Final Report

USEPA / South Florida Water Management District

Special Studies in the Florida Keys National Marine Sanctuary

"Reef Corals and Their Symbiotic Algae as Indicators of Nutrient Exposure"

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Project Title: Reef Corals and Their Symbiotic Algae as Indicators of Nutrient Exposure
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Date: November 3, 1997

STATEMENT OF THE PROBLEM AND APPROACH:

There is a widely held view that Florida Bay waters have "an inimical effect" on reef corals in the Florida Keys. This is based on the distribution of corals, which are generally lacking offshore of the wide passes between Keys, and on a sizable amount of largely anecdotal evidence. There is in fact little experimental evidence to document such effects, or to pinpoint what the "Florida Bay" effect would be. Possibilities include elevated nutrients, wide temperature and salinity range, and increased turbidity. We specifically examined nutrient exposure. We set up a transplant experiment to measure coral growth rates at both an inshore site directly impacted by Florida Bay water, and an offshore site that was not. Parameters of nutrient exposure of the symbiotic algae in the corals would be measured to determine nutrient exposure history.

Sites: We chose our two sites near Long Key, FL (Fig. 1). This location allowed us to use the boats and laboratory facilities of the Keys Marine Laboratory, as well as possibly coordinating with other Special Studies located in the area. GPS was used to locate both sites. The inshore site (CMS3) was sited at 24° 47.880' N, 80° 47.110' W, N of Marker 44 at a depth of 4m. Current meter studies by N. Smith and P. Pitts of Harbor Branch and aerial reconnaissance surveys by FDEP both indicated that this site should receive substantial flow from Florida Bay via Channel #5 during ebbing tides. The offshore site (CMS4) was located at 24° 45.475' N, 80° 46.370' W, NE of Tennessee Light at a depth of 6m. This site was on the western edge of the reef tract and was separated from CMS3 by Hawk Channel. We anticipated that net flow from NE to SW along Hawk Channel (N. Smith and P. Pitts, pers. comm.) would divert much of the water from Channel #5 away from this site. The distance between the two sites was 2.84 miles (4.52 km), as determined from the GPS coordinates.

Experimental Design. We chose the massive reef-building coral *Montastraea faveolata* for this study. This coral grows in large hemispherical colonies, and is one of the major reef-building species on the Florida reef tract. Our original proposal was to perform reciprocal transplants with corals from both the offshore and inshore sites, but budgetary and time considerations precluded this complete experimental design. Instead, we used four colonies from an inshore site off of Lower Matecumbe Key ("Coral Gardens", located at 24° 50.154' N, 80° 43.751' W; Fig. 1). Two-inch (diameter) cores of living coral were cut with a diamond-tipped coring bit and epoxied into PVC collars. The collars were mounted on stainless steel Coral Maintenance Structures (CMS) designed and fabricated by EMM. These units were deployed at both sites. Fig. 2 shows the design of these structures.

Each structure held 48 explants, comprising 12 cores from each of 4 donor colonies. The explants were deployed on the arrays such that corresponding pairs of explants from the same

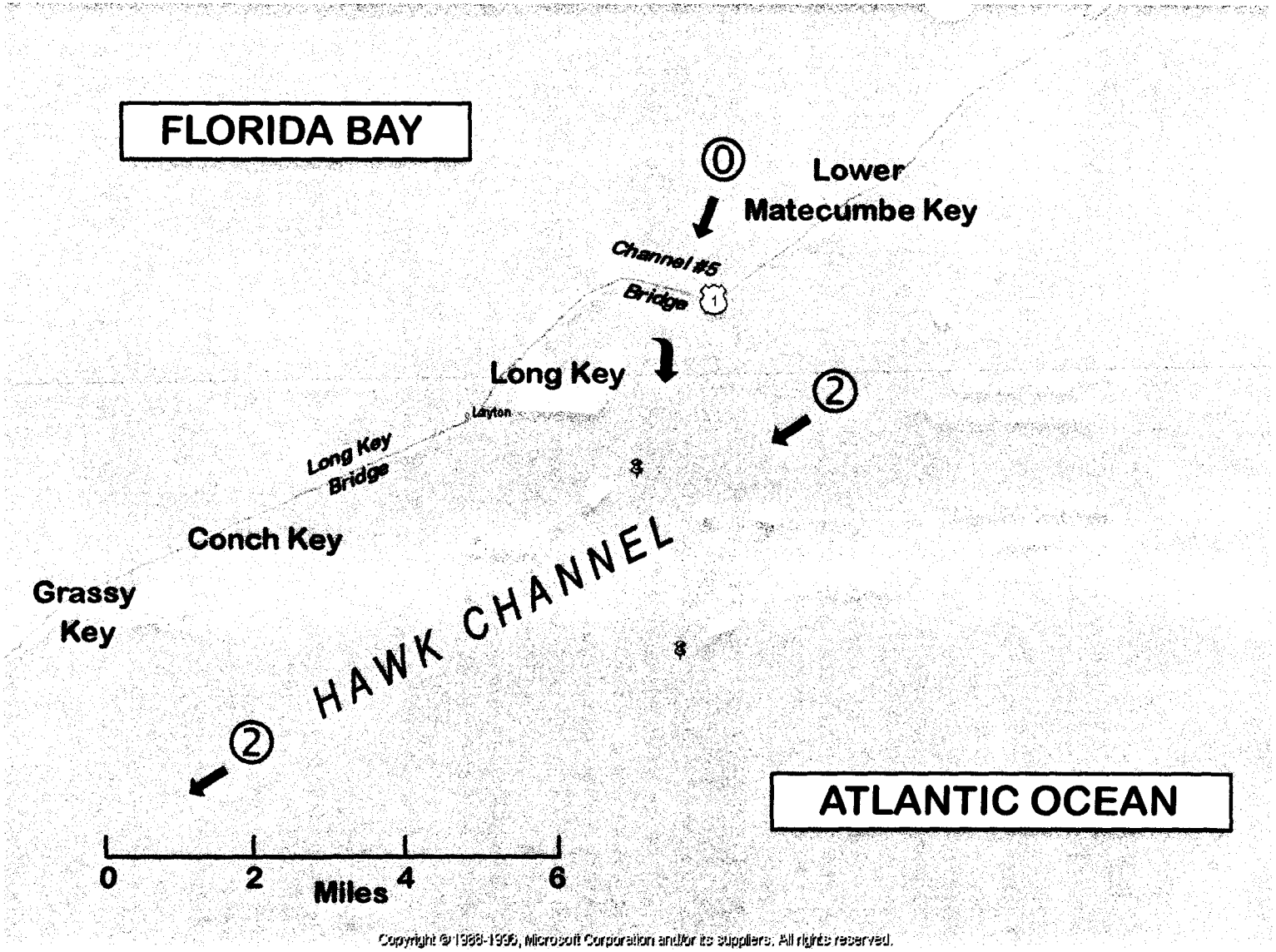
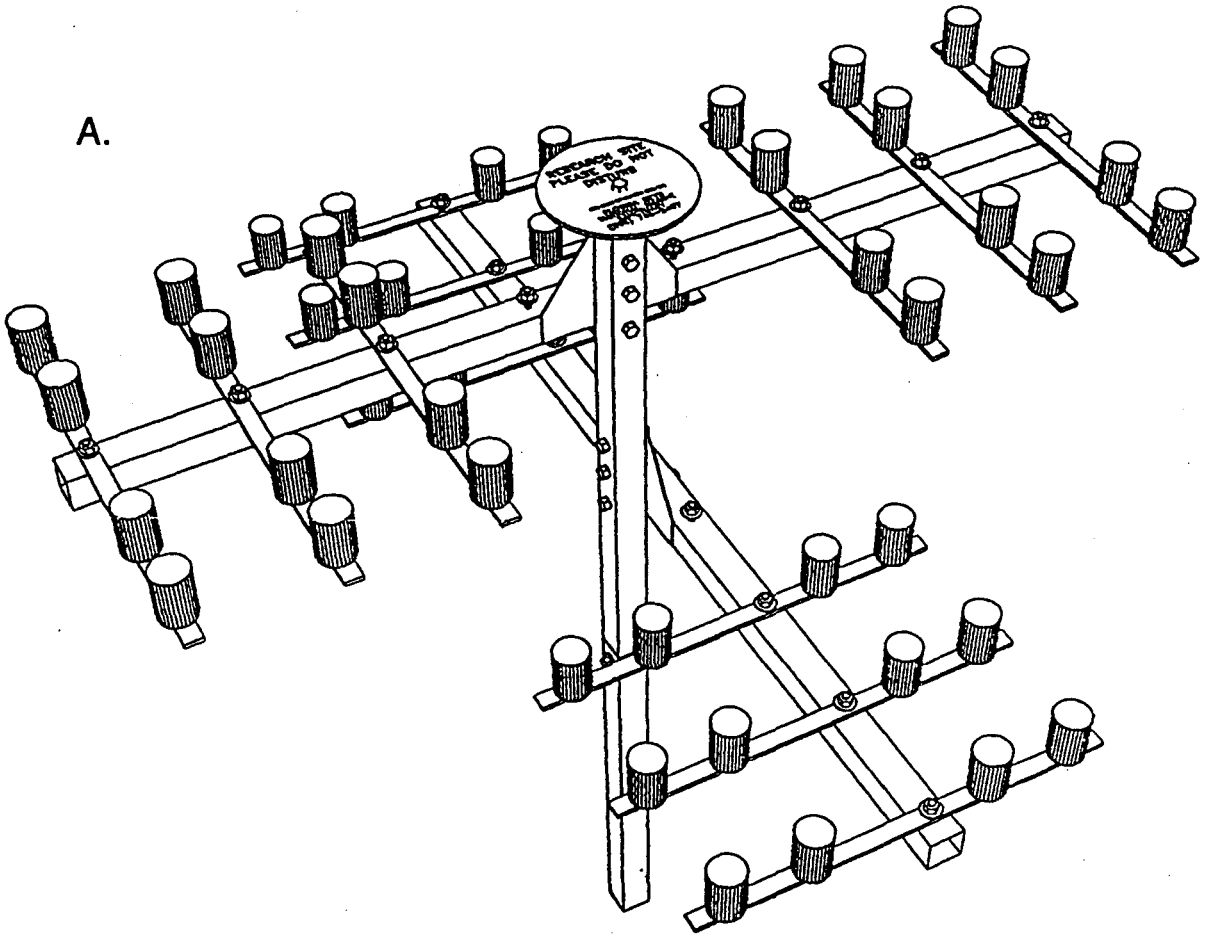


Figure 1. Location of coral maintenance structures (CMS3, CMS4) at sites south of Long Key, FL. The site of donor corals is also indicated. Dark arrows indicate (1) direction of ebb tidal flows through Channel #5 and (2) the net direction of flow through Hawk Channel. Data for (2) taken from a current meter operated by N. Smith and P. Pitts from 1993-1994, indicated on the map.



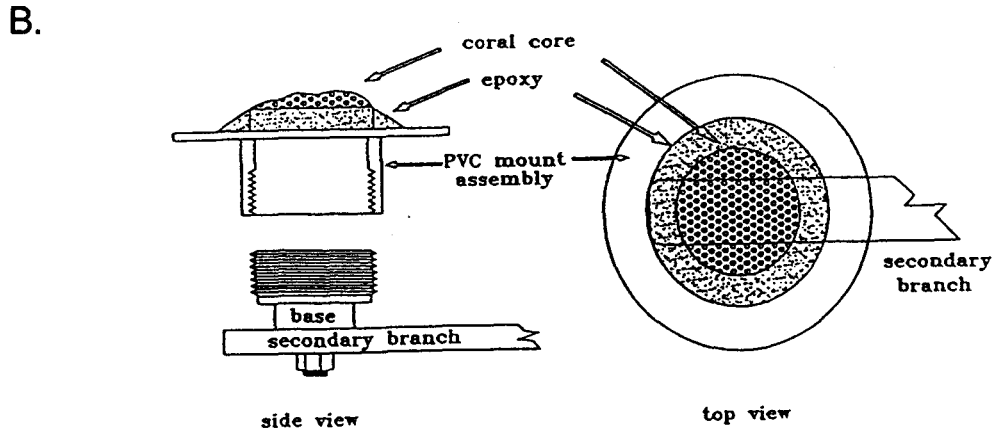


Figure 2. Diagram of the stainless steel coral maintenance structures (CMS) deployed at each site. A. Overview of the structure showing the central support, primary branches and secondary branches. Each of the primary branches is 60" in length. The cylinders indicate the position of the coral explants. B. Detail of a coral explant epoxied into a PVC collar; the collar attaches to a secondary branch by a screw-in fitting. Diameter of each explant is 2 cm.

colony were located at the same positions on each structure. Each explant was assigned a label based on site [3 or 4], donor colony [A,B,C,D] and position on each array [1-12]. During each quarterly sampling we took three corresponding pieces from each of the four colonies at each site, using previously assigned random numbers to determine the positions that were collected each time.

At the inshore site samples of colonies A and B were cored on February 15, 1996 and placed on CMS3 on February 20. Colonies C and D were cored on February 20, and placed on CMS3 on February 22. For the offshore site (CMS4) core samples were taken from the same colonies (A-D) on March 15, and placed on CMS4 on March 17. Subsequent quarterly sampling of both sites took place on the same days. Thus, samples taken from the inshore site had been in the water 24 or 26 days longer than those from the offshore site. Table 1 summarizes the dates of each collection:

Table 1. Summary of collection dates for the Florida Bay / EPA coral project

Collection	CMS3		CMS4
Date	Colonies A-B	Colonies C-D	Colonies A-D
[Date set out:]	02/20/96	02/22/96	03/17/96
Elapsed days	0	0	0
May 96	05/14/96	05/14/96	05/14/96
Elapsed days	84	82	58
Aug 96	08/08/96	08/08/96	08/08/96
Elapsed days	170	168	144
Oct 96	10/30/96	10/30/96	10/30/96
Elapsed days	253	251	227
Mar 97	03/11/97	03/11/97	03/11/97
Elapsed days	385	383	359

Measured parameters: coral growth rates. We compared the growth rates of coral explants at the two sites using four different measures. These procedures, discussed in detail in our QAPP, are summarized below. Other details will be given in the context of Results.

