

# Effects of nitrate, phosphate and iron on the growth of macroalgae and benthic cyanobacteria from Cocos Lagoon, Guam

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**ABSTRACT:** The observed high abundance of algae and cyanobacteria on Guam's coral reefs raises concern regarding a possible shift from coral- to algal-dominated communities. Possible increased nutrient supply to macroalgae and cyanobacteria via the watershed due to anthropogenic disturbance could be a partial cause. In this study, 2 outdoor microcosm experiments are used to test the effects of iron, nitrate and phosphate on 3 species of algae (*Halimeda incrustata*, *Padina tenuis* and *Dictyota bartayresiana*) and 3 species of cyanobacteria (*Tolypothrix* sp., *Schizothrix* sp. and *Lyngbya majuscula*) from Cocos Lagoon, Guam. The 6 species were cultured together sewn to an artificial substrate for 9 d with either nitrate- (~6  $\mu\text{M}$ ), phosphate- (~1  $\mu\text{M}$ ), iron- (~0.5  $\mu\text{M}$ ) enriched or control (ambient nutrients) conditions. Overall gram-specific growth was greatest for *L. majuscula*, which grew at 9 times the rate of the other species. Algae did not show statistically significant nutrient limitation, although results with *D. bartayresiana* and *P. tenuis* suggested iron and nitrate limited growth in the first and second experiment, respectively. Two species of cyanobacteria showed phosphate limitation. The growth of *L. majuscula* was enhanced with phosphate enrichment, whereas the release of hormogonia by *Tolypothrix* sp., not the growth of the colonies themselves, may also have been enhanced. Patterns of *Tolypothrix* sp. hormogonia release also suggested possible direct competition between algae and cyanobacteria; the hormogonia aggregated upon some species but not others. The results of this study suggest that *L. majuscula* may have more efficient growth and/or nutrient uptake mechanisms compared to the other species, and that it is capable of increased growth in response to phosphate in the water column.

**KEY WORDS:** Algae · Cyanobacteria · Iron · Nutrients · Phase shift

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## INTRODUCTION

Increased dominance of benthic cyanobacteria and macroalgae on coral reefs is a continuing phenomenon in near shore tropical environments, particularly in areas impacted by humans (Hughes 1994, McCook 1999). This type of community change, often termed a phase shift when accompanied by decreases in coral cover (Done 1992), may or may not be reversible (Hunter & Evans 1995, Aronson & Precht 2000). Factors

that control the abundance of macroalgae have been studied (Lapointe 1989, Larned 1998, Schaffelke & Klumpp 1998, Russ & McCook 1999), but controversy remains over the relative importance of top-down (herbivory) and bottom-up (nutrients) effects (Lapointe 1997, 1999, Hughes et al. 1999).

Growth of many species of algae is limited by the availability of nitrogen and/or phosphate (Lapointe 1989, Larned 1998, Russ & McCook 1999). Possible nutrient limitations of cyanobacteria have not been as thoroughly investigated, and have generally been restricted to laboratory culture experiments. Manipulative studies on benthic or mat-forming cyanobacteria are few, but suggest cyanobacterial mats may be nutrient limited, and that phosphate may be of particular

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importance (Fong et al. 1993a,b). Nutrient-enhanced (mixed N and P) cyanobacterial growth was also observed by Miller et al. (1999).

Historically, cyanobacteria have been grouped with 'turf algae' and subsequently underrepresented in ecological studies. Coral reef cyanobacteria are often small, cryptic and hard to collect, and the taxonomy is in a state of confusion (Littler & Littler 2000). Despite their being lumped together in ecological studies, there is little reason to expect cyanobacteria and algae to respond to environmental variables in a similar fashion. Many marine cyanobacteria can fix nitrogen; thus, cyanobacterial abundance may be limited by the availability of other nutrients, such as iron and phosphorus, both of which are required for the synthesis of nitrogenase (Paerl 1990). The physiological ecology of cyanobacteria differs from that of algae in many ways. The presence of oxygen inactivates nitrogenase, requiring that photosynthesis and nitrogen fixation is separated either in space or time. Some cyanobacteria localize nitrogenase within heterocysts (specialized thick-walled cells where no photosynthesis takes place), but many filamentous cyanobacteria do not have these specialized structures. Non-heterocystous nitrogen-fixing species have developed ways of compartmentalizing nitrogen fixation within the central regions of the trichomes (Janson et al. 1994), while others have strong diel patterns where photosynthetically derived oxygen suppresses nitrogen fixation during the day and nitrogenase is active at night (Paerl et al. 1996). In addition, cyanobacteria have specialized iron-uptake mechanisms involving the production of low molecular weight compounds called siderophores that are secreted to scavenge iron from the water column (Wilhelm & Trick 1994). Iron limitation has been demonstrated for the pelagic *Trichodesmium* spp. (Rueter et al. 1990, Paerl et al. 1994). *Anabaena variabilis* and *Anacystis nidulans* in culture have been shown to produce siderophores in nitrogen-limited conditions, but not in nitrogen-replete conditions (Kerry et al. 1988), suggesting that iron may be limiting only during phases of low nitrogen availability. The possibility of nutrient limitation among benthic filamentous cyanobacteria on coral reefs has not been examined to date.

