Vol. 24: 209–224, 2001 | AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol | Published July 18

Net growth rates of picocyanobacteria and nano-/microphytoplankton inhabiting shelf waters of the central (17° S) and southern (20° S) Great Barrier Reef

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ABSTRACT: Growth rates of diffusion cultures of nano- and microphytoplankton from Great Barrier Reef shelf water, especially diatoms (μ_{max} = 3.2 doublings d^{-1}), exceeded those of picocyanobacteria (*Prochlorococcus* and *Synechococcus*) when DIN ($NH_4^+ + NO_2^- + NO_3^-$) concentrations were ≥0.05 µM. The picocyanobacteria (μ_{max} = 1.1 and 1.6 doublings d^{-1} for *Prochlorococcus* and *Synechococcus* respectively) achieved higher relative and absolute growth rates when time-averaged DIN concentrations were <0.05 µM. Most estimates (71%) of *in situ* growth rates of nano-/microphytoplankton were ≤0.25 of μ_{max} when DIN concentrations were <0.1 µM, while only 18% of *in situ* picocyanobacteria growth rates were ≤0.25 of μ_{max} when DIN concentrations were <0.1 µM, the majority being ≥0.50 of μ_{max} at such DIN concentrations. Thus growth rates of *Synechococcus* and *Prochlorococcus* populations did not appear to be significantly nutrient (nitrogen)-limited under ambient concentrations, and were of similar order to those measured in the equatorial Pacific Ocean, where NO_3^- concentrations (>2 µM) are far above growth-saturating levels, and in the oligotrophic North Pacific Gyre. In contrast, for those nano-/microphytoplankton for which *in situ* or simulated *in situ* growth rates were estimated, growth rates appeared to be nitrogen-limited at DIN concentrations <0.1 µM. The results provide further support for the hypothesis that picocyanobacteria dominate oligotrophic marine water columns because of their superior ability to grow at low nutrient concentrations.

KEY WORDS: *Synechococcus* · *Prochlorococcus* · Tropical nano-/microphytoplankton · Growth rates

INTRODUCTION

Planktonic primary productivity of oceans and seas, as C fixed per unit volume or area, is increasingly dominated by autotrophic picoplankton, particularly picocyanobacteria, as systems tend towards oligotrophy (Agawin et al. 2000). As nutrients increase within the water column, however, larger phytoplankton, particularly diatoms, are observed to increase their relative contribution to primary productivity (e.g. Mousseau et al. 1996, Furnas & Mitchell 1997, Velduis et al. 1997). While these patterns in primary productivity have been consistently observed, providing important clues on the processes underlying phytoplankton dynamics, direct estimates of *in situ* growth rates are often lacking or, if available, are low in biological information, due to the poor taxonomic resolution of the method(s) used to measure growth (see reviews by Furnas 1990, Furnas & Crosbie 1999). Estimates of *in situ* growth rates are particularly important in ecological studies because individual phytoplankton cells usually quickly convert increases in the biomass resulting from photosynthesis to an increase in cell numbers through cell division (Furnas 1990). Phytoplankton growth rates therefore have a direct bearing on the rate at which phytoplankton biomass is channelled to higher trophic

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levels. As such, phytoplankton growth rates measured under existing conditions can be used to predict changes to phytoplankton standing stocks and primary productivity that might occur following increased nutrient concentrations and other changes to growth conditions. Bulk biochemical measurements provide one estimation of production, but cells, rather than carbon per se, are the major currency of phytoplankton-based food webs (Banse 1992, 1994). Additionally, the extrapolation of biochemical measurements to phytoplankton growth rates is notoriously difficult and the resulting estimates can be substantially different from the true *in situ* growth rates (Furnas 1990).

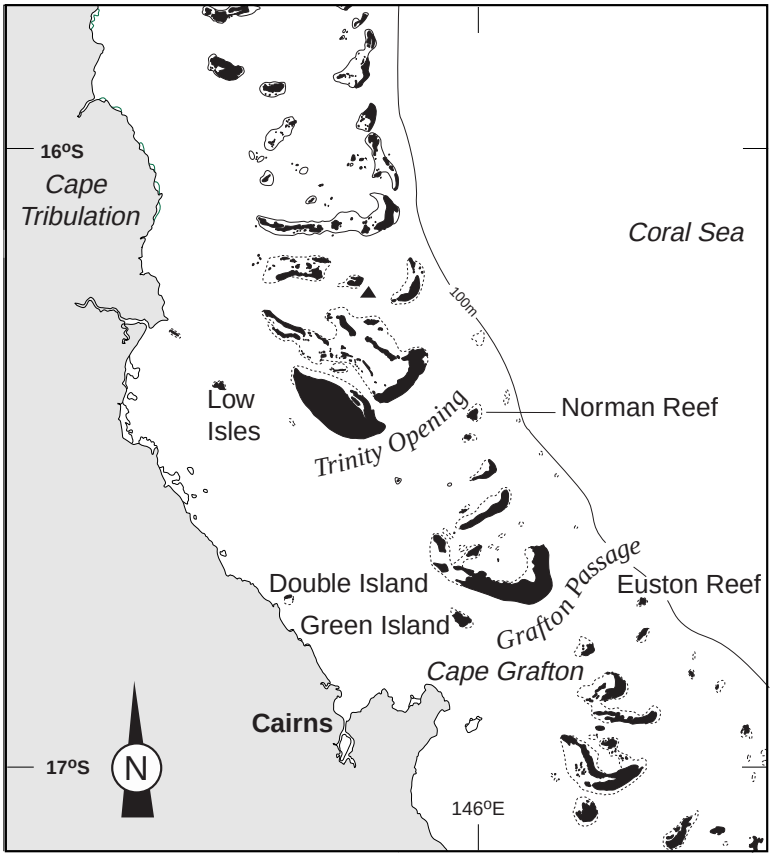
Picocyanobacteria- and diatom-based food chains co-exist in shelf waters of the Great Barrier Reef (GBR), but their relative importance varies in time and space (Furnas & Mitchell 1997). Resident phytoplankton populations have the capacity to develop significant blooms within 1 to 2 d when significant amounts of nutrients, particularly nitrogen, are introduced into shelf waters (Furnas & Mitchell 1997). During the months of monsoonal rainfall (November–April), the GBR shelf system may experience broad-scale, elevated water column nutrient concentrations (more than 10 times greater than 'normal') associated with cyclonically generated resuspension of shelf sediments (e.g. after cyclone 'Winifred', 1986) and very large run-off or monsoonal rainfall (e.g. after cyclone 'Joy', 1991) (Furnas & Mitchell 1997). Although such elevated nutrient concentrations are short-lived (days to weeks, Liston 1990, Jokiel et al. 1993), the nutrients, particularly nitrogen and phosphorus, are rapidly taken up by phytoplankton in the lagoon, resulting in significant phytoplankton blooms (2- to 5-fold increase) over broad areas (10^3 to 10^4 km^2) (Brodie & Furnas 1996). These and other phytoplankton blooms, resulting from significant nutrient input events such as shelf-break upwelling (Furnas & Mitchell 1986), are characteristically dominated by diatoms (>10 µm size fraction, Revelante & Gilmartin 1982, Furnas & Mitchell 1986), which are the preferred food of herbivorous copepods and planktonic larvae. Enhancements of diatom and zooplankton production resulting from upwelling, flood or cyclonic disturbance events are therefore likely to be important to the growth and survival of planktonic larvae, reef fish and other organisms. In the absence of significant nutrient input events, picophytoplankton (principally picocyanobacteria) dominate GBR shelf waters, accounting for 37 to 99% of integrated water column primary production and frequently >50% of the chlorophyll standing stock (Furnas & Mitchell 1986, 1987). Benthic and pelagic consumers may gain significant nutrition by direct consumption of picocyanobacteria prey (e.g. Ayukai 1995) or consumption of prey (e.g. ciliates) that feed on picocyanobacteria cells (e.g. Sorokin 1994, Ferrier-Pages & Gattuso 1998).

Prior to the commencement of this work, few estimates were available on the *in situ* growth rates of GBR phytoplankton (Furnas 1989, 1991, Ayukai 1992). Previous studies, using diffusion chamber techniques to estimate *in situ* growth rates of phytoplankton in GBR shelf waters (Furnas 1989, 1991), concluded that diatoms rather than picocyanobacteria, should consistently dominate phytoplankton blooms developing after short-term nutrient input events, because of their higher growth potentials. In this study, we used diffusion chambers to simultaneously estimate *in situ* growth rates of GBR picocyanobacteria and nano-/microphytoplankton under comparable growth conditions. Growth rates at the species, genus, and population levels were estimated from changes in cell concentrations in diffusion chambers incubated under low nutrient concentrations typical of non-perturbed conditions in shelf waters of the central and southern GBR, and also under enhanced nitrogen and phosphorus concentrations typical of upwelling, flood or cyclonic disturbance events. By this approach, we found evidence in support of the hypothesis that *in situ* growth of GBR *Synechococcus* and *Prochlorococcus* populations are not significantly nutrient (nitrogen)-limited under ambient concentrations, and are of similar order to those measured in the equatorial Pacific Ocean, where NO_3^- concentrations (>2 µM) are far above growth-saturating levels, and in the oligotrophic North Pacific Gyre. In contrast, our data suggest that growth rates of nano-/microphytoplankton under 'normal' (<0.1 µM) GBR shelf water nutrient concentrations are typically nutrient-limited. Of those nano-/microphytoplankton for which *in situ* or simulated *in situ* growth rates were estimated, growth rates appeared to be nitrogen-limited at DIN (= $NH_4^+ + NO_2^- + NO_3^-$) concentrations <0.1 µM.

METHODS

Study sites. Phytoplankton growth experiments in the central Great Barrier Reef (GBR) were conducted at inshore sites (Double Island: 16° 42′ S, 145° 41′ E), mid-shelf sites (Green Island: 16° 43′ S, 145° 59′ E), and outer-shelf sites (Euston Reef: 16° 45′ S, 146° 16′ E; Norman Reef: 16° 23′ S, 146° 01′ E; Fig. 1). In the southern GBR, growth experiments were carried out at an outer shelf site in the Pompey Reefs complex (Reef 20-345: 20° 46′ S, 150° 53′ E) (Fig. 2).

