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Nitrogen- versus phosphorus-limited growth and sources of nutrients for coral reef macroalgae

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Abstract Recent investigations of nutrient-limited productivity in coral reef macroalgae have led to the conclusion that phosphorus, rather than nitrogen, is the primary limiting nutrient. In this study, comparison of the dissolved inorganic nitrogen:phosphorus ratio in the water column of Kaneohe Bay, Hawaii, with tissue nitrogen:phosphorus ratios in macroalgae from Kaneohe Bay suggested that nitrogen, rather than phosphorus, generally limits productivity in this system. Results of nutrient-enrichment experiments in a flow-through culture system indicated that inorganic nitrogen limited the growth rates of 8 out of 9 macroalgae species tested. In 6 of the species tested, specific growth rates of thalli cultured in unenriched seawater from the Kaneohe Bay water column were zero or negative after 12 d. These results suggest that, in order to persist in low-nutrient coral reef systems, some macroalgae require high rates of nutrient advection or access to benthic nutrient sources in addition to nutrients in the overlying water column. Nutrient concentrations in water samples collected from the microenvironments inhabited or created by macroalgae were compared to nutrient concentrations in the overlying water column. On protected reef flats, inorganic nitrogen concentrations within dense mats of *Gracilaria salicornia* and *Kappaphycus alvarezii*, and inorganic nitrogen and phosphate concentrations in sediment porewater near the rhizophytic algae *Caulerpa racemosa* and *C. sertularioides* were significantly higher than in the water column. The sediments associated with these mat-forming and rhizophytic species appear to function as localized nutrient sources, making sustained growth possible despite the oligotrophic water column. In wave-exposed habitats such as the Kaneohe Bay Barrier Reef flat, water motion is higher than at protected sites, sediment nutrient concentrations are low, and zones of high nutrient concentrations do not develop near or beneath macroalgae, including dense *Sargassum echinocarpum* canopies. Under these conditions, macroalgae evidently depend on rapid advection of low-nutrient water from the water column, rather than benthic nutrient sources, to sustain growth.

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Introduction

Shifts from coral to algal domination of coral reefs have become common in tropical coastal regions, and the identification and control of nutrients which enhance algal productivity has become a priority for reef management (Smith and Buddemeier 1992; Richmond 1993). In the majority of cases, nitrogen and phosphorus limit productivity, although iron limitation has also been detected (Entsch et al. 1983). In several recent investigations, a conclusion has been drawn that phosphorus availability limits the productivity of fleshy macroalgae in oligotrophic reef waters (Lapointe et al. 1987, 1992, 1993; Littler et al. 1991). However, results of nutrient-enrichment experiments using coral reef macroalgae indicate that nitrogen limitation is also common (e.g. Lapointe et al. 1987; Lapointe 1989; Littler et al. 1991; McGlathery et al. 1992; Delgado and Lapointe 1994). The type and severity of nutrient limitation often varies spatially due to the presence of localized nutrient sources such as guano deposits (Littler et al. 1991), sediment patches (Littler et al. 1988), or groundwater seeps (Naim 1993). These observations suggest that the nutrient whose availability limits growth may be species-specific or habitat-specific.

Ratios of dissolved inorganic nitrogen:phosphate ($DIN:PO_4$) in seawater and ratios of total nitrogen:phosphorus (TN:TP) in algal tissues have each been used in lieu of experiments to assess nutrient limitation in algae, but these ratios may have little predictive power (Maestrini et al. 1984; Atkinson 1989). Water-column

DIN:PO_4 ratios do not provide information about the requirements of particular species; at a given water-column DIN:PO_4 ratio, some species may be nitrogen-limited and others phosphorus-limited (e.g. Fong et al. 1994). Predictions of limiting nutrients have also been made by comparing TN:TP ratios in algal tissues to TN:TP ratios that are thought to correspond to maximum growth rates (e.g. Lapointe et al. 1992; Wheeler and Björnsäter 1992). This approach is also unreliable, because different species have different nutrient-storage capacities and different rates and seasonal patterns of growth and nutrient storage (Rosenberg and Ramus 1981, 1982), and because it is usually unclear whether TN:TP ratios reflect nutrient-storage capacities or nutrient requirements for growth (Lobban and Harrison 1994). Comparisons of DIN:PO_4 ratios with corresponding TN:TP ratios may be more useful, because they can provide information about the availability of nitrogen and phosphorus relative to nitrogen and phosphorus requirements or storage capacity, if these are known. Under conditions of very low nitrogen and phosphorus availability, it is likely that tissue TN:TP ratios reflect the relative requirements of an alga for nitrogen and phosphorus. Conversely, when water-column concentrations of nitrogen or phosphorus are very high, as under conditions of upwelling or eutrophication, and algal growth rates are near maximal, then tissue TN:TP ratios may reflect the storage capacity of one or both nutrients (Wheeler and Björnsäter 1992; Pedersen and Borum 1996).

A comparison of water-column DIN:PO_4 and macroalgal TN:TP ratios suggests that benthic macroalgae in Kaneohe Bay, Oahu, Hawaii, will be limited by the availability of nitrogen rather than phosphorus. DIN and phosphate concentrations in the Kaneohe Bay water column near coral reef slopes are quite low (DIN $< 0.5\ \mu M$, phosphate $< 0.2\ \mu M$) (Laws and Allen 1996; Larned and Stimson 1997; present study) and the water-column DIN:PO_4 ratio averages 4:1. The mean TN:TP ratio in tissues of 30 macroalgae species from Kaneohe Bay is 44:1 (Atkinson and Smith 1983; Smith 1994). Therefore, phosphorus in the water column appears to be available far in excess of nitrogen, relative to the TN:TP ratio of algal tissues. The prediction that nitrogen is limiting assumes that (1) concentrations of DIN and PO_4 in the water column represent the availability of these nutrients for benthic macroalgae, and (2) TN:TP ratios in algae from nutrient-poor habitats reflect nitrogen and phosphorus requirements for growth, rather than storage capacity.

While comparisons of water-column and tissue-nutrient ratios are useful for generating hypotheses, controlled experiments are a more accurate method of assessing nutrient limitation. In this study, factorial nutrient-enrichment experiments were carried out with nine species of macroalgae from Kaneohe Bay and the results were used to evaluate three alternative predictions: (1) phosphorus generally limits macroalgal productivity in Kaneohe Bay, as in other oligotrophic reef systems (Lapointe et al. 1987; Littler et al. 1991); (2) nitrogen is generally limiting as predicted by the comparison of Kaneohe Bay water-column DIN:PO_4 and algal TN:TP ratios; (3) nutrient limitation is species-specific, and there is no system-wide pattern. Another objective of the study was to determine the appropriate microenvironments in which to measure nutrient concentrations in order to assess actual nutrient availability to algae (e.g. the well-mixed water column above algal thalli versus the water immediately adjacent to thalli or to reef substrata).

Materials and methods

Macroalgae species and collection sites

The nine macroalgae species used for experimental assays were *Padina japonica* Børgesen and *Sargassum echinocarpum* J. Agardh (Phaeophyceae), *Caulerpa racemosa* (Forsskål) Lamouroux, *C. sertularioides* (Gmelin) Howe, *Codium edule* Silva, *Dictyosphaeria versluysii* Weber van Bosse and *Ulva fasciata* Delile (Chlorophyceae), and *Gracilaria salicornia* (C. Agardh) Dawson and *Kappaphycus alvarezii* (Doty) Doty (Rhodophyceae). These species are abundant in Kaneohe Bay and represent a wide range of morphological forms. *Caulerpa racemosa* and *C. sertularioides* are coenocytic algae with horizontal axes that are partially buried in sediment and with unpigmented rhizoids that anchor thalli. *G. salicornia* and *K. alvarezii* have highly branched, intertwined thalli that form loose mats up to 2 m across. The remaining species occur as solitary thalli without rhizoids. Published results of experiments on the chlorophyte *Dictyosphaeria cavernosa* (Forskål) Børgesen, with the same methods used in the present study, are included here for comparison (Stimson et al. 1996). *D. cavernosa* forms dense mats over and between coral colonies on Kaneohe Bay reef slopes. The species used in this study can be assigned to three groups based on their morphologies and habitats: rhizophytic, sediment-dwelling species (*C. racemosa* and *C. sertularioides*), mat-forming species beneath which fine, organic matter-rich sediments accumulate (*D. cavernosa*, *G. salicornia* and *K. alvarezii*), and solitary species attached to limestone and associated with coarse carbonate sand (*Codium edule*, *Dictyosphaeria versluysii*, *P. japonica*, *S. echinocarpum* and *U. fasciata*).