Competition for space and nutrients may occur between and among different species of benthic cyanobacteria and macroalgae on coral reefs. Thacker & Paul (in press) have shown that the abundance of *Oscillatoria margaritifera* is inversely correlated with macroalgal abundance at 1 site on Guam. Caging experiments suggested that some species of cyanobacteria may be stronger competitors with macroalgae than others (Thacker et al. 2001). Work by Fong & Zedler (1993b) suggests that macroalgae and cyanobac-

teria may compete for attachment space in shallow coastal lagoons in southern California. Different plant traits can be advantageous in different environmental settings, so competitive ability to capture light, occupy space, and acquire nutrients may vary along environmental gradients (Carpenter 1990, Olson & Lubchenco 1990). If the environment is changed by human impacts, disruptions in the previously established competitive hierarchy could result and lead to blooms of previously rare species.

Macroalgal and cyanobacterial abundance in the benthic community undergoes major seasonal fluctuations in Cocos Lagoon off the southern tip of Guam (Paul et al. unpubl.). Guam's volcanic southern half contains abundant rivers, and the watershed system can deliver pulses of iron-laden sediments and nutrient-enriched runoff to the shallow reef environment after rains of several days magnitude (Matson 1989, 1991). The purpose of this study was to investigate whether nutrient supply has the potential to exhibit control over macroalgal and cyanobacterial abundance in Cocos Lagoon. The effects of several nutrients (nitrate, phosphate and iron) on the growth rates of 3 of the most abundant species of algae and 3 abundant species of cyanobacteria were tested in outdoor microcosms in hopes of revealing different competitive advantages of certain species under the different nutrient regimes.

## MATERIALS AND METHODS

Two experiments were conducted using macroalgae and cyanobacteria collected from Cocos Lagoon, Guam (13° 24' N, 144° 46' E). The first (Expt I) was conducted from March 30 to April 8, 2000 and the second (Expt II) from May 18 to 27, 2000. The purpose of conducting 2 experiments was replication; it was not possible to increase replication during a single experimental time period.

Monitoring of the shallow (1 to 4 m) Cocos Lagoon benthic community revealed fluctuations of cyanobacterial and macroalgal abundance over time (Paul et al. unpubl.). These data, along with general field observations, were used to select the most common species of cyanobacteria and algae to use in the nutrient enrichment experiments. We chose the algae *Halimeda incrassata*, *Padina tenuis* and *Dictyota bartayresiana*, and the cyanobacteria *Tolypothrix* sp., *Schizothrix* sp. and *Lyngbya majuscula*. These species have different patterns of growth and temporal abundance in the Cocos Lagoon community, but each has been observed to occupy large proportions of space during certain periods of time. *H. incrassata* forms dense clumps attached to hard substrate, and its abun-

dance does not seem to vary as greatly on a temporal scale as the other species used. Abundance of *P. tenuis* varies substantially on a temporal scale, and it is observed to change morphology from leafy, loosely attached mats to the more cryptic *Vaughaniella* stage when herbivory is high. *D. bartayresiana* forms loosely attached thickets where individual thalli are hard to identify, and abundance seems to vary seasonally. *Tolypothrix* sp. is a heterocystous cyanobacterium and forms button-like colonies which together form dense, carpet-like populations. *Tolypothrix* sp. grows epiphytically on *Padina* spp., *Halimeda* spp and *Schizothrix* spp. (I.B.K. pers. obs.). *Schizothrix* sp. does not form aggregations, rather it occurs in isolated soft, cushion-like colonies widely distributed across the reef. *L. majuscula* has the consistency of human hair and forms tangled mats wrapped around the reef structure, often blanketing macroalgae and dead coral rubble. Both *Schizothrix* sp. and *L. majuscula* are nonheterocystous filamentous cyanobacteria suspected to be nitrogen fixers (Paerl 1990, Dennison et al. 1999). All 3 species of cyanobacteria form well-defined thalli that are easy to handle as a single entity; they are not delicate or film-like.

Algae and cyanobacteria were collected from the shallow (1 to 4 m depth) lagoon and placed in flowing seawater at the University of Guam Marine Laboratory until pieces were selected for use. Set-up of the experiment spanned a 36 h time period. Small thalli or pieces of thalli of approximately equal size were selected on the basis of apparent health and appropriate size, cleaned of macroepiphytes and invertebrates as best as possible, and weighed fresh. Fresh weight was obtained after removing excess water via a specific number of spins (different for each species) in a salad spinner and blotting each piece on a paper towel. One piece of each species was then sewn to an artificial substrate (flexible plastic grid) with braided nylon fishing line. Each of the 6 thalli were sewn upright on each grid so that they were firmly attached at the base of the thallus. Species were organized in a consistent configuration as follows: starting at 12 o'clock and moving clockwise around the perimeter of the square grid, *Halimeda incrassata*, *Lyngbya majuscula*, *Padina tenuis*, *Tolypothrix* sp., *Dictyota bartayresiana*, and *Schizothrix* sp.