Inocula. Inocula for diffusion culture experiments were collected from incubation sites at 5 to 10 m depth with an acid-cleaned (0.6 M AR-grade HCl) Niskin bottle. Shortly after collection, the inoculum sample was



gently prescreened (Dodson & Thomas 1964) to reduce grazer populations. Nano-/microphytoplankton inocula were prepared by reverse flow gravity filtration (Dodson & Thomas 1964) through nylon mesh filters (10 or 35 µm). Picocyanobacteria inocula were prepared by reverse flow gravity filtration through Poretics® polycarbonate membrane filters with 1 or 2 µm pore diameters, or by gentle vacuum filtration through Poretics® polycarbonate membrane filters with 0.6 or 1 µm pore diameters. Pressure heads during the preparation of inocula by reverse flow gravity filtration and vacuum filtration were always < 0.4 kPa. Inocula were prepared under subdued light, after which aliquots (ca 67 to

Fig. 1. Central Great Barrier Reef (GBR) region showing shelf sites where phytoplankton growth experiments were conducted ('Norman Reef' site, 16° 23′ S, 146° 01′ E; depth ≈ 50 m. 'Euston Reef' site, 16° 45′ S, 146° 16′ E; depth ≈ 50 m. 'Double Island' site, 16° 42′ S, 145° 41′ E; depth ≈ 15 m. 'Green Island' site, 16° 43′ S, 145° 59′ E; depth ≈ 50 m)

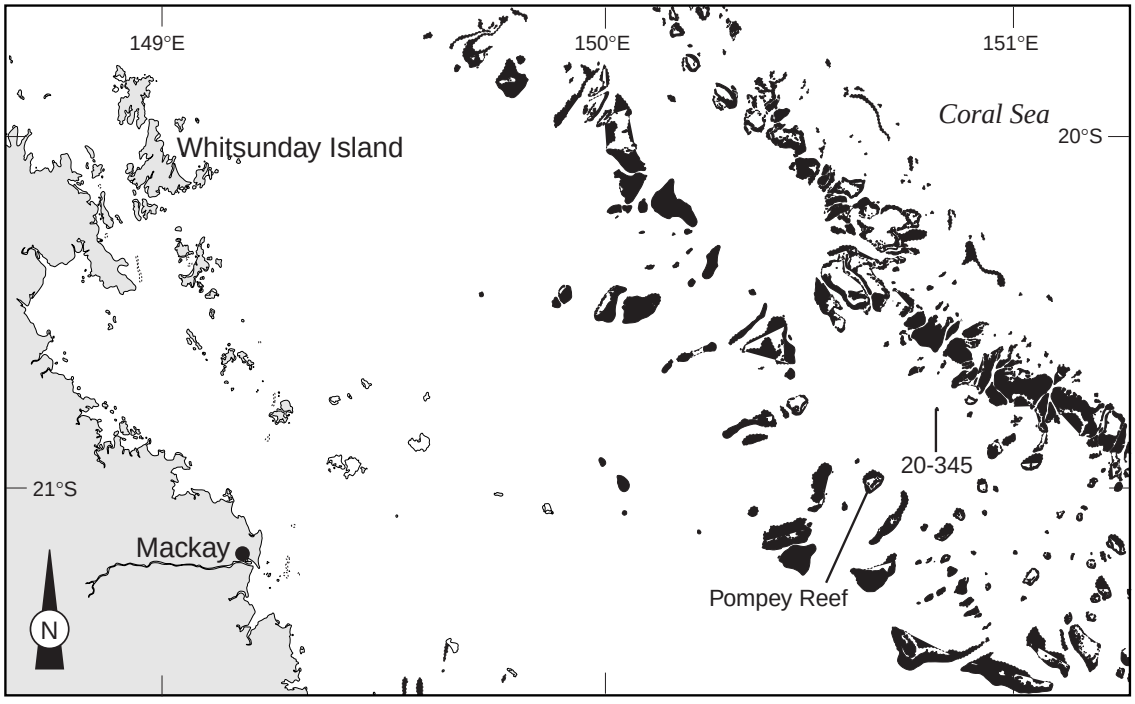


Fig. 2. Southern GBR region showing outer shelf site 'Reef 20-345' (20° 46′ S, 150° 53′ E; depth ≈ 50 m) where phytoplankton growth experiments were conducted

650 ml, depending on diffusion chamber volume) were gently poured into the diffusion chambers, which were incubated *in situ* or under simulated *in situ* conditions. Between 1 and 2 h elapsed between the collection of inocula and diffusion chamber deployment.

Diffusion chambers. The diffusion chambers used for *in situ* and simulated *in situ* phytoplankton growth experiments are based on those first described by McFeeters & Stuart (1972) and later modified for phytoplankton studies (Owens et al. 1977, Furnas 1982a,b). Three diffusion chamber designs were used (Fig. 3). Large and small chambers (Fig. 3A) used for *in situ* experiments were filled with nano-/microphytoplankton and picocyanobacteria inocula respectively, while diffusion chambers used for simulated *in situ* incubations ('shipboard' chambers) were filled with both types of inoculum. Large *in situ* chambers have a nominal volume of 137 ml and were fitted with 147 mm (filter diameter) 0.4 µm (pore diameter) membranes. Small *in situ* chambers have a nominal volume of 67 ml and were fitted with 90 mm 0.2 µm membranes. Both large and small *in situ* chambers have a nominal diffusive surface area to volume ratio of 1.57. Shipboard diffusion chambers (Fig. 3B) have a nominal volume of 650 ml and feature a side port stoppered by a clear silicone rubber bung for syringe sub-sampling. Shipboard chambers were fitted with 90 mm 0.2 µm membranes, and have a diffusive surface area to volume ratio of 0.16.

Attenuation of light by the body and containment membranes of diffusion chambers was determined by inserting a cosine quantum sensor (LI-190SA) through a slit in 1 membrane. Light supplied by cool-white fluorescent tubes above chambers positioned as during incubations was attenuated by ca 50% by large and small *in situ* chambers, and by ca 6% by shipboard chambers. Surface irradiance during incubations was measured as described below.

Laboratory trials indicated that the internal PO_4^{3-} concentration in large or small *in situ* chambers initially containing 1 mM PO_4^{3-} was reduced by 50% within ca 1 h of being bathed in continuously stirred deionized water. The time required for exchange of half the PO_4^{3-} in shipboard chambers was ca 5 h. For comparison, near-surface NH_4^+ depletion times estimated from Redfield C:N ratios and ^{14}C uptake rates have previously been reported to be within the range 3 to >10 h (Furnas & Mitchell 1984).

Potential intrusion or leakage of picocyanobacteria cells through diffusion chamber membranes was checked by immersing chambers filled with twice-filtered (0.2 µm membranes) surface oceanic seawater in unfiltered seawater containing *Prochlorococcus* cells at an abundance of 6.12×10^4 cells ml^{-1}. After 48 h immersion, no *Prochlorococcus* cells were detected in samples taken from within the chambers and analysed by flow cytometry.

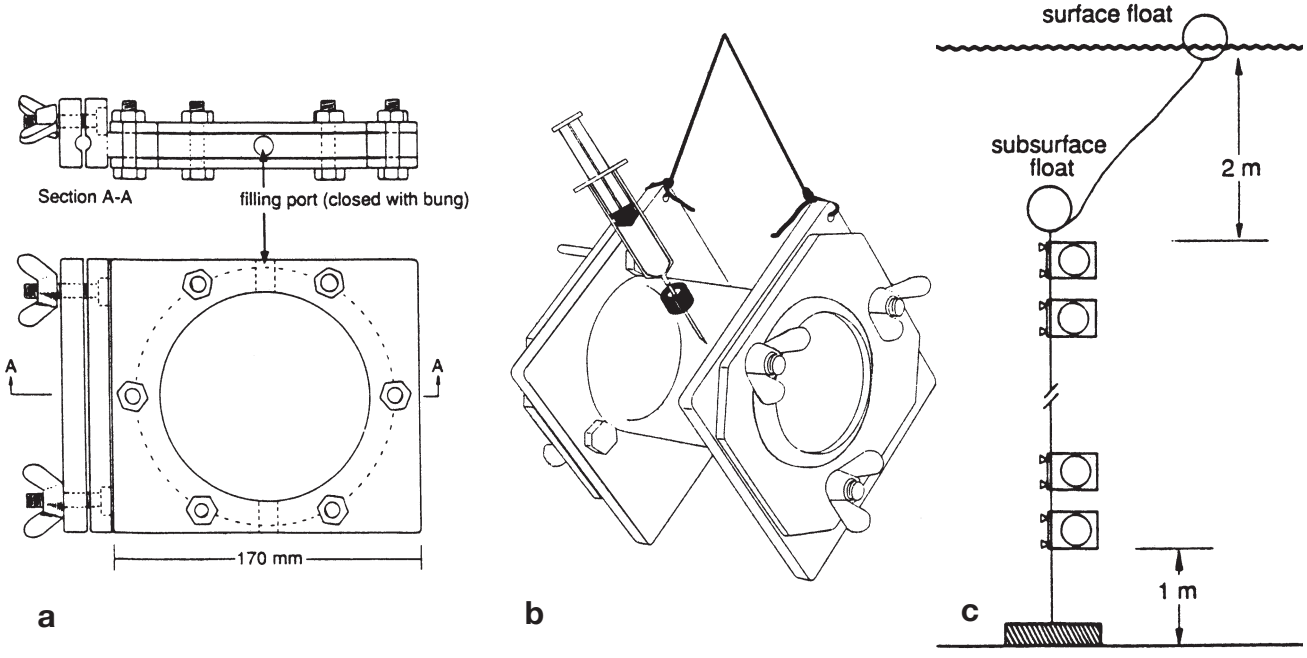


Fig. 3. (a) 'Large' (length = 17 cm) *in situ* diffusion chamber (adapted from Furnas 1991), (b) 'shipboard' diffusion chamber (length = 13.8 cm, width = 14.5 cm, height = 14.8 cm) with syringe-sampling port, and (c) *in situ* diffusion chamber mooring (adapted from Furnas 1991)

Before experiments, diffusion chambers were dismantled, washed with 'phosphate-free' laboratory detergent, rinsed thoroughly with deionized (Super-Q) water, soaked for several days in 1 N trace-metal grade HCl (Baker Instra-analyzed or Merck Suprapur), rinsed again with Super-Q, air-dried, reassembled, and stored in similarly cleaned polyethylene boxes. Fresh diffusive membranes were applied for each experiment. Between chamber filling and deployment and after recovery, filled chambers were kept in polyethylene boxes full of seawater to cushion the diffusion membranes from shocks and to prevent leakage of chamber contents.

***In situ* phytoplankton growth experiments.** Large and small *in situ* diffusion chambers were suspended in open water, at depths of up to 40 m, by clamping the chambers to a weighted mooring rope held taught by a sub-surface buoy (Fig. 3C). Replicate chambers (2 or 3) were deployed, usually before 09:00 h for each incubation depth and inoculum prescreening treatment. Salinity, temperature, and irradiance at incubation depths were measured once or twice per day by CTD casts using a Seabird SBE25 CTD mounted with a Biospherical QSP–200 underwater scalar (4π) irradiance sensor, the output of which was referenced to the output from a surface irradiance sensor in order to obtain percent surface irradiance.

Samples were taken at incubation depths for analysis of dissolved inorganic nutrients. These samples were filtered and frozen for later analysis (see below). At the end of each 1.5 to 2 d incubation period, the mooring was recovered and the chambers brought on board for end-of-experiment processing.

Simulated *in situ* phytoplankton growth experiments. Shipboard diffusion chambers (volume = 650 ml, see Fig. 3B) were suspended in grey polyethylene incubation tanks supplied with near-surface seawater pumped continuously through PVC pipe, garden hose and non-metallic fittings. Incubation tanks were acid-cleaned with 0.6 M AR-grade HCl and rinsed thoroughly with Super-Q deionized water prior to each cruise. The external plumbing and incubation tanks were always flushed with near-surface seawater pumped from the incubation sites for at least 24 h before experiments were started. Ambient light reaching the diffusion chambers was attenuated by covering the incubation tanks with black shade cloth or, for 'dark controls', totally eliminated by covering the incubation tanks with opaque black plastic. Water temperatures in the incubation tanks were measured with a mercury thermometer at least twice daily.

