acidified with 6N HCl to evolve unincorporated $^{14}CO_2$, and were neutralized before the addition of scintillation fluid (Ecolume; ICN).

Effects of feeding and starvation

The effect of host feeding on ammonium enhancement of dark carbon fixation was examined using a single colony of *Madracis mirabilis* collected from 6 m at Bailey's Bay Flats in January 1991. The colony was transferred to a shaded aquarium (maximum irradiance from a sunny window = 100 μmol m^{-2} s^{-1}) supplied with running seawater. The following day, zooxanthellae were isolated from several 2 to 3 cm-long branches and assayed for ammonium enhancement of dark carbon fixation. Other coral branches were placed in individual capped Nunc tubes and starved in 20 ml of 0.22 μm-filtered FSSW that was changed weekly.
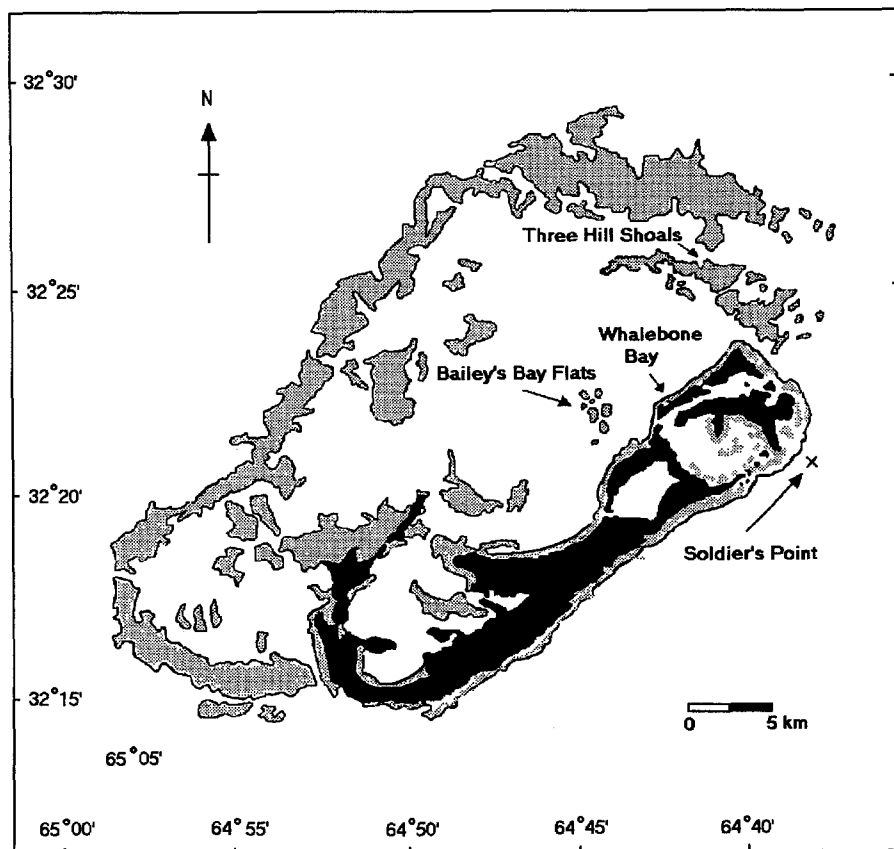

Fig. 1. Map of Bermuda showing sites of coral collections used in this study. Stippled area marks 4 m depth contour; black area, land mass

The remaining pieces of the colony were kept in the aquarium and fed daily rations of *Artemia* sp. nauplii (Argent Chemical Co., Redmond, Washington); during feeding, the seawater supply was shut off. During the first week this feeding period lasted from 30 min to 2 h. Subsequently the feeding period was extended to ~15 h to allow the corals to feed ad libitum overnight. Tubes with unfed corals were kept in the same aquarium and thus experienced the same conditions of light and temperature as did the fed corals. Zooxanthellae from fed and starved branches of the colony were sampled every 7 to 9 d for a month; each sample contained symbionts from a single branch.

The coral pieces maintained in tubes appeared to be surprisingly healthy, despite the lack of water flow and nutrient input. Per cell photosynthetic rates of isolated algae were comparable after 4 wk in each treatment (tube corals = 1.08 ± 0.17 pg C h^{-1}; aquarium corals: 1.27 ± 0.08 pg C h^{-1}, mean $\pm$ SD). Only one of 32 corals in the tubes died during the experiment, and none of the other branches showed signs of tissue necrosis.

Symbiont densities

We determined algal densities (cells cm^{-2} coral surface) in some specimens of *Montastrea annularis*. Water-pikked areas were measured by the aluminum foil method (Marsh 1970). Algal concentrations in each homogenate were determined from at least eight replicate hemocytometer counts. To yield areal densities, the total number of cells in the homogenate was normalized to the area that was water-pikked.