Specimens of macroalgae were collected at four sites in southern and central Kaneohe Bay (Fig. 1). *Codium edule*, *Padina japonica* and *Sargassum echinocarpum* were collected from a high-energy reef-flat on the leeward side of the Kaneohe Bay Barrier Reef. *Caulerpa racemosa* and *C. sertularioides* were collected from a fringing reef flat at Heeia which is occasionally exposed to oceanic swell through a channel in the Barrier Reef. *Dictyosphaeria versluysii*, *Gracilaria salicornia* and *Ulva fasciata* were collected from the windward reef flat of Moku o Loe (Coconut Island), in southern Kaneohe Bay. *Kappaphycus alvarezii* was collected from the windward reef slope of Moku o Loe. Additional specimens of each species were collected for wet:dry weight determination and tissue nitrogen and phosphorus analyses.

Macroalgae preparation and culture

Whole macroalgae were transported in seawater to the Hawaii Institute of Marine Biology on Moku o Loe within 1 h of collection, and then rinsed and divided by hand or cut into experimental units. Apical portions of branched species (*Codium edule*, *Gracilaria salicornia*, *Kappaphycus alvarezii*) were used, except for *Sargassum echinocarpum*, from which only the apical portion of the main axis was used. The *Caulerpa racemosa* and *C. sertularioides* specimens each had an apical rhizome and 3 to 4 upright blades. Thalli of non-branched species (*Dictyosphaeria versluysii*, *Padina japonica*, *Ulva*

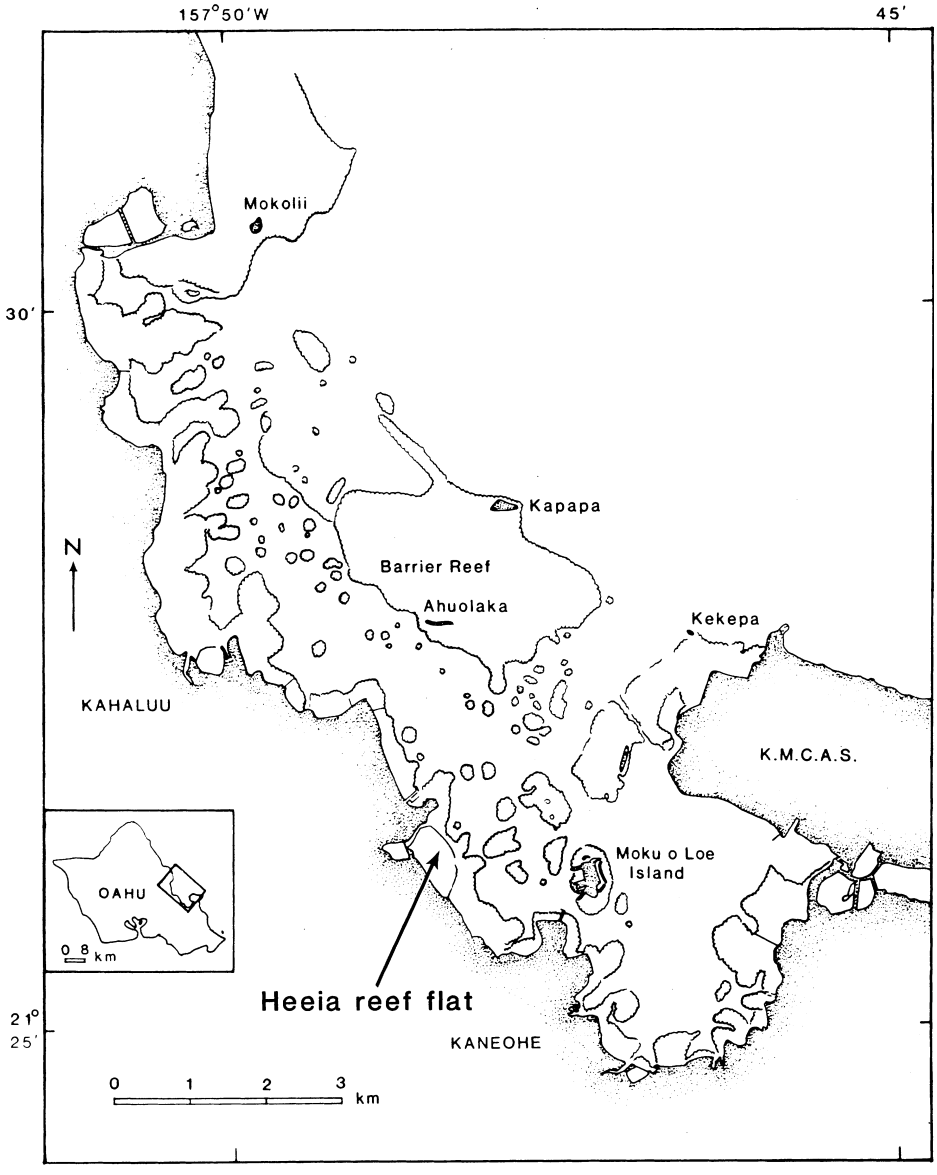

Fig. 1 Map of Kaneohe Bay, Oahu, Hawaii, showing four sites (Barrier Reef flat, Heeia reef flat, Moko o Loe reef flat and reef slope) at which macroalgae and nutrient samples were collected and relative flow rates were measured (*Open contour lines of Barrier Reef* indicate that substrata in northern portion is largely unconsolidated and shifting; *K.M.C.A.S.* Kaneohe Marine Corps Air Station)

fasciata) were used whole. Within 2 h of collection, thalli were placed in a flat (3 cm high) vinyl-mesh cage to exclude grazers and minimize self-shading, and then preconditioned for 3 to 4 d on the Moku o Loe reef slope at 2 m depth. Preconditioning served two purposes: thalli which had been cut to size were thus given sufficient time for healing, and the variability of initial thallus growth rates within species was reduced. Results from a pilot study with *C. racemosa*, *C. sertularioides*, *D. versluysii*, and *K. alvarezii* indicated that variance in growth rates was significantly reduced for each species after preconditioning. Following preconditioning, thalli were rinsed in unfiltered seawater, spun dry in a salad spinner, weighed, and then assigned at random to experimental treatments.

The nutrient-enrichment experiments were factorial in design, with two levels of nitrogen (enriched and unenriched) and two levels of phosphorus (enriched and unenriched). Each of the four treatments was replicated 4 to 8 times for each species during three runs of the experiment. A total of 12 experimental runs was carried out from January to November 1995. Not all species were used in each run, but the species were haphazardly distributed across the runs to minimize seasonal effects. Experimental runs were 11 to 12 d long, and thalli were cleaned of epiphytes and weighed at 2 to 3 d intervals. For all species except *Ulva fasciata*, experimental thalli were grown outdoors in opaque white 2-liter containers, each housing three thalli. For these species, ratios of algal biomass to seawater volume ranged from 1.5 to 8 g wet wt l^{-1} during experiments. In order to maintain low ratios of biomass:seawater volume with the fast-growing *U. fasciata*, 8-liter aquaria were used instead of 2-liter containers. Biomass:seawater volume ratios for *U. fasciata* ranged from 0.2 to 3 g wet wt l^{-1} during experiments.

Culture containers and aquaria were held in a single fiberglass tank and were independently supplied with flowing seawater, aeration, and – for thalli assigned to enrichment treatments – added nutrients. The seawater used for experiments was from the Hawaii Institute of Marine Biology seawater system. This water is pumped from the water column $\simeq$2 m from the windward reef slope of Moku o Loe at 1.5 to 2.5 m depth, depending on tide level. Before reaching the experimental setup, the seawater was filtered (100 μm) to reduce sediment accumulation in water lines and culture containers. Filtered seawater was then pumped to a headbox to maintain a constant flow rate of 350 ml min^{-1} $container^{-1}$, and (for *Ulva fasciata*) 500 ml min^{-1} $aquarium^{-1}$.