In 7 experiments, seawater pumped through incubation tanks was enriched with ammonium and phosphorus using a peristaltic pump which delivered a concentrated mixture of NH_4Cl and KH_2PO_4. In order to minimise fluctuations in the supply of nutrients to the incubation tanks, the input of near-surface seawater to the incubation tanks was controlled by means of a header tank. The concentration of dissolved inorganic nutrients [NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, $Si(OH)_4$] in the incubation tanks was determined from samples taken periodically during the incubations and frozen for later analysis, or, for PO_4^{3-} and NH_4^+, determined by manual analysis of fresh samples (see below). Depending on available processing time, samples for manual analysis were collected from the incubation tanks at up to 2-hourly intervals. At other times, especially during rough sea conditions, only 2 or 3 samples were taken, spaced at regular intervals over the incubation period.

The simulated *in situ* growth experiments typically commenced before 9.00 h, usually at dawn. Samples for phytoplankton cell counts were collected from shipboard diffusion chambers at the beginning and end of each experiment, which were run for 1 to 1.5 d. Growth rates of picocyanobacteria incubated for a period of >1 d are given only in the case of *Synechococcus*, and result from a single experiment conducted at an inshore location where time series data (Crosbie 1999) suggested that growth of the population was poorly synchronized to the light-dark cycle.

Surface irradiance. Time-averaged daytime fluxes (μmol quanta $m^{-2}\,s^{-1}$) during growth experiments were calculated by averaging the trapezoidal integration (AREA.XFM transform, SigmaPlot® 4.0 for Windows®, SPSS Inc., 1997) of dawn-to-dusk (04:00 to 20:00 h) minute-averaged values from a 4π surface quantum sensor (Biospherical QSR–240) that had been calibrated against a 2π quantum sensor (Li-Cor LS-190).

Dissolved inorganic nutrients. Concentrations of NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}, and $Si(OH)_4$ in frozen samples were determined using a segmented flow analyzer (Treguer & LeCorre 1975). The lower limits of detection for analysis of dissolved inorganic nutrients by this method were 0.02 μM for NO_2^-, NO_3^-, PO_4^{3-}, 0.1 μM for NH_4^+ (higher detection limit due to problems with contamination of stored samples; see Furnas & Brodie 1996), and 0.3 μM for $Si(OH)_4$ (Ryle et al. 1981).

For measurements of PO_4^{3-} and NH_4^+ concentrations in fresh samples, duplicate 5 and 20 ml samples were collected and processed immediately according to Parsons et al. (1984) (for PO_4^{3-}) and Solorzano (1969) (for NH_4^+, using reagents modified from Dudek et al. 1986), respectively. After color development, absorbance was measured on a Hitachi Spectrophotometer (1 cm cuvette for PO_4^{3-} analysis; 5 cm cuvette for NH_4^+ analysis). The lower detection limit for the analysis of NH_4^+ was 0.02 μM (i.e. 3 × instrumental detection limit), and 0.03 μM for the analysis of PO_4^{3-} (Parsons et al. 1984).

Flow cytometry. Duplicate or triplicate 3 ml samples for flow-cytometric counts of picocyanobacteria were

taken from inoculum samples, and from diffusion chambers at the end of each experiment. Samples were then treated with glutaraldehyde for 10 min (0.1% final conc.), stored in liquid nitrogen (Vaulot et al. 1989) for periods of generally <4 wk, then counted by flow cytometry as described below.

Samples were thawed in a water bath at 37°C just prior to cytometric analysis, then counted immediately on a Becton Dickinson FACScan flow cytometer. The flow cytometer was equipped with an argon laser (power = 15 mW, at 488 nm). For each cell, 2 light scatter (side scatter and forward light scatter) and 3 fluorescence signals were recorded on 4-decade logarithmic scales. The photomultipliers were set up to quantify: red fluorescence from chlorophyll (wavelength > 650 nm), orange fluorescence from phycoerythrin (564–606 nm), and green fluorescence from phycourobilin (515–545 nm).

Procedures for flow cytometry generally followed those of Olson et al. (1993). Instrument settings appropriate for the analysis of picocyanobacteria populations in natural samples were chosen using GBR-derived cultures of *Synechococcus* and *Prochlorococcus*. Aged near-surface oceanic water filtered through 0.2 µm polycarbonate filters was used as sheath fluid. Sheath fluid quality was checked periodically by running blanks through the FACScan in the same manner as picocyanobacteria samples. *Synechococcus* and *Prochlorococcus* abundance were calculated by reference to known additions of calibration grade, 0.92 µm yellow-green fluorescent beads (Fluoresbrite™, Polysciences). Before and after running each sample set, flow rate was determined using a sample with a known concentration of beads. Working stock bead concentration was determined by the gravimetric method of Olson et al. (1993). Samples were run on the flow cytometer until 20 000 events were recorded (typically 100 to 150 µl of sample were analyzed). The resulting LYSYS II (Beckton Dickinson) data files were converted to DOS format with HP-READER (Verity Software House). The program MFI (E. Martz, University of Massachusetts) was used to convert file header information to MS Excel (Microsoft Inc., Redmond) format. WinMDI flow cytometric analysis software (J. Trotter, BD PharMingen) was subsequently used to define picocyanobacteria populations.

Using WinMDI, *Synechococcus* and *Prochlorococcus* populations were defined using a process known as

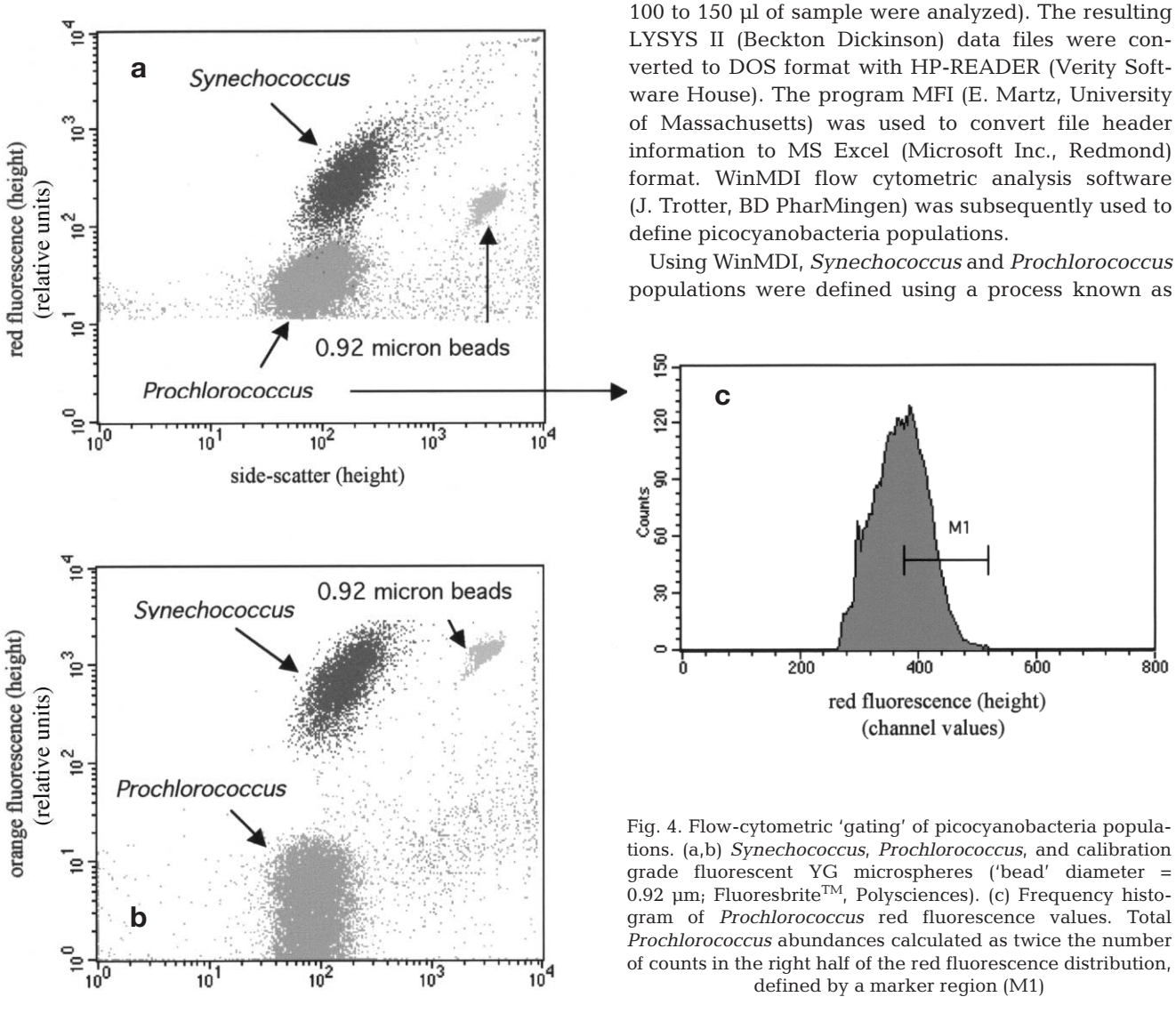


Fig. 4. Flow-cytometric 'gating' of picocyanobacteria populations. (a,b) *Synechococcus*, *Prochlorococcus*, and calibration grade fluorescent YG microspheres ('bead' diameter = 0.92 µm; Fluoresbrite™, Polysciences). (c) Frequency histogram of *Prochlorococcus* red fluorescence values. Total *Prochlorococcus* abundances calculated as twice the number of counts in the right half of the red fluorescence distribution, defined by a marker region (M1)

'gating', after which, so-defined populations are said to have been 'gated'. This was done by Boolean combinations of regions drawn in different bivariate dotplots, mainly red fluorescence versus side scatter, red fluorescence versus forward light scatter, and orange fluorescence versus side scatter (Olson et al. 1993). *Synechococcus* cells were distinguished by high orange fluorescence (phycoerythrin), and *Prochlorococcus* cells by their low forward light scatter and red fluorescence (chlorophyll, Fig. 4). Where necessary, gating of *Synechococcus* and *Prochlorococcus* populations was tailored to account for shifts in red and orange fluorescence signatures resulting from presumed photoacclimative changes in cellular phycoerythrin and chlorophyll content. Coefficients of variation for same-sample triplicate analysis of *Synechococcus* and *Prochlorococcus* populations were <6% for counts and mean cellular fluorescence parameters.

As configured, the FACScan was incapable of resolving the entire *Prochlorococcus* population in some samples incubated under simulated near-surface condition, due to the weak red fluorescence signature of cells in such *Prochlorococcus* populations. Since, however, the mode of the frequency distribution of red fluorescence values for the *Prochlorococcus* population (hereafter 'red fluorescence distribution') was visible, the total *Prochlorococcus* abundance was calculated to be twice the number of counts in the right half of the *Prochlorococcus* red fluorescence histogram, defined by a marker region (Fig. 4). This approach assumes that the *Prochlorococcus* red fluorescence distribution has a normal or Gaussian shape (i.e. is not skewed). This was checked by fitting a Gaussian function to the red fluorescence distribution of gated *Prochlorococcus* populations, where the Gaussian function (y) is given by the formula:

$$y = \mathrm{a}\mathrm{e}^{\left[-0.5\left(\frac{X-X_0}{\mathrm{b}}\right)^2\right]}$$

where x = red fluorescence value, y = number of *Prochlorococcus* cells per red fluorescence value, and a and b are constants. In surface (<1 m depth) samples containing *Prochlorococcus* populations with weak red fluorescence signatures, 'observed' (gated populations) and 'predicted' (from curve area of the Gaussian function fitted to gated *Prochlorococcus* populations) *Prochlorococcus* abundance agreed to 7.8 ± 4.5% (mean ± 1 SD, n = 5), and to < 5% (n = 14) and < 2% (n = 12) for non-surface samples and bright (high red fluorescence) *Prochlorococcus* populations respectively.