Initial fresh weights ranged from 0.075 to 1.75 g, but individuals were size-matched within replicate arrays and randomly assigned to treatments. The arrays were placed individually in plastic 1 l graduated beakers previously soaked in seawater for 24 h. Five arrays were prepared for each treatment for each experiment ( $n = 5$ ). To each beaker, 750 ml of filtered seawater (filtered with a 10  $\mu\text{m}$  nominal rating filter bag) was added. Adding the appropriate concentrated stock sol-

ution to the ambient filtered seawater imposed 1 of 4 treatments, each aimed at bringing the nutrient to a level 1 order of magnitude above the ambient level measured at Cocos Lagoon. Stock solutions were prepared with Milli-Q 18 M $\Omega$  deionized water so that adding 1 ml (or 0.5 ml in the case of Fe-enrichment) of stock solution resulted in the desired level of nutrient enhancement. NaNO<sub>3</sub> stock solution was added so that the final NO<sub>3</sub> concentration was 5  $\mu\text{M}$  above ambient (see Table 1) in the N+ treatment. NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O was added so that the final concentration was 1  $\mu\text{M}$  PO<sub>4</sub> above ambient concentration in the P+ treatment. An equimolar stock solution of FeSO<sub>4</sub>·7H<sub>2</sub>O and EDTA was added to bring final concentrations of Fe to 0.5  $\mu\text{M}$  above ambient concentration for the Fe+ treatment. The control treatments received 1 ml of distilled water. Water was exchanged and treatments were re-imposed in each microcosm on every third day (see below).

Water samples were taken from the filtered seawater source at the time of each water exchange to measure ambient nutrient and iron levels in the marine laboratory seawater system. Samples were taken in 125 ml pre-soaked acid washed HDPE Nalgene bottles and frozen until analysis (nutrient samples) or kept at room temperature (iron samples). Water sample nutrient analyses (NH<sub>4</sub>-N, NO<sub>x</sub>-N and PO<sub>4</sub>-P) were performed using a Lachat QuikChem<sup>®</sup> FIA+ 8000 automated ion analyzer. Iron analyses were conducted using the reagent ferrozine according to Stookey (1970) and Murray & Gill (1978). In addition to the exchange water, water samples were taken from the microcosms immediately after nutrient addition (to confirm treatments) and after the last 3 d period of the second experiment (to measure uptake of nutrients and iron).

Microcosms were kept outdoors in natural sunlight. A clear Plexiglas cover (transparent to ultraviolet radiation) was placed over the experimental set-up to keep rainwater from entering the microcosms. After the third day of the first experiment, 1 layer of neutral density screening was added to the rain cover because slight bleaching was observed in *Halimeda incrassata*. The screening + rain cover resulted in a 55 % reduction in the intensity of ambient photosynthetically active radiation (PAR), and a 51 % reduction in ambient UVA intensity. Light measurements were made with an IL1700 Research Radiometer (International Light Inc., Newburyport, MA) alternately fitted with a PAR sensor (410 to 770 nm) and a UVA sensor (330 to 375 nm). These % light reductions were similar to those measured at 4 m at Cocos Lagoon on October 3, 2000.

Microcosms were placed upon a grate in a vigorously flowing open seawater bath so that the water level within the microcosms was slightly higher than the surrounding flowing seawater. This arrangement

Table 1. Measurement of nutrients and iron in water samples taken before, during and after the 2 experiments. 'Exchange' samples are from the filtered seawater used during water exchanges, representing background levels of nutrients to which concentrated stock solutions were added. 'Before' samples are levels measured just after the addition of stock solutions and before uptake by algae and cyanobacteria commenced. 'After' samples are levels measured after 3 d of uptake by the organisms. Values are means with (SE) where applicable. na = data not available

	n	$\mu\text{M NO}_x\text{-N}$	$\mu\text{M NH}_4\text{-N}$	$\mu\text{M PO}_4\text{-P}$	$\mu\text{M Fe}$
<b>Cocos Lagoon</b>					
(1998–1999)	12	0.24 (0.06)	na	0.06 (0.01)	na
28 Mar 2000	3	na	na	na	0.036 (0.003)
16 May 2000	1	0.23	<0.15	<0.03	0.101
<b>Expt I</b>					
Exchange 1	1	0.57	<0.15	<0.03	0.025
Exchange 2	1	0.43	<0.15	<0.03	0.025
Exchange 3	1	0.93	0.64	<0.03	<0.015
<b>Expt II</b>					
Exchange 1	1	1.49	<0.15	<0.03	0.073
Exchange 2	1	1.15	<0.15	<0.03	0.079
Exchange 3	1	1.56	<0.15	<0.03	0.061
<b>Before</b>					
Control	3	0.71 (0.03)	<0.15	<0.03	0.044 (0.002)
+N	3	5.86 (0.24)	<0.15	<0.03	na
+P	3	0.84 (0.13)	<0.15	0.85 (0.02)	na
+Fe	3	na	na	na	0.38 (0.020)
<b>After</b>					
Control	3	<0.07	2.18 (1.91)	<0.03	0.13 (0.005)
+N	3	<0.07	0.38 (0.12)	<0.03	na
+P	3	0.08 (0.01)	0.38 (0.23)	<0.03	na
+Fe	3	na	na	na	0.19 (0.007)