Nutrient solutions were continuously supplied to thalli assigned to enrichment treatments. Ammonium and phosphate solutions were prepared with filtered seawater and reagent-grade NH_4Cl and KH_2PO_4, respectively. Concentrations in the enrichment treatments were calculated to provide assigned thalli with 6 μ*M* ammonium

and/or 2 μM phosphate; concentrations in the ammonium + phosphate treatments were close to the N:P ratio of the water column. Actual nutrient concentrations in the enriched and unenriched seawater were measured during each experimental run (see following paragraph). A peristaltic pump was used to pump nutrient concentrates from carboys to 4-liter nutrient–seawater mixing chambers downstream from the headbox and upstream from the culture containers. Aeration was used to mix solutions in the chambers, and fluorescein dye was used to confirm that complete mixing had taken place before enriched seawater reached the aquaria and culture containers. Identical mixing chambers were used for treatments receiving unenriched seawater. Nutrient-enriched and unenriched seawater ran from the mixing chambers into 2 m-long PVC manifolds. The manifolds ran the length of the fiberglass tank holding culture containers and aquaria, and had plastic nozzles spaced $\simeq$20 cm apart. Tygon water lines were attached to each nozzle. Four water lines carrying ammonium-enriched, phosphate-enriched or unenriched seawater entered the top of each culture container. Experimental nutrient treatments were provided to the culture containers assigned them by varying the ratio of ammonium-enriched to phosphate-enriched to unenriched seawater lines. Mixing within the containers was enhanced by aeration. The arrangement of containers in the fiberglass tank was changed every 2 to 3 d to minimize position effects.

Water samples were collected from the culture containers during the experiment to monitor nutrient concentrations in unenriched seawater, and to determine actual enrichment levels. Mean ($\pm$1 SD) ammonium, nitrate + nitrite and phosphate concentrations in containers receiving unenriched seawater from the laboratory system were 0.52 $\pm$ 0.22, 0.34 $\pm$ 0.22 and 0.12 $\pm$ 0.03 μM, respectively (n = 23 samples). The mean ammonium concentration was significantly higher in the unenriched seawater used in the experiment than in the water column near the mouth of the seawater-system intake-pipe (0.19 $\pm$ 0.17, n = 79 samples, see "Results – Water-column nutrients"). This difference probably arises from excretion by invertebrates inhabiting the seawater-system pipes (Larned and Stimson 1997). Differences between nitrate + nitrite and phosphorus concentrations of unenriched seawater in the experimental containers and in the water column were not significant. Mean ($\pm$1 SD) ammonium and phosphate concentrations in the enrichment treatments were 6.08 $\pm$ 0.75 and 2.12 $\pm$ 0.30 μM, respectively (n = 18 samples).

Culture containers and aquaria were shaded to $\simeq$30% of full sun. Irradiance levels in the containers corresponded to these at reef-slope depths of 2 to 4 m, based on irradiance profiles measured between May and December 1994. Laboratory and field irradiance-measurements were made using a Biospherical QSI-140 meter and a 4π sensor. Rapid flow rates minimized heating in the culture containers. Daytime water temperatures in the containers during the study ranged from 22.5 to 28.0 °C, within the range measured on the Moku o Loe reef slope during the same period.

Analysis of algae growth

Growth rates of algal thalli were based on changes in wet weight because dry weight-based calculations would have required the use of predicted dry weights at the second-to-last weighing. Repeatability of wet weights was assessed by weighing six thalli of each species six times, keeping the thalli in seawater between weighings. Coefficients of variation were calculated for the wet weights of each thallus; these coefficients of variation were all $<$2%. To determine if there was a non-linear relationship between thallus weight and growth, the growth in wet weight (Δg d^{-1}) of 3 or 4 thalli from all nine species in each treatment was calculated after 5 or 6 d and regressed on initial wet weight. For each species, growth showed a positive linear relationship to initial wet weight and a Y-intercept that was not significantly different from zero. Thereafter, growth rates of experimental thalli were calculated as specific growth in wet weight (g g^{-1} d^{-1}).

To minimize the effects of initial responses to treatments on subsequent growth rates, the last 2 or 3 d growth increment calculated for each experimental run was used for comparing growth rates among treatments. Growth rates of the three thalli in each container were averaged and treated as a single data point. Two-way ANOVAs were used to compare differences among experimental treatments. Replicate experimental runs were treated as blocks in the ANOVAs.

Tissue carbon, nitrogen and phosphorus concentrations

Thalli collected for tissue carbon, nitrogen and phosphorus determination were rinsed in deionized water and frozen within 1 h of collection. Thalli were then dried to constant weight at 65 °C, lyophilized for 18 h, and ground with a mortar and pestle. Carbon and nitrogen concentrations in 10 to 20 mg aliquots of powdered tissue were measured using a Perkin-Elmer 2400 CHN Analyzer. Phosphorus concentrations in the remaining powdered tissue were measured after dissolution in 1 M HCl using a Perkin-Elmer 6500 inductively-coupled plasma spectrophotometer.

Water-column nutrient availability

Seawater samples for inorganic nutrient analysis were collected from the water column adjacent to the laboratory seawater-system intake-pipe, 1 m horizontally from the surface of the windward reef slope of Moku o Loe, at a depth of 1.5 to 2.5 m. These samples were collected two to four times a month from January 1994 to May 1997. Additional samples were collected on seven dates from September 1995 to September 1996 at the other sites from which macroalgae were collected. Reef-flat samples were collected at depths of 0.5 to 1 m, $\sim$0.5 m above the reef surface. All water samples were collected with acid-washed (10% HCl) and sample-rinsed syringes, filtered through Whatman GF/F filters into acid-washed, sample-rinsed Nalgene bottles, and then frozen until analysis. Ammonium, nitrate + nitrite, phosphate, total dissolved nitrogen and total dissolved phosphorus concentrations were measured with a Technicon Autoanalyzer II (see Walsh 1989 for details of analytical methods). The inorganic nutrient concentrations from the Moku o Loe data set were analyzed for seasonal trends using a cosine-regression model with a 12 mo period (Cryer 1985).

Localized nutrient availability at study sites

Several of the macroalgae used in the study occur in areas of high sediment cover and low coral cover. To determine if fine organic-matter rich sediments below *Caulerpa racemosa* and *C. sertularioides* on the Heeia reef flat, and coarse carbonate sand near *Codium edule*, *Padina japonica*, and *Sargassum echinocarpum* thalli on the Barrier Reef flat, were potential sources of localized nutrient enrichment, sediment porewater samples were collected and analyzed for nutrient concentrations. Porewater collectors (2.6 cm diam aquarium airstones attached to 20 cm of plastic tubing) were inserted 5 cm into sediment patches. Porewater collecters were left in place for a week to minimize effects of disturbing the sediment. At the time of collection, 10 ml of seawater were withdrawn by syringe through the plastic tubing and discarded, then another 40 ml were withdrawn, filtered through GF/F filters, and frozen. Additional seawater samples were collected 10 cm above sediment surfaces for comparison with porewater samples. To determine if nutrients accumulate within and beneath *S. echinocarpum* canopies, seawater samples were collected from beneath canopies (10 to 20 cm above the substratum) and from the water column $\sim$10 cm above canopies. Samples were collected by inserting the tip of a 10 cm-long Pasteur pipette attached to a syringe into the space beneath canopies to avoid disturbing thalli. To determine if nutrients accumulate within *Gracilaria salicornia* and *Kappaphycus alvarezii* mats, samples were collected from the approximate center and from 10 cm above the mats, again using pipettes attached to syringes to avoid disturbing thalli or sediments.

Water motion at study sites and in laboratory experiments

The dissolution of plaster cubes or "clod cards" was used to measure relative water motion at the four study sites over entire tidal cycles and in the containers and aquaria used for experiments. Clod cards were calibrated by measuring their dissolution rates in a flume at varied water velocities. Methods for clod card use and calibration are detailed in Larned and Stimson (1997). Estimates of water motion in the field were "relative" because the clod cards relate multidirectional flow in the field to an equivalent unidirectional flow in the flume. Estimated water motion in the field fell within the range used for calibration (0 to 25 cm s^{-1}). Field measurements using 10 to 11 clod cards per site were made in July and December 1995 and in March, April and September 1996. In the laboratory, clod cards were placed in culture containers and aquaria for 24 h, and exposed to the same seawater flow rate (350 to 500 ml min^{-1}) and aeration used during the experiments.