Data analysis

Dark carbon-fixation rates were calculated as fg C $cell^{-1}$ h; ammonium enhancement of dark carbon fixation was expressed either as the ammonium-enhancement ratio (dark ammonium rate: dark control rate) or as $V_{D'}:V_L$, where $V_{D'} = $ (dark ammonium rate $-$ dark FSSW rate), and V_L is the light carbon-fixation rate (Goldman and Dennett 1986).

The mean value of duplicate samples of each cell suspension was used for statistical procedures. Rate data from time-course experiments were analyzed by linear regression, with comparisons of rates made by fitting confidence limits to slopes. In other experiments, samples were taken only at the end of incubations, with mean rates estimated from these samples. The effect of ammonium on these rates was analyzed with two-sample Student's t-tests. In some cases, a single colony was sampled several times in the field over a 1 to 2 wk period; each sample was treated as separate for statistical analysis.

Comparisons of ratios (dark enhancement and $V_{D'}:V_L$) were performed with nonparametric Kruskal-Wallis and Mann-Whitney procedures.

Results

Nutrient concentrations of seawater at reef sites

Dissolved inorganic nitrogen (nitrate plus nitrite, ammonium) was generally quite low at the sites where we collected *Madracis mirabilis* and *Montastrea annularis*, and most phosphate levels were near detection limits (Table 1). We did not collect seawater samples from Bailey's Bay in 1990, but we include data from 1988 for comparison. No nutrient samples were taken from the reef waters at Whalebone Bay.

Table 1. Dissolved inorganic nitrogen and phosphorus concentrations of seawater samples taken from reef sites in this study; all values are μM

Site, date	$NO_3^- + NO_2^-$	NH_4^+	PO_4^{-3}
Bailey's Bay Flats, 5 m			
6 Oct. 1988	0.30	ND	0.20
Three Hill Shoals, 7 m			
31 Aug. 1989	0.71	0.5	0.05
9 Sep. 1989	0.55	0.3	0.05
13 Sep. 1989	0.21	0.2[a]	0.02
Three Hill Shoals, 7 m			
30 Aug. 1990	0.18	2.2	0.01
31 Aug. 1990	0.38	0.8	0.01
5 Sep. 1990	0.46	0.2[a]	<0.01[a]
6 Sep. 1990	0.22	0.5	0.01
7 Sep. 1990	0.21	0.2[a]	0.01
South Shore, 25 m			
21 Aug. 1990	0.44	0.4	0.01
23 Aug. 1990	0.37	1.2	0.01

[a] Limit of detection

Time-course of dark carbon fixation

We performed time-course experiments with zooxanthellae isolated from single coral colonies of *Madracis mirabilis* and *Montastrea annularis* to monitor dark carbon uptake both with and without added ammonium. The rates of dark carbon fixation were linear for all experiments between 30 min and 4 h ($r > 0.92$, $P < 0.01$); (Fig. 2) except for *Madracis mirabilis* in seawater without added ammonium ($P > 0.05$).

Both 20 and 50 μM NH_4^+ markedly stimulated dark carbon fixation by zooxanthellae from the colony of *Madracis mirabilis* collected from the Whalebone Bay reef (Fig. 2A; $P < 0.001$ for both compared to seawater controls). After 4 h, zooxanthellae with 20 μM NH_4^+ had fixed 3.8 times as much carbon in the dark as those in seawater alone, while those in 50 μM had fixed 5.0 times as much. The addition of either 20 or 50 μM NH_4^+ had marginally significant effects on the rate of dark carbon uptake by zooxanthellae from the Bailey's Bay colony of *Montastrea annularis* used in this experiment (Fig. 2B; $0.1 > P > 0.05$ for 20 μM; $P < 0.05$ for 50 μM; confidence limits of slopes).

The y-intercepts in Fig. 2 are greater than zero, suggesting that uptake rates during the first 30 min exceeded subsequent rates. To minimize the influence of these initial rates, we routinely used 4 h incubations in subsequent experiments. As there were no significant differences in the rates produced by 20 or 50 μM NH_4^+, 20 μM was employed in all other experiments reported in this paper.

Effects of feeding and starvation of *Madracis mirabilis* on ammonium enhancement of dark carbon fixation by its zooxanthellae