was intended to provide temperature control. Temperatures inside the microcosms matched those of the surrounding bath water with the exception that on cloudless, hot days for about 1 h in the afternoon, microcosm temperatures were 1 to 2°C above the bath water. Water movement within the microcosms was created with aeration delivered via glass pipettes. Intensity of bubbling was adjusted so that thalli gently waved back and forth. Salinity was adjusted within the microcosms each day by adding Milli-Q 18 M $\Omega$  deionized water to obtain the original volume of 750 ml. Microcosms lost approximately 25 ml d<sup>-1</sup> to evaporation, which corresponded to a 0.5 ppt rise in salinity. Positions of microcosms within the experimental set-up were rotated daily to minimize positional effects created by differences in shading, bubbling and other position-sensitive factors.

Water within the microcosms was exchanged every 3 d with filtered water from the marine laboratory seawater system and enriched with respective stock solutions. The artificial substrates were wedged into the bottom of the containers in a way that allowed the water to be poured out without disturbing the algae and cyanobacteria. After the third 3 d enrichment period, thalli were removed from the grids and weighed fresh as described above. *Tolypothrix* sp. released large quantities of hormogonia (= modified filaments associated with reproduction and dispersal)

during the experiments. This output was measured using digital photography of the container bottoms and calculating the area of the container covered with hormogonia using SigmaScan Pro 5.

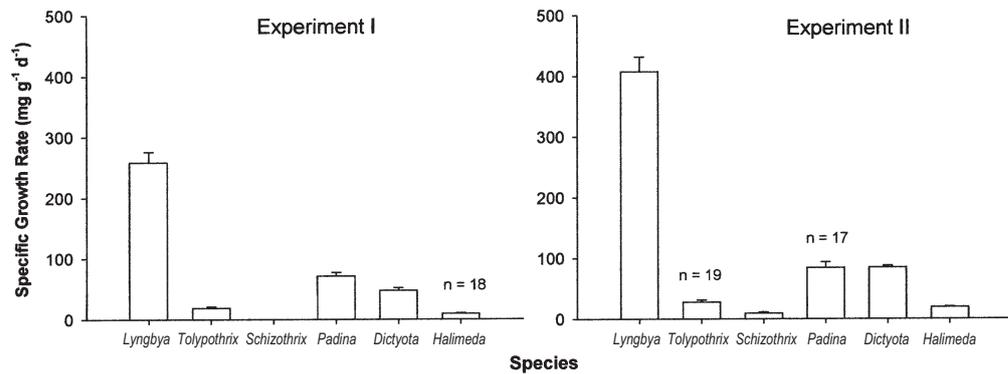
Growth rates for the 2 experiments plus the area of hormogonia released by *Tolypothrix* sp. were analyzed in 2-way ANOVA using SAS version 6.12 and Statistix<sup>®</sup> version 2.0. The factors were treatment, with 4 levels, and experiment, with 2 levels. Normality of the data was confirmed using normal probability plots, and equality of variances was confirmed with the Hartley test (Neter et al. 1990). ANOVA *F*-statistics were adjusted for unequal sample sizes when data sets were unbalanced by using Type III sums of squares reported by SAS or by hand calculation as specified in the Statistix<sup>®</sup> user's manual.

## RESULTS

### Confirmation of treatments

Ambient seawater from the laboratory seawater system used to fill the microcosms at each water exchange had an average nitrate/nitrite-N level of 0.64  $\mu\text{M} \pm 0.15$  SE during the first experiment and 1.4  $\mu\text{M} \pm 0.13$  SE during the second (Table 1). These NO<sub>x</sub> values are slightly above those measured in Cocos Lagoon where

Fig. 1. Mean ( $\pm$ SE) gram-specific growth rate for the 6 species averaged over the 4 nutrient treatments for Expts I and II.  $n = 20$  except where marked otherwise. For simplicity, the genus is used to label the different species



the algae and cyanobacteria originated (Table 1). Ammonium and phosphate levels in the seawater system were at or below detection limits ( $<0.15$  and  $<0.03$   $\mu\text{M}$  respectively). Ambient iron in the seawater system was also low during the first experiment ( $<0.025$   $\mu\text{M}$ ), but was slightly higher in the second experiment ( $0.071$   $\mu\text{M}$ ). Nutrient enhancement was confirmed for  $\text{NO}_x$ ,  $\text{PO}_4$  and Fe during the third water exchange of the second experiment (Table 1). After the addition of stock solutions,  $\text{NO}_x$  was  $5.86$   $\mu\text{M} \pm 0.24$  SE,  $\text{PO}_4$  was  $0.85$   $\mu\text{M} \pm 0.02$  SE, and Fe was  $0.38 \pm 0.02$  SE ( $n = 3$ ).

### Growth of algae and cyanobacteria

In general, the pigmentation and appearance of the 6 species appeared normal over the course of the experiment. Because *Halimeda incrassata* became slightly bleached during the first couple of days of the first experiment, a neutral density shade cloth was added to the Plexiglas rain cover to reduce light intensity. *H. incrassata* returned to original pigmentation by the next day with the exception of 2 of the samples that died, necessitating their removal from the data set. During the second experiment, blade material was lost from 3 *Padina tenuis*, and associated sediments were lost from a *Tolypothrix* sp. individual. As change in mass could not be measured for these replicates, they were removed from the data analysis as well.