Results

Nutrient-limited growth

In each of the macroalgae species assayed, specific growth rate in either ammonium- or phosphate-enriched seawater was significantly higher than in unenriched seawater (Fig. 2, Table 1). For 8 of the 9 species, thalli supplied with ammonium-enriched seawater grew at significantly higher rates than thalli in unenriched or phosphate-enriched seawater (Fig. 2, Table 1). Mean specific growth rates of these species in ammonium-enriched seawater ranged from 0.003 g g^{-1} d^{-1} in *Dictyosphaeria versluysii*, to 0.08 g g^{-1} d^{-1} in *Ulva fasciata*. These growth rates are equivalent to biomass-doubling times of 231.0 and 8.7 d, respectively. Growth in a single species, the chlorophyte *Codium edule*, was enhanced by phosphorus-enrichment; the specific growth rate of *C. edule* thalli in phosphate-enriched seawater (0.023 g g^{-1} d^{-1}) was significantly higher than that of thalli in unenriched seawater (0.016 g g^{-1} d^{-1}). Ammonium enrichment did not have a significant effect on *C. edule* growth. Interactions between ammonium and

Fig. 2 Mean (±1 SD) specific growth rates (in wet wt) of macroalgae in nutrient-enrichment experiments and in controls (unenriched). Continuous nutrient-enrichment treatments raised ammonium and phosphorus concentrations to ≃6.1 and 2.1 μ*M*, respectively

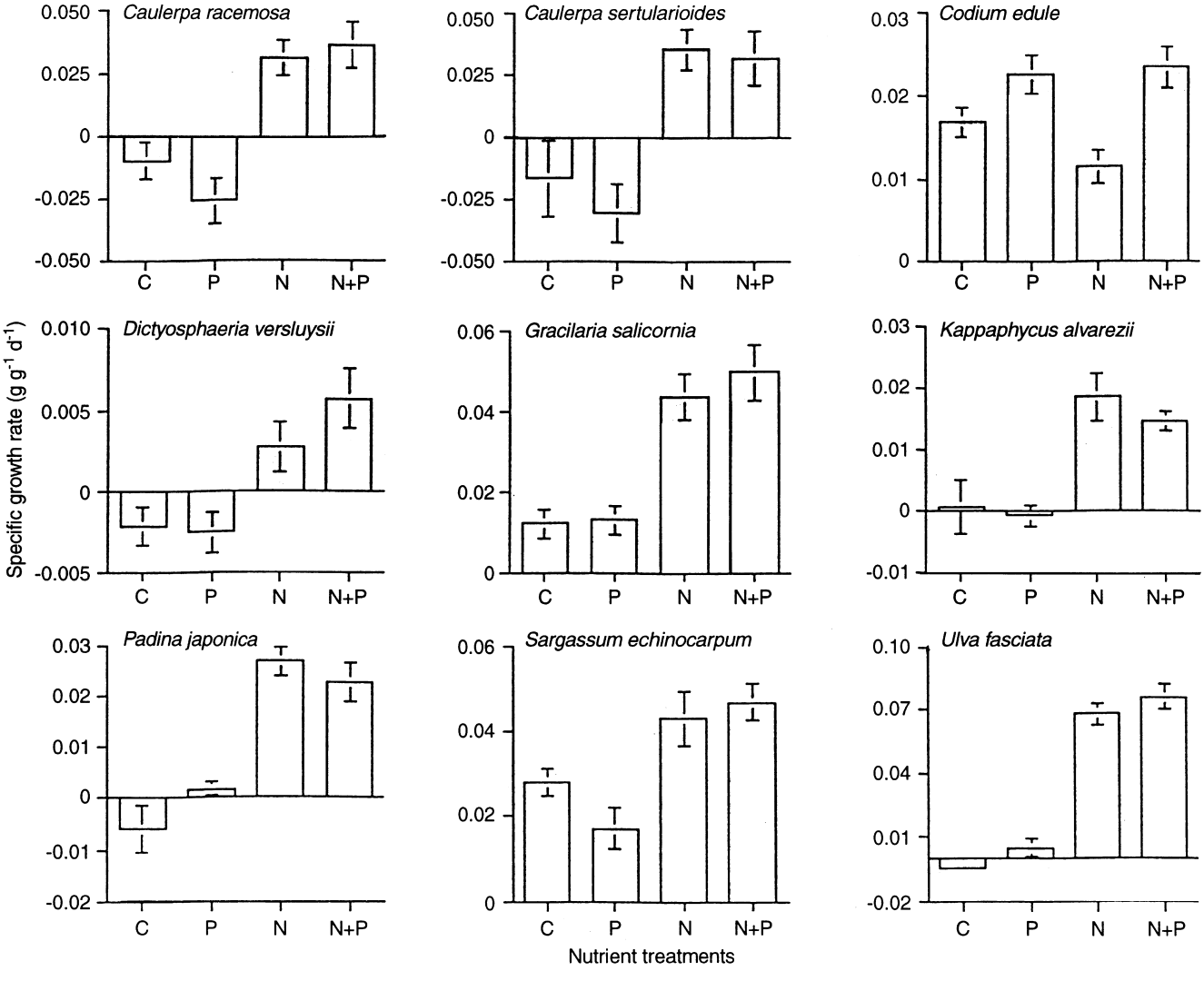

Table 1 Summary of factorial ANOVA results from growth bioassays (species here and in later tables are presented in alphabetical order). Mean specific growth rates are shown in Fig. 2 (*MS* mean square)

Species	Ammonium	Phosphate	Interaction	Run	Error	Total
Caulerpa racemosa						
df	1	1	1	2	75	80
MS	$5.41 \cdot 10^{-2}$	$1.02 \cdot 10^{-3}$	$2.03 \cdot 10^{-3}$	$1.72 \cdot 10^{-2}$	$1.10 \cdot 10^{-3}$	
p	< 0.001	> 0.10	> 0.10	< 0.001		
Caulerpa serratularioides						
df	1	1	1	2	49	54
MS	$4.15 \cdot 10^{-2}$	$9.98 \cdot 10^{-4}$	$4.60 \cdot 10^{-4}$	$7.61 \cdot 10^{-4}$	$1.66 \cdot 10^{-3}$	
p	< 0.001	> 0.10	> 0.10	> 0.10		
Codium edule						
df	1	1	1	2	74	79
MS	$7.37 \cdot 10^{-5}$	$1.51 \cdot 10^{-3}$	$2.01 \cdot 10^{-4}$	$5.83 \cdot 10^{-4}$	$6.57 \cdot 10^{-5}$	
p	> 0.10	< 0.001	> 0.05	< 0.001		
Dictyosphaeria versluysii						
df	1	1	1	2	51	55
MS	$6.94 \cdot 10^{-3}$	$2.34 \cdot 10^{-5}$	$4.18 \cdot 10^{-5}$	$2.17 \cdot 10^{-6}$	$1.58 \cdot 10^{-3}$	
p	< 0.05	> 0.10	> 0.10	> 0.10		
Gracilaria salicornia						
df	1	1	1	2	51	55
MS	$1.63 \cdot 10^{-2}$	$1.69 \cdot 10^{-4}$	$8.60 \cdot 10^{-5}$	$6.20 \cdot 10^{-3}$	$2.57 \cdot 10^{-4}$	
p	< 0.001	> 0.10	> 0.10	< 0.001		
Kappaphycus alvarezii						
df	1	1	1	2	77	82
MS	$7.34 \cdot 10^{-3}$	$1.10 \cdot 10^{-4}$	$5.39 \cdot 10^{-5}$	$5.44 \cdot 10^{-3}$	$2.03 \cdot 10^{-4}$	
p	< 0.001	> 0.10	> 0.10	< 0.001		
Padina japonica						
df	1	1	1	2	30	35
MS	$5.24 \cdot 10^{-3}$	$5.70 \cdot 10^{-6}$	$2.81 \cdot 10^{-4}$	$9.64 \cdot 10^{-5}$	$8.45 \cdot 10^{-5}$	
p	< 0.001	> 0.10	> 0.05	> 0.10		
Sargassum echinocarpum						
df	1	1	1	2	76	81
MS	$9.85 \cdot 10^{-3}$	$3.03 \cdot 10^{-4}$	$9.64 \cdot 10^{-4}$	$2.12 \cdot 10^{-3}$	$4.42 \cdot 10^{-4}$	
p	< 0.001	> 0.10	> 0.10	< 0.05		
Ulva fasciata						
df	1	1	1	2	30	35
MS	$9.51 \cdot 10^{-2}$	$5.00 \cdot 10^{-6}$	$2.98 \cdot 10^{-3}$	$6.21 \cdot 10^{-3}$	$1.69 \cdot 10^{-3}$	
p	< 0.001	> 0.10	> 0.10	< 0.05		

phosphate-enrichment treatments were not significant for any species (Table 1).