Total calcification (skeletal mass accretion) was determined by the buoyant weight technique, in which explants were weighed in seawater. Each explant was weighed in its PVC collar before it was placed on a CMS array, and again after collection. This yielded the change in skeletal mass ($CaCO_3$) over this time interval. Any encrusting organisms that would contribute to the buoyant weight were removed prior to weighing.

Areal growth was measured by the aluminum foil method, in which aluminum foil of known density is fit to the coral surface and then weighed. In the later samples coral tissue and skeleton overgrew portions of the PVC collars. The areas of these "skirts" were included in these measurements. This procedure is appropriate for cleaned coral skeletons but not for live specimens, so that changes in individual explants were not determined.

Polyp numbers were determined from photographs (Time 0 [when first mounted in the PVC collars] and first quarterly samples) or cleaned coral skeletons (all subsequent samples). The original (Time 0) counts included any polyps that were damaged by the coring bit. The number of **polyps per unit area** was derived from these counts and the areal measurements.

Vertical extension (linear growth) was determined with the use of the alizarin technique. Alizarin red S is a stain that is incorporated into skeletons containing calcium.

Immediately after initial collection and buoyant weighing, all coral explants were stained with alizarin red S for approximately 24 hours, incorporating a "time line" in the skeleton. After the quarterly sampling, cleaned skeletons were removed from the PVC collars and bisected with a diamond saw. One half was haphazardly designated as the "right" half and used for measurements. In those cases when the "right" half was not suitable for examination due to damage or other factors, the other half was used. All of the "right" halves were lightly sanded (600 grit; last 3 samplings) or ground with carbide powder (also 600 grit - first sampling only) on a glass plate to remove material damaged by the saw blade. Measurements were made on calibrated video images taken through an Olympus stereo microscope.

Measured parameters: nutrient status of symbiotic zooxanthellae. The live coral samples were transported on the day of collection in an aerated carrier to the Harbor Branch Oceanographic Institution (HBOI), and assays of the nutrient status of the isolated zooxanthellae performed over the next 48 h. The corals were maintained in the aerated collection seawater outdoors under open shade until analysis. Samples were processed in a pairwise fashion so that corresponding pairs from each site (e.g. 3A1 and 4A1) were processed at the same time. The procedures for isolating the algae and the assays of nutrient status are outlined in our QAPP. Samples of algae for elemental and free amino analyses were collected on filters and stored at -17°C until analyzed. Samples for chlorophyll *a* content were collected on GF filters and immediately extracted with 100% acetone. Centrifuged samples were analyzed spectrophotometrically 24h later. The assays included:

Elemental ratios (C:N:P), analyzed by the Analytical Services Department of the Chesapeake Biological Laboratory. The data included per cell content as well as C:N:P ratios.

Free amino acids (FAA). FAA were extracted and analyzed by HPLC by MDF at Hood College. Ratios indicative of nitrogen status (glutamine:glutamate, basic FAA:total FAA) and total FAA content per cell were calculated.

Chlorophyll *a* and C_2 content, analyzed by CBC at HBOI.

Ammonium enhancement of dark carbon fixation, analyzed by CBC at HBOI (only on samples from May 96, August 96, October 96)..

Statistical analyses:

Calculations. All data calculation, reduction and storage were done using Excel and Quattro Pro spreadsheets. For each sampling, between-sites comparisons of corresponding pairs (i.e. 3A1 and 4A1) were performed using paired *t*-tests using the routines supplied with the spreadsheet programs. Unequal variances were assumed for all comparisons. For other statistical analyses (seasonal, colony and overall effects) reduced data from the spreadsheets were imported into SYSTAT for multi-way ANOVA and correlation analyses. Post-hoc between-groups comparisons with Tukey's HSD procedure were used to examine some seasonal and inter-colony differences at each site.

Samples: Prior to statistical analyses some corals were deleted from the datasets. Five corals from the inshore site showed signs of damage or mortality when collected. One coral in May 96 was covered with black material which appeared to be both the product of reduction and filamentous material. Two corals were omitted from the Oct 96 collections: one core had entirely disappeared from its PVC collar, and 75% of the other coral was missing. Two corals

were omitted from the Mar 97 collection: both had bare areas of the skeleton where tissue had died, and were overgrown with algae (cyanobacteria?).

A loose buoy rope damaged some corals at the inshore site during the last quarter of sampling. These corals showed areas of abrasion where some tissue was lost, mostly from inter-polyp areas. This appeared to be less than 25% of the total surface area. The affected areas were less than 25% of the total, and these corals were used for all analyses except total biomasses.

Since the paired *t* comparisons required matching pairs, incomplete pairs were excluded from the between-sites comparisons. However, incomplete pairs were included in the overall SYSTAT analyses.

RESULTS

Coral Growth Rates

1. Mass accretion (total calcification) Mass accretion is measured by changes in buoyant weight. This assumes that all changes in buoyant weight are the net result of deposition and dissolution of calcium carbonate, and that tissues are neutrally buoyant in seawater. During each of the quarterly samplings, coral explants from the offshore site (CMS4) exhibited consistently higher calcification rates as measured by buoyant weight (Fig. 3). Rates at CMS3 ranged from 54.1% (Aug 96) to 74.8% (Oct 96) of those at the offshore site. It is important to note that the data in Fig. 3 represent the integrated growth from the time explants were first weighed to the time of collection (Table 1), and do not reflect necessarily reflect seasonal differences in growth rates. One would expect that corals would calcify at slower rates during cooler seasons, but this is not evident in the data of Fig. 3.

The results for May 96 deserve particular comment. As seen in Table 1, the coral explants at CMS3 were deployed 24-26 days longer than the explants at CMS4. The extra time meant both that the corals at CMS3 had additional time to recover from experimental manipulation (coring, staining and other handling procedures), and that they were exposed to an additional period of presumably cool water temperatures between mid-February and mid-March. This additional exposure might in part explain the differences in growth rates between the two sites during this period. However, any such effect is likely to be swamped out by overall growth during subsequent sampling periods.

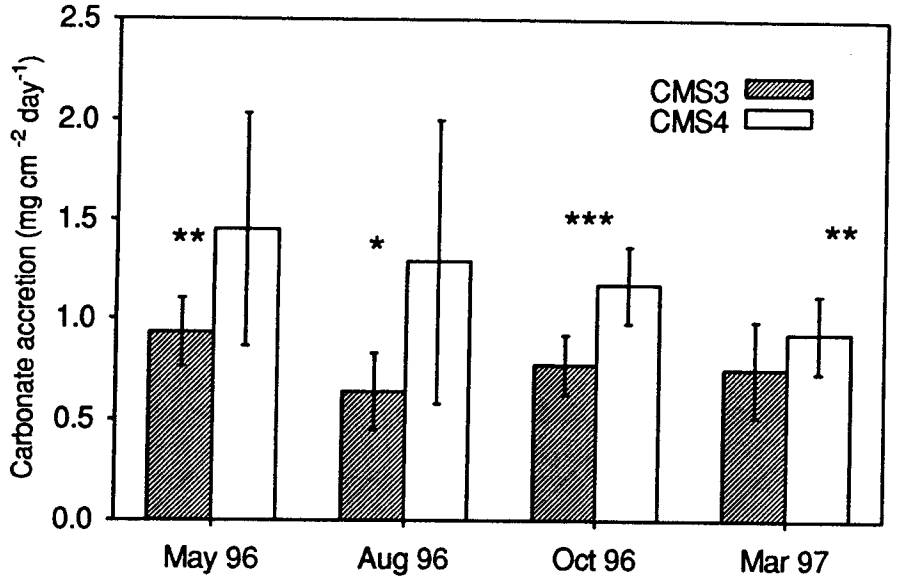


Figure 3. Paired *t*-test comparisons of mass accretion rates (skeletal carbonate, buoyant weight data) by coral explants at inshore (CMS3) and offshore (CMS4) sites. Vertical bars are one standard deviation. N = 10 (May 96, Oct 96, Mar 97) or 12 (Aug 96). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

A complete *t*-test including all paired samples showed a strong effect between sites (CMS4 > CMS3, $p < 0.0001$, $t = -6.571$, d.f = 41). Mean and standard deviations for all samples are given in Table 2. Overall, rates at CMS3 were 60.3% of those at CMS4 (Table 2).

Table 2. Summary of mass accretion rates (buoyant weight) for all corals in the study.

Site	CaCO3 accretion (mg day-1)	S.D.	N*
CMS3	21.07	7.59	43
CMS4	34.95	11.78	47

*Includes unpaired samples

A three-way ANOVA for the entire dataset (site, date and colony) also showed a strong site effect and a slight effect of date (Table 3). There were no significant interactions between these factors, and a two-way ANOVA showed no effect of date or colony. When the data for each site were examined, there were significant effects of colony and date at CMS3 but not CMS4 (Table 4).

Table 3. 3-way ANOVA for effects of site, date and colony on mass accretion rates.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	502.644	3	167.548	1.822	0.153
DATE	721.355	3	240.452	2.615	0.060
SITE	4049.901	1	4049.901	44.038	**0.000**
COLONY*DATE	670.16	9	74.462	0.81	0.609
COLONY*SITE	291.833	3	97.278	1.058	0.374
DATE*SITE	125.068	3	41.689	0.453	0.716
COLONY*DATE*SITE	1037.968	9	115.33	1.254	0.281
ERROR	5333.855	58	91.963		

Table 4. 2-way ANOVA tables for the effects of date (season) and colony on carbonate accretion rates at each site.

A. CMS3:

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	441.013	3	147.004	3.197	**0.039**
DATE	460.084	3	153.361	3.335	**0.034**
COLONY*DATE	259.709	9	28.857	0.627	0.763
ERROR	1241.696	27	45.989		

B. CMS4:

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	377.502	3	125.834	0.953	0.427
DATE	377.659	3	125.886	0.954	0.427
COLONY*DATE	1505.623	9	167.291	1.267	0.293
ERROR	4092.159	31	132.005		

2. Areal growth (x-y axis growth) Our original plan was to use video analysis of projected 3-D images of slides of each core for these measurements. However, the likely errors arising from the 2-D projection of 3-D surfaces convinced us to use the more laborious aluminum foil method. (Even this method does not account for the surface area of living tissue, only of skeleton). As noted above, measurements could only be taken of the explants after final collection, so rates of areal increase for individual explants could not be determined.

The between-site comparisons are shown in Fig. 4. There were no differences in the skeletal surface areas of the explants in the first three sampling periods, but in the fourth quarter the surface areas of the offshore corals at CMS4 were 30% greater than those at CMS3 ($p \leq 0.05$). When the paired t-test included all samples, there was no overall difference between the sites ($t = -1.602$, $p > 0.1$, d.f. = 43).

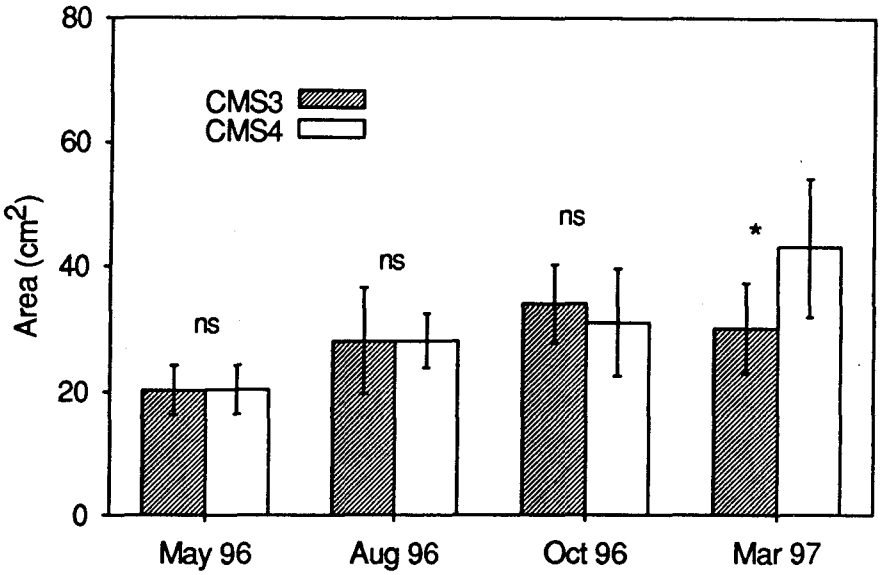


Figure 4. Paired comparisons of skeletal surface areas of harvested coral explants at the two study sites. Statistical parameters as in Fig. 3, except that N = 11 for May 96. ns, not significant ($p > 0.05$).

Over the time of the experiment corals at both sites increased in surface area, as indicated by regression analyses. For CMS3 the regression of area against time yielded an overall rate of 0.034±0.02 cm^2 day^{-1} ($r^2 = 0.212$, n = 44; p [Pearson correlation] < 0.01; mean ± 2 SE). For CMS4 the regression of area against time yielded an overall rate of 0.072±0.02 cm^2 day^{-1} ($r^2 = 0.509$, n = 48; p [Pearson correlation] < 0.001). The regressions for both sites with 95% confidence limits around the slopes are shown in Fig. 5. Much of the difference in overall rates was due to the increase in area of CMS4 corals during the last quarterly sampling (Fig. 4). Most of these corals actually had grown over the PVC collars by this time, and this extension accounted for most of the areal increase in these samples. In contrast, little of this growth was seen in the corals at CMS3. We believe that this was at least partly due to the increase in fouling organisms (algae, hydroids, other epibionts) which was evident on the collars of corals at CMS3 during all periods of sampling. In addition, some of the explants at the inshore site appeared to have lost some tissue during the March 97 sampling; these were not included in our statistical analyses.