Overall gram-specific growth rate of *Lyngbya majuscula* was approximately 9 times greater than the average growth of the other 5 species during both experiments, regardless of nutrient regime (Fig. 1). By the end of the first 9 d experiment, the mats of *L. majuscula* had more than doubled their original fresh weights. During the second experiment, the mats nearly quadrupled their original fresh weights.

Nutrient treatment was not statistically significant in affecting the growth of *Padina tenuis*, *Dictyota bartayresiana* or *Halimeda incrassata* (Fig. 2, Table 2). However, the p-value for the *D. bartayresiana* treat-

ment effect was  $<0.056$ , suggesting that treatment may have affected growth (Table 2). Least squares means analyses revealed that the enhanced N treatment resulted in the highest growth followed by the iron,

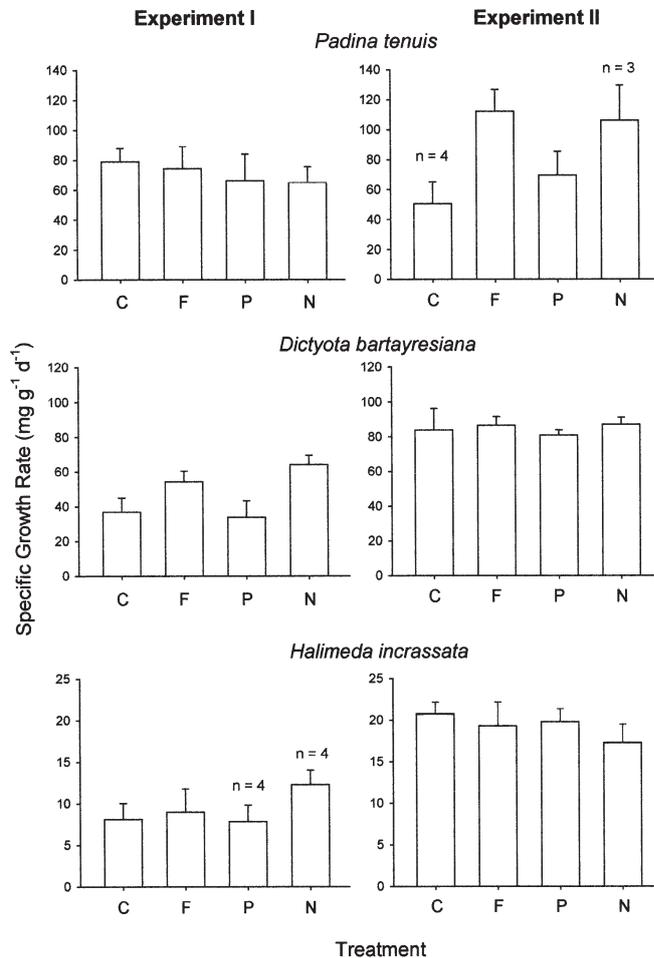


Fig. 2. Mean ( $\pm$ SE) gram-specific growth rate of algae in Expts I and II.  $n = 5$  except where marked otherwise. Treatments are C = control, F = Fe-enrichment, P =  $\text{PO}_4$ -enrichment, and N =  $\text{NO}_3$ -enrichment. Note different y-axis scaling for each species

Table 2. Summary of ANOVA results for growth of 3 species of algae and 3 species of cyanobacteria, and the area covered by hormogonia released by *Tolypothrix* sp. p-values less than 0.05 highlighted in bold

	df	MS	F	p
<b><i>Padina tenuis</i></b>				
Treatment	3	1781	1.77	0.176
Experiment	1	1657	1.64	0.210
Interaction	3	2354	2.34	0.095
Error	29	1008		
<b><i>Dictyota bartayresiana</i></b>				
Treatment	3	740	2.79	0.056
Experiment	1	13867	52.3	<b>&lt;0.0001</b>
Interaction	3	350	1.32	0.284
Error	32	265		
<b><i>Halimeda incrassata</i></b>				
Treatment	3	1.53	0.07	0.976
Experiment	1	940	42.2	<b>&lt;0.0001</b>
Interaction	3	27.5	1.24	0.313
Error	30	22.3		
<b><i>Lyngbya majuscula</i></b>				
Treatment	3	45472	8.37	<b>0.0003</b>
Experiment	1	221801	40.8	<b>&lt;0.0001</b>
Interaction	3	7701	1.42	0.256
Error	32	5432		
<b><i>Schizothrix</i> sp.</b>				
Treatment	3	4.07	0.06	0.983
Experiment	1	1450	19.7	<b>0.0001</b>
Interaction	3	45.9	0.62	0.605
Error	32	73.6		
<b><i>Tolypothrix</i> sp.</b>				
Treatment	3	296	1.59	0.212
Experiment	1	728	3.91	0.057
Interaction	3	161	0.87	0.469
Error	31	186		
<b>Hormogonia of <i>Tolypothrix</i> sp.</b>				
Treatment	3	53458	2.08	0.122
Experiment	1	84787	3.31	0.078
Interaction	3	3418	0.13	0.940
Error	32	25651		