When grown in unenriched seawater specific-growth rates of all nine species declined over time (Fig. 3). Six species, *Caulerpa racemosa*, *C. sertularioides*, *Dictyosphaeria versluysii*, *Kappaphycus alvarezii*, *Padina japonica* and *Ulva fasciata* were unable to sustain positive growth (specific growth rates < 0 g g^{-1} d^{-1}) in unenriched seawater by the end of the experimental runs.

Algal TN:TP ratios

The tissue TN:TP ratios of all macroalgae were higher than the water-column N:P ratios at the study sites; the average for the nine species tested was 65.8:1 (Table 2). TN:TP ratios for *Caulerpa racemosa* (148.7:1) and *C. sertularioides* (198.9:1) were among the highest reported for macroalgae under field conditions. When the *Caulerpa* spp. values are excluded, the mean percent nitrogen, percent phosphorus and TN:TP ratio of the remaining species was 1.3, 0.08 and 34.9:1, respectively.

Water-column nutrients

DIN concentrations in the water column at the collection sites were generally < 0.5 μ*M* and phosphate concentrations were < 0.2 μ*M* (Table 3). There were no significant differences in water-column ammonium, nitrate + nitrite and phosphate concentrations among sites (one-way ANOVA, $p > 0.2$ for each comparison). DIN and phosphate concentrations in the water column at the Moko o Loe reef slope over a 39 mo period are shown in Fig. 4. The cosine regression-analysis indicated that ammonium and nitrate + nitrite concentrations at this site varied seasonally, with higher values in winter and lower values in autumn; $R^2 = 8.2\%$, $p < 0.05$ for ammonium, and $R^2 = 8.3\%$, $p < 0.05$ for nitrate + nitrite. The amplitudes of the seasonal cycles in

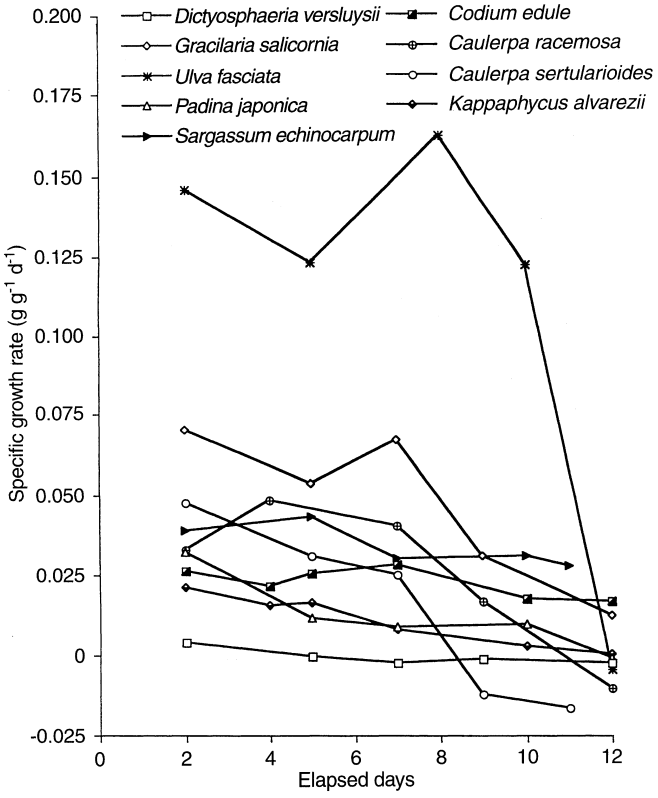

Fig. 3 Time courses of growth of macroalgae in control treatments (unenriched seawater from laboratory seawater system) during nutrient-enrichment experiments. Data points are mean specific growth rates calculated over previous time increment. Mean nutrient concentrations in control treatments during experiments were 0.52 μ*M* ammonium, 0.34 μ*M* nitrate + nitrite, and 0.12 μ*M* phosphate.

ammonium and nitrate + nitrite concentrations were ~0.1 and 0.2 μ*M*, respectively. No seasonality was detected in either untransformed or natural log-transformed phosphate concentrations at the same site. Mean dissolved organic nitrogen (DON) and dissolved organic phosphorus (DOP) concentrations in the water column at the Moko o Loe reef slope were 6.73 and 0.19 μ*M*, respectively. DON concentrations at this site were ~10 times higher than DIN concentrations, and DOP concentrations were ~1.5 times higher than phosphate concentrations. Water-column DIN:PO_4 ratios, based on the mean DIN and phosphate concentrations in Table 3, were 4.1:1 at the Barrier Reef flat, 4.5:1 at the Heeia reef flat, 4.9:1 at the Moku o Loe reef flat and 4.2:1 at the Moko o Loe reef slope. The average DIN:PO_4 ratio for all sites was 4.4:1.

Table 2 Nitrogen and phosphorus concentrations (% dry wt) and total nitrogen:phosphorus (TN:TP) ratios in macroalgae tissues under field conditions. Values are means of four samples ± 1 SD

Species	Nitrogen	Phosphorus	TN:TP
Caulerpa racemosa	5.6 ± 3.3	0.08 ± 0.03	148.7:1
Caulerpa sertularioides	12.7 ± 1.13	0.14 ± 0.08	198.9:1
Codium edule	1.46 ± 0.13	0.10 ± 0.01	33.4:1
Dictyosphaeria versluysii	0.78 ± 0.03	0.06 ± 0.01	27.2:1
Gracilaria salicornia	0.93 ± 0.13	0.07 ± 0.03	29.5:1
Kappaphycus alvarezii	0.63 ± 0.02	0.05 ± 0.01	29.7:1
Padina japonica	1.32 ± 0.09	0.08 ± 0.01	38.2:1
Sargassum echinocarpum	1.32 ± 0.08	0.08 ± 0.01	38.2:1
Ulva fasciata	2.69 ± 0.29	0.12 ± 0.01	48.3:1

Localized nutrient enrichment at collection sites

Ammonium and nitrate + nitrite concentrations within mats of both *Gracilaria salicornia* and *Kappaphycus alvarezii* were significantly higher than in the water column 10 cm above the mats (Student's *t*-tests, $p < 0.05$; Table 4). No differences were detected between phosphate concentrations within algal mats and in the overlying water column. Ammonium and nitrate + nitrite concentrations in the shallow (5 cm depth) porewater below *Caulerpa racemosa* and *C. sertulariodes* was significantly higher than in the water column 10 cm away (Student's *t*-tests, $p < 0.05$; Table 4). No differences were detected between porewater and water-column phosphate concentrations. No differences were detected between ammonium or nitrate + nitrite concentrations beneath *Sargassum echinocarpum* canopies, the water column above the canopies and the sediment porewater near the canopies. Phosphate concentrations in the sediment porewater

Table 3 Dissolved inorganic and organic nitrogen and phosphorus concentrations (μ*M*) in water column at sites of macroalgae collections. Reef-slope samples were collected at depth of 1.5 to 2.5 m, ~1 m from reef surface; reef-flat samples were collected at depth of 0.5 to 1 m, ~0.5 m from reef surface. Values are means ± 1 SD, sample size in parentheses (*nm* no measurement made; *DON* dissolved organic nitrogen; *DOP* dissolved organic phosphorus)

Site	NH_4	$NO_3 + NO_2$	PO_4	DON	DOP
Barrier Reef flat	0.25 ± 0.11 (7)	0.19 ± 0.09 (7)	0.11 ± 0.02 (7)	nm	nm
Heeia reef flat	0.18 ± 0.09 (7)	0.28 ± 0.37 (7)	0.11 ± 0.09 (7)	nm	nm
Moko o Loe reef flat	0.31 ± 0.21 (7)	0.19 ± 0.14 (7)	0.11 ± 0.05 (7)	nm	nm
Moko o Loe reef slope	0.19 ± 0.17 (79)	0.25 ± 0.31 (79)	0.11 ± 0.04 (79)	6.73 ± 1.58 (11)	0.19 ± 0.08 (11)