Tukey's post-hoc HSD test was used to compare within-site differences at each quarterly sampling. Explants at CMS4 in Aug 96, Oct 96 and Mar 97 had added significantly more skeletal area than those in the preceding period. However, despite the overall trend shown by the regression analysis, only the surface areas of the inshore corals in Aug 96 showed significantly increased growth over the preceding collection ($p < 0.05$).

The strong effect of date is evident in the 3-way ANOVA of the entire dataset (Table 6). There was no overall effect of site in this analysis, nor of donor colony ($0.08 > p > 0.07$ for both). The significant interaction between site and date was probably due to the influence of March 97 samples.

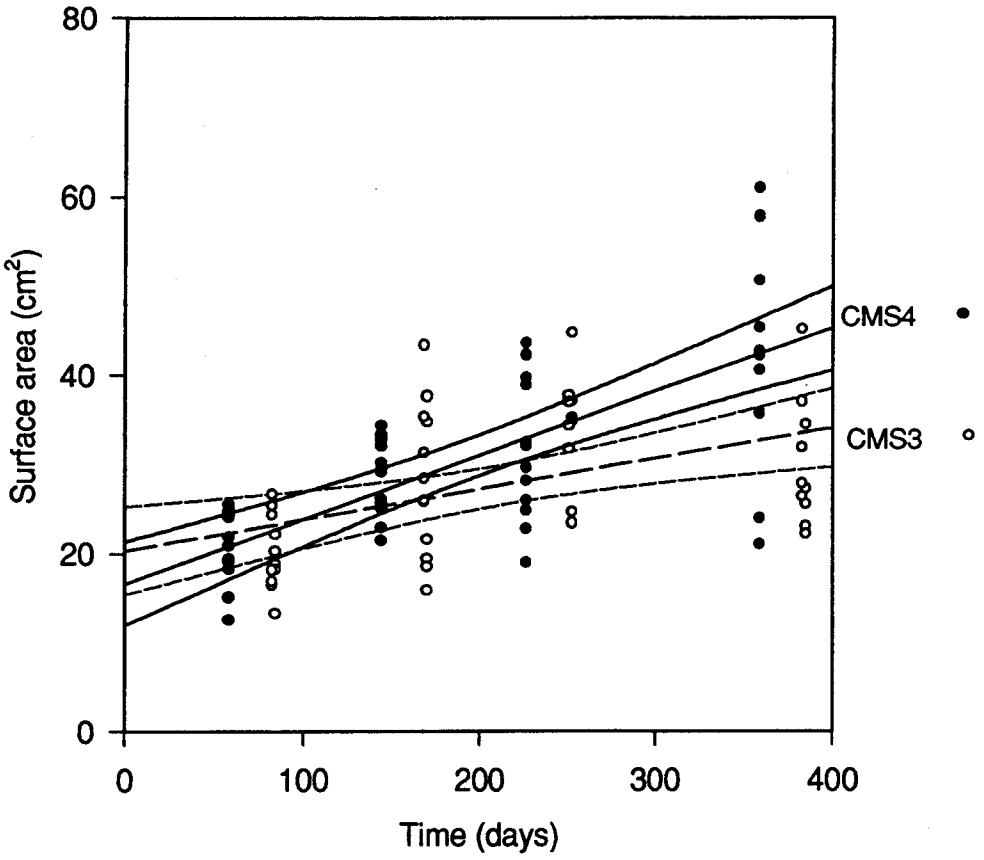


Figure 5. Regressions of areal growth of coral explants at the two study sites. For each site the least-squares linear regression line is plotted, together with the 95% confidence limits. Dashed lines: CMS3; solid lines: CMS4.

Table 6. 3-way ANOVA for effects of site, date and colony on skeletal area.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	377.061	3	125.687	2.42	0.075
DATE	3322.075	3	1107.358	21.325	**0.000**
SITE	170.927	1	170.927	3.292	0.075
COLONY*DATE	301.327	9	33.481	0.645	0.754
COLONY*SITE	321.186	3	107.062	2.062	0.115
DATE*SITE	779.494	3	259.831	5.004	**0.004**
COLONY*DATE*SITE	435.742	9	48.416	0.932	0.504
ERROR	3115.715	60	51.929		

3. Polyp numbers. Polyps are the tentaculate feeding portions of coral colonies, and increase in polyp numbers is clearly related to increase in surface area. New polyps are added as coral

tissue and skeleton are added at the periphery of colonies. However, environmental and other factors can stimulate the formation of new polyps at other sites on the coral surface.

The pair-wise between sites comparisons are shown in Fig. 6. Initial polyp counts (Feb/Mar 96) are given in this figure, and include any damaged polyps. There was no difference between the two sites in the May 96 sampling, but subsequent samples in August 96 and Mar 97 showed that the offshore corals at CMS4 added significantly more polyps than the inshore site. This was perhaps marginally true for the Oct 96 samples ($0.07 \geq p \geq 0.06$). The greatest difference was seen in the Mar 97 corals, when the offshore corals had on average 36% more polyps than those at the inshore site. The May 96 samples at both sites were probably influenced by the tissue damage produced by the drilling procedure, as repair processes probably reduced the production of new polyps. The overall paired *t*-test for all quarterly samples (May 96 through Mar 97) did not show a significant between-sites effect ($p > 0.1$, t=-1.065, d.f. = 43)

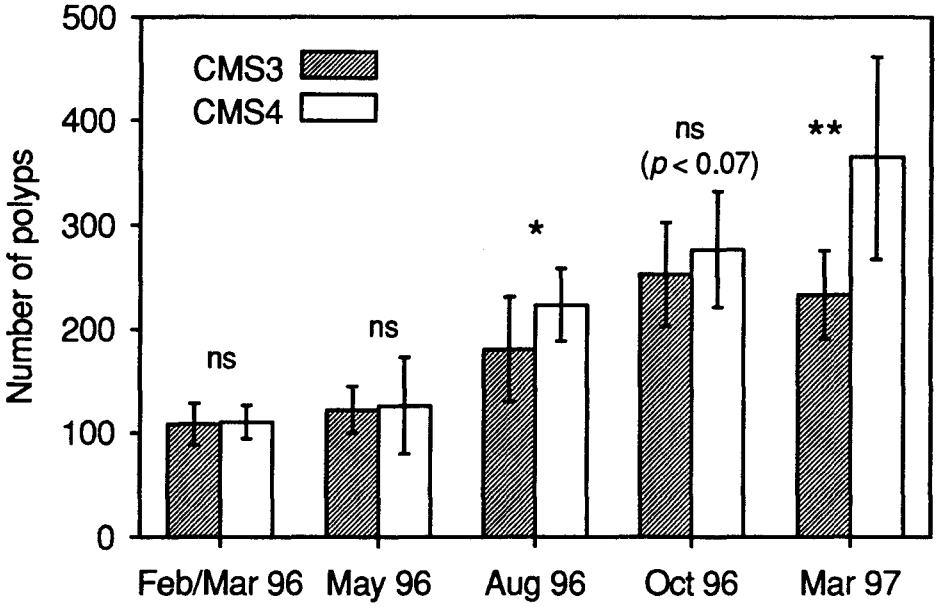


Figure 6. Paired comparisons of total polyp numbers for the coral explants at the two study sites. All of the initial explants are included in the Feb / Mar 96 sample. Statistical parameters as in Fig. 5; N = 48 for the initial samples.

As with surface area, corals at both sites progressively added polyps: polyp number was highly correlated with time (Pearson; $p < 0.001$) at both CMS3 ($r^2 = 0.448$, n = 44) and CMS4 ($r^2 = 0.663$, n = 48). Regressions yielded overall rates of 0.41 ±0.17 polyps day^{-1} at CMS3 and 0.76 ± 0.17 polyps day^{-1} at the offshore site (slope ± 2 SE). These rates are different as indicated by 95% confidence limits (Fig 7). Thus, the overall rate of polyp addition by corals at

the offshore site was significantly greater than that at the inshore site. As with surface areas, post-hoc between-dates analysis of the corals at CMS4 showed that corals had added significantly more polyps at each quarterly sampling (Aug 96, Oct 96, Mar 97). Corals at the inshore site exhibited this progressive growth only in August and October; there was no significant difference in polyp numbers between the October and Mar 97 samples ($p > 0.7$).

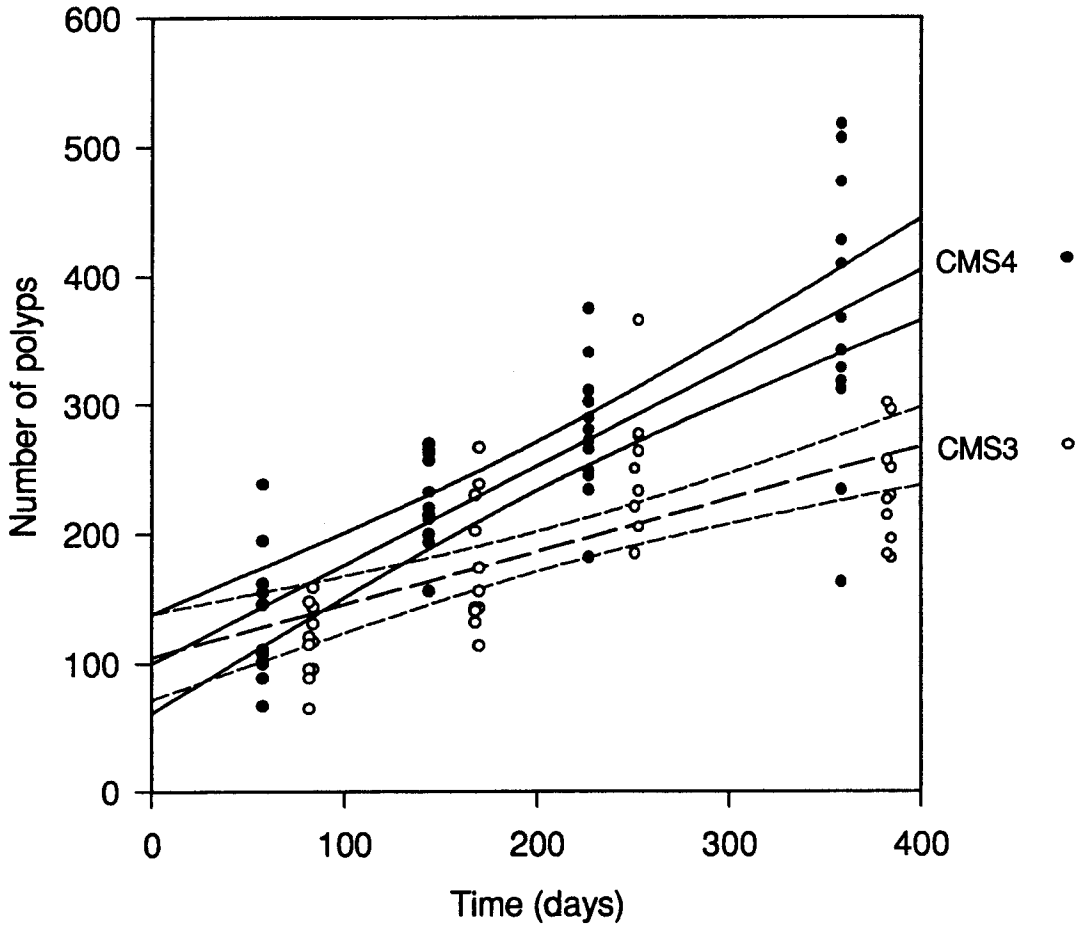


Figure 7. Regressions of polyp growth of coral explants at the two study sites. For each site the least-squares linear regression line is plotted, together with the 95% confidence limits. Dashed lines: CMS3; solid lines: CMS4.

The effects of data and site are evident in the three-way ANOVA of the entire dataset (Table 6). There were no effects due to donor colony.

Table 6. 3-way ANOVA for effects of site, date and colony on total polyp number.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	6338.4	3	2112.8	0.608	0.613
DATE	401918.6	3	133972.9	38.524	**0.000**
SITE	68432.9	1	68432.9	19.678	**0.000**
COLONY*DATE	9230.7	9	1025.6	0.295	0.974
COLONY*SITE	12807.9	3	4269.3	1.228	0.308
DATE*SITE	46557.2	3	15519.1	4.462	**0.007**
COLONY*DATE*SITE	29809.6	9	3312.2	0.952	0.488
ERROR	208660.0	60	3477.7		

4. Polyp density. This derived parameter (polyps cm^{-2}) was calculated from the preceding two quantities. Fig. 8 summarizes the paired comparisons for the between-sites samples. There was no difference in polyp density of explants at the two sites after the first quarterly sampling, but in each of the three subsequent samples corals at the offshore site had significantly higher

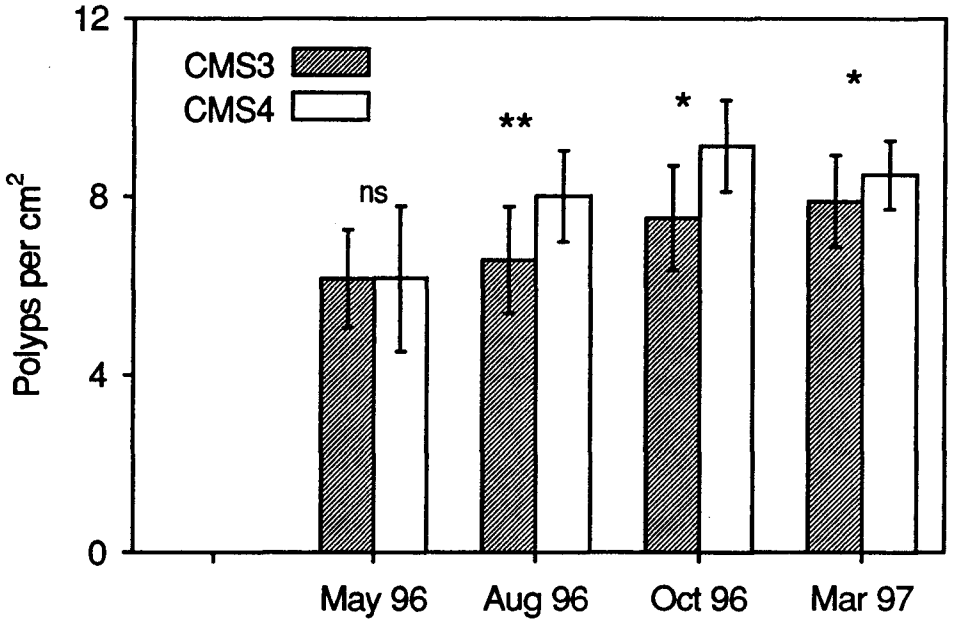


Figure 8. Pairwise comparisons of polyp density of coral explants at the two study sites. Statistical parameters as in Fig. 6.

densities of polyps. When all of the paired comparisons over the entire experiment are included in the analysis, corals at CMS4 had significantly more polyps per unit area than did corals at the inshore site ($p < 0.001$, $t = -5.124$, 43 d.f.). The increased density of polyps at both

sites may have been due to the addition of new but smaller and more closely spaced polyps at the growing edges of the colonies.