We isolated zooxanthellae from individual branches of a single colony of *Madracis mirabilis* that were either fed or
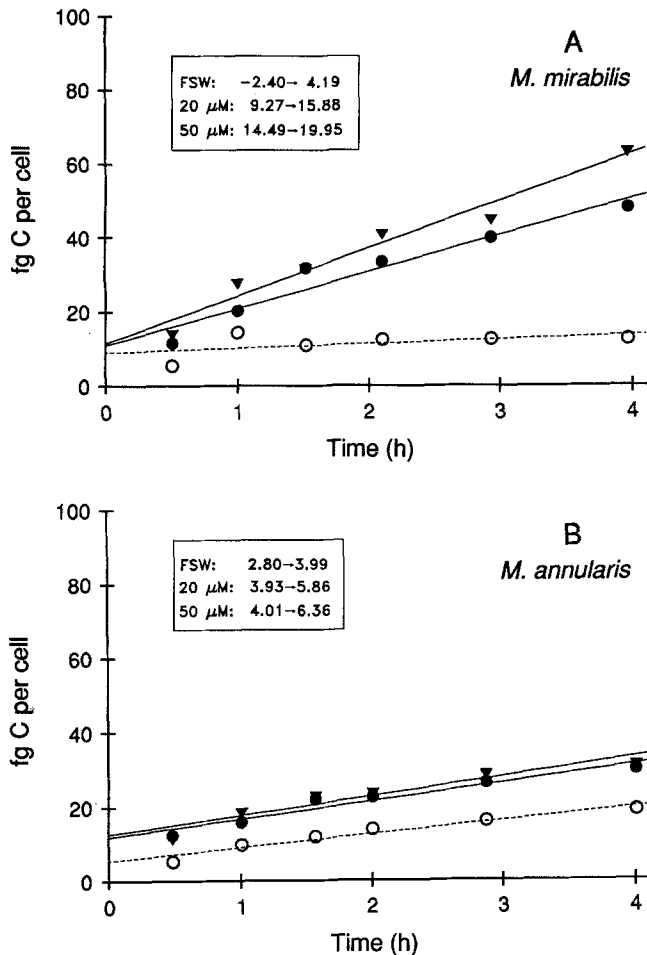
Fig. 2. *Madracis mirabilis* and *Montastrea annularis*. Time-course of dark carbon uptake by zooxanthellae isolated from single colonies collected from Whalebone Bay on 23 May 1990 (A) and Bailey's Bay Flats on 17 May 1990 (B). Linear regression lines are given for seawater (o), and for additions of 20 (●) and 50 μM (▼) NH_4^+. 95% confidence limits for rates (fg C cell^{-1} h^{-1}) are given in insets (fg = 10^{-15} g). FSW: filtered seawater with no added NH_4^+
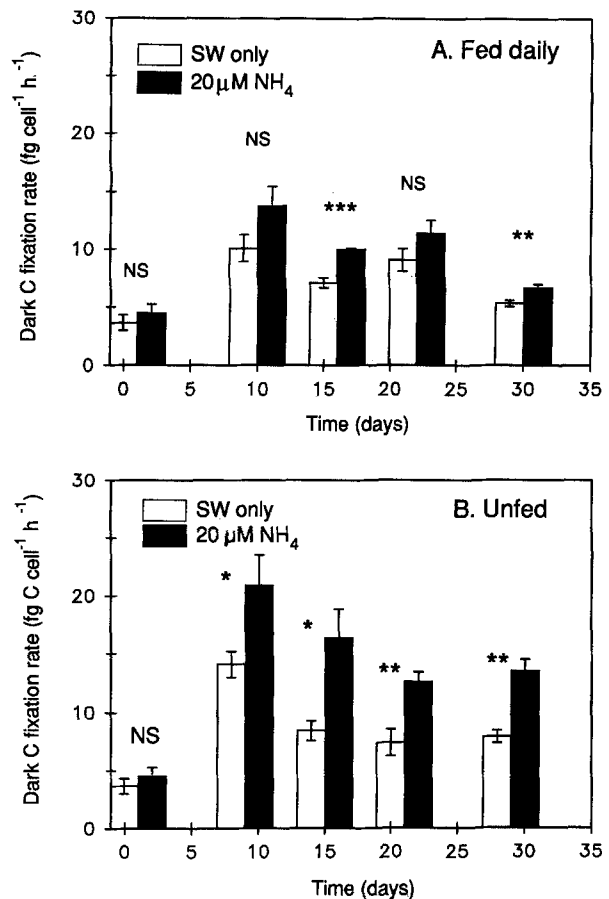
Fig. 3. *Madracis mirabilis*. Dark carbon fixation by zooxanthellae from a single colony as function of time since collection (Bailey's Bay, 23 January 1991). (A) Corals exposed to food in flowing seawater aquarium; (B) isolated "branches" of corals kept in closed tubes without food. Vertical bars indicate one standard error of mean; $N = 6$ for all samples. Statistical significance between FSW and NH_4^+ groups was determined by two-sample Student's t-test of cell suspensions from individual branches. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$; NS: $P > 0.05$

starved in the laboratory to assess the effect of host feeding on the enhancement of dark carbon fixation by 20 μM ammonium. The initial samples were analyzed on the day after collection; mean rates of dark carbon fixation by these algae are given as the zero-time points in Fig. 3. The addition of ammonium had no significant effect on dark carbon fixation ($P > 0.4$), although the ammonium-enhancement ratio for these algae was 1.23 (Fig. 3 B).

Zooxanthellae from the fed portions of the colony showed moderate ammonium enhancement of dark carbon fixation throughout the experiment (Fig. 3 A). Dark C fixation was stimulated by ammonium in 2 of the 4 weekly samples. When all the data for these corals were pooled, the addition of ammonium increased dark C-fixation rates by 24.3% ($P < 0.01$, $N = 48$, Student's t-test). Zooxanthellae from the unfed portions of the colony exhibited a greater ammonium effect (Fig. 3 B). Significant stimulation was observed in each weekly sample, with a mean increase for all samples of 39.6% ($P < 0.001$, $N = 48$, Student's t-test).