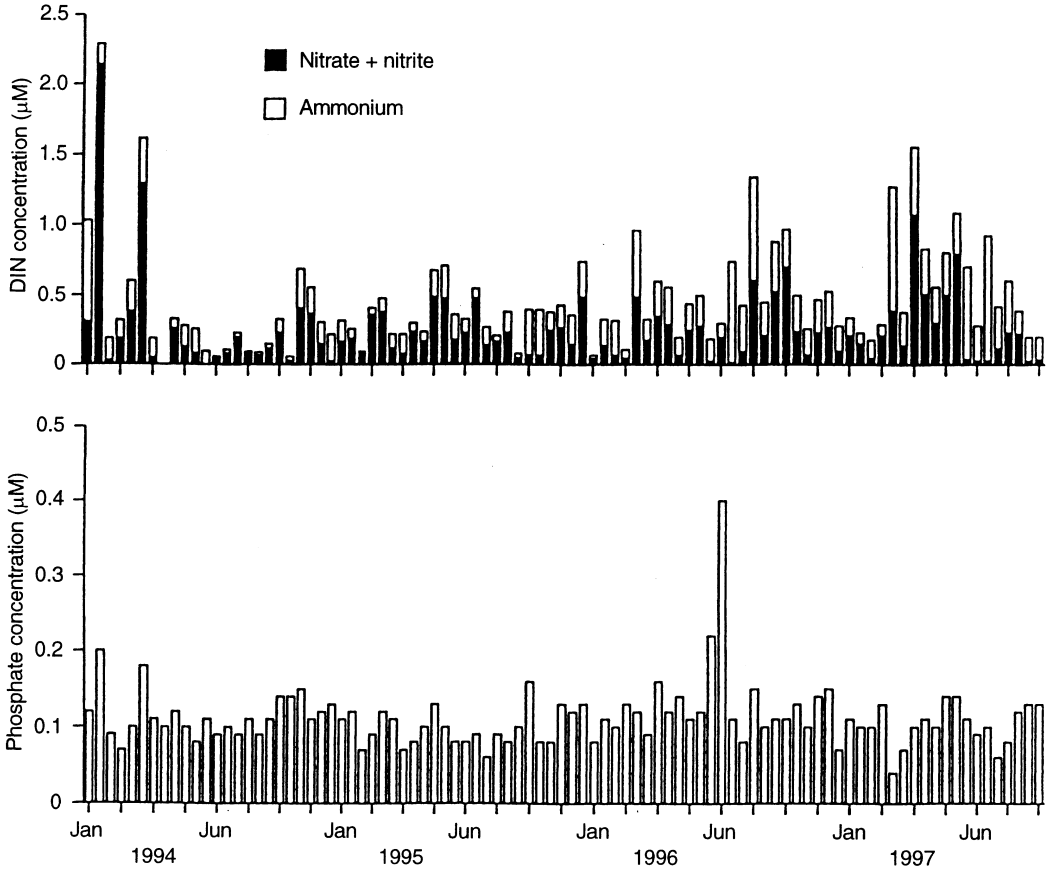

Fig. 4 Inorganic nitrogen (*DIN*) and phosphate concentrations in water column at 1.5 to 2.5 m depth. Samples were collected 2 to 4 times per month near Hawaii Institute of Marine Biology seawater-intake pipe, 1 m horizontally from surface of windward reef slope of Moku o Loe

were significantly higher than in the water beneath or above *S. echinocarpum* canopies (one-way ANOVA, $p < 0.025$; Table 4).

Water motion at study sites and in laboratory experiments

Relative water-flow rates, as measured by clod cards, were highest at the Barrier Reef flat and the Heeia fringing-reef flat, the sites with the greatest exposure to oceanic swell, and lowest on the reef flat and reef slope of Moku o Loe (Table 5). Moku o Loe is protected from swells by the Mokapu Peninsula (Fig. 1). Average flow rates for the seven sampling dates at each site were

Table 4 Localized nutrient concentrations (μM) associated with canopy-forming, mat-forming and rhizophytic algae. Porewater concentrations are from samples collected 5 cm below sediment surface. Values are means ± 1 SD, sample sizes in parentheses

Species	Water column above thalli			Under canopy or within mat			Porewater		
	NH_4	NO_3+NO_2	PO_4	NH_4	NO_3+NO_2	PO_4	NH_4	NO_3+NO_2	PO_4
Caulerpa racemosa and *C. sertularioides*	0.14 ± 0.06 (6)	0.13 ± 0.10 (6)	0.13 ± 0.11 (6)	nm	nm	nm	12.6 ± 12.2 (6)	0.74 ± 0.69 (6)	0.85 ± 0.83 (6)
Gracilaria salicornia	0.42 ± 0.21 (4)	0.27 ± 0.32 (4)	0.14 ± 0.02 (4)	1.43 ± 0.15 (4)	1.88 ± 0.29 (4)	0.29 ± 0.08 (4)	nm	nm	nm
Kappaphycus alvarezii	0.51 ± 0.37 (4)	0.25 ± 0.17 (4)	0.13 ± 0.02 (4)	1.21 ± 0.39 (4)	1.02 ± 0.34 (4)	0.24 ± 0.08 (4)	nm	nm	nm
Sargassum echinocarpum	0.26 ± 0.14 (5)	0.18 ± 0.10 (5)	0.11 ± 0.03 (5)	0.31 ± 0.06 (5)	0.23 ± 0.10 (5)	0.12 ± 0.06 (5)	0.37 ± 0.12 (4)	0.43 ± 0.31 (4)	0.44 ± 0.25 (4)

Table 5 Relative water-flow rates (cm s^{-1}) at sites of macroalgae collections. Values are means ± 1 SD, number of clod cards in parentheses

Date	Barrier Reef flat	Heeia reef flat	Moku o Loe reef flat	Moku o Loe reef slope
1995				
29–30 July	nm	nm	4.4 ± 2.6 (10)	1.3 ± 0.3 (11)
21–22 Dec	14.7 ± 0.9 (11)	7.9 ± 0.3 (11)	nm	nm
28–29 Dec	15.3 ±0.5 (10)	8.5 ± 0.4 (10)	5.0 ± 0.4 (10)	1.4 ± 0.1 (10)
1996				
2–3 Mar	18.8 ± 2.5 (11)	4.6 ± 0.8 (11)	5.2 ± 1.8 (11)	1.0 ± 0.2 (11)
21–22 Mar	16.7 ± 2.6 (11)	4.2 ± 1.0 (11)	3.8 ± 0.9 (11)	3.6 ± 1.1 (11)
22–23 Aug	12.84 ± 0.42 (11)	6.67 ± 0.38 (11)	4.17 ± 0.36 (11)	0.92 ± 0.24 (11)
29–30 Sep	15.96 ± 0.34 (11)	5.48 ± 0.5 (11)	4.73 ± 0.3 (11)	1.04 ± 0.7 (11)

15.7 cm s^{-1} at the Barrier Reef flat, 6.2 cm s^{-1} at the Heeia reef flat, 4.7 cm s^{-1} at the Moku o Loe reef flat and 1.5 cm s^{-1} at the Moku o Loe reef slope. The average flow rate in the 2-liter containers used for the nutrient-enrichment experiments was 9.8 ± 3.3 cm s^{-1} (mean ± 1 SD, $n = 28$). The average flow rate in the 8-liter aquaria used for *Ulva fasciata* was 7.9 ± 2.8 cm s^{-1} (mean ± 1 SD, $n = 7$).