The 3-way ANOVA of the entire dataset shows that site, date and source colony all affected polyp density (Table 7). The effect of sampling date is clear in Fig. 7: there is a trend of increasing polyp density at both sites ($p < 0.001$ for both; $r^2\{CMS3\} = 0.304$, N = 44; $r^2\{CMS4\} = 0.289$, N = 48). However, when successive samples at each site (e.g., May 96 vs. Aug. 96) were compared by the Tukey post-hoc procedure, there were no significant differences.

Table 7. 3-way ANOVA for effects of site, date and colony on polyp density (polyps cm^{-2}).

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
DATE	67.126	3	22.375	31.527	**0.000**
COLONY	60.775	3	20.258	28.544	**0.000**
SITE	23.828	1	23.828	33.574	**0.000**
DATE*COLONY	6.209	9	0.690	0.972	0.472
DATE*SITE	7.092	3	2.364	3.331	**0.025**
COLONY*SITE	2.355	3	0.785	1.106	0.354
DATE*COLONY*SITE	1.220	9	0.136	0.191	0.994
ERROR	42.583	60	0.710		

There were consistent differences in polyp density between donor colonies at both sites (Table 8). At CMS3, colonies A and B had similar densities, as did colonies C and D. However, explants from colonies A and B consistently had higher densities than did those from C and D (Table 8A). When data for the two groups were pooled (A plus B vs. C plus D) there was a highly significant difference (two sample *t*-test; Table 8A). Colonies at CMS4 showed a similar relationship, although the differences were not as pronounced (Table 8B).

Table 8. Intercolony comparisons of polyp density.

A. Matrix of probabilities of intercolony comparisons of polyp density of corals at CMS3. Significant differences highlighted in boldface.

COLONY:	A	B	C	D
A	____			
B	0.640	____		
C	**0.000**	**0.007**	____	
D	**0.000**	**0.001**	0.858	____

Two sample t-test of pooled groups (AB vs. CD) (polyps cm^{-2}).

GROUP	N	MEAN	±	SD	*P*
AB	23	7.843	±	0.943	**<0.001**
CD	21	5.943	±	1.016	

B. Matrix of probabilities of intercolony comparisons of polyp density of corals at CMS4. Significant differences highlighted in boldface.

COLONY	A	B	C	D
A	____			
B	0.991	____		
C	0.328	0.201	____	
D	**0.051**	**0.025**	0.781	____

Two-sample *t*-test of pooled groups (AB vs. CD; polyps cm^{-2})

GROUP	N	MEAN	±	SD	*p*
AB	24	8.658	±	1.326	<0.01
CD	24	7.321	±	1.449	

5. Vertical extension (radial growth). Vertical extension of the skeleton (radial growth along the z-axis) was measured as the linear amount of skeleton deposited on initial alizarin red S stain lines. "Boulder" or plate-like corals typically have greater growth in areal (X-Y) dimensions than the z-axis, in contrast to branching corals. In contrast to both the areal and total calcification (buoyant weight) results, there were no between-site differences in radial growth in any of our samples (Fig. 9). Overall, the vertical extension rates at the two sites were remarkably similar, especially after the first sampling period.

The 3-way ANOVA of the effects of site, date and colony (Table 9) revealed significant effects of donor colony and date; as expected from the paired comparisons, there was no effect of site ($p > 0.9$). The effects of date were due to the increased extension rate during the initial

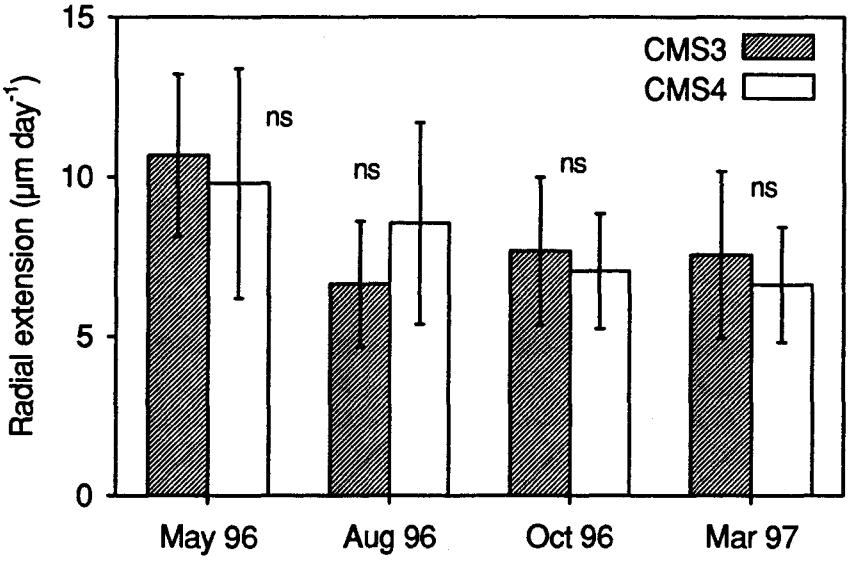


Figure 9. Pairwise comparisons of linear extension (z-axis growth, alizarin red S staining) of coral explants from the two study sites. Statistical parameters as in Fig. 3

2 -3 months. At CMS3, the extension rate of the May96 samples was greater than for the subsequent samples ($p < 0.05$), while the later samples were not different from each other (Tukey post-hoc comparison). At CMS4, a similar trend was observed: the extension rates of the May 96 corals was greater than those from the Oct 96 and Mar 97 samples, but were not significantly different from those in August (Table 10).

Table 9. 3-way ANOVA for effects of site, date and colony on vertical extension (alizarin growth)

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
COLONY	135.841	3	45.280	9.870	**0.000**
DATE	150.428	3	50.143	10.930	**0.000**
SITE	0.007	1	0.007	0.002	0.968
COLONY*DATE	47.864	9	5.318	1.159	0.338
COLONY*SITE	23.700	3	7.900	1.722	0.172
DATE*SITE	55.197	3	18.399	4.010	0.012
COLONY COLONY*DATE*SITE	36.884	9	4.098	0.893	0.537

Table 10. Comparisons between sampling dates of vertical extension (linear growth).

A. Matrix of probabilities of interdate comparisons of vertical extension of corals at CMS3. Significant differences highlighted in boldface

SAMPLE	Aug-96	Mar-97	May-96	Oct-96
Aug-96	____			
Mar-97	0.636	____		
May-96	**0.001**	**0.022**	____	
Oct-96	0.565	1.000	**0.029**	

B. Matrix of probabilities of interdate comparisons of vertical extension of corals at CMS4. Significant differences highlighted in boldface

SAMPLE	Aug-96	Mar-97	May-96	Oct-96
Aug-96	____			
Mar-97	0.084	____		
May-96	0.866	**0.013**	____	
Oct-96	0.205	0.970	**0.039**	____

Analysis of the within-site colony effects on linear growth showed that at CMS3, corals from the AB group had faster extension rates than those from the CD group (Table 10A). However, this was not seen in the corals from the offshore site (Table 10B). Since there were no intercolony differences in total carbonate deposition (Table 3), this suggests that the AB corals made less dense skeletons, and that this is effect is more pronounced at the in shore site receiving the greater ebb tide flow from Florida Bay. This may relate to our observations when the coral colonies were cored that skeletons of the A and B colonies were easier to cut than those of the C and D colonies.

Table 10. Intercolony comparisons of vertical extension.

A. Matrix of probabilities of intercolony comparisons of linear growth of corals at CMS3. Significant differences highlighted in boldface

COLONY	A	B	C	D
A	____			
B	0.320	____		
C	**0.044**	**0.001**	____	
D	0.120	**0.002**	0.971	____

Two-sample *t*-test of pooled groups:

GROUP	N	MEAN (μm day^{-1}) ± SD	*p*
AB	23	9.543 ± 2.313	<0.001
CD	20	6.307 ± 2.309	

B. Matrix of probabilities of intercolony comparisons of linear growth of corals at CMS4. Significant differences highlighted in boldface

COLONY	A	B	C	D
A	____			
B	0.994	____		
C	0.422	0.284	____	
D	0.939	0.836	0.768	____

Two-sample *t*-test of pooled groups:

GROUP	N	MEAN (μm day^{-1}) ± SD	*P*
AB	24	8.713 ± 3.516	ns
CD	24	7.324 ± 1.903	

Summary: Site-dependent effects on coral growth

The differences in growth at our two study sites – CMS3, exposed to direct tidal flow from Florida Bay, and CMS4, on the edge of the Florida Reef tract and generally protected from tidal flow, can be summarized as follows:

Growth parameter	Effect
• **Rates of mass accretion (total calcification)**	• Reduced at all sampling times at CMS3.
• **Skeletal surface area**	• Surface areas of corals at CMS4 exceeded those at CMS3 in last sampling. Overall rates of areal growth slightly higher at CMS4.
• **Polyp numbers**	• Polyp numbers greater at CMS4 in two of the sampling periods. Overall rate of polyp addition greater at CMS4 than at CMS3.
• **Polyp density**	• Greater at CMS4 in the last three quarterly samples; CMS4 corals overall had greater polyp density.
• **Rates of vertical (linear) extension**	• No difference between sites.

Conclusions Regarding Coral Growth

One of the objectives of this study was to assess how Florida Bay water emanating from Channel #5 affected coral growth. Measurements of areal growth were generally similar up to the last sampling, and vertical extension was virtually identical at both sites. Thus, spatial or volumetric growth was the same. However, rates of $CaCO_3$ accretion was consistently higher in the coral explants placed offshore. This suggests that the density of skeletons made by the offshore corals was higher than the density of inshore corals. This may have consequences for the structural integrity of the corals or the ability of the skeletons to withstand bioerosion.

There were no obvious seasonal effects on $CaCO_3$ accretion, but the experimental design did not allow analysis of any such effects. Given the integrated growth data and the fact that the explants had constantly increasing circumferences, dissection of seasonal effects from the datasets is difficult. We did find that corals collected from the offshore site during the last sampling (Mar. 97) had greater tissue areas and polyp numbers than those at the inshore site. Since these parameters were generally similar up to this time, it is not clear whether this was a seasonal effect, or if it resulted from particular conditions during the last quarter that negatively affected the corals at the inshore site. The inshore explants appeared to be healthy when observed in Oct. 96, but were clearly impacted over the winter. The winter of 1996/97 was not particularly severe, and was in fact warmer than usual. We do not know what caused the mortality and reduced tissue growth at the inshore site, but this observation may indicate that episodic events there may reduce spatial growth over time.

Although surface areas increased at about the same rate at both sites during the first three quarters, corals at the offshore site had higher rates of polyp addition, and thus, polyp density. This may be indicative of the greater growth potential of the offshore corals which may have been constrained in their ability to expand areally because of the PVC collar mounting system. The rate of area increase did jump offshore during the last quarter and substantial growth was observed down the sides of the collars at this time. This expansion is also reflected in lower polyp densities on CMS4 corals collected Mar., 97. The vertical extension data shows a trend to lower rates. This, too, may be related to the mounting system. The corals had to grow up to some degree before they could expand in area.

When coring the original colonies, we noticed that colonies A and B were much easier to drill than colonies C and D. Colonies C and D also had bright green oral disks, in contrast to those of A and B which were uniformly brown. Although we observed no strong effects of colony on areal growth or on the number of polyps, polyp densities were generally greater on corals from A and B colonies than from C and D. At CMS3 (but not CMS4), A and B had greater vertical extension rates than C and D. Since there were no inter-colony differences in skeletal mass accretion, the observation suggests that, at the inshore site, the A and B corals were making a less dense skeleton than the C and D corals. This perhaps relates to the differences we observed during the drilling process.

The taxonomy of the *"Montastraea annularis"* complex (which includes *M. faveolata*) has been recently revised. Since polyp and skeletal density are two of the characters that are used to separate these sometimes confusing corals, it is possible that there were significant genetic difference between our donor colonies. However, we planned all of our between-site statistical tests as paired comparisons between explants taken from the same colony, so that any inter-colony differences would not have affected these statistical analyses.

Indices of Nutrient Sufficiency in Symbiotic Zooxanthellae

1. Chlorophyll content. The chlorophyll *a* content of algae is a response both to ambient light levels and to nitrogen supply, and can be used as an indicator of nutrient history. All other factors being equal, algae with increased nitrogen sources will contain higher amounts of chlorophyll. We extracted total chl *a* and chl c_2 from the symbiotic algae of our coral samples. Figure 10 summarizes the pairwise comparisons of cellular chl *a* content between our two sites on either side of Hawk Channel.

At every quarterly sampling, zooxanthellae from corals at the inshore site contained more chl *a* per cell than corresponding explants at the offshore site. When all pairs of explants were included in the analysis, this effect was highly significant ($p < 0.0001$; $t = 5.64$, d.f. = 38). The effect of site on chl a content was evident in the three-way ANOVA of the entire dataset, as well as a highly significant effect of sampling date (Table 11). The pattern of high per cell values seen in the May and October quarterly samples were also seen in chl c_2 content (Fig. 11), as well as in the C:N:P and FAA data (see below). Whether this effect represented seasonal differences in cellular content or cell size or was due to some anomaly in cell counts between samples is not clear. There were no effects due to inter-colony differences.