Utermöhl counts. Samples for inverted microscope counts of nano-/microphytoplankton and microheterotrophs were preserved in glutaraldehyde (2 ml of 25% fixative in 60 ml) and stored at 4°C. Prior to counting by inverted microscopy (Utermöhl 1958), samples were warmed to room temperature and settled in chambers for 48 h. Abundances of nano-/microphytoplankton and microheterotroph taxa in inoculum samples were estimated from duplicate or triplicate 50 ml settled samples, and those in final or time-series samples from similarly treated duplicate samples. Diatoms and dinoflagellates, microflagellates, and nonmotile ultraplankton were counted by strip-counting the full or half-chamber bottom (Venrick 1978). Identifications were at least to genus level and often to species level, by reference to Tomas (1997).

Mean inoculum abundances of those nano-/microphytoplankton taxa for which growth rates could be calculated ranged from <100 cells l^{-1} to 4.2×10^4 cells l^{-1}, the latter recorded for *Pseudo-nitzschia* 'straight-chain' spp. during a growth experiment in the southern GBR. Species or groups were detected at the end of growth experiments that were not recorded in the inoculum, as their initial abundances were below detection; in such cases, growth was not calculated.

Growth rate calculations. Growth rates (μ) of phytoplankton and microheterotrophs were expressed as doublings d^{-1}:

$$\mu = (1/T)\log_2(X_t/X_0) \text{(Guillard 1973)} \quad (1)$$

where T is the incubation period (d) and X_0 and X_t are the cell numbers at the beginning and end-point respectively. Because growth of picocyanobacteria largely ceases at night (Furnas & Crosbie 1999) cell losses over time in dark incubated controls were used to correct picocyanobacteria growth rates recorded in light-incubated diffusion chambers in several simulated *in situ* growth experiments (see Table 5).

Uncertainties associated with mean phytoplankton growth rate estimates. Uncertainties associated with mean phytoplankton growth rate estimates are a combination of 'counting error' (E_A) and between-chamber variations (E_B), the latter arising from differing growth rates in replicate diffusion chambers. Maximum uncertainties ($E_{A_{max}}$ and $E_{B_{max}}$) associated with mean phytoplankton growth rate estimates were estimated from the general relationship:

$$E_{A_{max}} \text{ and } E_{B_{max}} = \mu_{max} - \mu \text{ (doublings d}^{-1}\text{)} \quad (2)$$

where μ was calculated from Eq. 1, but where X_0 and X_t were defined as follows:

For counts based on settlement chambers ($E_{A_{max}}$):

$$X_0 = I_{mean} - \overline{\left(\frac{I_{single} - I_{mean}}{I_{mean}}\right)} \quad (3)$$

$$X_t = F_{calculated} + \overline{\left(\frac{F_{single} - F_{mean}}{F_{mean}}\right)} \quad (4)$$

For counts based on flow-cytometric data ($E_{A_{max}}$):

$$X_0 = I_{mean} - CV_{mean} \quad (5)$$

$$X_t = F_{calculated} + CV_{mean} \quad (6)$$

For chamber errors ($E_{B_{max}}$):

$$X_t = F_{calculated} + \left(\frac{C_{single} - C_{replicate}}{C_{replicate}}\right) \quad (7)$$

where I_{mean} is the mean of duplicate or triplicate inoculum counts, and F_{mean} is the mean of duplicate final counts; I_{single} and F_{single} are individual inoculum and final counts respectively; $F_{calculated}$ is an estimated final cell abundance calculated using Eq. 1 assuming a mean inoculum count, μ_{max} (from Table 2) and a 2 d incubation; CV_{mean} is the mean coefficient of variation for triplicate flow-cytometric counts; C_{single} are individual chamber counts; $C_{replicate}$ are means of replicate chambers counts.

RESULTS

A summary of conditions for growth of phtoplankton in diffusion culture experiments is given in Table 1.

Nano-/microphytoplankton taxa and their abundance in growth experiments

A diverse range of nano-/microphytoplankters were observed in settled samples, including larger diatom taxa such as *Lioloma* (individuals resembling *L. delicatulum* and *L. pacificum*) and *Thalassionema frauenfeldii* (maximum length of ca 2 mm), which can pass lengthwise as individual cells through pores in membranes with relatively small holes. Small (longest axis < 10 µm) solitary pennate diatoms resembling *Navicula* and several capitate *Nitzschia* species were aggregated into 1 operational taxon, and referred to as 'Naviculoid' diatoms. Several chain-forming species of the genus *Pseudo-nitzschia* were frequently encountered. *Pseudo-nitzschia* cells were operationally classified as *Pseudo-nitzschia* 'straight-chain' spp. or *P. subcurvata*. Cells identified as *Thalassiosira* spp. were generally <10 µm (longest axis), and solitary or joined by single threads into straight-chain colonies of 4 to 10 cells. Small hylachaete *Chaetoceros* spp. occurred in samples, and at times exhibited rapid growth, but growth rates of individual species could not be reliably established because of difficulty in making consistent species identifications.

A number of dinoflagellate taxa were identified, including thecate and non-thecate taxa but, with the exception of several small (longest axis typically <20 µm) *Gymnodinium* spp., they were usually poorly preserved. Coccolithophorids were also poorly preserved, precluding growth rate estimates for this group.

Maximum counting and between-chamber variability associated with nano-/microphytoplankton mean growth rates ranged from 0.1 to 0.5 doublings d^{-1}. For picocyanobacteria, maximum growth rate uncertainties associated with counting errors and between-chamber variability were ca 0.1 and 0.1 to 0.2 doublings d^{-1}, respectively. Estimates of chamber errors were generally lower for simulated *in situ* experiments than for *in situ* experiments with nano-/microphytoplankton inocula, likely due to lower levels of environmental variability between replicate chamber deployments. Total maximum uncertainties associated with single-chamber growth rate estimates ($E_{A_{max}} + E_{B_{max}}$) ranged from 0.4 to 0.9 doublings d^{-1} for nano-/microphytoplankton, and 0.3/0.2 doublings d^{-1} for *Synecho-*

Table 1. Time-averaged nutrient concentrations and irradiances measured at incubation depths (*in situ* growth experiments) and in incubation tanks (shipboard growth experiments). *Median values given in parentheses

Type of growth experiment	Location of experiment	$[NH_4^+]$	[DIN]	$[PO_4^{3-}]$	$[Si(OH)_4]$	Irradiance (µmol quanta m^{-2} s^{-1})
		(µmol l^{-1})				
In situ	Central–inshore	0.02–0.07 (0.04)	0.03–0.09 (0.06)	0.01–0.02 (0.01)	3.92–10.57 (8.75)	175–852 (483)
	Central–offshore	0.02–0.03 (0.03)	0.05–0.07 (0.06)	0.01–0.02 (0.02)	1.26–2.10 (1.81)	76–698 (460)
	Southern GBR	0.01–0.40 (0.22)	0.02–0.41 (0.23)	0.01–0.03 (0.01)	0.19–2.40 (1.20)	17–232 (88)
Shipboard: Non-enriched	Central–inshore	0.13–0.19 (0.15)	0.20–5.05 (4.24)	0.09–0.21 (0.11)	7.96–36.14 (31.63)	298–827 (429)
	Central–offshore	0.32–0.98 (0.61)	1.23–5.26 (3.94)	0.04–0.26 (0.16)	9.38–35.38 (23.97)	339–418 (383)
	Southern GBR	0.00–1.73 (0.69)	0.03–2.15 (0.86)	0.01–0.39 (0.12)	0.06–2.89 (1.63)	346–426 (381)
Shipboard: Nutrient-enriched	Central–inshore	7.22–13.87 (9.62)	12.08–16.37 (13.61)	0.09–0.76 (0.20)	7.96–36.14 (31.02)	298–412 (310)
	Central–offshore	5.90–31.62 (12.95)	6.15–36.56 (14.94)	0.26–6.53 (3.86)	9.38–35.38 (19.01)	339–403 (388)
	Southern GBR	17.430	17.46	2.62	0.06	551

coccus/Prochlorococcus respectively. For the nano-/microphytoplankton, these uncertainties were often greater than or equal to observed growth-rate means and standard deviations, but were less than the observed growth-rate means and standard deviations in the case of the picocyanobacteria. For paired comparisons between nano-/microphytoplankton growth rate means derived from different prescreening treatments (see below), significant differences of 0.2 to 0.5 doublings d^{-1} would be detected with a power of 0.80 (Cohen 1988).

Grazers and their abundance in growth experiments using nano-/microphytoplankton inocula

Favella-like tintinnids and *Strombidium*-like oligotrichous ciliates in nano-/microphytoplankton inocula occurred at <100 cells l^{-1}. Small heterotrophic dinoflagellates and microflagellates were occasionally observed, but may have been underestimated by poor preservation (Tomas 1997) and the difficulty of distinguishing autotrophic from heterotrophic forms (Lessard & Swift 1986). Foraminifera, radiolarians, larvaceans, and copepod nauplii were also occasionally observed, but always at ≤40 cells l^{-1}.

Growth of nano-/microphytoplankton

Growth rates (μ, doublings d^{-1}) were estimated for 24 nano-/microphytoplankton taxa (Table 2). Most nano-/microphytoplankton taxa for which *in situ* growth rates were recorded had maximum growth rates of ≥1. Mean growth rates were typically <1, irrespective of taxonomic affiliation (Table 2). Maximum growth rates of centric diatoms varied from 0.7 (*Eucampia zodiacus* f. *cylindrocornis*) to 2.8 (*Dactyliosolen fragilissimus*), and for pennate diatoms from 0.5 (*Lioloma*) to 3.2 (*Cylindrotheca closterium*). Non-diatoms, with the exception of the aggregate 'microflagellate spp.' (μ = 2.3) for which underestimation of inoculum counts is strongly suspected, typically achieved absolute growth rates of <2 (Table 2).

Under low nutrient conditions which typify the GBR (DIN concentrations ≤ 0.03 µM), *Cylindrotheca closterium* and *Thalassiosira* spp. achieved the highest relative growth rates for eukaryotes (μ/μ_{max} = 0.3). No positive growth rates of non-diatom nano-/microphytoplankton were recorded under these conditions (Table 3). Although most of the nano-/microphytoplankton taxa were capable of high relative growth rates ($\mu/\mu_{max} \geq 0.5$) at DIN (mostly NH_4^+) and PO_4^{3-} concentrations close to 0.1 µM (Table 4), 64 % of these growth rates measured at DIN < 0.1 µM were ≤0.25 of μ_{max}. Most (70 %) of these low relative growth rates were measured at growth saturating light levels (i.e. > 300 µmol quanta m^{-2} s^{-1}; see Furnas 1991, Crosbie 1999), implying that growth rates of common and bloom-forming nano-/microphytoplankton taxa are typically nutrient (nitrogen) limited at DIN concentrations < 0.1 µM. In combination with Redfield ratios (Redfield 1934, Redfield et al. 1963), and the low DIN concentrations and DIN:PO_4^{3-} ratios (mean = 0.89; SE = 0.10) measured in GBR shelf waters, these low relative growth rates imply that growth of common and bloom-forming nano-/microphytoplankton taxa are typically nutrient (nitrogen)-limited at DIN concentrations <0.1 µM.