Discussion

Nitrogen vs phosphorus-limited growth

Results of the nutrient-enrichment experiments indicate that inorganic nitrogen availability limited the growth rates of 8 out of 9 Kaneohe Bay macroalgae tested in the laboratory-culture system. Nutrient-limited growth in a tenth species from Kaneohe Bay reefs, the chlorophyte *Dictyosphaeria cavernosa*, had been assayed in two previous studies using the same culture system (Stimson et al. 1996; Larned and Stimson 1997). Results of those assays indicated that growth in *D. cavernosa* was nitrogen-limited and could not be sustained in unenriched seawater from the laboratory system. In the *D. cavernosa* studies and in the present study, ammonium concentrations were higher in the unenriched laboratory seawater than in the Kaneohe Bay water column, and average flow rates were higher in the laboratory experiments (8 to 10 cm s^{-1}) than at any field site except the Barrier Reef (Table 5). These observations suggest that the rates at which nutrients were supplied to macroalgae in the unenriched laboratory treatments were higher than at most field sites. Consequently, nitrogen-limited species without access to elevated nutrients in their microenvironments may be even more severely nutrient-limited in the field than in the laboratory. However, for species that create nitrogen-enriched microenvironments (e.g. *Gracilaria salicornia*, *Kappaphycus alvarezii*), nitrogen limitation in the laboratory is not conclusive evidence of nitrogen limitation in the field. For example, the DIN concentrations within *G. salicornia* and *K. alvarezii* mats were generally higher than the DIN concentrations in the unenriched laboratory controls. For these species, the identity of the limiting nutrient in the field would be best substantiated with field experiments. Results of a field experiment and laboratory culture at a range of DIN enrichment levels from 0.5 to 33 μM demonstrated that *D. cavernosa* is nitrogen-limited in the field as well as in the laboratory, despite the fact that this mat-forming alga creates a microenvironment in which DIN concentrations are higher than in the laboratory seawater (Larned and Stimson 1997).

That nitrogen appears to be generally limiting is consistent with a comparison of the average DIN:PO_4 ratio in the Kaneohe Bay water column (4.4:1) and the average tissue TN:TP ratio of 30 macroalgae from Kaneohe Bay (43.7:1) (Atkinson and Smith 1983; Smith 1994). However, growth of the chlorophyte *Codium edule* was phosphorus-limited (Fig. 2), and there may be other phosphorus-limited macroalgae among the approximately 90 species that occur in Kaneohe Bay (Soegiarto 1973). The fact that *C. edule* had a tissue TN:TP ratio within the range of values for the nitrogen-limited species (Table 2) illustrates the need for experimental determination of the type and severity of nutrient limitation.

Examination of tissue TN:TP ratios has been recommended as a rapid method for determining if nitrogen or phosphorus limits productivity in the field, independent of nutrient availability (Wheeler and Björnsäter 1992). High TN:TP ratios (>15 in temperate regions, >30:1 in tropical regions) have been considered to be an indication of phosphorus limitation and luxury nitrogen storage in macroalgae, because maximum growth rates have been measured at TN:TP ratios between 10:1 and 20:1 (Björnsäter and Wheeler 1990; Littler et al. 1991; Lapointe et al. 1992; Wheeler and Björnsäter 1992). Despite the high TN:TP ratios in the species used in the present study, nitrogen storage appears to be limited in these species, as indicated by decreasing growth rates in unenriched seawater during the experimental runs (Fig. 3). In six species, nitrogen reserves were apparently exhausted after 10 to 11 d in unenriched seawater, as growth rates declined to zero and thalli began to lose weight. Furthermore, those nitrogen-limited species which maintained positive growth rates without nitrogen enrichment (*Gracilaria salicornia*, *Sargassum echinocarpum*) did not have higher than average TN:TP ratios, while some of the severely nitrogen-limited species (e.g. *Caulerpa* spp.) had TN:TP ratios >100:1 (Table 2). These observations suggest that high TN:TP ratios in Kaneohe Bay macroalgae are generally an indication of high nitrogen requirements, not high levels of nitrogen storage.

Broad generalizations about the identity of growth-limiting nutrients for coral reef macroalgae are probably unwarranted, since nutrient concentrations and fluxes

Table 6 Summary of nitrogen (N)- and phosphorus (P)-enrichment bioassays using macroalgae from tropical, oligotrophic [≤1.5 μ*M* dissolved inorganic nitrogen (DIN) and ≤0.2 μ*M* PO_4] ecosystems, and DIN:PO_4 ratios in water column at collection sites. In each investigation, bioassays were carried out in situ, or in a laboratory system shortly after collection [*Class*: *P* Phaeophyceae, *R* Rhodophyceae, *C* Chlorophyceae; *Assay*: *P* photosynthesis (oxygen evolution), *G* tissue growth; for N and P treatments: + main effect of nutrient enrichment significantly greater than control, − significant inhibitory effect of nutrient enrichment, *O* effect of nutrient enrichment not significant; for N + P treatments: + significant postive interaction, − significant negative interaction, *O* interaction not significant; *na* not applicable (N + P treatment not included or interaction term not provided)]

Species	Class	Assay	N	P	N + P	DIN:PO_4	Source
Acanthophora spicifera	R	P	0	+	na	18.5	Lapointe et al. (1987)
Dictyota divaricata	P	P	0	+	na	18.5	
Halimeda opuntia	C	P	+	0	na	18.5	
Gracilaria tikvahiae	R	G	+	+	+	< 10	Lapointe (1989)
Laurencia poitei	R	G	0	+	0	< 10	
Sargassum polyceratium	P	G	0	+	0	< 10	
Penicillus capitatus	C	P	+	0	na	> 30	McGlathery et al. (1992)
Halymenia sp.	R	P	0	+	na	7.0	Littler et al. (1991)
Microdictyon montagne	C	P	0	0	na	10.8	
Caulerpa cupressoides	C	P	0	0	na	10.8	
Caulerpa sp.	C	P	0	+	na	?[a]	
Gracilaria crassa	R	P	−	−	na	3.5	
Lobophora variegata	P	P	0	0	na	3.5	
Halimeda micronesica	C	P	0	+	na	3.0	
Halimeda opuntia	C	P	+	0	na	3.0	
Avrainvillea sp.	C	P	+	+	na	5.8	
Codium sp.	C	P	+	+	na	5.8	
Udotea argentea	C	P	+	+	na	5.8	
Udotea orientalis	C	P	+	+	na	5.8	
Udotea palmata	C	P	+	+	na	5.8	
Udotea sp.	C	P	0	+	na	5.8	
Penicillus capitatus	C	P	+	0	na	3.1	Delgado and Lapointe (1994)[c]
Penicillus dumetosus	C	P	+	0	na	3.1	
Halimeda opuntia	C	P	+	0	na	3.1–9.2[b]	
Dictyota cervicornis	P	P	0	0	na	9.2	
Hydroclathrus clathratus	P	P	+	+	na	3.1	
Laurencia intricata	R	P	+	0	na	3.1–9.2	
Ulva sp.	C	P	+	+	na	3.1–9.2	
Dictyosphaeria cavernosa	C	G	+	0	0	4.2	Larned and Stimson (1997)
Caulerpa racemosa	C	G	+	0	0	4.5	Present study
Caulerpa sertularioides	C	G	+	0	0	4.5	
Codium edule	C	G	0	+	0	4.1	
Dictyosphaeria versluysii	C	G	+	0	0	4.9	
Gracilaria salicornia	R	G	+	0	0	4.9	
Kappaphycus alvarezii	R	G	+	0	0	4.2	
Padina japonica	P	G	+	0	0	4.1	
Sargassum echinocarpum	P	G	+	0	0	4.1	
Ulva fasciata	C	G	+	0	0	4.9	

[a] PO_4 concentration below limits of detection
[b] Seasonal range
[c] Site described as eutrophic, but mean seasonal DIN concentrations range from 0.33 to 0.59 μ*M*, mean seasonal PO_4 concentrations range from 0.07 to 0.11 μ*M*

are variable among coral reef habitats (Johannes et al. 1983; D'Elia and Wiebe 1990; Table 4 of present study) and since macroalgae have diverse strategies for acquiring nutrients and allocating nutrients to growth, reproduction and storage (Rosenberg and Ramus 1981, 1982). A survey of results from nutrient-enrichment experiments using coral reef macroalgae bears this out: of 36 species tested in seven studies, inorganic nitrogen enrichment enhanced growth in 22 species and inorganic phosphorus enhanced growth in 17 species (Table 6). There is no apparent pattern of nitrogen or phosphorus limitation attributable to experimental methods, e.g. measurements of growth or photosynthetic rate. Distinct patterns of nutrient limitation within taxonomic classes are not apparent either: 4 rhodophytes listed in Table 6 were nitrogen-limited and 4 were phosphorus-limited, 3 phaeophytes were nitrogen-limited and 3 were phosphorus-limited, 15 chlorophytes were nitrogen-limited and 10 were phosphorus-limited. Thus, neither nitrogen nor phosphorus appears to be generally limiting across coral reef ecosystems.