Comparisons of the ammonium enhancement and $V_{D'} : V_L$ ratios are shown in Fig. 4. The ammonium-enhancement ratios of the fed corals were similar to those of the field corals throughout the experiment, with mean enhancement values between 1.2 and 1.4 (Fig. 4 A). The lowest values for both enhancement and $V_{D'} : V_L$ occurred during the last two weeks of the experiment, when the feeding period was increased (see "Materials and methods – Effects of feeding and starvation"). These values indicate nitrogen-sufficient algae. Enhancement ratios of algae from the unfed corals exceeded those of algae from fed corals in each sample, with mean values of 1.7 to 1.9. $V_{D'} : V_L$ values for the fed corals gradually decreased during the experiment, while $V_{D'} : V_L$ values for algae from the unfed corals clearly exceeded those from fed corals in the last three samples (Fig. 4 B). At the end of the experiment, these values were four times higher than those for zooxanthellae from the fed corals.
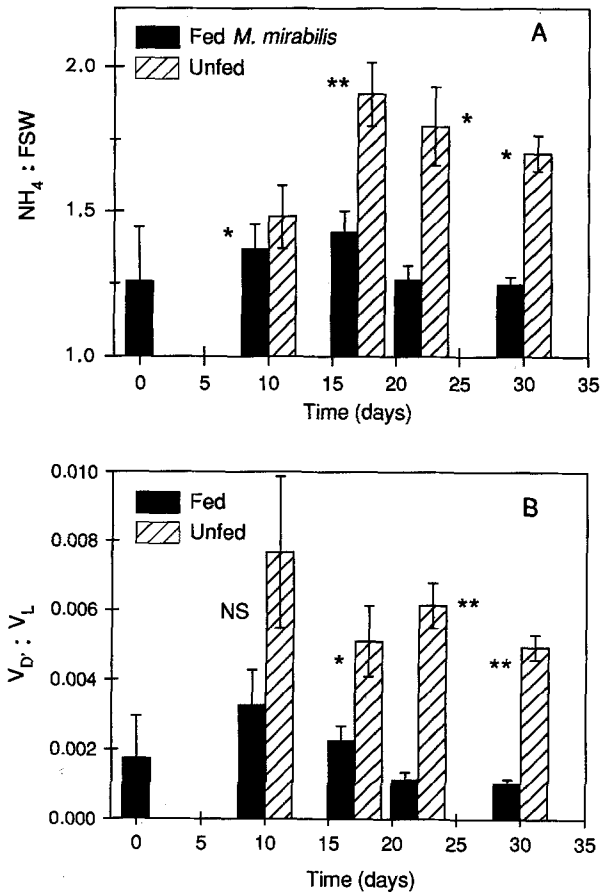
Fig. 4. *Madracis mirabilis.* Parameters of ammonium enhancement of dark carbon fixation by zooxanthellae from corals in Fig. 3. Vertical bars as in Fig. 3; significance values between fed and unfed groups determined from Kruskal-Wallis/Mann-Whitney procedures; significance levels as in Fig. 3. (A) Ammonium-enhancement ratios (ammonium rates : seawater only rates). (B) $V_{D'} : V_L$ [(ammonium dark rate − seawater dark rate) : light rate in seawater]

Zooxanthellae from field populations of *Madracis mirabilis*

Fig. 5 summarizes the effects of 20 μM ammonium on dark carbon fixation by zooxanthellae from samples of *Madracis mirabilis* collected from patch reefs at Three Hill Shoals and Bailey's Bay Flats (Fig. 1). Ammonium had no significant effect on samples taken from Three Hill Shoals in 1989 or 1990 (Student's t-test, $P > 0.05$ for both; Fig. 5A), although the 1990 samples were only single branches from two colonies. The data from the single colony collected from Bailey's Bay in January 1991 (no effect; Fig. 3A) are included in Fig. 5A. The ammonium-enhancement ratios of zooxanthellae from all of these corals (mean values: 1.3 to 1.5; Fig. 5B) were not different from those of the fed corals in the laboratory experiment (Fig. 4A; Mann-Whitney, $P > 0.05$). This was also true for $V_{D'} : V_L$ values (Fig. 5C), except for zooxanthellae from two 1990 Three Hill Shoals corals; these samples yielded higher values than any of the fed corals.