Table 2. Summary statistics for growth rates (doublings d^{-1}) of nano/microphytoplankton, picophytoprokaryotes, and microheterotrophs. Growth rates for all *in situ* and simulated *in situ* diffusion chamber incubations irrespective of prescreening treatment were pooled. n = number of individual diffusion chambers. When net mortality occurred, growth was assumed to equal zero. SD = standard deviation

	μ_{max}	μ_{mean}	1 SD	n
Centric diatoms				
Bacteriastrum spp.	1.3	1.3	–	1
Chaetoceros peruvianus	1.7	1.7	–	1
Coscinodiscus spp.	0.4	0.4	–	1
Dactyliosolen fragilissimus	2.8	1.1	1.1	7
Eucampia zodiacus f. *cylindricornis*	0.7	0.6	0.1	2
Leptocylindrus danicus	2.6	0.9	0.8	11
Leptocylindrus minimus	1.3	0.7	0.5	7
Probiscia alata	1.0	1.0	–	1
Rhizosolenia setigera	2.0	0.7	0.9	9
Skeletonema costatum	1.9	0.5	0.8	6
Thalassiosira spp.	2.6	0.8	0.8	22
Pennate diatoms				
Cylindrotheca closterium	3.2	1.1	0.9	52
Lioloma spp.	0.5	0.3	0.4	2
Naviculoid diatoms	3.0	0.5	0.8	7
Phaeodactylum tricornutum-like	0.5	0.1	0.2	11
Pleurosigma/Gyrosigma	1.4	1.4	–	1
Pseudo-nitzschia subcurvata	2.6	0.7	1.0	7
Pseudo-nitzschia (straight-chains)	2.4	0.9	0.7	27
Thalassionema frauenfeldii	2.0	0.6	0.7	19
Thalassionema nitzschioides	2.1	0.8	0.8	14
Other nano-/microphytoplankton				
Gymnodinium spp.	1.7	0.4	0.6	30
Microflagellate — 'kidney' shaped	1.7	0.7	0.7	12
Microflagellate spp.	2.3	1.6	0.9	6
Non-motile ultraplankton	1.6	0.8	0.7	7
Picophytoprokaryotes				
Synechococcus	1.6	0.6	0.4	37
Prochlorococcus	1.1	0.5	0.2	6
Microheterotrophs				
Strombidium-like ciliate	1.7	0.5	0.6	19
Favella-like tintinnid	1.8	0.9	0.9	3

Table 3. Maximum relative growth rates (μ/μ_{max}) measured under time-averaged nutrient concentrations of <0.01, 0.01 to <0.1, 0.1 to <1, and ≥1 µM NH_4^+, DIN and PO_4^{3-}. Growth rates for all *in situ* and simulated *in situ* diffusion chamber incubations irrespective of prescreening treatment were pooled. When net mortality occurred, growth rates were assumed to equal zero. Relative growth rates italicized if [NH_4^+] and [PO_4^{3-}] ≤0.2 µM and fresh samples analysed. Relative growth rates in bold if measured during *in situ* diffusion culture experiments. Corresponding [DIN] (in parentheses) given for maximum relative growth rates recorded under [NH_4^+] and [DIN] categories <0.01 µM, and 0.01 to <0.1 µM respectively

	[NH_4^+]				[DIN]			[PO_4^{3-}]			
	<0.01	0.01 to <0.1	0.1 to <1	≥1	0.01 to <0.1	0.1 to <1	≥1	<0.01	0.01 to <0.1	0.1 to <1	≥1
Centric diatoms											
Dactyliosolen fragilissimus	**0.0** (0.02)	–	0.8	1.0	**0.0** (0.02)	0.8	1.0	**0.0**	*1.0*	0.2	–
Leptocylindrus danicus	–	*0.5*	0.6	1.0	0.5 (0.05)	0.6	1.0	*0.5*	*0.6*	–	0.5
Leptocylindrus minimus	0.0 (0.02)	**0.7**	1.0	0.9	**0.7** (0.06)	1.0	1.0	**0.0**	*1.0*	–	–
Rhizosolenia setigera	**0.0** (0.02)	0.4	0.0	1.0	0.4 (0.05)	**0.1**	1.0	*0.4*	*1.0*	0.0	0.8
Thalassiosira spp.	*0.3* (0.02)	*0.3*	1.0	1.0	0.3 (0.05)	**1.0**	1.0	**1.0**	*0.9*	1.0	0.5
Pennate diatoms											
Cylindrotheca closterium	***0.3*** (0.02)	**0.9**	0.9	0.8	**0.9** (0.09)	0.9	0.8	**0.8**	**1.0**	0.0	0.8
Naviculoid diatoms	0.1 (0.02)	***0.1***	0.4	1.0	**0.1**(0.05)	**0.4**	1.0	**0.4**	*0.2*	1.0	0.5
Pseudo-nitzschia subcurvata	*0.0* (0.02)	–	0.6	1.0	**0.1**(0.05)	0.6	1.0	**0.1**	0.0	1.0	0.0
Pseudo-nitzschia (straight-chain)	*0.1* (0.02)	**0.8**	1.0	0.9	**0.8** (0.05)	1.0	1.0	*0.7*	***0.8***	1.0	0.9
Thalassionema frauenfeldii	*0.1* (0.03)	*0.5*	0.6	0.9	0.5 (0.05)	0.6	1.0	*0.5*	*0.7*	1.0	0.0
Thalassionema nitzschioides	**0.1** (0.03)	*0.9*	**0.5**	1.0	0.9 (0.05)	**0.5**	1.0	*1.0*	*1.0*	0.9	0.0
Other nano-/microphytoplankton											
Gymnodinium *spp.*	–	**0.6**	1.0	1.0	**0.6** (0.05)	1.0	1.0	**0.6**	***0.2***	1.0	0.1
Microflagellate – 'kidney' shaped	–	**0.7**	0.9	1.0	**0.7** (0.09)	0.9	1.0	–	***0.7***	0.9	1.0
Microflagellate spp.	–	–	0.9	1.0	–	0.9	1.0	–	*0.9*	0.0	1.0
non-motile ultraplankton	–	0.0	0.8	1.0	0.0 (0.07)	0.8	1.0	–	*0.8*	–	1.0
Picophytoprokaryotes											
Prochlorococcus	*1.0* (0.02)	***0.6***	**0.3**	–	***1.0*** (0.02)	**0.3**	–	**0.6**	*0.5*	**1.0**	–
Synechococcus	*0.7* (0.02)	***1.0***	0.7	0.5	*1.0* (0.02)	0.7	0.5	*1.0*	*0.8*	**0.6**	0.4

Visual examination of the data did not indicate any obvious relationship between growth rates and either temperature or salinity within the relatively small observed ranges of these variables (26.0 to 29.1°C; 33.71 to 35.56‰), although insufficient data precluded testing such relationships statistically.

Growth and mortality of picocyanobacteria

Picocyanobacteria exhibited high absolute and relative (>0.5 μ_{max}) growth rates down to the lowest nutrient concentrations observed (Table 4). In experiments where positive growth rates were recorded, growth rates ranged from 0.3 to 1.1 for *Prochlorococcus* and 0.2 to 1.6 for *Synechococcus*.

Grazing impact on picocyanobacteria appears to have been reduced by prefiltering inocula through membranes with small pore diameters. In paired comparisons, *Synechococcus* populations pre-screened through membranes with a 1 µm pore diameter achieved 2.3 times higher mean growth rates than *Synechococcus* populations pre-screened through membranes with 2 µm diameters (*t*-test, p = 0.001, df = 5; mean μ = 0.7, 0.3 respectively). *Synechococcus* inoculum populations prescreened through membranes with 0.6 µm pore diameters achieved 3.5 times higher mean growth rates than *Synechococcus* populations prescreened through membranes with pore diameters of 1 µm (*t*-test, p = 0.010, df = 6; mean μ = 0.7, 0.2 respectively).

Despite efforts to reduce grazing by prescreening, mortality nonetheless accounted for 42 to 140% of corrected picocyanobacteria growth rates achieved during simulated *in situ* growth experiments that incorporated a 'dark control' (Table 5).

DISCUSSION

In comparison to nano-/microphytoplankton, high relative growth rates (μ/μ_{max}) of autotrophic picoplankton can be expected during oligotrophic conditions because nutrient acquisition by large cells is limited by molecular diffusion at the low nutrient concentrations (Fogg 1986, Raven 1986, 1998). The constraint on nutrient acquisition imposed by diffusive boundary layers is much lower for a picoplankton-size cell than for a nano- or, especially, a microplankton size cell. To achieve a given fraction of μ_{max} under nutrient limiting

Table 4. Time-averaged dissolved inorganic nutrient concentrations (µM) and irradiances (µmol quanta m^{-2} s^{-1}) at which maximum growth rates (μ_{max}) recorded, and time-averaged minimum irradiances and minimum dissolved inorganic nutrients for which relative growth rates (μ/μ_{maxx}) ≥0.5 to <0.75 (A) and ≥0.75 (B) to <1.0 recorded. Growth rates pooled for all *in situ* and simulated *in situ* diffusion chamber incubations irrespective of prescreening treatment. When net mortality occurred, growth rates assumed to equal zero. $[NH_4^+]$ and $[PO_4^{3-}]$ ≤ 0.2 µM italicized if measured by analysis of fresh samples; $[NH_4^+]$ and $[PO_4^{3-}]$ in bold if measured during *in situ* diffusion culture experiments