Localized sources of nutrients

Positive growth could not be sustained in a number of macroalgae species cultured in unenriched seawater from the Kaneohe Bay water column, despite their

persistence on Kaneohe Bay reefs. These observations suggest that the water column is not the sole source of nutrients for these species in the field; nutrients acquired from benthic sources may be necessary for sustained growth. Concentrations of DON and DOP in the Kaneohe Bay water column are higher than DIN and phosphate (Table 3), and it is possible that some macroalgae use dissolved organic nutrients for growth; however, the lack of sustained growth in unenriched seawater argues against this. Laboratory experiments with *Dictyosphaeria cavernosa* from Kaneohe Bay indicated that DON (as urea) enrichment did not enhance growth (Larned and Stimson 1997).

Macrofaunal excretion (Meyer and Schultz 1985; Williams and Carpenter 1988), groundwater seeps (D'Elia et al. 1981; Lewis 1987) and remineralized organic matter in reef cavities and sediment patches (Andrews and Müller 1983; Williams et al. 1985; Hansen et al. 1987; Johnstone et al. 1989; Capone et al. 1992; Boucher et al. 1994) have been identified as sources of localized nutrient enrichment in coral reef systems. When dissolved nutrients are released from these sources at rates higher than the rate of mixing into the water column, the water adjacent to the benthos becomes enriched and macroalgae may acquire nutrients from this enriched zone (Lapointe and O'Connell 1989; Lavery and McComb 1991; Krause-Jensen et al. 1996; Larned and Stimson 1997). In this study, it was determined that concentrations of ammonium and nitrate + nitrite within *Gracilaria salicornia* mats on reef flats and *Kappaphycus alvarezii* mats on the reef slopes were significantly higher than in the overlying water column (Table 4). It is probable that the accumulation of nutrients in the algal mats results from efflux from sediment patches beneath the mats.

If efflux from sediments constitutes an important source of nutrients for benthic macroalgae, then the ratio of the rates at which DIN and phosphate are released from the sediment is a relevant measure of relative nutrient availability. In Kaneohe Bay, DIN and phosphate efflux from Moku o Loe reef slope sediments were measured during the same period as the present study (Stimson and Larned unpublished data). The mean DIN and phosphate-efflux rates at this site were 704 and 94 $\mu M\ m^{-2}\ d^{-1}$, respectively ($n = 21$ measurements from 1994 to 1997). The mean efflux DIN:PO_4 ratio from this data set was 7.5:1, and ranged from 0.1:1 to 31:1. The variability observed in efflux DIN:PO_4 ratios suggests that macroalgae that use sediment-derived nutrients could undergo shifts from nitrogen- to phosphorus-limited growth as the relative availability of DIN and phosphate changes. This phenomenon has been described for phytoplankton (Cowen et al. 1996). However, tissue TN:TP ratios for most of the macroalgae used in the study (Table 2) and for *Dictyosphaeria cavernosa* (Larned and Stimson 1997), are greater than the maximum efflux DIN:PO_4 ratio so temporary shifts from nitrogen to phosphorus limitation in these species are probably infrequent. The range of water-column DIN:PO_4 ratios (Table 3) in the 1994 to 1997 Moku o Loe data set is 0.5:1 to 15:1. Thus, for macroalgae that rely on the water column as a primary nutrient source, the DIN:PO_4 ratio in the water column is less variable than the efflux DIN:PO_4 ratio, so shifts from nitrogen to phosphorus limitation in these species are probably even more infrequent. These observations suggest that, for species shown to be nitrogen limited in this study, nitrogen limitation is likely to be a persistent condition in the field.

In wave-exposed habitats such as the Kaneohe Bay Barrier Reef flat, sediments are composed of coarse carbonate sand, and porewater nutrient concentrations are low. High water-flow rates may compensate for the lack of sediment-derived nutrients by generating high nutrient-advection rates (the product of water-flow rate and nutrient concentration). Inorganic nutrient concentrations under *Sargassum echinocarpum* canopies on the Barrier Reef flat were not higher than in the overlying water column. This lack of enrichment beneath canopies was expected, as water flow over the Barrier Reef flat is typically rapid (14 to 20 cm s^{-1}) and turbulent, and the *S. echinocarpum* canopies are swept back and forth by passing wave orbitals. Water beneath the canopies is rapidly flushed, preventing the accumulation of nutrients. *Codium edule*, and *Padina japonica*, also common on the Barrier Reef, do not form mats, canopies, or other structures that could impede the mixing of sediment-derived nutrients into the water column. Porewater nutrient concentrations are low in the coarse, porous carbonate sediments of the Barrier Reef, which precludes high rates of nutrient efflux. It is therefore likely that rapid advection from the water column is the primary source of nutrients for *S. echinocarpum*, *C. edule* and *P. japonica* on the Barrier Reef flat.

In contrast to the other macroalgae investigated here, growth in the chlorophyte *Codium edule* was phosphorus-limited. Nitrogen-fixing endophytic cyanobacteria have been reported on the photosynthetic surfaces of several species of *Codium* (Dromgoole et al. 1978; Rosenberg and Paerl 1981; Gerard et al. 1990), and transfer of fixed nitrogen from the nitrogen-fixing endophytes to host tissues could explain, in part, phosphate-limited growth in these species. Heterocystous cyanobacteria are also abundant in *C. edule* thalli from the Kaneohe Bay barrier reef (author's personal observation). The presence of nitrogen-fixing cyanobacteria suggests, but does not in itself prove, that these endophytes function as localized nutrient sources. Dromgoole et al. (1978) and Rosenberg and Paerl (1981) calculated that fixed nitrogen released by symbiotic cyanobacteria constituted only a small fraction (4 to 6%) of the nitrogen budgets of *Codium* spp. As with the other Barrier Reef species, *Padina japonica* and *Sargassum echinocarpum*, nutrient advection from the water column may be the primary source of nutrients for *C. edule*. The fact that *P. japonica* and *S. echinocarpum* were nitrogen-limited while *C. edule* was phosphorus-limited may be due to dissimilar nutrient requirements for growth and dissimilar storage

capacities, rather than the presence of endophytic cyanobacteria.

Rhizophytic macroalgae such as *Caulerpa* spp. may have direct access to nutrient-rich sediment porewaters in addition to other localized sources. Williams (1984) showed that *C. cupressoides* translocates ammonium from subsurface rhizoids to photosynthetic regions of the thallus above the sediment, and reasoned that porewater was the primary nitrogen source for *C. cupressoides*. Ammonium, nitrate + nitrite and phosphate concentrations in sediment porewater beneath *C. racemosa* and *C. sertularioides* were 90, 5.7 and 6.5 times higher, respectively, than in the overlying water (Table 4).

Kaneohe Bay may be viewed as a mosaic of macroalgal habitats ranging from wave-exposed sites, like the Barrier Reef flat to very protected sites such as patch-reef slopes in southern Kaneohe Bay. At the most exposed sites, advection from the water column is likely to be the primary source of nutrients for macroalgae, while at protected sites the contribution of water-column nutrients to algal requirements decreases and the contribution of nutrients from benthic sources increases. A simple model relating flow rate and water-column DIN and phosphate concentrations to growth in *Dictyosphaeria cavernosa* predicted that, at sites were the average flow rate is >5 cm s^{-1}, the alga can sustain long-term growth with no additional nutrients from the benthos (Larned and Atkinson 1997). At sites where the average flow rate is <5 cm s^{-1}, the model predicted that growth in *D. cavernosa* cannot be sustained without access to benthic nutrients. Because these protected habitats are also sites at which turbulence is low, organic matter-rich sediments accumulate on the reef substrata. Following the remineralization of organic matter, dissolved inorganic nutrients are released from these sediments and enrich the benthic environments inhabited by macroalgae. On the basis of observations made in this study, it may be suggested that distributions of macroalgae species in oligotrophic coral reef systems depend in part on patterns of benthic nutrient availability and water flow, on the nutrient requirements of individual species, and on the mechanisms by which those species acquire nutrients.

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