In contrast, zooxanthellae from six coral colonies collected from Bailey's Bay Flats on 31 May 1990 showed
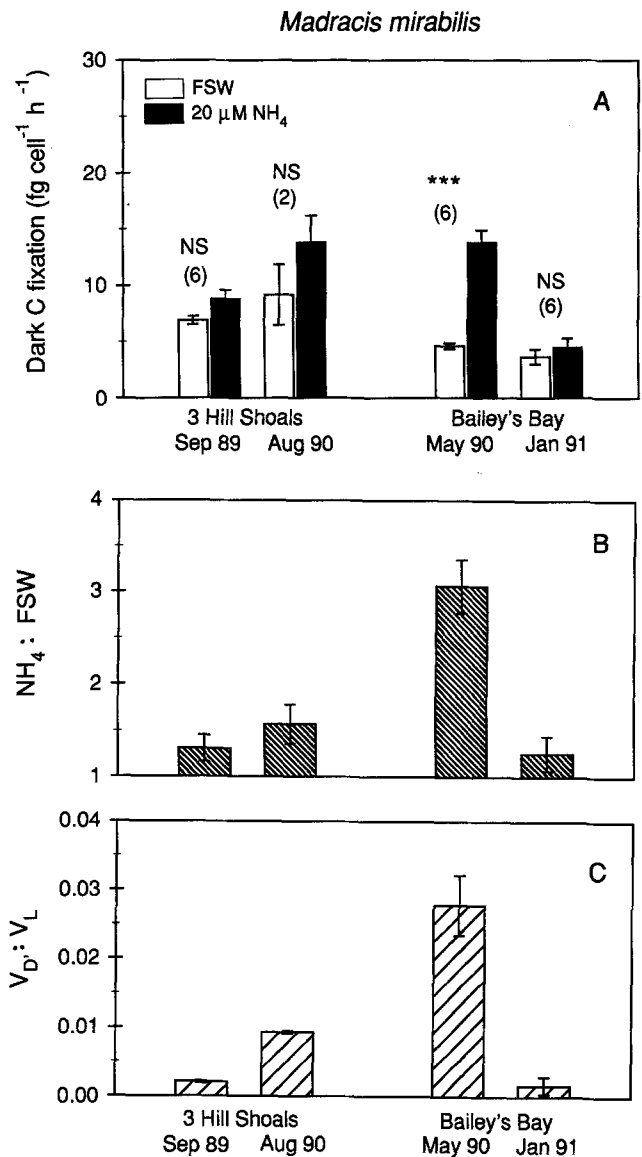

Fig. 5. *Madracis mirabilis.* Ammonium enhancement of dark carbon fixation by zooxanthellae isolated from various field populations. (A) Rates of dark carbon fixation with and without added 20 μM NH_4^+; each bar is mean of values from several branches, with sample sizes in parentheses; 1989 Three Hill Shoals samples were taken from three colonies sampled twice over 6 to 12 d, 1990 samples from this site were single branches from two colonies, May 1990 samples were single branches from 6 different colonies, and January 1991 samples were taken from single colony. Statistical conventions as in Fig. 3. (B) Ammonium-enhancement ratios based on data of (A). (C) $V_{D'} : V_L$ values for these samples

strong enhancement of dark C fixation by ammonium ($P < 0.001$; Fig. 5A), with a mean ammonium-enhancement ratio > 3 (Fig. 5B). Both the enhancement ratios and $V_{D'} : V_L$ (> 0.01; Fig. 5B, C) were greater than those of any of the unfed corals in the laboratory experiment. The ammonium-enhancement ratios of these algae were greater than any of the field populations that we sampled, excepting the Whalebone Bay population of zooxanthellae from *Madracis mirabilis* in the time-course study (Fig. 2A). The Whalebone Bay sample was collected one week earlier (23 May 1990).
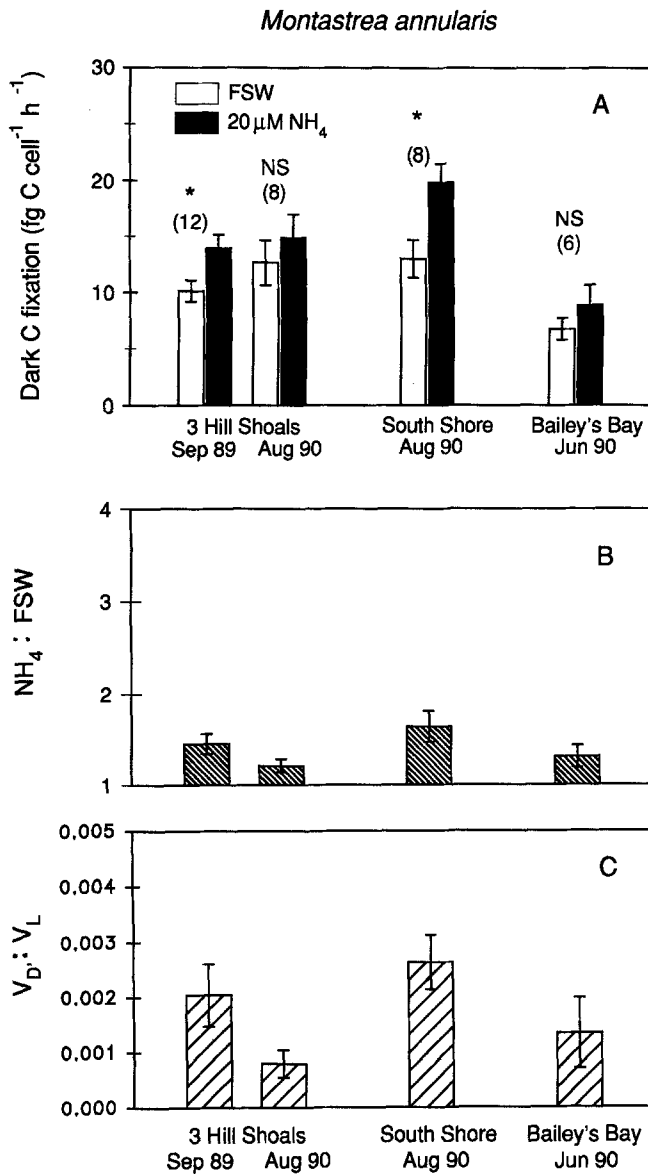
Fig. 6. *Montastrea annularis*. Ammonium enhancement of dark carbon fixation by zooxanthellae isolated from field populations. (A) Rates of dark carbon fixation with and without added 20 μM NH_4^+; each bar is mean of samples taken from individual pieces of coral; 1989 Three Hill Shoals samples came from 6 colonies sampled twice over a 1 wk period, all other samples are single samples from different colonies; Bailey's Bay corals were collected on 5 June 1990; statistical conventions as in Fig. 3. (B) Ammonium-enhancement ratios based on data of (A). (C) $V_{D'} : V_L$ calculations based on data of (A); note that Y-axis is expanded relative to that in Fig. 5 C