	$[NH_4^+]$			$[DIN]^{\%\ [NH4^+]}$			$[PO_4^{3-}]$			Irradiance		
	μ/μ_{max}		μ_{max}	μ/μ_{max}		μ_{max}	μ/μ_{max}		μ_{max}	μ/μ_{max}		μ_{max}
	A	B		A	B		A	B		A	B	
Centric diatoms												
Dactyliosolen fragilissimus	0.13	0.18	7.22	5.00^{3}	5.05^{4}	12.09^{60}	0.09	*0.09*	0.09	298	298	298
Leptocylindrus danicus	*0.03*	8.10	8.10	$*0.05*^{60}$	8.35^{97}	8.35^{97}	*0.01*	6.53	6.53	403	403	403
Leptocylindrus minimus	**0.05**	7.22	*0.18*	$\mathbf{0.06}^{80}$	12.09^{60}	5.05^{4}	***0.02***	*0.09*	*0.09*	**199**	298	298
Rhizosolenia setigera	0.03	0.98	7.22	0.05^{60}	5.00^{3}	12.09^{60}	0.01	*0.09*	*0.09*	298	–	298
Thalassiosira spp.	**0.16**	*0.13*	1.73	$\mathbf{0.17}^{94}$	$\mathbf{0.29}^{96}$	2.15^{80}	**0.03**	***0.01***	*0.11*	**17**	298	378
Pennate diatoms												
Cylindrotheca closterium	**0.02**	**0.04**	**0.07**	$\mathbf{0.05}^{80}$	0.05^{80}	**0.09**	**0.01**	**0.01**	**0.01**	**199**	**199**	**426**
Naviculoid diatoms	0.19	1.73	1.73	0.20^{95}	2.15^{80}	2.15^{80}	0.01	*0.11*	0.11	339	378	378
Pseudo-nitzschia subcurvata	0.32	1.73	1.73	5.25^{6}	2.15^{80}	2.15^{80}	0.26	0.11	*0.11*	358	378	378
Pseudo-nitzschia (straight-chain)	**0.03**	**0.02**	0.32	0.05^{80}	$\mathbf{0.05}^{40}$	5.25^{6}	*0.01*	**0.01**	0.26	378	**266**	339
Thalassionema frauenfeldii	*0.03*	1.73	1.73	$*0.05*^{80}$	2.15^{80}	2.15^{80}	*0.01*	*0.11*	*0.11*	298	378	378
Thalassionema nitzschioides	*0.03*	*0.03*	7.22	$*0.05*^{80}$	$*0.05*^{60}$	12.09^{60}	0.01	*0.01*	*0.09*	298	378	298
Other nano-/microphytoplankton												
Gymnodinium spp.	**0.04**	0.32	0.32	0.05^{60}	5.25^{6}	5.25^{6}	**0.01**	0.26	0.26	339	339	385
Microflagellate – 'kidney' shaped	**0.02**	0.32	8.10	$\mathbf{0.05}^{40}$	5.26^{6}	8.35^{97}	**0.01**	0.26	6.53	**266**	385	403
Microflagellate spp.	0.98	0.98	8.10	1.23^{80}	1.23^{80}	8.35^{97}	0.04	*0.04*	6.53	403	403	403
non-motile ultraplankton	0.98	0.98	8.10	1.23^{80}	1.23^{80}	8.35^{97}	0.04	*0.04*	6.53	403	403	403
Picophytoprokaryotes												
Prochlorococcus	***0.01***	***0.01***	***0.01***	$***0.02***^{50}$	–	$***0.03***^{33}$	***0.01***	–	*0.12*	384	–	357
Synechococcus	*0.01*	***0.01***	***0.01***	$***0.02***^{50}$	$\mathbf{0.04}^{100}$	$***0.02***^{50}$	***0.01***	***0.01***	*0.01*	**169**	**169**	817

conditions, autotrophic picoplankton cells require a lower nutrient flux per unit plasmalemma area than larger cells (Raven 1986, 1998). However, although autotrophic picoplankton can theoretically grow at rates approaching nearly 3 doublings d^{-1} at nutrient concentrations of 20 nM and still be below the threshold for diffusion-limited growth (Chisholm 1992), diel growth rates of *Synechococcus* and *Prochlorococcus* exceeding 1 are rarely recorded from marine environments, irrespective of the approach taken to measure growth rates (Furnas & Crosbie 1999). One possible explanation is that small cells, such as *Synechococcus* and *Prochlorococcus*, may be unable to store enough energy reserves to do more during the dark period than complete cell division processes that are already well advanced (Odum et al. 1963, Raven 1998). Strong diel coupling of cell division in picocyanobacteria populations may also constrain absolute growth rates of some picocyanobacteria to 1 division per diel cycle (Raven 1994, 1998).

Although there are constraints on the maximum achievable growth rates of picocyanobacteria, *Synechococcus* and *Prochlorococcus* are at times capable of rapid growth. In tropical, sub-tropical and temperate coastal waters, it has been shown that light-saturated *Synechococcus* and *Prochlorococcus* sub-populations are capable of dividing more than once per day as a result of multiple (asynchronous) cell divisions within a diel period or ultradian growth (Waterbury et al. 1986, Shalapynok et al. 1998, H. Liu pers. comm.). In the GBR shelf, maximum growth rates of 1.1 and 1.6 were recorded for *Prochlorococcus* and *Synechococcus*, respectively (results herein).

If one assumes that the highest picocyanobacteria mortality measured herein also occurred in the growth experiments which gave the maximum uncorrected picocyanobacteria growth rates (Table 5), then the corrected growth rates would approach the maximum *in situ* growth rates reported for these genera (3 and 2 doublings d^{-1} for *Synechococcus* and *Prochlorococcus* respectively, Furnas & Crosbie 1999). In the growth experiments conducted herein, however, the removal of carnivorous predators which prey upon the microplankton that graze on picocyanobacteria, may sometimes have resulted in higher losses to picocyanobacteria from microplankton grazers than normally occur in the field (see Reckermann & Veldhuis 1997).

Despite its greater abundance at oligotrophic sites (e.g. outer central GBR shelf sites and Coral Seas sites), there was no evidence that absolute growth rates of

Table 5. Net and mortality-corrected (bold values, correction in parentheses) *Prochlorococcus* (Pro) and *Synechococcus* (Syn) growth rates (μ, doublings d^{-1}) estimated from *in situ* (I) and simulated *in situ* (S) diffusion culture experiments. [NH_4^+] and [DIN] concentrations (µM) and irradiances (µmol quanta m^{-2} s^{-1}) time-averaged over period for which growth and grazing rates expressed. Growth and mortality values mean of duplicate/triplicate chamber deployments, except where otherwise indicated. Results pooled for *in situ* and simulated *in situ* experiments conducted in shelf waters near Double Island (CI), Green Island, Euston and Norman Reefs (CO), or Reef 20-345 (So). Inocula prefiltered through polycarbonate membrane filters with pore diameters of 0.6, 1, or 2 µm, or through nylon mesh filters with pore diametere of 10 µm. Incubations for 1.5 to 2 d. Growth and grazing rates from simulated *in situ* incubations calculated for first diel period of incubation

Taxa	Area	Inoc. (µm)	μ	Light	[NH_4^+]	[DIN]	°C	Date	I or S
Pro	CI	1	0.3	852	0.07	0.09	–	Apr 1996	I
Pro	CO	1	**1.0** (–1.4)	357	0.02	0.04	26.9	Nov 1995	S
Pro	CO	2	0.4	76	0.06	0.09	27.1	Nov 1994	I
Pro	CO	2	0.9	398	0.02	0.04	27.1	Nov 1995	I
Pro	So	2	0.3	232	0.40	0.42	26.5	Feb 1996	I
Syn	CI	10	0.7[a]	622	0.40	0.42	26.1	Jun 1995 (A)	S
Syn	CI	0.6	0.1[a]	600	0.40	0.42	26.1	Jun 1995 (A)	S
Syn	CI	0.6	0.9[a]	373	0.40	0.42	26.1	Jun 1995 (A)	S
Syn	CI	0.6	1.0[a]	360	0.40	0.42	26.1	Jun 1995 (A)	S
Syn	CI	0.6	0.4[a]	404	0.40	0.42	26.1	Jun 1995 (A)	S
Syn	CI	0.6	1.5	827	0.45	0.47	26.3	Jun 1995 (B)	S
Syn	CI	0.6	0.5	496	0.45	0.47	26.3	Jun 1995 (B)	S
Syn	CI	1	1.6	817	0.02	0.02	28.8	Apr 1995	S
Syn	CI	1	1.0	852	0.07	0.09	–	Apr 1996	I
Syn	CO	1	**0.7** (–0.4)[a]	406	0.02	7.50	26.5	Apr 1996	S
Syn	CO	1	**0.5** (–0.3)	406	0.02	0.11	26.5	Apr 1996	S
Syn	CO	1	**0.2** (–0.3)	357	0.02	0.04	26.9	Nov 1995	S
Syn	CO	2	1.2	521	0.02	0.04	26.8	Nov 1994	I
Syn	CO	2	1.2	169	0.04	0.04	26.4	Nov 1994	I
Syn	CO	2	0.6	76	0.06	0.09	27.1	Nov 1994	I
Syn	CO	2	1.0	398	0.02	0.04	27.1	Nov 1994	I
Syn	CO	10	1.0	521	0.04	0.04	26.8	Nov 1994	I
Syn	CO	10	0.3	169	0.04	0.04	26.4	Nov 1994	I
Syn	So	10	**1.2** (–0.5)	551	0.02	0.04	28.0	Feb 1996	S
Syn	So	10	0.4	232	0.40	0.42	26.5	Feb 1996	I

[a]Single chamber deployment

Prochlorococcus were significantly greater than those of *Synechococcus* under oligotrophic conditions (DIN concentrations < 0.1 µM) (see Table 5). Liu et al. (1995) likewise found that *Synechococcus* can often grow at higher rates (μ = 1.6) than *Prochlorococcus* (μ = 1.0) at DIN concentrations as low as 0.02 µM. In oligotrophic waters of the GBR, equatorial Pacific Ocean and North Pacific Central Gyre, for which appropriate nutrient concentration data are available (this study, JGOFS EqPac[1] and HOT data archives[2]), both *Synechococcus* and *Prochlorococcus* exhibit high relative growth rates at low (≤0.03 µM) concentrations of NH_4^+ (Landry et al. 1995a,b, Liu et al. 1995, 1997, Vaulot et al. 1995, results herein). In fact, in most settings, macronutrient availability does not appear to strongly limit growth rates of *Synechococcus* and *Prochlorococcus* (Furnas & Crosbie 1999). For *Synechococcus* and *Prochlorococcus* in the equatorial Pacific Ocean, where NO_3^- concentrations (>2 µM) are far above growth-saturating levels (Landry et al. 1995a,b, Vaulot et al. 1995, Shalapyonok et al. 1998), *in situ* growth rates are of the same order as those measured in the tropical shelf waters of the GBR (Furnas 1991, results herein) and in the oligotrophic North Pacific Gyre (e.g. Liu et al. 1995, 1997).

Estimation of phytoplankton growth rates *in situ* under the low nutrient concentrations that typically occur in tropical waters requires that phytoplankton remain in close chemical contact with surrounding waters. This can be achieved by using diffusion culture techniques (Sakshaug & Jensen 1978) where phytoplankton are enclosed in permeable-membrane chambers. Growth can thus continue for periods long enough to average out, or measure, diurnal fluctuations in growth rates. Many phytoplankton incubated in diffusion chambers have been shown to achieve growth rates equal to, or in some cases greater than, those that have been recorded in batch or chemostat cultures (see review by Furnas 1990). Significantly, diffusion chambers deployed in low nutrient environ-

[1]http://ads.smr.uib.no/jgofs/Science/Regional/EP.htm
[2]http://hahana.soest.hawaii.edu/hot/hot_jgofs.html

ments have been shown to sustain high rates of phytoplankton growth (Landry et al. 1984, Furnas 1989, 1991, Ferrier-Pages & Gattuso 1998). The ratio of the size of the transported nutrient to the pore size of the diffusion chamber membranes (e.g. 0.001 for PO_4^{3-}) suggests that membrane hindrance of mass transport is probably minimal (Deen et al. 1981, Deen 1987, Nakao 1994). Any stirring would further weaken membrane-associated boundary layer resistance to solute convection (Deen 1998). Transport of nutrients across the diffusion chamber membranes is therefore largely through entrainment in the bulk flow of water. Relatively efficient replacement of water within diffusion chambers (cf. bottles, dialysis bags) with outside water can be expected to have minimized changes in nutrient availability caused by containment and prescreening of the inocula in the growth experiments conducted herein. Nonetheless, further studies using, for example, flow cytometric and/or molecular techniques (e.g. Gonzalez-Gil et al. 1998, La Roche et al. 1999, Scanlan & Wilson 1999) are needed to verify that the nutrient status of GBR phytoplankton grown within diffusion chambers approaches that of GBR phytoplankton growth under (non-contained) natural conditions.