control, and phosphate treatments. *P. tenuis* showed possible Fe- and N-limited growth during the second experiment but not the first, as suggested by the p-value of 0.095 for the interaction term (Fig. 2, Table 2). If the *P. tenuis* data from the second experiment are analyzed separately with a non-parametric Kruskal-Wallis 1-way ANOVA, the treatment effect is significant ( $p < 0.04$ ). Comparisons of mean ranks by treatment revealed that growth was enhanced by Fe-enrichment compared to controls at the  $\alpha = 0.10$  level. *D. bartayresiana* and *H. incrassata* both grew more during the second experiment compared to the first ( $p < 0.0001$ , Table 2).

The cyanobacteria as a group did not respond in a consistent manner to the different treatments. The growth of *Lyngbya majuscula* was significantly affected by treatment ( $p < 0.0003$ , Table 2), showing

enhanced growth rates in the phosphate treatment (Tukey's Studentized Range test,  $\alpha < 0.05$ ). Also, *L. majuscula* grew more during the second experiment than it did in the first ( $p < 0.0001$ ; Table 2, Figs 1 & 3). Growth of *Schizothrix* sp. was the lowest of all the species, with no appreciative gain in mass during the first experiment, and only slight positive growth in the second experiment (Figs 1 & 3). The effect of experiment was significant ( $p < 0.0001$ ), but treatment was not (Table 2).

Increase in fresh weight may not have been the optimal way of tracking the growth of *Tolypothrix* sp. because of 2 observations. First, colonies of *Tolypothrix* sp. were heavily epiphytized and were home to diverse and cryptic fauna that were extremely difficult to remove, including small polychaetes, foraminifera, gastropods, and various crustaceans. Since the growth of these organisms and the growth of the epiphytes

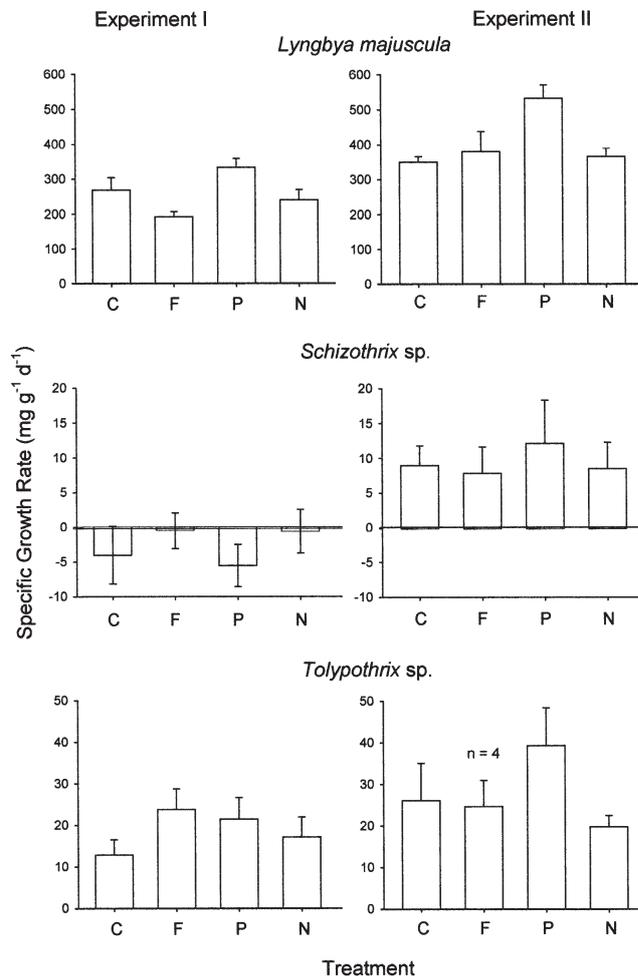


Fig. 3. Mean ( $\pm$ SE) gram-specific growth rate of cyanobacteria in Expts I and II.  $n = 5$  except where marked otherwise. Treatment abbreviations as in Fig. 2. Note different y-axis scaling for each species

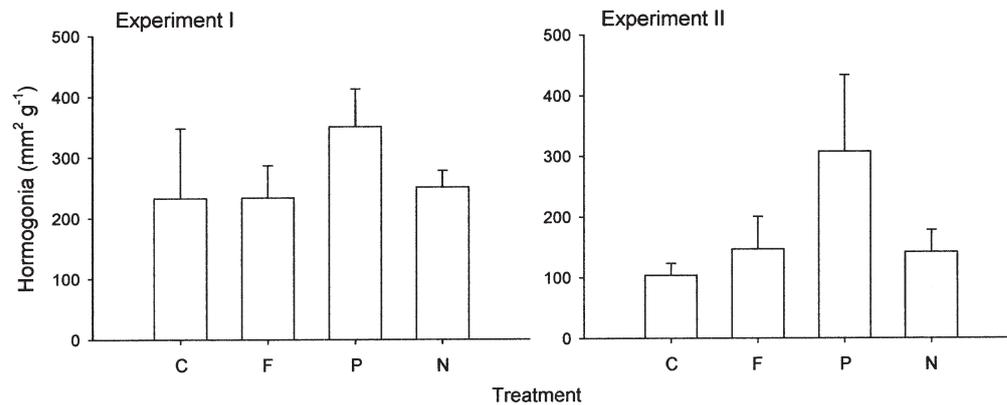


Fig. 4. Mean ( $\pm$ SE) area of microcosms ( $n = 5$ ) covered by hormogonia of *Tolypothrix* sp. for Expts I and II. Treatment abbreviations as in Fig. 2