Fig. 7. *Montastrea annularis*. Relationship between symbiont density and parameters of ammonium enhancement (20 μM ammonium) of dark carbon fixation for zooxanthellae from Three Hill Shoals (o, ●) and Soldier's Point on the South Shore (∗). Regressions include only Three Hills Shoals data. (A) Ammonium-enhancement ratios (NH_4^+ : seawater); least-squares regression line is significant at $P < 0.01$ ($r = 0.701$, $N = 14$). (B) $V_{D'} : V_L$ calculations; least-squares regression line is significant at $P < 0.01$ ($r = 0.680$, $N = 14$)

Zooxanthellae from field populations of *Montastrea annularis*

Zooxanthellae obtained from *Montastrea annularis* collected at various sites and dates in Bermuda displayed moderate stimulation of dark carbon fixation in response to 20 μM ammonium (Fig. 6 A). Samples taken from Bailey's Bay in June of 1990 did not show a significant effect of ammonium addition; the mean ammonium enhancement ratio for these algae was 1.31 (Fig. 6 B). The Bailey's Bay sample of zooxanthellae used for the time-
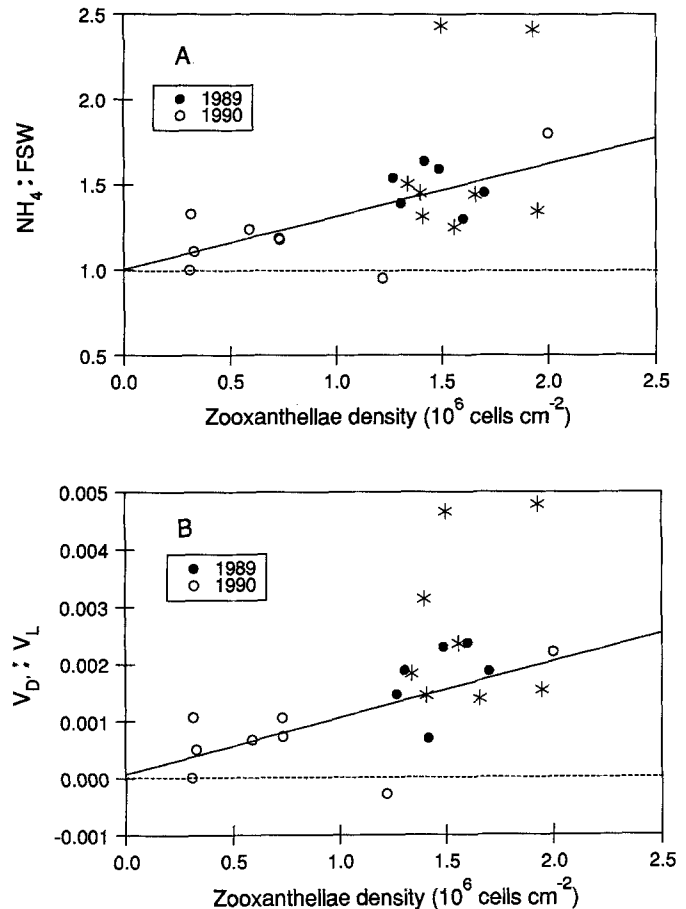
course study with this species was collected one month earlier from the same reef and yielded a similar result (Fig. 2 B).