This study provides evidence to support the predictions of Raven (1986, 1998) that marine picocyanobacteria achieve higher relative and absolute growth rates than nano-/microphytoplankton when DIN concentrations are low (<0.05 µM). Our results extend previous studies on the growth rates of GBR phytoplankton (Furnas 1989, 1991), which found no obvious difference between the relative growth responses (μ/μ_{max}) of both nano-/microphytoplankton and picocyanobacteria at DIN concentrations down to 0.1 µM. Although we present growth data for only a small proportion of the total number of nano-/microphytoplankton taxa that have been recorded in GBR shelf waters (see Revelante & Gilmartin 1982, Hallegraeff & Jeffrey 1984), 16 of the 24 nano-/microphytoplankton taxa (e.g. *Cylindrotheca closterium*, *Leptocylindrus danicus*, *Thalassionema nitzschioides*) are important contributors to phytoplankton blooms arising from significant nutrient input events in GBR shelf waters (Furnas & Mitchell 1986, Furnas 1989). All are commonly observed in GBR shelf waters (Revelante & Gilmartin 1982, Furnas & Mitchell 1986, Furnas 1989).

As with picocyanobacteria, low nutrient concentrations do not prevent nano-/microphytoplankton in tropical oligotrophic waters from achieving high absolute (μ_{max}) and relative (μ/μ_{max}) growth rates (Goldman et al. 1979, Landry et al. 1984, Laws et al. 1984, 1987, Furnas 1991). In fact, the results presented herein show that common bloom-forming diatom taxa had the potential to grow at absolute rates exceeding those of picocyanobacteria at DIN concentrations as low as 0.05 µM.

Though most important GBR species or groups are capable of high relative growth rates ($\mu/\mu_{max} > 0.5$) at NH_4^+ concentrations ≤0.1 µM and DIN concentrations ≤0.2 µM (Furnas 1991, results herein), growth rates of most nano-/microphytoplankton taxa were apparently nitrogen-limited for much of the time. Thus, 71% of nano-/microphytoplankton growth rates estimated from *in situ* growth experiments were ≤0.25 of μ_{max} when DIN concentrations were <0.1 µM. In comparison, only 18% of picocyanobacteria growth rates estimated from *in situ* growth experiments were ≤0.25 of μ_{max} when DIN concentrations were <0.1 µM, the majority being ≥0.50 of μ_{max} at these DIN concentrations. Even when nutrients were naturally or artificially elevated (e.g. DIN concentrations > 1 µM) relative to typical ambient concentrations (i.e. DIN concentrations < 0.1 µM), picocyanobacteria growth rates showed no signs of being significantly stimulated.

Recurrent high growth rates of diatoms suggest that prescreening was effective in relieving grazing pressure. Inocula prescreened through nylon mesh filters with pore diameters of 10 µm generally contained fewer and smaller ciliates than inocula prescreened through 35 µm nylon mesh filters. Given that ciliates, heterotrophic dinoflagellates and raptorial microflagellates were the most abundant grazers in nano-/microphytoplankton inocula, grazing pressures would probably have been greatest on microflagellates and non-motile ultraplankton (Hansen et al. 1997), but there were insufficient data for a statistical comparison of the growth rates of microflagellates and non-motile ultraplankton prescreened through the 10 and 35 µm filters. No differences were found between growth rates of *Gymnodinium* spp., *Cylindrotheca closterium*, and Naviculoid diatoms prescreened through the 10 and 35 µm filters (paired *t*-tests), implying that grazing of those taxa was either negligible or at least similar for the 2 prescreening treatments. Since fewer micrograzers were present in inocula prescreened through the 10 µm filters, and those present were typically smaller in size, the former suggestion is the more likely.

Even under highly oligotrophic conditions (DIN ≤ 0.03 µM), small changes in nitrogen concentrations (10s of nanomoles) were enough to promote growth of nano-/microphytoplankton over that of the picocyanobacteria. According to steady-state diffusion calculations of Chisholm (1992), however, a spherical cell of radius 5 µm growing at 1 doubling d^{-1} should be diffusion-limited at nitrogen concentrations <0.1 µM. Since there is good evidence that nano-/microphytoplankton can achieve higher growth rates at DIN concentrations ≤0.1 µM (Laws et al. 1984, 1987, Furnas 1991, results

herein), how might these high growth rates be achieved at such low DIN concentrations?

Several ideas have been proposed to reconcile the co-occurrence of fast growth rates and low ambient nutrient concentrations, including saturating uptake rates (e.g. McCarthy 1980, Harrison et al. 1996), access to small parcels of water where the concentration of nutrients is considerably higher than in the rest of the water column, as for example, due to micro(protozoan)- and macro(metazoan)-zooplankton excretion (McCarthy & Goldman 1979, Lehman & Scavia 1982a,b, Caron & Goldman 1990), or in association with 'Marine snow' (flocs of detrital, bacterial and other organic matter, see Shanks & Trent 1980, Alldredge & Silver 1988). Additionally, phytoplankton may supplement dissolved inorganic nutrient uptake with uptake of dissolved organic matter (see Antia et al. 1991, Palenik & Henson 1997). Large zooplankton (e.g. copepods) and marine snow would be most affective in supplying nutrients when they are in close proximity (10s of microns) to phytoplankton (Jackson 1980, Shanks & Trent 1980, Alldredge & Silver 1988). Due to the absence or low abundance of large zooplankton and marine snow within diffusion chambers, these potential nutrient sources are unlikely to have contributed significantly to the growth of phytoplankton in the diffusion culture experiments conducted herein.

Most high relative growth rates of phytoplankton at nanomolar DIN concentrations were recorded when NH_4^+ concentrations were ≥60% of DIN concentrations (Table 4), although NH_4^+ is the most abundant inorganic nitrogen species in GBR surface waters at nanomolar DIN concentrations. Under certain conditions, the forms of inorganic nitrogen available to phytoplankton also influences achievable growth rates (Levasseur et al. 1993). When phytoplankton are grown under saturating light conditions, higher growth rates are achieved on an ammonium source than a nitrate source (Levasseur et al. 1993). Preferential storage of nitrate by larger phytoplankton, however, can lead to higher growth rates of these phytoplankton than is achievable on an ammonium source (Stolte 1996).

Acknowledgements. This manuscript forms part of a dissertation submitted in partial fulfilment of the requirements for the PhD degree at James Cook University of North Queensland. Comments by D. Griffiths and T. Weisse improved the manuscript. Funding and material support was provided by the Australian Institute of Marine Science, James Cook University of North Queensland, the Australian Coral Reef Society, and the Co-operative Research Center Program through the Co-operative Research Center for the Ecological Sustainability of the Great Barrier Reef. Shore-based analyses of dissolved inorganic nutrients were conducted by Neil Johnson and Jane Wu Won.

LITERATURE CITED

Agawin NSR, Duarte CM, Agusti S (2000) Nutrient and temperature control of the contribution of picoplankton to phytoplankton biomass and production. Limnol Oceanogr 45:591–600

Alldredge A, Silver M (1988) Characteristics, dynamics, and significance of marine snow. Prog Oceanogr 20:41–82

Antia NJ, Harrison PJ, Oliveira L (1991) The role of dissolved organic nitrogen in phytoplankton nutrition, cell biology and ecology. Phycologia 30:1–89

Ayukai T (1992) Picoplankton dynamics in Davies Reef lagoon, the Great Barrier Reef, Australia. J Plankton Res 14:1593–1606

Ayukai T (1995) Retention of phytoplankton and planktonic microbes on coral reefs within the Great Barrier Reef, Australia. Coral Reefs 14:141–147

Banse K (1992) Grazing, temporal changes of phytoplankton concentrations, and the microbial loop in the open sea. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum, London, p 409–440

Banse K (1994) Grazing and zooplankton production as key controls of phytoplankton production in the open ocean. Oceanography 7:13–20

Berges JA, Falkowski PG (1998) Physiological stress and cell death in marine phytoplankton: induction of proteases in response to nitrogen or light limitation. Limnol Oceanogr 43:129–135

Brodie J, Furnas M (1996) Cyclones, river flood plumes and natural water quality extremes in the Central Great Barrier Reef. In: Hunter HM, Eyles AG, Rayment GE (eds) Downstream effects of land use. Department of Natural Resources, Brisbane, p 367–374

Caron DA, Goldman JC (1990) Protozoan nutrient regeneration. In: Capriulo GM (ed) Ecology of marine protozoa. Oxford University Press, Oxford, p 283–306

Chisholm SW (1992) Phytoplankton size. In: Falkowski PG, Woodhead AD (eds) Primary productivity and biogeochemical cycles in the sea. Plenum, London, p 213–237

Cohen J (1988) Statistical power analysis for the behavioral sciences. Lawrence Erlbaum Associates, Hillsdale, NJ

Crosbie ND (1999) Environmental and ecological controls on picophytoprokaryotes and nano-/microphytoplankton in shelf waters of the central (17° S) and southern (20° S) Great Barrier Reef. PhD thesis, James Cook University of North Queensland

Deen WM (1987) Hindered transport of large molecules in liquid-filled pores. AICHE J 33:1409–1425

Deen WM (1998) Analysis of transport phenomena. Oxford University Press, New York

Deen WM, Bohrer MP, Epstein NB (1981) Effects of molecular size and configuration on diffusion in microporous membranes. AICHE J 27:952–959

Dodson AN, Thomas WH (1964) Concentrating plankton in a gentle fashion. Limnol Oceanogr 9:455–456

Dudek N, Brzezinski M, Wheeler P (1986) Recovery of ammonium nitrogen by solvent extraction for the determination of relative ^{15}N abundance in regeneration experiments. Mar Chem 18:59–69

Ferrier-Pages C, Gattuso JP (1998) Biomass, production and grazing rates of pico- and nanoplankton in coral reef waters (Miyako, Japan). Microb Ecol 35:46–57

Fogg GE (1986) Picoplankton. Proc R Soc Lond B 228:1–30

Furnas MJ (1982a) An evaluation of two diffusion culture techniques for estimating phytoplankton growth rates *in situ*. Mar Biol 70:63–72

Furnas MJ (1982b) Growth rates of summer nanoplankton (<10 µm) populations in Lower Narragansett Bay, Rhode Island, USA. Mar Biol 70:105–115

Furnas MJ (1989) Cyclonic disturbance and a phytoplankton bloom in a tropical shelf ecosytem. In: Okaichi, Anderson, Nemoto (eds) Red tides: biology, environmental science, and toxicology. Elsevier, Amsterdam, p 273–276

Furnas MJ (1990) *In situ* growth rates of marine phytoplankton: approaches to measurement, community and species growth rates. J Plankton Res 12:1117–1151