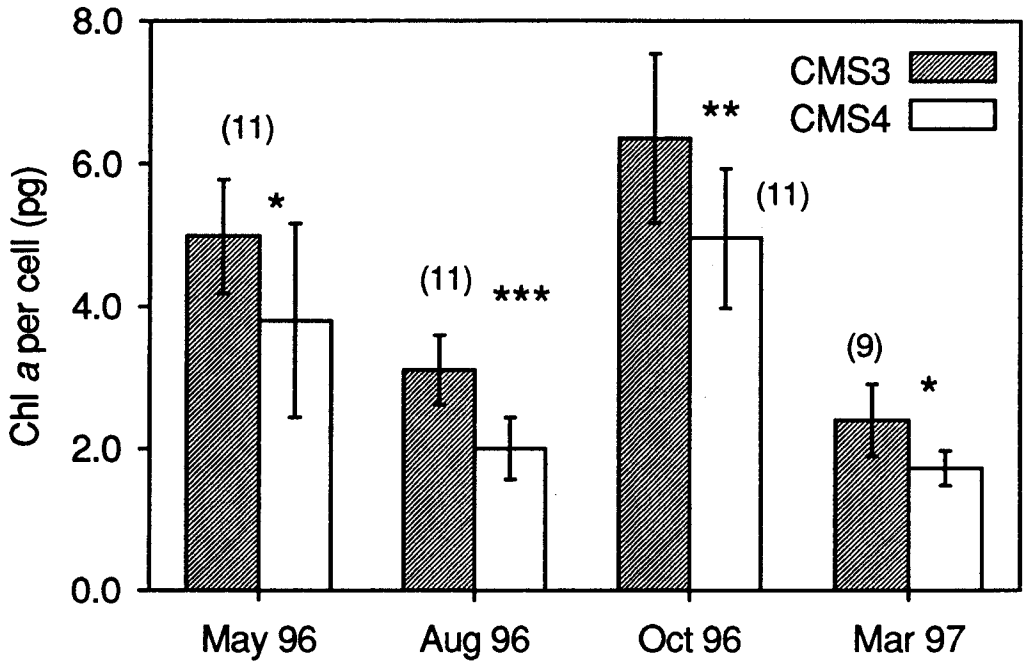


Figure 10. Between site pairwise comparisons (paired *t*) of chlorophyll *a* content of zooxanthellae from coral explants at the two study sites. Statistical parameters as in Fig. 3; sample sizes in parentheses.

Table 11. Three-way ANOVA of the complete dataset for effects of site, date and colony on chlorophyll *a* content of zooxanthellae.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
DATE	160.082	3	53.361	72.031	**0.000**
COLONY	2.065	3	0.688	0.929	0.433
SITE	27.959	1	27.959	37.741	**0.000**
DATE*COLONY	3.681	9	0.409	0.552	0.830
DATE*SITE	2.104	3	0.701	0.947	0.425
COLONY*SITE	0.997	3	0.332	0.448	0.719
DATE*COLONY*SITE	12.811	9	1.423	1.921	0.068
ERROR	40.744	55	0.741		

Chlorophyll c_2 is an accessory photosynthetic pigment of dinoflagellates, the algal group to which zooxanthellae belong. It is thought to be synthesized in response to low light levels, and levels of chl c_2 may also track with nitrogen supply. Zooxanthellae from explants at the inshore site had significantly higher chl c_2 levels in two of the four quarterly samples (Fig. 11). When all samples were included in the comparison, chl c_2 levels at CMS3 were significantly higher than those at CMS4 ($p < 0.01$, $t = 2.729$, d.f. = 36).

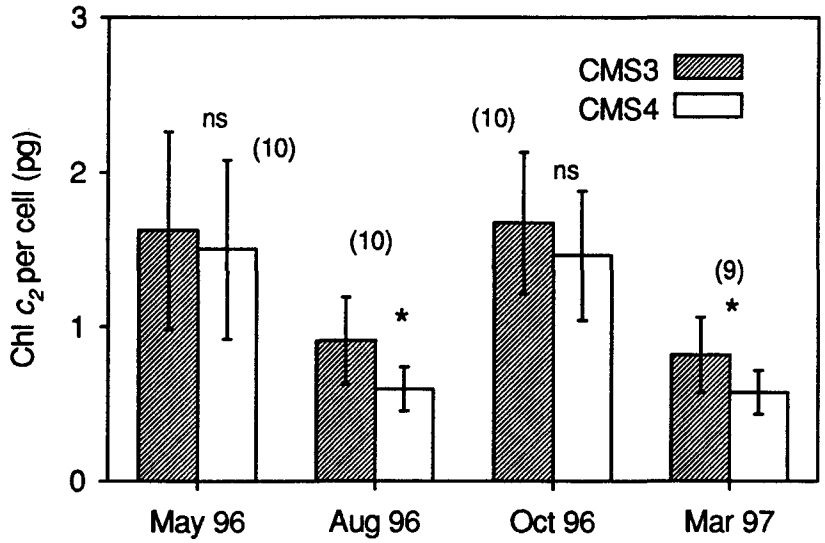


Figure 11. Paired between-site comparisons of chl c_2 content of coral zooxanthellae from the two study sites. Statistical parameters as in Fig. 10.

The ratios of chl *a* to chl c_2 may reflect light history of corals and zooxanthellae. There may be increased synthesis of *c2* as part of a photoadaptive response, and ratios have been reported to increase with reduced light intensity in a few (but not most) studies of adaptation

of these algae to variations in light intensity. The absolute levels of chl c_2 our seasonal samples track with those of chl a (Figs. 10 and 11), and the seasonal samples show no intersite difference in a / c_2 ratios (Table 12). A paired t-test including all of the samples showed no overall difference between the sites ($p > 0.1$, $t = 1.334$, d.f. 36).

Table 12. Pairwise comparisons of chl a / chl c_2 ratios of zooxanthellae from the inshore and offshore sites (paired t-tests). Data expressed as mean ± one standard deviation.

Sample	CMS3	N	CMS4	N	p
May 96	3.10 ± 0.76	(10)	2.66 ± 0.50	(10)	ns
Aug 96	3.97 ± 1.23	(10)	3.42 ± 1.18	(10)	ns
Oct 96	3.73 ± 0.51	(10)	3.44 ± 0.79	(10)	ns
Mar 97	2.99 ± 0.30	(9)	3.03 ± 0.44	(9)	ns

As noted above, the inter-site differences in chl a content of coral zooxanthellae could reflect either differences in light levels or in nitrogen supply. As is clear from all of the following sections on nutrient limitation / sufficiency, there were no between-site differences in the nitrogen status of zooxanthellae in any of our quarterly samples, using any of our other assays of N-status. N was in at least sufficient supply at both of our sites throughout the study. Thus, we believe that the differences in chl a levels between our study sites is indicative of reduced light levels at the inshore site.

2. Elemental Ratios. Elemental ratios (carbon:nitogen:phosphorus; C:N:P) are standard indicators of past nutrient supply for plants. Algae (phytoplankton) growing under nutrient-replete conditions typically exhibit "Redfield ratios" (C:N:P = 106:16:1). We determined the elemental composition of zooxanthellae from the corals in our study.

a. C:N ratios: Fig. 12 summarizes the between-sites pairwise comparisons for the four quarterly sampling periods. There was clearly no difference in C:N between sites at any time. The values for C:N were remarkably similar throughout the study, generally ranging between 6 and 7. Since the Redfield ratio for C:N is 6.6:1 for N-sufficient algae, these data indicate that zooxanthellae from corals at both sites were exposed to sufficient (if not excess) nitrogen. These values are among the lowest reported for coral zooxanthellae, and are lower than the majority of published values for nutrient-sufficient algae. However, the overall paired t-test, including all four quarterly samples, yielded a slightly higher C:N at CMS4 (Table 13). The overall mean value at CMS4 is still indicative of N-sufficiency.

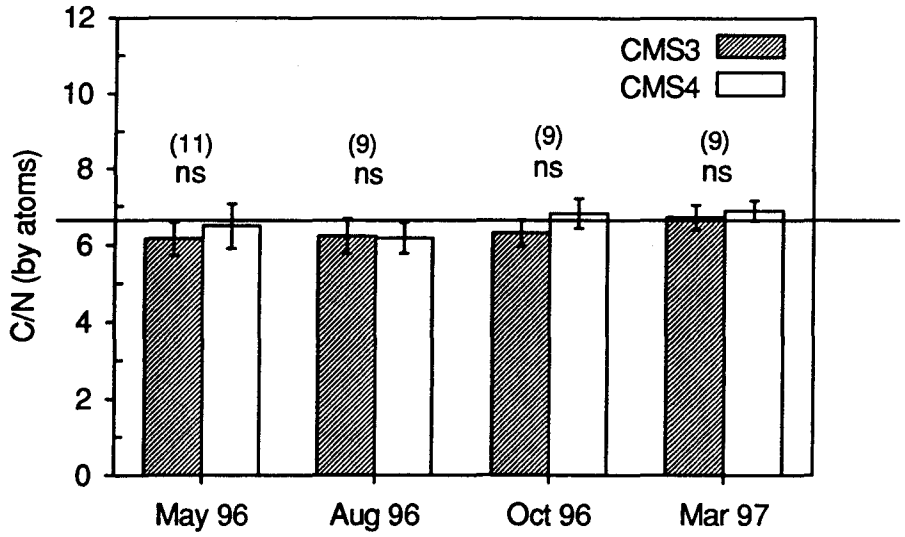


Figure 12. Pairwise between-site comparisons of C:N ratios of zooxanthellae from corals at the two study sites. The horizontal line indicates the N-sufficient Redfield ratio of 6.6:1. Statistical parameters as in Fig. 10

Table 13. Paired *t*-test between site comparison of C:N ratios for zooxanthellae of all paired corals in the study.

SITE	MEAN		ST. DEV.	N	*P*
CMS3	6.518	±	0.515	38	<0.05
CMS4	6.819	±	0.481	38	

The three-way ANOVA for the complete dataset revealed both this slight effect of site and an effect of sampling date (Table 14). At CMS3, the Aug 96 samples had the lowest C:N ratios (lower than any of the others; $p < 0.05$, Tukey HSD); none of the other samples differed from each other. At CMS4, the Aug 96 samples tended to have lower C:N ratios than the other samples, although the only significant difference was with the May 96 corals ($p < 0.05$). As with the overall site effects, these differences between sampling dates were small (Fig. 12).

Table 14. Three-way ANOVA of the complete dataset for effects of site, date and colony on C:N ratios of zooxanthellae.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*P*
DATE	4.989	3	1.663	8.113	**0.000**
COLONY	0.042	3	0.014	0.068	0.977
SITE	1.958	1	1.958	9.555	**0.003**
DATE*COLONY	0.709	9	0.079	0.385	0.937
DATE*SITE	0.599	3	0.200	0.974	0.412
COLONY*SITE	0.320	3	0.107	0.520	0.670
DATE*COLONY*SITE	2.510	9	0.279	1.361	0.230
ERROR	10.658	52	0.205		

b. C:P ratios. Fig. 13 summarizes the between-sites comparison for C:P ratios of zooxanthellae in the quarterly samples. In three of the four samplings there were no significant differences between the sites. In the Aug 96 samples, C:P ratios of zooxanthellae from the inshore site were significantly higher than those of the offshore site, indicating relatively more P-limitation at CMS3 at this time. The overall paired t-test (all samples) did not show a significant between-site difference (Table 15). However, all of the mean values for both sites appear to be elevated over the Redfield ratio for P sufficiency of 106. This indicates that zooxanthellae from both sites experienced slight P-limitation.

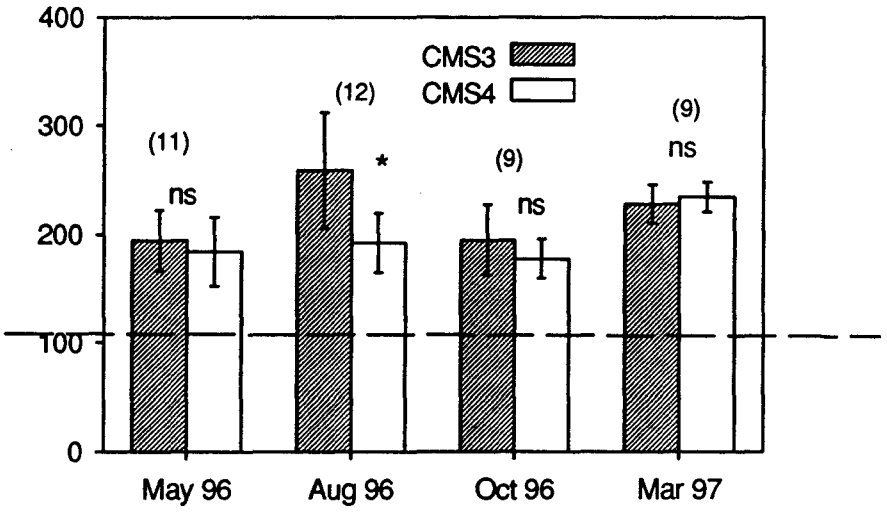


Figure 13. Pairwise between-site comparisons of C:P ratios of zooxanthellae from corals at the two study sites. Statistical parameters as in Fig. 10. The dashed line indicates the Redfield ratio for P-sufficiency (C:P = 106).

Table 15. Paired *t*-test between site comparison of C:P ratios for zooxanthellae of all paired corals in the study.

SITE	MEAN		ST. DEV	N	*p*
CMS3	214.417	±	55.596	41	>0.05
CMS4	195.825	±	32.064	41	

Table 16 contains the three-way ANOVA for the entire dataset, examining the effects of site, date and colony. All three factors had a significant effect on C:P ratios, with sampling date being the most significant. At CMS3, the Aug 96 samples were significantly higher than the May and Oct samples ($p < 0.05$ for both; Tukey HSD), indicating increased P-limitation at that time. There were no other significant between-date differences at this site. At CMS4, the Mar 97 values were significantly higher than any of the other samples ($p < 0.05$ for all; Tukey HSD), indicating increased P-limitation at the offshore site at that time. No other comparisons were significant.