There was a significant ammonium effect on dark carbon fixation in samples collected from Three Hill Shoals in 1989 ($P < 0.05$, Student's t-test), but not in those collected in 1990 ($P > 0.4$; Fig. 6 B). However, there were no differences between the two years in ammonium-enhancement ratios (Fig. 6 B) or $V_{D'} : V_L$ values (Fig. 6 C, Mann-Whitney U-test, $P > 0.05$). Both NH_4:filtered seawater and $V_{D'} : V_L$ values for the 1990 samples were lower than those for fed *Madracis mirabilis* ($P < 0.05$, Mann-Whitney), while the 1989 samples were not different from the fed samples (Mann-Whitney, $P > 0.05$).

Samples of zooxanthellae taken from the 24 m colonies of *Montastrea annularis* in 1990 showed a pronounced enhancement of dark carbon fixation by ammonium (Fig. 6 A). Both the ammonium-enhancement ratios (Fig. 6 B; mean = 1.64; $P < 0.05$, Mann-Whitney) and $V_{D'} : V_L$ values (Fig. 6 C; $P < 0.01$) were greater than those of the shallow specimens of *M. annularis* sampled during

the same month. Zooxanthellae from two of the 24 m colonies yielded enhancement ratios >2.4; these were the highest values that we obtained for this species.

Symbiont density and ammonium enhancement in *Montastrea annularis*

Our sampling of *Montastrea annularis* at Three Hill Shoals in August 1990 included some corals that were involved in a minor coral bleaching event in Bermuda and contained a reduced population of zooxanthellae compared to the 1989 corals collected from this site. Expressed as a function of colony surface, the 1990 corals had a symbiont density of $0.78 \pm 0.58 \times 10^6$ cells cm^{-2}, while the 1989 samples had a density of $1.46 \pm 0.17 \times 10^6$ cells cm^{-2} ($P < 0.05$, Student's t-test). Ammonium enhancement and $V_{D'}:V_L$ values increased with symbiont density (circles in Fig. 7; $P < 0.05$ for both). The data for the 1990 specimens from the 24 m colonies are included in Fig. 7 (asterisks), but not in the regression analyses. They provide further support for the conclusion that the nitrogen status of these algae, as indicated by ammonium effects on dark carbon fixation, is density-dependent.

Discussion

The technique of ammonium enhancement of dark carbon fixation appears to be a useful indicator of nitrogen status for zooxanthellae symbiotic with corals. This approach was originally developed for free-living phytoplankton (Morris et al. 1971) and was adapted for use with symbionts from the sea anemone *Aiptasia pallida* (Cook et al. 1992). It is relatively simple and convenient, and can be applied to symbionts from corals freshly collected from the field. The ammonium-enhancement ratio appears to have more utility for comparisons between species and within species at different sites than the $V_{D'}:V_L$ ratio, since it is independent of photosynthetic rate. The $V_{D'}:V_L$ ratio is a function of the per cell photosynthetic rate which, in turn, is influenced by algal size (varying between species or strains of symbionts) and the light history (photoadaptation) of corals, which varies with depth and shading. The $V_{D'}:V_L$ ratio shows a more pronounced effect of host nutritional history than the enhancement ratio (Fig. 4), and may be more useful for repeated studies of the same species at a given site.

The use of the dark carbon technique yields three conclusions concerning nitrogen supply and limitation of zooxanthellae in the reef corals *Madracis mirabilis* and *Montastrea annularis*. First, we have shown that host feeding is an important factor in nitrogen supply for zooxanthellae of *Madracis mirabilis*: ammonium enhancement of dark carbon fixation is significantly greater in algae from starved corals than in algae from fed corals. Secondly, zooxanthellae from field collections of *Madracis mirabilis* and *Montastrea annularis* in Bermuda appear to show temporal variability in nitrogen sufficiency. The third conclusion is that nitrogen sufficiency in

field populations of *M. annularis* appears to be density-dependent, being lowest in corals with the highest densities of symbionts.

Role of host-feeding in supplying nitrogen for zooxanthellae of corals

Our laboratory experiments with *Madracis mirabilis* produced results that were similar to those found with zooxanthellae from the sea anemone *Aiptasia pallida* (Cook et al. 1992). Ammonium-enhancement ratios of algae from both fed corals and fed anemones were generally between 1.2 and 1.4, and these values may be taken to indicate the most nitrogen-sufficient algae in these hosts. Other parameters have also indicated that well-fed anemones contain nutrient-sufficient symbionts, with limitation occurring as the hosts are starved (Cook et al. 1988, D'Elia and Cook 1988, Muller-Parker et al. 1990).

Both enhancement ratios and $V_{D'}:V_L$ values of zooxanthellae increased with starvation in *Aiptasia pallida* and corals. However, a comparison of the dark carbon data indicates that withholding food had a more pronounced effect on the sea anemone symbionts. In the sea anemone study, the major increases in ammonium enhancement and $V_{D'}:V_L$ values occurred during the first week of starvation, while these parameters increased only slightly in algae from corals sampled 10 d after starvation. The ammonium-enhancement ratios of zooxanthellae from starved anemones (means: 2.3 to 3.2) were consistently higher than those of symbionts from unfed corals. The comparison suggests that the algae from the anemones are more dependent upon host feeding as a nitrogen supply.