Furnas MJ (1991) Net *in situ* growth rates of phytoplankton in an oligotrophic, tropical shelf ecosystem. Limnol Oceanogr 36:13–29

Furnas MJ, Brodie J (1996) Current status of nutrient levels and other water quality parameters in the Great Barrier Reef. In: Hunter HM, Eyles AG, Rayment GE (eds) Downstream effects of land use. Department of Natural Resources, Brisbane, p 9–21

Furnas MJ, Crosbie ND (1999) *In situ* growth dynamics of photosynthetic prokaryotic picoplankters *Synechococcus* and *Prochlorococcus*. In: Charpy L, Larkum AWD (eds) Marine cyanobacteria. Bulletin de l'Institut Océanographique, Monaco, Spécial 19, p 387–417

Furnas MJ, Mitchell AW (1984) Phytoplankton productivity measurements in Palm Passage (central Great Barrier Reef) during 1983. AIMS Data Rep. 05-84-2. Australian Institute of Marine Science, Townsville

Furnas MJ, Mitchell AW (1986) Phytoplankton dynamics in the central Great Barrier Reef. I. Seasonal changes in biomass and community structure and their relation to intrusive activity. Cont Shelf Res 6:363–384

Furnas MJ, Mitchell AW (1987) Phytoplankton dynamics in the central Great Barrier Reef. II. Primary production. Cont Shelf Res 7:1049–1062

Furnas MJ, Mitchell AW (1997) Biological oceanography of the Great Barrier Reef. In: Proceedings of the Great Barrier Reef: Science, Use and Management National Conference, Townsville, 25–29 November 1996. Great Barrier Reef Marine Park Authority, Vol 1, p 75–87

Goldman JC, McCarthy JJ, Peavey DG (1979) Growth rate influence on the chemical composition of phytoplankton in oceanic waters. Nature 279:210–215

Gonzalez-Gil S, Keafer RVM, Jovine A, Aguilera A, Lu S, Anderson DM (1998) Detection and quantification of alkaline phosphatase in single cells of phosphorus-starved marine phytoplankton. Mar Ecol Prog Ser 164:21–35

Guillard RRL (1973) Division rates. In: Stein JR (ed) Handbook of phycological methods. Culture methods and growth methods. Cambridge University Press, Cambridge, p 289–311

Hallegraeff GM, Jeffrey SW (1984) Tropical phytoplankton species and pigments of continental shelf waters of north and north-west Australia. Mar Ecol Prog Ser 20:59–74

Hansen P, Bjornsen P, Hansen B (1997) Zooplankton grazing and growth: scaling within the 2–2000 micron body size range. Limnol Oceanogr 42:687–704

Harrison WG, Harris LR, Irwin BD (1996) The kinetics of nitrogen utilization in the oceanic mixed layer: nitrate and ammonium interactions at nanomolar concentrations. Limnol Oceanogr 41:16–32

Jackson GA (1980) Phytoplankton growth and zooplankton grazing in oligotrophic oceans. Nature 284:439–440

Jokiel PL, Hunter CA, Taguchi S, Watarai L (1993) Ecological impact of a fresh-water 'reef kill' in Kaneohe Bay, Hawaii. Coral Reefs 12:177–184

Landry MR, Haas LW, Fagerness VL (1984) Dynamics of microbial plankton communities: experiments in Kaneohe Bay, Hawaii. Mar Ecol Prog Ser 16:127–133

Landry MR, Constantinou J, Kirshtein J (1995a) Microzooplankton grazing in the Central Equatorial Pacific during February and August, 1992. Deep-Sea Res II, Top Stud Oceanogr 42:657–671

Landry MR, Kirshtein J, Constantinou J (1995b) A refined dilution technique for measuring the community grazing impact of microzooplankton, with experimental tests in the central equatorial Pacific. Mar Ecol Prog Ser 120: 53–63

La Roche J, McKay RML, Boyd P (1999) Immunological and molecular probes to detect phytoplankton responses to environmental stress in nature. Hydrobiologia 401: 177–198

Laws EA, Redalje DG, Haas LW, Bienfang PK, Eppley RW, Harrison WG, Karl DM, Marra J (1984) High phytoplankton growth and production rates in oligotrophic Hawaiian coastal waters. Limnol Oceanogr 29:1161–1169

Laws EA, DiTullio GR, Redalje DG (1987) High phytoplankton growth and production rates in the North Pacific subtropical gyre. Limnol Oceanogr 32:905–917

Lehman JT, Scavia D (1982a) Microscale nutrient patches produced by zooplankton. Proc Natl Acad Sci USA 79: 5001–5005

Lehman JT, Scavia D (1982b) Microscale patchiness of nutrients in plankton communities. Science 216:729–730

Lessard EJ, Swift E (1986) Dinoflagellates from the North Atlantic classified as phototrophic or heterotrophic by epifluorescence microscopy. J Plankton Res 8:1209–1215

Levasseur M, Thompson PA, Harrison PJ (1993) Physiological acclimation of marine phytoplankton to different nitrogen sources. J Phycol 29:587–595

Liston PW (1990). Spatial variability and the co-variability of chlorophyll and zooplankton on the Great Barrier Reef. PhD thesis, James Cook University of North Queensland

Liu H, Campbell L, Landry MR (1995) Growth and mortality rates of *Prochlorococcus* and *Synechococcus* measured with a selective inhibitor technique. Mar Ecol Prog Ser 116:277–287

Liu H, Nolla A, Campbell L (1997) *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical North Pacific Ocean. Aquat Microb Ecol 12:39–47

McCarthy J (1980) Nitrogen. In: Morris I (ed) The physiological ecology of phytoplankton. Blackwell Science, Oxford, p 195–233

McCarthy JJ, Goldman JC (1979) Nitrogenous nutrition of marine phytoplankton in nutrient-depleted waters. Science 203:670–672

McFeeters GA, Stuart DG (1972) Survival of coliform bacteria in natural waters: field and laboratory studies with membrane filter chambers. Appl Microbiol 24:805–811

Mousseau L, Legendre L, Fortier L (1996) Dynamics of size-fractionated phytoplankton and trophic pathways on the Scotian shelf and at the shelf break, northwest Atlantic. Aquat Microb Ecol 10:149–163

Nakao S (1994) Determination of pore size and pore size distribution 3. Filtration membranes. J Membr Sci 96: 131–165

Odum HT, Beyers RJ, Armstrong NE (1963) Consequences of small storage capacity in nanoplankton pertinent to measurement of primary production in tropical waters. J Mar Res 21:191

Olson RJ, Zettler ER, Durand MD (1993) Phytoplankton analysis using flow cytometry. In: Kemp PF, Sherr BF, Sherr EB, Cole JJ (eds) Handbook of methods in aquatic microbial ecology. Lewis Publishers, Boca Raton, p 175–186

Owens O, Dresler P, Crawford CC, Tyler MA, Seliger HH (1977) Phytoplankton cages for the measurement *in situ* of the growth rates of mixed natural populations. Chesapeake Sci 18:325–333

Palenik B, Henson S (1997) The use of amides and other organic nitrogen sources by the phytoplankton *Emiliania huxleyi*. Limnol Oceanogr 42:1544–1551

Parsons TR, Maita Y, Lalli CM (1984) A manual of chemical and biological methods for seawater analysis. Pergamon Press, Oxford

Raven JA (1986) Physiological consequences of extremely small size for autotrophic organisms in the sea. In: Plat T, Li WKW (eds) Photosynthetic picoplankton. Department of Fisheries and Oceans, Ottawa, p 1–70

Raven JA (1994) Why are there no picoplanktonic O_2 evolvers with volumes less than 10^{-19} m^3? J Plankton Res 16: 565–580

Raven JA (1998) The twelfth Tansley lecture—small is beautiful—the picophytoplankton. Funct Ecol 12:503–513

Reckermann M, Veldhuis MJW (1997) Trophic interactions between picophytoplankton and micro- and nanozooplankton in the western Arabian Sea during the NE monsoon 1993. Aquat Microb Ecol 12:263–273

Revelante N, Gilmartin M (1982) Dynamics of phytoplankton in the Great Barrier Reef Lagoon. J Plankton Res 4: 47–76

Ryle VD, Meuller HR, Gentian P (1981) Automated analysis of nutrients in tropical seawater. Oceanography Series Report, AIMS-OS-82–2, Australian Institute of Marine Science, Townsville

Sakshaug E, Jensen A (1978) The use of cage cultures in studies of the biochemistry and ecology of marine phytoplankton. Oceanogr Mar Biol Annu Rev 16:81–106

Scanlan DJ, Wilson WH (1999) Application of molecular techniques to addressing the role of P as a key effector in marine ecosystems. Hydrobiologia 401:149–175

Shalapyonok A, Olson RJ, Shalapyonok LS (1998) Ultradian growth in *Prochlorococcus* spp. Appl Environ Microbiol 64:1066–1069

Shanks A, Trent J (1980) Marine snow: sinking rates and potential role in vertical flux. Deep-Sea Res 27A:137–143

Solorzano L (1969) Determination of ammonia in natural waters by the phenolhypochlorite method. Limnol Oceanogr 14:799–801

Sorokin YI (1994) Role of plankton in the turnover of organic matter on the Great Barrier Reef, Australia. Hydrobiologia 308:35–44

Stolte W (1996) Size-dependent restrictions on competition for nutrients by marine phytoplankton. PhD thesis, Reijksuniversitet, Groningen

Tomas CR (ed) (1997) Identifying marine phytoplankton. Academic Press, San Diego

Treguer P, LeCorre P (1975) Manuel d'analyse des nutrififs dan l'eau de mer (Utilisation de l'Autoanalyzer II Technicon). Laboratoire d'Oceanologie Chemistrie, University de Bretagne Occidentale, Brest

Utermöhl H (1958) Zur Vervollkommnung der quantitativen phytoplankton-methodik. Mitt Int Ver Theor Angew Limnol 9:1–38

Vaulot D, Courties C, Partensky F (1989) A simple method to preserve oceanic phytoplankton for flow cytometric analyses. Cytometry 10:629–635

Vaulot D, Marie D, Olson RJ, Chisholm SW (1995) Growth of *Prochlorococcus*, a photosynthetic prokaryote, in the Equatorial Pacific Ocean. Science 268:1480–1482

Veldhuis MJW, Kraay GW, Vanbleijswijk JDL, Baars MA (1997) Seasonal and spatial variability in phytoplankton biomass, productivity and growth in the northwestern Indian Ocean—the southwest and northeast monsoon, 1992–1993. Deep-Sea Res Part I—Oceanogr Res Pap 44: 425–449

Venrick EL (1978) Estimating cell numbers. In: Sournia A (ed) Phytoplankton manual. UNESCO, Paris, p165–179

Waterbury JB, Watson SW, Valois FW, Franks DG (1986). Biological and ecological characterization of the marine unicellular cyanobacterium *Synechococcus*. In: Platt T, Li WKW (eds) Photosynthetic picoplankton. Department of Fisheries and Oceans, Ottawa, p 71–120

Editorial responsibility: William Li, Dartmouth, Nova Scotia, Canada

Submitted: November 1, 2000; Accepted: March 28, 2001
Proofs received from author(s): July 12, 2001