Table 16. Three-way ANOVA of the complete dataset for effects of site, date and colony on C:P ratios of zooxanthellae

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*P*
DATE	30102	3	10034	8.408	**0.000**
COLONY	15398	3	5133	4.301	**0.008**
SITE	7755	1	7755	6.498	**0.014**
DATE*COLONY	4126	9	458	0.384	0.938
DATE*SITE	21178	3	7059	5.915	**0.001**
COLONY*SITE	11516	3	3839	3.217	**0.029**
DATE*COLONY*SITE	16496	9	1833	1.536	0.158
ERROR	68027	57	1193		

The intercolony differences appeared to be minor. At CMS3, zooxanthellae from colony D had slightly higher C:P than those from colony C ($0.05 > p > 0.04$); there were no other intercolony differences at the inshore site. None of the samples from CMS4 showed any colony effect.

c. N:P ratios. As suggested by the C:N and C:P data, N:P ratios at the inshore site were higher in August they were at the offshore site (Fig. 14); they were also significantly higher in the October samples. The overall paired t-test including all samples showed that N:P ratios were higher at CMS3 ($p < 0.01$; $t = 3.371$, 35 d.f.) Given the Redfield ratio of 16:1 for N:P, the values in Fig. 14 indicate N-sufficiency / P-limitation at both sites.

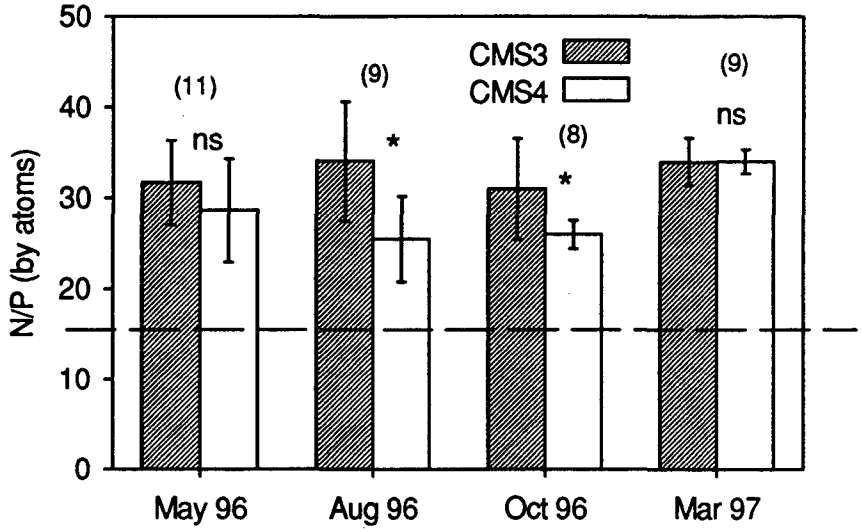


Fig. 14. Pairwise between-site comparisons of N:P ratios of zooxanthellae from corals at the two study sites. Statistical parameters as in Fig. 10. The dashed line indicates the Redfield ratio for N and P-sufficiency.

The three-way ANOVA of the entire dataset for the effects of site, date and donor colony for N:P ratios showed the same trends as C:P: there were significant effects of date and colony in addition to site (Table 17). At CMS3, the Aug 96 samples had significantly higher N:P than did the May and October samples ($p < 0.05$ for each). There were no other significant differences, even though the data in Fig. 14 indicated elevated N:P in Mar 97. At CMS4, the N:P ratios of the Mar 97 corals were significantly higher than the other three seasonal samples; the other three groups did not differ from each other. These seasonal results indicate that at both sites, N was always available in sufficient if not excess amounts. At the inshore site P-limitation appeared during the summer of 1996 (and possibly Mar 97), while this condition appeared at the offshore site in Mar 97.

Table 17. Three-way ANOVA of the complete dataset for effects of site, date and colony on NP ratios of zooxanthellae.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	p
DATE	342.732	3	114.244	7.393	**0.000**
COLONY	218.580	3	72.860	4.715	**0.006**
SITE	435.807	1	435.807	28.204	**0.000**
DATE*COLONY	144.850	9	16.094	1.042	0.421
DATE*SITE	266.156	3	88.719	5.742	**0.002**
COLONY*SITE	204.467	3	68.156	4.411	**0.008**
DATE*COLONY*SITE	154.996	9	17.222	1.115	0.369
ERROR	803.507	52	15.452		

d. CNP content of zooxanthellae. Fig. 15 summarizes the data for the actual (per cell) CNP content of zooxanthellae from these corals. The pairwise between-sites comparisons indicate that in the initial (May 96) sampling, zooxanthellae from the offshore site had higher levels of all three elements than did those at the inshore site, even though the elemental ratios during this time did not differ (cf. Figs. 12-14). Whether this was due to differences in cell size or to other factors is not clear. The only other inter-site difference was in the P-content of zooxanthellae from in Aug 96 (Fig. 15C), when samples from CMS3 had lower P content than zooxanthellae of the offshore samples ($p < 0.05$). This indicates that the trends in C:P and N:P ratios seen at the inshore site during the summer of 96 were primarily due to P-limitation.

The between site effects seen in the paired comparisons are reflected in the three-way ANOVA's of the entire dataset for cellular content of C, N and P (Table 18). There were no overall between-site differences in carbon or nitrogen content (Tables 18A, B), providing further indication of the N-sufficiency at both sites. The increased P-limitation of the inshore site in summer resulted in a significant overall effect (Table 18C)

The data in Fig. 15 suggest that there were differences between sampling dates at both sites. This is seen in the three-way ANOVA (Table 18) There was a strong effect of date for all three elements, even though there were few between-site differences. Post-hoc between-dates analyses at each site revealed that the Mar 97 samples were significantly less than the other samples at both sites for all three elements. The May and October samples were not different (both sites, all three elements), but the Aug values tended to be less than these at both sites. This is the same pattern seen on the pigment per cell data. There were no significant effects of colony on CNP content of the zooxanthellae.

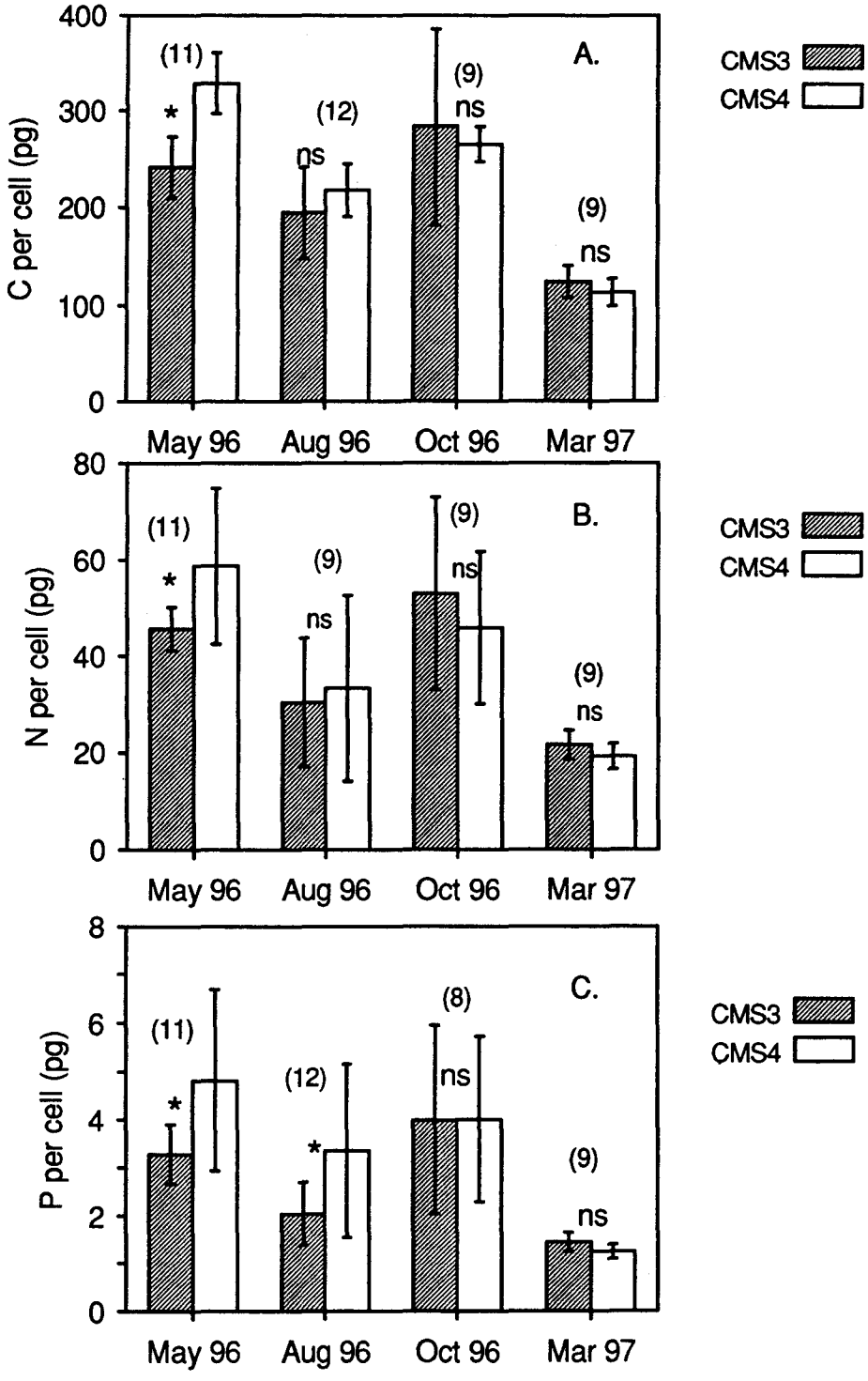


Fig. 15. Pairwise comparison of elemental content of zooxanthellae from corals at the two study sites. Statistical conventions as in Fig. 10. A, C per cell; B, N per cell; C, P per cell

Table 18. Three way ANOVA's of the entire dataset for cellular content of carbon, nitrogen and phosphorus of zooxanthellae at the two sites.

18A: Carbon per cell:

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*p*
DATE	356850	3	118950	25.775	**0.000**
COLONY	7944	3	2648	0.574	0.635
SITE	17036	1	17036	3.691	0.060
DATE*COLONY	44632	9	4959	1.075	0.395
DATE*SITE	22344	3	7448	1.614	0.196
COLONY*SITE	3058	3	1019	0.221	0.882
DATE*COLONY*SITE	91907	9	10212	2.213	0.034
ERROR	267669	58	4615		

Table 18B. Nitrogen per cell:

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*p*
DATE	12054.36	3	4018.12	30.437	**0.000**
COLONY	422.70	3	140.90	1.067	0.371
SITE	167.53	1	167.53	1.269	0.265
DATE*COLONY	2006.32	9	222.93	1.689	0.115
DATE*SITE	794.87	3	264.96	2.007	0.124
COLONY*SITE	336.80	3	112.27	0.850	0.473
DATE*COLONY*SITE	3625.06	9	402.78	3.051	0.005
ERROR	6996.74	53	132.01		

Table 18C. Phosphorus per cell:

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*P*
DATE	95.03	3	31.678	20.222	**0.000**
COLONY	2.28	3	0.761	0.486	0.693
SITE	11.49	1	11.486	7.332	**0.009**
DATE*COLONY	15.10	9	1.678	1.071	0.398
DATE*SITE	7.67	3	2.556	1.632	0.192
COLONY*SITE	3.21	3	1.068	0.682	0.567
DATE*COLONY*SITE	33.37	9	3.708	2.367	0.024
ERROR	89.29	57	1.567		

3. Free amino acid composition. The composition of the free amino acid (FAA) pool of algae provides a number of indices of nitrogen sufficiency or limitation. Two ratios are of interest, and both depend on the relative abundance of free amino acids that are high in nitrogen content. One is the glutamine - glutamate (Gln:Glu) ratio, and the other is the proportion of basic free amino acids (arginine, glutamine, lysine, histidine, ornithine) in the total FAA pool. Both of these ratios increase with N-sufficiency.

a. Glutamine:glutamate ratios: A Gln:Glu ratio of > 0.5 commonly indicates N sufficiency, while values < 0.1 are thought to indicate N stress. The between-sites comparisons

of zooxanthellae from the paired explants are shown in Fig. 16. There were no differences in this ratio in any of our quarterly samples: the overall paired *t*-test (including all samples) showed absolutely no difference ($p > 0.9$. $t = 0.041$, 28 d. f.). All of the mean values shown in Fig. 16 exceed 0.5; means for Oct 96 and Mar 97 are exceptionally high. These values corroborate the elemental ratio data and indicate that N supplies for the coral zooxanthellae were either sufficient or in excess at both sites throughout our study.