Madracis mirabilis is an active feeder (Sebens and Johnson 1991), and feeding in the field probably provides a significant amount of nitrogen for its symbionts. This also may be true for the temperate coral *Astrangia danae* ($=A.$ *poculata*; Szmant-Froelich and Pilson 1984). Zooxanthellae from other corals may be less dependent upon host feeding for nitrogen. In a study of the effects of feeding and dissolved inorganic nitrogen (DIN) enrichment on zooxanthellae in *Stylophora pistillata*, host feeding had little effect on growth rates of the algae (Muscatine et al. 1989 a). However, the addition of ammonium stimulated zooxanthellar growth, indicating that DIN can be a major nitrogen source for these algae, and that the algae in these corals are normally nitrogen-limited. Ammonium addition also stimulated the growth of zooxanthellae in *Pocillopora damicornis* (Stambler et al. 1991, Stimson and Kinzie 1991) and in *Seriatopora hystrix* (Hoegh-Guldberg and Smith 1989).

Nitrogen status of zooxanthellae from field populations of *Madracis mirabilis* and *Montastrea annularis*

If we consider the ammonium-enhancement values of zooxanthellae from fed corals and sea anemones as indicating nitrogen sufficiency, it would appear that most of the zooxanthellae from shallow-water (<9 m) corals

were relatively nitrogen-sufficient. This was particularly true for the zooxanthellae from most collections of *Madracis mirabilis* and from bleached specimens of *Montastrea annularis*. Shallow-water colonies of *M. annularis* with "normal" symbiont densities ($>10^6$ cm^{-2}) contained algae which exhibited moderate nitrogen limitation, as did those from the 24 m colonies of *M. annularis*. The 24 m samples yielded the highest ammonium-enhancement values that we observed for this species.

We suggest that the differences observed in the nitrogen sufficiency of zooxanthellae relate to either the feeding rate or physiological state of their hosts. Zooxanthellae *in hospite* can utilize DIN. However, our DIN measurements (Table 1), although limited, do not show any obvious relationship to ammonium-enhancement patterns (Figs. 5 and 6). Nutrient concentrations were generally low; the one high value for [NH$_4^+$](2.2 μM on 30 August 1990) appears to be anomalous. Thus, major differences in nitrogen supply for the system are likely to result from variations in host feeding, rather than from variations in DIN supply. Other factors might be related to temporal changes in the physiological state of the host. One possibility is the cost of reproduction (e.g. Szmant 1986), which might reduce nitrogen fluxes to the algae. How these possibilities relate to the exceptionally high values of ammonium enhancement in some samples of zooxanthellae from *Madracis mirabilis* (Figs. 2 A and 5 B) is not clear. These corals were collected within 8 d of each other from different patch reef areas, but we know of no relevant environmental or physiological event that occurred during this intervening period. Zooxanthellae from *Montastrea annularis* collected from the same reef within a month of these samples (Figs. 2, 6) showed no such effects.

Symbiont density and nitrogen sufficiency

Our data for zooxanthellae from *Montastrea annularis* clearly show that nitrogen sufficiency of symbiotic algae, as indicated by our techniques, is inversely correlated with the density of symbionts within host tissue. Hoegh-Guldberg and Smith (1989) and Muscatine et al. (1989 a) reported that the chlorophyll *a* content of symbiotic zooxanthellae is inversely correlated with symbiont density. Their results, which are the opposite of a self-shading photoadaptive effect (Dubinsky et al. 1990), also indicate a density-dependent relationship between nutrient status and symbiont density. Both their findings and our data are consistent with the view that zooxanthellae living at naturally high densities in host tissue may be limited by nitrogen, or perhaps other nutrient elements (Cook and D'Elia 1987). Conversely, zooxanthellae from corals with reduced symbiont populations would be more nutrient-sufficient. This may in part explain observations of increased symbiont growth rates in hosts with reduced populations (Hoegh-Guldberg et al. 1986, Smith 1986). Another possibility is that CO$_2$ limitation at high symbiont densities (Muscatine et al. 1989 b, Dubinsky et al. 1990) may impose a limit on nutrient utilization by zooxanthellae.

If, as we propose, nutrient-sufficiency is related to symbiont density in corals, these density-dependent effects could have important consequences for bleached corals. Following episodic bleaching events, a coral may lose a major percentage of its population of zooxanthellae (e.g. Porter et al. 1989, Williams and Bunkley-Williams 1991). Nutrient sufficiency and resultant higher symbiont growth rates would favor the repopulation of such corals when environmental conditions are more suitable. Bleaching itself may be a response by corals to promote survival during periods of environmental stress, in part by reducing symbiont demands upon host resources (Buddemeier and Fautin 1993).

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