cannot be separated from the growth of the cyanobacterial colony itself, the measurement of growth represents the entire community living within the *Tolypothrix* sp. colony. Growth of the *Tolypothrix* sp. communities was not affected by treatment, but overall growth was higher in the second experiment compared with the first (experiment effect  $p < 0.057$ ; Fig. 3, Table 2). Second, *Tolypothrix* sp. shed a substantial quantity of hormogonia into the water column. The hormogonia settled onto the bottom surfaces of the microcosms, as well as on to *Padina tenuis*, *Halimeda incrassata*, and to some extent *Schizothrix* sp., but did not seem to adhere to *Dictyota bartayresiana* or *Lyngbya majuscula*. Digital photographic analyses of hormogonia release revealed that more hormogonia were released during the first experiment compared to the second, but this was not statistically significant ( $p < 0.078$ ; Fig. 4, Table 2). Treatment was not statistically significant in determining hormogonia release either ( $p < 0.122$ ). However, there were 2 outliers in the data; 1 control microcosm in the first experiment, and 1 phosphate treatment microcosm in the second experiment, which released exceptionally large quantities of hormogonia. If these 2 outliers are removed from the data set, the treatment effect becomes significant ( $p < 0.025$ ), as does the experiment effect ( $p < 0.005$ ), suggesting that release of hormogonia may have been stimulated by phosphate enrichment.

## DISCUSSION

Under all nutrient regimes, *Lyngbya majuscula* was the most competitive species among the 6 chosen for this study, growing 9 times faster than the other 5 species. *L. majuscula* also displayed the only convincing treatment effect, with enhanced growth in the phosphate-enriched microcosms. The release of hormogonia by *Tolypothrix* sp. also may have been enhanced by phosphate enrichment, although further investigation is warranted.

Cyanobacteria did not show enhanced growth in the iron-enriched microcosms as expected. When nitrogen availability is low and iron availability high, it might be expected that cyanobacteria would be favored due to the availability of iron for the production of nitrogenase to fix nitrogen. However, the results of this study do not support this scenario. Perhaps the cyanobacteria here were iron-replete, or the production of siderophores was sufficient to chelate enough iron from the water column in all treatments. Another possibility is that nitrogen was not limiting in our experiments, especially in Expt II (Table 1), and thus nitrogenase (and hence iron) was not required in large amounts. In addition, Kudo & Harrison (1997) suggest that competing algae may have receptors for cyanobacteria-produced siderophores. In support of this, *Padina tenuis* displayed possible enhanced growth in the Fe-enriched microcosms during the second experiment. However, the presence of siderophores was not confirmed in our study.

Low nitrogen and high phosphorus conditions often favor cyanobacteria in fresh and saltwater systems (Sellner 1997). In our study, the growth of *Lyngbya majuscula*, and possibly the release of hormogonia by *Tolypothrix* sp., was phosphate-limited. Whole colony growth of *Tolypothrix* sp. and *Schizothrix* sp. were not affected by nutrient treatment. Previous examinations of nutrient limitation of benthic marine cyanobacteria have revealed mixed results. Dizon & Yap (1999) found that the biomass of sediment-associated microphyto-benthic communities (dominated by diatoms and cyanobacteria) from a reef flat in the Philippines was enhanced by the addition of nitrate, but not by phosphate. Fong et al. (1993b) revealed that phosphate is of particular importance to cyanobacterial mats in the Tijuana River estuary in southern California.

The 3 species of algae investigated here did not show the expected nitrogen and/or phosphate limitation often observed in other studies (Lapointe 1989, 1997, Larned 1998, Schaffelke & Klumpp 1998). Larned (1998) reported higher growth in ammonium-

enriched conditions compared to controls for *Padina japonica* in Kaneohe Bay, Hawaii. However, ambient nitrate levels were 0.25  $\mu\text{M}$  in that study, which is one third to one sixth of those reported in the ambient seawater used in our experiment. Specific growth rates ( $\text{g g}^{-1} \text{d}^{-1}$ ) of *P. tenuis* reported for the controls in our experiment were about double those reported by Larned (1998) for the ammonium-enriched treatment for *P. japonica*. It is possible that our algae were nitrogen-replete. This could have been from storage of nitrogen taken up in the field prior to collection, or from uptake during the 36 h preparation time spent in the marine laboratory seawater system. Schaffelke & Klumpp (1998) found that the magnitude of nutrient-enhancement depends greatly on initial levels of nutrients stored in the thalli of *Sargassum baccularia*. A nitrogen replete state could also have been a result of uptake during the experiment, as background levels of nitrate in the laboratory sea water system were slightly higher than those reported at Cocos Lagoon where the algae were collected (Table 1). However, *Padina tenuis* suggested N-limitation during the second experiment, when nitrate levels were higher, rather than during the first experiment (Table 1). Another possibility is that the algae were benefiting from the release of fixed nitrogen (usually glutamine; Paerl 1990) by the cyanobacteria, and thus did not exhibit nitrate limitation. In support of this, levels of ammonium in the microcosms were higher than ambient levels by the end of the 3 d 'uptake' period in the control, nitrogen- and phosphate-enriched microcosms (Table 1). Yet another point to consider for the calcified species is that they were growth limited by calcium and/or carbonate depletion in the beaker; however, the thalli used were quite small (<1 g fresh weight) compared to the volume of water (750 ml).