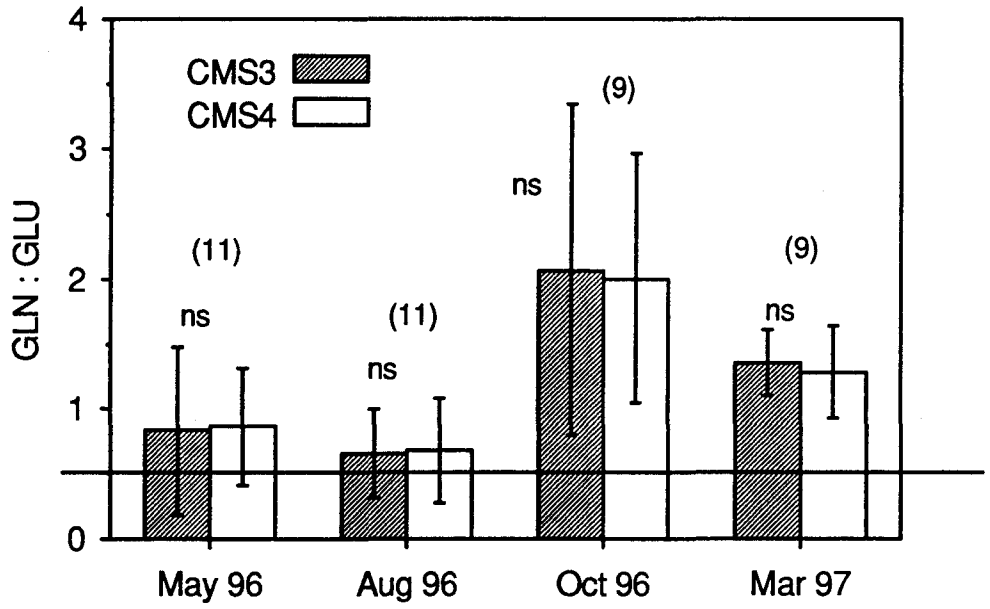


Fig. 16. Paired comparisons of glutamine:glutamate ratios of zooxanthellae at the two study sites. Statistical parameters as in Fig. 10. The horizontal line indicates N-sufficiency (Gln:Glu = 0.5).

The three-way ANOVA for the effects of site, date and source colony showed a significant effect only for date (Table 19.) This effect was largely due to the elevated values at both sites in Oct 96. At both the inshore and offshore sites the Oct 96 values were significantly higher than those in Aug and May 96, while the Mar 97 values were intermediate.

Table 19. Three way ANOVA of the entire dataset for glutamine:glutamate ratios of zooxanthellae at the two sites.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*P*
DATE	14.815	3	4.938	11.086	**0.000**
COLONY	0.872	3	0.291	0.653	0.585
SITE	0.004	1	0.004	0.009	0.924
DATE*COLONY	0.772	9	0.086	0.192	0.994
DATE*SITE	0.309	3	0.103	0.231	0.874
COLONY*SITE	1.916	3	0.639	1.434	0.243
DATE*COLONY*SITE	1.330	9	0.148	0.332	0.960
ERROR	23.163	52	0.445		

b. Basic FAA : Total FAA ratios: The results for the analysis of basic FAA to total FAA are shown in Fig. 17. As with the Gln:Glu ratios, there were no between-site differences in any of the sampling periods. With the exception of the Aug 96 samples, zooxanthellae from both sites had values approaching or exceeding 0.25, indicative of N-sufficiency in cultured zooxanthellae. The Oct 96 samples did not exhibit elevated values, in contrast to the Gln:Glu ratio. The Aug 96 samples were somewhat lower.

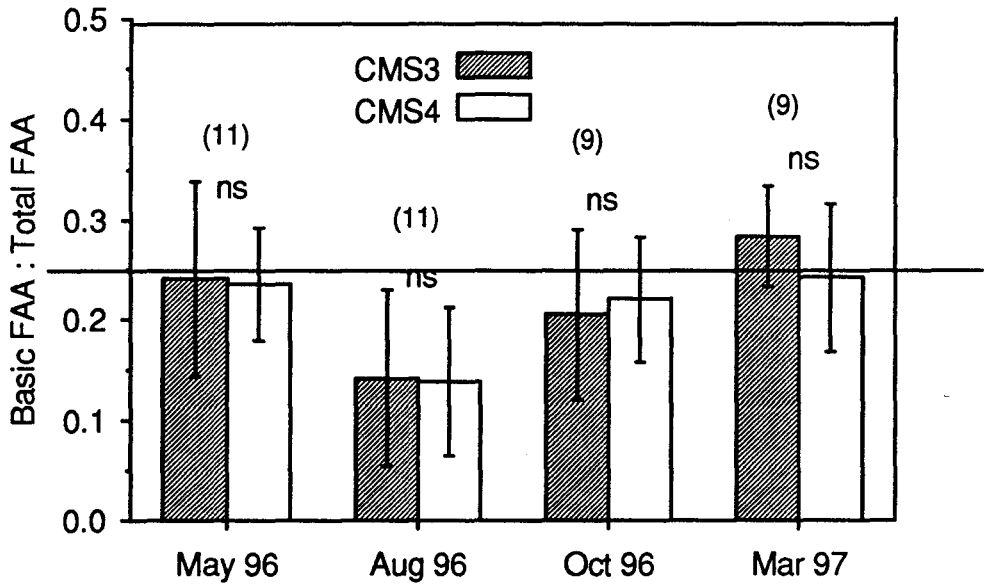


Fig. 17. Paired comparisons of basic FAA:total FAA ratios of zooxanthellae at the two study sites. Statistical parameters as in Fig. 10. The horizontal line indicates N-sufficiency (Basic FAA:Total FAA = 0.25).

The three-way ANOVA for the effects of site, date and source colony using the entire dataset reiterated the lack of difference in N-status of the zooxanthellae from corals at the two

sites (Table 20). As with the Gln:Glu ratios, there was an effect of date; in addition, there was an effect of source colony. However, post-hoc comparisons of the seasonal data at CMS3 showed no significant differences between any of the sampling periods, although the Aug 96 samples at CMS4 were significantly lower than the other samples at that site. None of the inter-colony comparisons at each site revealed a significant effect.

Table 20. Three way ANOVA of the entire dataset for basic:total free amino acid ratios of zooxanthellae at the two sites.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	*p*
DATE	0.141	3	0.047	10.486	**0.000**
COLONY	0.059	3	0.020	4.387	**0.008**
SITE	0.002	1	0.002	0.509	0.479
DATE*COLONY	0.057	9	0.006	1.413	0.207
DATE*SITE	0.014	3	0.005	1.065	0.372
COLONY*SITE	0.018	3	0.006	1.314	0.280
DATE*COLONY*SITE	0.027	9	0.003	0.675	0.728
ERROR	0.234	52	0.004		

c. Total FAA content of zooxanthellae. The total free amino acid content of zooxanthellae is not an indicator of nitrogen status, but we include these data to point out the consistent between-sampling trends in data expressed per cell There were no consistent differences between sites (Fig. 18). Using the complete dataset of paired comparisons,

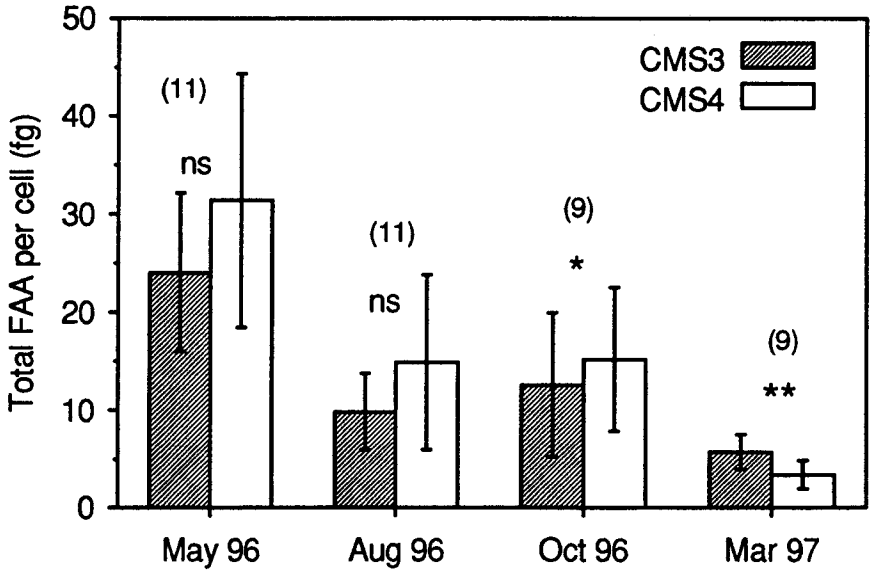


Fig. 18. Pairwise between-site comparisons of the total free amino acid content of zooxanthellae from the two study sites. Statistical parameters as in Fig. 10.

zooxanthellae from the offshore site had slightly more FAA per cell than those from the inshore site (20.5±13.0 vs. 15.8±9.0; $P < 0.05$, paired t; $t = 2.27$, 29 d.f.), but a site effect was not significant in the three way ANOVA (Table 21). However, the trends seen in the chl a per cell and CNP per cell data are clear in this figure. There was a clear effect of sampling date on the entire dataset (Table 21), but none of either site or donor colony. At both CMS3 and CMS4, the values for May 96 were again higher than during the other periods, and the Mar 97 values were the smallest (Tukey post-hoc comparisons).

Table 21. Three way ANOVA of the entire dataset for total free amino acid content of zooxanthellae at the two sites.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	p
DATE	5260.651	3	1753.550	25.697	**0.000**
COLONY	122.311	3	40.770	0.597	0.619
SITE	161.145	1	161.145	2.361	0.130
DATE*COLONY	489.005	9	54.334	0.796	0.621
DATE*SITE	127.461	3	42.487	0.623	0.604
COLONY*SITE	160.897	3	53.632	0.786	0.507
DATE*COLONY*SITE	255.230	9	28.359	0.416	0.921
ERROR	3616.676	53	68.239		

4. Ammonium enhancement of dark carbon fixation: This technique also is an indicator of nitrogen status (limitation or sufficiency) of marine algae. It involves the non-photosynthetic fixation of CO_2 by algae. Algae that are nitrogen-limited typically respond to the addition of ammonium in the dark with increased CO_2 fixation over seawater rates. (This results in the synthesis of amino acids with multiple carboxyl groups.) Ammonium addition has no effect on dark CO_2 fixation in nitrogen-sufficient algae. This effect is usually expressed as the ammonium enhancement ratio (CO_2 fixation rate with ammonium / CO_2 fixation rate in seawater.) We have previously shown that zooxanthellae from healthy colonies of *Montastraea* in Bermuda show a typical N-limited enhancement ratio of about 1.5.

We assayed ammonium enhancement only in the May 96, Aug 96 and Oct 96 samples. In Aug 96, some samples had to be discarded due to incorrect ammonium concentrations. There were no differences in enhancement ratios in the between-sites paired comparisons (Fig. 19). When all of the paired comparisons were pooled, there was no difference between the sites (CMS3: 1.15±0.19; CMS4: 1.06±0.16; $p > 0.05$, $t = 1.967$, 23 d.f.). The mean values are only slightly above unity for both sites. A paired t-test for the effect of ammonium on dark carbon fixation rates over the entire dataset for each site showed no difference vs. rates in seawater (Table 22). The lack of ammonium enhancement throughout this experiment is yet another indication that N supplies were sufficient, if not in excess, at both sites.

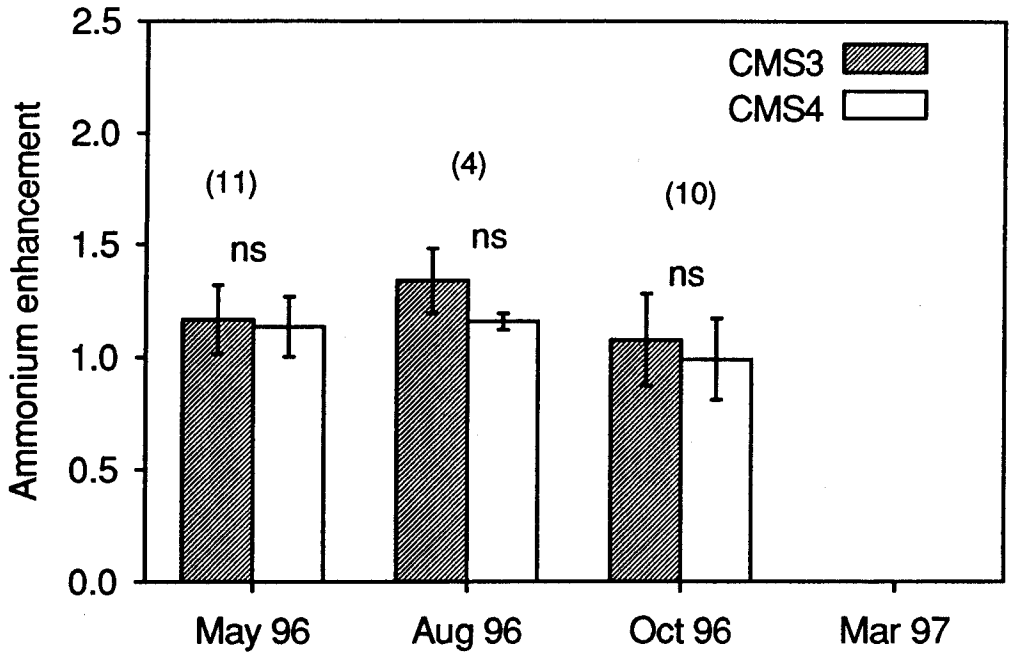


Fig. 19. Pairwise between-site comparisons of ammonium enhancement of dark carbon fixation by zooxanthellae from the two study sites. Statistical parameters as in Fig. 10.

Table 22. Dark carbon fixation rates of isolated zooxanthellae in seawater and seawater + 20 μ*M* ammonium chloride. Rates given as pg C fixed cell^{-1} h^{-1}. Data include all available corals. *p*-values from paired *t*-tests.

CMS3:	Mean		sd	n	*p*	CMS4:	Mean		sd	n	*p*
FSW:	0.041	±	0.037	26	ns	FSW	0.037	±	0.027	28	ns
NH4:	0.044	±	0.032			NH4	0.038	±	0.021		

The three-way ANOVA for the effects of site, date and donor colony on ammonium enhancement showed a significant effect only for date (Table 23). In Tukey post-hoc between-groups comparisons this effect showed up in the Aug 96 samples. At CMS3 these had significantly higher ammonium enhancement ratios than the Oct 96 samples, while at CMS4 the Aug samples were greater than the May 96 samples. Whether this result is an artifact of the small sample size or whether it indicates slightly higher N availability for algae in Aug 96 is not clear. Both the GLN:GLU and basic FAA:total FAA data also suggest lower N supply during this time. However, the elemental data indicate that N supplies may have been somewhat higher during this time (C:N not different, N:P elevated).

Table 23. Three way ANOVA of the entire dataset for ammonium enhancement of dark carbon fixation by zooxanthellae at the two sites.

SOURCE	SUM-OF-SQUARES	DF	MEAN-SQUARE	F-RATIO	P
DATE	0.386	2	0.193	5.392	**0.010**
COLONY	0.023	3	0.008	0.217	0.883
SITE	0.096	1	0.096	2.665	0.114
DATE*COLONY	0.030	6	0.005	0.140	0.990
DATE*SITE	0.036	2	0.018	0.497	0.614
COLONY*SITE	0.016	3	0.005	0.148	0.930
DATE*COLONY*SITE	0.064	6	0.011	0.295	0.934
ERROR	1.003	28	0.036		

5. Zooxanthellae density. One other parameter that can serve as an indicator of nutrient status in corals is the density of zooxanthellae, usually expressed as total zooxanthellae per unit area of coral surface. The density of zooxanthellae has been shown to increase when corals are exposed to elevated ammonium.

We measured the densities of zooxanthellae in our corals, and found no differences between sites in the pairwise comparisons of each collection (Fig. 20.) However, there is high variance in these data, and we fairly certain that these calculations are underestimates of the symbiont density of these corals. Published densities of non-bleaching *Montastraea* from the Florida Keys (and our published data from Bermuda) are typically greater than 10^6 cells cm^{-2}, and our sectioned skeletons from the alizarin work clearly show brownish material just below the surface. This indicates that some zooxanthellae were not removed by Water-Pikking.

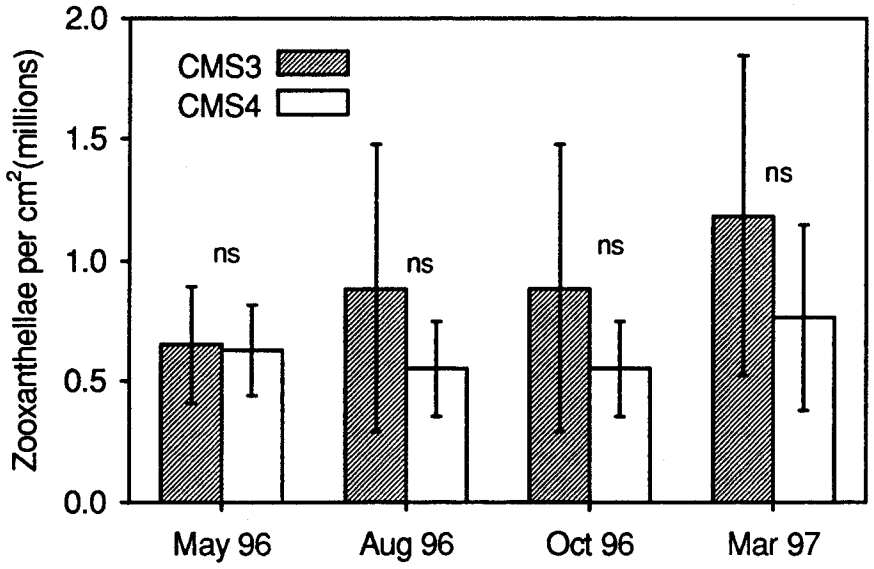


Figure 20. Densities of zooxanthellae at the two study sites. N's range from 6 (Mar 97) to 9 (May . Aug 96). Other statistical parameters as in Fig. 10.

Summary: Site-dependent effects on nutrient status of coral zooxanthellae

The differences in the nutrient status of symbiotic algae from corals at our two study sites -- CMS3, exposed to direct tidal flow from Florida Bay, and CMS4, on the edge of the Florida Reef tract and generally protected from tidal flow, are summarized as follows:

Nutrient parameter	Effect
• **Chlorophyll *a* content**	• Elevated at all times at CMS3.
• **Elemental ratios**	• **C:N:** No differences between sites at any time; values very low at both sites. • **C:P:** Higher at CMS3 than at CMS 4 in August; no difference at other sampling periods. Values relatively high at both sites. • **N:P:** Higher at CMS3 than CMS4 in August and October; no difference at other times. Values relatively high at both sites.
• **Free amino acid ratios**	• **GLN:GLU:** No differences between sites at any time. Values generally high. • **Basic FAA:Total FAA:** No differences between sites at any time. Values tend to be high.
• **Ammonium enhancement of dark carbon fixation**	• No difference between sites. Values very low, essentially no enhancement.

Conclusions Regarding Nutrient Sufficiency of Coral Zooxanthellae.

1. Nitrogen. It is generally agreed that the zooxanthellae of reef corals from "normal" oligotrophic reefs are nitrogen-limited. This has been shown in numerous studies involving the addition of ammonium or nitrate to corals. The typical response is an increase in the density of zooxanthellae, i.e., the algae respond to added nitrogen by growing. Indicators of N-sufficiency of zooxanthellae from "normal" reefs support this: C:N ratios tend to be elevated over Redfield ratios, and the algae exhibit significant increases of dark carbon fixation when exposed to ammonium.

Virtually all of the samples that we examined in this study exhibited N-sufficiency, and did not follow this classical pattern of N-limitation. C:N ratios were remarkably low at both sites at all times, as were ratios of ammonium enhancement of dark carbon fixation. Free amino acid ratios showed high levels of nitrogen-rich free amino acids in zooxanthellae from corals at both sites at all times during the year. Thus, it appears that the zooxanthellae in the corals at both sites were exposed to high levels of nitrogen throughout the one-year period of this study.

The sources and amounts of nitrogen available to organisms on the Florida reef tract have been matters of some controversy. Our data do not clarify this, but they do show that nitrogen sources available to corals on both sides of Hawk Channel in the vicinity of Long Key appear to be sufficient, if not excessive. It should be realized that the algal symbionts of corals have a "multi-trophic" mode of nutrition, as they occupy a specialized niche within the tissue of a carnivorous animal. The zooxanthellae may obtain nitrogen from (a) dissolved sources in seawater (NH_4^+, NO_3^-, possibly DON), (b), from zooplanktonic food ingested by the coral, or (c) from metabolic wastes of the animal (primarily NH_4^+). It would appear that, for the coral zooxanthellae, the sum of all of these sources was equally sufficient at both CMS3 (receiving the direct tidal flow from Florida Bay) and at CMS4.

Measurements of nutrient concentrations along a transect running from Channel #5 past Tennessee Light indicated declining dissolved nitrogen sources across Hawk Channel in 1990 / 91 (Szmant and Forrester, *Coral Reefs* 15:32). R. Jones of the SouthEast Research Laboratory analyzed samples of seawater that we took during our first three sampling periods (Table 24). Although we view such samples -- taken infrequently and without respect to tide or wind conditions -- to be mere snapshots of dissolved nutrient conditions, they do indicate greater N supplies at the inshore site. We have no measurements of the particulate food available to our corals at these sites, but we have indirect evidence that more of this was likely to have been available at CMS3. We routinely cleaned fouling material from each of the coral maintenance structures during each visit, and consistently found that more organisms had settled on the inshore structure than the offshore one. This observation suggests that more larvae (zooplanktonic food) might have been available at the inshore site than the offshore one.

The observation that N supplies were sufficient at our offshore site requires comment. We do not know whether this nitrogen was transported across Hawk Channel, if it had an offshore source (e.g., the Pourtales Gyre), if it was being generated on the reef tract itself, or resulted from some combination of all three. What is clear is that if the corals at this site are receiving all of the N that they can use, increases in N supplies will be in excess, and this N likely would be utilized by other algae -- particularly benthic macro-algae.

Table 24. Measured dissolved nitrogen concentrations at the two study sites. Seawater samples were collected at the time of coral collection and analyzed by R. Jones of SERC. All values in μM. Values given for replicate samples.

A. Ammonium:

Date	CMS3		CMS4	
May 14, 1996	0.54		0.96	
Aug 8, 1996	0.82,	0.85	0.47	0.53
Oct 30, 1996	0.75,	0.76	0.09	0.31

B. Nitrate:

Date	CMS3		CMS4	
May 14, 1996	0.35,		0.18,	
Aug 8, 1996	0.27,	0.30	0.07,	0.07
Oct 30, 1996	0.71,	0.72	0,	0

C. Dissolved organic N:

Date	CMS3		CMS4	
May 14, 1996	13.4,		6.9,	
Aug 8, 1996	14.1,	18.2	13.9,	12.6
Oct 30, 1996	11.76,	11.98	8.17,	8.18

2. Phosphorus. Typically the waters around carbonate-rich coral reefs are P-limited, in part due to the adsorption of inorganic phosphate to carbonate sediments. Our elemental data suggest that the zooxanthellae from our corals at both experimental sites generally exhibited moderate P-limitation. We did find evidence for increased P-limitation of zooxanthellae from corals at the inshore site in the summer of 1996 (Aug 8 samples) and possibly the fall (Oct 30 samples). Other studies have shown that other algae and seagrasses follow a similar seasonal pattern, particularly in the Upper Keys and the northeast portion of Florida Bay. There is some question as to whether this summer pattern is due to decreased P availability, increased N availability or to a combination of both. Since we found no significant seasonal patterns in the N-signals from our coral zooxanthellae, it appears that decreased P availability was responsible. Our "snapshots" of soluble reactive and total phosphorus concentrations tend to support this conclusion (Table 25), although the caveats regarding these data (noted above) must be considered.

Table 25. Measured seawater phosphorus concentrations at the two study sites. Seawater samples were collected at the time of coral collection and analyzed by R. Jones of SERC. All values in μM. Values given for replicate samples.

A. Soluble reactive phosphorus:

Date	CMS3		CMS4	
May 14, 1996	0.23,		0.27	
Aug 8, 1996	0.08,	0.08	0.09	0.10
Oct 30, 1996	0.16,	0.18	0.03	0.03

B. Total phosphorus:

Date	CMS3		CMS4	
May 14, 1996	0.72,		0.95,	
Aug 8, 1996	0.11,	0.13	0.12,	0.13
Oct 30, 1996	0.24,	0.25	0.15,	0.15

3. Chl *a* content of zooxanthellae. The only consistent between-site difference that we found in our algal nutrient analyses was the increased chlorophyll *a* content of the zooxanthellae from corals at the inshore site. As discussed above, it is most unlikely that this difference was due to nitrogen supply. Rather, we think that the increased chl *a* content at the inshore site was probably a photoadaptive response to decreased light levels there. Depth was not a factor: CMS3 was actually located in shallower water than CMS4 (4m vs. 6m). The amount of suspended material in the water column was more likely to be responsible. Although we were not able to make turbidimetric measurements at our sites, two qualitative kinds of observations made during dives suggested this. The first was that water was always murkier (and, in onsite photographs, greener) at CMS3 than at CMS4. The second was noted above: the amount of debris that accumulated on the structure at CMS3 was always greater than the amount at CMS4. Presumably this suspended material originated in Florida Bay and was transported through Channel #5 by ebbing tides and wind-driven currents (cf. Fig. 1). Much of this material probably settled out or was diverted by the long shore current along Hawk Channel before reaching CMS4 (Fig. 1).

4. Effects of site on coral growth. Our growth measurements clearly showed that total calcification by corals was reduced at the inshore site by an average of 40% over the entire experiment. A number of factors are known to affect coral calcification: these include both nutrients and light levels. Both elevated ammonium and phosphate are known to inhibit the process; phosphate in particular is a crystal poison of calcium carbonate. However, none of our nutrient sufficiency assays indicated any difference in exposure at the two sites, and, if anything, P supplies were limiting for a portion of the year at the inshore site.

We believe that the main effect of Florida Bay water on coral growth at our sites resulted from increased turbidity, and not elevated nutrients, in the waters emanating from the Bay. The most direct effect of this increased turbidity would be reduced light transmission. Calcification in corals with zooxanthellae is light-enhanced, and it has been shown in staghorn corals that it is the infilling of skeletal mass, and not skeletal extension, that is light-dependent. This would be consistent with what we have observed in our explants: total calcification was reduced, but skeletal extension was not affected. Other effects of increased turbidity are also possible. One effect could be the "smothering" of coral polyps and interference with feeding mechanisms by sediment. This has several direct costs to corals: it reduces the energy available from photosynthesis by the zooxanthellae, while at the same time increasing respiration rates due to the energy expended in clearing sediments from the coral surface. The interference with feeding clearly reduces both energetic and other nutritional inputs. Both of these effects will reduce calcification in corals: this growth process is highly energy-dependent.

A second possibility is competition with other organisms (particularly hydroids, possibly algae) that had settled on the PVC collars. These organisms could compete for space or actively inhibit the peripheral growth of coral tissue; such an effect might be responsible for the differences in areal and polyp growth that we found at the two sites.

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November 4, 1997

Dr. William L. Kruczynski
US Environmental Protection Agency
Florida Keys National Marine Sanctuary
5500 Overseas Highway Main House
Marathon, FL 33050

Dear Bill:

On behalf of Erich Mueller and Drew Ferrier I am pleased to send you the Final Report for our EPA / SFWMD project, "Reef Corals and Their Symbiotic Algae as Indicators of Nutrient Exposure".

I apologize for the delay in getting this report to you. We have been concerned that our procedures might have been in part responsible for the high nitrogen signals we have found in the coral symbionts from both of our study sites, and have performed a number of experiments to check this. Unfortunately the results of some of these assays are not yet available. I have submitted this report now, rather than delay submission any longer. I would hope to have the results of these studies in time for the TAC meeting later this month, when I will discuss some of the possible problems. For now, we tentatively conclude that the N signals are high at both sites, but I caution that this is preliminary until we have checked our procedures. I would be pleased to include this information in the revised version, pending EPA review.

I hope you will find this interesting reading. I have sent copies of the report to Susan Olson at the District, and to Fred McManus at the Atlanta office.

Sincerely,

Clayton B. Cook, Ph. D.
Senior Research Scientist

cc Dr. Erich Mueller, FKMRL
Dr. Drew Ferrier, Hood College
John Jansen, Dir. of Contracts and Business Development, HBOI