The effect of 'experiment' was quite prominent in this study for both algae and cyanobacteria. *Dictyota bartayresiana*, *Halimeda incrassata*, *Lyngbya majuscula* and *Schizothrix* sp. grew faster during the second experiment compared to the first. A number of factors could have contributed to this effect, including slightly longer day length, warmer temperatures and higher ambient levels of nitrate (Table 1). It is also possible that the algae/cyanobacteria were in different phases of growth (e.g., reproductive, vegetative, senescent) during the different experiments.

Observations of hormogonia release by *Tolypothrix* sp. suggest direct competition among species of cyanobacteria and algae. Epiphitism is often observed in the field, with *Tolypothrix* sp. colonies growing firmly attached to and on top of *Padina tenuis*, *Halimeda incrassata*, and *Schizothrix* sp. Before we observed the release of hormogonia in the microcosms, the mode of propagation in this undescribed species of *Tolypothrix*

was unknown to us. In the microcosms, hormogonia blanketed the plastic grid, the bottom of the microcosm, as well as *Padina tenuis*, *Halimeda incrassata*, and *Schizothrix* sp., suggesting this as the mechanism that allows *Tolypothrix* sp. to dominate large areas of the benthos in Cocos Lagoon. By the end of the 9 d experiments, the hormogonia had aggregated into 'protocolonies' at the base of each adult colony. This observation could help explain the occurrence of tightly packed monospecific mats of *Tolypothrix* sp. often observed at several sites on Guam. However, the alternative hypothesis that hormogonia release was a 'bail out' response in this study also needs to be entertained.

The *Tolypothrix* sp. hormogonia did not seem to settle on *Dictyota bartayresiana* or *Lyngbya majuscula*. This observation suggests that these species may have defense mechanisms against being fouled by *Tolypothrix* sp. hormogonia. Allelopathic interactions and chemical defenses have been found in several species of macroalgae. *Dictyota menstrualis* produces compounds that deter organisms from fouling its surface, and has been shown to be less heavily fouled in nature than other species of algae (Schmitt et al. 1995). Secondary metabolites from *Dictyota menstrualis* and *Dictyota ciliolata* have been found to cause larval mortality, abnormal development and reduced growth rates for 3 species of invertebrates common in the co-occurring fouling community (Schmitt et al. 1998). Walters et al. (1996) found 2 species in the genus *Dictyota* to be the most toxic and deterrent to bryozoan and polychaete larvae out of 12 algal species tested.

A striking observation made during the experiments was the substantial increase in volume of *Lyngbya majuscula*. By the end of each 9 d experiment, *L. majuscula* took up a much larger proportion of space within the microcosm than at the start. The overall dominance of *Lyngbya majuscula* with regards to space occupation within the microcosms indicates that this species may have superior mechanisms in obtaining space and/or nutrient resources. There may be a hierarchy in nutrient uptake, with some species exhibiting more efficient nutrient uptake kinetics than others. Thickness of cell walls, morphology of thallus/colony, presence of epiphytes, or anything that would affect the movement of water across the organism could affect uptake rates of nutrients. Many studies that report nutrient limitation for algae tested 1 species at a time in isolation. Research here and by others (Fong & Zedler 1993) suggest that these results may not be realistically extrapolated to nature where interactions such as competition between species occur.

*Lyngbya majuscula* forms mats around the bases of corals and often blankets rubble areas of the reef. However, the mats are not omnipresent in the benthic

community of Cocos lagoon, suggesting that the high capacity for greater short-term growth observed in this study does not necessarily translate to competitive advantage in the field. In addition to competing for light and attachment space by the addition of biomass, algae can expend energy fortifying the thallus against physical disturbance, fending off predators with noxious chemicals and calcium carbonate, and dispersing via production of vegetative spores and gametes. The allocation of resources towards these different aims will influence competitive interactions between algal and cyanobacterial species, the outcomes of which will vary along gradients in environmental variables (Carpenter 1990), herbivory in particular (Miller et al. 1999, Thacker et al. 2001).

From this study, we conclude that the cyanobacteria *Lyngbya majuscula* and *Tolypothrix* sp. could respond to phosphate enrichment by enhanced growth rates and increased dispersal, respectively. The next step in this research would be to investigate these same hypotheses in the field where complex interactions between all members of the benthic community, as well as environmental gradients, are in place. Factorial studies examining possible synergistic effects of nitrate, phosphate and iron would also be insightful.

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